I, Zexin Qian, hereby submit this original work as part of the requirements for the degree of Master of Science in Chemical Engineering.

It is entitled:
The Impact of Humidity on an Optical Chemical Sensing Device for Non-invasive Exhaled Gas Monitoring

Student’s name: Zexin Qian

This work and its defense approved by:

Committee chair: Anastasios Angelopoulos, Ph.D.

Committee member: Jonathan Bernstein, M.D.

Committee member: Rakesh Govind, Ph.D.
The Impact of Humidity on an Optical Chemical Sensing Device for Non-invasive Exhaled Gas Monitoring

A thesis submitted to the Graduate School Of the University of Cincinnati in partial fulfillment of the requirements for the degree of Master of Science

In the Chemical Engineering Program of School of Energy, Environmental, Biological and Medical Engineering (College of Engineering and Applied Science)

BY

ZEXIN QIAN

B.S. Chemical Engineering, Sichuan University, Sichuan, Chengdu, China 2014

Committee Members:
Dr. Anastasios Angelopoulos (Chair)
Dr. Joanthan Bernstein
Dr. Rakesh Govind
Abstract

A novel technique for the detection of acetone in human breath was previously developed as a non-invasive medical diagnostic tool for potential use in the monitoring blood glucose level of diabetic patients. Perfluorosulfonic acid (PSA) polymer membrane, commercially available as Nafion® 117, was used as the heterogeneous catalyst for the condensation reaction of ambient acetone and imbibed immobilized resorcinol reagent. The condensation reaction product, a flavon compound, yielded a unique colorimetric response, which can be measured over time using UV/VIS spectroscopy. The response signal was significantly reduced by the presence of water vapor. This effect was hypothesized to be associated with the rapid deprotonation of the PSA groups in the high relative humidity condition as a result of losing the catalytic activity.

Previous studies have shown that by introducing a weak organic acid such as tiglic acid into the membrane prior to exposure, it is possible to preserve the catalytic activity of the PSA groups. The hypothesis is that the tiglic acid did not catalyze the condensation reaction itself, but will alter the local solvent environment of the acid. An increase in light absorbance due to the reaction was observed in the presence of 100% relative humidity as the concentration of the tiglic acid imbibed increased. Comparison of the light absorbance of exposed membrane after a fixed exposure time between different concentration of tiglic acid imbibed membranes under both ambient humidity and 100% relative humidity confirmed that membrane soaked into 50 g/L tiglic acid solution for 18 hours prior exposure to acetone can provide a similar absorbance signal. This was the first time this colorimetric response was shown to provide comparable light absorption under both ambient humidity and 100% relative humidity condition.

The focus of the research described this thesis was to develop an optical sensing device for real time detection in practical clinical applications. For clinical diagnostic purposes, the sensing system should be able to provide an appreciable signal immediately (which in monitoring patients breath should be less than 1 minute rather than 15 minutes exposure). In order to determine whether the condensation reaction previously described was able to provide a rapid response when exposed to acetone, an in-situ flow cell was used in this work. This system permitted controlled flow of a given concentration of acetone into a flow cell containing a membrane sample. Using the flow cell system, dynamic response of different concentration of
tiglic acid imbibed membrane exposure to both ambient humidity and 100% relative humidity was determined. The dynamic response obtained in this research confirmed that acid based membrane was able to provide us a signal within 100 seconds under both dry and wet condition. The results suggested that the acid based membrane can be used in uncontrollable humidity environment such as human breath but only in the kinetic-controlled region of acetone transport into the membrane.

As part of this work, evaluation and optimization of a bench-top prototype was conducted to evaluate the effects of temperature, flow rate and pre and post humidification of both membrane and sample chamber. A strong linear correlation (typically, $R^2 > 0.9$) of the optical signal from the device was observed at a given exposure to acetone at early times as long as steady humidification in the sample chamber was maintained. Steady humidification was inferred from the strong influence of flow rate (which affected pressure), temperature, and sample chamber pre-conditioning. The slope of this optical signal exhibited a strong linear correlation to the acetone concentration, $R^2 > 0.99$.

A small group of clinical trials were conducted assess the prototype response under real-world conditions. The exhaled breath of a small group of diabetic patients was monitored using the device during 30 to 60 seconds of exhalation. As in the case of constant acetone exposure, linear correlation (typically, $R^2 > 0.9$) was observed between the optical response and exposure at early times. Consistent with previous work that suggested correlation of breath acetone and blood glucose, we observed a linear correlation between the slope of the optical response and breath acetone concentrations and blood glucose concentrations for diabetics. Such excellent correlations motivate further research on uses of this device to assess the exhaled breath composition for various disease biomarkers.
Acknowledgements

I would like to express my sincere gratitude to Dr. Anastasios Angelopoulos, the most supportive professor, for giving me the opportunity to work in his research group. His dedication, his encouragement, his generosity and limitless patience in helping me at all times has contributed immensely in completion of this research work.

I would also like to thank Dr. Jonathan Bernstein for providing helps in clinical trials with diabetes patients, and with Dr. Rakesh Govind for serving in my committee and providing useful reviews and suggestions.

I am thankful to Dr. Adam Worrall for helping me got familiar with the previous works on the development of the optical sensing device. I am also equally thankful to Zhipeng Nan and my other lab mates for sharing their technical knowledge and providing technical assistance throughout the different stages of this work.

Thanks to my family for their unconditional support and motivation which helped me to remain focused on my studies.

Finally, Thanks to all my friends in Cincinnati and back in China, especially to Ce Gao, Chia-I Ko and Ting Yu, for their constant encouragement and for ensuring that I stay in good spirits during tough times.
# Table of Content

Abstract ........................................................................................................................................... i
Acknowledgements ........................................................................................................................... iv
Table of Content ............................................................................................................................... v
List of Figures .................................................................................................................................... vii
List of Tables ..................................................................................................................................... x

## Chapter 1 - Introduction .................................................................................................................. 1
1.1 General ......................................................................................................................................... 1
1.2 Development of optical chemical sensing device for diabetes monitoring............................... 1
1.3 Resorcinol and tiglic acid imbedded membrane for acetone detection in high relative humidity 19 ................................................................................................................................. 3
1.4 Resorcinol and tiglic acid imbedded membrane for acetone detection in high relative humidity 21 ................................................................................................................................................. 5
1.5 Optical sensing device for real time acetone detection ............................................................... 6
1.6 Objective of Study ....................................................................................................................... 7

## Chapter 2 - Materials and Methodology ...................................................................................... 9
2.1 Membrane Preparation ................................................................................................................ 9
2.1.1 12g/L resorcinol imbibed membrane preparation ................................................................. 9
2.1.2 Acid based membrane preparation ....................................................................................... 9
2.2 Experimental Methods ................................................................................................................ 10
2.2.1 Tiglic acid concentration calibration .................................................................................... 10
2.2.1.1 Absorbance determination of different concentration of tiglic acid in ethanol ........ 10
2.2.1.2 Absorbance determination of different concentration of tiglic acid in Nafion membrane ................................................................................................................................................. 10
2.2.2 Acetone exposure experiments ............................................................................................. 10
2.2.2.1 12g/L resorcinol imbibed membrane ............................................................................. 10
2.2.2.2 Different concentration of acid based membrane ......................................................... 11
2.2.3 Flow cell acetone exposure experiments ............................................................................ 11
2.2.4 Second Generation CRMAD prototype for real time acetone detection ......................... 12
2.2.4.1 Different relative humidity detection ............................................................................ 15
2.2.4.2 Different flow rate detection ....................................................................................... 15
2.2.4.3 Temperature variation experiments .......................................................... 15
2.2.4.4 Different acetone concentration detection .............................................. 15
2.3 Clinical test ........................................................................................................ 15

Chapter 3 – Results and Discussion .................................................................. 17
3.1 Tiglic acid concentration optimization .......................................................... 17
  3.1.1 Extinction coefficient of tiglic acid in ethanol ........................................... 17
  3.1.2 Determination of the membrane absorbance with different tiglic acid uptake.... 19
3.2 Acetone exposure at different humidity level .................................................. 20
  3.2.1 12 g/L resorcinol imbibed membrane 4ppmv acetone exposure ................. 20
  3.2.2 Acid based membrane 4ppmv acetone exposure ........................................ 21
  3.2.3 Effect of tiglic acid uptake ........................................................................ 23
3.3 Flow cell dynamic response study .................................................................. 28
  3.3.1 Acid based membrane dynamic study ....................................................... 29
  3.3.2 Control study .......................................................................................... 30
  3.3.3 Tiglic acid concentration effect ................................................................ 33
  3.3.4 Humidity impact on membranes after exposure ........................................ 36
3.4 Optical sensing device study ........................................................................... 39
  3.4.1 Effect of temperature ................................................................................ 40
  3.4.2 Pre and post treatment ............................................................................... 44
  3.4.3 Effect of flow rate ................................................................................... 45
  3.4.4 Sensing device acetone concentration testing ........................................... 47
3.5 Clinical breath analysis .................................................................................... 49

Chapter 4 – Conclusion and Future Works ......................................................... 52
4.1 Conclusions ....................................................................................................... 52
4.2 Future work ....................................................................................................... 53
Bibliography ........................................................................................................... 55
List of Figures

