Improving Spectrophotometric Carbon System Measurements

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Improving Spectrophotometric Carbon System Measurements

by

Mark C. Patsavas

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy College of Marine Science University of South Florida

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ABSTRACT

This work provides improved procedures for spectrophotometric carbon system measurements. Indicator dyes used for routine spectrophotometric pH measurements in seawater suffer from impurity issues, which introduce vendor-specific systematic errors in pH determinations. The magnitude of these errors for several vendors was investigated for meta Cresol Purple (mCP) and Cresol Red (CR). Flash chromatography procedures were developed to obtain purified mCP and CR on a bulk scale in order to supply the oceanographic research community with the indicators. Easy access to the purified indicators ensures global intercomparability of spectrophotometric pH determinations.

Internal consistency of marine inorganic carbon system measurements was studied using datasets obtained on two large coastal ocean acidification research cruises. In both cases, purified mCP was used to obtain the pH measurements, thereby improving accuracy relative to previous studies in which measurements were obtained with unrefined mCP. Based on this internal consistency study, recommendations are made for selecting the parameter pairs used for saturation state calculations.

Direct spectrophotometric methods for measuring carbonate ion concentrations in seawater were improved by (a) using a higher concentration of lead as the carbonate indicator and (b) altering the carbonate computational algorithm based on high quality field data. Measurements of DIC and pH (using purified mCP) were used to calculate carbonate ion
concentrations for comparison with spectrophotometrically measured carbonate ion concentrations (i.e., via spectrophotometric measurements of Pb(II) spectra in the ultraviolet). Minor changes in the computational algorithm substantially improved agreement between measured and calculated carbonate ion concentrations.
CHAPTER ONE:

INTRODUCTION

1.1 Atmospheric Carbon Dioxide

In May of 2013 the concentration of atmospheric carbon dioxide (CO$_2$) at the Mauna Loa observatory reached 400 ppm.\textsuperscript{1} This concentration is 27\% higher than when measurements started at Mauna Loa in 1958 (Figure 1.1) and 43\% higher than since the onset of the industrial revolution, when atmospheric CO$_2$ was 280 ppm.\textsuperscript{2} Gas bubbles trapped in ice provide evidence that the concentration of CO$_2$ in the atmosphere before the onset of the industrial revolution oscillated between 180 - 280 ppm for the past 650,000 years.\textsuperscript{3} More concerning than the increase in CO$_2$ concentration is that the rate of increase of anthropogenic CO$_2$ in the atmosphere observed over the last half century is unprecedented in the Anthropocene.\textsuperscript{4}

Carbon dioxide acts as a greenhouse gas, trapping incoming solar radiation, thereby warming the planet. Changing the heat-budget of the earth alters the climate system and, besides the obvious threat of increased land and sea temperatures, the increased CO$_2$ in the atmosphere eventually dissolves into the surface of the oceans. It is estimated that 28\% of the anthropogenic CO$_2$ released since the industrial revolution began has dissolved in the oceans.\textsuperscript{5,6} The ocean is sequestering CO$_2$ from the atmosphere, thereby reducing the greenhouse effect, but there is a negative side to this process. The lesser-publicized fact, “the other CO$_2$ problem,” is that CO$_2$ acts as an acid in seawater, decreasing the pH of the oceans.\textsuperscript{7-9} This downward trend in oceanic
pH, which is referred to as ocean acidification, has profound effects on marine biogeochemistry.\textsuperscript{10-19}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{atmospheric_co2.png}
\caption{Atmospheric CO$_2$ at Mauna Loa Observatory.\textsuperscript{1}}
\end{figure}

Although there are global compensatory mechanisms for buffering the effects of high atmospheric CO$_2$ perturbations (e.g., dissolution of carbonate sediments and increased terrestrial weathering), these mechanisms act on a timescale of millennia.\textsuperscript{20} The biogeochemical changes initiated by the uptake of anthropogenic CO$_2$ in the ocean will persist for millennia, even if all
CO₂ emissions were immediately cut to zero. The most efficient way to mitigate ocean acidification is to reduce CO₂ emissions.

1.2 Dissolution of CO₂ in Seawater

Carbon dioxide in seawater exists in three forms: CO₂, HCO₃⁻, and CO₃²⁻. The relative proportion of each species is pH dependent. The dissolution of CO₂ in seawater is described by the following equilibrium:

\[
\text{CO}_2(g) \rightleftharpoons \text{CO}_2^*(aq)
\]  \hspace{1cm} 1.1

The CO₂* (where CO₂* includes both CO₂ and H₂CO₃) then reacts with water to form bicarbonate:

\[
\text{CO}_2^* + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+
\]  \hspace{1cm} 1.2

and some portion of the bicarbonate dissociates into a carbonate ion and a hydrogen ion:

\[
\text{HCO}_3^- \rightleftharpoons \text{CO}_3^{2-} + \text{H}^+
\]  \hspace{1cm} 1.3

Reactions 1.1, 1.2, and 1.3 can be described by equilibrium constants, \(K_0\), \(K_1'\), and \(K_2'\). These constants are functions of temperature, salinity, and pressure.

The dissolution of CO₂ in seawater causes a reduction in carbonate ion concentrations, which negatively affects the production of calcium carbonate shells or tests by marine calcifiers. The formation of calcium carbonate is described as:

\[
\text{Ca}^{2+} + \text{CO}_3^{2-} \rightleftharpoons \text{CaCO}_3(s),
\]  \hspace{1cm} 1.4

and the equilibrium relationship between Ca²⁺, CO₃²⁻ and solid calcium carbonate is given as:

\[
K'_{sp} = [\text{Ca}^{2+}]_T[\text{CO}_3^{2-}]_T
\]  \hspace{1cm} 1.5
where $K'_{sp}$ is the apparent solubility product of calcium carbonate at a given temperature, salinity and pressure. The two principal polymorphs of calcium carbonate, calcite and aragonite, have solubility products that differ by a factor of 1.51 at a temperature of 25°C such that aragonite is more soluble than calcite.\textsuperscript{18} Marine organisms such as pteropods, coccolithophorids and scleractinian corals depend on an ability to form calcium carbonate shells. Other organisms, in turn, are dependent on shelled organisms as a food source (e.g., pteropods are a food source for Pacific salmon).\textsuperscript{8,23}

1.3 Saturation States

It is convenient to describe the formation or dissolution of calcium carbonate polymorphs in terms of saturation states ($\Omega$). The saturation state is the product of the calcium concentration and the in situ carbonate ion concentration divided by the apparent solubility product of the particular polymorph:

$$\Omega_{\text{aragonite}} = \frac{[\text{Ca}^{2+}]_T[\text{CO}_3^{2-}]_T}{K_{sp,\text{aragonite}}} \quad \text{and} \quad \Omega_{\text{calcite}} = \frac{[\text{Ca}^{2+}]_T[\text{CO}_3^{2-}]_T}{K_{sp,\text{calcite}}}$$

In open ocean waters, calcium ion concentrations are substantially conservative and can be estimated from salinity.\textsuperscript{24} Because riverine concentrations of calcium differ from open ocean calcium concentrations, direct measurements of calcium concentrations by techniques such as ion chromatography or mass spectrometry must be employed for systems with a strong riverine influence.\textsuperscript{25} Carbonate ion concentrations are typically calculated using paired $\text{CO}_2$ system parameters (defined in the next section). If $\Omega<1$, the system is undersaturated, indicating that dissolution of calcium carbonate can occur and that more energy is required by organisms to
calcify. If $\Omega > 1$, the system is supersaturated, the precipitation of calcium carbonate is favored, and less of an organism’s energy budget is required for the calcification process. In view of the significance of saturation states to oceanic biogeochemical processes, many ocean acidification studies aim to determine the depths of aragonite and calcite saturation horizons (the depths of the three-dimensional surfaces where $\Omega = 1$) and, as well, changes in these surfaces with time.

Because decreased solution temperature promotes gas dissolution, enhanced CO$_2$ dissolution in polar oceans naturally lowers saturation states. At least 50% of Arctic Ocean surface waters are projected to become undersaturated with respect to aragonite when atmospheric CO$_2$ reaches 490 ppm, and the Southern Ocean is projected to become undersaturated when atmospheric CO$_2$ reaches 580 ppm.$^{21}$ Depending on CO$_2$ emission scenarios, these levels of CO$_2$ could be reached by the year 2100.$^{21}$ Although the tropical ocean is supersaturated with respect to aragonite, it will experience the greatest change in aragonite saturation state -- from 4.2 (year 1820) to 2.3 by the year 2100 (when atmospheric CO$_2$ is projected to reach 850 ppm) following the IPCC SRES A2 CO$_2$ emission scenario.$^{21,23}$ If the rate of change of saturation state is a critical factor influencing the viability of marine calcifiers, then the tropical oceans might show enhanced biological responses to ocean acidification even though the polar oceans will become undersaturated sooner.$^{22}$ Veron et al.$^{26}$ suggested that 350 ppm CO$_2$ was the tipping point for tropical coral reefs since corals are negatively affected both by ocean acidification and the thermal stress caused by global warming. While absolute tipping points are difficult to define, it is likely that the levels of CO$_2$ projected by the year 2100 will have substantial effects on the biogeochemistry of the oceans.$^{16,21,27,28}$

The biological responses to reduced saturation states (manifested as shoaling saturation horizons and exposure of organisms to reduced saturation states) are difficult to quantify. For
most calcifying species studied to date, an overall net decrease in calcification in undersaturated waters has been observed.\textsuperscript{16,29} It is important to note that organisms may become stressed not only under undersaturated conditions (i.e., $\Omega<1$), but also when the overall saturation state is decreasing.\textsuperscript{16,30} In order to design mesocosm experiments for investigation of the biological effects of reduced saturation states, it is useful to consider both the natural range of saturation states currently encountered by specific organisms and the saturation state conditions that will be encountered as a result of ocean acidification. Research expeditions that document current saturation state conditions generally occupy specific locations for a short time, thereby providing a ‘snapshot’. If a particular region is susceptible to wind-induced upwelling, saturation states determined from direct measurements of carbon system parameters might vary greatly on short time scales.\textsuperscript{31,32} With current atmospheric CO\textsubscript{2} levels close to or even exceeding estimated tipping points for marine calcifiers,\textsuperscript{21,26,27,33} it is becoming increasingly important to have high frequency documentation of the ocean’s saturation state conditions.

