A miniaturized spectrophotometric in situ pH sensor for seawater

Reuben C. Darlington

The University Of Montana

Follow this and additional works at: http://scholarworks.umt.edu/etd

Part of the Chemical Engineering Commons, Environmental Chemistry Commons, Geochemistry Commons, and the Mechanical Engineering Commons

Recommended Citation
http://scholarworks.umt.edu/etd/11028

This Thesis is brought to you for free and open access by the Graduate School at ScholarWorks at University of Montana. It has been accepted for inclusion in Graduate Student Theses, Dissertations, & Professional Papers by an authorized administrator of ScholarWorks at University of Montana. For more information, please contact scholarworks@mail.lib.umt.edu.
A MINIATURIZED SPECTROPHOTOMETRIC IN SITU pH SENSOR

FOR SEAWATER

By

REUBEN CURTIS DARLINGTON

B.A. Physics, The University of Montana, Missoula, MT, 2002

Thesis

presented in partial fulfillment of the requirements
for the degree of

Master of Interdisciplinary Studies

The University of Montana Missoula, MT

Official Graduation Date Spring 2017

Approved by:

Scott Whittenburg, Dean of The Graduate School
Graduate School

Michael D. DeGrandpre, Chair
Chemistry

J.B.A. Sandy Ross
Chemistry

Bradley E. Layton
Applied Computing and Engineering Technology

James C. Beck
Sunburst Sensors, LLC
A Miniaturized Spectrophotometric in situ pH Sensor for Seawater

Chairperson: Michael D. DeGrandpre

Since the Industrial Revolution, the world’s oceans have absorbed increasing amounts of CO$_2$ and the resultant changes to the marine carbonate chemical system have reduced the pH by > 0.1 units (∼ 30%) in surface waters. This acidification of the oceans has many far reaching impacts on marine life and there is great need of quality instrumentation to assess and follow the changing carbonate system. This MIS project aims to develop a low cost pH sensor with high precision and accuracy for open sea measurements with special emphasis on reduced size and cost. Design effort is based on the commercially available in situ ocean pH sensor, the SAMI-pH. Emphasis on small size and low cost will allow deployment of the sensors on a much wider variety of platforms than are currently viable thus greatly extending the spatial and temporal resolution of ocean acidification measurements. One such platform is NOAA’s Global Drifter Program, a network of non-recovered drifting buoys that has potential for ocean carbon cycle research. A prototype instrument was designed, the inexpensive SAMI-pH or iSAMI-pH. This instrument was entered into the Wendy Schmidt Ocean Health (WSOH) XPRIZE. This was an incentivized global competition to spur innovation in pH sensor technology with both accuracy and affordability prize purses totaling $2 million dollars. The affordability purse consisted of three phases of testing that explored accuracy, precision and stability using a variety of tests that spanned 6 months. It progressed from bench testing in a temperature controlled chamber and a 60 day tank test at the Monterey Bay Aquarium Research Institute (MBARI), to a month long deployment in a specially designed tank at the Seattle Aquarium that used the highly variable waters of Puget Sound. In lab testing, the iSAMI showed ∼0.01 accuracy. In the MBARI test tank, the iSAMI showed precision of ±0.004 pH units and stability of 0.008 pH units per month with validation uncertainty of ±0.009 pH units. In the coastal trials, the iSAMI again showed a precision of ±0.004 pH units and a stability of 0.011 pH units per month with a validation uncertainty of ±0.012 pH units. Stability or drift was statistically indistinguishable from that of the validation measurements. The iSAMI was in excellent agreement with the commercially available SAMI-pH which won the accuracy prize purse of the WSOH XPRIZE. The iSAMI won the affordability prize purse exceeding the performance metrics by several fold.
Acknowledgements

This research was supported by grants funded through the National Oceanographic and Atmospheric Administration Small Business Innovation Research program.

Gratitude to the Wendy Schmidt Foundation and the XPRIZE Foundation for sponsoring the Wendy Schmidt Ocean Health XPRIZE which spurred development of the iSAMi.

I wish to thank the crew at Sunburst Sensors and the DeGrandpre Lab. Jim Beck gave sage advice on mechanical design and whose maddening attention to grammar finally drove me to learn to punctuate. Reggie Spaulding was a wealth of knowledge and I subjected her to numerous discussions on chemistry and ocean acidification. Dave Podrasky offered encouragement, professional advice, countless edits and kept a sense of humor. Professor Mike DeGrandpre has been an excellent teacher and mentor. His enthusiasm and encouragement to be excited, not terrified, about presenting this work made all the difference.

Mark O’Day (Big Sky Machining) was a massive help in machining prototypes and offered excellent advice on communicating mechanical drawings.

I thank my wife, Stacy Houge, and my children, Constance Darlington, Ayla Baca and Oliver Darlington for supporting me through all the kvetching and late nights throughout the writing process.

This work is dedicated to the memory of Harrison Tide Darlington. I love you more.
Continuous improvement is better than delayed perfection.
– Mark Twain
## Contents

1 Introduction and background 1

1.1 Overview and motivation ................................. 1
1.2 The marine inorganic carbon cycle ........................ 6
1.3 Measuring pH in seawater ................................. 8
1.3.1 pH scales ........................................... 8
1.3.2 Luminescence methods ................................. 9
1.3.3 Potentiometric methods ............................... 9
1.3.4 Spectrophotometric methods .......................... 11
1.4 Spectrophotometric theory ............................... 13
1.5 Overview of SAMI-pH ................................. 15
1.6 Combined $p$CO$_2$ and pH .............................. 21
1.7 Research objectives ................................. 22

2 Methods and materials 24

2.1 Designs: mixers ........................................ 24
2.2 Designs: optofluidic cells ............................... 27
2.2.1 3D-printed cells .................................... 28
2.2.2 Ghosting correction ................................... 29
2.2.3 Clamshell cells ....................................... 30
2.2.4 Integrated beam combiner cell ....................... 33
2.3 iSAM-pH ........................................... 34
2.4 Wendy Schmidt Ocean Health XPRIZE .................... 37

3 Results 41

3.1 Designs: mixers ........................................ 41
3.2 Designs: optofluidic cells ............................... 46
3.2.1 3D printed cells .................................... 47
3.2.2 Clamshell cells ....................................... 48
3.2.3 Integrated beam combiner cell ....................... 53
3.3 iSAM-pH ........................................... 55
3.4 Wendy Schmidt Ocean Health XPRIZE .................... 56
3.4.1 Phase 2a - lab accuracy trials ....................... 57
3.4.2 Phase 2b - precision and stability trials ............ 61
3.4.3 Phase 3 - coastal trials at the Seattle Aquarium ... 64

4 Discussion 68

4.1 WSOH XPRIZE discussion ............................... 68
4.1.1 Possible sources of error ........................... 69
4.1.2 Phase 2a - lab accuracy trials ....................... 71
4.1.3 Phase 2b - precision and stability trials ............ 74
## List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>The Keeling Curve</td>
<td>2</td>
</tr>
<tr>
<td>1.2</td>
<td>Speciation of DIC</td>
<td>4</td>
</tr>
<tr>
<td>1.3</td>
<td>Spatial coverage status of the Global Drifter Array</td>
<td>6</td>
</tr>
<tr>
<td>1.4</td>
<td>Schematic of an Ion Sensitive Field Effect Transistor</td>
<td>11</td>
</tr>
<tr>
<td>1.5</td>
<td>Absorbance spectra of meta-Cresol Purple</td>
<td>13</td>
</tr>
<tr>
<td>1.6</td>
<td>Schematic layout of the SAMI-pH</td>
<td>16</td>
</tr>
<tr>
<td>1.7</td>
<td>SAMI-pH intensity data</td>
<td>17</td>
</tr>
<tr>
<td>1.8</td>
<td>SAMI-pH absorbance data</td>
<td>18</td>
</tr>
<tr>
<td>1.9</td>
<td>SAMI-pH point-pH curve</td>
<td>19</td>
</tr>
<tr>
<td>1.10</td>
<td>SAMI-pH indicator perturbation correction</td>
<td>20</td>
</tr>
<tr>
<td>2.1</td>
<td>The cartridge mixers</td>
<td>25</td>
</tr>
<tr>
<td>2.2</td>
<td>Hypersheer and tube mixers</td>
<td>26</td>
</tr>
<tr>
<td>2.3</td>
<td>The SAMI Z, a flow-cell</td>
<td>28</td>
</tr>
<tr>
<td>2.4</td>
<td>Model for 3D printing Z-cells with optical windows</td>
<td>29</td>
</tr>
<tr>
<td>2.5</td>
<td>Lateral displacement through a plate</td>
<td>29</td>
</tr>
<tr>
<td>2.6</td>
<td>Drawing of clamshell 1</td>
<td>31</td>
</tr>
<tr>
<td>2.7</td>
<td>Drawing of clamshell 2</td>
<td>32</td>
</tr>
<tr>
<td>2.8</td>
<td>The integrated beam combiner flow-cell</td>
<td>34</td>
</tr>
<tr>
<td>2.9</td>
<td>The inexpensive SAMI-pH</td>
<td>35</td>
</tr>
<tr>
<td>2.10</td>
<td>WSOH XPRIZE competition guidelines</td>
<td>39</td>
</tr>
<tr>
<td>3.1</td>
<td>Of mixers and membranes</td>
<td>42</td>
</tr>
<tr>
<td>3.2</td>
<td>Best laid plans lead to smaller volumes</td>
<td>43</td>
</tr>
<tr>
<td>3.3</td>
<td>Dilution curves for two cartridge mixers</td>
<td>45</td>
</tr>
<tr>
<td>3.4</td>
<td>3D printed flow-cells in ABS</td>
<td>48</td>
</tr>
<tr>
<td>3.5</td>
<td>Clamshell 1 machined in PC</td>
<td>49</td>
</tr>
<tr>
<td>3.6</td>
<td>Clamshell 2 optical window detail</td>
<td>51</td>
</tr>
<tr>
<td>3.7</td>
<td>IBC flow-cell on a SAMI control board</td>
<td>54</td>
</tr>
<tr>
<td>3.8</td>
<td>IBC exploded view</td>
<td>54</td>
</tr>
<tr>
<td>3.9</td>
<td>WSOH XPRIZE accuracy trials</td>
<td>59</td>
</tr>
<tr>
<td>3.10</td>
<td>iSAMI at MBARI test tank</td>
<td>61</td>
</tr>
<tr>
<td>3.11</td>
<td>WSOH XPRIZE precision trials</td>
<td>63</td>
</tr>
<tr>
<td>3.12</td>
<td>iSAMI at the Seattle Aquarium</td>
<td>65</td>
</tr>
<tr>
<td>3.13</td>
<td>WSOH XPRIZE coastal trials</td>
<td>66</td>
</tr>
<tr>
<td>4.1</td>
<td>$pK_a$ difference plot for mCP</td>
<td>70</td>
</tr>
<tr>
<td>4.2</td>
<td>Blank measurements for phase 2a</td>
<td>72</td>
</tr>
<tr>
<td>4.3</td>
<td>Difference plot for iSAMI and tSAMI in phase 2b</td>
<td>74</td>
</tr>
<tr>
<td>4.4</td>
<td>iSAMI-002 raw unfiltered signal intensities for 600 measurement sequences during the precision trials (578 nm in red 434 nm in blue, x-axis is pump cycles, See Section 1.5)</td>
<td>75</td>
</tr>
</tbody>
</table>

vi
4.5  Blank raw intensity values measured at 434 nm for iSAMI-2 and tSAMI-3 during Phase 2b of the WSOH XPRIZE. The iSAMI is prone to bubble induced blank instability.  
76
4.6  tSAMI-3 raw unfiltered signal intensities for 600 measurement sequences during the precision trials (578 nm in red 434 nm in blue, x-axis is pump cycles, See Section 1.5).  
77
4.7  tSAMI-3 point-pH curves for measurement sequences 260 to 270. Measurements 260 to 270 occur during some large spikes in tSAMI-3 on about 10/16/2014 in Figure 3.11 (x-axis is pump cycles, See Section 1.5).  
78
4.8  Difference plot for iSAMI and tSAMI in phase 3  
79
4.9  Average blanks for phase 3  
80
4.10 iSAMI-1 raw unfiltered signal intensities for 600 measurement sequences of Phase 3 (578 nm in red 434 nm in blue, x-axis is pump cycles, See Section 1.5).  
81
4.11 iSAMI-1 filtered signal intensities for 600 measurement sequences of Phase 3 (578 nm in red 434 nm in blue, x-axis is pump cycles, See Section 1.5).  
81
4.12 tSAMI-2 raw unfiltered signal intensities for 600 measurement sequences of Phase 3 (578 nm in red 434 nm in blue, x-axis is pump cycles, See Section 1.5).  
82

5.1  The next generation iSAMI-pH. Potential names for the instrument include: SAMin or Submersible Autonomous Miniaturized instrument for pH, µSAMI, or iSAMI².  
87

A.1  Phase 2a final pH data calculated using the SAMI Client v1.28. This highlights the necessity of the MATLAB™ data filters.  
94
A.2  iSAMI-001 raw unfiltered signal intensities for phase 2a.  
95
A.3  iSAMI-001 filtered signal intensities for phase 2a.  
95
A.4  iSAMI-001 point pH curves for phase 2a.  
96
A.5  iSAMI-001 final tris pH and temperature for phase 2a.  
96
A.6  iSAMI-002 raw unfiltered signal intensities for phase 2a.  
97
A.7  iSAMI-002 filtered signal intensities for phase 2a.  
97
A.8  iSAMI-002 point pH curves for phase 2a.  
98
A.9  iSAMI-002 final seawater pH and temperature for phase 2a.  
98
A.10 iSAMI-003 raw unfiltered signal intensities for phase 2a.  
99
A.11 iSAMI-003 filtered signal intensities for phase 2a.  
99
A.12 iSAMI-003 point pH curves for phase 2a.  
100
A.13 iSAMI-003 final tris pH and temperature for phase 2a.  
100
A.14 tSAMI-001 raw unfiltered signal intensities for phase 2a.  
101
A.15 tSAMI-001 filtered signal intensities for phase 2a.  
101
A.16 tSAMI-001 point pH curves for phase 2a.  
102
A.17 tSAMI-001 final tris pH and temperature for phase 2a.  
102
A.18 tSAMI-002 raw unfiltered signal intensities for phase 2a.  
103
A.19 tSAMI-002 filtered signal intensities for phase 2a.  
103
A.20 tSAMI-002 point pH curves for phase 2a.  
104
A.21 tSAMI-002 final seawater pH and temperature for phase 2a.  
104
A.22 tSAMI-003 raw unfiltered signal intensities for phase 2a.  
105
A.23 tSAMI-003 filtered signal intensities for phase 2a.  
105
A.24 tSAMI-003 point pH curves for phase 2a.  
106
A.25 tSAMI-003 final tris pH and temperature for phase 2a.  
106
A.26 iSAMI-002 raw unfiltered signal intensities for 600 measurement sequences.  
107
A.27 iSAMI-002 filtered signal intensities for 600 measurement sequences.  
108
A.28 iSAMI-002 point pH curves for 600 measurement sequences.  
108
A.29 iSAMI-002 final seawater pH and temperature for 600 measurements.  
109
A.30 iSAMI-002 least squares fit for indicator perturbation correction for 600 measurements.  
109
A.31 iSAMI-002 raw unfiltered signal intensities for measurement sequences 260 to 270.  
110
A.32 iSAMI-002 filtered signal intensities for measurement sequences 260 to 270.  
110
## List of Tables

3.1 Summary statistics of mixer testing .......................................................... 44  
3.2 iSAM II and tSAM II validation data ......................................................... 56  
3.3 WSOH XPRIZE accuracy trials validation team results .............................. 60  
3.4 WSOH XPRIZE precision trials validation team results ............................ 64  
3.5 WSOH XPRIZE coastal trials validation team results ............................... 67
Chapter 1

Introduction and background

Ocean acidification is an anthropogenic phenomenon that is changing the fundamental biogeochemistry of seawater throughout the world’s oceans. This chapter introduces how anthropogenic atmospheric carbon dioxide interacts with seawater to reduce the pH and reviews methods for quantifying this change. Spectrophotometric methods are reviewed and the theory is discussed in detail. This chapter concludes with an in depth discussion of the SAMI-pH and the goals of this research: to miniaturize a spectrophotometric in situ pH sensor for seawater.

1.1 Overview and motivation

The global average concentration of atmospheric carbon dioxide has been increasing at a geologically unprecedented rate since the industrial revolution due to fossil fuel combustion. Antarctic ice cores at Vostock and Dome C show atmospheric concentrations of CO$_2$ have fluctuated between 170 and 300 parts per million by volume (PPMV) for the last 800,000 years (Luthi et al., 2008). Humans are carrying out the largest geophysical experiment in history, one that will never be reproduced (Revelle and Suess, 1957). It is of great importance to attempt to record this experiment which will yield insight into the processes which determine our climate. Principally, it is critical to understand how carbon dioxide released into the atmosphere is divided between land and the sea.

Prior to the pioneering work of C.D. Keeling in the late 1950’s (Keeling, 1958) there was no scientific consensus regarding the atmospheric level of CO$_2$ (Keeling, 1998). Keeling’s work revealed for the first time the
dramatic annual cycle of CO₂ in the atmosphere: the breath of the earth. Not only had Keeling discovered the seasonal fluctuation of CO₂ due to annual plant growth, but also the increase of CO₂ in the atmosphere. Figure 1.1 shows the monthly mean concentration of atmospheric CO₂ measured atop Mauna Loa, HI, from March 1958 to November 2nd, 2016.

The global ocean has absorbed up to 48% of an estimated 118 ±19 petagrams of carbon released since the industrial revolution (Sabine and Tanhua, 2010). This increase in aqueous CO₂ in the surface ocean has led to a significant decrease in sea surface pH (∼ 0.1 pH units nearly %26), a sustained and long term process that is termed Ocean Acidification, (OA) (Raven et al., 2005). It should be noted that although the oceans are currently alkaline, OA is the process and trend of decreasing ocean pH.