Fig. 1-1 Scheme of the condensation reaction of acetone and resorcinol\textsuperscript{21} ........................................... 3
Fig. 1-2 UV-vis spectra of resorcinol imbibed Nafion membrane prior and after exposure to 4ppmv acetone in a sealed flask........................................................................................................ 4
Fig. 1-3 Schematic of hypothesized equilibrium occurred inside the PSA clusters of the Nafion membrane and responsible for preservation of PSA catalytic activity in humid environments without or with tiglic acid\textsuperscript{19} ........................................................................................................... 5
Fig. 1-4 First generation CRMAD hand-held device\textsuperscript{21} ................................................................................. 6
Fig. 1-5 First generation CRMAD hand-held device ........................................................................................................ 7
Fig. 2-1 Schematic of the flow cell system used in determine the dynamic response......................................................... 12
Fig. 2-2 Second generation CRMAD prototype .................................................................................................................. 13
Fig. 2-3 Color measurement of the second generation CRMAD .......................................................................................... 14
Fig. 2-4 Windows based software HMI for 2nd generation prototype device ................................................................. 14
Fig. 3-1 UV/VIS spectra of different concentration of tiglic acid-ethanol solution ............................................................. 17
Fig. 3-2 Correlation about average UV/VIS absorbance maximum peak wavelength around 220 nm and different concentration of tiglic acid-ethanol solution......................................................................................... 19
Fig. 3-3 UV/VIS spectra of 50g/L tiglic acid imbibed membrane before and after 24h extraction in ethanol................................................................................................................................. 19
Fig. 3-4 UV/VIS spectra of a membrane exposed to different concentration of tiglic acid-mineral oil solution for 18 hours....................................................................................................................... 20
Fig. 3-5 UV/VIS spectra of 12 g/L resorcinol imbibed membrane 4ppmv acetone exposure at different humidity level......................................................................................................................... 21
Fig. 3-6 UV/VIS spectra of acid based membrane and resorcinol imbibed membrane ....................................................... 22
Fig. 3-7 UV/VIS spectra for membranes exposed to 4ppmv acetone with and without tiglic acid (TA) and in the presence of ambient humidity or 100% relative humidity ........................................... 23
Fig. 3-8 UV/VIS spectra of different concentration of TA imbibed membranes exposed to 4ppmv acetone at 100% relative humidity.......................................................................................................... 24
Fig. 3-9 Absorbance of different concentration of tiglic acid imbibed membrane exposed to 4ppmv acetone in the presence of 100% relative humidity at wavelength at 400 nm (Error bars indicates +/- on standard deviation.) ......................................................................................................................... 25
Fig. 3-10 UV/VIS spectra of different concentration of TA imbibed membranes exposed to 4ppmv acetone at ambient relative humidity ....................................................................................................... 26
Fig. 3-11 UV/VIS spectra for membranes treated with 50 g/L tiglic acid exposed to 4ppmv acetone at ambient humidity and 100% relative humidity after 15 minutes................................................................. 27
Fig. 3-12 Comparison of absorbance of different concentration of tiglic acid treated membranes exposed to 4ppmv acetone in ambient humidity and 100% relative humidity .................. 28

Fig. 3-13 Dynamic response at wavelength 400.69 nm of acid based membrane exposed to 4ppmv acetone under ambient humidity and 100% relative humidity for 15 minutes .................. 29

Fig. 3-14 Dynamic response at wavelength 400.69 nm of resorcinol imbibed membranes exposed to 4ppmv acetone under ambient humidity and 100% relative humidity for 15 minutes .................. 31

Fig. 3-15 Dynamic response at wavelength 400.69 nm of tiglic acid imbibed membrane exposed to 4ppmv acetone under ambient humidity and 100% relative humidity for 15 minutes .................. 32

Fig. 3-16 Dynamic response at wavelength 400.69 nm of nafion membrane with no immobilization exposed to 4ppmv acetone under ambient humidity and 100% relative humidity for 15 minutes ............................................................................................................ 33

Fig. 3-17 Dynamic response at wavelength 400.69 nm of different concentration of tiglic acid treated membranes exposed to 4ppmv acetone under 100% RH for 15 minutes .................. 34

Fig. 3-18 Dynamic response at wavelength 400.69 nm of different concentration of tiglic acid treated membranes exposed to 4ppmv acetone under Room RH for 15 minutes .................. 34

Fig. 3-19 Dynamic response at wavelength 510.73 nm of different concentration of tiglic acid treated membranes exposed to 4ppmv acetone under 100% RH for 15 minutes .................. 35

Fig. 3-20 Dynamic response at wavelength 510.73 nm of different concentration of tiglic acid treated membranes exposed to 4ppmv acetone under 4% RH for 15 minutes .................. 36

Fig. 3-21 UV/VIS spectra of the dynamic response of 100% relative humidity and ambient humidity exposure cycle in 100 s interval on 4ppmv acetone exposed acid based membrane at absorbance wavelength at 400.69nm .................................................................................................................. 37

Fig. 3-22 Hypothesized reaction mechanism for synthesis of 4-(3,4-Dihydro-7-hydroxy-2,4,4-trimethyl-2H-1-benzopyran-2-yl)-1,3-benzenediol from acetone and resorcinol22 .................. 38

Fig. 3-23 UV/VIS spectra of the dynamic response of 100% relative humidity and ambient humidity exposure cycle in 100 s interval on 4ppmv acetone exposed acid based membrane at absorbance wavelength at 510.73nm .................................................................................................................. 39

Fig. 3-24 UV/VIS spectra of acid based membrane exposed to 0ppmv and 4ppmv acetone under ambient and 100% RH at 40 °C ........................................................................................................... 41

Fig. 3-25 UV/VIS spectra of acid based membrane exposed to 0ppmv and 4ppmv acetone under ambient and 100% RH at 60 °C ........................................................................................................... 42

Fig. 3-26 UV/VIS spectra of acid based membrane exposed to 0ppmv and 4ppmv acetone under ambient and 100% RH at 80 °C ........................................................................................................... 43

Fig. 3-27 Comparison of device signal of acid based membrane exposed to pure nitrogen and 100% relative humidity air purge cycle in a 30s interval and with no purge .................. 44

Fig. 3-28 Comparison of device signal of Nafion membrane with no additive exposed to pure nitrogen and 100% relative humidity air purge cycle in a 30s interval and with no purge ....... 45

Fig. 3-29 Comparison of device signal of acid based membrane exposed to 100% relative humidity air inlet with different flow rate .................................................................................................................. 46
Fig. 3-30 Correlation of device signal and 2ppm acetone exposure time. Error bar indicate ± one standard deviation from the data collected ................................................................. 47

Fig. 3-31 Slope of the response of the device containing acid based membranes to various concentration of acetone during 50 seconds. Error bar indicate ± one standard deviation from the data collected ........................................................................................................... 48

Fig. 3-32 Patient 1 test result. Correlation of device signal and patient's breath time. Error bars indicate ± one standard deviation from the data collected ................................................................. 49

Fig. 3-33 Correlation of slope of device signal change during patients’ breath and the blood glucose of the subject. Error bars indicate ± one standard deviation from the data collected ...... 50

Fig. 3-34 Correlation of slope of device signal during patients’ breath and the blood glucose of the subject. Error bars indicate ± one standard deviation from the data collected ................. 51
List of Tables

Table 3-1 UV/VIS absorbance of different tiglic acid-ethanol solution around 220 nm............ 18
Table 3-3 Blood glucose information from diabetic patients used in human breath testing ........ 49
Chapter 1 - Introduction

1.1 General

Diabetes is one of the most common, noninfectious chronic diseases threatening human health and modern medicine has no cure for it. In 2015, one in 11 adults has diabetes and the number will rise to one in 10 adults in 2040.\(^1\) It is estimated by International Diabetes Federation that 193 million people with diabetes are undiagnosed and are therefore more at risk of developing complications. Patients need to take medicines punctually and keep monitoring blood glucose level to maintain a healthy life. Nowadays diagnostic or monitoring methods are based on invasive blood sampling and will cause certain pain to patients and may lead to cross infections.

The exhaled gas exchange in the blood and air interface in the lungs has been considered as the top air of blood, which can reflect the metabolism of human body in some extent. Human breath is a mixture of nitrogen, oxygen, carbon dioxide, water vapor, and inert gases. The rest fraction consists of more than 1000 volatile compounds that either endogenous or exogenous.\(^2\) Some of the endogenous compounds including inorganic gases and volatile organic compounds (for example acetone, ethanol, pentane) can be used as biomarker to aid in the diagnosis of diseases.\(^3\) In recent years, breath analysis has become one of the least invasive and painless technique attracting more and more attention and has been used as a tool for clinical early diagnosis and monitoring of many diseases.

For diabetic patients, their body cannot produce or use insulin properly. Insulin is known as a hormone that converts sugar and food into energy needed for daily life. In response to a lack of insulin, diabetic patients will burn of fatty acids into ketone bodies in the liver to act as an additional energy source. One of the characteristics of diabetic ketoacidosis is the accumulation of acetoacetate (AcAc), beta-hydroxybutyrate (\(\beta\)-OHB) and acetone. \(\beta\)-OHB reflects the ketoacidosis level in blood and AcAc in urine. Produced acetone will travel through the blood and is excreted through either urine or exhaled breath.\(^4\)

1.2 Development of optical chemical sensing device for diabetes monitoring

The first quantitative technique suitable for measuring acetone in diabetic patients’ breath was described in 1898 by Muller.\(^5\) And in 1920, Hubbard\(^6\) and Widmark\(^7\) successfully measured the concentration of acetone in the breath of normal humans. In 1964, gas chromatography with
flame ionization detection was applied to breath acetone measurements by Levery, Stewart and Boettner.