1.4 Marine CO\textsubscript{2} System Characterization

While saturation states must be calculated and cannot be directly measured using current technology, other important CO\textsubscript{2} system parameters can be both directly measured and interrelated (calculated) via a thermodynamic model. The latter parameters include pH, dissolved inorganic carbon (DIC), total alkalinity (TA), and CO\textsubscript{2} fugacity ($f$CO\textsubscript{2}), and carbonate ion concentrations ([CO\textsubscript{3}\textsuperscript{2-}]\textsubscript{T}). Measurements of any two of these parameters allows for calculations of others. State of the art measurement methods, as well as the thermodynamic relationships that relate them, are described in Dickson.\textsuperscript{34}
1.4.1 Dissolved Inorganic Carbon

Dissolved inorganic carbon (DIC) is the summation of the three species of carbon dioxide in seawater:

\[
\text{DIC} = [\text{CO}_2^*] + [\text{HCO}_3^-]_T + [\text{CO}_3^{2-}]_T
\]

where \([\text{CO}_2^*] = [\text{CO}_2] + [\text{H}_2\text{CO}_3].\]

DIC is measured by first converting all forms of carbon to \(\text{CO}_2\) via acidification, purging the \(\text{CO}_2\) from the sample with an inert gas (\(\text{N}_2\)), and then measuring the \(\text{CO}_2\) concentration by coulometry.\textsuperscript{34,35} Certified reference materials (CRMs) are used to assess the accuracy of the coulometer. Coulometric determination of DIC is considered as the state of the art measurement procedure and is free of matrix effects, such as high levels of organics. The desired accuracy is \(\pm 1\ \mu\text{mol kg}^{-1}\) while the practical accuracy is closer to 2-4 \(\mu\text{mol kg}^{-1}.\textsuperscript{34}

1.4.2 Total Alkalinity

Total alkalinity (TA) is the summation of proton acceptors minus proton donors.\textsuperscript{36,37}

\[
\text{TA} = [\text{HCO}_3^-]_T + 2[\text{CO}_3^{2-}]_T + [\text{B(OH)}_4^-]_T + [\text{OH}^-]_T + [\text{HPO}_4^{2-}]_T + 2[\text{PO}_4^{3-}]_T + [\text{SiO(OH)}_3^-]_T - [\text{H}^+]_T - [\text{H}_3\text{PO}_4^0] + [\text{organic bases}]
\]

Typically, TA is determined via a closed-cell or open-cell acidimetric titration.\textsuperscript{34,37,38} Spectrophotometric measurements of TA eliminate the use of problematic electrodes and offer improved measurement precision.\textsuperscript{36} Both potentiometric and spectrophotometric determinations of TA require the use of CRMs to ensure accuracy.\textsuperscript{37,39} The desired accuracy is \(\pm 2\ \mu\text{mol kg}^{-1}\) while the practical accuracy is closer to 2-4 \(\mu\text{mol kg}^{-1}.\textsuperscript{34} Coastal areas and highly productive waters can have high contributions of organic bases that contribute significantly to the organic alkalinity.\textsuperscript{40-43} As such, the use of measured TA in \(\text{CO}_2\) system calculations can significantly
reduce internal consistency with the other parameters since current CO₂ system models do not account for organic bases.

1.4.3 CO₂ fugacity

Carbon dioxide fugacity (f/CO₂) is the escaping potential of a gas in solution and is numerically closely identical to the mole fraction of CO₂ that would be observed in air that is equilibrated with the solution. The partial pressure of CO₂ (pCO₂) in the gas phase differs from the fugacity in that it does not account for the non-ideal behavior of the gas. Fugacity calculations in the gas phase account for interactions such as CO₂-CO₂ and H₂O-CO₂. Fugacity can be determined by equilibrating air with seawater and measuring the CO₂ concentration in the air with IR spectroscopy or gas chromatography in conjunction with use of standard reference gasses.

1.4.4 pH

The pH of seawater is the negative log of the concentration of hydrogen ions in solution:

\[ \text{pH}_T = -\log[H^+]_T \]  

1.9

Although considered a master variable which influences speciation and chemical stability, the pH is controlled by the TA/DIC ratio. The pH of a solution can be determined on various scales (e.g., NBS, SWS, free, or total). Spectrophotometric pH measurements are generally determined on the total hydrogen ion scale, the use of which implicitly accounts for the ion pairing of hydrogen and sulfate. The pH of seawater can be measured with glass electrodes, ion selective field effect transistors or spectrophotometrically. While potentiometric measurements involving glass electrodes often suffer from accuracy issues and drift, spectrophotometric measurements are simple, fast, precise, and calibration free. Spectrophotometric measurements employ a sulfonephthalein indicator dye (I), which changes color depending on the pH of the solution:
A derivation from first principles can be used to provide quantitative assessments of pH based on color change:\textsuperscript{46, 50-52}

\[
\text{pH}_T = -\log(K_2^T r) + \log \left( \frac{R - e_1}{1 - R e_3/e_2} \right)
\]

where \( \text{pH}_T \) is defined on the total hydrogen ion concentration scale. \( R \) is the ratio of indicator absorbances (\( A \)) measured in seawater at the wavelengths of maximum absorption.

The \( K_2^T \) in equation 1.11 describes the dissociation of the HI\(^-\) form of the dye. This transition occurs roughly over the range of typical seawater pH. Each sulfonephthalein indicator dye has a unique \( pK_2 \) which gives the dye a unique indicating range. The most commonly used indicator is meta Cresol Purple (mCP) which has a \( pK_2 = 8 \) (\( S=35, T=25^\circ C \)). This makes the indicating range of mCP \( 7.2 \leq \text{pH} \leq 8.2 \) which is well suited for surface to deep oceanic profiles. Cresol red (CR) has a slightly lower indicating range (\( pK_2 = 7.8 \) at \( S=35, T=25^\circ C \)) and is sensitive in low pH areas (e.g., the Arctic or areas of upwelling) where \( 6.8 \leq \text{pH} \leq 7.8 \).

The parameters \( e_1, e_2, \) and \( e_3 \) are ratios of molar absorptivities of the HI\(^-\) and I\(^2-\) forms of the dye at the wavelengths of maximum absorption.

\[
e_1 = \frac{z \epsilon_{H^1}^{\text{H}_2}}{z \epsilon_{H^1}^{\text{H}_2}}, e_2 = \frac{z \epsilon_{I^2}^{\text{I}_2}}{z \epsilon_{I^2}^{\text{I}_2}}, e_3 = \frac{1 \epsilon_{I^2}^{\text{I}_2}}{1 \epsilon_{H^1}^{\text{H}_2}}
\]

These parameters are determined experimentally as functions of temperature and salinity. Field measurements of pH only require \( T, S, P \) and \( R \). As such, spectrophotometric pH measurements in seawater are calibration-free.
1.4.5 Carbonate Ion

Until recently, direct measurements of carbonate ion concentrations in seawater were unattainable. Byrne and Yao\textsuperscript{53} pointed out that carbonate ion concentrations could be determined spectrophotometrically by observing lead carbonate complexation via spectrophotometric measurements in the ultraviolet. Subsequent efforts have been devoted to improvements in accuracy by relating measured carbonate ion concentrations to calculated $[\text{CO}_3^{2-}]_T$ obtained via the pH and DIC pair.\textsuperscript{54} The simple and fast nature of spectrophotometric measurements allows use of the pH and $[\text{CO}_3^{2-}]_T$ pair as a reasonable option for marine CO\textsubscript{2} system calculations when equipment resources are limited. The spectrophotometric determination of $[\text{CO}_3^{2-}]_T$ in seawater is analogous to pH measurements.\textsuperscript{53,54} Instead of using sulfonephthalein indicators, as is the case of pH, the titrant for $[\text{CO}_3^{2-}]_T$ measurements is lead perchlorate. The ratio of absorbances (R) of lead chloride and lead carbonate species are used to obtain $[\text{CO}_3^{2-}]_T$ with an algorithm similar to that used for pH measurements:

\[
-\log[\text{CO}_3^{2-}]_T = \log \left( \frac{\text{CO}_3 \beta_1}{e_2} \right) + \log \left( \frac{R - e_1}{1 - R e_1} \right)
\]

(1.13)

where,

\[
e_1 = \frac{250 \varepsilon_{\text{PbCO}_3}}{234 \varepsilon_{\text{PbCO}_3}}, e_2 = \frac{250 \varepsilon_{\text{Pb}}}{234 \varepsilon_{\text{PbCO}_3}}, e_3 = \frac{234 \varepsilon_{\text{Pb}}}{250 \varepsilon_{\text{Pb}}}
\]

(1.14)

The $\text{CO}_3\beta_1$ term is the stability constant for the formation of lead carbonate. The $e_x$ terms are ratios of molar absorptivities ($\varepsilon_x$) and are functions of salinity. These constants are also functions of temperature but the current experimental definitions are limited to 25°C.
1.5 Research Rationale

The overarching theme of this dissertation was to improve spectrophotometric CO$_2$ system measurements in seawater in order to facilitate increased understanding of ocean acidification. Measurements of pH were improved by addressing the accuracy issues that are created by the use of unpurified sulfonephthalein indicator dyes. Using purified indicators, the internal consistency of carbon system measurements on a large ocean acidification cruise was investigated. Based on this work recommendations were made for optimal calculation of carbonate saturation states. In addition, the accuracy of direct [CO$_3^{2-}$]$_T$ measurements was improved by linking the computational algorithm for spectrophotometric [CO$_3^{2-}$]$_T$ measurements to calculated [CO$_3^{2-}$]$_T$ values based on pH and DIC field data.

1.5.1 Improving Spectrophotometric pH Measurements in Seawater

Yao et al.\textsuperscript{55} pointed out that impurities in the indicator dyes used for spectrophotometric pH measurements could introduce systematic errors in pH calculations, especially at high pH. In 2011, I quantified the errors that are created by use of indicators obtained from various mCP manufacturers. This work revealed that errors as large as 0.018 pH units could be encountered by using impure mCP at pH=8.2, Figure 1.2. This work was published in Liu et al.\textsuperscript{51}

The original method for purifying mCP\textsuperscript{51} produced small amounts of product and required the use of large amounts of hazardous solvents over the period of a day to obtain only 0.02 g of purified indicator. Even though Liu et al.\textsuperscript{51} characterized purified mCP, creating the basis for substantial improvements in pH measurements on a global basis, the small quantities of purified product obtained by HPLC greatly limited the use of mCP for use by oceanographic research groups other than our own. As such, I set out to purify mCP and CR on a scale that would allow widespread use by the oceanographic research community. In addition, since
purified CR had not previously been utilized for seawater pH measurements, characterization of the physical-chemical properties of purified CR was undertaken as an important extension of the purification work.