It is unknown how the consequences of this continuing process will be manifested. However, it is becoming clear that the resultant changes in ocean and atmosphere geochemistry, are disrupting entire ecosystems – from the coral reefs (Edmunds et al., 2013) to the human food supply – with both beneficial and detrimental impacts (Doney et al., 2009).

Coral reefs provide protection for coastal zones and crucial habitat essential for the survival of commercially harvested species. Under the pressures of OA and climate change, reefs are being threatened by a reduc-
tion in calcification rates, increased dissolution of the skeletal structure, ocean warming and deoxygenation leading to bleaching (Hall-Spencer et al., 2015). Recent research suggests that the decrease in pH of bulk seawater under OA will require more energy for coralline organisms to remove ions from their calcifying fluid (Cyronak et al., 2015a). Magnesian calcite and aragonite are the more soluble of the calcium carbonate species and marine organisms that precipitate them to form shells could be affected more severely (Millero, 2007).

These multiple stressors are accompanied by a host of microbial changes occurring in concert with acidifying seas. O’Brien et al. (2016) recently reviewed the effects of OA on marine microorganisms. Marine microorganisms provide essential functions such as nutrient cycling for coral reefs and serve complex symbiotic roles to help maintain the health of a wide ranging list of reef invertebrates. This work suggests that studying reef microbial ecosystems in natural CO$_2$ seeps will give clues as to prevalence of disease associated microbes, structural diversity, and alternative dominant species in other habitats in a high CO$_2$ world.

Across taxa it is the larval stages of calcifying organisms, such as pteropods, oysters and other commercially valuable bivalves, that are most at risk (Kwiatkowski et al., 2016). Pteropods are pelagic-shell forming gastropods that serve as a crucial component of the marine food web. They serve as prey for important species either for conservation or commercial harvest, such as pink salmon. They also serve as important biological indicators of environmental conditions (Bednaršek et al., 2016). However, emerging research shows the complexity of studying the effects of OA on marine ecosystems. MacLeod and Poulin (2016) recently showed that parasitic infection can modify the host response to OA. Pteropods were screened for trematode infection and maintained in either acidified or unmodified seawater for 90 days. Those pteropods infected showed little difference while the uninfected pteropods showed a marked increase in mortality in acidified seawater.

As discussed above, the larval stages of all carbonate mineral forming organisms are most at risk from OA. OA is changing the naturally occurring proportions of dissolved inorganic carbon species in the ocean. As pH decreases the carbonate species (CO$_3^{2-}$) will decline (See Figure 1.2). This is traditionally thought to be the driver impeding calcification rates. The saturation state, $\Omega$, of calcite and aragonite – which are important
minerals for all calcifying organisms such as corals and larval oysters – is thermodynamically defined as

\[ \Omega = \frac{[\text{Ca}^{2+}][\text{CO}_3^{2-}]}{K_{sp}} \]  

where \( K_{sp} \) is the apparent solubility product constant of either calcite or aragonite. Values of \( \Omega > 1 \) indicate supersaturation and favor formation (precipitation) while values of \( \Omega < 1 \) favor dissolution. Aragonite is the more soluble of the two forms of calcium carbonate since it has a larger \( K_{sp} \).

![Figure 1.2: Speciation of dissolved inorganic carbon (DIC) as a function of pH. (Holmén, 1992)](image)

There is much debate about whether the reduced \( \text{CO}_3^{2-} \) substrate availability (i.e. \( \Omega < 1 \)) is really the driver for reduced calcification rates under OA. The colorful exchange between Cyronak et al. (2015a) and Waldbusser et al. (2015) (See Cyronak et al. (2015b) for the response) is a good example. What is clear is that with decreasing ocean pH there is an increased energy expenditure for calcifying organisms.

The shellfish hatcheries of the west coast of the US are among the first to see a significant impact of OA.
This is driven by coastal upwelling. Upwelling brings deep ocean water high in CO$_2$ onto the continental shelf and occurs due to prevailing winds displacing surface waters along the coastline (Feely et al., 2012; Harris et al., 2013). The advection of high CO$_2$ waters poses risks to the marine economy as the dramatic collapse of the hatchery larval oysters between 2005 and 2009 shows. This disrupted oyster seed supplies for shellfish farms coast wide. Washington state alone provides 85% of west coast sales (including Alaska) with over a $270 million economic impact. These seed production failures should be seen as the canary in the coal mine as the impacts will increase over time (Adelsman and Whitely Binder, 2012).

Despite a large body of carbon data, there is uncertainty in certain mechanisms of the global carbon cycle. For example, global air-ocean flux has an uncertainty greater than 50% (Takahashi et al., 2009). Such uncertainties limit our ability to make model-based predictions of the impacts of increased atmospheric and oceanic CO$_2$. The overwhelming size of the oceans and many inhospitable regions, result in sparse temporal and spatial resolution of carbon measurements. Due to this sparse resolution, there remains a great need to quantify the changing oceanic geochemistry.

One way to increase the temporal and spatial resolution is to take advantage of the Global Drifter Program (GDP). The GDP is a network of non-recovered surface drifting buoys that provide near real-time measurement of a suite of oceanographic parameters. The array is used primarily for calibrating satellite measurements of sea surface temperature and is the main source of ocean current measurements which are used to validate climate and ocean forecast models. This impressive array is an underutilized tool for carbon cycle measurements because current in situ instrumentation is both too large and too expensive to implement on a global scale in a network where instruments are not recovered.
The purpose of this work was to develop in situ pH instrumentation both small and affordable enough to be implemented on the GDP network. Utilizing commercially available spectrophotometric pH sensor technology as the core of this effort; an instrument with a significant reduction in size, complexity and cost was developed. Extensive lab, tank and coastal trials compared this miniaturized instrument to the commercial instrument upon which it was based. These instruments were judged against a global community of top researchers developing pH sensors. This testing took place as part of the Wendy Schmidt Ocean Health (WSOH) XPRIZE, an incentivized global competition to spur innovation in oceanographic pH sensor technology. This opportunity provided a comparison of the leading pH sensor technologies that would otherwise have been too great a challenge for any one research group to undertake.

1.2 The marine inorganic carbon cycle

Carbonate chemistry is the dominant chemical system in the oceans, and quality, high frequency measurement parameters are important for understanding relative sources and sinks of atmospheric CO$_2$. The uptake of atmospheric CO$_2$ into the oceans results in the formation of carbonic acid. At the ocean surface, atmospheric
CO₂ is in thermodynamic equilibrium with aqueous CO₂

\[
\text{CO}_2(\text{g}) \rightleftharpoons \text{K}_H \times \text{CO}_2(\text{aq}) .
\] (1.2)

The aqueous CO₂ reacts with water to form carbonic acid,

\[
\text{CO}_2(\text{aq}) + \text{H}_2\text{O} \iff \text{H}_2\text{CO}_3 .
\] (1.3)

Since CO₂(aq) and H₂CO₃ are nearly indistinguishable the sum of those species is denoted as H₂CO₃*

\[
\text{CO}_2(\text{g}) + \text{H}_2\text{O} \rightleftharpoons \text{K}_H \times \text{H}_2\text{CO}_3^* .
\] (1.4)

where \(K_H\) is the Henry’s Law constant for CO₂. Carbonic acid is diprotic which further dissociates into bicarbonate and carbonate in the following equilibria:

\[
\text{H}_2\text{CO}_3^* \rightleftharpoons \text{K}_{a1} \times \text{HCO}_3^- + \text{H}^+ ,
\] (1.5)

\[
\text{HCO}_3^- \rightleftharpoons \text{K}_{a2} \times \text{CO}_3^{2-} + \text{H}^+ .
\] (1.6)

The equilibrium constants are derived from the expressions above:

\[
\text{K}_H = \frac{[\text{H}_2\text{CO}_3^*]}{[\text{pCO}_2]},
\] (1.7)

\[
\text{K}_{a1} = \frac{[\text{HCO}_3^-][\text{H}^+]}{[\text{H}_2\text{CO}_3^*]},
\] (1.8)

\[
\text{K}_{a2} = \frac{[\text{CO}_3^{2-}][\text{H}^+]}{[\text{HCO}_3^-]}.
\] (1.9)

The marine inorganic carbon cycle can be characterized by any two of the four measurable parameters: pH,
partial pressure of CO$_2$ (pCO$_2$), dissolved inorganic carbon (DIC), and total alkalinity ($A_T$),

\[ \text{DIC} = [\text{H}_2\text{CO}_3^{\ast}] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}] \]  
\[ (1.10) \]

\[ A_T = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{OH}^-] + [\text{B(OH)}_4^-] - [\text{H}^+] - [\text{H}_2\text{SO}_4^-] - [\text{HF}] \].  
\[ (1.11) \]

Using the above equilibrium expressions along with DIC and $A_T$, the system can be reduced to two equations and four unknowns. Thus, any two parameters can be measured to characterize the entire system (Millero, 2007). Sea surface salinity is directly correlated with alkalinity in the open ocean and has a well documented relationship (Lee et al., 2006). Using this relationship to derive $A_T$, along with measuring pH, it is possible to characterize the entire carbon system. The GDP will have up to 300 drifters with sea surface salinity measurement capability and this combined with an accurate pH instrument could enable a fleet of drifting buoys to characterize the entire carbonate system in the surface ocean in near real-time. This capability would be very beneficial for climate modeling and OA researchers.

1.3 Measuring pH in seawater

1.3.1 pH scales

In chemical oceanography, the pH scale differs on the basis of the buffers used to calibrate the measurement system and hence, defines the pH. The scales on which seawater pH can be measured are: the National Bureau of Standards (NBS) scale; the free hydrogen ion scale; the total hydrogen ion scale; and the seawater scale. There exist ionic strength differences between seawater and the NBS buffers. This results in differences in pH between calibration and seawater measurements. The free hydrogen ion scale concerns just the free or unbound hydrogen ion concentration. The total hydrogen ion scale defines pH as a combination of free hydrogen ion and sulfate concentrations. The seawater scale defines pH as a combination of free ion, sulfate and fluoride concentrations (See e.g. Dickson (1984, 1993)).

Synthetic seawater buffers exist for the total hydrogen ion scale and have been used extensively to determine various equilibrium constants of the carbon cycle. Therefore, it is the most commonly used and accepted standard scale for oceanographic pH measurements (Dickson et al., 2007).
1.3.2 Luminescence methods

Optical pH sensors (optodes) use pH sensitive dyes immobilized in a polymer matrix film that are luminescent. Phosphorescence is defined by the excited state lifetime being on the microsecond timescale whereas fluorescence is defined by excited state lifetimes on the nanosecond timescale. These dyes change either their intensity or their excited state lifetime, on the order of microseconds, when exposed to variations in hydrogen ion concentration. Intensity techniques (emission based) for pH sensing suffer from photo-bleaching and ambient light contamination of the signal.

One approach uses a combination of two dyes with only one species being sensitive to pH, while a longer lifetime dye is insensitive to pH. The technique, time domain dual-lifetime referencing, is ratiometric and is combined with excited state lifetime measurements (Liebsch et al., 2001). Another approach uses a resonance energy transfer from the excited state of the pH insensitive ruthenium complex (donor) to the excited state of the pH sensitive indicator (acceptor) (Kosch et al., 1998). This approach still has limitations such as baseline drift, photo-bleaching of the dye which limits deployment duration and displacement or delamination of the film.

Though there have been great strides with dual lifetime referencing techniques (Korostynska et al., 2007; Moore et al., 2009; Clarke et al., 2015), and at least one instrument inspired by the WSOH XPRIZE (http://www.analytchem.tugraz.at/xprize/), there remain no commercial in situ instruments suitable for OA research as of this writing. Byrne (2014) and Martz et al. (2015) summarize the available technology and needs of researchers studying OA yet make no mention of pH optodes in their critical reviews. As shown in Clarke et al. (2015) there are commercially available pH sensor spots (films) available to researchers wanting to explore the possibilities for this technology.

1.3.3 Potentiometric methods

Potentiometric determination of pH relies on an electric potential difference between a reference electrode and an electrode formed from an ion selective membrane. A ubiquitous example is the hydrogen ion sensitive glass electrode. Though proven useful in short term studies of systems with naturally high temporal variability in pH changes (> 0.01) they are not considered suitable for inter-annual OA studies (Rérolle et al., 2012). Further, frequent recalibration is necessary to account for drift between the reference and pH sensing
A relatively new application of the potentiometric measurement of pH is the ion sensitive field effect transistor or ISFET (See Figure 1.4). The sensor chip contains an internal reference electrode and an ion sensitive conduction gate. Current flowing between the source and drain is controlled by the concentration of hydrogen ion in the conduction gate. The pH is determined by the potential difference between the conduction gate and the reference electrode. For detailed explanation of the operating principles see Sandifer and Voycheck (1999) as well as a review covering 30 years of development work with ISFETs (Bergveld, 2003).

An in situ ISFET pH instrument for seawater has been developed using the commercially available Durafet® pH sensor from Honeywell (Martz et al., 2010). It required extensive calibration procedures and long conditioning times. The system also posed similar problems to the glass electrode with drift in the internal reference electrode. This system was suitable to the surface waters, at 1 atmosphere. A deep water version has also been developed and has been used on the Argo profiling system. This version takes advantage of known stable pH at depth as a proxy to determine drift and back calculate calibrated surface pH measurements (Johnson et al., 2016). A recent evaluation of ISFET sensors was conducted along the California coast in a network context by comparison to discrete (bottle samples) measurements of seawater pH (McLaughlin et al., 2017). Results showed the average difference between discrete samples and in situ ISFET pH of 0.005. This pH difference ranged from 0.083 to -0.030 where the authors attributed the range to user specific handling. Yet even with the most experienced operators the ISFET systems only achieved $\pm 0.02$ pH unit accuracy as compared to the discrete measurement.
1.3.4 Spectrophotometric methods

Spectrophotometric determination of seawater pH is a direct measurement based on the ratio of absorbance maxima of a pH sensitive sulfonephthalein dye (For detailed explanation of the theory see Section 1.4 and Dickson et al. (2007)). Systems based on this method come in three basic types of measurement configurations: benchtop spectrophotometer, shipboard underway systems at semi in situ conditions and fully autonomous at in situ conditions. The method is routinely carried out manually with a benchtop spectrophotometer and is used for discrete sampling of seawater collected by rosettes either on board ship during transects or back on land based laboratories (Dickson et al., 2007). This method is highly accurate but the spatial and temporal resolution is still quite low.

Various sulfonephthalein indicators have been successfully employed for the analysis of pH with many different instrument configurations (DeGrandpre et al., 2014). Phenol red has been characterized with discrete samples for seawater measurements (Robert-Baldo et al., 1985). Thymol blue has also been characterized in a similar manner at in situ conditions (Zhang and Byrne, 1996). A more recent study using thymol blue in a shipboard underway autonomous configuration suggested an improvement on the technique by fitting the absorbance maxima over a broader wavelength range (Olilie et al., 2007). Cresol red was characterized ship-
board using discrete samples and a benchtop spectrometer (Byrne and Breland, 1989). The recommended indicator for carbon system measurements is meta-cresol purple (mCP) (Dickson et al., 2007) and has been used extensively for seawater pH from discrete laboratory samples (Clayton and Byrne, 1993) to shipboard service underway systems (Rérolle et al., 2012, 2013) to fully in situ instruments (Seidel et al., 2008; Gray et al., 2011; Harris et al., 2013). It is the most useful primarily because it has been extensively characterized and evaluated in both impure and purified forms as compared to the other indicators mentioned.

The method is robust and has even been adapted to a DIY Arduino microcontroller based photometer with 0.01 pH unit accuracy for discrete samples (Yang et al., 2014). Though this unit is suitable for citizen science and limited coastal monitoring, the method still requires the use of a high quality analytical spectrophotometer to calibrate the system. Recent steps have been taken towards a miniaturized spectrophotometric pH sensor using a microfluidic approach (Rérolle et al., 2012, 2013). This system uses a flow through seawater sample chamber to house the absorbance flow-cell. This allows measurements to be made at near in situ temperature (+0.2 °C). Though the system uses very little reagent per measurement (on the order of 17 µL) it relies on the use of syringe pumps which would be difficult to use in situ and consume more power than would be practical for an in situ instrument.

Commercially available autonomous in situ spectrophotometric instruments are limited in number. There are shipboard underway and in situ spectrophotometric sensors available from Sunburst Sensors, LLC, (USA) and Sensor Lab (Spain) (See e.g. Moore et al. (2009); Byrne (2014); Martz et al. (2015)). These systems are automated and suitable for moorings, buoys and shipboard laboratories but do not extend the temporal or spatial resolution more than any systems mentioned above. This thesis project took advantage of unrestricted access to the SAMI-pH, or Submersible Autonomous Moored Instrument for pH (Sunburst Sensors). This commercially available instrument is designed to make these measurements in situ at depths of up to 600 m for as long as a year. Spectrophotometric theory and the SAMI-pH are described in detail in Sections 1.4 & 1.5.
1.4 Spectrophotometric theory

The use of indicators for pH measurements of seawater are based on the ratio of absorbance maxima of the distinctly colored acid [HL\(^-\)] and base [L\(^2-\)] forms of the diprotic family of sulfonephthaleins (Clayton and Byrne, 1993). Figure 1.5 shows the absorption spectra of meta-Cresol Purple (mCP) in both an acid (green curve) and base form (purple curve).