Since 1964, more and more investigators have applied breath acetone measurements. Gas chromatography-mass spectrometry (GC-MS) has been proven to be an effective breath analysis technique. But in order to improve the accuracy and sensitivity of determination, pre-concentration of exhaled breath will be needed. However, these sample preparation procedures were tedious and time consuming. To overcome the problem, a method using GC-MS and solid-phase microextraction (SPME) with on-fiber derivatization has been developed to determine acetone in human breath.

Selected ion flow tube mass spectrometry (SIFT-MS) has shown great potential in real time monitoring the concentration of few breath biomarkers such as acetone and ammonia. SIFT-MS shows high selectivity, sufficient sensitivity and low limit of detection. However, the high cost and low portability make it less possible to become a standard diagnostic tool.

With the potential for highly sensitive absorption measurements, cavity ring down spectroscopy (CRDS) is a powerful technique for detecting trace gas species. And has been reported to be used in studying the breath acetone analyzer for diabetes monitoring. However, the requirement of laser systems and high reflectivity mirrors always makes CRDS very expensive.

All previously mentioned breath analysis methods have been shown to be accurate in the measurement of concentration of acetone in breath samples. Although these methods are effective, the extensive equipment and required training for using limit their function only in hospital or research center. Recently, an optical approach using metalloporphyrin derivatives has been demonstrated in characterizing different VOCs.

More recent solid oxide potentiometric techniques have been developed by Pratsinis. The chemo-resistive metal oxide gas sensor in the report rely on changes of electrical conductivity due to a change in the surrounding atmosphere. A portable prototype breath sampler was developed by integrating a Si:WO$_3$ based acetone sensor. However, these require substantial time to develop appreciable signal and are thus not suitable for real time application. Furthermore, inconsistent results have been observed. Thus, dynamics of the response need more careful analysis.
1.3 Resorcinol and tiglic acid imbedded membrane for acetone detection in high relative humidity.\textsuperscript{19}

The ideal real time breath analysis sensor must have some of their characteristics to be modified and optimized during the synthesis and processing. The first requirement is that the sensor should have a high sensitivity to the low concentrations of the detecting gases that are present in the breath, ranging from ppt to ppm. Second, the sensor should be targeting a specific analyte (biomarker) due to the large amount of similar compounds present in the breath. Third, the sensor has to be able to work at the high relative humidity of the breath which is about 90% RH. Last but not least, to achieve real time monitoring, the sensor needs to exhibit rapid response for onsite measurements.

In a previous study, Prof. Angelopoulos’ group demonstrated a sensing mechanism involves chemical reaction between a dye molecule immobilized within a polymer membrane catalyst and VOCs can be used in real time detection.\textsuperscript{20} The acid catalyzed Fridel-Crafts acylation of toxic anhydrides with immobilized resorcinol was shown to produce quinones which shows highly optical characteristics selectivity in the visible region of electromagnetic spectrum. Perfluorsulfonic acid (PSA) polymer, Nafion\textsuperscript{®} 117, was used as the heterogeneous solid acid catalyst. Resorcinol was used as the immobilized reagent molecule to produce a flavan compound as the condensation product of the reaction with acetone according to the scheme shown in Figure 1.1.\textsuperscript{21}

\textbf{Fig. 1-1 Scheme of the condensation reaction of acetone and resorcinol\textsuperscript{21}}

Resorcinol in solution with ethanol shows two characteristic peaks in the UV absorption region at 219.38 nm and 271.01 nm wavelength as shown in figure 1.2.\textsuperscript{20} The higher intensity (at 219.38
nm) attains signal saturation very quickly; therefore, the lower intensity peak (at 271.01 nm) can be best used to quantify the resorcinol concentrations in the membrane.

In the previous study in Dr. Angelopoulos’ group, the condensation reaction was found to be mass transport limited, and the light absorbance response is being driven by the uptake of acetone into the membrane followed by the rapid reaction of the acetone and resorcinol to form the colored product. The reaction product can provide us with a unique colorimetric response that can be measured using UV-vis spectroscopy in real time. Upon exposure to acetone, a very noticeable signal can be observed at the near UV to visible range (380-420 nm). A strong visible response was recorded at 400.69 nm upon exposure to acetone as shown in Figure 1.2 due to the formation of the flavan product.

![UV-vis spectra of resorcinol imbibed Nafion membrane prior and after exposure to 4ppmv acetone in a sealed flask](image)

However, the response signal was found to be significantly compromised with the presence of water vapor. This is hypothesized to be caused by the rapid deprotonation of the PSA groups that results the loss of activity of the catalyst.
1.4 Resorcinol and tiglic acid imbedded membrane for acetone detection in high relative humidity.\textsuperscript{21}

Previous work has shown that by introducing a weak organic acid, trans-2-Methyl-2-buenoic acid (tiglic acid) into membrane prior to exposure can effectively preserve the catalytic activity of the PSA group. The appearance of water can create a heterogeneous gas-solid-liquid catalyst system. And as the ionization of acids is known to be lower in acidic solvents than in water \textsuperscript{23}, the presence of tiglic acid in the membrane can increase the pKa of the PSA group. Without tiglic acid, as shown in Figure 1.3(a), PSA is a super acid that can be readily deprotonated in the presence of water that can no longer be used in catalyzing the condensation of resorcinol and acetone in its reaction sites. With tiglic acid, as shown in Figure 1.3(b), PSA is a weak acid with enough protonation to catalyze the condensation reaction.

\textit{Fig. 1-3 Schematic of hypothesized equilibrium occurred inside the PSA clusters of the Nafion membrane and responsible for preservation of PSA catalytic activity in humid environments without or with tiglic acid}\textsuperscript{19}
1.5 Optical sensing device for real time acetone detection

In 2013, Mound Technical Solutions was commissioned to automate the data acquisition and control features of the first generation prototype of the Chromatically Responsive Membrane Analysis Device (CRMAD). This first prototype was a hand held device as shown in figure 1.4.

![First generation CRMAD hand-held device](image)

Added to this portable unit was a tethered data acquisition and control box that connected to a PC to present the human machine interface (HMI) and log data for record and future analysis. The software also provided management of test protocols of time duration and membrane chamber temperature as well as real time display of RGB color measurement.

The first generation CRMAD measures each RGB light intensity through membrane. Three photodiodes were placed inside the device, as shown in figure 1.5, each of the three photodiodes absorbs photons and generates a proportional current. A small amount of current is also produced when no light is present. Each photodiode reacts individually to photons received. And each photodiode receives photons from a unique red, green and blue LED.
1.6 Objective of Study

Although the previous studies have demonstrated the benefit of using TA for optical detection in humid environments, only the equilibrated response was investigated in detail. Specifically, a hold time of 15 minutes was used to allow full development of the signal. However, in any practical, real time clinical application, breath exhalation will typically not exceed 1 minute. Furthermore, while TA permitted signal observation under 100% RH conditions, maintaining full humidification will likely be difficult at the reaction temperature (60°C) in real world device applications. Consequently, understanding the dynamic response of the sensing element both ex-situ (using laboratory-scale UV/Vis apparatus, well defined RH and acetone levels) and in-situ (using the clinical device previously described and more ill-defined acetone and RH levels in human breath) is essential. Developing such understanding is the objective of my thesis research.

Introducing tiglic acid into the resorcinol imbedded membrane prior exposure to acetone will provide mitigation of interference of humidity in human breath. In this work the dynamic (or time dependent) response will be investigated during actual patent use.

The condensation reaction temperature of resorcinol and acetone is at 60°C. Human breath has relatively high humidity, but at 60°C, it is hard to be measured accurately. Hence it is important to find the proper concentration of tiglic acid that can provide us with close UV-vis signal after exposure to acetone at both room and 100% relative humidity. In order to achieve real time monitoring diabetes using our optical sensing device, the interference from the appearance of water in humid environment need to be ruled out.
With the help from Mound Technical Solutions, we were able to developed a second generation CRMAD prototype. In this work, bench top tests will be conducted to optimize the device in order to find the suitable parameters affecting the dynamic response. These include temperature, flow rate, and prototype pre-conditioning under specific humidity condition. Clinical trials will also be conducted to determine the prototype response in real-world applications. Based on the work of previous investigators, a correlation of diabetes patients’ blood glucose level with the optical signal from our prototype was attempted.

