Contributions Toward Urinary Carbon Dioxide Monitoring: Sensor Development, Validation, and Utilization

James Gilbert Atherton

Follow this and additional works at: https://digitalcommons.memphis.edu/etd

Recommended Citation

This Dissertation is brought to you for free and open access by University of Memphis Digital Commons. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of University of Memphis Digital Commons. For more information, please contact khggerty@memphis.edu.
CONTRIBUTIONS TOWARD URINARY CARBON DIOXIDE MONITORING: SENSOR DEVELOPMENT, VALIDATION, AND UTILIZATION

by

James G. Atherton

A Dissertation
Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

Major: Biomedical Engineering

The University of Memphis
May 2022
Copyright © James Gilbert Atherton
ALL RIGHTS RESERVED
DEDICATION

To the One who reveals mysteries
AKNOWLEDGEMENTS

I am grateful to many for enabling this project. Thank you to . . .

- My parents, Jim and Loretta Atherton, I never doubt your love.
- My siblings, Jessica, Daniel, and Emily for your friendships.
- Dr. Ernő Lindner. Your skill in your craft is obvious, and I benefitted from your guidance. Your insightful test questions demonstrate your ability as a teacher to verify not only comprehension but also stimulated my thinking, they are among the best I have had.
- Dr. John Bissler for giving me the opportunity to conduct my research and being a never ending source of knowledge and practical advice in both engineering and medicine.
- Dr. Bradford Pendley for your example as a physician scientist. I always learned from our discussions and admire your teaching ability.
- Dr. Eugene Eckstein. You broadened my perspective by introducing me to relevant research and increased the rigor of my thinking.
- Dr. Marcin Guzinski for your friendship and help. You were my lab partner, and I appreciate you listening to my ideas and helping me brainstorm. On several occasions your scientific aptitude advanced my research, and you taught me much about chemistry and Poland.
- Thank you Dr. Bradley Hambly and Dr. Jenny Jarvis; you were exemplary graduate students.
ABSTRACT

The measurement of urinary CO₂ (U-CO₂) may provide timely recognition of changes in the microcirculatory status of patients, which may correlate better with the prognosis of a patient in septic shock than traditional measures like mean arterial pressure (MAP). We describe the development of a U-CO₂ measurement system with a miniature planar CO₂ probe that could be used to evaluate the relationship between U-CO₂, the microcirculation, and patient outcome. Features of the probe like gas permeable membrane material and thickness, as well as inner hydrogel bicarbonate concentration and volume, are optimized. Parameters of the system, including sample chamber size and flow rate, are also tested. The CO₂ response of the probe has been validated in standard buffer solutions with known CO₂ levels. The utility of the measurement system is demonstrated through the simultaneous monitoring of U-CO₂ in a model bladder using the planar CO₂ probe and a commercial CO₂ probe. The agreement between the measured U-CO₂ values in healthy volunteer urine samples projects the possibility of using this CO₂ measurement system in clinical testing to determine its utility in bedside monitoring.
TABLE OF CONTENTS

List of Tables viii
List of Figures ix
Keys to Symbols or Abbreviations xi

Chapter 1 Contributions Toward Urinary Carbon Dioxide Monitoring:

Sensor Development, Validation, and Utilization 1
  Sepsis 1
  Urinary Carbon Dioxide 2
  Prior Studies on the Correlation Between Urinary CO₂ and Shock 4
  Electrochemical CO₂ probes 5
  Laboratory Performance of Commercial CO₂ Probe 10
  Clinical Performance of Commercial CO₂ Probe 12
  Utilization of Planar U-CO₂ Probe 26

Chapter 2 Specific Aims 28

Chapter 3 Development of a Planar Severinghaus-type U-CO₂ Probe 31
  Solid Contact pH Sensors on Screen Printed Electrodes 33
  Flow cell volume and the U-CO₂ Probe’s transient response 36
  The U-CO₂ Measurement System 38
  Gas Permeable Membrane Material and U-CO₂ Probe Performance 38
  Gas Permeable Membrane Thickness and U-CO₂ Probe Performance 42
  Solution Flow Rate and U-CO₂ Probe Performance 43
  Hydrogel Layer NAHCO₃ Concentration and U-CO₂ Probe Performance 45
  Lifetime of U-CO₂ Probe 46
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 3-1. Drift planar CO₂ probes according to volume of hydrogel</td>
<td>49</td>
</tr>
<tr>
<td>Table 3-2. Measured CO₂ according to NaCl concentration</td>
<td>51</td>
</tr>
<tr>
<td>Table 3-3. Performance characteristics of planar and commercial CO₂ probe</td>
<td>54</td>
</tr>
<tr>
<td>Table 4-1. Comparison of performance characteristics of the U-CO₂ probe using the CFA and SFA protocols</td>
<td>59</td>
</tr>
<tr>
<td>Table 4-2. RMSDs of data points in the scatter plot in different concentration ranges of CO₂</td>
<td>73</td>
</tr>
<tr>
<td>Table 7-1. Contents of tested pH membrane cocktails.</td>
<td>82</td>
</tr>
</tbody>
</table>
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-1</td>
<td>Relevant equilibria influencing the CO₂ concentration in the urinary bladder</td>
<td>3</td>
</tr>
<tr>
<td>1-2</td>
<td>Flow through measurement system for the planar and commercial CO₂ probes</td>
<td>11</td>
</tr>
<tr>
<td>1-3</td>
<td>Potential data collected during the calibration of the CO₂ probe and determination of the CO₂ concentration in urine samples</td>
<td>12</td>
</tr>
<tr>
<td>1-4</td>
<td>Urinary CO₂ and mean arterial pressure in hemodynamically stable subjects over time</td>
<td>16</td>
</tr>
<tr>
<td>1-5</td>
<td>Urinary CO₂ and mean arterial pressure in unstable subjects over time</td>
<td>18</td>
</tr>
<tr>
<td>1-6</td>
<td>Urinary CO₂ and mean arterial pressure in unstable subjects on vasopressors over time</td>
<td>23</td>
</tr>
<tr>
<td>3-1</td>
<td>Screen printed electrochemical cell with pH sensing capabilities</td>
<td>33</td>
</tr>
<tr>
<td>3-2</td>
<td>Calibration transients of the planar pH sensor in phosphate buffers</td>
<td>34</td>
</tr>
<tr>
<td>3-3</td>
<td>Stability of the pH sensor potential in phosphate buffers</td>
<td>35</td>
</tr>
<tr>
<td>3-4</td>
<td>Calibration curve of planar pH sensor in phosphate buffers</td>
<td>36</td>
</tr>
<tr>
<td>3-5</td>
<td>Flow cells used for testing planar Severinghaus U-CO₂ probes</td>
<td>37</td>
</tr>
<tr>
<td>3-6</td>
<td>Response times measured with large or small volume flow chambers</td>
<td>37</td>
</tr>
<tr>
<td>3-7</td>
<td>Cross section view of planar U-CO₂ probe with associate hardware</td>
<td>40</td>
</tr>
<tr>
<td>3-8</td>
<td>Response time curves recorded with CO₂ probes using SR or PVC as GPMs</td>
<td>42</td>
</tr>
<tr>
<td>3-9</td>
<td>Effect of GPM thickness on response time.</td>
<td>43</td>
</tr>
<tr>
<td>3-10</td>
<td>Flow rate dependence of the response times of CO₂ probes.</td>
<td>44</td>
</tr>
<tr>
<td>3-11</td>
<td>The slope of the calibration curves of CO₂ probes assembled with different concentrations of HCO₃⁻</td>
<td>45</td>
</tr>
</tbody>
</table>
Figure 3-12. Response slope and response time values of a planar probe over 23 days 47

Figure 3-13. Response time curves of planar CO$_2$ probes with different volumes of hydrogel 48

Figure 3-14. Potential vs. time transients of a planar CO$_2$ probe with different sample osmolarities 50

Figure 4-1: Arrangement for monitoring urinary CO$_2$ in a model bladder simultaneously with commercial and planar CO$_2$ probes for CFA and SFA modes of operation. 56

Figure 4-2. Potential vs. time transients recorded in CFA mode during the calibration and use of a U-CO$_2$ probe 58

Figure 4-3. Potential vs. time transients recorded in SFA mode during the calibration and use of a U-CO$_2$ probe with graphical display of SFA protocol 61

Figure 4-4 Traces of CO$_2$ levels recorded in pooled urine with a commercial CO$_2$ probe and the planar U-CO$_2$ probe during a monitoring experiment in the model bladder 67

Figure 4-5. Examples of monitoring experiments in the model bladder filled with CO$_2$ containing buffer solution and pooled urine 70

Figure 4-6. Correlation between the CO$_2$ values measured in CO$_2$ containing buffer solutions and pooled urine with a commercial and three planar U-CO$_2$ probes 72
## KEYS TO SYMBOLS OR ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_i$</td>
<td>ion activity</td>
</tr>
<tr>
<td>Ag</td>
<td>AgCl</td>
</tr>
<tr>
<td>Au</td>
<td>gold</td>
</tr>
<tr>
<td>$c_i$</td>
<td>ion concentration</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>CP</td>
<td>conductive polymer</td>
</tr>
<tr>
<td>D</td>
<td>diffusion coefficient</td>
</tr>
<tr>
<td>DI</td>
<td>deionized</td>
</tr>
<tr>
<td>E</td>
<td>potential</td>
</tr>
<tr>
<td>$E^o$</td>
<td>standard potential</td>
</tr>
<tr>
<td>$E^{\text{diff}}$</td>
<td>cell voltage of differential CO₂ probe</td>
</tr>
<tr>
<td>GPM</td>
<td>gas permeable membrane</td>
</tr>
<tr>
<td>H⁺</td>
<td>hydrogen ion</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>bicarbonate ion</td>
</tr>
<tr>
<td>H₂CO₃</td>
<td>carbonic acid</td>
</tr>
<tr>
<td>IFS</td>
<td>inner filling solution</td>
</tr>
<tr>
<td>ISE</td>
<td>ion selective electrode</td>
</tr>
<tr>
<td>ISM</td>
<td>ion selective membrane</td>
</tr>
<tr>
<td>K</td>
<td>chemical equilibrium constant</td>
</tr>
<tr>
<td>k</td>
<td>partition coefficient</td>
</tr>
<tr>
<td>KCl</td>
<td>potassium chloride</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>mV</td>
<td>millivolts</td>
</tr>
<tr>
<td>Na⁺</td>
<td>sodium</td>
</tr>
<tr>
<td>NaCl</td>
<td>sodium chloride</td>
</tr>
<tr>
<td>pCO₂</td>
<td>partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>PVC</td>
<td>poly-vinyl-chloride</td>
</tr>
<tr>
<td>R</td>
<td>molar gas constant</td>
</tr>
<tr>
<td>m</td>
<td>slope</td>
</tr>
<tr>
<td>SC</td>
<td>solid contact</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SIRS</td>
<td>systemic inflammatory response syndrome</td>
</tr>
<tr>
<td>SR</td>
<td>silicone rubber</td>
</tr>
<tr>
<td>T</td>
<td>temperature</td>
</tr>
<tr>
<td>T-CO₂</td>
<td>tissue carbon dioxide</td>
</tr>
<tr>
<td>U-CO₂</td>
<td>urinary carbon dioxide</td>
</tr>
<tr>
<td>Uₖ-CO₂</td>
<td>urine carbon dioxide from kidney</td>
</tr>
</tbody>
</table>
CHAPTER 1 CONTRIBUTIONS TOWARD URINARY CARBON DIOXIDE MONITORING: SENSOR DEVELOPMENT, VALIDATION, AND UTILIZATION

Sepsis

As the most frequent diagnosis in medical intensive care units, sepsis is one of hospitals’ “top killers.” Cellular metabolism, influenced by the microcirculation, plays a critical role in its occurrence. Hidden microcirculatory dysfunction and subsequent delayed medical intervention drives excessive human and monetary costs. Sepsis was the leading US hospital cost at $23 billion in 2015 and septic shock, the most severe type of sepsis, has a mortality rate exceeding 30%. Survivors have profound impairments, including reduction in activities of daily living, cognitive function, and quality of life. Reexamining current modes in the assessment of sepsis may uncover reasons why sepsis mortality has not improved in 20 years.

Current standards for diagnosing and managing sepsis are based on global hemodynamic parameters, like mean arterial pressure (MAP) and blood lactate concentration. Unfortunately, these standards fail to provide an “early” warning of metabolic-cellular (oxidative biology) dysfunction. In fact, the ability of global parameters to reflect the microcirculation has been rigorously questioned. On the other hand, microcirculatory assessment methods utilized for monitoring tissue CO₂, e.g., sublingual capnometry, laser doppler flowmetry, near-infrared spectroscopy, video microscopy, and gastric tonometry can reveal hypoperfusion-associated oxidative dysoxia. Compared to current methods, microcirculatory monitoring tools provide more timely warning of microcirculatory dysfunction and impending global hemodynamic crash. But their clinical implementation is challenging. In combination with a cumbersome nature, monetary cost also limits the use of such “microcirculation monitoring tools.” Thus, their routine application remains unadvised.
Urinary Carbon Dioxide

Impaired microcirculation alters U-CO₂ through complex processes. In fact, the interplay of factors determining even healthy urine’s final CO₂ concentration has puzzled physiologists for decades and is the subject of in-depth academic discussions.²³-²⁴ Of particular ambiguity is the high variability of U-CO₂ measured in the urine of healthy subjects, ranging 20 to 150 mmHg.²⁵-²⁷ Causes of this wide-ranging U-CO₂, including the role of urine HCO₃⁻ concentration, renal hydrogen ion excretion, urine acidification etc., are discussed in detail by others.²³-²⁴ Broadly, known factors influencing U-CO₂ include a) CO₂ from the renal system and b) gaseous CO₂ from gradients established around the urinary bladder (for example, local tissue hypercapnia). The urinary bladder exchanges gas with surrounding tissue and is able to be modeled as a hollow organ with a wall permeable to CO₂ gas.²⁸-²⁹ During shock, we understand that local tissue hypercapnia becomes the predominant factor influencing U-CO₂. In contrast, during periods of normal tissue perfusion, other elements prevail (like the renal acidification and urine bicarbonate content²³-²⁴). During periods of normal bladder perfusion, local tissue CO₂ gradients remain low, and CO₂ gas would not significantly diffuse through the bladder into urine. During sepsis and septic shock, a mismatch develops between tissue CO₂ delivery, generation, and removal so that tissue CO₂ (T-CO₂) accumulates and a CO₂ gradient develops. Urinary bladder CO₂ (U-CO₂) is a function of kidney-derived urinary CO₂ (Uₖ-CO₂) and bladder tissue generated CO₂ (T-CO₂). T-CO₂ is a function of arterial CO₂ (CO₂ delivery), venous CO₂ (CO₂ removal/efflux), and CO₂ generation in local tissue cells. Uₖ-CO₂ is influenced by renal H⁺ ion excretion and HCO₃⁻ content, as stated above. Overall, during shock, as T-CO₂ increases, its contribution to U-CO₂ disproportionately enlarges. Therefore, in shock, we suggest T-CO₂ becomes the predominant factor determining U-CO₂ in the bladder and can be followed to know
the status of a patient’s microcirculatory perfusion. The relevant equilibria influencing bladder U-CO$_2$ are shown in **Figure 1-1**.

**Figure 1-1.** Relevant equilibria influencing the CO$_2$ concentration (U-CO$_2$) in the urinary bladder (circle). T-CO$_2$ represents the tissue CO$_2$ level while U$_k$-CO$_2$ the CO$_2$ concentration in the urine leaving the kidneys and emptying into urinary bladder. Dashed lines indicate equilibrium through gas diffusion. If U$_k$ is constant, or if changes in T-CO$_2$ are sufficiently large, changes in U-CO$_2$ will relate to changes in tissue CO$_2$ concentration around the bladder.

Due to advantages of clinical feasibility, bladder tonometry has been proposed as a microcirculatory monitoring tool for shock. For close monitoring of urine output, catheterization of shock patients is routine. This provides the chance to sample urine frequently with quantitative measurement possibilities for urinary CO$_2$ (U-CO$_2$) assessment with no added risk. The relationship between the microcirculation and U-CO$_2$ has been explored, but for lack of appropriate investigative tools it remains insufficiently described. To expand the current knowledge of U-CO$_2$’s capacity to serve as a microcirculatory indicator and further study the
relationship between U-CO$_2$ levels and hypoperfusion, this dissertation concerns the development and validation of a bedside U-CO$_2$ monitoring system based on a new planar CO$_2$ probe.