![Graph](image)

**Figure 1.2.** Comparison of pH values obtained using mCP from seven different vendors in strongly buffered solutions. pH Difference = pH(purified sample) − pH(reference), where pH(reference) is purified Acros mCP.

### 1.5.2 Internal Consistency of Marine CO₂ System Measurements

With the improved accuracy of pH measurements created by purified indicators, as a natural next step I undertook examination of the internal consistency of CO₂ system measurements (e.g., pH, DIC, TA, and fCO₂) that were obtained on large seagoing expeditions. In 2011 I participated in the West Coast Ocean Acidification Cruise (W1108C) and in 2012 I participated in the Gulf of Mexico East Coast Carbon cruise (GOMECC-2).
1.5.3 Improving Spectrophotometric $[\text{CO}_3^{2-}]_T$ Measurements in Seawater

Easley et al.\textsuperscript{54} demonstrated that minor changes in the $[\text{CO}_3^{2-}]_T$ computational algorithms could improve the correspondence between spectrophotometrically measured $[\text{CO}_3^{2-}]_T$ and $[\text{CO}_3^{2-}]_T$ calculated from pH and DIC. This work was performed using lead chloride (PbCl\textsubscript{2}) as the titrant in the Pacific and Arctic oceans. It was subsequently discovered that measurement precision could be improved by doubling the concentration of lead added to the sample. This was accomplished by using lead perchlorate (PbClO\textsubscript{4}) as the titrant since it has improved solubility characteristics relative to lead chloride. The improved measurement method was utilized on the GOMECC-2 cruise. Through this work I found that measurements with the increased lead concentration necessitated a correction for the perturbation caused by formation of lead carbonate in seawater. Whereas the work of Easley et al.\textsuperscript{54} involved empirical alteration of the experimentally well-defined $e_3/e_2$ parameter that is obtained in acidified seawater (Eqs. 1.13 and 1.14), using the GOMECC-2 data set, I was able to modify the algorithm for $[\text{CO}_3^{2-}]_T$ computation in a manner that did not create an inconsistency between the measurement algorithm and direct experimental measurements of $e_3/e_2$.

1.6 Overview of Dissertation

Some chapters presented in this dissertation have been published in or submitted to peer-reviewed journals. Each published or submitted manuscript is presented as an individual chapter with embedded figures and tables. References for each chapter are displayed at the end of the chapter.

- Chapter two introduces methodology for bulk-scale purification of two indicators that are routinely used for spectrophotometric pH measurements in seawater. Use of purified
indicator ensures globally uniform and accurate pH measurements. This work has been published in *Marine Chemistry*. \(^{56}\)

- Chapter three details the physical-chemical characterization of purified cresol red for use in spectrophotometric pH measurements in seawater. This work has been published in *Marine Chemistry*. \(^{52}\)

- Chapter four examines CO\(_2\) system internal consistency of two large ocean acidification cruise datasets and shows how choices of parameter pairs used can influence saturation state calculations. This work has been submitted for publication.

- Chapter five introduces updated algorithms for direct spectrophotometric measurements of carbonate ion concentrations in seawater based on a large cruise data set in the Gulf of Mexico and east coast US waters. This work will be submitted for publication.

- Chapter six outlines future studies.

1.7 *References*


33. Intergovernmental Panel on Climate Change, Climate change 2007: the physical science basis. Contribution of working group 1 to the fourth assessment report of the Intergovernmental Panel on Climate Change (eds Solomon, S. et al.) (Cambridge University Press, **2007**).


CHAPTER TWO:

PURIFICATION OF META CRESOL PURPLE AND CRESOL RED
BY FLASH CHROMATOGRAPHY: PROCEDURES FOR ENSURING ACCURATE SPECTROPHOTOMETRIC pH MEASUREMENTS

Note to Reader

This chapter has been published in full,¹ and is included with the permission of the publisher.

2.1 Abstract

Impurities in sulphonephthalein indicator salts can result in significant errors in seawater pH determinations. To ensure suitable measurement accuracy and intercomparability on a global basis, impurities must be removed from all indicators used for oceanographic CO₂ system analyses. Previous work has described an effective HPLC (high-performance liquid chromatography) procedure for purification of meta cresol purple, but the technique is labor-intensive, with each HPLC run producing only a small batch of purified indicator. This work describes the use of flash chromatography to more efficiently produce large batches of purified meta cresol purple (mCP) and cresol red (CR), the preferred indicators for direct water column determinations of seawater pH.
Several batches of unrefined mCP and CR of independent origin were prepared by flash chromatography. Indicator purity was then assessed in two ways: by (a) HPLC verification and (b) pH measurements of highly buffered solutions. HPLC chromatograms of the various flash-prepared mCPs indicated that the process did not always result in a completely pure product. In terms of performance, however—i.e., pH measurements of highly buffered solutions—no differences were observed between an HPLC-purified reference mCP and the flash-purified mCPs. HPLC examination of the flash-purified CRs indicated that every product was free of detectable impurities. No differences were seen in comparative pH measurements made with the purified CRs. The flash chromatography procedures outlined in this work are suitable for producing bulk quantities of mCP and CR for use in high-precision spectrophotometric pH measurements in seawater.

2.2 Introduction

High-precision carbon system measurements are required to document the changes in seawater chemistry that accompany the oceanic uptake of anthropogenic atmospheric CO$_2$. Prior work has shown that spectrophotometric measurements of pH are simple, fast, and precise. However, the accuracy of spectrophotometric pH measurements can be adversely affected by impurities in the sulfonephthalein indicator dyes that are used for such measurements.

The indicator meta cresol purple (mCP) is currently well suited for water column measurements of seawater pH in most ocean areas, but as the upper ocean continues to acidify an indicator with an indicating range slightly lower than that of mCP will be required. In this
case, and in regions where the seawater pH is already low (e.g., the Arctic Ocean or Southern Ocean), cresol red (CR), which has a pK lower than that of mCP, is a suitable choice.

The use of unrefined mCP for spectrophotometric measurements can result in systematic errors as large as 0.018 at typical surface ocean pH values. Errors due to the use of unrefined CR have not been quantified, but offsets of similarly large magnitude could reasonably be expected. Purification is therefore recommended for both indicators.

A method for purifying mCP by high-performance liquid chromatography (HPLC) has been previously established, but the method lacks large-scale production capability. The present work focuses on developing methods for producing large batches of purified mCP and CR for use in high-precision spectrophotometric seawater pH measurements.

2.3 Methods

2.3.1 Reagents

Sodium salts of mCP and CR of independent origin were used for the purification study. The mCP salts were from Acros (Lot# A0182569), Aldrich (Lot# 07005HH), and Ricca (Lot# 4003124). The CR salts were from Acros (Lot# A0255180), Alfa Aesar (Lot# L09754), Biosynth (Lot# 220307/11), MP Bio (Lot# 2045F), and Ricca (Lot# 2011271). Sodium chloride, TRIS (tris(hydroxymethyl)-aminomethane), EPPS (3-[4-(2-hydroxyethyl)-piperazin-1-yl]propane-1-sulfonic acid), MOPS (3-morpholinopropane-1-sulfonic acid), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), trifluoroacetic acid (TFA), and acetonitrile (MeCN, HPLC grade) were obtained from Fisher Scientific and were used without further purification.

A series of highly buffered solutions was prepared by adding 0.08 moles of TRIS, EPPS, MOPS, or HEPES to 0.04 moles of either HCl (TRIS, HEPES) or NaOH (EPPS, MOPS). The solutions were brought to 0.7 mol (kg-H₂O)⁻¹ ionic strength by the addition of NaCl.
2.3.2 Determination of the Effects of CR Impurities on Seawater pH Measurements

Each of the five unrefined (i.e., off-the-shelf) cresol reds was used to independently measure the pH of the highly buffered solutions described in section 2.3.1. For each pH measurement, the buffer solution was weighed (~102.3 g) into a custom-made quartz wide-top 10 cm pathlength spectrophotometric cell (NSG Precision Cells, Inc.). The cell was fitted with a motor-driven stirrer and a lid with a built-in space for a digital thermometer probe. Absorbance measurements were made using a Varian Cary 400 UV-Vis spectrophotometer fitted with a water-jacketed cell holder connected to a recirculating water bath. The solution temperature was maintained at 25.00 ±0.03 °C and monitored with a VWR digital thermometer (accuracy ±0.01 °C).

After a blank spectrum was obtained, indicator (25 µL of 10 mM stock solution) was added to the cell. Absorbance was recorded for six replicate scans, which were subsequently averaged. Triplicate measurements were made at each pH point for each indicator batch. Solution pH values were calculated following published protocols for CR.\textsuperscript{11}

2.3.3 Development of the Flash Purification Procedure

For chromatographic purification, it is generally useful to consult the literature for guidance in selecting a column and a mobile phase appropriate to the molecular weight, solubility, and hydrophobic character of the analyte.\textsuperscript{12} However, no protocols have been published for flash purification of mCP or CR. Empirical determination of an appropriate procedure was therefore one of the first steps of this work. The solvent system consisted of water, MeCN, and trifluoroacetic acid (TFA). Investigative separations were first conducted using HPLC under isocratic (constant concentration) conditions. The organic phase concentration was determined by trial and error, first starting at 30% MeCN with 0.05% TFA.
and then increasing by 5% MeCN increments until the organic phase concentration was 80% MeCN. For MeCN concentrations equal to or greater than 80%, no separation of impurities was achieved. The optimal concentration of the TFA mobile phase modifier was determined by incrementally increasing the concentration to attain a general understanding of how the addition of TFA affected the main peak retention time and separation of related impurities.

For the flash chromatographic procedure, a gradient mobile phase profile was used. For mCP, solvent $A_{mCP}$ was water and 0.05% trifluoroacetic acid (TFA), and solvent $B_{mCP}$ was acetonitrile (MeCN) and 0.05% TFA. For CR, solvent $A_{CR}$ was water and 0.2% TFA, and solvent $B_{CR}$ was MeCN and 0.2% TFA. For both indicators, the gradient profile was determined by manually increasing the percentage of solvent B until an impurity began to elute from the top of the column. The percentage of solvent B was then held constant until the impurity was entirely eluted from the column. The process was repeated for all impurities and the pure fraction. After a general gradient was determined, the gradient was optimized to increase efficiency. Table 2.1 summarizes the gradient steps for mCP and CR. This optimized procedure was used for all subsequent work.