The specific objectives are:

- Optimize the Tiglic Acid concentration for detection of acetone concentration under high humidity environments.
- Determine the dynamic response of PSA membrane sensing element.
- Determine the key parameters (flow rate, temperature, pre-conditioning) on the response from optical sensing prototype device.
- Clinical test of the optical sensing device prototype with diabetes patients.
Chapter 2 - Materials and Methodology

2.1 Membrane Preparation

2.1.1 12g/L resorcinol imbibed membrane preparation
Resorcinol was imbibed into PSA membrane as a dye molecule. A 12g/L dye solution was made by dissolving resorcinol (Acros Organics, 98%) into ethanol (Acros Organics, ≥95% purity, ACS spectrophotometric grade). This concentration of the dye solution is well below the solubility limit for resorcinol, which is 58.4 wt% to 61 wt% at 20 °C. 24

Nafion membrane (The Fuel Cell Store, Nafion 117, protonated perfluorosulfonic membrane, 0.007 in. thick) was cut into the dimensions of 2cm width x 0.6cm length x 0.0178cm thickness, and will be maintained constant for all measurements. Resorcinol dye was imbibed into PSA membrane by immersing Nafion membrane into the 12g/L dye solution for 31 minutes. The membranes were rinsed with Deionized (DI) water. The DI water was obtained by passing the house water supply through a Millipore Synthesis unit until 18.2 MΩ cm resistivity was achieved. Then the membranes were put into a dish for air drying at room temperature.

2.1.2 Acid based membrane preparation
Acid solution was made by dissolving tiglic acid (Sigma-Aldrich, >98%) in food grade mineral oil (Howard). Mineral oil was utilized to avoid loss of the resorcinol in the membrane during the organic acid uptake.

The dried resorcinol imbibed membranes were soaked in the acid-oil solution for 18 hours. Then the excess oil solution was rinsed off and the membrane were placed into a dish allowed to air dry.
2.2 Experimental Methods

2.2.1 Tiglic acid concentration calibration

2.2.1.1 Absorbance determination of different concentration of tiglic acid in ethanol

0.00117g/L, 0.00585g/L, 0.0117g/L, and 0.02925g/L tiglic acid-ethanol solutions were made by dissolving different amount of tiglic acid in ethanol at room temperature. UV/VIS spectrometer (Ocean Optics HR 2000+ CG-UV-NIR High Resolution Spectrometer, 1cm path length through a quartz cuvette) was employed for ex-situ determination of the absorbance of the solutions. Three sets of sample solutions were prepared to provide average absorbance at each concentration.

The characteristic absorbance peak associated with tiglic acid in the acid-ethanol solution spectra is at 225 nm.

2.2.1.2 Absorbance determination of different concentration of tiglic acid in Nafion membrane

Different concentration of tiglic acid-mineral oil solutions were made by dissolving different amount of tiglic acid in mineral oil. Nafion membranes were then soaked in the acid-oil solutions at room temperature for 18 hours.

After rinsed off the excess oil and the air drying, the absorbance of different concentration of tiglic acid imbibed membranes was determined ex-situ by UV/VIS.

2.2.2 Acetone exposure experiments

2.2.2.1 12g/L resorcinol imbibed membrane

The resorcinol imbibed membranes were suspended in a sealed round bottom flask with 4ppmv acetone (Acros Organics, ACS spectroscopic grade, ≥99%). For samples in the presence of water, an additional amount of DI water was added to the flask using a gas-tight syringe to reach the desired relative humidity for the experiment. Ambient conditions were measured prior to addition of water to account for the water content of the air in the flask ensuring that the proper relative humidity level was present in the flask when heated. As the condensation reaction of resorcinol and acetone happens at 60°C. 4%, 25%, 75% and 100% RH in the flask at 60°C were used in the experiment. The flask was immersed in a water bath at 60°C for 15 minutes. After the
allotted equilibration time for the exposure, the membrane was removed and the signal response was observed ex-situ using the UV/VIS.

2.2.2.2 Different concentration of acid based membrane

In order to provide a more stable exposure environment for the condensation reaction, they flow cell system has been used during the acetone exposure of different concentration of acid based membranes. The scheme of the flow cell system is shown in figure 2.1. Different concentration of acid based membrane was placed in a holder inside the sealed quartz cuvette maintained at 60°C. Acetone vapor with the concentration fixed at 4ppmv under ambient and 100% relative humidity will be continuous applied to the system for 15 minutes. Then the absorbance of the exposed membranes will be determined by using the UV/VIS.

2.2.3 Flow cell acetone exposure experiments

An in-situ flow cell system allowing monitoring of the UV/VIS spectrum during exposure of the membrane has been constructed for the determination of the dynamic response of the sensing element. The membrane was placed in a holder inside the sealed quartz cuvette maintained at 60°C. Acetone vapor with different relative humidity levels were flowed continuously through the quartz chamber allowing a constant concentration exposure to the membrane. UV/VIS was acquired during the exposure at 10s intervals to provide the evolution of the visible region as 4ppmv of acetone is exposed to the membrane for 15 minutes.
Control experiments of 12g/L resorcinol, 50g/L tiglic acid, 12g/L resorcinol + 50g/L tiglic acid imbibed membranes were used in the flow cell system to determine the dynamic response at both ambient humidity and 100% relative humidity level with 4ppmv acetone continuously flowing through the membrane.

2.2.4 Second Generation CRMAD prototype for real time acetone detection
In 2014, Mound Technical Solution was commissioned to develop a second generation CRMAD device, as shown in figure 2.2, to provide better sample management, wider exposure temperature range, flow measurement and improved membrane color measurement with enhancement of both reproducibility and precision.
This second generation CRMAD device prototype based on reflecting white color measurement into RGB, as shown in figure 2.3. The camera inside the device can record an image by collecting photons in Red, Greed and Blue sites. The more photons collected, the higher the intensity for that red, green or blue site. Each pixel on the sensor has a color filter that only lets in one color. And captured image only records the brightness of the red, green and blue pixels separately. Then the camera’s processor calculates, or interpolates the actual color of each pixel by looking at the brightness of three colors recorded by it and others around it. Only the response in blue region will be observed in the experiments as the absorbance peak wavelength for the blue LED residing at near UV to visible range (380-430 nm). The absorbance of the exposed membrane can be instantaneously measured. This device is an improvement over that used in previous research in our lab in that it permits evaluation and storage of a wider range of parameters, for example, flow rate, temperature, dynamic response.
This second generation CRMAD device is a table top device that is tethered to a PC which provides the HMI and data recording functions. The software also provided management of test protocols of time duration and membrane chamber temperature as well as real time display of RGB color measurement. The software interface is depicted in figure 2.4.

The sample of a person’s exhaled breath is provided via a standard soda straw attached to the sample inlet at the front of the device. The sample membrane is also installed at the front panel. The second generation device was purposefully made larger than the first generation device to allow for the addition of added features such as flow measurement, higher temperature and better control of temperature, and enhanced color measurement all using commercial off the shelf (COTS) components. This enabled cost reduction, ability to modify, and evaluation of technology response to numerous variations in operating conditions.
2.2.4.1 Different relative humidity detection

Acid based membranes were prepared for the detection experiments. Two sets of detection experiments were performed using bare Nafion membranes and acid base membranes at 60°C. Pure Nitrogen and 100% relative humidity air inlet cycle was used to purge through the membrane, the time interval of each relative humidity level inlet continued for around 30s, and with just the membrane sitting in the prototype chamber for 3 minutes as comparison.

2.2.4.2 Different flow rate detection

Acid based membranes were prepared for the detection experiments. As the flow rate will impact pressure and further influence the relative humidity in the sample chamber, four sets of detection experiments with different flow rates were performed to determine the desired detection flow rate. 100% relative humidity inlet air with flow rate of 1.2-1.8 LPM, 1.8-2.2 LPM, 2.2-2.8 LPM, and 2.8-3.2 LPM were used in purging through the prototype chamber for 10 minutes.

2.2.4.3 Temperature variation experiments

Acid based membranes were prepared for the detection experiments. Three sets of 4ppmv acetone exposure flask experiments have been conducted at 40 °C, 60 °C and 80 °C for 15 minutes. The membranes after exposure have been tested under UV/VIS to determine the light absorbance.

2.2.4.4 Different acetone concentration detection

Acid based membranes were prepared for the detection experiments. Five sets of detection experiments using 0ppm, 2ppm, 4ppm, 6ppm, and 8ppm acetone flow with pre and post humidification treatment were performed to determine the correlation of device signal and the acetone concentration.

2.3 Clinical test

In order to correlate acetone concentrations in exhaled breath with blood glucose measurements in diabetic patients. Patients with a history of physician diagnosed diabetes mellitus (DM) were randomly recruited to participate in this study from a large community medical practice. After signing an informed consent approved by the University of Cincinnati Institutional Review
Board, each patient was asked to breathe into the prototype of our sensing device for 50s after 3 minutes pre humidification treatment of the membrane, and followed by 3 minutes post humidification treatment of the membrane. Blood glucose measurements were obtained by the nurses in the clinic using a One Touch Ultra 2 blood glucometer (Lifescan Inc.).
Chapter 3 – Results and Discussion

3.1 Tiglic acid concentration optimization

3.1.1 Extinction coefficient of tiglic acid in ethanol

There is one peak associated with the presence of tiglic acid in the solution spectra at 220 nm as shown in Figure 3.1. In figure 3.1, we note that the wavelength of the absorbance peak shifts from 215 nm to 220 nm. This bathochromic shift is associated with a change in the polarity of the environment.