![Figure 1.5: Absorbance spectra of meta-Cresol Purple showing both the acid form [HL\(^-\)] in green and base form [L\(^2-\)] in purple (DeGrandpre et al., 2014).](image)

Generally, the dissociation is written as

\[
\text{HL}^- \xrightleftharpoons[K'_a]{K'_a} \text{H}^+ + \text{L}^{2-}
\]  

(1.12)

since at seawater pH (~8) the H\(_2\)L form is not present. The second apparent dissociation constant, \(K'_a\), includes the temperature and ionic strength dependence (Seidel et al., 2008) and so can be expressed as

\[
K'_a = \frac{[\text{H}^+][\text{L}^{2-}]}{[\text{HL}^-]}
\]

(1.13)

Converting this expression to log form and solving for pH yields

\[
pH = pK'_a + \log \left( \frac{[L^{2-}]}{[HL^-]} \right)
\]

(1.14)
The Beer-Lambert Law \( (A = \epsilon cl) \) is used to quantify the concentrations, \([c]\), of the acid and base forms, taking into account the possibility of both forms having some concentration at each absorbance maxima,

\[
A_{\lambda 1} = \epsilon_{a\lambda 1}[HL^-]l + \epsilon_{b\lambda 1}[L^2^-]l, \tag{1.15}
\]

\[
A_{\lambda 2} = \epsilon_{a\lambda 2}[HL^-]l + \epsilon_{b\lambda 2}[L^2^-]l, \tag{1.16}
\]

where the \(\epsilon\)'s are the molar absorptivities for the acid \((a)\) and base \((b)\) forms at wavelengths \(\lambda_1\) and \(\lambda_2\) respectively, and \(l\) is the optical path length. Absorbance is determined with \(A_\lambda = -\log(I/I_0)\), where \(I\) is the intensity of light transmitted through the indicator sample mixture and \(I_0\) is the intensity of light transmitted through the sample without indicator, a blank measurement. Solving these absorbance formulas for \([L^2^-]\) and \([HL^-]\) yields

\[
[L^2^-] = \frac{A_{\lambda 2}\epsilon_{a\lambda 1} - A_{\lambda 1}\epsilon_{a\lambda 2}}{\epsilon_{a\lambda 1}\epsilon_{a\lambda 1} - \epsilon_{a\lambda 1}\epsilon_{a\lambda 1}}, \tag{1.17}
\]

\[
[HL^-] = \frac{A_{\lambda 1}\epsilon_{b\lambda 2} - A_{\lambda 2}\epsilon_{b\lambda 1}}{\epsilon_{a\lambda 1}\epsilon_{a\lambda 1} - \epsilon_{a\lambda 1}\epsilon_{a\lambda 1}}, \tag{1.18}
\]

where total indicator concentration is given as,

\[
[I]_T = [HL^-] + [L^2^-]. \tag{1.19}
\]

In order to simplify the pH calculation, let \(R\) represent the ratio of absorbances and consider the following re-parameterization of the molar absorptivities,

\[
R = \frac{A_{\lambda 1}}{A_{\lambda 2}}, \tag{1.20}
\]

\[
e_1 = \frac{\epsilon_{a\lambda 1}}{\epsilon_{a\lambda 2}}, \quad e_2 = \frac{\epsilon_{b\lambda 1}}{\epsilon_{a\lambda 1}}, \quad e_3 = \frac{\epsilon_{b\lambda 2}}{\epsilon_{a\lambda 2}}. \tag{1.21}
\]

Substituting 1.17 and 1.18 into 1.14 using the above parameters, a convenient form of 1.14 is derived,

\[
pH = pK'_a + \log \left( \frac{R - e_1}{e_2 - Re_3} \right). \tag{1.22}
\]

It should be noted that the molar absorptivities are instrument specific and it is implicit in the derivation that \(R\) be measured with the same bandpass and analytical wavelengths as used to determine the \(\epsilon\)'s (DeGrandpre et al., 2014).
1.5 Overview of SAMI-pH

The SAMI-pH (Submersible Autonomous Moored Instrument for pH) is an in situ spectrophotometric pH instrument that was previously developed for natural waters (Martz et al., 2003), and later optimized for marine research (Seidel et al., 2008). The primary difference between the two being the choice of sulfonephthalein indicator: for marine applications mCP is the recommended choice as its $pK_a$ more closely matches the pH of seawater (Dickson et al., 2007). The SAMI-pH has since been redesigned with completely integrated control electronics and uses light emitting diodes (LED) with bandpass filters centered at the absorption peaks of the acid and base forms of mCP. The SAMI-pH is calibration-free and is validated by measurement of the pH of tris at 25 °C according to Dickson et al. (2007). The raw data is stored with a chip-based data logger and later downloaded to be processed by custom software.

Seawater is drawn into a solenoid pump through the normally open port of a 3-way solenoid valve using a 50 µL solenoid pump. Indicator is drawn into the system through the normally closed port when the solenoid valve is energized. The indicator and seawater are passively mixed as the solution is pushed through a hyper-shear mixer and through an optical flow-cell. The LED light sources are fitted with 10 nm bandpass filters. These shine through the 50/50 beam splitter (BS) and onto both a reference photodiode (PD) and an optical fiber (Figure 1.6). Fiber optics convey light from the BS assembly through the flow-cell to the signal PD. The reference PD is fitted with a neutral density filter and is used to monitor and correct for any intensity changes in the LEDs.
Each SAMI-pH measurement proceeds as follows: fifty-five 50 µL aliquots of seawater are pumped through the static mixer and absorbance flow-cell to clear bubbles, remove any remaining indicator and fill the system with fresh sample. One 50 µL aliquot of indicator is drawn into the pump via the valve and pumping of seawater resumes for 27 more, 50 µL aliquots. Indicator and sample water are mixed along the flow path to the optical flow-cell.

At each of these 27 remaining pump cycles light intensity measurements are taken at 434 nm and 578 nm, the peak absorbance wavelengths for the acid and base forms of mCP. The first four of these intensity measurements are indicator-free and are averaged together for a blank intensity, $I_0$. The next 23 intensity measurements, $I$, observe the seawater-indicator mixture pass through the flow-cell. This raw data forms
the backbone of the calculations discussed in Section 1.3.4. This sequence is shown graphically in Figure 1.7 as intensity versus pump cycle. This curve is referred to as the dilution curve.

Figure 1.7: Intensity data from one pH measurement sequence. Intensity measurements from the 578 nm and 434 nm LEDs are shown in red and blue, respectively. The first four measurements, shown with square markers, are the blanks with the remaining intensity measurements showing the reagent bolus enter the flow-cell and as it leaves the signals return to near blank levels. Intensity is measured immediately after each pump cycle.

With $I_0$ and $I$ measured, data filters in the MATLAB™ are applied to the raw data. The first data filter compares the blank intensities for 434 nm and 578 nm against the first 434 nm signal intensity. If any of these values are 10% lower than this value, or are saturated (i.e. > 4096 on a 12 bit analog to digital converter), this filter assigns the blank value from the previous measurement sequence. The second data filter checks for outliers in the signal intensities (i.e. the signals measured after reagent injection), which are larger than twice the standard deviation of the surrounding values. Outlying signals are assigned an intensity signal from the previous measurement sequence.

(Note: this filtering scheme was used in the MATLAB™ routines used for the XPRIZE so is described here.)
These routines have been improved as a result of the XPRIZE. These filtering routines are not currently used in the SAMI-Client software developed at Sunburst Sensors. See Appendix A Section A.1 for a comparison of data calculated both ways.

Using the blank and signal intensites, absorbance values \( A_\lambda = -\log(I/I_0) \) are calculated for both wavelengths. Figure 1.8 shows absorbance versus pump cycle. Here the reagent bolus is seen to enter the flow-cell where the maximum absorbance occurs around the 10th pump cycle and is almost completely flushed out by the 27th pump cycle.

![Figure 1.8: Absorbance calculated from one pH measurement sequence. Absorbance values at 578 nm and 434 nm are shown in red and blue, respectively. The blanks are not shown as they are used for the absorbance calculation and this is represented in the Pump Cycle axis. The relative peak absorbance values are set by the concentration of absorbing species \([HL^-]\) and \([L^{2-}]\).](image)

A pH value is determined (Equation 1.22) for each point in the measurement sequence (not for blanks) using the absorbance ratio \( R \) (Equation 1.20), the temperature-dependent molar absorptivity ratios (Equation 1.21) and the temperature and salinity dependent \( pK'_a \). This data is termed the point-pH curve and is shown in Figure 1.9.
It is generally the case that the point-pH values are quite variable at either end of the measurement sequence. This occurs because there is low indicator concentration in the flow-cell at the head or tail of the indicator bolus. As can be seen in the sulfonephthalein pH equation (Equation 1.14), as the concentration of either species approaches zero the log term becomes asymptotic and the pH becomes erratic.

The minimum (dip) in the curve, centered around the 10th pump cycle, is caused by the pH of the indicator perturbing the pH of the seawater sample. This dip does not occur in systems employing a 10 cm optical cell since the total indicator concentration is an order of magnitude less ($A = \epsilon cl$). To obtain an equivalent absorbance value using different path length, ($l$), optical cells, the indicator concentration must change by the same factor as the path length since the molar absorption coefficient, ($\epsilon$), doesn’t change.
To account for this pH perturbation, a linear region is taken on the point pH curve past the perturbation. This ensures that points are taken from the region of the curve with the least gradient of indicator across the optical cell. The suitable region is found in a stepwise fashion, using 5 points at time, and taking a linear regression through those point pH values. The region with the best coefficient of determination, \((R^2)\), is used to linearly extrapolate to the pH of the sample water to zero indicator concentration. This is acquired from a least squares fit of total indicator concentration \([mCP]_T\) (Equation 1.19) versus pH for the sequence to obtain the unperturbed final pH value for seawater (Figure 1.10). Tris is well buffered and its pH is not perturbed by the reagent bolus. The pH of tris calculated by the SAMI is the mean of the point-pH as determined by the region with the lowest standard deviation.

This perturbation correction was a major innovation from Seidel et al. (2008) that allowed the use of a 1 cm path length absorbance cell and a simple static mixing scheme. A 10 cm path length flow-cell is more difficult to fabricate than a 1 cm cell. The shorter path length reduces the total amount of pump cycles needed to flush the system completely to obtain blank intensities. This power savings is critical for in situ instrumenta-
tion and allows for longer deployment times. The 1 cm path length also has the advantage of decreased gains on the LEDs and photodiodes. With these types of flow through absorbance systems, the longer path length also leads to decreased light throughput and increased gradients. Throughput is roughly proportional to the path length, so \( \sim 10 \) times the LED gain would be needed for a 10 cm cell over a 1 cm cell (Seidel et al., 2008).

This discussion represents the full sequence for one single SAMI-pH measurement and will be referenced heavily in the Results and Discussion.

### 1.6 Combined \( p\text{CO}_2 \) and pH

The initial concept for this project was a miniaturized instrument to measure both \( p\text{CO}_2 \) and pH using a single indicator and optical cell. As noted in Section 1.2, measuring any two out of the four carbon parameters allows for the calculation of the remaining two.

Phenol Red could be used because its \( pK_a \) (\( \sim 8.0 \)) is a good compromise between Bromothymol Blue (\( pK_a \) \( \sim 7.3 \)) and mCP (\( pK_a \) \( \sim 8.3 \)), two indicators commonly used for \( p\text{CO}_2 \) and pH measurements, respectively. Though this approach sounds attractive and simple, it belies complexity: not only is the reagent solution composition different for \( p\text{CO}_2 \) and pH, but so also is the nature of its use in the measurement sequence.

For pH measurements, the reagent solution pH is kept constant. In use, concentrated indicator solution is mixed with seawater and so, is diluted. Therefore, absorbance measurements are taken with a mixture of seawater and indicator and a blank measurement using seawater is most appropriate (DeGrandpre et al., 1999).

For \( p\text{CO}_2 \) measurements, the reagent solution composition fixes the alkalinity so that the pH of the solution freely changes as it equilibrates with the \( p\text{CO}_2 \) of the seawater. The indicator is separated from the seawater by a gas permeable membrane and a more dilute solution is used. Therefore, absorbance measurements are taken on the indicator solution only and a blank measurement using pure water allows for consistent blank values during calibration and subsequent use.
Solution concentrations are chosen to obtain absorbance measurements in a linear range. Absorbance values $0.1 < 1.0$ ensure a linear range since this region has the least noise. This choice requires that indicator concentration be an order of magnitude greater for pH measurements compared to $pCO_2$ measurements. $pCO_2$ measurements require the periodic flushing of indicator solution with blank solution to obtain a blank measurement requiring the storage of more indicator for $pCO_2$ compared to that of pH. The SAMI-$pCO_2$ system is not as readily miniaturized as the SAMI-pH system since the membrane equilibrator is external to the SAMI-$pCO_2$. It was not determined whether using seawater for $pCO_2$ blanks would affect the $pCO_2$ accuracy, precision, or drift.

Therefore, a single system measuring both $pCO_2$ and pH would require two concentrations of the same indicator and a blank solution for $pCO_2$ measurements. This increases complexity and points of failure. Taken alone, the necessity of two separate reagent bags nearly precludes a miniaturized instrument. Additionally, $pCO_2$ and pH are covariant and don’t create the most accurate combination of the four carbon system master variables. A combination of pH and $A_T$ produce less error in modeled $pCO_2$ over other system pairs. (Millero, 2007; Gray et al., 2011).

The resulting complexity of the system is such that employing parallel systems makes much more sense and has the added benefit that the failure of one system does not cause the failure of the second. As an example, the pH system is open to seawater and is therefore susceptible to clogging whereas the $pCO_2$ system is closed to seawater and not prone to this type of failure. For these reasons, the decision was made to pursue a miniaturized pH system based on the spectrophotometric method, and the technology of the SAMI-pH. The remainder of this writing will focus on that effort.

### 1.7 Research objectives

The overarching goal of this project was to dramatically reduce the size, cost and complexity of the SAMI-pH. As discussed in Section 1.1, the GDP is an ideal platform for a low cost non-recoverable pH instrument. The current SAMI-pH design is too large and expensive to meet this need on a global scale. Further, the SAMI-pH is designed as a core instrument that functions as the central storage and control unit able to host multiple auxiliary instruments such as oxygen optodes, CTDs (conductivity temperature depth), PAR (photosynthetically active radiation), and fluorometers. Whereas a miniaturized SAMI-pH could function
as an auxiliary sensor for use on other carbon system instruments.

To reduce the cost, complexity and cost elimination of the fiber optics and static mixer would be ideal starting places. For use on the GDP fleet, and other surface water platforms, the instrument would not require pressure compensation, further reducing cost and complexity. The following points emphasize important considerations for this miniaturization effort: identification and reduction of expensive components; identification and replacement of components that limit the overall size and shape; decrease power consumption; design of a housing specific for surface measurements; design of the instrument as a slave device.

This thesis research focused on two main aspects of the instrument: the fiber optics and the static mixer (Figure 1.6). The static mixer is one of the most expensive components of the instrument. It is essential that its function be replicated in a smaller form factor and at a reduced cost. A lower volume mixer would reduce the number of pump cycles required to perform a measurement sequence which would decrease overall power consumption. Using microfluidic techniques could enable complex mixing schemes with increased configurability, functionality and scalability. These types of devices are relatively cheap and readily mass producible which would be a fraction of the cost of commercial chromatography mixers.

The optical fibers are time consuming to manufacture for the SAMIs, and one of the main failure points in situ. Failures include flooding of the pressure housing through the fiber penetration and fracture of the fiber. The fibers can be broken through improper handling either in shipping or on deployment. Currently the fibers’ minimum bend radius dictates the minimum diameter and, to an extent, length of the SAMI-pH. Integrating the optics into the flow-cell would completely eliminate the optical fibers, and simplify the design parameters.

In the following chapters, designs for new mixing approaches and for integrating the optics with a flow-cell will be discussed along with results of extensive field testing of a miniaturized prototype SAMI-pH.
Chapter 2

Methods and materials

Spectrophotometric in situ pH sensors require a mechanism for mixing indicator with seawater and a flow-cell to make absorbance measurements. Seawater and indicator are passively mixed in the SAMI-pH and alternatives for the mixer are explored in this chapter. Three different approaches to eliminate the SAMI-pH optical fibers are also presented. This chapter concludes with the development of the inexpensive SAMI-pH (iSAMI) and the competition details of the WSOH XPRIZE which provided a thorough evaluation of its performance.

2.1 Designs: mixers

The standard mixer (See Figures 1.6 & 2.2) on the SAMI-pH is a commercially available static mixer (SM) designed for high pressure liquid chromatography (HPLC) from Analytical Scientific Instruments (421-0350B). All wetted surfaces are polyether ether keytone (PEEK) and the mixer has an internal volume of 350 µL.

A microfluidic approach was taken to construct alternative mixers with varying and reduced internal volumes. Cartridge mixers (Figure 2.1) were custom made for Sunburst Sensors by MicroPlumbers, LLC, (Minneapolis, MN). These cartridges contain a convoluted fluidic path that mix the seawater sample and indicator bolus as they pass through to the flow-cell. The cartridges were connected to the pump outlet and inlet of the flow-cell with a manifold clamp. The cartridges were constructed in layers using sheets of silicone adhesive film (3M 91022) with machined fluidic channels in the rigid capping materials. The capping materials were clear polycarbonate and black acetal copolymer.
The membrane mixer was composed of two capping layers, two layers of adhesive film, and a central layer of gas permeable 4 mil (mili-inches) silicone film (Figure 2.1). The gas permeable film is exposed to the surrounding environment. Fluidic channels are 5 mil deep by 50 mil wide comprising a nominal total volume of $\sim 138 \mu L$. Recall from Section 1.6 that there was an attempt to create a combination $pCO_2$ and pH instrument. The membrane mixer would have served as both the $pCO_2$ equilibrator and mixer for the pH system in this application (See Section 1.6).

Based on this design, four other cartridges were made without a gas permeable membrane. The first had the same nominal internal volume ($\sim 138 \mu L$) and the remaining three had decreasing internal volumes of 103 μL, 70 μL and 35 μL.