**2.3.4 Flash Purification of meta Cresol Purple and Cresol Red**

Batches of mCP and CR were purified using a Teledyne ISCO Combiflash Rf-200 UV-VIS automated flash chromatography system. This system includes a touch screen controller capable of controlling gradients with up to four solvents, two positive displacement pumps (5–200 mL min$^{-1}$), an internal fraction collector, a solvent waste management system, and a UV-VIS detector.

The flash chromatography column was a 150 g reversed phase Teledyne ISCO RediSep Gold C18Aq with an average particle size of 20–40 microns. This column prevents C18 chain
collapse in highly aqueous conditions and was specifically designed for separation of water-soluble dyes. For storage periods longer than a few hours, a solution of 80% MeCN and 20% water was pumped through the column for 4-6 column volumes; the column was then removed from the system, capped, and stored.

Table 2.1 Gradient separation procedure for meta cresol purple and cresol red.

<table>
<thead>
<tr>
<th>mCP</th>
<th>Time (min)</th>
<th>% Solvent B&lt;sub&gt;mCP&lt;/sub&gt;</th>
<th>Time (min)</th>
<th>% Solvent B&lt;sub&gt;CR&lt;/sub&gt;</th>
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<td></td>
<td>0 - 4</td>
<td>5</td>
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<td>4 - 5</td>
<td>5 - 10</td>
<td>5 - 18</td>
<td>21</td>
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<tr>
<td></td>
<td>5 - 8</td>
<td>10</td>
<td>18 - 21</td>
<td>100</td>
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<td>8 - 10</td>
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<tr>
<td></td>
<td>18 - 21</td>
<td>5</td>
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</tr>
</tbody>
</table>

* Solvent A<sub>mCP</sub> was water and 0.05% TFA, and solvent B<sub>mCP</sub> was MeCN and 0.05% TFA.

† Solvent A<sub>CR</sub> was water and 0.2% TFA, and solvent B<sub>CR</sub> was MeCN and 0.2% TFA.

Multiple stock solutions of unrefined mCP and CR were prepared by dissolving the sodium salts in MilliQ water (70 mM). The flash column was removed from the Combiflash system, 25 mL of stock solution was injected into the top of the column from a plastic syringe, and the column was returned to the system. The purification procedure then proceeded according to Table 2.1. (A detailed description of the results of our methods development is given in section 2.4.2.) The purified solid (acid forms) of the indicators were obtained by vacuum rotary evaporation of the eluate (indicator in a solution of water, TFA, and MeCN) using a Buchi Rotavapor-R. The evaporation flask was partially submerged in a 40 °C waterbath. One batch of unrefined indicator (MP Bio Lot #2054F) was excluded from purification because the salt had
formed a white precipitate in solution, and when the solution was injected into the flash column, the column became clogged and over-pressurized.

Finally, stock solutions of purified mCP and CR were prepared for use in spectrophotometric pH analyses. The acid form of each indicator batch was dissolved by sonication in MilliQ water and 1N NaOH. The final concentration of each stock solution was 10 mM, and the pH was adjusted to approximately pH=7.9 by adding 1N NaOH or 1N HCl as necessary.

2.3.5 HPLC Verification of mCP and CR Purity

For all of the flash-purified products, HPLC chromatograms were examined for the presence of peaks other than pure indicator. The mCPs were analyzed spectrophotometrically using the HPLC setup and mobile phase outlined by Liu et al. For CR, 20 µL of a 10 mM stock solution made from flash-purified product was injected into the HPLC. The procedure then followed Liu et al. except that the mobile phase modifier concentration was 0.20% TFA (rather than 0.05% TFA as for mCP).

2.3.6 Comparative Measurements of pH in Highly Buffered 0.7 m NaCl Solutions

The performance of the three mCP batches purified by flash chromatography were compared to the HPLC-purified mCP used by Liu et al. Each indicator was used to independently measure the pH of the TRIS, EPPS, and HEPES buffer solutions according to the procedure outlined in section 2.2. Solution pH values were calculated following published protocols for mCP. The performance of the four CR batches purified by flash chromatography was also assessed by comparing independent measurements of pH in the highly buffered solutions of TRIS, EPPS, and MOPS. Solution pH values were calculated following published protocols for CR.
2.4 Results and Discussion

2.4.1 Effects of Cresol Red Impurities on High-Precision pH Measurements

Previous work has documented the necessity to purify unrefined meta cresol purple and thymol blue if the application is to be high-precision measurements of seawater pH\(^8,10\). Our work confirms that unrefined cresol red must also be purified before use for this application.

Figure 2.1 shows a chromatogram of an unrefined cresol red. The large peak at approximately \( t = 11 \) min represents pure CR. The smaller peaks represent other materials (impurities) in the indicator salt. Chromatograms of the other four unrefined CRs similarly document the presence of impurities, though with fewer peaks or peaks of smaller magnitude than those shown in Figure 2.1.

![Figure 2.1. HPLC chromatogram of unrefined cresol red.](image)

The mere presence of impurities does not necessarily translate to compromised performance for a given application. To assess whether the impurities evident on the HPLC chromatograms would affect indicator performance for seawater pH measurements, the five
unrefined cresol reds were used to measure pH in a series of highly buffered solutions with an ionic strength similar to that of seawater (Figure 2.2). Accuracy of the spectrophotometric method is approximately 0.001 pH units, but Figure 2.2 shows that differences of >0.1 in reported pH can result if unrefined salts are used. As was seen for mCP, the effects of impurities on pH errors are greatest at higher pH. To obtain accurate spectrophotometric seawater pH measurements, only purified CR should be used.

![Figure 2.2](image)

**Figure 2.2.** Comparison of pH measurements made using unrefined CR.

### 2.4.2 Procedure for Flash Purification of meta Cresol Purple and Cresol Red

The optimized procedures developed for purification of mCP and CR by flash chromatography utilizes a gradient mobile phase profile. Table 2.1 summarizes the gradient steps for the two indicators. For mCP, solvent $A_{mCP}$ was water and 0.05% trifluoroacetic acid (TFA), and solvent $B_{mCP}$ was acetonitrile (MeCN) and 0.05% TFA. Figure 2.3a outlines the gradient profile. At 30% Solvent $B_{mCP}$, the pure mCP fraction was collected with a retention time of 11.0 minutes. For CR purification, the mobile phase was the same as for mCP except that a higher
concentration was used for the TFA mobile phase modifier. Solvent $A_{CR}$ was water and 0.2% TFA, and Solvent $B_{CR}$ was MeCN and 0.2% TFA. Figure 2.3b outlines the gradient profile for purification of CR. At 23% Solvent $B_{CR}$, the pure CR fraction was collected at a retention time of 14.7 minutes. For all indicator batches, after the pure fraction eluted, the column was washed with 100% Solvent B for 4–6 column volumes and then equilibrated to 5% Solvent B for four column volumes for subsequent purifications.

![Figure 2.3](image)

Figure 2.3. (a) mCP gradient separation profile. (b) CR gradient separation profile.

Each gradient step in Figure 2.3a allows for the distinct separation of impurities from the pure indicator fraction. Because the flash column housing is clear, the separations can be easily visualized. Figure 2.4 shows a representative example of the elution of mCP and impurities originally present in the unrefined salt. In Figure 2.4a, the three distinct impurity bands (yellow,
orange, and red) in the middle of the column were eluted between 4 and 10 minutes. The bright red to dark red band at the top of the column is the pure fraction of mCP. Once the impurities were distinctly separated from the pure mCP on the column, the organic phase percentage was increased to 30% solvent B_{mCP}, and the pure mCP was collected at 10–14 minutes. Figure 2.4b shows the pure mCP band about to elute from the column bottom, with impurities above. After the pure mCP was collected, the remaining four impurities seen in Figure 2.4c were washed out of the column with 100% solvent B_{mCP}. The visual appearance of the CR purification sequence is similar to that of mCP.

Depending on the purity of the starting material, each run of 25 mL of 70 mM unrefined stock solution resulted in approximately 0.7 g of indicator loaded per injection. Each purification batch took approximately 30 minutes to complete.

Figure 2.4. Separation of mCP and seven impurities as seen through a clear flash chromatography column. Mobile phase flow is top to bottom. (a) Initial separation of three impurities (yellow, orange, red) at mid-column, with pure mCP fraction on top. (b) Pure mCP at the bottom, one visible yellow impurity in the middle, and a mix of impurities at the top. (c) The yellow impurity of photo (b) at the bottom; the mix of photo (b) impurities, now separated into purple, orange, and yellow impurities, toward the top.
2.4.3 HPLC Verification of mCP and CR Purity

To assess whether flash chromatography could achieve results comparable to HPLC purification, we compared HPLC chromatograms of our flash-purified mCPs to a chromatogram of the Liu et al.\textsuperscript{13} HPLC-purified mCP. Figure 2.5a shows the chromatogram for the HPLC-purified reference product, and Figure 2.5b shows the corresponding flash-purified product derived from the same starting batch of off-the-shelf mCP. The two chromatograms show no differences. For the other two indicator batches, however, differences were observed between the HPLC-purified reference (Figure 2.5a) and the flash-purified dyes. Figure 2.5c shows, for example, a small impurity peak that eluted at approximately 32 minutes. No indication of other impurities is evident.

For cresol red, the four different batches of flash-purified CR produced identical HPLC chromatograms with no impurity peaks (e.g., Figure 2.6). Flash purification was adequate to achieve a pure product.

2.4.4 Comparative Measurements of pH in Highly Buffered 0.7 m NaCl Solutions

The performance of the indicators for pH measurements is more important than HPLC verifications of their purity. For seawater pH measurements, for example, the presence of an impurity (e.g., Figure 2.5c) does not necessarily imply inadequate performance. We therefore assessed indicator adequacy by comparing pH measurements made with each purified indicator in a range of highly buffered solutions at an ionic strength equal to that of seawater. If the pH values measured by each indicator (HPLC- or flash-purified) are identical within the measurement precision, then for the purposes of measuring seawater pH, the products are operationally the same.
Figure 2.5. (a) HPLC-purified mCP (Ricca Lot# 4003124). (b) Flash chromatography–purified mCP from the same starting batch (Ricca Lot# 4003124). (c) Flash chromatography–purified mCP from a different starting batch (Aldrich Lot# 07005HH). (The view in (c) has been zoomed in to show the impurity at $t = 32\text{ min.}$)
We used the mCP that had been purified by flash chromatography to measure spectrophotometric pH values in highly buffered 0.7 m NaCl solutions over the typical ranges of absorbance ratios and solution pH values encountered in ocean waters (Figure 2.7). The pH values obtained using the HPLC-purified mCP of Liu et al.\textsuperscript{13} served as reference values: $\Delta p\text{H} = p\text{H}_{\text{HPLC}} - p\text{H}_{\text{Flash}}$. Over the pH range of 7.4–8.2, all pH differences were within ±0.0004 of the reference pH. As such, all observed pH differences were within the normal analytical precision of spectrophotometric seawater pH measurements.\textsuperscript{6} The flash chromatography purification procedure resulted in mCP that performs as well as HPLC-purified mCP in seawater pH measurements.