Calibration of the peak intensity was used to quantify the solution concentrations in subsequent extraction studies to permit application to a broader range of concentrations without signal saturation. Table 3.1 and Figure 3.2 shows the correlation of the absorbance value at wavelength at 225 nm and the tiglic acid concentration in solution. The maximum peak shift was caused by the change of the concentration in the solution, which indicated a change of the environment. The extinction coefficient of tiglic acid in the solution is determined by using the Beer-Lambert Law expression given by:

\[ A = \varepsilon \cdot l \cdot C \]
Where $A$ is the absorbance, $C$ is the molar concentration of absorbing species (mol), $l$ is the path length of the membrane (m) and $\varepsilon$ is the extinction coefficient of the absorber (m$^2$/mol).

To determine the extinction coefficient, the unit calculation is:

$$\varepsilon = \frac{A}{l \times C} = \frac{1}{1 \text{cm} \times \text{mol/L}} = \frac{1}{10^{-2} \text{m} \times \frac{\text{mol}}{10^{-3} \text{m}^3}} = 0.1 \text{ m}^2/\text{mol}$$

The absorbance of the different concentration of the tiglic acid-ethanol solutions are listed in table 3.1.

<table>
<thead>
<tr>
<th>Conc. (M)</th>
<th>Abs 1</th>
<th>Abs 2</th>
<th>Abs 3</th>
<th>Ave Abs</th>
<th>STDEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.17E-05</td>
<td>0.081</td>
<td>0.071</td>
<td>0.078</td>
<td>0.076667</td>
<td>0.005132</td>
</tr>
<tr>
<td>5.84E-05</td>
<td>0.354</td>
<td>0.366</td>
<td>0.361</td>
<td>0.360333</td>
<td>0.006028</td>
</tr>
<tr>
<td>0.000117</td>
<td>0.679</td>
<td>0.685</td>
<td>0.646</td>
<td>0.67</td>
<td>0.021</td>
</tr>
<tr>
<td>0.000292</td>
<td>1.426</td>
<td>1.426</td>
<td>1.448</td>
<td>1.433333</td>
<td>0.012702</td>
</tr>
</tbody>
</table>

*Table 3.1 UV/Vis absorbance of different tiglic acid-ethanol solution around 220 nm*

Extinction coefficient has meaning only if Beer-Lambert Law is obeyed. The highest concentration shown in the table 3.1 deviates from Beer-Lambert Law linearity and need to be removed. One possible source of this deviation is signal saturation. From table 3.1 we found the correlation of the solution absorbance and the solution concentration as shown in figure 3.2. A strong linear correlation can be found in the result figure, with $R^2=0.9967$ and yielded a slope of 5826, therefore an extinction coefficient can be found as 582.6 (m$^2$/mol).
Correlation about average UV/VIS absorbance maximum peak wavelength around 220 nm and different concentration of tiglic acid-ethanol solution

3.1.2 Determination of the membrane absorbance with different tiglic acid uptake

Figure 3.3 shows the UV/VIS spectra of a membrane originally exposed to 50g/L tiglic acid solution before and after extraction as well as the spectra of the solution into which tiglic acid was extracted (Nafion membrane as background has been subtracted). From Figure 3.3 we note that after 24 hours soaked in ethanol, imbibed tiglic acid has been completely extracted.

Fig. 3-3 UV/VIS spectra of 50g/L tiglic acid imbibed membrane before and after 24h extraction in ethanol
Figure 3.4 shows the UV/VIS spectra of a membrane exposed to 0.1 g/L, 1 g/L, 10 g/L, 25 g/L, 50 g/L and 80 g/L tiglic acid-mineral oil solution at room temperature for 18 hours. During the experiment, we noticed that concentration of tiglic acid in mineral oil substantially greater than 50 g/L has reached the saturation limit. And from the figure we can observe the absorbance of membranes treated with 50 g/L and 80 g/L tiglic acid solutions are close to each other indicating the membrane uptake of tiglic acid has reached to a maximum value. TA absorption from mineral oil solution was performed at room temperature.

![UV/VIS spectra of a membrane exposed to different concentration of tiglic acid-mineral oil solution for 18 hours](image)

*Fig. 3-4 UV/VIS spectra of a membrane exposed to different concentration of tiglic acid-mineral oil solution for 18 hours*

A previous study in Dr. Angelopoulos’s group on the determination of the partition coefficient of tiglic acid in the membrane indicated a one-to-one partitioning between the membrane and solution phases.\(^{22}\)

### 3.2 Acetone exposure at different humidity level

#### 3.2.1 12 g/L resorcinol imbibed membrane 4ppmv acetone exposure

12g/L resorcinol imbibed membranes have been exposed to 4ppmv acetone in a sealed flask at relative humidity level of 4% RH, 25% RH, 75% RH and 100% RH at 60 °C. Figure 3.5 shows the UV/VIS spectra of the membrane prior and after exposure to acetone at each humidity level.
From figure 3.5, we note that with the increasing relative humidity level of the exposure environment, a significant drop of the signal at absorbance peak wavelength at 400 nm can be observed. The signal at 400.69 nm and the broad UV peak to the immediate left is significantly diminished at 25% RH. The signal is reduced to that prior to acetone exposure at relative humidity greater than or equal to 75%. This signal drop can be explained by the hypothesis proposed in section 1.4. The appearance of water will cause the deprotonation of the membrane catalyst and further impeding the condensation reaction of resorcinol and acetone at its reaction site. The two sharp peaks in the UV region to the far left are associated primarily with the immobilized resorcinol reagent and, being present well in excess of the acetone concentration and flavan product, change only slightly.

![Fig. 3-5 UV/VIS spectra of 12 g/L resorcin imbibed membrane 4ppmv acetone exposure at different humidity level](image)

**3.2.2 Acid based membrane 4ppmv acetone exposure**

Tiglic acid has been used to immobilize into the ionic clusters of the resorcinol imbibed PSA membrane in order to mitigate the water interference on heterogeneous catalysis in gas-solid systems. Figure 3.6 shows the UV/VIS spectra of 50 g/L tiglic acid + 12 g/L resorcinol and 12 g/L resorcinol imbibed membrane.
4ppmv acetone exposure experiments have been conducted on both acid based membrane and resorcinol imbibed membrane. Figure 3.7 plots the spectra of 12 g/L resorcinol imbibed membrane with and without tiglic acid exposure at 100% RH level for 15 minutes. Exposure of resorcinol imbibed membrane at ambient humidity environment was used as comparison.

From figure 3.7 we can also observe an absorbance peak at wavelength at 510.73 nm from the spectra of membrane with tiglic acid. As tiglic acid has been imbibed into the membrane, this peak is hypothesized to associate with the dimerization of tiglic acid.
3.2.3 Effect of tiglic acid uptake

From section 3.2.1 we have confirmed that without tiglic acid, resorcinol imbibed membranes showed no response under 100% RH when exposed to 4ppmv acetone. However, human breath consists of a significant concentration of water vapor that will impeding the application of the dye immobilized membrane to be used in clinical diagnosis without humidification of the breath sample. The acid based membrane should be able to provide same signal under any relative humidity level.

The effect of tiglic acid concentration on the condensation reaction of resorcinol and acetone is investigated by increasing the tiglic acid concentration from 0.1 g/L to 50 g/L. 4ppmv acetone exposure experiments have been conducted using different concentration of tiglic acid imbibed membranes under ambient humidity and 100% relative humidity environment. Figure 3.8 illustrates the UV/VIS spectra of acid based membranes that have been soaked in 0.1 g/L, 1 g/L, 10 g/L, 25 g/L and 50 g/L for 18 hours exposed to 4ppmv acetone for 15 minutes. From the plots, it is clear that there is a strong statistically significant increase in the absorbance response as the concentration of tiglic acid present in the membranes is increased. The absorbance at
wavelength at 400 nm increased from around 0.5 of 0.1 g/L tiglic acid treated membrane to around 1.9 of 50 g/L tiglic acid treated membrane. This observation illustrated that with the increasing concentration of tiglic acid imbibed into the membrane will cause the increasing of the signal at absorbance peak wavelength of 400 nm due to the higher uptake of tiglic acid in the membrane.

![UV/VIS spectra of different concentration of TA imbibed membranes exposed to 4ppmv acetone at 100% relative humidity](image)

*Fig. 3-8 UV/VIS spectra of different concentration of TA imbibed membranes exposed to 4ppmv acetone at 100% relative humidity*

Figure 3.11 plots the absorbance value for membranes with different concentration of tiglic acid uptake from figure 3.9. The absorbance is the average value of two sets of exposure experiments with each concentration of tiglic acid treated membranes. The error bar analysis indicates the positive and negative on standard deviation.
Fig. 3.9 Absorbance of different concentration of tiglic acid imbibed membrane exposed to 4ppmv acetone in the presence of 100% relative humidity at wavelength at 400 nm (Error bars indicates +/- on standard deviation.)

4ppmv acetone exposure experiments under ambient humidity have been conducted as comparison set. Figure 3.10 illustrates the UV/VIS spectra of the absorbance of the same concentration of tiglic acid imbibed membranes after 15 minutes exposure under ambient humidity environment. We note that signals at absorbance around 1.93 at wavelength at 400 nm have been observed from each concentration. Membranes imbibed with different concentration of tiglic acid are able to provide the same absorbance signal under ambient humidity environment.
However, with the increasing concentration of tiglic acid imbibed into the membrane, an increasing signal at absorbance wavelength at 510 nm has also been observed. A color change of the membrane from bright yellow to orange after exposure associated with the increasing of the signal. The absorbance signal increased at wavelength at 510 nm could be attributed to the hypothesized dimerization of imbibed tiglic acid. Inserted picture in figure 3.10 shows the color of the membrane treated with 50 g/L tiglic acid prior to exposure (left) and after the exposure (right) and the membrane treated with 0.1 g/L tiglic acid after the exposure (middle).