Figure 2.1: Cartridge mixers from left to right, top to bottom: Mem4, M4, M3, M2 and M1. Mixer Mem4 contains an exposed gas permeable membrane and is shown connected to the input/output manifold. M4 (3 in x 3.5 in) has the same nominal internal volume (138 μL) and is constructed without exposed membrane. M3, M2 and M1 have nominal internal volumes of 103 μL, 70 μL and 35 μL, respectively.
Tubing mixers made in house from 5.5 in of 1/8 in O.D. by 1/16 in inner diameter PEEK tubing bent into a U-shape had a nominal internal volume of $\sim 280 \ \mu\text{L}$. Fittings are Upchurch Scientific $1/4-28$ Super Flangeless™ nuts and ferrules with Delrin™ unions.

![Figure 2.2: Left to right: Static mixer (2.25 in x 1 in diameter), t-Tube and i-Tube. Tube mixers are 1/8 in diameter x 5.5 in long.](image)

Functionality of the mixers was evaluated by comparison with the ASI static mixer for accuracy and precision of tris-pH measurement on a SAMI-pH. The SAMI-pH was thermostatted at 25 °C in a recirculated submersion tank. Accuracy was determined as the mean difference between the SAMI measurement and temperature dependent tris-pH calculation (Equation 2.7) where the precision is the standard deviation of the differences. In all, 7 mixers including 5 cartridge mixers, and 2 tube mixers, were compared to the static mixer.
2.2 Designs: optofluidic cells

All of the following optical flow-cell designs were based on a 1 cm long by 1 mm diameter absorbance path with a z-pattern flow, similar to that used in the SAMI-pH and SAMI-CO$_2$. Figure 2.3 shows a drawing of the standard z-cell with this configuration.

On the SAMI-pH the flow path is connected to the fluidic system with 1/16 in O.D. by 0.030 in I.D. tubing. The absorbance path is sealed from the environment by fiber optics seated with tubing fittings. The z-pattern flow path creates more “sweep” at the fiber interface than a flow path with a 90° configuration which helps to remove air bubbles from the system (Seidel et al., 2008). SolidWorks™ parametric modeling software was used for designing and modeling of parts, assemblies and mechanical drawings.

The dimensions of the SAMI flow-cell were chosen through trial and error since there are many trade offs in the design requirements of optical flow-cells. The optical path diameter is dictated by both flush volume and light throughput. By reducing the optical path diameter the flush volume is also reduced but so too is the amount of light that reaches the detector. The optical pathlength affects light throughput in reverse fashion: as the pathlength is increased the light throughput decreases roughly proportionally. Optical pathlength also affects the indicator concentration and thus the magnitude of indicator pH perturbation (Seidel et al., 2008). An order of magnitude reduction in optical pathlength, e.g. from 10 cm to 1 cm, requires an order of magnitude increase in indicator concentration which causes a significant increase in indicator pH perturbation. (See Seidel et al. (2008) and Section 1.5 for a description of indicator pH perturbation correction).
The following sections describe three simultaneous efforts to innovate the SAMI-pH optical design. 3D printing was explored as a manufacturing technique with potential to embed optical elements during a print. This is a rapid prototyping technique that allows quick turnaround times between design iterations. Microfluidic techniques were also explored to embed optics and the flow path in a sealed layer sandwich or clamshell. Finally, a more traditional approach explored combining an plate beam splitter, a flow-cell using machined components and a mechanically sealed flow-cell.

### 2.2.1 3D-printed cells

Acuity Design (Missoula, MT) manufactured absorbance cells in acrylonitrile butadiene styrene (ABS) plastic using their custom designed Helix™ dual print head fused filament fabrication 3D printer. This was the proof of concept print to attempt to reproduce the standard machined SAMI-pH z-cel (Figure 2.3). An alternative design, Figure 2.4, incorporated optical windows printed in polyethylene terephthalate (PET), which is semitransparent. The intent was to print a flow path encapsulated in the cell and seal the liquid from potential optical elements without need of mechanical fasteners and separate optical windows.
2.2.2 Ghosting correction

Optical designs like the SAMI-pH incorporate the use of a 50/50 plate beam splitter to combine light from two LED sources. Since the beam splitter has some nominal thickness, any light ray traveling through the substrate will be refracted and, upon, exit will be laterally displaced from the incident beam by a distance $d$, Figure 2.5 and Equation 2.1.
In Figure 2.5, $\alpha$ is the angle of incidence, $n_{\text{air}}$ and $n_g$ are the indices of refraction in air and glass substrate. Letting $t$ represent the thickness of the beam splitter, the lateral displacement $d$ is calculated as

$$d = t \sin \alpha \left( (1 - \cos \alpha)/\sqrt{(n_g/n_{\text{air}})^2 - \sin^2 \alpha} \right).$$  \hspace{1cm} (2.1)

In the SAMI instruments, the BK7 float glass beam splitter has a thickness $t = 1$ mm, with index of refraction $n_g = 1.527$ at 434 nm and $n_g = 1.517$ at 578 nm. With the angle of incidence $\alpha = 45^\circ$, the lateral displacement is $t = 377 \mu$m at 434 nm and $330 \mu$m at 578 nm. This lateral displacement may seem small but so too is the core diameter of the optical fiber, 800 $\mu$m. Not only did this change improve light throughput, it made it easier to balance the reference and signal photodiode intensities.

A plate beam splitter in use as an optical combiner must take this lateral displacement into account for both LEDs to maximize signal throughput and reduce noise. The following optical designs, incorporating a beam splitter as optical combiner, used the above calculation to adjust the position of the LEDs to ensure spatial overlap of emitted light.

### 2.2.3 Clamshell cells

Polycarbonate clamshell optical cells were designed in SolidWorks™ and fabricated for Sunburst Sensors by MicroPlumbers (Figure 2.6). The 50/50 beam-splitter and ball lens were purchased from Edmund Optics (Barrington, NJ). Hamamatsu Photonics K.K. (Japan) silicon photodiodes were used for both signal and reference detectors. The absorptive neutral density filter from Thorlabs (Newton, NJ) in front of the reference detector was cut by Intor Inc (Middle Rio Grande, NM). LED light sources from Roithner LaserTechnik GmbH Vienna, Austria) used interference band pass filters for the mCP absorbance maxima (Intor Inc). These filters are centered at 434 nm and 578 nm with 50% transmittance at the peak and a 10 nm band pass (full width at half maximum). The pockets for the ball lens and the detector photodiode were designed to mechanically seal the fluid path using the ball lens and the lens of photodiode. These elements were to then act as optical windows. The assembly was designed to interface with the mixing cartridges described in Section 2.1.
The second clamshell design, Figure 2.7, also designed in SolidWorks™ and fabricated for Sunburst Sensors by MicroPlumbers utilized the same optical components as the first design except for the ball lens and the LED filters were mounted in an aluminum sleeve that slipped over the LEDs. A custom Delrin™ photodiode detector pinhole cover was fabricated by Big Sky Machining (Superior, MT). The pinhole had a 1 mm diameter and slipped over the detector in an attempt to limit stray light. Both designs used Upchurch Scientific 10-32 Vacutight™ fittings. The pocket machining was greatly simplified over the first design since mounted filters were used resulting in less individual optical element pockets. The machining was further simplified by lowering the entire optical plane into the bottom plate of the cell. This meant that the flow path was no longer split between the two halves and only machined in the bottom plate. The assembly was designed to interface with the mixing cartridges described in Section 2.1.
With the omission of the ball lens there remained 2 mm of sealing surface between the fluid path and the optical element pocket machining. This space is transparent and acted as an optical window to separate the optical elements from the fluid path.

Both cells were tested for bubble retention and stray light. A SAMI-pH control board (Sunburst Sensors) with wire leads for the LEDs and photodiodes was used to collect intensity signals. The cells were filled with deionized water and the intensity signals were compared to intensity signals when the cell was filled with indicator. The transparent nature of the cells allowed for the visual inspection of bubble retention.
2.2.4 Integrated beam combiner cell

The integrated beam combiner flow-cell, Figure 2.8, was designed in SolidWorks™ and manufactured for Sunburst Sensors by Big Sky Machining (Superior, MT) in acetal copolymer (Delrin™). The LEDs, band-pass filters, neutral density filter, beam splitter, and photodiodes were the same as described in Section 2.2.3. MicroPlumbers cut polycarbonate windows from 15 mil polycarbonate film, which were each secured with either a 1/4−28 Delrin™ flanged fitting nut (Upchurch Scientific) or a M5.5 x 0.5 retaining ring (Thorlabs). The 1/4−28 Delrin™ nut was drilled to accommodate the detector photodiode and was sheared off once the window was secured. The beam splitter was secured by screwing the two halves of the cell together. High purity fluoroaniline (HPFA) tubing connections for inlet and waste were made with Upchurch Scientific VacuTight™ fittings.

The cell was mounted directly to the SAMI control board and the LEDs and reference photodiode were soldered to the board. The detector photodiode was connected to the board with 2 cm wire leads. The design allowed for direct integration with the current revision of the SAMI control board and mounting of the assembly on the SAMI electronics chassis (See Figure 3.7).
2.3 iSAMI-pH

The iSAMI-pH (i for inexpensive) utilized the integrated beam combiner cell above (Section 2.2.4), SAMI control board (Sunburst Sensors) and i-Tube PEEK tubing mixer (Section 2.1) in a 6” diameter polyvinyl chloride (PVC) housing for a complete in situ autonomous, spectrophotometric pH prototype instrument (Figure 2.9a) for surface measurement. The PVC housing and o-ring sealed top lid were machined by Big Sky Machining. A clamped rubber boot for 6” pipe was used as an inexpensive end cap on the reagent housing end. A communication and power bulkhead (Teledyne Impulse XSG-6MP) was installed in the o-ring sealed end cap along with thermistor fitting (Idaho Valve & Fitting) and stainless steel housed thermistor (YSI). The solenoid pump (BioChem) is connected to a 3-way solenoid valve (Neptune Research) via HPFA tubing and Upchurch Scientific fluidic fittings. The 8 D-cell alkaline battery pack is custom assembled (Batteries Plus) and is the stock battery for the SAMI line up.
Figure 2.9: (a) Prototype of the miniaturized spectrophotometric pH instrument, the iSAMI. The iSAMI inner workings shown in (b) with beam combiner flow-cell, control board, solenoid pump and valve, battery pack and PEEK tubing mixer. Indicator pouch was housed in a protective yellow plastic sleeve. This version of the iSAMI had a second battery pack at the base to increase deployment duration.

The minimum size of the instrument was constrained by the size of the SAMI control board and size of the internal battery pack. (See also design requirements under the WSOH XPRIZE Section 2.4) Purified mCP indicator was stored in a gas impermeable pouch (Pollution Measurement Corporation) that was housed internally at the bottom of the housing.

Seawater samples were collected from Yaquina Bay, OR and stored in 5 gal water jugs. NIST-traceable tris buffer in synthetic seawater was prepared according to DelValls and Dickson (1998) using ACS-grade salts without further purification. mCP was purified as H$_2$L according to Liu et al. (2011). Stock purified mCP solutions were prepared following DeGrandpre et al. (2014). Salts, solvents and indicators were obtained from Sigma Aldrich.
The iSAMI was validated for accuracy and precision using tris seawater buffer. The iSAMI was thermostated to 25 °C in a recirculating submersion tank. Accuracy was determined as the mean difference between the SAMI measurement and temperature and salinity dependent tris-pH calculation (Equation 2.7) where the precision is the standard deviation of the differences.

The following equations were used to calculate all XPRIZE pH data on the iSAMI-pH and the tSAMI-pH using a custom MATLAB™ script (Sunburst Sensors, reproduced with permission). The $pK_\alpha'$ equation uses temperature in Kelvin and is valid for salinity over the range of 20-40 practical salinity units (PSU) and temperature over the range 273K to 313K. The $pK_\alpha'$ of purified mCP is dependent on both temperature, $T$, and salinity, $S$. Temperature dependent molar absorptivities (Section 1.3) were determined according to DeGrandpre et al. (2014).

$$pK_\alpha' = -241.46 + 7085.7/T + 43.833ln(T) - 0.080641T + 0.00211(35 - S)$$  \hspace{1cm} (2.2)

$$\epsilon_{\alpha_{434}} = 17372 + 20.162(T_{ea} - T_{SAMI})$$  \hspace{1cm} (2.3)

$$\epsilon_{\beta_{434}} = 94.1 - 1.0177(T_{eb} - T_{SAMI})$$  \hspace{1cm} (2.4)

$$\epsilon_{\alpha_{578}} = 2284.1 - 6.3863(T_{ea} - T_{SAMI})$$  \hspace{1cm} (2.5)

$$\epsilon_{\beta_{578}} = 38676.5 - 66.808(T_{eb} - T_{SAMI})$$  \hspace{1cm} (2.6)

Where $T_{SAMI}$ is the in situ temperature, $T_{ea}$ and $T_{eb}$ are the temperatures that the molar absorptivities were determined, 24.80 °C and 24.84 °C, respectively. Seawater pH was calculated using these molar absorptivities according to Equations 1.21 and 1.22 and the method outlined in Section 1.5. Temperature, $T$ in Kelvin, and salinity dependent tris pH calculations from DelValls and Dickson (1998)

$$pH = (11911.08 - 18.2499S - 0.039336S^2)(1/T)$$

$$+(-366.27059 + 0.53993607S + 0.00016329S^2)$$

$$+(64.52243 - 0.084041S)ln(T) - 0.11149858T.$$
2.4  Wendy Schmidt Ocean Health XPRIZE

Three iSAMI prototypes were constructed and entered into the WSOH XPRIZE (See http://oceanhealth.xprize.org/). This global competition was designed to promote awareness of OA and to spur innovation in affordable and accurate pH sensors. The competition was divided into four phases with two prize purses: an affordability prize purse and accuracy prize purse (See Figure 2.10). Phase 1 was the innovation phase where teams registered their entries, and put together design ideas. Phase 2 was broken into two parts: phase 2a was lab accuracy trials using tris buffer; phase 2b was tank stability and precision trials. Phase 3 was the coastal field trials. Phase 4 was a deep ocean trial profiling to 3000 meters in which the iSAMI did not participate because it was designed for surface measurements and had not been designed with differential pump pressure compensation. All pH data reported to the XPRIZE judging panel were processed with a MATLAB™ script developed at Sunburst Sensors.

The first testing phase of the competition, phase 2a, was a lab test for accuracy. The testing took place in a refrigerated, walk-in cooler at the Monterey Bay Aquarium Research Institute (MBARI). The iSAMIs were temperature equilibrated in the cooler over night. They were then submersed in a 5 gal bucket of tris standard, which had also temperature equilibrated in the same cooler. There were no other means of temperature equilibration. The bucket was equipped with a mixing paddle and a pump to collect samples for lab comparison. As per the competition guidelines, pH measurements were performed by the entry instruments once per minute. The i & t SAMI one minute sampling frequency was achieved through the use of fewer flushing pumps and shortening the pump duty cycle. This represented the fastest sampling frequency achieved to date with SAMI technology. After at least 10 pH measurements, an aliquot of hydrochloric acid (HCL) was added to the test bucket to lower the pH, and sampling continued. Two samples of the tris standard before acid addition and two samples after acid addition were taken from the test buckets by the XPRIZE testing personnel for spectrophotometric determination of pH in a laboratory, deemed the true pH of the tris, and termed the validation pH. Entry pH measurements were compared to the validation pH and judged for accuracy with a minimum standard of \( \leq 0.04 \) pH units from validation pH (Affordability Prize See Figure 2.10).
The second testing phase of the XPRIZE competition, phase 2b, was a stability and precision trial which took place in the MBARI test tank facility. The tank is 10 m deep and holds 375,000 gal of seawater. Instruments were suspended at a depth of 5 m and were required to measure pH every two hours for up to 90 days. It appeared that some instruments began corroding and the trial was suspended at day 50. The tank is designed to be extremely stable over long time frames and was deemed an ideal location to test the stability and precision of the entries. The XPRIZE guidelines stated a target precision of $\leq 0.02$ pH units expressed as 1 standard deviation of successive measurements. Target stability was $\leq 0.013$ pH units per month. This was calculated from the daily differences between the entry and validation pH values and quantified as the spread of these values expressed as a standard deviation with units of pH/month.

The third and final testing phase for the iSAMI in the affordability prize purse, Phase 3, was a “field trial” utilizing the coastal waters off the pier at the Seattle Aquarium. A special tank was constructed at Pier 59 that allowed Puget Sound seawater to flow across all entries such that the entries could experience the natural variation of a real world coastal environment. Puget Sound water was pumped into and out of the tank. CTD instruments were placed at several locations along the tank and the entries were submerged $<1$ meter. Testing took place over $\sim 25$ days with a sampling frequency of once every hour. Target precision and stability were the same as in Phase 2b: $\leq 0.02$ pH units in the first standard deviation and $\leq 0.013$ pH units/month respectively.
C. STABILITY: Determines a sensor’s consistency over time and is defined as the rate of drift estimated over periods of a month or more, expressed as the root mean square error (deviation from the conventional true value) estimated from sensor measurements made over a specified period of time.

D. AFFORDABILITY: Determines the cost of using individual sensors to deliver accurate pH data over a given amount of time. This will be directly assessed from the materials cost estimate for the manufacture of Team’s Entry. A specific process for allocating points in this area will be set forth and defined in the Rules and Regulations.

E. EASE-OF-USE: Determines the ease with which devices can be calibrated (or self-calibrated), deployed, maintained, and data can be accessed, taking into consideration physical size, weight, durability, accessibility, and related characteristics. A specific process for allocating points in this area will be set forth and defined in the Rules and Regulations.

Table 1. Percentage of Points and Minimum Standards for Each Phase.