Similar comparisons were made for the flash-purified cresol reds (Figure 2.8). In this case, one of the CR products was arbitrarily chosen to serve as a reference for the difference calculations. (The choice was arbitrary because the purified CR products were all chromatographically identical.) The use of unrefined CR to measure seawater pH had produced apparent pH differences as large as 0.1 (Figure 2.2). For the subset of salts that underwent flash separation, the purification process diminished the maximum reported pH difference from ~0.03
(unrefined) to ~0.0004 (flash-purified). In other words, subsequent to flash purification, the CR produced pH measurements that fell within the expected analytical precision of the method (±0.0004). These results are consistent with the chromatographic verifications in indicating that flash purification is adequate to achieve a pure cresol red indicator.

Figure 2.7. Comparison of pH values obtained by HPLC and flash-purified mCP. All pH differences are defined relative to the HPLC-purified mCP (Ricca Lot #4003124).

Figure 2.8. Comparisons of pH measurements made using flash-purified CR. All pH differences are defined relative to an arbitrarily chosen flash-purified reference indicator, Ricca Lot #2011271.
2.5 Summary and Future Work

Because of the potentially large pH inaccuracies introduced by dye impurities, it is imperative that investigators utilize purified indicators for all oceanic CO$_2$ system characterizations. The flash chromatography procedures outlined in this work provide a method for purifying large batches of mCP and CR appropriate for use in high-precision applications.

Compared to HPLC purification of pH indicators, flash chromatography provides a number of advantages. Flash chromatography requires lower quantities of hazardous solvent and provides much higher loading capacities. In terms of rate of production of purified mCP, use of the flash chromatographic methods and column described in this work offers a 10-fold improvement over the HPLC methods and column of Liu et al.$^{13}$ The flash instrument described in this work can be fully automated and is designed for large-scale production with available column sizes as large as 415 g.

Purified sulphonephthalein indicators are required not only for modern seawater pH measurements but also for correction of historical measurements made using off-the-shelf indicator salts. The procedure for retrospective correction is outlined in Liu et al.$^{13}$ For all spectrophotometric CO$_2$ system analyses, it is recommended to keep records of vendor and lot numbers of the indicator dyes, as well as data records of absorbance ratios ($R$ ratios), measurement temperatures and pressures, and sample salinities. With this basic suite of information, historical measurements of the $R$ values can be used to recalculate pH should refinements in the sulphonephthalein indicator equations be developed.

At this point, absorbance characteristics for purified mCP have been described in terms of their dependence on sample salinity ($20 \leq S \leq 40$) and temperature ($278.15 \leq T \leq 308.15$) at a pressure of 1 atm.$^{10}$ When pressure effects have been fully characterized (Soli and Byrne, in
review), in situ pH values can also be calculated. For CR, the equation used to convert absorbance ratios to seawater pH\textsuperscript{11} is only provisional, as the underlying experimental work relied on an unpurified indicator salt. Future work (currently underway) will provide the physical–chemical characteristics of purified cresol red for seawater pH measurements, as well as the characteristics of other sulfonephthalein indicators used routinely in CO\textsubscript{2} system characterizations (e.g., bromocresol purple and bromocresol green for total alkalinity,\textsuperscript{14,15} bromocresol purple for dissolved inorganic carbon,\textsuperscript{16} and phenol red for CO\textsubscript{2} fugacity).\textsuperscript{17}

Researchers interested in comparing their own purification products with mCP or CR purified according to this work are invited to contact the corresponding author.

2.6 Acknowledgement

This work was supported by NSF Award OCE-0727082. Support for M. Patsavas was provided by the C.W. Bill Young Fellowship and the Paul L. Getting Endowed Memorial Fellowship of the USF College of Marine Science. The advice and insightful comments from Dr. Tonya Clayton are greatly appreciated.

2.7 References


CHAPTER THREE:

PHYSICAL-CHEMICAL CHARACTERIZATION OF PURIFIED CRESOL RED
FOR SPECTROPHOTOMETRIC pH MEASUREMENTS IN SEAWATER

Note to Reader

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3.1 Abstract

The use of impure cresol red in spectrophotometric seawater pH measurements can introduce systematic inaccuracies greater than 0.1. Cresol red has been purified on a bulk scale to address this problem, but a characterization of the dye’s physical–chemical properties has not been provided to date. This work reports the physical–chemical characteristics of purified cresol red for use in spectrophotometric seawater pH measurements over a range of temperatures and salinities. Seawater pH is expressed on the total hydrogen ion concentration scale (pHₜ) in terms of the ratio (R) of cresol red absorbances (A) at 433 and 573 nm (Rᵣᵣ = 573A/433A):

\[
pHₜ = -\log(K₂ e₂) + \log \left( \frac{R - e₁}{1 - R e₂} \right)
\]
where \(- \log(K_2 e_2) = a + b / T + c \ln T - d T\)

\[ a = -859.326051 + 0.146165S + 7.81164 \times 10^{-4} S^2 \]
\[ b = 22969.9366 + 8.04468S - 0.20512S^2 \]
\[ c = 152.209523 - 0.0317821S \]
\[ d = 0.259915 \]

and cresol red molar absorptivity ratios are expressed as:

\[ e_1 = -0.00413 + 1.814 \times 10^{-5}T \]
\[ e_3/e_2 = -0.021683 + 1.8107 \times 10^{-4} T + 3.163 \times 10^{-5} (S - 35) \]

for 278.15 \leq T \leq 308.15 K and 20 \leq S \leq 40.

We recommend using cresol red to measure the acidity of seawater that has (at 298.15 K) a pH\(_T\) of 6.8–7.8. This range might be encountered in ocean areas such as oxygen minimum zones or, hydrothermal vent fields, or it might be imposed in controlled laboratory studies. Ocean acidification will make cresol red an increasingly important indicator in coming decades as waters within ever larger ocean areas shift into its optimal indicating range.

### 3.2 Introduction

Absorption of anthropogenic atmospheric CO\(_2\) into the upper ocean lowers seawater pH and exerts a profound effect on ocean biogeochemistry. This uptake influences the entire carbon system of the earth.\(^2\)\(^,\)\(^3\) Accurate and precise measurement of ocean acidification is essential for documenting the extent of changing oceanic chemistry and its implications.

The ocean CO\(_2\) system can be fully characterized using two of four commonly measured parameters: total alkalinity, total carbon, pH, and CO\(_2\) fugacity.\(^4\) Although only two parameters are required for characterization, it is best practice to measure as many as possible to ensure internal consistency. Because spectrophotometric pH measurements are simple, fast, and
precise, \textsuperscript{5-8} spectrophotometric measurements of seawater pH have become routine and are often one of the two preferred directly measured variables when measurement redundancy is impractical.\textsuperscript{9,10} The most common sulfonephthalein indicators used for water column measurements of pH are thymol blue for measurements near the surface,\textsuperscript{11} meta-cresol purple (mCP) for surface to deep profiles,\textsuperscript{12} and cresol red for low-pH areas (e.g., upwelling waters, porewaters, waters influenced by hydrothermal vents, cold surface waters, and oxygen minimum zones).\textsuperscript{13}

Prior work has shown that impurities in indicator salts can result in systematic pH errors.\textsuperscript{14} To date, only meta cresol purple has been purified and characterized.\textsuperscript{12} The original characterization of cresol red (CR) for seawater pH measurement was based on the use of unrefined CR salts and was limited to $T = 298.15 \text{ K}$.\textsuperscript{13} This work describes the physical–chemical characteristics of purified cresol red for use in measurements of seawater pH over a range of temperatures and salinities.

\textbf{3.2.1 Analytical Background}

Sulfonephthalein indicators (I) exist in three protonation states:

\[
\begin{align*}
H_2I & \rightleftharpoons K_{I_1} HI \rightleftharpoons K_{I_2} I^2- \\
\end{align*}
\]

where $K_i$ is the dissociation constant of the indicator. For any indicator (e.g., cresol red, meta cresol purple, thymol blue), the pH indicating range is generally between $pH \approx pK_2$ and $pH \approx pK_2 - 1$, and is dependent on indicator molar absorbance characteristics. For CR at 298.15 K, $pK_2 \approx 7.8$, and this indicator is most appropriate for measurements in the range $6.8 \leq pH_T \leq 7.8$.

The $H_2I$ form of CR is brilliant red, the $HI^-$ form is a bright yellow, and the $I^2-$ form is a rich purple. The $HI^- \leftrightarrow I^2-$ equilibrium in seawater therefore produces a magenta-to-orange color
change as pH decreases from 7.8 to 6.8. The pH of a solution containing the indicator can be calculated from a quantitative assessment of color according to the following relationship:

$$\text{pH}_T = -\log(K_2 e_2) + \log \left( \frac{R - e_1}{1 - R \frac{e_1}{e_2}} \right)$$

where \(\text{pH}_T\) is defined on the total hydrogen ion concentration scale (i.e., \(\text{pH}_T = -\log([H^+]_T)\)), with \([H^+]_T\) being the total hydrogen ion concentration expressed in moles/kg seawater. \(R\) is the ratio of indicator absorbances \((A)\) measured in seawater at the wavelengths of maximum absorption.

For cresol red, \(R = R_{\text{CR}} = \frac{A_{573}}{A_{433}}\), the ratio of absorbances at 573 nm and 433 nm (Figure 3.1).

![Absorbance spectra of cresol red at pH = 0.0, pH = 4.5, and pH = 12.](image)

Figure 3.1 Absorbance spectra of cresol red at pH = 0.0, pH = 4.5, and pH = 12.