Figure 3.11 plots the UV/VIS spectra of the light absorbance data of membranes treated with 50 g/L tiglic acid exposure to 4ppmv acetone under both ambient humidity and 100% relative humidity. We observed from figure 3.10, that the absorbance signals are very close at wavelength at 400 nm. This observation confirmed that membrane treated with 50 g/L tiglic acid (which is the maximum uptake of tiglic acid of the membrane) is able to provide an accurate signal at both relative humidity environment.
The absorbance peak at 510.73 nm is hypothesized to be caused by the dimerization of imbibed tiglic acid. We observed that the absorbance peak under ambient humidity is higher than that under 100% relative humidity. With a higher relative humidity, water which has a higher electronegativity and will hinder the dimerization from happening.

For comparison, figure 3.12 plots the absorbance data from figure 3.9 and the absorbance value from for membranes with different tiglic acid uptake exposed in ambient humidity at wavelength at 400 nm. We observe from figure 3.12, that mitigation of water interference is strongly correlated to the membrane tiglic acid uptake. This result is also consistent with mixed solvent effects on the local pKa of the PSA sites that has been proposed in previous study.
In order to get a desirable response under ambient humidity and 100% relative humidity condition, 50 g/L tiglic acid-mineral oil solution would be used to get the acid based membrane as the membrane have shown a similar absorbance response at wavelength at 400.69 nm.

3.3 Flow cell dynamic response study

After exposure to acetone in the flask experiments, it was found that the acid based membrane was able to catalyze the condensation reaction of resorcinol and acetone at varies humidity environment and to produce a similar response with the maximum tiglic acid uptake of the membrane.

Current studies on using breath analysis for real time diabetes monitoring require substantial time to develop appreciable signals. And in order to be used in clinical real time diagnosis, the sensor should be able to perform a rapid response, which in monitoring patients in real time is less than 1 minute rather than 15 minutes. Control experiments of 12 g/L resorcinol, 50 g/L tiglic acid, and 12 g/L resorcinol + 50 g/L tiglic acid treated membranes have been performed using the flow cell system.
3.3.1 Acid based membrane dynamic study

The acid based membrane dynamic response studies were carried out with membranes imbibed with 12 g/L resorcinol and 50 g/L tiglic acid at 4ppmv and 0ppmv acetone concentration and at 60 °C. UV/VIS absorbance response at wavelength at 400.69 nm was recorded in a 10 seconds interval during the 15 minutes exposure.

Figure 3.13 shows the 4ppmv and 0ppmv exposure data of acid based membranes at 60 °C under ambient humidity and 100% relative humidity. And is plotted between absorbance wavelength at 400.69 nm and the exposure time. The plot illustrates that the acid based membrane was able to provide a signal within 100 seconds at both humidity level. And after 400 seconds exposure, UV/VIS absorbance at 400.69 nm approaches to near constant value.

![Graph](image)

*Fig. 3-13 Dynamic response at wavelength 400.69 nm of acid based membrane exposed to 4ppmv acetone under ambient humidity and 100% relative humidity for 15 minutes*

However, with tiglic acid, we were able to observe a response at each humidity level under 100 seconds, a difference in the dynamic response shown in figure 3.13 can be observed. Exposure experiments with 4ppmv and 0ppmv acetone concentration at both ambient humidity and 100% relative humidity need to be performed on resorcinol, tiglic acid imbibed membranes as well as the Nafion membrane with no additive as control sets to provide more details of the impact of
humidity to the dynamic response. Temperature, flow rate (which will cause the pressure change in the reaction chamber) studies also been performed during the optimization of the sensing device.

### 3.3.2 Control study

For comparison, dynamic response studies have been performed on 12 g/L resorcinol imbibed membrane, 50 g/L tiglic acid imbibed membrane and Nafion membrane with no additive under the same procedure.

Figure 3.14 shows the dynamic response at absorbance wavelength at 400.69 nm of 12 g/L resorcinol imbibed membrane exposed to 0ppmv and 4ppmv acetone concentration at 60 °C under ambient humidity and 100% relative humidity. The plot illustrates that resorcinol immobilized membrane was able to provide a signal under ambient humidity environment, but the presence of water in the flow cell system will impede the condensation reaction of resorcinol and acetone. This observation is consistent with all previous study and results.

The absorbance signal increase at 4% and 100% relative humidity without the presence of acetone is associated with the dimerization of resorcinol, which will cause an absorbance signal at wavelength at 430 nm.
Figure 3.15 shows the dynamic response at absorbance wavelength at 400.69 nm of membranes treated with 50 g/L tiglic acid exposed to 0ppmv and 4ppmv acetone concentration under ambient humidity and 100% relative humidity at 60 °C. The scale used for the plot is identical to the resorcinol controls above. We note that when the tiglic acid immobilized membranes were exposed to 4ppmv acetone, at both humidity level, an absorbance signal slightly increased to around 0.12 at wavelength at 400.69 nm. The response is comparable to that observed when only resorcinol is present in the absence of acetone. Nevertheless, high humidity can nearly eliminate this background signal altogether.
Figure 3.16 shows the dynamic response at absorbance wavelength at 400.69 nm of the Nafion membrane with no additive exposed to 0ppmv and 4ppmv acetone concentration under ambient humidity and 100% relative humidity at 60 °C for 15 minutes. We can observe that under each humidity level and with or without the presence of acetone, the membranes shown no absorbance response. This observation confirms that any response change of the immobilized membrane during the exposure is caused by the reactions or dimerization between immobilized resorcinol, tiglic acid and acetone.
3.3.3 Tiglic acid concentration effect

As from previous sections, we note that different relative humidity level has an impact on the absorbance signal response at wavelength at 510.73 nm. Therefore, it seemed proper to test whether the imbibed tiglic acid concentration would affect the absorbance signal change at wavelength at 510.73 nm and compare with the absorbance signal change at 400.69 nm. Membranes were imbibed with 12 g/L resorcinol and different concentration of tiglic acid, then exposed to 4ppmv acetone in the flow cell system at 60 °C under ambient humidity and 100% relative humidity condition to get the dynamic response.

The 4ppmv acetone exposure dynamic responses at wavelength at 400.69 nm of different concentration of tiglic acid imbibed membranes under 100% relative humidity level were shown in figure 3.17. From the plot we can observe that the increasing of the concentration of imbibed tiglic acid would cause the increasing of absorbance at 400 nm. After 400 seconds continuous exposure, the absorbance at 400nm reached to a near constant value indicates that the condensation reaction has reached to equilibrium.
Fig. 3-17 Dynamic response at wavelength 400.69 nm of different concentration of tiglic acid treated membranes exposed to 4ppmv acetone under 100% RH for 15 minutes.

Fig. 3-18 Dynamic response at wavelength 400.69 nm of different concentration of tiglic acid treated membranes exposed to 4ppmv acetone under Room RH for 15 minutes.
Figure 3.18 plots the plots exposure dynamic responses under ambient humidity level. Acid based membranes with different tiglic acid uptake after exposure to 4ppm acetone showed similar absorbance responses at 400nm. After 400 seconds continuous exposure, the absorbance at 400nm reached to a near constant value indicates that the condensation reaction has reached to equilibrium.

Figure 3.19 plots the 4ppmv acetone exposure dynamic responses of different concentration of tiglic acid imbibed membranes under ambient humidity level. From the plot we were able to observe that the increasing of the concentration of imbibed tiglic acid would cause the increasing of absorbance at wavelength at 510.73 nm. The absorbance signal reached to maximum at 0.3 after 200 seconds of exposure, then start dropping. After 400 seconds continuous exposure, the signal slowly reached to the equilibrium point. The increase and decrease of the absorbance is hypothesized to be caused by the water interference to the dimerization of tiglic acid.

Figure 3.20 plots the 4ppmv acetone exposure dynamic responses of different concentration of tiglic acid imbibed membranes under ambient humidity level. From the plot we note that with an increase of the concentration of imbibed tiglic acid, the signal at absorbance wavelength at
510.73 nm increased. After 100 s exposure, the signal started to reach the equilibrium. Under ambient humidity, there’s no signal drop been observed at each concentration of tiglic acid imbibed membranes.

The signal change at absorbance wavelength at 510.73 nm seemed to be a unique response due to the imbibed tiglic acid concentration and the relative humidity of the exposure environment. And the hypothesized dimerization may have a different reaction rate compared with the condensation reaction.