**Prior Studies on the Correlation Between Urinary CO$_2$ and Shock**

A small body of literature describes urinary CO$_2$ levels and perfusion state. In a clinical study exploring the influence of hemodynamic status on U-CO$_2$, Lin et al. found strong correlation between U-CO$_2$ and hemodynamic instability.$^{31}$ The authors reported statistically significant difference (p<0.0001) between U-CO$_2$ values measured in hemodynamically stable (U-CO$_2$ = 43.1± 1.7 mmHg) and unstable (U-CO$_2$ = 78.6± 9.9 mmHg) subjects. Such large discrepancy in stable/unstable U-CO$_2$ subjects is attributable to alterations in tissue CO$_2$ around the bladder from microcirculatory dysfunction induced by low mean arterial pressure (MAP). Most, but not all,$^{32}$ studies show correlation between U-CO$_2$ and microcirculatory perfusion,$^{30, 33-34}$ with a common sentiment “monitoring urinary bladder pCO$_2$ . . . may provide a simple and reliable means of monitoring tissue perfusion.”$^{30}$

Changes in U-CO$_2$ during shock were monitored in animals. For example, the aorta was clamped in swine to evaluate the effect of ischemia on bladder mucosal CO$_2$.\textsuperscript{30} Within 30 minutes of the insult, CO$_2$ increased from 57± 4.7 to 117± 7.1 mmHg before declining to baseline 30 minutes after perfusion was restored. In a separate swine study, bladder mucosal CO$_2$ was compared to jejunum mucosal CO$_2$ during hemorrhagic shock.\textsuperscript{33} After the insult, CO$_2$ levels displayed a step-like increase from 49± 6 to 71± 19 mmHg then stepped down to baseline after reperfused for 60 minutes. There was strong correlation between bladder and jejunum mucosa CO$_2$. A third study induced hemorrhagic shock in sheep.\textsuperscript{34} Changes in urinary and ileal CO$_2$ were compared. Both gut and bladder CO$_2$ increased during ischemia. Finally, septic shock was induced in canines to
study correlation between gastric and bladder CO₂. Both bladder and gastric CO₂ significantly increased after shock (p < 0.007, p = 0.026 respectively), but when bladder CO₂ was plotted against gastric CO₂ the correlation was weak ($r^2 = 0.16$).

**Electrochemical CO₂ probes**

U-CO₂ probes are potentiometric gas sensors where the pH behind a gas permeable membrane is measured using a pH sensitive indicator electrode and a reference electrode (Severinghaus design). In this dissertation the terms “sensor” and “electrode” refer to individual indicator, working or reference half-cells. The term “probe” refers to complete electrochemical cells that house both an indicator and reference electrode, e.g., a CO₂ probe that consists of pH and reference electrode in a solution behind a gas permeable membrane.

The pH of a solution is a function of its CO₂ concentration ([Equation 1-1]), a relationship between CO₂ concentration and potential response can be derived ([Equation 1-2]).

**Equation 1-1**

$$\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$$

$$K = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{CO}_2]}$$

$$[\text{H}^+] = \frac{K[\text{CO}_2]}{[\text{HCO}_3^-]}$$
$E_{CO_2} = E_{pH}^o + \frac{RT}{2F} \ln[H^+] = E_{pH}^o + 2.303 \, m \, \log[H^+]$

$= E_{pH}^o + 2.303 \, S \, \log \frac{K}{a_{HCO_3^-}} + m \, \log[CO_2]$

$= E_{CO_2}^o + 2.303 \, m \, \log[CO_2]$  \hspace{1cm} \text{Equation 1-2}

$E_{CO_2}$ is the measured potential of the CO$_2$ probe, $E_{pH}^o$ is standard potential of the pH sensor, $E_{CO_2}^o$ is standard potential of the CO$_2$ probe ($E_{CO_2}^o = E_{pH}^o + 2.303 S \, \log K / a_{HCO_3^-}$);

$m$ is the slope of the response function in mV/log[CO$_2$], and $K$ is the equilibrium constant of the reaction described in \textbf{Equation 1-1}. As seen in \textbf{Equation 1-2}, as long as $E_{CO_2}^o$ is constant, $E_{CO_2}$ is a linear function of the logarithm of the sample CO$_2$ concentration. $E_{CO_2}^o$ is a function of $E_{pH}^o$ (constant) and the HCO$_3^-$ concentration. In the Severinghaus type CO$_2$ probes a relatively concentrated HCO$_3^-$ solution is used as filling solution behind the gas permeable membrane, so the change in the HCO$_3^-$ concentration is generally negligible. However, when osmotic imbalance between the sample and the filling solution of the probe prompts water transport across the gas permeable membrane, both the HCO$_3^-$ concentration behind the gas permeable and $E_{CO_2}^o$ may change. A change in the filling solution composition may also affect the potential of the reference electrode which is used in combination of the pH electrode for the pH measurement behind the gas permeable membrane.

A CO$_2$ probe intended for bedside monitoring of urine CO$_2$ levels must be sturdy and small. Commercial CO$_2$ probes use glass electrodes for pH measurement. Small size glass electrodes
are characteristically fragile, and their manufacturing process is challenging. Fundamentally, the electrical resistance of smaller-size glass electrodes is larger, and contributes more noise in the potentiometric measurement. Instead of a glass electrode one can use an ionophore-based pH sensor with a plasticized polyvinyl chloride (PVC) sensing membrane. The resistance of these sensors is significantly smaller than that of the glass electrode and they are readily miniaturized. The disadvantage of using a pH sensor with a plasticized PVC membrane instead of a glass membrane is that CO$_2$ may diffuse across the PVC membrane and change the pH on the backside of the sensing membrane. This pH change on the backside of the pH membrane results in biased pH values measured with polymeric membrane pH sensors. The biased pH measurement is recognized through reduced response slope and a gradual signal drift. The recorded drift is especially significant in miniaturized sensors with only a few µL inner solution (e.g., a hydrogel layer) on the backside of the pH membrane because the fixed amount of CO$_2$ induces larger pH change in a few µL compared to a few mL of solution behind the sensing membrane. It has been assumed that drift experienced with miniature pH sensors in CO$_2$ containing samples would not occur if the solution on the backside of the membrane were buffered or replaced by a solid internal contact. Unfortunately, for most solid contact electrodes, e.g., coated wire electrodes, or electrodes with PEDOT-PSS (polyethyleneoxythiophene-polystyrene sulfonate) as solid contact, the expected benefits may not be realized because with time, a thin aqueous solution film, a water layer, is formed between the pH sensing membrane and its expected solid contact, again causing drift. The formation of such water layer can be prevented through the utilization of superhydrophobic solid contacts such as PEDOT-C$_{14}$(TPFPhB), i.e., (poly(2-n-tetradecyl-2,3-dihydrothieno-[3,4-b ][1,4] dioxine) – terakis(pentafluorophenyl)borate.$^{37}$
Instead of the Severinghaus-type CO₂ probe Mayerhoff et. al. recommended a novel concept for potentiometric CO₂ measurement, the differential CO₂ probe. The differential CO₂ probe exploits the error (bias) in the pH measurement related to the CO₂ transport across the pH sensitive membranes of polymeric membrane pH sensors. In the differential CO₂ probe the potential difference between two pH sensors is used to assess the sample CO₂ concentration. One of the pH sensors has a sensing membrane which is impenetrable to CO₂ (or has a highly buffered solution on the back side of the membrane with constant pH even if CO₂ diffusing into it) while the other has a sensing membrane through which CO₂ can diffuse into the solution on the back side of the membrane and induce a proportional pH change. In the differential measurement mode, the sample pH cancels out and the pH change on the backside of the electrode with CO₂ permeable membrane is the analytically relevant signal. An important advantage of the differential CO₂ probe lays in its capacity for miniaturization. Its advantageous properties have been shown for the assessment of CO₂ levels in whole blood. The differential sensor’s response function can be seen in \textbf{Equation} 1-3.

\[
E_{\text{diff}} = E_{pH^{-1}} - E_{pH^{-2}} = E_{\text{o, diff}} - m_{CO_2}^{\text{diff}} \log[CO_2]
\]

where \( E_{\text{diff}} \) is the cell voltage of the differential CO₂ probe, \( E_{pH^{-1}} \) is the potential of the pH sensor with unbuffered inner filling solution, \( E_{pH^{-2}} \) is the pH electrode potential with buffered inner filling solution, \( E_{\text{o, diff}} \) is the standard potential of the differential CO₂ probe, i.e., the sum of constant interfacial potentials of the two electrodes, \( m_{CO_2}^{\text{diff}} \) is the differential CO₂ probe’s response slope.
Lipophilic substances in urine partition into the plasticized PVC membrane(s), alter its composition, and impart excessive signal drift to a differential probe fabricated with plasticized PVC membrane.\textsuperscript{39-40} Consequently, the differential CO\textsubscript{2} probe utilizing plasticized PVC membrane-based pH electrodes cannot be used for urine measurement. Since the differential CO\textsubscript{2} probe fails in urine samples, while the commercial Severinghaus-type probe generated promising results,\textsuperscript{41} we built miniature Severinghaus-type planar CO\textsubscript{2} probe with ionophore-based pH sensor behind the gas permeable membrane. The ionophore-based pH sensor was built with a PEDOT-C\textsubscript{14}(TPFPhB) solid contact that prevents CO\textsubscript{2} interference on the pH response. Furthermore, according to the Severinghaus design, the polymeric membrane of the pH sensor is shielded from the urine by the CO\textsubscript{2} probe’s gas permeable membrane. Due to the extra barrier, lipophilic compounds in the urine are not expected to interfere with the response of the CO\textsubscript{2} probe.

The response time is a key performance parameter of any sensor aimed for continuous monitoring. If a sensor monitors concentration changes in a flowing media, the minimum sample volume required for reaching equilibrium for accurate measurement is partly determined by the response time. The response time of a CO\textsubscript{2} probe is defined as the time necessary to reach a certain percentage (e.g., 90\%, or 99\%) or accepted residual deviation from the expected potential change corresponding to a step change in the solution CO\textsubscript{2} concentration. Sluggish or long response time is a limitation of the Severinghaus type CO\textsubscript{2} probe. It limits the number of samples that can be analyzed in a certain time period with a given accuracy. There are several expressions for response time. For example, Ross et al. and Bailey provided expressions for the response time of a Severinghaus probe as a function of the bicarbonate concentration in the internal filling solution and the and thickness of the gas permeable membrane (GPM).\textsuperscript{42-43} Another expression is
more general. With the assumption that the CO$_2$ diffusion through the gas permeable membrane is the rate limiting step in the transient response of a CO$_2$ probe, the response time ($t(\varepsilon)$),

**Equation 1-4** can be expressed as function of the gas permeable membrane thickness ($\delta$), the diffusion coefficient of CO$_2$ in the membrane ($D$), the CO$_2$ concentrations in the solution before ($c_1$) and following ($c_2$) an abrupt change in the CO$_2$ levels in the solution. In **Equation 1-4**, $\varepsilon$ ($\varepsilon = \frac{E(t)-E_2}{E_1-E_2}$) represents the percentage of the potential change for which the response time is determined, where $E_1$, $E_2$ and $E(t)$ are potential values measured with the probe before, after and at certain percentage of the equilibration process.$^{44}$

$$t(\varepsilon) = \frac{\delta^2}{2D} \left[ \ln \frac{1 - \frac{c_1}{c_2}}{1 - \left(\frac{c_1}{c_2}\right)^\varepsilon} + 0.24 \right]$$

**Laboratory Performance of Commercial CO$_2$ Probe**

In our preliminary work related to this dissertation we studied the performance characteristics of commercially available Severinghaus-type CO$_2$ probe$^{45}$ and developed a measurement protocol for the quantitative assessment of U-CO$_2$ in human urine samples.$^{41,46}$ The purpose of this preliminary work was to formulate required performance criteria for the planar CO$_2$ probe we intended to develop and validate the performance criteria in comparison to the commercial probe. To establish its performance, the commercial CO$_2$ probe was implemented in a wall-jet type flow cell in series with calibration standards and samples (**Figure 1-2(b)**). During the calibration of the probe and the measurement of the urine samples we used 1.5 mL/minute flow rate. In this set up the commercial CO$_2$ probe had 59.0 ± 0.5 mV/log[CO$_2$] response slope, ±0.4 mV single day repeatability, 0.5 mV/hour drift while the 100% equilibration time was 10 minutes. The sensor also worked properly in tonometered urine samples (**Figure 1-3**). Using a
calibration curve recorded with 66.5 mmHg and 33.0 mmHg CO₂ saturated standards, the CO₂ levels in urine samples, tonometered with 61and 38 mmHg CO₂ containing gas mixtures, were measured as 61.5 and 40.1 mmHg (urine 1) and 60.7 (urine 2) mmHg, respectively. The CO₂ levels in citrate/phosphate buffers, tonometered with the same gas mixtures, were determined as 61.4 and 40.4 mmHg, respectively. In a pilot study, the CO₂ measurement protocol developed for the commercial CO₂ probe, was applied to track U-CO₂ together with the mean arterial pressure (MAP) during the treatment of septic shock patients for testing the hypothesis that U-CO₂ increases with impaired global circulation and decreases upon restoration.

**Figure 1-2.** Flow through measurement system for the planar U-CO₂ and commercial (Orion) CO₂ probes. (a) schematic of the flow cell and planar probe used in Chapter 3 for system optimization experiments and in Chapter 4 for testing continuous flow and stopped flow modes of operation. (b) schematic for the commercial probe used. The probes are not to scale. The planar probe’s dimensions are 0.5 x 10 x 32 mm. The commercial probe is 2 cm diameter, 15 cm length.
Figure 1-3. Potential data collected during the calibration of the CO$_2$ probe as well as during the determination of the CO$_2$ concentration in urine samples (Urine 1&2) and pH 4.8 citrate/phosphate (C&P) buffer solutions saturated with CO$_2$/N$_2$ gas mixtures with 61 mmHg and 38 mmHg CO$_2$ partial pressure. Filled symbols represent data collected with the CO$_2$ probe in contact with commercial calibration standard solutions (●) or the CO$_2$ saturated citrate/phosphate buffer (■). Open symbols show data collected when the probe was in contact with the Urine 1 (○) or 2 (◇) samples saturated with the same gas mixtures. Measurements were taken about 15 minutes apart.

**Clinical Performance of Commercial CO$_2$ Probe**

Our study was intended uncover design parameters for a bedside CO$_2$ measurement system with a new type of Severinghaus CO$_2$ probe. In our view, continuous or semi-continuous monitoring would be necessary to confidently describe the relationship between U-CO$_2$ and perfusion.
With respect to specifications for a future miniature CO₂ sensor, our pilot study was expected to delineate: 1) the necessary range for U-CO₂ changes in septic shock versus stable participants. The CO₂ sensor’s performance characteristics, e.g., response range and the precision and accuracy of the measured data should be adequate for providing quantitative information in this CO₂ range. 2) the required sampling frequency for following the expected U-CO₂ changes. The response time of the CO₂ sensor should be short for distortion free tracking of U-CO₂ changes without delay. Finally, our pilot study would identify: (i) clinical confounders that might bias the measured U-CO₂ and global perfusion data, (ii) additional critical parameters able to complement the information of U-CO₂ data.

Adult subjects diagnosed with systemic inflammatory response syndrome (SIRS) and the possibility for sepsis/septic shock with an indwelling urinary catheter were included. Subjects were considered septic when they exhibited SIRS with a positive source for infection. They were considered in shock when mean arterial pressure (MAP) dropped below 65mmHg with clinical correlate.

**Association between Urine CO₂ and Hemodynamic Status**

**Healthy U-CO₂:**

U-CO₂ has a wide normal range in healthy individuals. The commercial CO₂ probe with the setup shown in Figure 1-2(b) was used to study healthy urine CO₂. It was secured in a wall-jet type cell, and solutions (standard or sample) were pumped onto the electrode surface at 1.5 ml/minute using a syringe pump. The CO₂ levels in the healthy volunteer samples were determined with the help of a calibration curve recorded with commercial standard solutions provided by Instrumentation Laboratory (IL) (Bedford, MA). According to literature, healthy volunteer U-CO₂ levels range between 20 and 150 mmHg.²⁵⁻²⁷ In agreement with that data, we
measured U-CO$_2$ values in 26 samples from 6 healthy volunteers in the range between 35.3 and 129.8 mmHg (mean value of 7.3 ± 2.6 x 10$^1$ mmHg).\textsuperscript{41} Considering such a broad normal range, U-CO$_2$ trend, rather than the absolute value and its deviation from the mean, is the critical parameter indicating deteriorating or improving bladder microvascular perfusion. Consequently, the direction and magnitude of the change in the U-CO$_2$ levels is considered as the key parameter for understanding changes in U-CO$_2$ and perfusion status. The tracking of U-CO$_2$ over time in the urine of an at-risk individual is assumed to be the best approach to identifying the worsening patient.

In line with this assumption the commercial CO$_2$ probe and setup in Figure 1-2(b) was used in an IRB approved study at a tertiary intensive care unit to study the changes in U-CO$_2$ in subjects at risk for septic shock.