<table>
<thead>
<tr>
<th>Measurement Criterion</th>
<th>ACCURACY PRIZE PURSE</th>
<th>AFFORDABILITY PRIZE PURSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percentage of Points</td>
<td>Minimum Standards</td>
</tr>
<tr>
<td>Phase 2a</td>
<td>20% of total</td>
<td>20% of total</td>
</tr>
<tr>
<td>Accuracy</td>
<td>20.0%</td>
<td>≤0.02 pH units from “accepted value”</td>
</tr>
<tr>
<td>Phase 2b</td>
<td>15% of total</td>
<td>15% of total</td>
</tr>
<tr>
<td>Stability</td>
<td>10.0%</td>
<td>≤0.0067 pH units/month</td>
</tr>
<tr>
<td>Precision</td>
<td>5.0%</td>
<td>1 std. dev. ≤0.01 pH units</td>
</tr>
<tr>
<td>Phase 3</td>
<td>30% of total</td>
<td>65% of total</td>
</tr>
<tr>
<td>Stability</td>
<td>10.0%</td>
<td>≤0.0067 pH units/month</td>
</tr>
<tr>
<td>Precision</td>
<td>10.0%</td>
<td>1 std. dev. ≤0.01 pH units</td>
</tr>
<tr>
<td>Ease-of-Use</td>
<td>5.0%</td>
<td>--</td>
</tr>
<tr>
<td>Affordability</td>
<td>5.0%</td>
<td>≤ $15,000</td>
</tr>
<tr>
<td>Phase 4</td>
<td>35% of total</td>
<td>0% of total</td>
</tr>
<tr>
<td>Accuracy</td>
<td>15.0%</td>
<td>≤0.02 pH units from “accepted value”</td>
</tr>
<tr>
<td>Precision</td>
<td>15.0%</td>
<td>1 std. dev. ≤0.01 pH units</td>
</tr>
<tr>
<td>Ease-of-Use</td>
<td>5.0%</td>
<td>--</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2.10: Table taken from the competition guidelines of the WSOH Health XPRIZE. Accuracy prize purse minimum standards were half that of the affordability prize purse. Affordability prize purse participants, as was the iSAMI, did not compete in the deep oceanal trials of phase 4.

All entries were judged in five categories: accuracy, precision, stability, affordability and ease-of-use. Accuracy was defined as the difference between entry sensor pH and the conventional true pH of a reference sample of the same seawater or tris. Precision was determined as the standard deviation of successive measurements of pH. Stability was determined as the standard deviation of the daily differences between entry instruments and validation pH. Affordability was judged by the materials cost at production scale. Ease-of-use assessed the ease at which a non-developer could use the system. The competition guidelines specified reference sample pH to be on the total hydrogen ion concentration scale. Reference sample pH values were determined spectrophotometrically using the calibration values of Liu et al. (2011) and purified mCP.

A maximum of three instruments were accepted per each entry. The design characteristics of the entry
instruments were a maximum of one meter for the largest dimension and twenty centimeters in diameter with a maximum weight of 10 kg in air and 2 kg in water. All instruments were required to have an on-board power source. It was permissible to house the power source in a separate module. The power source was required to provide enough power to complete a phase or sub-component of a phase without need of external sources. The instrument was required to be self contained and as such all data was to be stored on-board. While real time data was not required, ease-of-use points were awarded for the capability.
Chapter 3

Results

Use of microfluidic cartridge mixers showed a marked reduction in flushing volume without a sacrifice in accuracy or precision. The experimental design of these cartridge mixers and the results of testing them on a SAMI-pH are explored in this chapter. The SAMI-pH measures absorbance of a mixture of seawater and indicator through a flow-cell connected to a light source and a detector with fiber optics. Three distinct approaches for integrating the flow-cell and optoelectronics are also explored. This chapter concludes with the validation results of the iSAMI-pH and the results of the WSOH XPRIZE.

3.1 Designs: mixers

The SAMI-pH uses the ASI static mixer (Figure 2.2), which has proven good performance in situ (Seidel et al., 2008), where the SAMI-pH achieves a laboratory accuracy of $\leq 0.003$ pH units and precision of $< \pm 0.001$ pH units in a salinity range of 20-40 PSU and pH range of 7-9.

For a cost-efficient miniaturized system for seawater pH measurements, a mixer must have similar if not better performance as compared to the ASI static mixer. Here, the goals are to find an economical solution that is simple and cheap to manufacture, is scalable in size, and adaptable to different instrument configurations. Microfluidic techniques are suited to all of these goals and were used to prototype five mixing cartridges.

Design effort first went into the construction of the membrane mixing cartridge (Figures 2.1 & 3.1a) meant to function as both a mixing cartridge and a CO$_2$ equilibrator. Recall from Section 1.6 that the initial
The concept for this project was a single, miniaturized instrument to measure both $pCO_2$ and pH using a single indicator and optical cell.

The first prototype design utilized a serpentine fluid path. There were five spaces where the channel depth was reduced from 5 mil to 2 mil where the fluid left the machined path and entered a path made by the adhesive layer only, en route to the next machined path. This intermittent serpentine fluidic path construction was used because a continuous cutout of the two adhesive layers would result in excessively flexible, hard to seal layers. These constrictions between layers may have aided mixing, but restricted flow and were overly sensitive to pressure from the manifold connection.

![Figure 3.1: The first design membrane mixing cartridge (a) showing fluid path in aquamarine and five constriction sites in darker shade of blue. The second design (b) membrane mixing cartridge eliminated the restriction sites. (Source MicroPlumbers)](image)

The second design (Figure 3.1b) eliminated these constrictions by machining long parallel fluid paths in one capping layer and short connecting paths in the other capping layer. This simpler design leaves only vias (through holes) for the membrane, long parallel cutouts in one adhesive layer and short parallel cutouts in the other adhesive layer. This design was copied and scaled to make four cartridge mixers with diminishing nominal internal volume. Eliminating the membrane resulted in a simplified design with only one adhesive
Tube mixers (Figure 2.2) were available at this time as alternatives to the static mixer and so were included in the evaluation below. The i-Tube mixer was used on the first miniaturized SAMI-pH, discussed in Section 2.3. The t-Tube was used on a deep water SAMI-pH constructed with a titanium pressure housing which was also entered into the XPRIZE. The i-Tube has a much tighter bend radius and was coupled directly to the optical cell, in this test, whereas the t-Tube has a broad bend radius and an approximately 5 in piece of tubing intermediary to the optical cell.

![Figure 3.2: Evaluation of various mixer performance comparing accuracy and precision for tris pH measurement at 25 °C for approximately 10 measurements on a SAMI-pH. Mixers were the ASI static mixer (SM 350 µm), a membrane mixing cartridge (Mem4 138 µL), an equal internal volume cartridge without membrane (M4). The remaining three had decreasing internal volumes of 103 µL (M3), 70 µL (M2) and 35 µL (M1). The orange dots are the accuracy and the error bars are the precision calculated as the measurement standard deviation (1 SD for n=10).](image)

Figure 3.2 shows that all of the mixers evaluated compared very favorably to the SM in terms of accuracy and precision of pH measurement. This suggests that a wide variety of shapes, sizes and materials were shown to make an effective mixer at a substantial reduction in flush volume and cost.
Tris was an ideal test solution for these experiments since the pH is set by the temperature (Equation 2.7) and is well buffered against environmental contamination of CO$_2$. Real seawater is not well buffered against CO$_2$ and it is therefore difficult to constrain its pH for the duration of testing. The pH perturbation of seawater could potentially be altered by different mixer geometries, however, and was not evaluated in this research (See Section 1.5).

Aside from accuracy and precision, the other parameter evaluated for the different mixers was the total volume required to flush the system with fresh sample and rid the system of residual reagent. As a general rule, 8 full-volume flushes are needed to effectively flush the mixer. For the static mixer (SM) this is a relatively large 2.8 mL or 56 pump cycles. The tubing mixers weigh in at a comparable 2.24 mL or 45 pump cycles. With the mixing cartridges, the flush volumes required for Mem4 and M4 are 1.1 mL or 22 pump cycles and M3 thru M1 are a much lower 0.83 mL, 0.55 mL and 0.28 mL, with corresponding 16, 11 and 6 pump cycles respectively.

<table>
<thead>
<tr>
<th>Mixer</th>
<th>Volume (µL)</th>
<th>Wetted Material</th>
<th>size (in)</th>
<th>cost (US$)</th>
<th>min. flush pumps</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM</td>
<td>350</td>
<td>PEEK</td>
<td>1 x 2.25</td>
<td>580</td>
<td>56</td>
</tr>
<tr>
<td>i-Tube</td>
<td>280</td>
<td>PEEK</td>
<td>1/8 x 5.5</td>
<td>25</td>
<td>45</td>
</tr>
<tr>
<td>t-Tube</td>
<td>280</td>
<td>PEEK</td>
<td>1/8 x 5.5</td>
<td>25</td>
<td>45</td>
</tr>
<tr>
<td>Mem4</td>
<td>138 nom</td>
<td>acetal/silicone/3M</td>
<td>3 x 3.5</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>M4</td>
<td>138</td>
<td>acetal/polycarb/3M</td>
<td>3 x 3.5</td>
<td>50</td>
<td>22</td>
</tr>
<tr>
<td>M3</td>
<td>103</td>
<td>acetal/polycarb/3M</td>
<td>3 x 2.5</td>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td>M2</td>
<td>70</td>
<td>acetal/polycarb/3M</td>
<td>3 x 1.75</td>
<td>50</td>
<td>11</td>
</tr>
<tr>
<td>M1</td>
<td>35</td>
<td>acetal/polycarb/3M</td>
<td>3 x 1</td>
<td>50</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 3.1: Summary statistics from Figure 3.2 for internal volume, types of wetted materials, general size, cost and the minimum number of pumps to flush the system based on 8 full volume flushes. The cartridge mixers, when purchased at scale, would be significantly cheaper than the prototype price listed.

While the tube mixers showed excellent accuracy and comparable precision, in addition to being cost effective they are not very scalable and still require a relatively large volume of seawater for an effective flush: meaning more power consumed per pH measurement. The mixing cartridges proved to be both accurate and precise while dramatically reducing both cost and flush volume as compared to the SM. The cartridge
mixers are mass producible and at scale the price could reduced even further. The mixers have proven reliable through submersion is saltwater for up to one year with hundreds of saltwater pH samples. These results show that there are many acceptable approaches to mixing a bolus of indicator with seawater yielding accurate pH. The cartridge mixers were not only effective, they are also highly configurable and adaptable to many different types of instrumentation and auxiliary equipment such as manifold mounted pumps and valves.

Figure 3.3: Dilution curves for two cartridge mixers, M1 and M4, measured at 434 nm for one pH measurement sequence (See Section 1.5). Blank intensities, labeled in red, are used to calculate absorbances using the remaining signal intensities. M4 has in internal volume of 138 µL and M1 has an internal volume of 36 µL.

Care must be taken in the design of mixing cartridges (Figure 3.3). These dilution curves represent one pH measurement sequence with intensities measured at 434 nm for mixers M1 (36 µL) and M4 (138 µL), respectively. With M1, the mixing path volume is only 25% that of M4 and the reagent bolus enters the flow-cell much earlier in the sequence and also has a shorter residence time. The longer residence time of M4 results in a more dispersed reagent bolus.

Depending on instrument configuration, there can be few blank measurements available, as with M1, or many, as with M4 (Figure 3.3). To test a new mixing cartridge design, the reagent bolus must enter the flow-cell within the measurement window. This can be adjusted to suit the configuration of the particular instrument to some degree with changes to the default measurement sequence or instrument configuration.
One could force the system to wait a programmable number of pumps before recording the measurement sequence in the case of M4, or a configurational change could add tubing between the mixer and the flow-cell to add more blank measurements as in the case of M1.

Further work with the mixing cartridges could include other microfluidic manufacturing techniques such as those that utilize solvent vapor to both polish the machined surfaces and weld the layers together (Ogilvie et al., 2010). The solvent vapor not only creates an irreversible bond but restores optical quality and surface wettability (De Marco et al., 2012) of the machined substrates. Other types of mixing chambers and geometries could be considered, such as the double heart vortex mixer from Fu et al. (2014). These adjustments to mixer geometries must be balanced against power use as the number of pumps required for a measurement sequence is the greatest in the power budget (flushing and sample sequence).

3.2 Designs: optofluidic cells

Criteria for a successful optical cell include a compact footprint, pathlength, minimal internal volume, good light throughput, minimal bubble retention, material inertness to avoid sample contamination and ease of production. Bubbles are a constant problem in optical fluidic systems and lead to a significant loss of precision.

The current SAMI-pH uses a Z shaped flow path (Figure 2.3 in Section 2.2). The Z shape reduces bubble retention compared to the 90° flow path cells previously used (DeGrandpre et al., 1999; Seidel et al., 2008). The sharp angle of the entry and exit arms of the flow path create sweep across the optical fiber faces at the terminal ends of the optical path. This design has proven reliable and tends to flush bubbles quickly.

For a miniaturized system, the ideal flow-cell should contain the entire optical system including light source optics, detection optics, flow-cell and fluidic connections in a compact footprint. Ideally the cell should be mass producible and allow for a simple assembly process with little or no manual preparation or alteration. The cell should not require extensive use of custom components. It should be adaptable to a changing supply of off the shelf components.
In the SAMI-pH, the optical system is isolated from the flow path with optical fibers and the optical fibers isolate the flow path from the surrounding environment. The challenge to integrating the optical system into the flow-cell is separating the fluid path from the optics and surrounding environment while still allowing light to pass through the optical path. As part of this effort, several different optical designs and fabrication methods were examined for fabricating integrated optical flow-cells. While only one of these optical designs was ultimately used in the iSAMI prototype, the remainder of the designs and fabrication methods hold potential and are presented.

### 3.2.1 3D printed cells

One approach to fabricating an optical flow-cell investigated the use of 3D printing. 3D printing is an emerging technology that allows geometry designed in the digital world to be rendered into physical form. With 3D printing, very complex internal geometries can be produced that could not be easily machined. This is accomplished in part by bridging, or filling voids with a soluble material that is later dissolved. This could allow for very complex fluidic paths within an optical flow-cell and eliminate mechanical sealing of mated parts. A printer with dual print heads could also print optical windows directly into an otherwise opaque optical flow-cell thus negating mechanical sealing of discrete windows. 3D printing also has the potential to expand the structure of an optical flow-cell to integrate mixing chambers and manifold fluidic connections for pumps and valves all within the same print. Potentially, the optical elements could also be embedded in the optical flow-cell as the print is underway fully sealing all elements (Willis et al., 2012).

To create a 3D printed optical flow-cell, the assistance of a local company, Acuity Design, with expertise in Fused Filament Fabrication (FFF) was used. FFF is a 3D-printing technique utilizing a heated print head moving in three dimensional space. Filament are spools of various thermoplastic polymers. Filament heated to fluid form and extruded from the print head is deposited on a heated base plate in layers. In this way material is built up into a 3-dimensional structure. This type of rapid prototyping allows small design changes to be made and a new prototype printed in as little as a few hours. The design team at Acuity attempted to print the standard SAMI-pH z-cell in acrylonitrile butadiene styrene (ABS) plastic (Figure 3.4a). A second proof of concept design incorporated polyethylene terephthalate (PET) optical windows in the cell and was the simplest innovation to the z-cell (Figure 3.4b).
Printing optical windows integral to the flow-cell proved too difficult with either the equipment available to Acuity Design or the programming of the second print head. During the print, some layers of PET were alternated with ABS layers and the resultant print had PET printed in random spots. Figure 3.4b shows the poor quality of the print. Though the print without windows was much cleaner, the quality of the product could not match that of a machined cell. Further, this did not innovate the manufacturing process of the z-cell and still would have required extensive post print processing to make it usable. The print time made the part even more expensive than its machined counterpart.

Given the vast potential of 3D printing, it may be worth investigating further. Future work with printing cells should look to other technologies such as Stereolithography (SLA) which has much higher spatial resolution with a host of rapid prototyping services available on the web. Research will need to focus on the chemical compatibility of individual polymers with the pH indicator, the potential composite part porosity and mechanical stability.

### 3.2.2 Clamshell cells

Another approach to fabricating an optical flow-cell investigated micro-machining in rigid layers. Features such as fluid flow paths, and fluid connection ports are machined in one or more layers. When these layers are bonded, complex fluid paths connecting micropumps and valves can be formed. The layered approach
allows for very flexible design parameters and fairly rapid prototyping. Micro-machining pockets for optical elements and the z-shaped flow path in two layers formed a clamshell integrated optical flow-cell.

The initial design intent for the clamshell optical cell, (Figure 3.5), was to eliminate the optical fibers and enclose all the remaining SAMI optics in a submersible package that would directly couple with the mixing cartridges. The first iteration was constructed in clear polycarbonate layers to allow visualization of the interior. If the design proved effective then it could be fabricated in an opaque material to eliminate stray light. (Stray light is any light reaching the detector that does not go through the absorbance path). A ball lens was used as an optical window on the light source side of the absorbance path and the lens cap of the detector was used at the opposite end for the same purpose. Using a ball end mill, all features were machined at equal depth in both halves of the clamshell so the flow path had a circular cross section.

![Figure 3.5: First prototype clamshell optical flow-cell connected to the M4 mixing cartridge (located under the optical cell). Fabrication by MicroPlumbers.](image)

Light source optics (A) 434 nm LED and (B) 578 nm LED with corresponding band pass filters direct light on the 50/50 beam splitter at (D). Half of the light is directed at the reference photodiode at (C) with a 2
absorbance unit neutral density filter. The remaining half is focused through the ball lens (E) and through the optical path onto the detector photodiode (F). Sample is introduced to the mixing card at port (G) and enters the fluid path at (H) where it flows through the optical path and to waste (W). The curved section of the fluid path serves as a smooth transition to the mixing card while maintaining a 45° entry to the optical path.