![Graph showing dynamic response at wavelength 510.73 nm of different concentration of tiglic acid treated membranes exposed to 4ppmv acetone under 4% RH for 15 minutes.](image)

**Fig. 3-20 Dynamic response at wavelength 510.73 nm of different concentration of tiglic acid treated membranes exposed to 4ppmv acetone under 4% RH for 15 minutes**

3.3.4 Humidity impact on membranes after exposure

To better understanding the humidity impact to UV/VIS absorbance response. Experiments of repeating 100% relative humidity exposure and ambient humidity exposure cycle in 100 s interval for three times has been conducted on the 4ppmv acetone exposed acid based membranes. Figure 3.21 plot the dynamic response of membrane exposed to 4ppmv acetone for 15 minutes under ambient and 100% relative humidity condition and then started the humidity exposure cycle at absorbance wavelength at 400.69 nm. From the plot, we note that the relatively
stable absorbance signal in the 4ppmv acetone exposure decreased as the 100% relative humidity exposure started. When changed to the ambient humidity exposure, the signal started increasing.

![UV/VIS spectra of the dynamic response of 100% relative humidity and ambient humidity exposure cycle in 100 s interval on 4ppmv acetone exposed acid based membrane at absorbance wavelength at 400.69nm](image)

From previous study on the mechanism of the condensation reaction of resorcinol and acetone, it has been proven that a formation of a colorless stable intermediate charged compound from step 3 in the hypothesis of the condensation reaction shown in Figure 3.22. The drop in response at 400.69 nm, which indicates the breakdown of the ring structure of the condensation product molecule back to its intermediate form. The oscillation of signal observed is due to the presence and loss of water in the exposure environment when switched the inlet air relative humidity, which could result in the reversible decomposition of the product molecule.
Figure 3.23 shows the dynamic response of membrane exposed to 4ppmv acetone for 15 minutes under ambient and 100% relative humidity condition and then started the humidity exposure cycle at absorbance wavelength at 510.73 nm. From the plot, we note that the relatively stable absorbance signal in the 4ppmv acetone 100% relative humidity exposure decreased as the 100% relative humidity exposure started. When changed to the ambient humidity exposure, the signal started increasing. The 4ppmv acetone ambient humidity exposure signal kept increasing before the humidity exposure started. The 100% relative humidity exposure caused the signal drop and
when switched to ambient humidity exposure, the signal increased immediately. The oscillation of signal occurred at 510.73 nm can be caused by the dissociation of the dimerization reaction. This could also confirm that with a higher relative humidity, water molecules have a higher electronegativity which may hinder the hypothesized dimerization of imbibed tiglic acid from happening.

![UV/VIS spectra of the dynamic response of 100% relative humidity and ambient humidity exposure cycle in 100 s interval on 4ppmv acetone exposed acid based membrane at absorbance wavelength at 510.73nm](image)

The humidity impact on the dynamic response of the exposed acid based membrane indicates that even at the exposure environment with no acetone presence, the signal at each absorbance wavelength will change based on the relative humidity change. So in order to use the membrane in the optical sensing device, we need to maintain the humidity level in the sample chamber to provide a more accurate signal.

### 3.4 Optical sensing device study

Now that we have established a viable way to reduce the effects of humidity on the response of the condensation reaction, it is possible to develop a means for detection of acetone in real-world
environments. To do this, we first need an optimization to our optical sensing device to make sure it can provide us an accurate signal then can be used in clinical diagnosis. From section 3.3 we have proved that with tiglic acid imbibed into the membrane, we were able to get a similar absorbance response at UV to visible region. But humidity still has an impact on the membrane during exposure. In order to further explore the potential application of our acid based membrane in the sensing device, we first need to maintain the membrane at a stable condition, which means a relative humidity stable environment. Therefore, pre and post sampling treatment schemes have been introduced prior and after the exposure of membrane. When switched the inlet air from the 100% relative humidity conditioning air inlet to the exposure air, the air mixing effect which depends on the flow rate and the volume of the sample chamber is negligible.

Second, from fundamental thermodynamic considerations, temperature and pressure change will also have an impact on relative humidity. Temperature is also a factor on the reaction rate due to Arrhenius’ Law. Thus, temperature and flow rate (which will cause the pressure change in the sample chamber) studies will be included in the device testing study.

3.4.1 Effect of temperature
The majority of reactions depend on thermal activation, so the main factor to consider is the fraction of the molecules that possess enough kinetic energy to react at a given temperature. Temperature is considered a major factor that affects the rate of a chemical reaction. As increase the temperature the rate of reaction increases. And for a rough approximation, for many reactions happening at around room temperature, the rate of reaction doubles for every 10 °C rise in temperature.

This phenomenon is related to the collision theory. Particles can only react when they collide, if you heat a substance, the particles move faster and so collide more frequently. From Arrhenius equation:

$$k = A \exp \left( \frac{-E_a}{RT} \right)$$

Molecules only react if they have sufficient energy for a reaction to take place. When the temperature increases, the molecular energy levels also increase, causing the reaction to proceed faster.
So in order to get a more rapid response, a higher reaction temperature may facilitate the condensation reaction of resorcinol and acetone. The effect of temperature on the optical response in UV/VIS is investigated. The experiments were done by exposing the acid based membrane to both ambient humidity and 100% relative humidity with and without acetone at 40 °C, 60 °C and 80 °C. At each temperature, the ambient air humidity is decreased to 2% RH, 4% RH and 10% RH, respectively.

Figure 3.23.24 6 plots the UV/VIS spectra of acid based membrane after exposure to 0ppmv and 4ppmv acetone at both humidity level at 40 °C. The UV/VIS spectra for 4ppmv acetone exposure at both humidity level showed that the absorbance are only about 0.25 under 40 °C. And a small absorbance peak can be observed from the spectra of the 4ppmv acetone exposure under ambient humidity, which may indicate the hypothesized dimerization of tiglic acid and resorcinol occurred. The UV/VIS spectra for 0ppmv acetone exposure looks similar to each other.

Figure 3.25 plots the UV/VIS spectra of acid based membrane after exposure to 0ppmv and 4ppmv acetone at both humidity level at 60 °C. We can observe that for 4ppmv acetone
exposure, under each humidity level, the absorbance signal from UV to visible region are very similar to each other. For 0ppmv acetone exposure, under both humidity level, the absorbance at wavelength 400.69 nm are very close around 0.4. Also, we can observe a small peak at wavelength 430 nm from the spectra of 0ppmv acetone exposure under ambient humidity, this can be associated with the dimerization of the imbibed resorcinol.

Figure 3.26 plots the UV/VIS spectra of acid based membrane after exposure to 0ppmv and 4ppmv acetone at both humidity level at 80 °C. We note that the 4ppmv acetone exposure spectra under both humidity level can provide us a close absorbance signal at wavelength at 400.69 nm. However, an absorbance signal peak can be observed at wavelength at 545 nm which was hypothesized to associate with the tiglic acid dimerization. And the spectra of the 0ppmv acetone exposure under each relative humidity level shows the resorcinol dimerization giving a strong peak at 430nm. And at 80 °C, the rate of dimerization of tiglic acid becomes increasingly prominent and is reflected in the absorbance peak formed at 545 nm as a result of the bathochromic shift.
From the above UV/VIS spectra of the acid membrane acetone exposure experiments under different temperature, with a temperature lower than the condensation reaction temperature 60 °C, the optical response has been significantly impeded as the absorbance at wavelength 400.69 nm decreased from around 1.9 at 60 °C to around 0.3 at 40 °C. When increased the temperature to 80 °C, the optical response remains sensitive to the given acetone concentration and was able to provide an absorbance signal at wavelength at 400.69nm. However, with a reaction temperature higher than 60 °C, peaks at 430 nm and 545nm which corresponds to the dimerization of resorcinol and hypothesized dimerization of tiglic acid became increasingly prominent.

Although with current technology, we could mitigate these side reactions by introducing inhibitors to prevent the dimerization reactions to occur, and get a more rapid response at a higher temperature, the PSA membrane tends to have a color change from transparent to black with the increase of exposure temperature.
3.4.2 Pre and post treatment.

From the flow cell dynamic study, we confirmed that the appearance of tiglic acid can mitigate the humidity influence to the condensation reaction. With 50 g/L tiglic acid imbibed into the membranes, we were able to observe a close absorbance signal at wavelength at 400.69 nm under different relative humidity levels. However, we can also observe a gap between the spectra of the 4ppmv acetone exposure under ambient humidity and 100% relative humidity form figure 3.13. And from section 3.3.4 we confirmed that even without the appearance of acetone, humidity will have an impact on the absorbance signal.

![Graph showing device signal comparison](image)

*Fig. 3-27 Comparison of device signal of acid based membrane exposed to pure nitrogen and 100% relative humidity air purge cycle in a 30s interval and with no purge*

As shown in figure 3.27, a significant drop of device signal was observed when using pure nitrogen purge (which should be more close to 0% RH) compared with the device signal of just the acid base membrane sitting inside the sample chamber. The nitrogen purge would lower the water content present in the sample chamber, driving off the water from the membrane and
producing a relative humidity value to dry conditions. When switched the humidity of the purging air to 100% relative humidity, the device signal became more stable.

Same procedure has been conducted on the Nafion membrane with no additive. As shown in figure 3.28, same oscillation of signal can be observed under dry and humidified purge cycle. However, the reason for the oscillation still remains uncertain, and need further investigation in the future study.

Therefore, pre and post treatment to the membrane for the optical sensing device use is necessary in order to provide more details of the humidity impact and to better optimize the device operating condition.

### 3.4.3 Effect of flow rate

Relative humidity is the ratio of two pressures:

\[
\%RH = 100 \times \frac{p}{P_s}
\]
Where $p$ is the actual partial pressure of the water vapor present in the ambient and $p_s$ is the saturation pressure of water at the temperature of the ambient.