*Subjects with Systemic Inflammatory Response Syndrome (SIRS) Negative for Sepsis and Shock.*

- 71-year-old female recovered after being diagnosed with pneumonia-related sepsis 5 days prior to first urine collection. At time of first collection there was concern for recurrence of sepsis, as she had quickly resolved altered mental status. She only received 0.5 L normal saline bolus without cardiac vasopressors and had normal time-averaged MAP, negative lactate, negative cultures. In retrospect, there was no evidence of sepsis. As seen in Figure 1-4(a), her U-CO$_2$ varied less than 10 mmHg over the 65 hours of monitoring.

- 52-year-old male quadriplegic developed supraventricular tachycardia and was given 2 doses of adenosine and diltiazem (Figure 1-4(b)). As patient was chronically catheterized, there was concern for urosepsis and 3 liters of fluid were administered, but urine failed to grow a pathogen and no pyuria on urinalysis. Participant’s lactate was
elevated but time-averaged MAP was never <65 mmHg. Additionally, both diltiazem and adenosine lower MAP.
**Figure 1-4.** Urinary CO$_2$ (•), mean arterial pressure (MAP; ●), time averaged mean arterial pressure (moving average of 5 MAP points) (=) and serum lactate levels (○) recorded during the treatment of 71-year-old female (a) and a 52-year-old male patient (b). Primary y-axis (blue) = urine pCO$_2$, secondary y-axis (red) = systemic mean arterial pressure, tertiary y-axis (green inset) = blood lactate levels. Dotted red horizontal lines as well as the red shaded bars on the secondary and tertiary y-axis indicate suboptimal systemic perfusion (MAP < 65) and abnormal lactate levels (Lactate > 2 mmol/dm$^3$), respectively. The measured data points for urinary CO$_2$ and blood lactate levels are connected by dotted or solid lines to help visualize the trends in the changes. In the figure the time period of fluid infusion is also indicated.

*Subjects in Septic Shock Responsive to Fluid Resuscitation:*

- 82-year-old female found unresponsive at home with sepsis from a urinary source. Her MAP was less than 65mmHg and she was treated with fluid resuscitation and broad-spectrum antibiotics. As seen in **Figure 1-5(a)**, U-CO$_2$ remained elevated until time averaged MAP stabilized.

- 63-year-old male with recent hospitalization for pneumonia developed septic shock from pneumonia. He was treated with broad-spectrum antibiotics and fluid resuscitation. As seen in **Figure 1-5(b)**, U-CO$_2$ decreased as MAP increased. Though not a trough, final U-CO$_2$ sample remained over 20 mmHg lower than time of hemodynamic compromise.

- 59-year-old female with systemic lupus erythematosus developed urosepesis. She was placed on broad-spectrum antibiotics and fluid resuscitated. According to **Figure 1-5(c)**, as mean arterial pressure improves, urinary CO$_2$ decreases.
Figure 1-5. Urinary CO₂ (●), mean arterial pressure (MAP; ○), time averaged mean arterial pressure (moving average of 5 MAP points) (=) and serum lactate levels (○) recorded during the treatment of a 82-year-old female (a), 63-year-old male (b), and 59-year-old female patient (c). Primary y-axis (blue) = urine pCO₂, secondary y-axis (red) = systemic mean arterial pressure, tertiary y-axis (green inset) = blood lactate levels. Dotted red horizontal lines as well as the red shaded bars on the secondary and tertiary y-axis indicate suboptimal systemic perfusion (MAP < 65) and abnormal lactate levels (Lactate > 2 mmol/L), respectively. The measured data points for urinary CO₂ and blood lactate levels are connected by dotted or solid lines to help visualize the trends in the changes. In the figure the time period of fluid infusion is also indicated.
Subjects in Septic Shock Requiring Pressor Support:

- A 29-year-old male with a remote history of infra-renal mycotic abdominal aortic aneurysm (AAA) s/p repair was admitted with a ruptured AAA. Two weeks after endovascular repair, he developed septic shock from a nosocomial pneumonia that required mechanical ventilation, broad-spectrum antibiotics, fluid resuscitation, and high-dose norepinephrine. As seen in Figure 1-6(a), around time of pressor support U-CO₂ and MAP poorly correlate, though U-CO₂ decreases once vasopressor has been cleared.

- A 48-year-old female, history of HTN and depression, found down from benzodiazepine overdose, CPR for 6 minutes, arrived at hospital intubated. Diagnosed with aspirational pneumonia (infiltrate on CXR, BAL with candida and α-hemolytic strep, no growth on blood culture). Patient treated with vancomycin and Zosyn and required both norepinephrine and vasopressin. Results are shown in Figure 1-6(b). No apparent correlation between MAP and U-CO₂.

- A 70-year-old male with imperforated bowel developed septic shock from the resultant bacteremia. He received broad-spectrum antibiotics, fluid resuscitation, and a low dose of norepinephrine. As seen in Figure 1-6(c), the patient gradually became hypotensive and was given intravenous (IV) fluids. Despite IV fluids, the mean arterial pressure dropped below 65 mmHg and his urinary pCO₂ increased. Low dose norepinephrine was initiated. Subsequently, his mean arterial pressure increased and his urinary pCO₂ decreased.

- A 56-year-old female with systemic lupus erythematosus and congestive heart failure developed septic shock with *Methicillin-resistant Staphylococcus aureus* blood infection and *E. Coli urinary tract infection*. She was placed on broad-spectrum antibiotics. Due to CHF she received low volume fluid resuscitation and was also placed on dobutamine.
She developed anuria and began hemodialysis after hour 45. As seen in Figure 1-6(d), lactate and U-CO₂ are both elevated while MAP is low, but U-CO₂ decreases as MAP improves.

- A 61-year-old female with history of recent necrotic bowel resection who developed septic shock from a catheter related *Pseudomonas aeruginosa* bacteremia. She was treated with broad-spectrum antibiotics, fluid resuscitation, and low-dose norepinephrine. As seen in Figure 1-6(e), in the first 24 hours both urinary CO₂ and lactate decreased with addition of norepinephrine. However, after 24 hours there was dissociation between urinary CO₂ and mean arterial pressure, with both increasing.
Figure 1-6. Urinary CO\(_2\) (●), mean arterial pressure (MAP; ●), time averaged mean arterial pressure (moving average of 5 MAP points) (—) and serum lactate levels (○) recorded during the treatment of a 29-year-old male (a), 48-year-old female (b), 70-year-old male (c), 56-year-old female (d) and 61-year-old female (e) patient. Primary y-axis (blue) = urine pCO\(_2\), secondary y-axis (red) = systemic mean arterial pressure, tertiary y-axis (green inset) = blood lactate levels. Dotted red horizontal lines as well as the red shaded bars on the secondary and tertiary y-axis indicate suboptimal systemic perfusion (MAP < 65) and abnormal lactate levels (Lactate > 2 mmol/dm\(^3\)), respectively. The measured data points for urinary CO\(_2\) and blood lactate levels are connected by dotted or solid lines to help visualize the trends in the changes. In the figure the time periods of infusion of fluid, norepinephrine, dobutamine, and vasopressin is also indicated.
Discussion

We found 3 patterns of U-CO₂ in the setting of SIRS and septic shock. Group A included two study participants who were in SIRS without MAP compromise who demonstrated relatively stable U-CO₂. This group represents U-CO₂ hemodynamically stable patients and could be considered a control. The U-CO₂, MAP and lactate values recorded during the treatment of these patients are featured in Figure 1-4. Group B included three participants who were septic with decreased MAP but responded to fluid resuscitation and required no pressor support. As we show in Figure 1-5 U-CO₂ was trending downward as MAP increased. Finally, group C included septic shock patients requiring vasopressor(s) for hemodynamic support. As we show in Figure 1-6, the changes in the U-CO₂ during hemodynamic improvement was arbitrary, particularly during times of vasopressor support in this group.

Confounding Effect of Vasopressors on Tissue CO₂:

The first two patterns of U-CO₂ support the association of higher U-CO₂ during poorly perfused states with lower U-CO₂ as perfusion was restored (perfusion indicated by MAP). U-CO₂ was stable in well-perfused group A (Figure 1-4) but was increased in group B during poor perfusion (low time-averaged MAP) (Figure 1-5). In all participants of Group B, U-CO₂ decreased as perfusion improved. The last scenario was group C, (Figure 1-6), septic shock treated with vasopressors. Similar to findings in studies with canines⁴⁷, this group failed to clearly demonstrate a relationship between MAP and U-CO₂.

Murakawa and Kobayashi monitored the effect of vasopressors in canines during hemorrhagic shock.⁴⁷ Dopamine initially increased renal tissue CO₂, but CO₂ levels decreased after effects of dopamine resulted in MAP restoration. Epinephrine and norepinephrine caused persistently elevated renal tissue CO₂ even after MAP restoration. Similar to Figure 1-5, a canine control
group with shock but without vasopressor support displayed increased renal tissue CO₂ with decreasing MAP values. In a human vasopressor-shock study examining gastric microcirculation, participants treated with epinephrine were then given dobutamine.⁴⁸ The authors found dobutamine decreased the gastric pCO₂ gap (the difference between gastric pCO₂ and arterial pCO₂) that reversed upon its termination. Because dobutamine was seen to improve gastric mucosal perfusion, while global hemodynamic parameters were unchanged, it was suggested that 1) epinephrine impairs microcirculatory perfusion and 2) dobutamine improves the epinephrine-induced microcirculatory perfusion impairment. Thus, effect of vasopressors on microcirculatory perfusion appears to depend on the type of vasopressor used.

Primarily based on the data from groups A and B, our study maintains the possibility that U-CO₂ trends downward when global perfusion is restored, particularly in settings with no vasopressor administration.

In summary, general correlation of U-CO₂ and global perfusion was found. The preliminary results justify further testing on the correlation between microcirculation and U-CO₂ and evaluating the benefits of monitoring U-CO₂ during the management of sepsis at the clinical level. For unambiguous conclusions on the utility of U-CO₂ as a microcirculatory-monitoring tool at the clinical level the sampling frequency must be improved. Semi-continuous monitoring of U-CO₂, using a sampling frequency similar to the MAP measurements, would: (i) provide more information on the impact of medical interventions on U-CO₂ during patient management; (ii) allow better assessment on the impact of random medical events (e.g. patient decompensation) through recorded changes in U-CO₂; (iii) provide the possibility of using time averaged U-CO₂ values, i.e., improve the power of the statistical analysis assessing the correlation between U-CO₂ and MAP; and (iv) reduce the possibility of conclusions driven by
outliers. This pilot study provided important guidance to design a more focused study using U-CO$_2$ measurement in combination with microcirculatory methods like gastric tonometry or tissue capnometry (once the appropriate U-CO$_2$ monitoring system is developed).

The use of a commercial CO$_2$ sensor in the preliminary clinical study revealed the need for a miniature U-CO$_2$ sensor that would allow continuous monitoring or semi-continuous monitoring with frequent intermittent sampling. This study also helped us to formulate the required performance criteria for a urinary CO$_2$ probe considering that septic shock patients are often oliguric and producing minimal amounts of urine (less than 20 ml urine per hour). These criteria will be discussed in Chapter 3.

**Utilization of Planar U-CO$_2$ Probe**

This dissertation concerns the development and testing of tools and methods aimed to explore the general hypothesis that U-CO$_2$ can augment the information provided by traditional markers utilized for sepsis assessment, like MAP and blood lactate concentration. The specific hypothesis relevant to my dissertation is that U-CO$_2$ increases with impaired global circulation and decreases with restored circulation. While prior studies suggest a link between U-CO$_2$ and hemodynamic status, this research shows the development of a point of care measurement system to specifically measure CO$_2$ content of urine for the assessment and management of sepsis. Previously, to establish the need for developing a bedside U-CO$_2$ monitoring tool that could provide further insight into the relationship of U-CO$_2$ and hemodynamic status, we designed and validated an offsite U-CO$_2$ measurement system based on a commercial CO$_2$ probe.$^{41}$ This protocol was then applied in a pilot study in which U-CO$_2$ in septic shock patients were measured from disease identification to resolution. The data collected during this clinical study guided the design criteria for a new, planar, miniature U-CO$_2$ probe for real time,
continuous bedside monitoring of U-CO\textsubscript{2} to explore the utility of monitoring U-CO\textsubscript{2} in the treatment of sepsis.
CHAPTER 2 SPECIFIC AIMS

In sepsis, generalized infection triggers systemic inflammatory response. Interplay between pathogen and immune system imparts significant morbidity and mortality. Septic shock, the most severe form of sepsis, includes impaired cellular oxygen delivery. Mortality for septic shock exceeds 30% and, year after year, its monetary cost tops hospital rankings ($23 billion in 2015). Survivors have permanent sequela, including diminished activities of daily living, impaired cognitive function, and reduced quality of life.

Sepsis-induced microcirculatory hypoperfusion causes local hypoxia and deranged cellular metabolism. Ideally, physicians would monitor microcirculation to follow the disease at this most basic level. Due to excessive costs and cumbersome nature of microcirculatory monitors such recommendations oppose their routine application. Instead, clinicians evaluate sepsis by assessing global perfusion. In doing so, sometimes they inadvertently assume that global indicators adequately reflect cellular metabolism. But global and local perfusion states are often quite distinct.

Bladder tonometry has been studied as a microcirculatory-monitoring tool for shock. To assess the benefits of bladder tonometry and monitoring urinary carbon dioxide (U-CO$_2$), we analyzed urine samples collected from a urinary catheter and assessed the correlation between CO$_2$ measured with a commercial probe and the mean arterial pressure. Based on these results I propose the development of a miniature planar U-CO$_2$ probe to be implemented inside the urinary catheter or in a bypass of the urinary catheter for point-of-care, continuous or semi-continuous sampling and measurement (bedside monitoring). The goal of my work is to design, build, and characterize a sensor to measure urinary carbon dioxide to be used to one day test the
hypothesis that U-CO$_2$ increases with impaired global circulation and decreases upon restoration. An even more remote hypothesis would investigate U-CO$_2$ relationship to microcirculation.

U-CO$_2$ probes are potentiometric gas sensors where the pH behind a gas permeable membrane is measured using a pH sensitive indicator and a reference electrode (Severinghaus design). The measured pH is a function of sample CO$_2$. Commercial CO$_2$ probes use glass electrodes for the pH measurement. Instead of a glass electrode we considered miniaturized pH electrodes with a H$^+$ ionophore loaded membrane cast over a conductive polymer-based solid contact. As solid contact, electrochemically deposited, super hydrophobic conductive polymer PEDOT-C$_{14}$(TPFPPhB) (poly(2-n-tetradecyl-2,3-dihydrothieno-[3,4-b] [1,4]dioxine-terakis (pentafluoro(phenyl) borate) will be used. This pH sensor is envisioned as a part of a planar (screen-printed) electrochemical cell that mimics the layer structure of the Severinghaus CO$_2$ probe.

**Aim 1:** Development of a miniature planar Severinghaus-type U-CO$_2$ probe and measurement system for bedside urine monitoring.

The CO$_2$ probe will be built on a DropSens® screen-printed planar electrochemical cell with gold working and Ag/AgCl reference electrode. The gold working electrode will be transformed into a solid contact pH sensor. pH and reference electrodes in the DropSens® template will be coated by NaHCO$_3$/NaCl loaded hydrogel layer and a gas permeable membrane, transforming the planar cell assembly into a Severinghaus-type planar CO$_2$ probe (Figure 3-7b). The performance characteristics of the planar CO$_2$ sensor will be tested in various flow cells. During optimization of the planar CO$_2$ sensor, the influence of the gas permeable membrane (material and thickness), hydrogel layer (composition and thickness), and the experimental conditions
(flow rate) will be studied on the response slope, response time, potential stability of the CO₂ sensor and the reproducibility of the potential measurements in CO₂ containing standards.

**Aim 2:** Demonstration of the performance characteristics of the planar Severinghaus-type U-CO₂ probe in combination with various flow analytical techniques and in a model bladder / catheter system

For initial demonstration of the planar U-CO₂ probe’s performance characteristics with various flow analytical techniques (continuous flow analysis and stopped flow analysis), samples with constant concentration (stored in sealed mylar bags), will be analyzed. Based on the results, one flow technique will be chosen to analyze samples with gradually changing concentration in a model bladder/catheter system. This model bladder system will consist of an open container with urine, a peristaltic pump, sampling valve, and the electrochemical flow cell which will allow testing the U-CO₂ probe in aqueous CO₂ standard solutions and pooled urine from healthy volunteers. The CO₂ levels in the container will be measured simultaneously by a commercial CO₂ probe. The results of the CO₂ measurements with the commercial and the planar CO₂ probes will be compared.