The other terms on the right side of Equation 3.2 are salinity- and temperature-dependent physical–chemical characteristics of cresol red. Determination of solution pH therefore requires measurement of the absorbance ratio \((R_{\text{CR}})\), sample salinity \((S)\), and sample temperature \((T)\).
After indicator calibration (e.g., this work), the seawater pH method is “calibration-free” in the field.

\[ K_2^T \] in Equation 3.2 describes the dissociation of the HI\(^-\) form of the dye on the total hydrogen ion concentration scale:

\[
K_2^T = \frac{[I^2^-][H^+]_T}{[HI^-]} \tag{3.3}
\]

The parameters \(e_1\), \(e_2\), and \(e_3\) are ratios of molar absorptivities of the HI\(^-\) and I\(^2^-\) forms of the dye at the wavelengths of maximum absorption.

\[
e_1 = \frac{573 \varepsilon_{HI^-}}{433 \varepsilon_{HI^-}}, e_2 = \frac{573 \varepsilon_{I^2^-}}{433 \varepsilon_{HI^-}}, e_3 = \frac{433 \varepsilon_{I^2^-}}{433 \varepsilon_{HI^-}} \tag{3.4}
\]

The magnitude of \(e_3/e_2\) is determined as a function of temperature and salinity at pH = 12, where the I\(^2^-\) form is highly dominant. The magnitude of \(e_1\) can be determined as a function of temperature at pH = 4.5, where HI\(^-\) is dominant. At this pH, absorbance contributions from the H\(_2\)I and I\(^2^-\) forms of the dye must also be taken into account. The \(-\log(K_2^T e_2)\) term can be determined by using (a) meta cresol purple to precisely determine the pH of tris-buffered synthetic seawater, in conjunction with (b) measurements of cresol red absorbance ratios (\(R_{CR}\)) for the same synthetic seawater samples. \(K_2\) and \(e_1\) values are then iteratively refined.

3.3 Methods

3.3.1 Reagents

Cresol red (CR) sodium salt (Acros Lot# A0255180) and meta cresol purple (mCP) sodium salt (Ricca Lot# 4003124) were purified by flash chromatography.\(^{16}\) Acetonitrile (HPLC grade) and trifluoroacetic acid were obtained from Fisher Scientific. Stock solutions (10 mM) of purified CR or mCP were prepared by dissolving the purified indicator (acid form) in 0.014 M NaOH. All solutions were composed using Milli-Q water. The pH of each dye stock solution was
adjusted to pH = 7.8 by additions of 0.1 N HCl or 0.1 N NaOH. High-purity sodium chloride, potassium chloride, and sodium sulfate salts were obtained from Sigma-Aldrich. Tris acidimetric SRM 723e (tris(hydroxymethyl)aminomethane) was obtained from the National Institute of Standards and Technology (NIST). The salts (tris, NaCl, KCl, Na₂SO₄) were oven-dried and then stored in a desiccator with phosphorus(V) oxide to maintain dryness. Magnesium chloride hexahydrate and calcium chloride dihydrate were obtained from Fisher Scientific. Solutions of MgCl₂ and CaCl₂ (~1 M) were prepared, and the exact concentrations were determined by ICP-MS. Hydrochloric acid (Fisher Scientific) concentrations were determined by spectrophotometric titration with phenol red.

### 3.3.2 Experimental Setup

Absorbance measurements were made using a Cary 400 Bio UV-Vis spectrophotometer fitted with a sample cell holder that was attached to a recirculating waterbath (Lauda or Neslab). Wavelength accuracy of the Cary 400 was verified using NIST SRM 2034 holmium oxide, and linearity was verified with NIST SRM 930D glass filters. The custom-made sample cell (NSG Precision, Inc.) was a quartz-window, 10 cm pathlength open-top cell with an acrylic lid. A motor-driven stirrer and a digital temperature probe (VWR, accuracy ±0.01 °C) were inserted through the lid. Depending on sample temperatures and local humidity, dry nitrogen gas was passed over the quartz cell windows to prevent condensation. Baseline sample solution (i.e., sample solution with no added indicator) was equilibrated to the desired temperature, and a blank absorption spectrum was obtained. Indicator was then added (20 µL of 10 mM CR or 30 µL of 10 mM mCP, for a final concentration of 2 or 3 µM), and an absorbance spectrum of the colored, well-mixed sample was obtained. For all pH measurements, absorbances were recorded at six or more wavelengths: the H₂I, HI⁻, and I⁺⁻ absorbance maxima; the H₂I/HI⁻ and HI⁻/I⁺⁻ isosbestic
wavelengths; and a non-absorbing wavelength. Absorbance at the non-absorbing wavelength was measured to confirm that the sample cell did not shift in the cell holder during the experiments. Wavelength resolution was 0.1 nm.

### 3.3.3 Determination of Wavelengths of Interest

Isosbestic wavelengths were determined as a function of temperature by titrating 0.7 M NaCl solutions with HCl at high pH (pH near 8) to obtain the HI/I\(^{2-}\) isosbestic point; low-pH solutions (pH near 2) were titrated to obtain the H\(_2\)I/HI\(^-\) isosbestic point. The amounts of added HCl were determined gravimetrically, and absorbance measurements were corrected for dilution.

### 3.3.4 Determination of \(e_3/e_2\) as a Function of Temperature and Salinity

The \(e_3/e_2\) term in Equation 3.2 was obtained by determining the molar absorptivity ratio \(\frac{\epsilon_{433}}{\epsilon_{573}}\) of CR at pH = 12, where the I\(^2-\) form of the dye is highly dominant. In seawater of this pH, precipitation of magnesium and sulfate salts occurs. Therefore, a modified synthetic seawater (i.e., a solution containing salts of NaCl, KCl, and CaCl\(_2\)) was prepared wherein MgCl\(_2\) was replaced with CaCl\(_2\), and Na\(_2\)SO\(_4\) was replaced with NaCl. Sodium hydroxide (0.01 m) was added to the modified synthetic seawater to raise the pH to 12. Absorbance measurements were made over a range of salinities (20 \(\leq S \leq 40\)) and temperatures (278.20 \(\leq T \leq 308.22\)).

### 3.3.5 Determination of \(-\log(K_2^T e_2)\) as a Function of Temperature and Salinity

Combining the \(e_2\) term with the \(K_2^T\) term produces an equation (i.e., Equation 3.2) with fewer measured parameters\(^{12}\) and obviates the need for direct determinations of \(e_2\). To determine the \(-\log(K_2^T e_2)\) term of Eq. (2), sample solutions were characterized using paired mCP and CR absorbance measurements over a range of temperatures and salinities. For each sample, solution pH was first determined using mCP absorbance ratios (\(R_{mCP}\)) at a known \(T\) and \(S\).\(^{12}\) In another
aliquot of the same sample (same pH, T, and S), cresol red absorbance ratios ($R_{CR}$) were then measured.

The sample solutions consisted of tris-buffered synthetic seawater prepared gravimetrically; 0.06 m HCl was added to 0.08 mol of tris to achieve a 1:3 molal ratio of tris:tris-HCl. Reagent amounts and weights were specified via a spreadsheet provided by Dr. Andrew Dickson of UCSD-SIO. The spreadsheet calculates required amounts of salts to be added based on the amount of added HCl for each salinity and buffer ratio. This buffer ratio differs from the typical 0.04 m equimolal tris buffer preparation\textsuperscript{17} in order to achieve CR absorbance ratios in the range $1.088 \leq R_{CR} \leq 4.707$ and mCP absorbance ratios in the range $0.494 \leq R_{mCP} \leq 2.039$. These absorbance ratios correspond roughly to the range of CR absorbance ratios ($R$) encountered in oceanic measurements. The absorbance measurements used to determine the ratios were well within the linear-response characteristics of the Cary 400 spectrophotometer. The temperature and salinity ranges were $278.13 \leq T \leq 308.27$ K and $20 \leq S \leq 40$.

3.3.6 Initial Estimation of $e_1$ as a Function of Temperature

Initial estimates for the $e_1$ term in Equation 3.2 were obtained by determining the $e_1$ molar absorptivity ratio at a pH where the HI$^-$ form of the dye is dominant. Iterative calculations are necessary to account for absorbance contributions at 433 nm and 573 nm from the H$_2$I and I$^2$\textsuperscript{2} forms of the dye. The overlapping absorbance spectra of H$_2$I, HI$^-$ and I$^2$\textsuperscript{2} are shown in Figure 1. A speciation model for $T =$ 298.15 K and $S =$ 35 was constructed using the $K_1$ determined as described in section 3.3.7 and the $K_2$ reported by Byrne and Breland.\textsuperscript{13} At a pH of 4.5, HI$^-$ is near 99.91\% of the total CR concentration; the fractions of H$_2$I and I$^2$\textsuperscript{2} are 0.045\% and 0.046\%. Requisite $e_1$ absorbance data ($573A/433A$) were determined with a 0.02 m acetate buffer solution at ionic strength of 0.7 m NaCl. No salinity dependence was observed for the very small $e_1$ term.
During preparation of the acetate/acetic acid buffer solution, pH_{f} (free scale) was monitored with a ROSS combination electrode that had been calibrated on the free hydrogen ion scale by titrating a 0.7 m NaCl solution with standard HCl.

**3.3.7 Iterative Refinement of e_{1} and \(-\log(K_{2}^{T}e_{2})\)**

Because the HI\(^-\) absorbance signal includes contributions from the H\(_2\)I and I\(^2-\) forms of the dye, the following equation was used to account for these contributions (see also derivation of Liu et al.).\(^{18}\)

\[
e_{1} = \frac{573\varepsilon_{HI^-}}{433\varepsilon_{HI^-}} = \frac{573 A_{HI^-}/s[H]I}{433 A_{HI^-}/s[H]I} = \frac{573 A_{f} - 573 A_{H,I} - 573 A_{I^2}}{433 A_{f} - 433 A_{H,I} - 433 A_{I^2}}
\]

where \(\varepsilon_{HI^-}\) is the molar absorptivity at a given wavelength (\(\lambda\)) for the HI\(^-\) form of the indicator, \(\varepsilon_{x}\) is the absorbance at wavelength \(\lambda\) of total (\(T\)) indicator (all forms) or of individual indicator forms (H\(_2\)I, HI\(^-\), or I\(^2-\)), \(s\) is the cell pathlength, and \([H]I\) is the concentration of the HI\(^-\) form.