In our sensing device, the membrane was placed in a chamber with air flow constant purging through. This can be treated with an open space, at constant moisture level (based on the moisture level of the purging air) and temperature. Relative humidity is directly proportional to the total pressure. Increasing the flow rate of the inlet air will cause the increase of the total pressure in our sample chamber. Thus, the relative humidity of a higher flow rate of the inlet air will be higher too. However, the value of relative humidity is limited to 100% as $p$ cannot be greater than $p_s$.

Device flow rate study has been conducted using the acid based membrane exposure to 100% relative humidity air purge at different flow rate. Figure 3.29 shows the plots of device signal versus exposure time. From the plots we can observe that the inlet 100% relative humidity air flow rate at 2.2 to 2.8 LPM and 2.8 to 3.2 LPM are very close to each other. This could indicate the relative humidity of the sample chamber has reached to 100%. And the device signal becomes stable after exposure to the constant purge after 3 minutes. Therefore, in the future

![Fig. 3-29 Comparison of device signal of acid based membrane exposed to 100% relative humidity air inlet with different flow rate](image)
device testing pre and post treatment will be conducted prior and after the exposure to acetone. The flow rate of the treatment air inlet and the acetone-air mixture inlet will be controlled at 2.2 to 2.8 LPM. The pre and post treatment will be maintained at 3 minutes for the membrane to reach a stable state.

3.4.4 Sensing device acetone concentration testing
As the objective was to develop a fast and inexpensive method for acetone detection. It is necessary to find the correlation of device signal with the acetone concentration.

Figure 3.30 shows a sample of device signal when exposed to 2ppm acetone for 45 seconds. A strong linear correlation, typical R²>0.9, between device signal and exposure time can be found on both acetone concentrations. This linear time dependence is very different from that observed in the membrane and suggests that a mechanism other than membrane diffusion is limiting the signal. The most likely limitation is the need for the acetone concentration to accumulate in the sample chamber of the device.

![Graph](image)

*Fig. 3-30 Correlation of device signal and 2ppm acetone exposure time. Error bar indicate ± one standard deviation from the data collected*

Since each signal points are not measured at exact same time during the exposure, thus are not comparable to each other. However, the slope change can be treated as an integral of the signal
change during the exposure. For calculation, slope of the device signal change during exposure will be used to correlate with the acetone concentration, as the slope is completely determined by the change of signal and is identical under each acetone concentration. Figure 3.31 shows a strong linear correlation of the slope of the device signal change during 45 seconds exposure (which is based on the breath time a patient can maintain) and the exposure acetone concentration, which $R^2 > 0.95$. The source of this correlation requires further investigation but is likely related to the accumulation of acetone in the sample chamber. Error bars indicate positive/negative one standard deviation from the data collected.

![Graph showing the relationship between acetone concentration and slope of device signal change during exposure. Error bars indicate ± one standard deviation from the data collected.](image)

*Fig. 3-31 Slope of the response of the device containing acid based membranes to various concentration of acetone during 50 seconds. Error bar indicate ± one standard deviation from the data collected*

With such an optical sensing breath analysis system, the concentration of acetone present in human breath can be determined in future clinical breath tests without need for bulky equipment or off site laboratory testing. And can provide a real time response to the clinical breath tests. This method has the potential to reduce the work necessary for clinical studies that attempt to further validate breath analysis techniques as non-invasive surrogates for blood testing.
3.5 Clinical breath analysis

As the technique of introducing tiglic acid into the membrane can effectively mitigate the impact of the presence of water in gas samples, the next step was to test this on a real world application by evaluating whether acetone concentrations in breath samples could be correlated with blood glucose measurements in diabetic patients. Patients were instructed to keep breathing through a plastic straw connected to the sensing device for 45 seconds and keep the breath flow rate at 2.2 to 2.8 LPM. Three samples of each patient were collected. Blood glucose measurements were taken using a blood glucometer provided by the clinic. Table 3.2 shows the blood glucose for each patient.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG (mg/dl)</td>
<td>187</td>
<td>110</td>
<td>426</td>
<td>153</td>
<td>128</td>
<td>100</td>
<td>126</td>
</tr>
</tbody>
</table>

*Table 3.2 Blood glucose information from diabetic patients used in human breath testing*

Figure 3.32 shows a sample of the test result of patient 1. The plot is generated between the device signal and the patient’s exhalation time. A strong linear correlation can be found in the test results, $R^2 = 0.9356$.

*Fig. 3-32 Patient 1 test result. Correlation of device signal and patient’s breath time. Error bars indicate ± one standard deviation from the data collected*
Figure 3.33 shows the plot of the slope of device signal change we obtained during patients’ breath as detailed above versus the blood glucose concentration of the subject.

![Graph showing correlation](image)

*Fig. 3.33 Correlation of slope of device signal change during patients’ breath and the blood glucose of the subject. Error bars indicate ± one standard deviation from the data collected.*

Figure 3.34 plots the correlation of acetone concentration versus patients’ blood glucose concentration which is generated from figure 3.31 and 3.33. There was perhaps a linear correlation present for all but the final data point suggesting that it may possible to use this technique as a non-invasive alternative to the current glucometers. However, the literature is mixed on this and much more extensive investigation is needed to devise the metabolic and physiologic hypotheses that could underpin such a correlation.
Fig. 3-34 Correlation of slope of device signal during patients’ breath and the blood glucose of the subject. Error bars indicate ± one standard deviation from the data collected.
Chapter 4 – Conclusion and Future Works

4.1 Conclusions

During this research, optimization has been completed of a novel method to detect of acetone that is highly selective and sensitive. Colorimetric responses to low parts per million acetone concentrations under different relative humidity conditions were observed. This result was achieved by using perfluorosulfonic acid membrane (Nafion 117 membrane) as the substrate and catalyst for the acid condensation reaction of acetone and immobilized resorcinol as reagents. Tiglic acid was used to mitigate PSA membrane deprotonation in the presence of water for catalyzing the condensation reaction between immobilized resorcinol reagent and ambient acetone. The resulting product from the condensation reaction yielded a unique colorimetric response and was measured over time using UV/VIS. The optimization of the membranes was conducted by monitoring the light absorbance of exposed membranes at different concentrations of imbibed tiglic acid at ambient humidity and 100% relative humidity. Membranes with a maximum tiglic acid uptake were found to provide comparable responses with varying relative humidity at the near UV to visible region (380 nm to 420 nm). The dynamic response of the membrane to acetone exposure has been obtained and it has been found that this method can generate an appreciable response within 100 seconds.

While tiglic acid mitigates water interference, dynamic investigations show that humidity still has an impact on the light absorbance as well as the dimerization between imbibed tiglic acid and resorcinol at early times.

An optical sensing device prototype was developed for preliminary clinical use and optimized with respect to temperature, flow rate as well as pre and post sample chamber conditioning. Prototype testing demonstrated that the acid based membrane can be used to achieve acetone detection at ppm levels (breath acetone concentration can range from 1 ppm in healthy non-dieting subjects to 1250 ppm in diabetic ketoacidosis) in high relative humidity environment similar to conditions in the human breath.

Preliminary clinical trials with small group of patients with random age, gender and health condition have been conducted. The signal response was found to vary linearly with respect to exhalation time at early time. Furthermore, a linear correlation of the device signal to blood
glucose concentrations has been found in diabetic patients. The reason for this latter correlation is uncertain.

While treatment and technology exits for people with diabetes to monitor and manage their disease, the reality is that for millions of people in low and middle-income countries with poor hygiene condition modern treatment and technology is still a dream. The invasive measurements in these countries have great potential of developing fatal infections. Without the acknowledgement of their blood glucose condition, much of tragic loss is unpreventable. These findings in the breath analysis for exhaled gas monitoring provide strong rational for developing a device that can be used in a real time point of care setting to monitor exhaled breath acetone concentrations as a potential surrogate marker for disease.

4.2 Future work
In this study, we confirmed the ability of imbibed tiglic acid to mitigate of water interference in the use of PSA membrane catalysts for optical sensing. There are still unresolved questions regarding the precise mechanism by which this is accomplished. Using neutron scattering studies and isotopic labeling, the experiments conducted here should be repeated to gain a better understanding as to how and where the resorcinol and organic acid molecules imbibed into the PSA membranes as well as determine the associated mechanisms for their immobilization. In addition, this will lead us to a better understanding about whether the morphology of the PSA membrane has an effect on the system.

Also in this study, we observed that as the temperature will facilitate the reaction rate, it will also facilitate the dimerization. The absorbance peak at wavelength at 510.73 nm is hypothesized to be associated with the dimerization of tiglic acid. However, detailed studies are needed to confirm this hypothesis. Also needed is determination of why the optical signal in the prototype oscillates when the plain membrane exposure environment changed from a dry to a humidified purge.

Introduction inhibitors into the membrane could potentially prevent the side reactions such as dimerization from happening, and could provide a more rapid response. This approach provides considerable insight into the reactions and extend our ability to formulate more cost-effective
polymeric membrane catalyst for more accurate clinical uses and should be investigated in future work.
Bibliography

1. International Diabetes Federation. IDF diabetes atlas. . 2015.