If the experimental results demonstrate attainment of the required performance characteristics by the planar CO₂ probe, it could be combined with the flow analytical technique that exhibits the most promising results for implementation into clinical testing. The planar U-CO₂ probe can be implemented for bedside monitoring of U-CO₂ in the urine of septic shock patients to evaluate whether U-CO₂ measurement could be used as a surrogate for the assessment of microcirculatory derangement and sepsis related damage.
CHAPTER 3 DEVELOPMENT OF A PLANAR SEVERINGHAUS-TYPE U-CO₂ PROBE

Toward improving sepsis mortality, we identified urinary CO₂ as a surrogate for the microcirculation to help monitor patients at risk for hemodynamic decompensation. Tracking urinary CO₂ has the potential to enhance current sepsis management and improve outcomes because it may indicate worsening conditions of a patient in real time. A commercial CO₂ electrode was previously validated in an intensive care (ICU) pilot study at a tertiary medical center in Memphis, TN. In this institutional review board (IRB) approved study, urine samples were collected from ICU patients for analysis at the University of Memphis. The required specifications for the miniature U-CO₂ measurement system, including the planar CO₂ probe, were formulated based on the experience gained during that pilot study. In general, for adequate assessment and management of septic shock patients, and to see the impact of interventions on U-CO₂ levels, the measurement/monitoring system must allow a sampling frequency of at least 4 measurements per hour, i.e., one measurement every 15 minutes. The 4 measurements per hour sampling frequency is the same as the measurement frequency of other ICU vital signs, e.g., mean arterial pressure (MAP). Also, the performance characteristic of the planar U-CO₂ probe (sensitivity, stability, and reproducibility of the response) should be comparable to the commercial macro-CO₂ probe. Considering the range of U-CO₂ values published in the literature and our experience during the pilot study, an overall measurement accuracy of ± 10 mmHg would be adequate to indicate changes and trends in U-CO₂ and alerting physicians on the worsening patient. Finally, the U-CO₂ probe should meet the stated design criteria for at least 7 days, the duration of catheter in place as stipulated by standard hospital practice.

The path from early prototype to operational bedside CO₂ measurement system included testing the impact of various system characteristics on the sensor/measurement performance.
Optimization included testing specific planar CO\textsubscript{2} probe features like gas permeable membrane (GPM) material and thickness, hydrogel HCO\textsubscript{3}\textsuperscript{-} concentration, and hydrogel volume. Other measurement system variables were examined, like the sample flow rate and flow cell sample chamber size.

Performance outcomes for comparison of variables included the response time and the sensitivity of the response to changes in CO\textsubscript{2}, i.e., the slope of the calibration curve of the CO\textsubscript{2} probe. Response times (90 and 99\%) were measured to determine the influence of system parameters on the sampling rate. Even though Equation 1-4 can be applied to both Severinghaus probes and ISE’s, it is worth considering the terms involved do not represent the exact same thing for each sensor type. For example, a Severinghaus probe’s response time also depend on the equilibrium kinetics of a chemical reaction (Equation 1-1), while the ISE’s response time does not.

The 90\% and 99\% response times are defined as the time needed to reach 90\% or 99\% of the cell voltage difference at equilibrium, corresponding to a step change in the CO\textsubscript{2} concentrations from \(c_1\) to \(c_2\). A CO\textsubscript{2} probe is considered at equilibrium when the drift of the measured cell voltage, also noted as EMF (electromotive force), is less than 50 \(\mu\text{V/minute}\). Along with the response time and the slope of the calibration curves, the residual mean standard deviation (RMSD) of the data points around the fitted line of the calibration points were also determined. The RMSD value allows estimation of the expected uncertainty in the determination of the CO\textsubscript{2} concentration in unknown samples, without accounting for any time dependent drift.\textsuperscript{49} We considered the response times, the response slope and the RMSD value as the major measures of a probe’s ability to fulfill the design specifications. The performance of the selected measurement system is tested in Chapter 4 during the model bladder experiments where the accuracy and the
uncertainty of the measurements with the planar CO$_2$ probe is determined using the required 4 samples/hour sampling rate.

**Solid Contact pH Sensors on Screen Printed Electrodes**

The proposed planar Severinghaus-type CO$_2$ probe uses a recently developed, solid contact pH sensor with a superhydrophobic conductive polymer as ion-to-electron transducer layer below a ionophore-loaded pH sensitive membrane (Figure 3-1). The hydrophobicity of the conductive polymer prevents H$_2$O accumulation between the conductive polymer and the pH sensitive membrane and eliminates CO$_2$ interference on the response of the polymeric membrane pH sensor.

**Figure 3-1.** Magnified image of the 3-electrode DropSens® screen printed electrochemical cell with pH sensing capabilities. The 1.6 mm diameter Au electrode in the center of the cell has been layered with PEDOT-C$_{14}$(TPFPbB) and a pH sensitive plasticized PVC membrane (2 coats x 1.5 µL). The Ag electrode in the cell is coated with AgCl and used as reference electrode.
Otherwise, the pH sensor in a Severinghaus type CO$_2$ probe would experience CO$_2$ interference that induces systematic error in the measurement of the CO$_2$ levels in unknown samples. The solid contact pH sensor was implemented on DropSens® planar electrochemical cell.

**Performance characteristics of the solid contact pH sensor.** Figure 3-2 shows an example of the transients recorded during the calibration of a planar pH sensor while Figure 3-3 shows the stability of the potential measurements with the pH sensor in pH 7.40, 6.90, 6.40, and 5.90 buffer solutions.

![Figure 3-2](image)

**Figure 3-2.** Transients recorded during the calibration of the planar pH sensor in phosphate buffer solutions of pH 7.40, 6.90, 6.40, and 5.90.

The pH sensor drift was always less than 0.01 pH unit per hour. **Figure 3-4.** shows the calibration curve constructed from the transients in Figure 3-2. The response slope of the pH sensor was determined by least square regression as -58.8 mV/pH. The RMSD of the data points around a fitted line for the example pH sensor was determined as ±0.35mV or ±0.006 pH unit. Results demonstrate that the planar pH probe has close to theoretical response slope, adequate potential reproducibility and stability.
Figure 3-3. Stability of the pH sensor potential in pH 7.40, 6.90, 6.40, and 5.90 buffer solutions in the experiment shown in Figure 3.1. The plotted potential values represent averages calculated from 10 data points collected in the last 50 seconds before a pH change in the solution.

Out of 32 pH sensors, 31 were functional with an average slope of 58.4 mV/pH and RMSD value of ±0.77 mV. This means 0.013 pH unit uncertainty in the pH measurement or ~ 1% uncertainty in the H⁺ ion concentration. This is more than adequate to serve as the pH sensor in the planar CO₂ probe.
**Figure 3-4.** Calibration curve constructed from the potential vs. time transients recorded in phosphate buffers of pH 7.40, 6.90, 6.40, and 5.90 in Figure 3-1. Red dotted lines represent the 95% confidence interval of the fitted line (black line). The data points in the calibration curve represent average potential values calculated from 10 data points collected in the last 50 seconds in the transients before the pH change in the solution.

The measurements were performed with a planar probe with 110 μm thick SR (silicone rubber) GPM and 4% PVA containing hydrogel with 0.5 mmol/dm$^3$ NaCl and 20 mmol/dm$^3$ NaHCO$_3$ concentration. The solutions were pumped with a peristaltic pump at 1 ml/min flow rate. The
smaller chamber size unequivocally provided a faster response so the flow cell with the smaller sample chamber was chosen in optimized system.

Figure 3-5. Flow cells used for testing planar Severinghaus U-CO₂ probes. Home-made flow cells with overall dimensions of 1 cm x 3 cm x 1.5 cm and 120 µl (left) of 20 µl (right) flow chamber volume. The flow cell with 20 µl chamber volume was chosen for optimized system.

![Flow cells](image)

Figure 3-6. 90% and 99% response times measured with a flow through system using 120 µl (large) and 20 µl (small) volume flow chambers in combination with a planar CO₂ probe. Error bars represent 1 standard deviation. CO₂ Concentration steps were 2 X

22.8→60.8→152→60.8→22.8 mmHg so N =4 for each direction. The up and down arrows on the y axes label indicate the direction of the CO₂ concentration change.
**The U-CO₂ Measurement System**

**Figure 3-7** shows the Dropsens® sensor chip and the flow through cell in which the sensor chip is implemented for continuous or intermittent monitoring of U-CO₂. The Dropsens® electrochemical cell was used as the template on which the pH sensor was built. A PEDOT-C₁₄(TPFPhB) conductive polymer layer was deposited onto the disc-shaped gold electrode in the center of the sensor chip with an overlaying pH sensitive membrane (**Figure 3-1**). The Dropsens® sensor chip with the PEDOT-C₁₄(TPFPhB) and ion-selective membrane coated gold electrode in the center and a Ag/AgCl electrode in the lower right corner of the chip is shown **Figure 3-7a**. To transform the solid-contact pH sensor into a Severinghaus type CO₂ probe a HCO₃⁻ containing hydrogel layer was then deposited over the pH sensor and the adjacent Ag/AgCl electrode which served as a reference electrode for the pH measurement in the HCO₃⁻ loaded hydrogel. Then the hydrogel was covered by a gas permeable membrane (GPM), and the entire unit was inserted into the flow cell as it is shown at the top right of **Figure 3-7**, the same as **Figure 3-5** (right side). A cross sectional view of the layer structure of the CO₂ probe is shown in **Figure 3-7b**.

**Gas Permeable Membrane Material and U-CO₂ Probe Performance**

The type of GPM material impacts the performance of the planar U-CO₂ probe through the diffusion of two species across the membrane during equilibration and measurement, CO₂ and H₂O. GPM material affects CO₂ transport and therefore response time of the probe according to the relationship in **Equation 1-4**, in which \( t_e \propto \delta^2/2D \). Where \( t_e \) is response time, \( \delta \) is membrane thickness, \( D \) is CO₂ diffusion coefficient within the GPM (alternatively, some publications use overall diffusivity, which is the product of diffusion coefficient and partition coefficient, or \( D_k \)). GPM material also affects the rate of H₂O transport across the membrane,
which determines the time necessary for the hydration of the hydrogel behind the GPM before use and sensitivity of the CO₂ probes to samples of different osmolality. This is of prime importance during the measurement of CO₂ concentrations in urine, as urine’s osmolality ranges from under 100 mOsm/kg to larger than 1000 mOsm/kg. Water transport across the GPM is not significant during the CO₂ measurement in whole blood because blood’s normal osmolality range is between 275 and 295 mOsm/kg. This narrow range for blood is maintained because the kidney can excrete a broad range of ionic and uncharged species and thereby change the osmolality over a wide range.
Figure 3-7. Dropsens® sensor chip and the flow through cell in which the sensor chip is implemented for continuous or intermittent monitoring of U-CO$_2$.  

a) Top left: An image of the Metrohm DropSens® 3-electrode planar electrochemical cell on a ceramic base with dielectric insulation material, two gold and one silver electrode and connection pads.

Top right: Cross sectional view of the 20 μL volume flow-through cell used in combination of the U-CO$_2$ probe

(b) Cross-sectional view of the planar U-CO$_2$ probe. It contains the solid contact ionophore-based pH electrode and Ag|AgCl reference electrode coated by NaHCO$_3$/NaCl loaded polyvinyl alcohol (PVA) hydrogel and a silicone rubber-based gas permeable membrane.
Water transport across the GPM due to large osmotic range in samples will alter the water the 
HCO$_3^-$ and Cl$^-$ ion concentrations in the hydrogel layer behind the GPM and generate a drift in 
the measured potential with the CO$_2$ probe. SR has > 10,000 times higher diffusivity to CO$_2$ than 
H$_2$O (Dk 2.9 x 10$^{-5}$ versus 5.1 x 10$^{-9}$). Consequently, CO$_2$ probes with SR gas permeable 
membranes are relatively insensitive to changes in the samples osmolalities.$^{43}$ Differential CO$_2$
probes, which are commonly used in commercially blood gas analyzers to measure blood CO$_2$, 
use PVC membranes.$^{38}$ The diffusivity of H$_2$O exceeds that of SR, consequently the differential 
CO$_2$ probe is not recommended for CO$_2$ determinations where the range in osmolality is 
expected to be large, such as urine.

To test the impact of GPM material on the rate of response of a CO$_2$ probe potential vs. time, 
transients were recorded with CO$_2$ probes implemented with SR or PVC gas permeable 
membranes following a step change in the CO$_2$ concentration in the sample solution from 22.8 to 
60.8 mmHg. The transient measurements were performed in the flow configuration of Figure 
3-8(a) using 0.3 ml/min flow rate. The composition (0.5 mmol/dm$^3$ NaCl and 10 mmol/dm$^3$ 
NaHCO$_3$) and the volume (10 µl) of the hydrogel was the same for both probes. As shown in 
Figure 3-8(a) the probe with SR membrane had faster response, i.e., shorter response time, 
compared to the probe with plasticized PVC based GPM of similar thickness. As we show in 
Figure 3-8(b), the rate of response is also influenced by the flow rate of the sample solution. The 
response times measured at 1 ml/min flow rate are shorter compared to the response time values 
recorded at 0.3 mL/min flow rate. The effect of flow rate is more significant with the probe using 
SR as GPM. Based on these results SR was chosen as the GPM material for the planar U-CO$_2$
probe.
Figure 3-8. Response time curves recorded with CO$_2$ probes using SR or PVC gas permeable membranes at 0.3 ml/min (a) and 1 ml/min (b) flow rates following a step change in the CO$_2$ concentration in the sample solution from 22.8 to 60.8 mmHg (a) CPM thicknesses: PVC 340 µm, SR 330 µm. (b) CPM thicknesses: PVC 380 µm, SR 330 µm.

Gas Permeable Membrane Thickness and U-CO$_2$ Probe Performance

According to Equation 1-4 the response time of a CO$_2$ probe ($t_e \propto \delta^2/2D$) depends on the material properties of the membrane (D) and its thickness ($\delta$). Figure 3-9 shows how both 90 and 99% response times for CO$_2$ probes with 50, 122, and 380 µm thick plasticized PVC gas permeable membranes. The response times were measured at 1 ml/min flow rate following a step change in the CO$_2$ concentration in the sample solution from 22.8 to 60.8 mmHg. As expected, the response times increased with increasing GPM thickness.
Figure 3-9. Effect of GPM thickness on 90 and 99% response time. The response times were measured at 1 ml/min flow rate following a step change in the CO$_2$ concentration in the sample solution from 22.8 to 60.8 mmHg. Inset shows the 90% and 99% response times and their standard deviation in minutes for each thickness. The composition and the volume of the hydrogel behind the gas permeable membrane was the same for all probes (10 mmol/dm$^3$ NaHCO$_3$ and 0.5 mmol/dm$^3$ NaCl in 10 µl 4% PVA)

Solution Flow Rate and U-CO$_2$ Probe Performance

As we have shown in Figure 3-8 the sample flow rate affects the rate of response. At higher flow rate the unstirred solution layer on the GPM surface is thinner, i.e., the CO$_2$ mass transport rate through this solution layer is faster. In Figure 3-10(a) we show 99% response time values as function of the flow rate for CO$_2$ probes with 330 µm thick SR and 340/380 µm thick PVC membranes. In Figure 3-10(b) we show 90% and 99% response time values as function of the flow rate for CO$_2$ probes with 140 µm thickness. For measuring the response times the CO$_2$ concentration in the sample solution was changed from 22.8 to 60.8 mmHg and back from 60.8 to 22.8 mmHg. In all of these experiments the composition and the volume of the hydrogel was
the same (20 mmol/dm³ HCO₃⁻ and 0.5 mmol/dm³ NaCl in 10 µl volume). As it has been previously demonstrated the response times of the CO₂ probes with SR membranes are shorter than with PVC membranes of comparable membrane thicknesses. **Figure 3-10(a)** also shows that until ~ 1 ml/min flow rate the response times decrease with increasing flow rates for the CO₂ probes equipped with SR and PVC membranes. Above ~ 1 ml/min flow the response times level off. For the CO₂ probes with 140 µm thin membrane (**Figure 3-10b**) the levelling of is experienced at significantly lower flow rates.