Expressing the absorbance terms in Equation 3.6 in terms of molar absorptivities and total CR concentrations (\(I_{T}\)) via \(K_{1}\) and \(K_{2}\), \(e_{1}\) can be written as follows:

\[
e_{1} = \frac{573 A_{f} - 573 A_{H,I} I_{T} S \left[\frac{[H^+]^2}{K_{1} K_{2}} \left(1 + \frac{[H^+]}{K_{2}} + \frac{[H^+]^2}{K_{1} K_{2}}\right)\right]^{-1} - 573 \varepsilon_{f} I_{T} S \left(1 + \frac{[H^+]}{K_{2}} + \frac{[H^+]^2}{K_{1} K_{2}}\right)^{-1}}{433 A_{f} - 433 A_{H,I} I_{T} S \left[\frac{[H^+]^2}{K_{1} K_{2}} \left(1 + \frac{[H^+]}{K_{2}} + \frac{[H^+]^2}{K_{1} K_{2}}\right)\right]^{-1} - 433 \varepsilon_{f} I_{T} S \left(1 + \frac{[H^+]}{K_{2}} + \frac{[H^+]^2}{K_{1} K_{2}}\right)^{-1}}
\]

To obtain the \(K_{2}\) value required in this calculation, initial \(e_{1}\) estimates were used to obtain initial \(K_{2}^{T}\) estimates by solving Equation 3.2 for \(-\log(K_{2}^{T} e_{2})\). The \(e_{2}\) term, required to calculate \(K_{2}^{T}\) from \(-\log(K_{2}^{T} e_{2})\), was calculated as a function of temperature by using the HI\(^-\) absorbance at \(\lambda = 433 \text{ nm}\) in the solution used to determine \(e_{1}\) (i.e., acetate buffer of pH = 4.5 and 0.7 m ionic strength) and the absorbance at \(\lambda = 573 \text{ nm}\) in the solution used to determine \(e_{3}/e_{2}\) (i.e., modified synthetic seawater of pH = 12 and 0.7 m ionic strength). Indicator concentration, \([I_{T}]\), which was
constant in these determinations, was determined gravimetrically via a Gilmont microburette and was verified using isosbestic point absorbances. After solving for $K_2^T$ the term was converted to the free concentration scale from the total scale with

$$K_2 = \frac{\frac{K_2^T}{1 + \frac{S_T}{K_S}}}{3.8}$$

where $K_S$ is the dissociation constant of HSO$_4^{-19}$ and $S_T$ is the total sulfate concentration. Conversion from the free to total scale was necessary since Equation 3.7 is expressed on the free hydrogen ion concentration scale while $-\log(K_2^T e_2)$ is expressed on the total scale. The H$_2$I molar absorptivity terms in Equation 3.7 were determined in 1 M HCl, where the H$_2$I form of the dye is dominant; the I$^2^-$ molar absorptivity terms were determined in solutions at pH = 12, where I$^2^-$ is dominant. To determine $K_1$ values, an aqueous HCl–NaCl mixture (0.7 m NaCl, pH $\approx$ 2) was prepared and CR absorbances were recorded after additions of standardized HCl at constant ionic strength. The pH in these experiments ranged from pH $\approx$ 2 to pH $\approx$ 1. Absorbances were corrected for dilution, and pH was calculated via HCl–NaCl mixing ratios. The absorbance maximum for the H$_2$I form of the dye occurs at $\lambda = 518$ nm. Using $\varepsilon_{518} A$ (measured) and [H$^+$] (calculated), the following equation was fitted to obtain $K_1$ as a function of temperature (282.40 $\leq T \leq$ 307.91 K):

$$\frac{\lambda A}{I_{75}} = \frac{\lambda \varepsilon_{75} + \lambda \varepsilon_{75}[H^+] / K_1}{1 + [H^+] / K_1}$$

Refined $e_1$ estimates calculated via Equation 3.7 were subsequently used in Equation 3.2 to obtain refined estimates of $-\log(K_2^T e_2)$ and $K_2$. Iterative calculations using Equations 3.2 and 3.7 were repeated until the $-\log(K_2^T e_2)$ and $e_1$ values stabilized to $\pm 10^{-14}$ and $\pm 10^{-9}$ respectively.
Refinements of $-\log(K_2 e_2)$ through this process were extremely small; the final $-\log(K_2 e_2)$ value was within 0.0001 of the initial estimate.

### 3.3.8 Development of the Equation for pH Determinations Using CR

Subsequent to the $-\log(K_2 e_2)$ and $e_1$ determinations, SigmaPlot software was used to fit the pH$_{mCP}$ and $R_{CR}$ data to Equation 3.10, thus producing an equation for calculation of seawater pH$_T$ from measurements of the CR absorbance ratio ($R_{CR}$), sample temperature ($T$), and sample salinity ($S$):

$$
\text{pH}_T = -\log(K_2 e_2) + \log \left( \frac{R_{CR} - e_1}{1 - R_{CR} \frac{e_3}{e_2}} \right)
$$

where $-\log(K_2 e_2) = a + b / T + c \ln T - dT$ and the terms $a$, $b$, and $c$ are functions of salinity. This equation is appropriate for pH$_T$ measurements made at atmospheric pressure for $278.15 \leq T \leq 308.15$ K and $20 \leq S \leq 40$.

### 3.4 Results

#### 3.4.1 Cresol Red Wavelengths of Interest

H$_2$I, H$I^-$, and I$_2^-$ cresol red absorbance maxima were observed to occur at 518 nm, 433 nm, and 573 nm, respectively (Figure 3.1). These determinations of CR wavelengths for routine spectrophotometric pH measurements in seawater are consistent with those of Byrne and Breland.$^{13}$

Isosbestic point wavelengths as a function of temperature are well described with these equations, as shown in Figure 3.2:

$$
\lambda_{i_{os}}^{H_2/H^-} = 496.82 - 0.076T
$$

$$
\lambda_{i_{os}}^{H^-/I^2-} = 513.01 - 0.092T
$$
At 298.15 K, the H₂I/H⁻ isosbestic point occurs at 474.2 nm and the HI⁻/I²⁻ isosbestic point occurs at 485.6 nm. The H₂I/H⁻ isosbestic point wavelength decreases by 0.076 nm per degree of warming, while the HI⁻/I²⁻ isosbestic point wavelength decreases by 0.092 nm per degree warming.

The use of 730 nm for the non-absorbing wavelength used for quality control is consistent with Byrne and Breland.¹³

Figure 3.2. Cresol red isosbestic points wavelengths as a function of temperature.

3.4.2 e₃/e₂ as a Function of Temperature and Salinity

As noted previously (section 3.3.4), the e₃/e₂ ratio was determined in modified synthetic seawater at a pH sufficiently high that the I²⁻ form of the dye was dominant. Because the path length and indicator concentration terms cancel in the 433Å/573Å quotient, e₃/e₂ is identical to the 433Å/573Å absorbance ratio. Absorbance data are shown in Table 3.1 and Figure 3.3. The following equation summarizes the temperature and salinity dependence of e₃/e₂:
\( e_3/e_2 = -0.021683 + 1.8107 \times 10^{-4} T + 3.163 \times 10^{-5} (S - 35) \)

At \( T = 298.15 \) K and \( S = 35 \), \( e_3/e_2 = 0.03230 \).

Table 3.1. Cresol red \( e_3/e_2 \) as a function of temperature and salinity.

<table>
<thead>
<tr>
<th>( T ) (K)</th>
<th>( S )</th>
<th>( e_3/e_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>298.18</td>
<td>20</td>
<td>0.03181</td>
</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>278.20</td>
<td>35</td>
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</tr>
<tr>
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<tr>
<td>283.22</td>
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<tr>
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<td>35</td>
<td>0.02929</td>
</tr>
<tr>
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<td>35</td>
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<tr>
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<td>308.22</td>
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<tr>
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</tr>
<tr>
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Figure 3.3. Cresol red $e_3/e_2$ as a function of (a) salinity at $T=298.15$K (b) temperature at $S=35$.

3.4.3 Temperature Dependence of $pK_1$ and $pK_2$

The transition from $H_2I$ to the $HI^-$ form of the dye occurs in the range of $1.0 \leq pH \leq 2.0$, with the dye’s absorption characteristics being a function of temperature. The temperature dependence of $pK_1$ in 0.7 m NaCl is given as follows:

$$pK_1 = \frac{386.341751}{T} - 0.167222$$ \hspace{1cm} (3.14)

The temperature dependence of $pK_2$ (on the free hydrogen ion concentration scale), for use in iterative refinements of $e_1$, is given as:

$$pK_2 = \frac{838.872749}{T} - 5.021899$$ \hspace{1cm} (3.15)
3.4.4 Temperature Dependence of $e_1$

The initial estimate of the $e_1$ temperature dependence is given as:

$$\frac{573A}{433A} = -0.01047 + 4.377 \times 10^{-5}T$$  \hspace{1cm} (3.16)$$

Iterative refinement of the initial $e_1$ estimate, to account for $H_2I$ and $I^2-$ absorbance contributions to $\frac{573A}{433A}$, produced the following description of $e_1$ as a function of temperature:

$$e_1 = -0.00413 + 1.814 \times 10^{-5}T$$  \hspace{1cm} (3.17)$$

The initial $e_1$ estimates ($\frac{573A}{433A}$) and the final calculated $e_1$ results are compared in Figure 3.4 and Table 3.2. At 298.15 K, $e_1 = 0.00128$. No salinity dependence was observed for $e_1$.

Table 3.2 Cresol red $\frac{573A}{433A}$ (i.e., initial $e_1$ estimate at pH$_f$ = 4.5) and final $e_1$, determined in 0.7 m NaCl.