The filters were originally packaged in a housing that slipped over the LEDs. Since the fit was loose the choice was made to fit the LEDs and their corresponding filters in individually machined pockets. The machining was not a tight fit and all of the emission optics rattled around in their pockets. This was mitigated somewhat by placing thin silicone strips on the top and bottom of the elements.

The design intent was to use precise machining to make a mechanical fluid-tight seal around the ball lens and photodiode lens cap. In practice this level of machining was unachievable, and sealing was accomplished using adhesive strips. As sample was pumped through the system, fluid flowed up to and around the adhesive strips with an especially large pocket at the ball lens. These dead volumes cause poor flushing of reagent, unstable blank measurements, and serve as bubble traps illuminating another design flaw of this sealing approach.

An absorbance measurement with this device proved impossible due to the large amount of stray light hitting the detector. LED light was not directed solely along the absorbance path but throughout the clamshell construction (Figure 3.5). This would not be a concern if the design was built in opaque material. Future work with this design must address the stray light, better sealing of the fluid path and loose optical elements.

To address loose optical elements, the original LED filter housings could be used which would also simplify the machining process since instead of three distinct pockets only one cylindrical pocket would need to be machined for each LED/filter and the reference photodiode/filter. Further, the LED filter housings cover the bulk of the LED and would help to reduce stray light emitted from the sides of the LEDs. To further address stray light, a pinhole cover for the detector photodiode could be employed. To address sealing of the flow path from the optics in a clamshell design the ball lens could be removed and the flow path shifted so that the substrate material would fully seal around the path. In transparent material, this area would serve as de facto optical windows (Figure 3.6).
These design concepts were used to construct a second prototype clamshell optofluidic cell. A pinhole cover for the detector photodiode was machined with a 1 mm pinhole to match the diameter of the optical path. To further simplify the machining, the ball lens was removed and the fluid path was machined in only one half of the clamshell with a corresponding z-axis shift of the optical element pockets so that the optical axis was still centered on the axis of the optical path. Since the fluid path was machined in only one half the clamshell its cross section was D-shaped. The optical arrangement and flow path configuration remained the same as the previous revision (Figure 3.5).

Figure 3.6: Second clamshell optofluidic cell showing optical window detail: the section of clamshell sealing surface between the flow path and the optical elements which is 2 mm wide. The LEDs, band pass filters, photodiodes and the beamsplitter are as arranged in the previous optical flow-cell. (Fabrication by MicroPlumbers).

Initially, the two halves of the clamshell would not seat properly and were lapped flat on plate glass using 500 µm film. To enhance surface finish on the lapped surfaces and the machined pockets the two halves
were solvent vapor polished using methylene chloride. The solvent vapor polish was especially important for clarifying the optical windows. These actions solved the poor element seating and the adhesive layer was applied, the optical elements inserted and the system capped with the remaining half of the clamshell.

Stability of the optical elements was greatly improved though silicone strips were still used to enhance the fit. MicroPlumbers improved the machining algorithm for the beam splitter pocket and this provided a very snug fit: no movement could be discerned. The fluid path was sealed from the surrounding optical elements with no discernible leakage or creep from the path. The adhesive layer had a cutout for the fluid path that acted as a tenacious bubble trap. This is possibly due to a difference in surface energies in the machined acrylic, the virgin acrylic and the adhesive layer.

Stray light proved to be too great to obtain an absorbance reading with this design even when placed under black plastic sheeting (i.e. bin liner). This was tested the in the same manner as the first clamshell by first filling the system with deionized water and obtaining a blank intensity. The system was then filled with mCP reagent and the resultant intensity was virtually identical indicating a severe stray light problem. Overall, the design addressed several major shortfalls of the first clamshell. Namely, machining was simplified, optical elements were more secure, and the fluid path was much better isolated and sealed. However, the stray light problem was not eliminated and the fluid path still trapped bubbles.

It is important to note that this design could not be replicated with opaque material since the optical windows would inarguably be opaque. Sieben et al. (2010) used tinted poly-methyl methacrylate (PMMA) to construct a similar microfluidic absorbance flow-cell. In that implementation, the optical windows were only 250 µm thick. They assembled the layers of the flow-cell with a solvent bonding process and mounted an LED and a photodiode on the device centered on the optical path. The tinted PMMA absorbed source light outside of the optical path dramatically reducing stray light over that of clear PMMA. MicroPlumbers’s fabrication techniques preclude such thin optical windows: sealing surface required is about twice the feature size. It is not clear whether such an approach would yield the signal to noise necessary for high quality pH measurements of seawater.

Future work with layered substrate designs could also add additional features such as a mixing layer, manifold connections for pumps and valves, or embed electronic boards. The adhesive layer could be eliminated all
together in favor of a direct solvent or solvent vapor bonding process. It could prove difficult to solvent bond and insert the optics, which could potentially damage them. Solvent vapor bonding would ensure that the fluid being sampled touched only the one substrate, reducing any differences in surface energies. Solvent vapor also reduces the surface roughness of micromachined fluid channels to near virgin material properties (Ogilvie et al., 2010). The cost of these prototypes was expensive, even for small production runs: roughly $500 US unassembled. Other prototyping services available may be more cost effective. See, for example, the manifold prototyping and production services of IDEX Health and Sciences.

3.2.3 Integrated beam combiner cell

After the difficulties of working with the clamshell designs, a new approach was needed. A modification of the fiber optic beam combiner in the SAMI-pH to include a the flow-cell was undertaken (See Section 1.5 and Figure 3.7). Since this design could be built in opaque material, it would eliminate the stray light issues encountered in the clamshell designs. Another potential advantage could be bubble reduction since there would be no difference in material properties along the entire length of the absorbance path. The machining would be simplified compared to the pocket machining of the clamshell design. As an additional benefit, this design could be fully integrated with the current revision of the SAMI-pH control board.

Design effort began with the SolidWorks™ model of the fiber optic beam combiner of the SAMI-pH. The right hand side of the cell in Figure 3.8 is identical to that of the SAMI-pH beam combiner (Figure 3.7 (C)). The left hand side was modified to contain the fluid path and was not restricted to the x-y plane. Instead, one terminal end of the fluid path is tilted 45° to the x-y plane. The fluid path was sealed from the optical elements with polycarbonate windows secured on the detector end with a nut, and the beam splitter end with a lock ring.
Figure 3.7: Picture of the flow-cell half (A) next to the SAMI beam combiner halves (B and C) mounted to a SAMI control board.

Figure 3.8: Exploded view of the Integrated Beam Combiner cell. Signal photodiode (a) housed in nut (b) that secures optical window (c). The other optical window (e) is secured with lock ring (f). 434 (d) and 578 (i) LED and filter packages. 50/50 BS (g) and reference photodiode (h). (See Figure 2.8 to see the flow path).

With this design, the cell was mounted directly to the current revision of the SAMI-pH control board, eliminating the fiber optics. All optoelectronics were soldered to the board with the exception of the detector
photodiode which was connected via 2 cm lead wires. The cell demonstrated increased signal throughput compared to the fiber optic system and decreased gains on the LEDs and photodiodes.

The optical fluid path is sealed with polycarbonate optical windows are secured with a Delrin™ nut and lock ring. The nut is sheared off and the signal photodiode is inserted. Tubing connections are made at the VacuTight™ fittings. LED and filter assemblies and reference photodiode with neutral density are secured with set screws. The beam splitter is secured as the two halves are screwed together.

The design solved several failings of the previous designs (Section 3.2.2). The sealing of the fluid path from the optics is noteworthy in that it uses optical windows with mechanical seals. While this adds more parts, it also allows for using different types of windows such as optical interference filters and allows physical access to service the cell. Stray light was eliminated due to the use of opaque material for the construction. Bubbles continued to be problematic but not catastrophic. Extensive testing with this design was done in the miniaturized SAMI-pH prototype discussed in the following Sections.

Future work with this design could focus on size reduction and simplification for machining. Thickness of the cell is dictated by the 1/2 in diameter of the beam splitter, though the beam splitter can be purchased in much larger formats and cut to minimal dimensions. Reducing the footprint further, the out-of-plane (X-Y) section of the flow path would need to be lowered back in plane (X-Y) with the rest of the flow path for a thinner version.

3.3 iSAMI-pH

Three iSAMI-pH instruments (Section 2.3) were validated on tris buffer in synthetic seawater according to Section 2.3. Along with these iSAMI instruments, three commercial SAMI-pH instruments with titanium pressure housings were also validated in the same manner. This was the first real test of the Integrated Beam Combiner (IBC) flow-cell discussed in Sections 2.2.4 and 3.2.3. These results are tabulated in Table 3.2.
Table 3.2: Validation data for 3 iSAMIs and 3 commercial SAMI-pH instruments. Results are the average of a minimum of n=10 measurements.

<table>
<thead>
<tr>
<th>SAMI Unit</th>
<th>SAMI pH</th>
<th>Calc Tris pH</th>
<th>ΔpH</th>
<th>Precision</th>
<th>Temp °C</th>
<th>σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>iSAMI-01</td>
<td>8.1531</td>
<td>8.1538</td>
<td>0.0006</td>
<td>± 0.0094</td>
<td>23.08</td>
<td>± 0.26</td>
</tr>
<tr>
<td>iSAMI-02</td>
<td>8.1072</td>
<td>8.1012</td>
<td>0.0053</td>
<td>± 0.0076</td>
<td>24.73</td>
<td>± 0.01</td>
</tr>
<tr>
<td>iSAMI-03</td>
<td>8.1004</td>
<td>8.0994</td>
<td>0.0010</td>
<td>± 0.0052</td>
<td>24.81</td>
<td>± 0.01</td>
</tr>
<tr>
<td>tSAMI-01</td>
<td>8.1054</td>
<td>8.1041</td>
<td>0.0012</td>
<td>± 0.0008</td>
<td>24.66</td>
<td>± 0.01</td>
</tr>
<tr>
<td>tSAMI-02</td>
<td>8.1026</td>
<td>8.0993</td>
<td>0.0030</td>
<td>± 0.0052</td>
<td>24.81</td>
<td>± 0.01</td>
</tr>
<tr>
<td>tSAMI-03</td>
<td>8.1061</td>
<td>8.1048</td>
<td>0.0013</td>
<td>± 0.0053</td>
<td>24.64</td>
<td>± 0.01</td>
</tr>
</tbody>
</table>

SAMI pH is the average pH calculated by the SAMI and Calc Tris pH is the average pH calculated according to Equation 2.7. ΔpH is the mean of the difference (SAMI pH - Calculated Tris pH) and represents the accuracy of the SAMI pH measurement. Precision is determined by the first standard deviation of the average of the differences. Temperature is also reported as the mean temperature measurement of the SAMI along with the first standard deviation of the temperature (σ).

These validation measurements show that the IBC flow-cell had comparable signal to noise to the SAMI-pH fiber optic system with the same control electronics. That is to say, the molar absorptivity ratios, $pK_a$, LEDs and band pass filters were the same and only the absence of fiber optics in the iSAMI was different. Overall, the iSAMI compared very well with the commercial version of the instrument (tSAMI) in terms of accuracy and precision. iSAMI-02 and tSAMI-02 proved to be the least accurate. tSAMI-02 had a 2-fold decrease in accuracy over the remaining tSAMIs while iSAMI-02 had a 5-fold decrease in accuracy over the remaining iSAMIs. The larger σ in the temperature measurement for iSAMI-01 is due to a warming trend in the recirculating water bath. These accuracy and precision values exceeded the minimum standards set out in the WSOH XPRIZE (See Figure 2.10) by at least an order of magnitude.

### 3.4 Wendy Schmidt Ocean Health XPRIZE

At the time this research was underway on the miniaturization of the SAMI-pH, the WSOH XPRIZE was announced. Recall from Section 2.4 the competition had two prize purses; an affordability prize purse and an accuracy prize purse each totaling $1 million dollars US. For each prize purse, entry instruments were graded on precision, accuracy, stability, cost, and ease of use. Tests were carried out in three different experimental
environments. These results were validated and judged by independent and well established researchers. See Section 2.4 for specific competition guidelines.

As mentioned previously, the iSAMI-pH was designed using the Integrated Beam Combiner Cell (Section 3.2.3) and the i-tube mixer (Section 3.1) to enter the WSOH XPRIZE in the affordability prize purse. This instrument was designated the iSAMI-pH, for inexpensive SAMI-pH. The iSAMI is unique from the well established SAMI-pH (See e.g. Martz et al. (2003), Seidel et al. (2008), Gray et al. (2011), Harris et al. (2013)) as it does not use fiber optics for absorbance measurements. Further, the pump and valve are not pressure compensated and so the instrument is suitable for surface measurements only: roughly to a depth of 5 m (See Section 2.3 for construction details of the iSAMI).

In addition, three SAMI-pH instruments, modified with a titanium pressure housing, were entered into the WSOH XPRIZE accuracy prize purse. These SAMI’s were termed the tSAMI-pH for titanium SAMI-pH. This prize purse had the additional requirement that instruments needed to withstand depths of 3000 m. Since the tSAMIs are essentially identical to the commercially available SAMI-pH instruments (save the Ti electronics enclosure), and both the tSAMIs and iSAMIs were entered into the WSOH XPRIZE, the iSAMI concept instrument received a thorough and independent critique and inter-comparison of performance.

The XPRIZE provided an ideal test of the iSAMI and a convenient comparison to the SAMI-pH on which it is based. It would have been cost prohibitive to obtain as many comparative samples, in three different environments, as this global competition. It should be noted that the iSAMI won first place in the affordability prize and the tSAMI won first place in the accuracy prize. Presented below are the results from the first three phases of the WSOH XPRIZE in which both the tSAMI and the iSAMI participated.

### 3.4.1 Phase 2a - lab accuracy trials

The first testing phase of the competition assessed individual pH sensors ability to provide pH values that were in agreement with the conventional true values assigned to two solutions of differing pH. The first attempt of this assessment was thwarted by a lack of tris standard. The previous group testing instruments had requested that the same batch of tris be used for all 9 of their entries which left just enough tris to partially submerge the 3 iSAMIs. The decision was made to postpone the test until the following morning
when new tris would be available. This gave the iSAMIs > 24 hours to temperature equilibrate in the MBARI walk-in cooler.

While the iSAMIs were sampling tris in their individual 5 gallon buckets, HCL was added to reduce the pH of the tris in the test bucket. This acid addition allowed both the judging panel and the entry instruments to measure against two solutions of differing pH. In all, the judging panel took two samples of tris before acid addition and two samples of tris after acid addition. The average of the two samples of tris pH before acid addition as well as after acid addition were compared to the average high and low pH values measured by the iSAMI-pH instruments. Three tSAMIs were put through the same testing phase and provided a great opportunity to intercompare the iSAMIs with Sunburst Sensors’ flagship instrument. See Section 2.4 for specific details of the trial.

The processed data reported by the entry units are shown in Figure 3.9 and the final results from the judging panel are tabulated in Table 3.3. Recall that three iSAMIs and three tSAMIs were entered into the WSOH XPRIZE Affordability and Accuracy prize purses, respectively. The tSAMI data is included here as a important inter-comparison with the iSAMI and serves as a validation of the iSAMI performance.
Figure 3.9: Consistent temperature and pH response for accuracy trial (Phase 2a) of the WSOH XPRIZE with three entries for the iSAMI version (a) and three entries for the tSAMI version (b).

Figure 3.9 shows the final pH of the tris standard as reported on one minute time intervals at the temperature shown for 3 identical iSAMIs (top) and 3 identical tSAMIs (bottom). The iSAMIs were all tested with the same batch of tris and report similar starting pH values. After the addition of HCL there is a larger difference in ending pH values due to slight volume differences in the aliquots of HCL. Overall the
acid addition represented a drop in pH of approximately 0.3 pH units for the iSAMIs. All three instruments reported temperature values very closely matched to each other. tSAMIs 1 and 2 were tested with a separate yet identical batch of tris and report nearly identical starting and ending pH and temperature values. The acid addition reduced the pH by approximately 0.45 pH units for tSAMIs 1 and 2. tSAMI-3 was tested with a third batch of tris that was almost 2 °C warmer which made it difficult to compare its performance with the other t & i SAMIs.

Table 3.3: Summary of results from Phase 2a of the WSOH XPRIZE for all six entries. Entry column is the name of the instrument, SAMI-pH column reports the average tris pH of all 6 instruments before and after acid addition, Validation pH column is the average of two samples of tris before and after acid addition, Δ(E-V) reports the difference in pH between the SAMI (E) and the Validation (V). AveAbsDev column is the average absolute deviation of entry and entry. The minimum standard for the affordability and accuracy prize purses were ≤ 0.04 and ≤ 0.02 pH units from the validation pH value, respectively.

<table>
<thead>
<tr>
<th>Entry</th>
<th>SAMI-pH</th>
<th>Validation pH</th>
<th>Δ(E-V)</th>
<th>AveAbsDev</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iSAMI-01</td>
<td>8.260</td>
<td>8.252</td>
<td>0.007</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>7.919</td>
<td>7.931</td>
<td>-0.012</td>
<td></td>
</tr>
<tr>
<td>iSAMI-02</td>
<td>8.278</td>
<td>8.282</td>
<td>-0.004</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>7.957</td>
<td>7.970</td>
<td>-0.013</td>
<td></td>
</tr>
<tr>
<td>iSAMI-03</td>
<td>8.267</td>
<td>8.271</td>
<td>-0.004</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>7.927</td>
<td>7.945</td>
<td>-0.018</td>
<td></td>
</tr>
<tr>
<td>tSAMI-01</td>
<td>8.151</td>
<td>8.143</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>7.702</td>
<td>7.694</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>tSAMI-02</td>
<td>8.152</td>
<td>8.142</td>
<td>0.010</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>7.712</td>
<td>7.709</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>tSAMI-03</td>
<td>8.235</td>
<td>8.222</td>
<td>0.013</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>7.912</td>
<td>7.910</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3 is the final comparison of the entry pH and the validation pH used in the competition for Phase 2a and was provided by the validation team at the conclusion of the competition. The SAMI-pH column is the average pH reported by the SAMIs centered on the time the validation samples were collected before and after acid addition. The Validation team did not indicate how many points were used to compute this average nor did they provide a time stamp for their measurements. The Validation pH column reports the average pH of the two samples of tris collected before acid addition and the average of the two samples collected.
after acid addition. The $\Delta$ column is the difference of Entry (E) - Validation (V) pH. The AveAbsDev is the average absolute deviation from validation pH. The minimum standard for the affordability and accuracy prize purses were $\leq 0.04$ and $\leq 0.02$ pH units from the validation pH value, respectively (See WSOH Figure 2.10).