**Figure 3-10.** Flow rate dependence of the response times of CO₂ probes. For measuring the response times the CO₂ concentration in the sample solution was changed from 22.8 to 60.8 mmHg and back from 60.8 to 22.8 mmHg. (a) Effect of flow rate on 99% response time of CO₂ probes with SR and PVC gas permeable membranes. GPM thicknesses: SR 330 µm, and PVC 340/380 µm (b) Effect of flow rate on the 90 and 99% response time of CO₂ probes with 140 µm thick GPM. Inset shows 90 and 99% response times at the specified flow rates with how much volume consumed for the slowest step direction, 99% step down. The 90% and the 99% responses times were determined from the transients recorded during 3 consecutive cycles (N = 3).
**Hydrogel Layer NAHCO₃ Concentration and U-CO₂ Probe Performance**

According to the literature, increasing HCO₃⁻ concentration in the filling solution behind the GPM up to 10 mmol/dm³ increases CO₂ probe sensitivity. At low CO₂ concentrations (below 2 mmol/dm³ or 44 mmHg at 25°C), the HCO₃⁻ concentration affects its response time.⁴⁵,⁵⁰,⁵¹

The sensitivity of the planar CO₂ probe was assessed in calibration experiments with the planar

![Sensitivity of CO₂ probe to mmol/dm³ HCO₃⁻ concentration](image)

<table>
<thead>
<tr>
<th>HCO₃⁻ mmol/dm³</th>
<th>90% Ave. Min</th>
<th>99% Ave. Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5 ± 1.4</td>
<td>9.3 ± 2.8</td>
</tr>
<tr>
<td>15</td>
<td>10.2 ± 3.2</td>
<td>17.9 ± 6.6</td>
</tr>
<tr>
<td>20</td>
<td>17.9 ± 6.6</td>
<td>13.8 ± 3.5</td>
</tr>
</tbody>
</table>

**Figure 3-11:** The slope of the calibration curves of CO₂ probes assembled with hydrogels containing different concentrations of HCO₃⁻. The 10 μL hydrogel also contained 4% PVA and 0.5 mmol/dm³ of NaCl. For the calibrations of the probe’s standard solutions with 22.8, 60.8, and 152 mmHg CO₂ partial pressure was used. As gas permeable membrane 121 to 134 μm PVC film was used.
CO₂ probes in which the bicarbonate ion concentration in the hydrogel was 10, 15, or 20 mmol/dm³ and also contained 4% PVA and 0.5 mmol/dm³ of NaCl. As shown in Figure 3-11 the slope of the calibration curve increased with HCO₃⁻ concentration.

**Lifetime of U-CO₂ Probe**

A CO₂ probe aimed for U-CO₂ monitoring of catheterized patients must maintain its response characteristics for about one week, after which time the catheter (and probe) would be replaced as a matter of clinical practice. Consequently, the determination of the longest attainable lifetime of the planar CO₂ probe was not a goal of this dissertation. However, certain probes were used/tested far beyond the one week time frame stated above. During this week, the performance characteristic of the U-CO₂ probe should not change significantly, e.g., should remain within ±5%. Figure 3-12 shows the results of a 23-day long measurement sequence performed with a planar U-CO₂ probe. The probe used for these experiments was prepared with a 60 µm thick PVC GPM and 10 µL 4% PVA containing hydrogel behind the membrane with 20 mmol/dm³ HCO₃⁻ and 0.5 mmol/dm³. During these experiments the flow rate was always 1 ml/min. As shown in Figure 3-12 the response slope and the response time of the planar CO₂ probe hardly changes in the first 7 days of use. After 7 days, it appeared that both the slope and response time values appeared to gradually degrade; the slopes decreased, and the response times increased.

The optimized planar probe used in Chapter 4 was also tested beyond 7 days. It showed promising results up to 21 days, measuring 59.9 mmHg for a mock sample whose true value was 60.8 mmHg.
Figure 3-12. Response slope and response time values of a planar probe during intermittent calibration in a time period of 23 days. Left y axis is the slope of the calibration curves. Right y-axis is the response time (average for up/down steps). N for daily slopes ranged from 5 to 14 and N for daily response times ranged from 10 to 28. The U-CO₂ measurement system performed well. Based on its slope and RMSD it provided an acceptable 5% uncertainty on day 23.

**Drift of the U-CO₂ Probe**

Early in our study, we recognized that the planar CO₂ probe’s drift could be a factor to watch. The significant drift meant that, depending on its magnitude, repeated calibration appeared to be necessary for adequate measurement accuracy during monitoring experiments. To understand the source of the drift we performed experiments with planar CO₂ probes prepared with different volumes of hydrogel and hydrogels with the same volumes but different osmotic pressures.
Influence of the Hydrogel Volume on the Performance of the U-CO$_2$ Probe

We tested the response characteristics of U-CO$_2$ probes in which various volumes of hydrogel were implemented between the solid contact pH electrode and the GPM. Increasing volume of hydrogel also increases the separation distance between the GPM and the pH ISE. With increased distance the equilibration between the sample and the hydrogel behind the GPM through diffusion takes longer. As we show in Figure 3-13, indeed the response times of planar CO$_2$ probes increased with increased hydrogel volumes.

**Figure 3-13.** Response time curves recorded with planar CO$_2$ probes implemented with different volumes of hydrogel between the GPM and the planar pH electrode. Potential vs. time transients recorded following an instantaneous change in the sample CO$_2$ partial pressure from 60.8 to 152 mmHg (a), and from 60.8 to 22.8 mmHg (b). The mean 99% response times values and their standard deviation is also indicated for each hydrogel volumes (N = 18 response times). Hydrogel composition: 30 mmol/dm$^3$ NaHCO$_3$ 0.5 mmol/dm$^3$ of NaCl in 4% PVA. GPM: 50 µm SR. The transients were recorded with 1 mL/min flow rate.
As shown in Figure 3-13 among the planar CO$_2$ probes with different hydrogel volumes only those with 15µL hydrogel volume showed adequate 99% response time for our design specifications. In contrast to the response times, we did not find differences in the drifts of CO$_2$ probes implemented with different volumes of hydrogels. The mean drift values with their standard deviations are summarized in Table 3-1. Based on these data the planar CO$_2$ probes were used with 15 µL hydrogel.

**Table 3-1.** Mean drift and standard deviation of planar CO$_2$ probes according to volume of hydrogel between the GPM and the planar pH electrode.

<table>
<thead>
<tr>
<th>Hydrogel volume (µL)</th>
<th>15$^*$</th>
<th>27</th>
<th>33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drift (mV/hr)</td>
<td>-2.2 ± 0.5</td>
<td>-2.5 ± 0.2</td>
<td>-2.4 ± 0.3</td>
</tr>
</tbody>
</table>

$^*$N = 9 for each volume

Influence of osmolality on the performance of the U-CO$_2$ Probe

The osmolality of urine ranges between 100 and > 1000 mOsm/kg. The osmolality in urine is determined largely by its urea concentration. To test the influence of the sample osmolality on the drift of U-CO$_2$ probes, the cell voltage produced in the U-CO$_2$ probe was recorded in sample solutions with 60.8 mmHg CO$_2$ partial pressure but different osmolality, i.e., different NaCl concentrations, 0, 40 and 220 mmol/L. The hydrogel behind the GPM was made with 30 mmol/dm$^3$ NaHCO$_3$ and 0.5 mmol/dm$^3$ NaCl, i.e., its osmolality was 61 mOsm.

It was assumed that if osmotic pressure differences on the two sided of the GPM drive H$_2$O across the GPM membrane it would change the concentrations/activities in the hydrogel. These concentration/activity changes are expected to change the potential of both the pH and the Ag/AgCl electrodes. For example, an increase in the Cl$^-$ concentration in the hydrogel would make the Ag/AgCl electrode potential more negative, while a decrease in Cl$^-$ concentration...
would make more positive. Regardless, if osmotic forces generate significant H$_2$O transport across the GPM, the probe is expected to drift in opposite directions following an increase or decrease in sample osmolality. According to **Figure 3-14** the drift of the planar CO$_2$ probe is not influenced by the sample osmolality in the tested osmolality range.

**Figure 3-14.** Potential vs. time transients recorded with a planar CO$_2$ probe in solutions of 60.8 mmHg CO$_2$ and 0, 40 and 220 mOsm osmolality set by NaCl. The measurements were performed in flowing solutions at 1 mL/min flow rate. The individual curves represent transients recorded upon changing the sample solutions from 0 to 220, 0 to 40 and 220 to 0 osmolality. For better comparison, the curves were shifted along the potential axes (normalized). The probe was assembled with 50 µm thick SR membrane. Hydrogel volume: 20 µL; Hydrogel concentration: 30 mmol/dm$^3$ HCO$_3^-$ and 0.5 mmol/dm$^3$ NaCl, in 4% PVA.

Related to our studies on the influence of the sample osmolality on the sensor performance we evaluated the accuracy and precision of CO$_2$ measurements in mock samples with 60.8 mmHg CO$_2$ partial pressure but different osmolalities. The sample osmolalities were set to 0.5, 40, and 220 mmol/dm$^3$ with NaCl. Before the measurement of the individual samples the planar CO$_2$
probe was calibrated using 20.8 and 60.8 mmHg partial pressure standard solutions. The results of these measurements are summarized in Table 3-2. No statistically significant difference was found between the CO$_2$ concentrations of the samples with different osmolalities (p-values ranged from 0.35 to 0.87 for the 3 comparisons).

**Table 3-2.** Measured CO$_2$ according to NaCl concentration

<table>
<thead>
<tr>
<th>[NaCl] mmol/dm$^3$ (N = 3)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>40</td>
<td>220</td>
</tr>
<tr>
<td>pCO$_2$, mmHg</td>
<td>58.2±3</td>
<td>57.7±4</td>
<td>61.5±5</td>
</tr>
</tbody>
</table>

Based on these findings it appears that the osmolality differences on the two sides of the SR GPM are not the dominating factor which determines the planar CO$_2$ probe’s drift.

**Performance of the Optimized U-CO$_2$ Probe**

Key performance characteristics of the optimized measurements system (planar CO$_2$ probe within the flow through manifold) have been identified, including response time, response slope, and potential drift.

The required specifications for a system for monitoring the CO$_2$ levels in the urine of septic shock patients are:

- Response time ($t_{99\%}$): less than 10 minutes (which would allow the measurement of 4 samples /h)
- Accuracy: 10 mmHg maximum deviation from the true value.

To fulfill these requirements the following design parameters were selected for the planar CO$_2$ probe:

- Ionophore based pH sensor with PEDOT-C$_{14}$(TPFPB) conductive polymer solid contact
50 µm thick silicone rubber gas permeable membrane

15 µL 4% PVA containing hydrogel with 30 mmol/dm³ NaHCO₃ and 0.5 mmol/dm³ NaCl concentration

20 µL volume, home-made flow cell

1 ml/min flow rate during continuous monitoring or the combination of stopped flow measurement with 0.2 mL/min and 1 ml/min sampling (this protocol is established in Chapter 4).

The key performance characteristics of the optimized planar CO₂ probe are summarized in Table 3-3. For comparison in Table 3-3 we also show relevant data for the commercial macro-CO₂ probe (Orion Research) and analysis results of standard solutions with the commercial and planar CO₂ probes. The commercial data is also from our own experiments. Because measurement of U-CO₂ is not performed in hospital clinics, the closest hospital comparison available was data for blood CO₂. A Vitros 5600 machine is used by LeBonheur Children’s hospital to measure CO₂ with calorimetry and it provides a %CV in a range of 2 to 6%. According to Table 3-3, both commercial and planar probes fall on the low end of this range. Hopefully this provides a frame of reference for the clinical performance of the commercial and planar CO₂ probes in our laboratory.

As it can be seen in Table 3-3, in the CO₂ range between 22.8 and 152 mmHg the slopes of the both the planar and the commercial CO₂ probe are less than the theoretical 59.1 mV/decade value. The larger RMSD value for the planar CO₂ probe is related to its more significant time-related drift. Due to this larger drift, the planar CO₂ probe needs more frequent calibrating to achieve the same measurement accuracy as the commercial probe.
Addressing Drift

To decrease effects of drift, a 2-point calibration is planned for the planar CO$_2$ probe every 60 minutes, allowing at least 4 sample measurements an hour (with the design goal of a 10-minute sampling rate). With this calibration schedule, the U-CO$_2$ probe of the optimized design were used to test 57 mock samples over 17 months using 19 different probes returning a mean value of 60.1 versus the nominal value of 60.8 mmHg (column 6, Table 3-3). To measure performance of the commercial probe, 2 different probes were used to measure 35 mock samples over 2 years. The mean CO$_2$ was 60.7 mmHg. When EMF of the planar probe was adjusted for drift of -1.49 mV/hr the resulting mean CO$_2$ was 61 mmHg. This indicates the largest source of inaccuracy for the planar probe was drift. A mean of 60.1 mmHg provides 98.8 % accuracy while 61 provides a 99.7 % accuracy. Partial pressure of CO$_2$ for planar versus commercial at 60.1 versus 60.7 was P-value of 0.12, indicating no difference between groups (student’s unpaired t-test). These values are promising, but uncertainty must be considered before determining if the planar probe provides measurements within the 10-mmHg design target.

Despite its somewhat smaller sensitivity, the precision of the CO$_2$ measurements with the planar CO$_2$ probe were similar to those of the commercial CO$_2$ probe. Data in Table 3-3 suggests that the measurement system is ready for testing in the experiments of Chapter 4.
Table 3-3. Performance characteristics of planar CO$_2$ probes and a commercial Orion CO$_2$ probe and analysis results of standard solutions.

<table>
<thead>
<tr>
<th></th>
<th>Slope mV/log[CO$_2$] (N)</th>
<th>RMSD mV</th>
<th>Drift mV/h</th>
<th>$\tau_{99%}$ min</th>
<th>Mean CO$_2$ mmHg (N)</th>
<th>E mmHg (N)</th>
<th>SD mmHg</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimized Planar CO$_2$ probe</td>
<td>52.5 (69)</td>
<td>1.9</td>
<td>-1.5</td>
<td>8.5</td>
<td>60.1/61* (57)</td>
<td>-0.7/+0.2</td>
<td>1.95</td>
<td>3.2%</td>
</tr>
<tr>
<td>Orion CO$_2$ probe</td>
<td>55.5 (21)</td>
<td>1.2</td>
<td>0.02</td>
<td>5.0</td>
<td>60.7 (35)</td>
<td>-0.1 (35)</td>
<td>1.61</td>
<td>2.7%</td>
</tr>
</tbody>
</table>

Standard CO$_2$ is 60.8 mmHg; $\tau_{99\%}$: 99% response time; * is drift corrected value; E: \( \text{absolute error} = P_{CO_2, \text{sample}} - P_{CO_2, \text{standard}} \); SD: standard deviation; CV: coefficient of variation, (SD/mean value).
CHAPTER 4 SIMULTANEOUS MONITORING OF CO$_2$ CONCENTRATION IN BUFFER SOLUTION OR POOLED URINE BY PLANAR AND COMMERCIAL CO$_2$ PROBES

After its development, the CO$_2$ monitoring system was tested in a model bladder simulating a bedside monitoring environment. In the first group of experiments, we compared the utility of different flow analytical techniques for analyzing the urine of septic shock patients, based on their sample volume requirement and measurement frequency. Continuous flow analysis (CFA), stop flow analysis (SFA), and flow injection analysis (FIA) techniques were tested. FIA was particularly appealing for its small sample volume requirement and high sampling rate. However, FIA was abandoned due to the complexity of the measurement of CO$_2$ levels in samples like urine with largely divergent pH values. For testing the flow techniques, the setup in Figure 1-2(a) was used. After selecting the best flow technique, a second group of experiments tested the bedside CO$_2$ measurement system in buffer samples and then urine samples. For comparing our planar CO$_2$ probe to a reliable standard, CO$_2$ values were followed simultaneously with a validated commercial CO$_2$ probe.$^{41}$ For the setup of the second group of experiments, see Figure 4-1.
Figure 4-1: Arrangement for monitoring urinary CO₂ content in a model bladder simultaneously with a commercial CO₂ probe and the planar CO₂ probe for continuous flow and stopped flow modes of operation. An Erlenmeyer flask open to air and stirred with a magnetic stir bar was used as model bladder. The same commercial CO₂ probe used previously and validate in a separate study and is considered a reliable standard.

Characterization of the U-CO₂ Measurement System: U-CO₂ Probe in Combination with Different Flow Analytical Methods

Urine output for healthy individuals is approximately 60 ml/hr. Generally, patients in septic shock produce much less urine and can be anuric. A bedside urinary measurements system must function with minimal sample volumes for use in patients with low urine production (like
oliguria, less than 20 ml urine per hour). Based on our preliminary pilot study, a system requiring total ≤10 ml sample volume and at least 4 measurements per hour would be adequate to monitor U-CO₂ in most septic patients, even those with oliguria.

**Continuous flow mode of operation:**

In selecting an appropriate CFA measurement protocol, we consider two features. First, the time needed for an accurate measurement and second, the target patient’s available sample volume. The experimental conditions suitable to fulfill both features were established in **Chapter 3**. The influence of the sample solution flow rate on the response time is shown in **Figure 3-10**. In prior experiments the flow rate of 1.0 ml/min was chosen as optimal and a 10-minute response time was stated as a design goal. So, for CFA a 10-minute run time was imposed at 1 ml/min resulting in 10 ml required for each sample and calibration standard.

In **Figure 4-2** we show a potential vs. time recording captured during the CFA mode determination of the CO₂ level in a mock sample with 60.8 mmHg CO₂ level in the flow system in **Figure 1-2(b)**. For assessing the CO₂ level in the mock sample two-point calibration with standard solutions of 22.8, 152.0 mmHg CO₂ partial pressure in pH 4.5 citrate buffer were used. During calibration and the measurement standard solutions and the mock sample were pumped through the flow cell with the Gilson peristaltic pump at 1ml/min flow rate for 10-10-10 minutes (total 30 minutes). This calibration/measurement sequence was repeated 5 times a day with 30-minute “break” periods when the mock sample solution with 60.8 mmHg CO₂ partial pressure was pumped through the flow cell. During the entire calibration/measurement sequence (3×10 min) as well as during the 30-minute “break” periods, when the U-CO₂ probe was continuously in contact with the flowing mock sample solution, the potential of the U-CO₂ probe was recorded with 1 data point /5s sampling frequency. For the calibration of the U-CO₂ probe and for
calculating the CO₂ level in the mock sample solution the average of the last 10 potential values recorded before a change in the solution CO₂ levels was used, i.e., collected at the end of the 10-10-10 minutes pumping periods. The potential values recorded during the 30 minute “breaks” were used to determine the drift of the U-CO₂ probe. This calibration/measurement protocol was reproduced with 3 different probes over 5 nonconsecutive days and the reported slope values and the mock sample CO₂ levels are average values of the 5×5 (n=25) two-point calibrations and measurements.

**Figure 4-2.** Five cycles of potential vs. time transients recorded in continuous flow analysis mode during the calibration of a U-CO₂ probe and the measurement of the CO₂ concentration in a mock sample with 60.8 mmHg CO₂ partial pressure. The potential values measured in standard solutions with 22.8 and 152.0 mmHg CO₂ levels were used for calculating the CO₂ concentration in the mock sample. Solid (blue) line represents the experimentally recorded potential values while the (orange) dotted line shows the same values after offsetting the potential drift during the experiment (dotted blue line), i.e., by compensating the negative drift with linearly increasing potential values of the same slope but opposite in sign to the drift.
Response slopes of the probes in mV/log[CO₂] units were determined for each of the 5 cycles over the 5 hours long experiment using the potential values measured in the 22.8 and 152.0 mmHg CO₂ containing standards. The average slope and drift values of the U-CO₂ probe and the results of the assessment of the CO₂ levels in the mock sample are summarized in Table 4-1.

Table 4-1. Comparison of the performance characteristics of the U-CO₂ probe using the CFA and SFA protocols

<table>
<thead>
<tr>
<th></th>
<th>CFA (n=25)</th>
<th>SFA (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope (mV/log[CO₂])</td>
<td>51.0 ±2.7</td>
<td>47.5 ±2.3</td>
</tr>
<tr>
<td>Drift (mV/h)</td>
<td>-2.1±1</td>
<td>-0.5±0.5</td>
</tr>
<tr>
<td>Mock sample with 60.8 mmHg CO₂ using 2 points calibration</td>
<td>59.7 ± 1.4</td>
<td>62.3 ± 1.3</td>
</tr>
<tr>
<td>Mock sample with 60.8 mmHg CO₂ using 1 point calibration</td>
<td>59.8 ± 1.7</td>
<td>63.3 ± 2</td>
</tr>
</tbody>
</table>

*The sample concentration was calculated from the slope and intercept of a two points calibration performed every hour;
* The sample concentration was calculated from the slope of the U-CO₂ probe determined by a two points calibration at the beginning of a 5 to 6 hours long experiment and a single point calibration performed once every hour, i.e., it was calculated with the assumption of constant slope and considering the shift in the calibration curve due to drift.

Stopped flow mode of operation:

CFA measurement technique fulfilled most of the design requirements for U-CO₂ monitoring but it required large volume (10 ml) for the analysis of a single sample. At least 40 mL/hour of urine would be needed for the analysis of 4 samples every hour (the minimum number of samples for adequate monitoring). This volume requirement exceeds what many septic patients provide. To reduce the necessary sample volume for each measurement, the samples and standards were intermittently pumped through the flow cell and then flow was suddenly stopped until the next sample was ready to be processed (stopped-flow analysis or SFA).

The utility of the stopped flow mode of operation was investigated in the flow through system of Figure 1-2(a) to find a calibration/measurement protocol using 2 mL/measurement or less per sample. Based on a variety of tested measurement conditions (flow rates for sampling and
flushing the flow cell, length of stopped flow mode, etc.) the following protocol was chosen: Standard solutions and mock sample are pumped with 0.2 ml/min flow rate for 5 minutes then with 1 ml/min flow rate for 1 min and then the pumping is stopped for 4 minutes. The potential data collected at the end of the 4 minutes stopped flow periods were used for calibration of the U-CO₂ probe and measurement of the CO₂ levels in the mock samples. **Figure 4-3(a)** shows an example of the potential values recorded during a two points calibration and sample measurement sequence together with the flow rates applied during the same time-period. The calibration/measurement sequence (3×10 min) shown in **Figure 4-3(a)** was repeated 5 times a day with 30 minute “break” periods without pumping. An example of the transients recorded during an approximately 6 hours long measurement sequence in stopped flow mode is shown in **Figure 4-3(b)**. The same experiment was repeated in 6 consecutive days. Like during the continuous flow mode of operation the slope values and mock sample CO₂ levels are average values of the 5×6 (n=30) two-point calibrations and sample measurements.

**Performance characteristics of the U-CO₂ probe using the CFA and SFA protocols:**

In **Table 4-1** the performance characteristics of the U-CO₂ probe using the CFA and SFA protocols are compared. The reported data were calculated from the results of experiments performed with 3 probes for the CFA and 3 probes for the SFA experiments. Probes were used for as little as 5 or as many as 14 days. The experimentally determined slope values were higher in CFA then in SFA mode of testing. On the other hand, the U-CO₂ measurements had smaller drift in the SFA experiments. The standard deviation of the calibration slope values, determined with a single probe in one day, was ±0.6 mV/log[CO₂]. The slight differences in the slope and drift values in the CFA and SFA modes of operation hardly influenced the results of the analysis of the mock samples with 60.8 mmHg CO₂ partial pressure.
Figure 4-3. (a) Potential vs. time transient recorded during a single cycle of the stopped flow analysis protocol. Above the potential transient the corresponding solution flow rates (v) profile is shown. The red vertical bars at 0 mL/min flow rates indicate data collection periods used for calibration the U-CO₂ probe and the assessment of the CO₂ concentration in the sample. The CO₂ partial pressure in the standard solutions and mock sample were 22.8, 152.0 and 60.8 mmHg respectively.
Figure 4-3 (continue) (b) Six cycles of potential vs. time transients recorded during the calibration of the U-CO$_2$ probe and the measurement of the CO$_2$ concentration in a mock sample with 60.8 mmHg CO$_2$ partial pressure in stopped flow mode of operation. Between the individual cycles 30 minutes “break” periods were introduced while the U-CO$_2$ probe was in contact with the stopped mock sample solution.

As shown in Table 4-1, the measured CO$_2$ concentrations were either 1.8% below (CFA) or 2.5% above (SFA) of the nominal value. The data summarized in Table 4-1 suggests that the U-CO$_2$ probe is adequate for monitoring the U-CO$_2$ levels with four measurements/hour sampling frequency, i.e., performing a two-point calibration and four sample measurements every hour. Post hoc analysis of the data showed that the slopes of the calibration curves for individual electrodes remained constant for at least 5 hours. Consequently, after the determination of the response slope of an electrode at the beginning of a monitoring experiment, it is sufficient to have hourly one-point calibration to minimize the negative bias in the determined values due to the drift in the standard potential of the U-CO$_2$ probe. Using hourly one-point calibration the measurement frequency can be increased from 4 to 5 measurements/hour. The comparison of the analysis results obtained with two- or single-point calibration in Table 4-1 supports that the single point calibration protocol is a feasible alternative. The experimentally determined CO$_2$ levels in the mock sample determined by the two- and single point calibration protocols were very similar, and both were within 4% of the nominal CO$_2$ partial pressure value in the mock sample. It appears that the sampling rate could be increased further by reducing the length of an analysis cycles from 10 minutes in Figure 4-3(a) to 8 minutes without significant loss in the accuracy of the analysis. Using data points measured after 8 minutes in a sampling cycle with the
two-point calibration protocol provided 63.0 ± 1.4 mmHg mean CO\textsubscript{2} level in the mock sample which is hardly different from the mean value obtained by using the data points measured after 10 minutes (62.3 ± 1.3 mmHg). However, decreasing the length of analysis cycle to 6 minutes significantly increased the systematic error in the measured values (67.2 ± 2.7 mmHg vs. the 60.8 mmHg nominal value). Combining the 8 minutes sampling rate with 1-point hourly calibration (first hour 2 point) gave a CO\textsubscript{2} of 64.2 ± 2.4 mmHg. Overall, using SFA measurement protocol with sample/calibration readings every 8 minutes, a one-point calibration every hour with a 2-point calibration the fifth hour, in combination with a two-point calibration at the beginning of the monitoring experiment, would allow 37 measurements over 5 hours. Six of these measurements will be calibrations and 31 measurements will be samples, so roughly 6 samples measured an hour, or one urine sample measured on average every 10 minutes with CO\textsubscript{2} uncertainty of ± 2.4 mmHg, which is much better than the system requirements for adequate monitoring of urine CO\textsubscript{2} levels for our application.

**Continuous and Intermittent Monitoring of U-CO\textsubscript{2} in a Model Bladder / Catheter System**

In our monitoring experiments a 250 mL volume, continuously stirred open Erlenmeyer flask was used as the model bladder Figure 4-1. At the beginning of monitoring experiments, the model bladder was filled with 250 mL CO\textsubscript{2} containing buffer solution or CO\textsubscript{2} containing pooled urine and a previously calibrated, commercial CO\textsubscript{2} probe was immersed in the solution. The CO\textsubscript{2} response of the commercial CO\textsubscript{2} probe in urine was validated in our previous work.\textsuperscript{41} The composition and the pH of the buffer solution in the model bladder was the same as the calibration standards, while the composition and pH of the pooled urine was from healthy volunteers with pH values between 5.4 and 5.9 and normal physiological composition. The potential of the commercial CO\textsubscript{2} probe was continuously recorded throughout the entire
monitoring experiment and the CO₂ concentration in the model bladder was calculated from the measured potential data using its calibration curve recorded before the monitoring experiment. Simultaneous to the CO₂ measurements with the commercial CO₂ probe, the CO₂ levels were measured intermittently with the planar U-CO₂ probe in samples periodically taken from the model bladder. The buffer or urine samples were pumped from the model bladder into the 20 μL volume flow-through cell with the U-CO₂ probe and recycled back to the model bladder. For the repeated two-point calibration of the U-CO₂ probe the sampling from the model bladder was temporarily ceased and the standard solutions with 22.8 and 152 mmHg CO₂ were pumped through the flow cell.

Comparison of the CO₂ values measured in pooled urine with a commercial CO₂ probe and the planar U-CO₂ probe during continuous monitoring in a model bladder.

Figure 4-4 shows the results of two ~ 3h long continuous monitoring experiment using pooled urine. In these experiments the CO₂ concentrations were measured in the model bladder simultaneously with a commercial CO₂ probe and the planar U-CO₂ probe. In the experiment shown in Figure 4-4(a) the commercial CO₂ probe and the planar U-CO₂ probe were calibrated before the beginning and at the end of the monitoring experiment using standard solutions with 22.8, 60.8 and 152 mmHg CO₂ partial pressure. To construct calibration curves for both CO₂ probes all the calibration data collected before and after the monitoring experiment were considered. The parameters of this calibration curve and the potential values recorded during the experiment were used to calculate the CO₂ concentrations in the model bladder as function of time.

As shown in Figure 4-4(a) the CO₂ level in the urine drops from the beginning of the experiment until ~ 100 minutes due to the loss of CO₂ from the stirred model bladder. To mimic the
expected CO\textsubscript{2} increase in a patient with septic shock, between 100 and 130 minutes the urine CO\textsubscript{2} level was gradually increased by bubbling a 152-mmHg partial pressure CO\textsubscript{2} containing gas mixture into the model bladder. After 130 minutes, the introduction of the CO\textsubscript{2} containing gas mixture was stopped, and the CO\textsubscript{2} level decayed again towards equilibrium with the room air. According to Figure 4-4(a), the planar U-CO\textsubscript{2} probe tracks very well the CO\textsubscript{2} concentrations measured with the commercial CO\textsubscript{2} probe. The difference between the values measured with the two probes remained within ±7% during 97% of the time during the three hours long monitoring experiment.

In the experiment shown in Figure 4-4(b), similar to Figure 4-4(a), the potential of the commercial CO\textsubscript{2} probe was continuously recorded throughout the entire monitoring experiment following the CFA measurement protocol, but we followed the SFA display style (Figure 4-5) with the planar CO\textsubscript{2} probe. It means, that after a two-point calibration in CFA mode between 0 and 20 minutes, the urine CO\textsubscript{2} concentration in the model bladder was measured 4 times in CFA mode between 20 and 60 minutes and this measurement cycle was repeated 3 times. The average difference in the concentrations measured with the commercial and planar probes in this experiment was 1.9% ± 3.8 (N=12). As shown in Figure 4-4, the planar U-CO\textsubscript{2} probe very well tracks the CO\textsubscript{2} concentrations measured with the commercial CO\textsubscript{2} probe.
(a) Commercial CO2 probe — Planar CO2 probe

(b) Commercial CO2 Probe — Planar U-CO2 Calibration — Planar U-CO2-samples
Figure 4-4 (continued) (a). Traces of CO$_2$ levels recorded in pooled urine with a commercial CO$_2$ probe and the planar U-CO$_2$ probe during a monitoring experiment in the model bladder. From 0 to 100 and 130 to 190 minutes CO$_2$ levels decrease towards equilibrium with the room air. Between 100 and 130 minutes 152 mmHg CO$_2$ containing gas mixture was bubbled through the urine to increase the CO$_2$ concentration in the model bladder. The difference between the values measured with the two different probes remained within $\pm 7\%$ during 97% of the three-hour long monitoring experiment.

(b) Trace of the urine concentration measured with a commercial CO$_2$ probe (blue line) and intermittently measured CO$_2$ concentrations measured with the planar CO$_2$ probe in calibration standard solutions (•) and samples from the model bladder (♦).
Comparison of the CO\textsubscript{2} values measured in pooled urine with Commercial CO\textsubscript{2} probe and the planar U-CO\textsubscript{2} probe in a model bladder using the stopped flow analysis protocol.

As shown in Table 4-1, the accuracy of the determinations of the CO\textsubscript{2} levels in mock samples were quite similar. Absolute errors of mean CO\textsubscript{2}’s were 1.1 and 1.5 mmHg with CFA and SFA analysis, respectively. This is hardly a significant difference on our designated scale. However, only a 2 mL sample was needed for a single measurement with the SFA protocol while the required sample volume was 10 mL in the CFA mode of operation. Because patients in septic shock produce limited volumes of urine (generally less than 20 mL/h) the stop flow analysis protocol was chosen to show the utility of the planar U-CO\textsubscript{2} probe for monitoring the CO\textsubscript{2} levels in catheterized patients in septic shock. In these monitoring experiments the CO\textsubscript{2} levels were recorded in the model bladder continuously with the commercial CO\textsubscript{2} probe and intermittent measurements were done using the planar U-CO\textsubscript{2} probe in the flow through arrangement of Figure 4-1. In the SFA measurement protocol, the measurements are performed in cycles; every hour a two-point calibration is followed by four sample measurements. For the calibration, 22.8 and 152 mmHg CO\textsubscript{2} saturated standard buffer solutions were used. In Figure 4-5 we show two examples of the monitoring experiments using the SFA protocol for the intermittent measurements of the CO\textsubscript{2} levels with the U-CO\textsubscript{2} probe in CO\textsubscript{2} containing buffer solution (Figure 4-5(a)) and in pooled urine (Figure 4-5(b)). In each of these experiments the model bladder CO\textsubscript{2} levels were recorded for 5 hours (5 cycles). During the 5 hours monitoring experiment, the planar U-CO\textsubscript{2} probe was calibrated 5 times in calibration standards with 22.8 and 152 mmHg CO\textsubscript{2} (10 measurements) and 20 measurements were made in samples from the model bladder. Like in the bladder-CFA monitoring experiments, the CO\textsubscript{2} levels measured with the U-CO\textsubscript{2} probe in SFA mode were compared to values recorded with the commercial CO\textsubscript{2} probe. At the
beginning of the monitoring experiments the CO₂ levels in the model bladder were high (CO₂ saturated buffer solution or urine) and gradually decreased due to the loss of CO₂ from the stirred open container. After ~ 190 minutes in Figure 4-5(a) or ~120 minutes in Figure 4-5(b) a calculated mass of NaHCO₃ was added to the samples in the model bladder to step up the CO₂ concentrations and demonstrate that the U-CO₂ probe equally well tracks decreasing and increasing concentrations (spikes).
Figure 4-5. Examples of monitoring experiments in the model bladder filled with CO$_2$ containing buffer solution (a) and pooled urine (b).
**Figure 4-5 (continued) (b).** The continuous (blue) traces were recorded by commercial CO$_2$ probe inserted into the solution in the model bladder while the individual points were measured by the planar U-CO$_2$ probe in SFA mode of operation. The filled orange circles (●) represent measurements in the standard solutions with 22.8 and 152 mmHg CO$_2$ levels used for calibration at the beginning of each measurement cycles. The red stars (∗) represent the measurements in samples taken from the model bladder. The abrupt increase in the recorded CO$_2$ levels were achieved by spiking the CO$_2$ containing buffer solution (a) or pooled urine (b) with NaHCO$_3$.