### 3.4.2 Phase 2b - precision and stability trials

Phase 2b also took place at the Monterey Bay Aquarium Research Institute (MBARI). Testing was conducted in the MBARI test tank facility with the intended goal of assessing the repeatability and stability of the pH sensors in natural seawater. Measurement frequency was set to 2 hr and the test lasted 50 days. All instruments were suspended at 5 m in the 10 m tank. Temperature and salinity were measured at several locations in the tank using in situ CTD devices. Bottle samples were collected by the Validation Team and analyzed spectrophotometricly for pH.

![Figure 3.10: Photo of entry instruments hanging at 5 m depth in the MBARI test tank facility. In the foreground hang the tSAMi (A) and iSAMi (B) entered in phase 2b.](image-url)
The XPRIZE judging panel results for stability and precision of the entries for Phase 2b are tabulated in Table 3.4. The iSAM and tSAMi data calculated for the judging panel interpolated with validation data is shown in Figure 3.11. The regulations state the precision is calculated as the first standard deviation of the final pH values. In the available Rules and Regulations the stability calculation is given in less straightforward terms with several different wordings. There is mention of stability calculated as the root mean square error of the daily differences (assumed to be the same as the standard deviation of the daily differences), as the max change in pH per month, and as the trimmed mean of the interdecile range.

Phase 2b summary data with iSAMi and tSAMi final pH values calculated at a constant salinity of 34.25 are shown in the top panel of Figure 3.11. Average validation pH samples were taken twice daily typically over 30 minute long intervals. Spatial tank values averaged over 90 minutes generally once a week with an uncertainty of ± 0.009 pH units. The middle panel shows the SAMi temperatures interpolated with the commercial CTD temperature. The CTD salinity is shown in the bottom panel. Salinity increased in the tank approximately 0.13 PSU over the 50 days.
Figure 3.11: Time series pH and temperature data for iSAMI-2 and tSAMI-3 interpolated with CTD temperature and salinity. See Table 3.4 for stability and precision results. Validation pH from discrete bottle samples. Error bars represent a validation uncertainty of ± 0.009 pH units.
Table 3.4: Validation team final results for phase 2b. Stability is calculated as the drift from validation team bottle samples and precision calculated as the first standard deviation of the final pH values.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Stability (pH/Month)</th>
<th>Precision (pH units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iSAMI-02</td>
<td>0.008</td>
<td>± 0.004</td>
</tr>
<tr>
<td>tSAMI-03</td>
<td>0.003</td>
<td>± 0.004</td>
</tr>
</tbody>
</table>

At the conclusion of this phase the iSAMI was still tied for first place, presumably with the tSAMI though team rankings were not made public at this time. The following section describes the last phase of the competition in which the iSAMI design participated. The fourth and final phase was a deep sea test to 3000 m that only the tSAMI could complete.

3.4.3 Phase 3 - coastal trials at the Seattle Aquarium

The coastal trials provided a rigorous real-world test of the iSAMI in the challenging and highly variable coastal environment of Elliott Bay, Puget Sound. A special tank was constructed on Pier 59 at the Seattle Aquarium, Figure 3.12, that allowed all sensors in the competition to be tested simultaneously. Seawater from Elliot Bay was pumped into and out of this tank. Temperature and salinity were measured at several points along the tank using in situ CTD devices. Bottle samples were collected by the Validation Team and analyzed spectrophotometrically for pH. The trial lasted approximately one month and was designed to test the repeatability and calibration stability of pH sensors in a highly variable real world coastal environment.
Final summary results from Phase 3 are shown in Figure 3.13. Validation pH was reported with an uncertainty of ± 0.012 pH units. SAMI pH values were calculated at in situ salinity and temperature. The CTD temperature was in good agreement with the SAMIs showing nearly identical values to the tSAMI and a maximum difference with the iSAMI of 0.05 °C. The average pH difference between the iSAMI and tSAMI was 0.0063 pH units. The precision reported for the entries was ± 0.003 and ± 0.004 for the tSAMI and iSAMI, respectively. The stability was reported as 0.009 and 0.011 pH units/month for the tSAMI and iSAMI, respectively (See Table 3.5).
Figure 3.13: Phase 3 summary data with SAMI pH calculated at in situ salinity and temperature top panel. See Table 3.5 for stability and precision results. Average validation pH samples were taken twice daily from multiple locations in the tank with an uncertainty of ± 0.012. The middle panel shows the SAMI temperatures and the commercial CTD temperature with CTD salinity in bottom panel.
Table 3.5: Validation team final results for phase 3. Stability is calculated as the interdecile range and precision calculated as the first standard deviation of the final pH values.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Stability (pH/month)</th>
<th>Precision (pH units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iSAMI-01</td>
<td>0.011</td>
<td>± 0.004</td>
</tr>
<tr>
<td>tSAMI-02</td>
<td>0.009</td>
<td>± 0.003</td>
</tr>
</tbody>
</table>

The coastal trials phase marked the end of the WSOH XPRIZE competition for those entries competing for the Affordability Prize Purse. Though the iSAMI exceeded the metrics for accuracy and precision for the Accuracy Prize Purse, it was not designed for the 3000 m depth of the Deep-Sea Trials. The following section is a general discussion of the XPRIZE results and the iSAMI performance. Discussed quantitatively are possible sources of inaccuracy between the validation pH and the SAMI instruments. Finally, a thorough comparison of the tSAMI and iSAMI is discussed.
Chapter 4

Discussion

4.1 WSOH XPRIZE discussion

Miniaturization of the SAMI-pH instrument has not previously been attempted. The iSAMI uses a novel optofluidic flow-cell that removes the design restrictions imposed by the fiber optics of the SAMI-pH. The SAMIs are primarily deployed as moored instruments, in an Eulerian reference frame, with the ability to host up to 3 external sensors at up to 600 m depth. The iSAMI is intended as a surface instrument for buoys, moorings, reef studies, gliders and Lagrangian OA studies that can be used as an auxiliary or slave sensor.

The SAMI is a proven design suitable for OA research that has been adapted to measure $pCO_2$ and pH (Gray et al., 2011; Harris et al., 2013), as well as $A_T$ (Spaulding et al., 2014). The WSOH XPRIZE provided a rare opportunity to compare the SAMI-pH with other pH systems developed by top researchers and engineers around the globe. It also served as a rigorous and thorough inter-comparison between the fiber-less iSAMI and the SAMI-pH.

Presented in this chapter, some of the possible sources of inaccuracy between the XPRIZE validation results and those of the SAMIs are discussed. The following sections highlight the differences and similarities between the iSAMI and tSAMI and their comparison to the validation results. These issues will be explored through accuracy, precision and stability in the three phases of the WSOH XPRIZE in which the iSAMI participated.
4.1.1 Possible sources of error

It is not clear why the difference between the validation pH and the SAMI pH values are larger for phases 2b (∼ 0.02 pH units, Figure 3.11 & Table 3.4) and 3 (∼ 0.027 pH units, Figure 3.13 & Table 3.5) than for the accuracy phase 2a (< 0.01 pH units, Figure 3.9 & Table 3.3). This could partially be due to the large number of replicate discrete samples averaged for the time series of phases 2b and 3. It was not disclosed to XPRIZE participants how validation pH was constrained. For example, it may have been constrained by measuring other CO$_2$ system parameters: DIC, A$_T$, or pCO$_2$ (See Section 1.2). At the time of this writing, it was also not disclosed how pH at in situ temperature was calculated from the discrete sample pH measurement (Okazaki et al., 2017). Therefore, it is unclear which is the better data set, however, the SAMI methodology outperformed all other instruments in the competition.

As noted there were a large number of replicate discrete samples to measure validation pH. There was no information available to contestants as to which lab, or labs, performed the determination for some or all of the validation pH measurements. It was not also made available to contestants whether the validation pH was fully constrained and as has been shown (Bockmon and Dickson, 2015) spectrophotometric determination of the pH of seawater certified reference material (CRM) standards is quite variable from lab to lab. As stated in that manuscript, the reported “results for the analysis of pH were quite scattered, with little suggestion of a consensus value.” Ultimately, due to this many variables, it makes more sense to see the ∆s as margins of error rather than an absolute value.

The largest likely sources of error in pH come from indicator physical properties (DeGrandpre et al., 2014). The indicator purity can affect both the molar absorption ratios and the $pK_a$ determination. The XPRIZE validation team used purified mCP and spectrophotometrically determined pH according to Liu et al. (2011) using the molar absorption ratios and the $pK_a$ contained therein. The SAMIs determined pH using molar absorption ratios and $pK_a$ as determined by Sunburst Sensors personnel. The mCP used for the SAMIs was also purified according to Liu et al. (2011), but by Sunburst Sensors personnel. As was shown in DeGrandpre et al. (2014), small differences in the determination of the molar absorption ratios and tris preparation can lead to significant error in $pK_a$ which adds a baseline offset to pH values (Equation 1.22 & Figure 4.1).
Instrument parameters that affect pH accuracy are wavelength errors and absorbance errors. All the SAMIs have molar absorption ratios determined with 10 nm bandpass interference filters (FWHM). The molar absorption ratios of Liu et al. (2011) were determined on a high quality spectrophotometer with a 2 nm bandpass. Assuming the XPRIZE validation team used a high quality spectrophotometer that was calibrated with e.g. NIST traceable absorbance standards, the difference in band pass on the SAMIs could show a small negative bias in pH. Absorbance maxima is broader in the acid form than the base form of mCP and accordingly the absorbance decreases more for the basic form. This leads to a lower absorbance ratio and lower pH (DeGrandpre et al., 2014). See Equation 1.14. Small differences in LED spectra and bandpass filters could then suggest a source of error between individual SAMI units and could suggest a small difference between the SAMIs and the validation results.
4.1.2 Phase 2a - lab accuracy trials

There is a slight downward trend in the iSAMI final pH values (Figure 3.9) with a slight increasing trend in the temperature. The increase in temperature is also evident in the tSAMI data without a corresponding decreasing trend in the pH values. The pH of tris is highly temperature dependent but the increase in temperature over the course of the trial is not enough on its own to explain the downward trend in the iSAMI pH values. It is likely that heat generated inside the iSAMI enclosure from near constant firing of the solenoid pump influenced the temperature of the tris in the flow-cell. This is not the case with the tSAMI as the pump and valve enclosure is oil filled and well buffered from temperature changes from the pump. The tSAMI flow-cell is also external to the enclosure and better temperature equilibrated to the surrounding sample fluid. In future work with high sample frequency using the iSAMI, an internal thermistor should be added to simultaneously monitor internal and external temperatures.

The accuracy difference between the validation team and the SAMI units is lower than some laboratory accuracy values reported in the literature (See e.g. Seidel et al. (2008), DeGrandpre et al. (2014)). This is informed partially by the use of different $pK_a$ and also the replicate sample skill of the validation technician (Bockmon and Dickson, 2015). Still, the iSAMIs and tSAMIs showed excellent accuracy compared to each other and compared to the validation pH ($\pm 0.01$ pH units, Table 3.9). This was two fold better than the metric for the accuracy purse ($\leq 0.02$ pH units, Table 2.10) and four fold better than the metric for the affordability purse ($\leq 0.04$ pH units, Table 2.10). Although not assessed for prize points in this phase, the iSAMIs showed excellent precision with $\pm 0.004$ pH units. The tSAMIs were even more precise with $\pm 0.002$ pH units.
Figure 4.2: Average of 4 blank measurements for each measurement sequence for the duration of the accuracy trial for the three iSAMIs and three tSAMIs. See Figure 1.7.
The comparison between the iSAMI and the tSAMI, which is a validation of the iSAMI performance, is in the details of the measurement sequence. A critical part of these measurements are the blank measurements, $I_0$, at the beginning of each measurement sequence. If a bubble obstructs the absorbance path in the flow cell during the blank measurement but releases during the reagent injection measurement sequence and signals increase, the blank will distort the reading. That is to say, an inaccurate blank measurement will lead to inaccurate absorbance measurements during the measurement sequence and yield an inaccurate pH value.

Figure 4.2 shows the mean of four blank intensity measurements (434 nm) taken at the beginning of each pH measurement sequence (Phase 2a). This value was used to determine the absorbance at each point in the sequence. As long as the blank value is indicative of the measurement sequence it will yield accurate absorbance calculations regardless of variability from measurement sequence to measurement sequence (i.e. bubbles affect light throughput and can pass the system during a measurement sequence).

There is variability in the subsequent blank values of iSAMI-2 - 3 contrasted with a less variable iSAMI-1. All of the tSAMIs show drift in this blank value but not the degree of variability. All of the instruments in the accuracy trial exhibited a significant downward trending average blank value that would not be expected over such a short time. This could be attributed in part to a truncated and rapid measurement sequence designed to allow the SAMIs to make measurements on 1 minute intervals. SAMI-pH measurements during the Sunburst Sensors validation procedure are typically performed on 5 minute intervals and allow for a more thorough flushing of reagent. This trend could also be due in part to a build up of reagent in the sample bucket of tris since the setup was a closed system containing less than 3 gal of fluid. This could have been avoided by collecting the SAMI effluent in a reagent pouch.

The drift in blank values point to build up of reagent in the sample environment or in the SAMI flow cell and tubing. This appears to not affect the accuracy or precision in this pH measurement trial. Clearly if the SAMIs were to continuously sample their own effluent ad infinitum the small sample vessel would become contaminated and the accuracy would suffer. The drift in final iSAMI pH is likely due to warming of the tris in the electronics enclosure. The iSAMI is also more susceptible to systematic micro-bubble induced intensity fluctuations than the tSAMI. Taken together, this affects both the accuracy and precision of the iSAMI for this type of high frequency tris pH measurement. The accuracy of the tSAMI and iSAMI are clearly affected more by the difference in $pK_a$ and the molar absorption ratios between the validation pH
measurements and those determined for the SAMIs.

4.1.3 Phase 2b - precision and stability trials

Salinity and temperature remained very constant over the course of the precision trial with a change of $< 0.15$ PSU and $< 0.5$ °C, respectively (Figure 3.11). The steady change in salinity could be due to evaporation from the tank, as was suspected by XPRIZE personnel. The entry temperatures were very close to the CTD temperature which was used in the validation pH calculation, with a maximum difference with tSAMI-3 being 0.05 °C and iSAMI-2 roughly half that (Figure 3.11).

The validation pH reported a $< 0.02$ pH change over the phase but the reported uncertainty in the validation measurement was $\pm 0.009$ (Figure 3.11). This uncertainty nearly accounts for the slope of validation pH, indicating that there is not much of a trend. The tSAMI appears to better mirror the apparent validation pH change, though examination of Figure 4.3 shows the difference between the iSAMI and tSAMI to be quite small over all ($< 0.0068$ pH units). This deviation is smaller than the uncertainty in the validation measurements so it is not clear which is the better data set. Clearly the test tank was very stable in terms of pH, temperature and salinity. The iSAMI exceeded the metrics for stability by 2 fold and precision by 5 fold in the affordability purse and compared quite favorably with the tSAMI (See Table 2.10).

![Chart](image.png)

Figure 4.3: The difference between iSAMI-2 and tSAMI-3 final pH values for Phase 2b revealing an average difference of only $0.0068 \pm 0.004$ pH units.

Analysis of iSAMI-2 interpolated to the validation pH revealed a standard deviation in agreement with the
official precision results (See Table 3.4). Stability analysis yielded agreement in the 95% confidence interval of the least squares slope only for the full range of daily differences in pH. It is not clear from Figure 3.11 how the precision calculation yielded identical precision results for both SAMIs. It is clear from Phase 2b that the tSAMI final pH values contained more noise than the iSAMI. Examination of the blank intensity and signal stability will elucidate the difference in performance between the two instruments.

Examination of the signal intensities show that the tSAMI intensity signal curves were tightly grouped together for the whole deployment while the iSAMI intensity signal curves were more loosely grouped. This spread is mostly due to the blank intensity drift during deployment as shown in Figure 4.5. This is further borne out in that the reference signals show no similar trend in LED brightness. The iSAMI showed more susceptibility to micro-bubbles than the tSAMI (See Figures 4.4 & 4.6). The data filters in the MATLAB™ code (Section 1.5) proved to be fairly effective as well as necessary (See Appendix A Section A.3).