As shown in **Figure 4-5**, the data point measured with the planar U-CO$_2$ probe lay almost perfectly on the trace representing the CO$_2$ levels measured with the commercial CO$_2$ probe for both the CO$_2$ containing buffer and pooled urine solutions. The correlation between the CO$_2$ levels measured with the commercial CO$_2$ probe and three individual planar U-CO$_2$ probes in three monitoring experiments in SFA mode is shown in **Figure 4-6**. Each monitoring experiment was 5 hours long in the CO$_2$ containing buffer and pooled urine solutions with n=60 measurements in each sample type. The mean of the difference in the CO$_2$ levels measured with the probes (Commercial vs. planar U-CO$_2$) in CO$_2$ containing buffer solutions was -0.9% ± 5.1%, while in pooled urine it was -2.3% ± 6.2%. For CO$_2$ containing buffer sample, 96% of data points are within 10% of nominal value. For urine, it is 87%. Overall, the Residual Mean Standard Deviations (RMSDs) around the scatter plots in **Figure 4-6** were 3.3 and 3.9 mmHg for the measurements in the buffer solutions and urine samples, respectively. However, as it can be seen in **Table 4-2**, the RMSDs were smaller at low CO$_2$ levels and larger at larger CO$_2$ levels,
i.e., the agreement between the CO$_2$ concentrations measured with the commercial CO$_2$ probe and the planar CO$_2$ probe remained below 10% in each range. The RMSD of Table 4-2 also demonstrates our system is more than capable of providing an accuracy well within 10 mmHg.

![Graph](image)

**Figure 4-6.** Correlation between the CO$_2$ values measured in CO$_2$ containing buffer solutions (a) and pooled urine (b) with a commercial CO$_2$ probe and three planar U-CO$_2$ probes in 3 x five hours long monitoring experiments in each.
The CO$_2$ concentration in the mock samples could be determined with better than ± 4% accuracy, and the agreement between the values measured with the commercial and the planar U-CO$_2$ probe was better than 3% with ~6% uncertainty.

**Table 4-2.** Residual Mean Standard Deviations (RMSDs) of data points in the scatter plot in different concentration ranges of CO$_2$ in buffer solutions and urine samples.

<table>
<thead>
<tr>
<th>Concentration Range (mmHg)</th>
<th>Buffer (Number of Samples)</th>
<th>Urine (Number of Samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-130</td>
<td>3.3 (60)</td>
<td>3.8 (60)</td>
</tr>
<tr>
<td>20-57</td>
<td>1.8 (22)</td>
<td>1.5 (22)</td>
</tr>
<tr>
<td>57-94</td>
<td>3.6 (23)</td>
<td>3.8 (26)</td>
</tr>
<tr>
<td>94-130</td>
<td>3 (15)</td>
<td>3.7 (12)</td>
</tr>
<tr>
<td>20-75</td>
<td>1.8 (36)</td>
<td>2.9 (36)</td>
</tr>
<tr>
<td>75-130</td>
<td>4 (24)</td>
<td>3.9 (24)</td>
</tr>
</tbody>
</table>
CHAPTER 5 CONCLUSIONS

Because studies of U-CO$_2$ are not common, no standardized measurement schemes or protocols exist. The first portion of our study validated a commercially available Severinghaus CO$_2$ probe in a homemade wall-jet type flow cell for use in urine. Results from the subsequent clinical study informed the design of an improved CO$_2$ measurement system to track the time dependence of U-CO$_2$ and its correlation with global hemodynamic parameters, from the diagnosis of shock until its resolution. Such data are not available in literature and, in our view, support the necessity of investment into bedside U-CO$_2$ measurement devices.

To monitor CO$_2$ levels in the urine of catheterized septic shock patients, a planar U-CO$_2$ sensor was built on a screen-printed Dropsens® chip. The sensor was implemented in a ~20 µL flow cell and tested in a flow system both in continuous flow and stopped flow modes. The utility of the U-CO$_2$ sensor has been demonstrated in measuring the CO$_2$ levels in mock samples (buffer or urine) and in monitoring experiments performed in a stirred open container, model bladder, in which the CO$_2$ levels decayed due to the loss of CO$_2$ to the environment or could be increased by purging the solution with CO$_2$ containing gas mixtures or adding NaHCO$_3$ into the solution. In the monitoring experiments the CO$_2$ levels were recorded continuously with a commercial CO$_2$ probe and 4 to 6 measurements were made in 2 mL samples from the model bladder using the U-CO$_2$ probe with SFA measurement protocol.

The CO$_2$ concentration in the mock samples could be determined with better than ±4% accuracy, and the agreement between the values measured with the commercial and the planar U-CO$_2$ probe was better than 3% with ~6% uncertainty with sampling rate of 6 measurements per hour, well within the stated goal of 4 samples per hour. Planar CO$_2$ probe lifetime exceeded the 1 week minimum in our design criteria.
The central aim of this dissertation was the design of a CO₂ measurement system for providing continuous or semi-continuous U-CO₂ values during the treatment of septic shock patients. This requires a miniature CO₂ probe incorporated into a microfluidic manifold attached to a Foley catheter with a mechanism for providing 4 samples an hour and periodic recalibration.

We have successfully fabricated a U-CO₂ probe and provided a working measurement system ready to test how monitoring U-CO₂ could impact the management of septic shock patients and reduce mortality rates.
CHAPTER 6 RECOMMENDATIONS FOR FUTURE WORK

Strategies to improve probe and flow cell.

Improvements in the CO₂ monitoring system hardware are necessary prior to routine bedside implementation in a clinical study for evaluating U-CO₂ as an indicator of shock. The DropSens® template which we used is not ideal for building a planar CO₂ probe. It is a three-electrode electrochemical cell in which the distance between Ag and Au electrodes was too small to have reliable complete coverage of the conductive polymer coated Au electrode with the pH sensitive membrane without partially coating the Ag/AgCl electrode surface. A new template, with only two electrodes but larger separation between these electrodes would allow to achieve better yield in probe fabrication.

The layer-by-layer assembly of the planar probes was challenging. In particular, simplifying the final step of the assembly, the deposition of the 50 μm thin GPM and clamping the flow cell must be simplified. One possibility would be to use the planar U-CO₂ probe, preassembled in combination with a single use flow cell.

Automation

Transitioning to an automated system is expected to improve the precision of the measurements and the operator involvement would be reduced to troubleshooting. At the bedside, automation would allow the system to measure U-CO₂ 24/7 operation with the assumption that the monitored patient produces the required amount of urine (~ 2 ml/sample). Inclusion of temperature control system would allow sample to be run at 37°C and significantly improve response time through its effect on CO₂ diffusivity and the speed equilibration kinetics of Equation 1-1.
Determination of shelf and use lifetime

Studies were performed showing probe lifetime of at least 3 weeks, exceeding the 1-week design requirement. But no studies were performed on the shelf life of the CO\textsubscript{2} probe or the consequences of long-term storage of the probe. Storing the probes in a dry environment may lead to drying out of the hydrogel behind the GPM. It has to be determined how much time of “conditioning/hydration” is necessary for a probe before it can be used with the expected accuracy and precision.

Study Urinary CO\textsubscript{2} and Sepsis with Planar U-CO\textsubscript{2} Probe

As designed, the planar U-CO\textsubscript{2} probe and measurement system is adaptable to bedside monitoring application. This dissertation contains the evidence needed to justify efforts to further refine the system and utilize at the bedside of a septic shock patient. It is nearly ready for testing the main hypothesis of the dissertation in a clinic setting, that U-CO\textsubscript{2} increases with impaired global circulation and decreases upon restoration.
CHAPTER 7 EXPERIMENTAL

Clinical Performance of Commercial CO₂ Probe

A pilot study was conducted at the University Tennessee Methodist University Hospital ICU to evaluate the relationship between U-CO₂ levels and patient hemodynamic status. U-CO₂ levels were measured in urine samples collected from each participant as they moved through various hemodynamic phases. Samples were collected in 4 time periods: 1) at SIRS identification, 2) between 6-12 hours, 3) between 12-48 hours, and 4) after recovery.

A minimum of 10 mL of urine was collected for each sample. Before sampling, the urinary catheter tubing was cleared of urine and then clamped 30 cm distal to the access ports to allow urine to pool. Urine accumulated for an established 20 minutes maximum and then aspirated from the sampling port of the urinary catheter with a syringe. After sample collection, syringes were sealed with a luer lock valve until analysis. Syringes were transferred to the University of Memphis in a protective container and were analyzed within 24 hours of collection. The CO₂ probe allowed CO₂ measurements with a ±1.2 mmHg uncertainty. The overall uncertainty of CO₂ measurement (accounting for sample collection, sample storage, calibration, and measurement) was less than ±2.5 mmHg. An additional 3.4 to 5.7% systemic error may have biased the results due to the CO₂ loss through the wall of the Foley catheter tubing during the maximum 20-minute clamp for sample collection. We consider a sensor resolution of 10 mmHg necessary to assess patient status and to make informed clinical decisions based on the trends of change in the U-CO₂.

All U-CO₂ measurements were made with a Thermo Scientific 9502BNWP Severinghaus type CO₂ probe in a custom wall-jet type flow cell. The CO₂ probe was secured in a wall-jet type cell, and solutions (standard or sample) were pumped onto the electrode surface at 1.5 ml/minute...
using a Yale Apparatus YA-12 (Waltham, MA) syringe pump. The CO₂ levels in the ICU patient urine samples were determined with the help of a calibration curve recorded with commercial standard solutions provided by Instrumentation Laboratory (IL) (Bedford, MA). Further details of the experimental setup can be found in our validation study.⁴¹

IRB approval was obtained from University of Tennessee Health Sciences Center/Methodist University Hospital and University of Memphis, IRB#14-03355-XP UM (see appendix).

**Development of a Planar Severinghaus-type U-CO₂ Probe**

**Chemicals**

Sodium chloride, sodium citrate dihydrate, and hydrochloric acid were purchased from Fisher Scientific, potassium chloride was purchased from Alfa Aesar. Citric acid and sodium bicarbonate were from Sigma-Aldrich. Sodium phosphate monobasic was obtained from Acros Organics and sodium hydrogen phosphate anhydrous from Fluka Chemika. Poly(vinyl alcohol) 99.7% hydrolyzed (PVA; Mw: ~115,000 g/mol) from Scientific Polymer Products Inc.

The protocols for the chemical synthesis of monomer EDOT-C₁₄ (2-n-tetradecyl-2,3-dihydrothieno-[3,4-b][1,4]dioxine) and the procedure for the electrochemical deposition PEDOT-C₁₄ (tetrakis-(pentafluorophenyl)borate) conductive polymer (PEDOT-C₁₄(TPFPhB) are based on the works of Amemiya³⁷, ⁵² and were published previously.³⁷, ⁵² The components of the ion-selective membrane cocktail were purchased from Sigma-Aldrich. Tetrahydrofuran (THF) was obtained from Fisher Scientific.

**Electrodes/Instruments**

To build the planar U-CO₂ sensors, screen-printed, 3-electrode electrochemical cells (C223AT) from Metrohm DropSens® were used. For testing the planar pH electrodes configured on the disc
shaped gold electrode of the C223AT cell an Orion Model 900200 (Thermo Scientific, USA) double junction Ag|AgCl reference electrode was used. For setting the CO$_2$ levels in the standard solutions used for calibration and for validating the CO$_2$ response of the planar U-CO$_2$ probe an Orion™ Model 9502BNWP commercial carbon dioxide electrode (Thermo Scientific, USA) was used in combination with an Orion model 720A pH meter. For the pH measurements a Fisherbrand AccuTupH combination glass electrode (model 13-620-183) was used. A Lawson Lab (Malvern, PA) 16-channel high input impedance data acquisition system was used for potentiometric data acquisition. The data acquisition system has been connected to a computer equipped with the EMF Suite version 2.0.0.2 program. To deposit AgCl onto the silver electrode and PEDOT-C$_{14}$(TPFPhB) onto the gold disc electrode in the planar electrochemical cell a CHI 760C Electrochemical Workstation (CH Instruments Inc., TX) was used.

**Planar U-CO$_2$ Probe Design**

The layer structure of the planar U-CO$_2$ probe follows the design of a conventional Severinghaus CO$_2$ probe, i.e. the pH is measured in a bicarbonate solution behind a gas permeable membrane (GPM). In the planar U-CO$_2$ probe described in this paper, pH is measured with a solid contact pH electrode in combination with a Ag/AgCl reference electrode. The U-CO$_2$ probe is built on a DropSens® model C223AT 3-electrode electrochemical cell. The screen-printed planar electrochemical cell is configured on a 3.4 cm × 1.0 cm × 0.05 cm ceramic base (Figure 3-7) with two gold (Au) and one silver (Ag) electrode. Only one of the gold electrodes (the disc shaped Au electrode) and the silver electrode are used for the construction of the U-CO$_2$ probe.

**Solid Contact pH Sensor Fabrication:**

Template:
Solid contact pH electrodes were built on the gold working electrode of a commercially available DropSens® electrochemical cell. The screen-printed planar electrochemical cell was configured on a 3.4 cm × 1.0 cm × 0.05 cm ceramic base. The cell includes two Au electrodes and one Ag electrode. The Ag electrode is generally used as a reference electrode while one Au electrode is used as working and the other as counter electrode in voltammetric experiments. In the planar U-CO₂ probe only one of the Au electrodes was used along with the Ag electrode. The Au electrode was transformed to a solid contact pH electrode and the Ag electrode is transformed to an Ag/AgCl reference electrode of the second kind. The pH electrode was prepared with the published protocol, using PEDOT-C₁₄(TPFPhB) - based solid contact as ion-to-electron transducer between the gold electrode and the pH sensitive membrane.³⁷

PEDOT-C₁₄(TPFPhB) film deposition:
Using a 0.01 mol/dm³ solution of EDOT-C₁₄ and 0.03 mol/dm³ solution of [CH₃(CH₂)₁₆CH₂]₄N-(TPFPhB) in acetonitrile, the EDOT-C₁₄ monomer was electrochemically polymerized and deposited as PEDOT-C₁₄(TPFPhB) with cyclic voltammetry on the DropSens® Au electrode using 10 cycles with 0.1V/s scan rate in the potential range from -0.85 to 1.45 V. The polymerization was finished at -0.85 V, when the polymer is in its reduced form.

pH membrane deposition:
The PEDOT-C₁₄(TPFPhB)-based solid contact was coated by a pH sensitive ionophore loaded plasticized PVC membrane by drop casting. Various membrane cocktail proportions were tested (Table 7-1) with cocktail #2 chosen. The membrane ingredients were dissolved in 1.2 mL THF. From this membrane cocktail two x 1.5 µL aliquots, were dispensed onto the PEDOT-C₁₄(TPFPhB) coated Au electrode (1.6 mm diameter) surface. Figure 3-1 shows a completed pH sensor.
Solid Contact pH Sensor Characterization: The pH sensors were tested in four phosphate buffer solutions ranging from pH 5.8 to 7.4 for their response slopes, potential reproducibility and stability by dipping the planar pH sensors into the buffer solutions. One calibration cycle was defined as a sequence of potential measurements in the calibration solutions with pH values 5.8, 6.4, 6.9, 7.4 and 7.4, 6.9, 6.4 and 5.8. The planar pH sensor potential was measured against an Orion Model 900200 double junction Ag|AgCl reference electrode (Thermo Scientific, USA). The inner filling solution of the reference electrode was 4 mol/dm$^3$ KCl saturated with AgCl, while the outer filling solution was saturated KCl solution.

Table 7-1. Contents of tested pH membrane cocktails.

<table>
<thead>
<tr>
<th>Cocktail</th>
<th>PVC mg (wt %)</th>
<th>DOS</th>
<th>TDDA</th>
<th>KTkpClPhB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocktail 1</td>
<td>60 (32.7)</td>
<td>120 (65.5)</td>
<td>2 (1.1)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Cocktail 2</td>
<td>62.8 (31.4)</td>
<td>127 (63.5)</td>
<td>7.8 (3.9)</td>
<td>2.4 (1.2)</td>
</tr>
</tbody>
</table>

Ag/AgCl reference electrode: The Ag electrode in the DropSens® electrochemical cell was coated by AgCl using chronoamperometry in a 0.1 mol/dm$^3$ HCl solution in combination with single junction Ag|AgCl reference electrode with 0.1mol/dm$^3$ KCl filling solution and a glassy carbon counter electrode. The Ag electrode in the cell was converted to a Ag/AgCl electrode using chronoamperometry (applied potential 0.14 V, electrolysis time 900 s).

Hydrogel layer: Initial composition was polyvinyl alcohol (PVA) 4.2% (mass), 5 mmol/dm$^3$ NaHCO$_3$, and 0.5 mmol/dm$^3$ NaCl. Various NaHCO$_3$ concentration were tested, finally 30 mmol/dm$^3$ was chosen for the optimized planar CO$_2$ probe. During early investigations for impact of hydrogel volume on probe performance, 5, 10, 15, and 27 and 33 µL of bicarbonate-containing hydrogel solution were drop-cast into the recessed area of the planar electrochemical
cell to cover the pH sensitive ion selective electrode (ISE) and the Ag/AgCl reference electrodes with 15 μL chosen for optimized planar probe.

*Gas Permeable Membrane:* For gas permeable membrane silicone rubber (polydimethyl siloxane from BOC Sciences (CAS 63394-02-5) and plasticized PVC membranes were cast in molds for different thicknesses. SR GPMs were cast on plexiglass without solvent and cross linked with Siloprene Cross Linking Agent® (CL) (Siloprene Cross Linking Agent K-11, product #85418) in 1μL CL to 10 mg SR ratio. CL agent was mixed with SR on plexiglass with a spatula for 30 seconds and allowed to cure overnight. PVC cocktails contained Bis(2-ethylhexyl) sebaccate as plasticizer with PVC (2:1 mass ratio). The PVC and plasticizer mixture was dissolved in THF and cast in a glass ring secured on a glass plate. Both PVC and SR membranes were cast according to a determined mass to surface area ratio (using molds) for the desired membrane thickness. Precast SR films of 50 μm thickness were purchased from Wacker (product Elastosil®film 2030 250/50). The 50 μm SR GPM was chosen for the optimized planar probe.

*Flow through electrochemical cells:* Figure 3-5 (top) shows the methacrylate, wall-jet type flow cell obtained from DropSens®. Its open/close system with a magnetic lock provides secure fitting and easy replacement of the ceramic templates with the screen-printed electrochemical cells. A rubber gasket provides seal between the sample chamber and CO₂ probe and keeps the GPM in place. Home-made flow cells were also tested. The 20 μL cell had superior dimensions to the DropSens® cell and was chosen for the optimized planar U-CO₂ (Figure 3-5(b)). It was held in place with clamps on a ring stand.

**Assembly of Multilayer Planar U-CO₂ Probe**

To transform the planar electrochemical cell with a polymeric membrane pH and a Ag/AgCl reference electrode to a planar CO₂ sensor, the shallow well of the screen-printed cell was filled
with a bicarbonate-containing PVA hydrogel. The membranes (SR or PVC) were placed over the PVA hydrogel and gently pressed on top of the pH sensor on the ceramic electrochemical cell template. The unit composed of pH sensor, hydrogel, and GPM was then placed in the flow cell, either Dropsens® or homemade (Figure 3-5).

**System Optimization Experiments:**

*Influence of the GPM Material on the Performance of the U-CO₂ Probe*

The response time of planar U-CO₂ probes with PVC or SR as gas permeable membranes of the same thickness was assessed at 0.3 ml/min flow rate. PVC thickness was either 340 or 380 μm while SR thickness was 330. Hydrogel layer was 4% PVA and 10 mmol/dm³ HCO₃⁻ concentration with 10 μl volume in a 0.5 mmol/dm³ NaCl solution. Solution flow rate was 0.3 ml/min. The 90% and the 99% responses times were determined from the transients recorded during 2 points calibrations using standard solutions with 22.8 and 60.8 mmHg CO₂ levels in 3 consecutive cycles, i.e., by recording the transients during the CO₂ concentration changes 22.8 → 60.8 → 22.8 → 60.8 → 22.8 mmHg, so N = 3. To illustrate how steps up in CO₂ concentration differs from steps down, 90 and 99% response times were divided into descending and ascending steps. The CO₂ probe was considered in equilibrium once the signal drift drops below 50 μV/min change. To determine the equilibrium potential the last 10 potential values before a concentration change were averaged (at 5 second sampling intervals).

*Influence of the GPM Thickness on the Performance of the U-CO₂ Probe*

Planar U-CO₂ probes with a PVC GPM material with thicknesses of 50, 120, and 380 μm were constructed. Hydrogel was 4% PVA and 10 mmol/dm³ HCO₃⁻ concentration in a 0.5 mmol/dm³ NaCl solution with 10 μl volume and flow rates were 1 ml/min. The 90% and the 99% responses times were determined from the transients recorded during 2 points calibrations using standard
solutions with 22.8 and 60.8 mmHg CO\(_2\) levels in 3 consecutive cycles (N = 3). The CO\(_2\) probe was considered in equilibrium once the signal drift drops below 50 \(\mu\)V/min value. To determine the equilibrium potential the last 10 potential values before a concentration change were averaged (at 5 second sampling intervals).

**Influence of the Solution Flow Rate on the Performance of the U-CO\(_2\) Probe**

For the first experiment, Figure 3-10A, U-CO\(_2\) probes with PVC (thickness 340 and 380 \(\mu\)m) and SR gas permeable membranes (thickness 140 and 330 \(\mu\)m) were tested in the flow rate range from 0.12 ml/min to 2 ml/min. Hydrogel was 4% PVA and 10 mmol/dm\(^3\) HCO\(_3^-\) concentration in a 0.5 mmol/dm\(^3\) NaCl solution with 10 \(\mu\)l volume for all probe but the SR GPM with 140 \(\mu\)m thickness. Its only other difference was a [HCO\(_3^-\)] of 20 mmol/dm\(^3\). This effort was to establish a threshold flow rate value above which an increase in the flow rate does not improve the rate of response, i.e., the CO\(_2\) transport through the GPM and the hydrogel layer is the rate determining. The 90% and the 99% responses times were determined from the transients recorded during 2 points calibrations using standard solutions with 22.8 and 60.8 mmHg CO\(_2\) levels in 3 consecutive cycles (N = 3). The CO\(_2\) probe was considered in equilibrium once the signal drift drops below 50 \(\mu\)V/min value. To determine the equilibrium potential the last 10 potential values before a concentration change were averaged (at 5 second sampling intervals).

**Influence of the Hydrogel Layer Bicarbonate Concentration on the Performance of the U-CO\(_2\) Probe**

The influence of the NaHCO\(_3\) concentration in the PVA hydrogel on the response slope and response time of the planar U-CO\(_2\) probes was tested. Experiments using two, two, and two planar U-CO\(_2\) probes with 10, 15, and 20 mmol/dm\(^3\) HCO\(_3^-\) concentration in a 0.5 mmol/dm\(^3\) NaCl solution in 10 \(\mu\)L PVA hydrogel were performed with PVC planar U-CO\(_2\) probes of
thicknesses from 120 to 130 µm and flow rate of 1 ml/min. The response slopes and the response times were determined from the transients recorded during 3 points calibrations using standard solutions with 22.8, 60.8 and 152 mmHg CO\(_2\) levels in 3 consecutive cycles (N = 6). To determine the equilibrium potential the last 10 potential values before a concentration change were averaged (at 5 second sampling intervals).

*Influence of the sample chamber size on the Performance of the U-CO\(_2\) Probe*

Hydrogel with NaHCO\(_3\) concentration of 20 mmol/dm\(^3\) and 4% PVA content was deposited on the planar CO\(_2\) probe with a 110 µm GPM thickness in a 0.5 mmol/dm\(^3\) NaCl solution and peristaltic pump at 1 ml/min. Two homemade flow cells were used, one with small chamber size and the other with large chamber size as in Figure 3-5. CO\(_2\) measurement systems were calibrated with 3-point calibrations including 20.8, 66.8, and 152 mmHg in citrate buffer. Each flow cell was calibrated on one day with the same planar CO\(_2\) probe, 3 x 22.8 → 60.8 → 152 → 60.8 → 22.8 up-down cycles (N = 12) were performed for the small chamber size and 2 x 22.8 → 60.8 → 152 → 60.8 → 22.8 (N = 8) for the large chamber size.

*Lifetime of U-CO\(_2\) Probe*

To evaluated lifetime of U-CO\(_2\) probe, a probe with PVC GPM was made with 4% PVA hydrogel containing 20 mmol/dm\(^3\) HCO\(_3\)^- and 0.5 mmol/dm\(^3\) NaCl 10 µL volume with CO\(_2\) standards on 11 days over a range of 23 days.

For SR, a probe with SR GPM was tested as in *Flow Analytical Methods Tested in Combination of the U-CO\(_2\) Probe* on 8 days over a range of 23 days. Between measurements it was stored in the measurement system at low pump speed (0.07 ml/min) in 152 mmHg CO\(_2\) calibration standard. At this low rate the solution reaching the probe was around 10 mmHg.
Investigations into Drift of U-CO₂ Probe

Influence of the Hydrogel Volume on the Performance of the U-CO₂ Probe:

Hydrogel with NaHCO₃ concentration of 30 mmol.dm⁻³ and 4% PVA content was deposited on the planar CO₂ probe with a 50 µm GPM thickness in a 0.5 mmol.dm⁻³ NaCl solution and peristaltic pump at 1 ml/min. Tape was applied to the ceramic template with electrode cut-outs to form wells for hydrogel deposition. Various layers of tape corresponded to each hydrogel volume: no tape corresponded to 15 µL, 2 layers of tape (31µm well height) to 27 µL, and 3 layers of tape (47µm height) to 33 µL. Performance characteristics of the planar U-CO₂ probes were determined from the transients recorded during 3-point calibrations using standard solutions with 22.8, 60.8 and 152 mmHg CO₂ levels in 3 consecutive cycles. We assessed the influence of the deposited volume on the response time, response slope, signal stability and reproducibility of the planar U-CO₂ probes. A single probe was made for each hydrogel volume. 3 calibration cycles were tested each day. Each probe was tested for 3 days for total 9 cycles each volume, N = 18 for each volume. Single factor ANOVA and Bonferroni t-test was used to make statistical comparison.

Influence of sodium concentration on the drift of the optimized U-CO₂ Probe:

CO₂ probe was constructed with inner hydrogel content 4% PVA, 0.5 mmol.dm⁻³ NaCl and 30 mmol.dm⁻³ NaHCO₃. GPM was SR with 50 µm thickness. Three sample solutions were prepared, all with 8% CO₂. Sodium concentrations were: Deionized water (DI), 40, and 220 mmol in 10 mmol.dm⁻³ citrate buffer. The two solutions were pumped by continuous flow at 1 ml/min for 60 minutes and the drifts were compared.

To infer how NaCl concentration and osmolality affected measurement accuracy, another experiment was performed to find the CO₂ of mock sample solutions in various NaCl
concentrations. The probe had an inner hydrogel content of 4.2% poly-vinyl alcohol with 0.5 mmol NaCl and 30 mmol NaHCO₃ with SR GPM of 50 µm thickness. After calibration, the following sample solutions of various osmolarities were tested for the CO₂ content at equilibrium: 40 mmol NaCl, 220 mmol NaCl, and the standard 0.5 mmol NaCl was also measured again and included in analysis. Equilibrium was defined as < 0.05 mV change in EMF per minute. After measurements, signals were converted to mmHg using calibration curve. The measurement order was as follows: 3 x 220 mmol → 40 mmol → 0.5 mmol (N = 3 for each measurement).

**Simultaneous Monitoring in Buffer or Urine by Planar and Commercial CO₂ Probe**

**Measurement Manifold and Standard Solutions**

The response characteristics of the U-CO₂ probe were tested in the flow-through manifold of **Figure 3-5** consisting of the 20 µL volume flow-through cell, peristaltic pump (Minipuls 3, Gilson Inc.), modular valve positioner (Hamilton MVP) with 6-port distribution (HVXM 6-5) or a 6-ports Loop valve (HVXM 6-6) and standard solutions in metalized mylar bags sealed with rubber stoppers. The calibration standard and the sample solutions, stored in metalized Mylar bags (IMPAK corp.), were sequentially pumped through the flow through electrochemical cell. The sequence of the solutions was selected by the multi-position selection valve. The content of the model bladder (“samples”) was pumped through the flow-through electrochemical cell and back to the model bladder by the peristaltic pump. The response of the U-CO₂ probe was periodically tested by pumping calibration standards through the electrochemical cell.

*Standard solutions and mock sample:* The U-CO₂ probes were calibrated with 22.8, 60.8, and 152.0 mmHg CO₂ gas (Airgas®) saturated pH 4.15, 0.01 mol/dm³ citrate buffer solution (6.2 × 10⁻³ mol/dm³ citric acid and 3.8 x 10⁻³ mol/dm³ sodium citrate). For determining the precision
and accuracy of the CO\textsubscript{2} measurements with the planar CO\textsubscript{2} probe the standard solution, saturated with the 60.8 mmHg CO\textsubscript{2} gas was used as mock sample, and the U-CO\textsubscript{2} probe was calibrated only with the 22.8 and 152.0 mmHg CO\textsubscript{2} gas saturated standard solutions.

Flow Analytical Methods Tested in Combination of the U-CO\textsubscript{2} Probe

To select a flow analytical method and flow through manifold which is best suited for the continuous or semi-continuous assessment of U-CO\textsubscript{2} in the urine of septic shock patients in a bedside arrangement, continuous flow analysis (CFA), stopped flow analysis (SFA) and flow injection analysis (FIA) were considered. Among these possibilities FIA was particularly appealing for its small sample volume requirement and high sampling rate. However, FIA was abandoned due to the complexity of the measurement of CO\textsubscript{2} levels in samples like urine with largely divergent pH values.

For experiments regarding Simultaneous Monitoring of CO\textsubscript{2} Concentration in Buffer Solution or Pooled Urine by Planar and Commercial CO\textsubscript{2} Probes, further experimental details are provided in Chapter 4.
REFERENCES


APPENDIX IRB APPROVAL

October 20, 2014

Amado X Freire, MD, MPH.
UTHSC - COM - Medicine - Pulmonary
G228 Coleman College of Medicine Building
956 Court Avenue
Memphis, TN 38163

Re: 14-03355-XP UM
Study Title: Urine Carbon Dioxide as a prognostic indicator in Septic Shock

Corrected Letter

Dear Dr. Freire:

The Administrative Section of the UTHSC Institutional Review Board (IRB) has received your written acceptance of and/or responses dated 10/18/2014, and 10/1/2014 to the provisos outlined in our correspondence of 10/3/2014 and 9/9/2014 concerning the above referenced project. The IRB determined that your application is eligible for expedited review under 45 CFR 46.110(b)(1) categories (3) and (5). The IRB has reviewed these materials and determined that they do comply with proper consideration for the rights and welfare of human subjects and the regulatory requirements for the protection of human subjects. Therefore, this letter constitutes full approval by the IRB of your application Version 1.4 as submitted including:

Main consent form, dated 10/20/2014 (stamped IRB approved 10/20/2014). This stamped consent form must be used to enroll subjects in the study.

Approval of this study will be valid from 10/20/2014 to 8/29/2015.

This study may not be initiated until you receive approval from the institution(s) where the research is being conducted.

The IRB has determined that the informed consent form, incorporating the authorization of subjects to use their protected health information in research, complies with the federal privacy regulations as specified in 45 CFR 160 and 45 CFR 164.

In developing procedures and documents for use with legally authorized representatives and family members of the subject, investigators should use the following definitions. The LAR must be a competent adult who has exhibited special care and concern for the subject, who is familiar with the subject’s personal values, who is reasonably available, and who is willing to serve. No
person who is identified in a protective order or other court order that directs that person to avoid contact with the subject shall be eligible to serve as the subject's LAR. Identification of a LAR should normally be made using the following order of descending preference: conservator; guardian; attorney-in-fact; subject's spouse, unless legally separated; the subject's adult child; the subject's parent; the subject's adult sibling or any other adult relative of the subject; or any other adult who is familiar with the patient's personal values, who is reasonably available, and who is willing to serve. The term "family member" means any of the following legally competent persons who are not the legally authorized representative of the subject: spouse; parents; children (including adopted children); brothers; sisters, and spouses of brothers and sisters; and any individual related by blood or affinity whose close association with the subject is the equivalent of a family relationship.

In the event that subjects are to be recruited using solicitation materials, such as brochures, posters, web-based advertisements, etc., these materials must receive prior approval of the IRB. Any revisions in the approved application must also be submitted to and approved by the IRB prior to implementation. In addition, you are responsible for reporting any unanticipated serious adverse events or other problems involving risks to subjects or others in the manner required by the local IRB policy.

Finally, re-approval of your project is required by the IRB in accord with the conditions specified above. You may not continue the research study beyond the time or other limits specified unless you obtain prior written approval of the IRB.

Sincerely,

[Signature]

Signature applied by Margaret M Sularin on 10/20/2014 09:03:46 AM CDT

Signature applied by Terrence F Ackerman on 10/20/2014 09:04:23 AM CDT

Margaret M. Sularin, LMSW, RDN, CCRP, CIM, CIP                    Terrence F. Ackerman, Ph.D.
Regulatory Specialist                              Chairman
UTHSC IRB                                            UTHSC IRB