![Figure 4.4: iSAMI-002 raw unfiltered signal intensities for 600 measurement sequences during the precision trials (578 nm in red 434 nm in blue, x-axis is pump cycles, See Section 1.5).](image)
Again, the most interesting difference between the iSAMI and the tSAMI is the blank intensity stability (Figure 4.5). With the tSAMI, the blank intensity is steadily increasing while in contrast the iSAMI blank intensity is steadily decreasing during the deployment. This might suggest that the iSAMI was retaining more mCP reagent that was building up in the system or adhering to the polycarbonate optical windows. It could also indicate that micro-bubbles were adhering to the optical windows. It is important to note that while the blank measurements for the iSAMI continued to be representative of the measurement sequence, there would appear to be a point of no return where the blank signal might be too low to continue with the measurement sequence (noise floor). This would also suggest that the signal to noise ratio would decrease since the noise remains constant while the dynamic range decreases.
It is interesting to note that the iSAMI final pH values are slightly less variable than the tSAMI final pH values (Figure 3.11) despite the fact that the signal curves for the tSAMI were tightly grouped showing little variation and, in contrast, the iSAMI signal curves were more variable and slightly more broad (See Figures 4.4 & 4.6 & Appendix A Section A.3). The spread in the signal curves of the iSAMI is due to the drift in blank intensity. The signal curves of the tSAMI would not suggest such behavior and it is unclear what is driving this variation. The tSAMI signal curves on 10/16/2014 are smooth, tightly grouped and the corresponding point-pH curves are smooth (Figure 4.7). Though the point-pH curves are smooth, there is a significant jump in pH (>0.05) from measurement to measurement (2 hour intervals). The tSAMI final pH variation could be due to real environmental variation or an influence from other pH sensors in the tank. See Appendix A Section A.3 for more figures of iSAMI and tSAMI Phase 2b.
4.1.4 Phase 3 - coastal trials

Analysis of SAMI data interpolated to validation pH revealed agreements in reported precision and stability. The validation pH was reported with an uncertainty of ± 0.012. This uncertainty would suggest a pH stability of < 0.01 pH/month would fall within the error of the validation measurement. The reported stability (pH/month) for the iSAMI and tSAMI are well within this window. The iSAMI-1 and tSAMI-2 follow the trend in validation pH quite well with a consistent offset of < 0.027 pH units (Figure 3.13). This offset is similar to that of phase 2b.

The final pH difference plot, iSAMI-tSAMI, shown in Figure 4.8, shows an average difference of only 0.0063 pH units. The offset began at about the 10 day mark and remained in a steady state. The offset is not borne out by any sudden shift in signals as neither of the instruments show any abrupt change in LED brightness. Clearly the iSAMI compares quite favorably with the tSAMI even in a highly variable coastal environment. The iSAMI measured pH in with greater stability (> 15%) and precision 5-fold better than required for the
affordability purse (See Table 2.10).

Figure 4.8: Phase 3 pH difference plot of iSMAI-tSAMI. A slight pH difference appears at the 10 day mark but does not seem to become larger over time and bears an overall average difference of only 0.0063 ± 0.004 pH units.

The main difference between the iSAMI and the tSAMI is the blank intensity stability, shown in Figure 4.9. With the tSAMI, the raw blank intensity doesn’t show micro-bubble spikes while, in contrast, the iSAMI shows many large and sudden decreases in raw blank intensity during the deployment. Once filtered, the iSAMI blank intensity signals actually show a better trend than the tSAMI (Figure 4.9). Interestingly, the case here is the opposite of Phase 2b since the tSAMI blank intensity decreases more than the iSAMI (∼10%).
Figure 4.9: Average raw 434 nm blank signals for Phase 3 of the WSOH XPRIZE held at the Seattle Aquarium. Variability in blank signals are higher in the iSAMi than in the tSAMi. Large spikes pre-data-filter in the iSAMi indicate bubble adhesion and release.

It is important to note, again, that the blank measurements for the iSAMi continued to be representative of the measurement sequence once filtered. The bubble induced signal drops were severe and the MATLAB<sup>TM</sup> data filters did a decent job of smoothing the raw data for processing. Compare the raw unfiltered signal intensities of iSAMi-1 and tSAMi-2 and the idea is readily apparent, Figures 4.10 and 4.12, respectively. See Appendix A Section A.4 for more signal figures of iSAMi-1 and tSAMi-2 in this phase.
Figure 4.10: iSAMI-1 raw unfiltered signal intensities for 600 measurement sequences of Phase 3 (578 nm in red 434 nm in blue, x-axis is pump cycles, See Section 1.5).

Figure 4.11: iSAMI-1 filtered signal intensities for 600 measurement sequences of Phase 3 (578 nm in red 434 nm in blue, x-axis is pump cycles, See Section 1.5).
4.1.5 Accuracy and affordability

The tSAMIs slightly outperformed the iSAMIs in the Accuracy Trials (See Section 3.4.1 Table 3.3), the Precision Trials (See Section 3.4.2 Table 3.4) and the Coastal Trials (See Section 3.4.3 Table 3.5). In phase 2a the iSAMIs accuracy and precision were likely affected by the internal heating of tris. In phase 2b the iSAMIs had less noise in the final pH values but showed identical precision. In phase 3 the tSAMIs had a calculated stability slightly better than the iSAMIs by 0.002 pH units/month. The above exploration of the XPRIZE compared data across all three phases of the WSOH XPRIZE. This global competition judged pH sensors from top researchers and entrepreneurs for three different experimental environments: laboratory, test tank, and coastal waters. These testing environments highlight the differences and similarities between the iSAMIs and tSAMIs and points to one main difference: micro-bubble adhesion to the optical windows in the iSAMIs.

The main difference in the design of these instruments is that the tSAMIs uses fiber optics as a light delivery mechanism while the iSAMIs shines the LED emission directly through the absorbance path. With the iSAMI
the full 1 mm aperture of the absorbance path is illuminated by the LEDs whereas with the tSAMI only the core diameter of the optical fiber, being only 800 µm, shines into the absorbance path. The tSAMI uses optical fiber to collect the light transmitted through the flow cell to direct the light to the detector. This means that the tSAMI does not sense the transitional edges at the ends of the absorbance path whereas with the iSAMI these edges are fully illuminated and any micro-bubbles accumulating there are sampled thus affecting the signal integrity. Since these microbubbles can essentially hide at the interface of the fiber cladding and the absorbance path end walls of the flowcell with the tSAMI, they do not significantly affect the signal integrity nearly as much as with the iSAMI. This is somewhat speculative since it has not been physically observed in the fiber system, though it has been observed in the iSAMI flow-cells.

Emphasis in the data exploration was placed on the comparison in performance between the iSAMI and the instrument it was based on, the tSAMI, providing a thorough evaluation and validation of a fiber-less approach to spectrophotometric pH measurement of seawater. This analysis showed that the main difference between the iSAMI and tSAMI was that the iSAMI was prone to bubble induced signal fluctuations whereas the tSAMI was not significantly affected by bubbles (See Appendix A). These signal fluctuations, however, did not significantly impact the accuracy and precision of the iSAMI and blank intensity measurements were still representative of the measurement sequence. The challenging nature of the iSAMI micro-bubbles spurred development work with the SAMI Client and MATLAB™ routines.

These chronic micro-bubble induced signal fluctuations encountered by the iSAMI seem to not interfere with achieving excellent stability and precision in long term deployments. The iSAMI has been designed without pressure compensation for the pump and valve. This results in deployments limited to the surface ocean, ≥ 5 m, whereas the tSAMI was successfully deployed to 3000 m. This difference can be seen as an advantage for Lagrangian studies of OA, as proposed for the GDP, as elimination of the pump and valve housing also reduced the overall cost of the iSAMI.

The XPRIZE judging panel assessed the manufactured cost of 1000 units of the iSAMI and the tSAMI and gave conservative estimates of $800 and $4500 USD, respectively. This 82% reduction in cost can be attributed to several factors. Overall, the iSAMI was constructed with only 4 major components that were machined with the pressure housing made of tube stock. The tSAMI, by contrast, was constructed with 15 machined components and the pressure housing was machined from solid titanium stock. An additional
feature of the iSAMI was the reduced number of person hours to construct the instrument. With its simplified construction, elimination of pump and valve housing and reduced person hours the iSAMI will be a very affordable and accurate instrument.
Chapter 5

Conclusion and future work

5.1 Summary

This masters project set out to miniaturize the commercially available in situ spectrophotometric SAMI-pH sensor. Efforts were made to find ways to decrease power consumption, identify design limiting components, identify and reduce expensive components, and package the instrument in a housing designed for surface waters. The overarching goal was to design an instrument that could be implemented on the Global Drifter Program (GDP) as a non-recovered instrument. This effort focused primarily on finding an alternative approach to mix indicator dye with seawater and an alternative approach to fiber optics for light delivery through the flow cell.

This work has demonstrated a suite of alternatives to the static mixer (SM) on the SAMI-pH. All of the alternative mixers tested showed accuracy as good or better than the SM, while reducing the internal volume. The tube mixers were cheap and easy to make in house, though still had a relatively large internal volume. They are now being used for the commercial SAMI-pH as a replacement of the static mixer and have reduced the flushing pumps from 55 to 35. The mixing cards designed by Micro Plumbers proved effective with internal volumes as low as 37 µL. The mixing cards represent a new and highly flexible platform for mixing and could open the door to other novel applications.

This work also explored several novel approaches for creating flow cells. 3-D printing was sampled as a possible approach for embedded optics in a flow cell, however, the technology is not quite ready for such
a demanding application. Two clamshell designs were also explored where the optics and flow cell were embedded in a sandwich of polycarbonate. In the first design, the design intent used optical elements and micromachining to seal the flow cell path. This proved unsuccessful and a second design used the substrate material as optical windows. Though the second design improved on the first, and successfully demonstrated the sealing approach, both designs suffered from severe stray light issues. A third flow cell was constructed from opaque materials and used mechanical fasteners to secure liquid tight optical windows. This design incorporated the absorbance flow cell and the optical elements in a package that fit on the SAMI board as a direct replacement of the beam combiner assembly. This innovation led to the design of the iSAMI-pH which was entered into the WSOH XPRIZE.

The WSOH XPRIZE provided an ideal test of these innovations. The XPRIZE spurred the iSAMI design which incorporated these innovations into the first miniaturized SAMI-pH. The rigorous testing protocols allowed for a thorough side by side comparison of the iSAMI and the SAMI-pH and were judged along side a global community of sensor developers. The iSAMI proved to be accurate in laboratory testing, stable in long term seawater tank testing, as well as accurate, precise, and stable in a real world test with coastal waters of the Puget Sound. In all, the iSAMI-pH and the SAMI-pH won first place in the Affordability and Accuracy prize purses, respectively, for a grand total of $1.5 million US.

5.2 Current revision and future work

Post XPRIZE work continues on miniaturizing and refining the iSAMI-pH. Figure 5.1 shows the current state of the effort. The reagent box at the bottom can hold ~ 250 µL reagent pouch. The system employs a 25 µL manifold mounted solenoid pump and a 35 µL serpentine cartridge mixer. This will make for an 80% reduction in flush pumping over that of the SAMI-pH while still enabling ~ 10,000 measurements. The integrated beam combiner flow cell now uses a dual wavelength band pass filter and a hot mirror as optical windows. Overall dimensions are 2.5 in diameter by 12 in long. The logic board is a four layer two sided board with analog components and digital components on opposite sides. The system performs at the level of the iSAMI, showing good precision and accuracy and remains susceptible to micro-bubble adhesion.
The GDP will require an instrument that will not interfere with the core directives of the drifter (e.g. use little power, low cost, will not interfere with the Lagrangian path). While the iSAMI proved to be accurate
and precise, and compared very well with its predecessor in the WSOH XPRIZE, it remains too large to accompany a drifting buoy on a long journey. The size of the iSAMI design was driven by the requirement of an internal battery for the WSOH XPRIZE and the size of the available logic control board. With the GDP, the iSAMI would be powered by the buoy which will remove the need for an internal battery pack. The logic control board for the SAMI line-up is based on the minimum bend radius of the fiber optics. Since the iSAMI has proven the efficacy of the integration of the flow-cell and optical elements, thus eliminating the fiber optics, the board design can be reduced in size considerably. Taken together, the innovations of this research along with the dramatic reduction in the size of the control board and elimination of the internal battery pack, a miniaturized SAMI-pH considerably smaller than even the iSAMI has been possible, enabling the possible integration of a miniaturized spectrophotometric pH sensor with the Global Drifter Program.
Bibliography


Appendix A

WSOH XPRIZE: Supplemental

A.1 Software comparison

As a commercially available instrument, the SAMI-pH is supplied with a software Client that allows the user to obtain final pH values from their deployment. While not as powerful for data exploration as the custom MATLAB™ code, it is interesting to see how much the data filters in that code affect the performance of the iSAMI versus that of the tSAMI which is virtually identical to the commercial SAMI-pH. This exploration is detailed in the following figure.

Figure A.1: Phase 2a final pH data calculated using the SAMI Client v1.28. This highlights the necessity of the MATLAB™ data filters.

Figure A.1 shows the final pH of the accuracy trial of Phase 2a as calculated using the SAMI Client version 1.28. This software contains no internal data filters for blank signal and dilution curve smoothing. The difference in the average starting pH before acid addition and average ending pH after acid addition between the iSAMI and tSAMI are due to the fact that they were immersed in different batches of tris. The final pH curve for the tSAMI is very similar to Figure 3.9 whereas in stark contrast the iSAMI final pH values calculated without data filters are considerably noisier. The data smoothing filters offered in the MATLAB™ code are obviously very essential and should be implemented in any commercial application of the iSAMI.
A.2 Phase 2a - accuracy trials

Figures for iSAMI-001, 002, 003, and tSAMI-001, 002, 003 for the Accuracy Trials at MBARI.

Figure A.2: iSAMI-001 raw unfiltered signal intensities for phase 2a.

Figure A.3: iSAMI-001 filtered signal intensities for phase 2a.
Figure A.4: iSAMU-001 point pH curves for phase 2a.

Figure A.5: iSAMU-001 final tris pH and temperature for phase 2a.
Figure A.6: iSAMI-002 raw unfiltered signal intensities for phase 2a.

Figure A.7: iSAMI-002 filtered signal intensities for phase 2a.
Figure A.8: iSAMI-002 point pH curves for phase 2a.

Figure A.9: iSAMI-002 final seawater pH and temperature for phase 2a.
Figure A.10: iSAM-003 raw unfiltered signal intensities for phase 2a.

Figure A.11: iSAM-003 filtered signal intensities for phase 2a.
Figure A.12: iSAMI-003 point pH curves for phase 2a.

Figure A.13: iSAMI-003 final tris pH and temperature for phase 2a.
Figure A.14: tSAMI-001 raw unfiltered signal intensities for phase 2a.

Figure A.15: tSAMI-001 filtered signal intensities for phase 2a.
Figure A.16: tSAM-001 point pH curves for phase 2a.

Figure A.17: tSAM-001 final tris pH and temperature for phase 2a.
Figure A.18: tSAMI-002 raw unfiltered signal intensities for phase 2a.

Figure A.19: tSAMI-002 filtered signal intensities for phase 2a.
Figure A.20: tSAMI-002 point pH curves for phase 2a.

Figure A.21: tSAMI-002 final seawater pH and temperature for phase 2a.
Figure A.22: tSAMI-003 raw unfiltered signal intensities for phase 2a.

Figure A.23: tSAMI-003 filtered signal intensities for phase 2a.
Figure A.24: tSAM1-003 point pH curves for phase 2a.

Figure A.25: tSAM1-003 final tris pH and temperature for phase 2a.
A.3 Phase-2b precision trials

Presented below are a suite of figures to compare tSAMi-003 and iSAMi-002 for the Precision Trials. As was noted in the manuscript there is no apparent reason that the iSAMi was less noisy than the tSAMi at the start of the phase. The measurements from 260 to 270 were very noisy for the tSAMi and were separated from the whole to help assess the noise issue.

![Reference Signals and Unfiltered Intensity Signals](image)

Figure A.26: iSAMi-002 raw unfiltered signal intensities for 600 measurement sequences.
Figure A.27: iSAM-002 filtered signal intensities for 600 measurement sequences.

Figure A.28: iSAM-002 point pH curves for 600 measurement sequences.
Figure A.29: iSAMI-002 final seawater pH and temperature for 600 measurements.

Figure A.30: iSAMI-002 least squares fit for indicator perturbation correction for 600 measurements.
Figure A.31: iSAM-002 raw unfiltered signal intensities for measurement sequences 260 to 270.

Figure A.32: iSAM-002 filtered signal intensities for measurement sequences 260 to 270.
Figure A.33: iSAMI-002 point pH curves for measurement sequences 260 to 270.

Figure A.34: iSAMI-002 final seawater pH and temperature for measurement sequences 260 to 270.
Figure A.35: iSAMI-002 least squares fit for indicator perturbation correction for measurements 260 to 270.

tSAMI-003 Figures

Figure A.36: tSAMI-003 raw unfiltered signal intensities for 600 measurement sequences.
Figure A.37: tSAMI-003 filtered signal intensities for 600 measurement sequences.

Figure A.38: tSAMI-003 point pH curves for 600 measurement sequences.
Figure A.39: tSAMI-003 final seawater pH and temperature for 600 measurements.

Figure A.40: tSAMI-003 least squares fit for indicator perturbation correction for 600 measurements.
Figure A.41: tSAM-I-003 raw unfiltered signal intensities for measurement sequences 260 to 270.

Figure A.42: tSAM-I-003 filtered signal intensities for measurement sequences 260 to 270.
Figure A.43: tSAMI-003 point pH curves for measurement sequences 260 to 270.

Figure A.44: tSAMI-003 final seawater pH and temperature for measurement sequences 260 to 270.
Figure A.45: tSAMI-003 least squares fit for indicator perturbation correction for measurement sequences 260 to 270.

A.4 Phase 3 - coastal trials

Figure A.46: iSAMI-001 raw unfiltered signal intensities for 600 measurement sequences.
Figure A.47: iSAMI-001 filtered signal intensities for 600 measurement sequences.

Figure A.48: iSAMI-001 point-pH curves for 600 measurement sequences.
Figure A.49: iSAM-001 final seawater pH and temperature for 600 measurements.

Figure A.50: iSAM-001 least squares fit for indicator perturbation correction for 600 measurements.
Figure A.51: tSAMI-002 raw unfiltered signal intensities for 600 measurement sequences.

Figure A.52: tSAMI-002 filtered signal intensities for 600 measurement sequences.
Figure A.53: tSAMI-002 point pH curves for 600 measurement sequences.

Figure A.54: tSAMI-002 final seawater pH and temperature for 600 measurements.
Figure A.55: tSAMIC-002 least squares fit for indicator perturbation correction for 600 measurements.