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$\frac{573A}{433A}$</th>
<th>$e_1$</th>
</tr>
</thead>
<tbody>
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<td>278.09</td>
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<td>0.00093</td>
</tr>
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<td>0.00217</td>
<td>0.00112</td>
</tr>
<tr>
<td>288.28</td>
<td>0.00217</td>
<td>0.00112</td>
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<tr>
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<td>0.00260</td>
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<td>298.45</td>
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<td>298.47</td>
<td>0.00259</td>
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</tr>
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<td>0.00264</td>
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<tr>
<td>308.30</td>
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<td>0.00144</td>
</tr>
<tr>
<td>308.32</td>
<td>0.00300</td>
<td>0.00143</td>
</tr>
</tbody>
</table>
3.4.5 Calculation of seawater $pH_T$ from $R_{CR}$, $T$, and $S$

For salinities of $20 \leq S \leq 40$, temperatures of $278.15 \leq T \leq 308.15$ K, and measurements made at atmospheric pressure, seawater $pH_T$ is calculated from measured $R_{CR}$, $T$, and $S$, using Equation 3.10 with

$$a = -859.326051 + 0.146165S + 7.81164 \times 10^{-4}S^2$$

$$b = 22969.9366 + 8.04468S - 0.20512S^2$$

$$c = 152.209523 - 0.0317821S$$

$$d = 0.259915$$

The molar absorptivity ratios in Equations 3.2 and 3.10 are given as

$$e_1 = -0.00413 + 1.814 \times 10^{-5}T$$

$$e_3/e_2 = -0.021683 + 1.8107 \times 10^{-4}T + 3.163 \times 10^{-5}(S - 35)$$
Absorbance ratios ($R_{CR}$ and $R_{mCP}$), calculated pH values, and residuals (pH$_{CR}$ minus pH$_{mCP}$) determined over a range of $S$ and $T$ are shown in Figure 3.5 and Table 3.3. Investigators can use Table 3.3 to test their coding of Equation 3.10: entering the $S$, $T$, and $R_{CR}$ values in Table 3.3 should yield the pH$_{CR}$ values shown in the sixth column.

Table 3.3. Absorbance ratios and pH$_T$ values of tris synthetic seawater samples, obtained using mCP (Liu et al.$^{18}$ Equation 18) and CR (this paper, Equation 3.10) over a range of $T$ and $S$.

<table>
<thead>
<tr>
<th>S</th>
<th>T (K)</th>
<th>$R_{mCP}$</th>
<th>pH$_{mCP}$</th>
<th>$R_{CR}$</th>
<th>pH$_{CR}$</th>
<th>Residual</th>
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<tr>
<td>20</td>
<td>278.006</td>
<td>1.7567</td>
<td>8.2256</td>
<td>4.0697</td>
<td>8.2252</td>
<td>−0.00042</td>
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<tr>
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<td>287.963</td>
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</tr>
</tbody>
</table>
3.5 Discussion

3.5.1 Use of CR and mCP in Seawater pH Measurements

Cresol red (this paper) was linked to mCP\textsuperscript{12} over a range of temperatures and salinities to ensure that spectrophotometric pH determinations using the two indicators are internally consistent over their overlapping pH ranges. Figure 3.5 shows that the maximum difference between pH determined using CR and pH determined using mCP (i.e., pH\textsubscript{CR} minus pH\textsubscript{mCP}) is 0.0010. The average difference is –0.00002. The variance at 1σ is better than ±0.00045, and the variance at 2σ is better than ±0.00070; the analytical precision of spectrophotometric pH measurements is ±0.0004.\textsuperscript{6} As such, the overall uncertainty of the purified CR calibration relative to mCP is substantially better than 0.001.

Figure 3.5. Residuals of pH\textsubscript{T} determined using mCP (Equation 18, Liu et al.\textsuperscript{18}) and CR (Equation 3.10, this paper) in tris-buffered synthetic seawater over a range of $T$ and $S$. 

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The CR characterization in this work is intended for use only with absorbance ratios obtained using purified cresol red. For measurements made using unrefined CR and earlier characterization equations, the retrospective correction procedures outlined in Liu et al.\textsuperscript{18} should be followed. For all spectrophotometric pH measurements, records of indicator lot number, absorbance ratios, measurement temperatures and pressures, and sample salinities should be routinely archived so that pH values can be recalculated if indicator equations are refined in the future.

For investigators to choose indicators and concentrations appropriate for a particular environment or application, they must be aware of the pH range likely to be encountered under measurement conditions (not just in situ conditions) and they must be familiar with the linearity limitations of their spectrophotometer. Figure 3.6 shows CR absorbances (433 and 573 nm) and mCP absorbances (434 and 578 nm) as a function of pH\textsubscript{T}; indicator concentrations were 2.5 $\mu$M. Absorbances at the shorter wavelengths (solid lines) range between 0.24 and 0.65, behaving similarly as pH increases from 6.8 to 8.2.

This range of absorbance values is within the measurement limitations of most spectrophotometers. Absorbances at the longer wavelengths (broken lines) are substantially more sensitive to changing pH, with absorbance values ranging from as low as 0.08 (mCP) to as high as 1.59 (CR). $A > 1.0$ can be problematic due to nonlinear behavior at high absorbances, while $A < 0.1$ may reduce measurement precision due to low signal-to-noise ratios.

An assessment such as that depicted in Figure 3.6 can be used to guide the selection of an indicator (mCP or CR) and optimal indicator concentrations. For surface-to-deep profiles of typical ocean waters, with a seawater pH\textsubscript{T} range of 7.2–8.2 at 298.15 K, we advise the use of mCP at a concentration of 3 $\mu$M. For a 10 cm pathlength cell, this concentration produces
absorbances in the range of 0.20–0.97. For seawater with a higher acidity content, we recommend cresol red. A CR concentration of 2.5 µM results in absorbances of 0.21–0.95 over a pHₜ range of 6.8–7.8 (at 298.15 K). For pH > 7.8, the CR concentration can be reduced to ensure that absorbances do not exceed the linear range of the spectrophotometer. Figure 3.6 also shows that CR at higher concentrations can be used to measure pH well below 6.8.

For some waters, either indicator is suitable. Areas of the coastal Arctic, for instance, can have pH values ranging from 7.7–8.2 at in situ temperatures. At a measurement temperature of 298.15 K (typical of shipboard analyses), the pH range of these waters would be 7.3–7.8. Under these conditions with a typical benchtop spectrophotometer, mCP and CR would work equally well.

Figure 3.6 Absorbances for 2.5 µM CR (shown in red) at wavelengths of 433 and 573 nm and for 2.5 µM mCP (shown in purple) at 434 and 578 nm as a function of pH (T = 298.15 K and S = 35).
3.5.2 Summary and Future Work

This work describes the physical–chemical characteristics of purified cresol red for use in spectrophotometric seawater pH measurements over the temperature and salinity ranges of $278.15 \leq T \leq 308.15$ and $20 \leq S \leq 40$ (at atmospheric pressure). For seawater within the range of $6.8 \leq \text{pH}_T \leq 7.8$ (at a measurement temperature of 298.15 K), we recommend the use of CR at a concentration equal to $2.5 \mu M$. To ensure global intercomparability of measurements, investigators should use purified indicator only.

Cresol red is well suited for seawater with a relatively high hydrogen ion content—e.g., waters strongly influenced by atmospheric carbon dioxide, hydrothermal vents, or remineralization. Waters amenable to CR analysis would therefore include high-latitude surface waters, sediment porewaters, and oxygen-minimum zones.

Due to CO$_2$-driven ocean acidification, the average pH of the global surface ocean has decreased by 0.1 since the onset of the Industrial Revolution.$^{21}$ Over the 21$^{st}$ century, Arctic surface ocean pH is projected to decrease by 0.45.$^3$ Ocean acidification makes cresol red an increasingly important indicator, not only for characterization of seawater pH in the world’s oceans but also for laboratory studies of the biogeochemical effects of the phenomenon.

Future work will include purification and characterization of other sulfonephthalein indicator dyes used for CO$_2$ system analyses (e.g., thymol blue, bromocresol green, bromocresol purple, phenol red). The procedures used in the present investigation help ensure that measurements obtained with different indicators are made on an internally consistent pH scale.

3.6 Acknowledgements

This work was supported by NSF Award OCE-0727082. Support for M. Patsavas was partially provided by the Robert M. Garrels Memorial Fellowship and the C.W. Bill Young
Fellowship. Advice and insightful comments from Dr. T. Clayton are greatly appreciated. The authors gratefully acknowledge the comments and suggestions of two anonymous reviewers.

3.7 References


19. Dickson, A. G., Standard potential of the reaction AgCl(s)+1/2H_2(g)=Ag(s)+HCl(aq) and the standard acidity constant of the ion HSO_4^- in synthetic sea-water from 273.15K to 318.15K. *Journal of Chemical Thermodynamics* 1990, 22, (2), 113-127.

CHAPTER FOUR:

MARINE CO₂ SYSTEM CHARACTERIZATIONS OF US COASTAL WATERS:
INFLUENCE OF MEASUREMENT CHOICES ON
CALCULATED CALCIUM CARBONATE SATURATION STATES

Note to Reader

This chapter has been submitted for publication.

4.1 Abstract

This research compares calculated and directly measured marine CO₂ system parameters: dissolved inorganic carbon (DIC), total alkalinity (TA), pH, and CO₂ fugacity (fCO₂), based on an evaluation of data from 1890 water samples collected during two NOAA cruises along the west and east coasts of the United States. Despite recent analytical advances in CO₂ system measurements, calculations of in situ aragonite saturation states (Ωₐ) near the saturation horizon exhibited differences on the order of ±10% between predictions obtained using the (DIC, TA) pair vs. (pH, DIC), (fCO₂, DIC), or (fCO₂, pH). Differences of this magnitude are roughly equivalent to the magnitude of Ωₐ change expected to occur in ocean acidification projections over several decades. Uncertainties in the calculated depths of saturation horizons point to the importance of direct in situ validation of Ωₐ predictions.
Calculations involving pH, DIC, and TA show that internal consistency can be achieved if minor subtractions (4 µmol kg\(^{-1}\)) are applied to TA for samples with \(S < 35\), which could potentially be necessitated by the presence of organic bases. Calculations involving \(f\text{CO}_2\), TA, and DIC indicate a bias in \(f\text{CO}_2\) for \(f\text{CO}_2 > 500\ \mu\text{atm}\), which is consistent with a loss of CO\(_2\) during \(f\text{CO}_2\) measurement.

### 4.2 Introduction

Ocean biogeochemistry is being strongly influenced by the uptake of anthropogenic carbon dioxide (CO\(_2\)) from the atmosphere. The uptake of CO\(_2\) in seawater increases the hydrogen ion concentration (decreases pH) and alters the relative proportions of the inorganic carbon species, in particular decreasing the concentration of carbonate ions. Many pH-dependent biogeochemical processes in open and coastal waters are being affected by CO\(_2\) uptake.\(^{1-14}\) Documentation of the rapidly changing ocean chemistry is essential for understanding this perturbation and its consequences.

Four CO\(_2\) system parameters are routinely measured in seawater: inorganic carbon (DIC), total alkalinity (TA), pH, and carbon dioxide fugacity (\(f\text{CO}_2\)):

\[
\text{DIC} = [\text{CO}_2^*] + [\text{HCO}_3^-]_T + [\text{CO}_3^{2-}]_T
\]

where \([\text{CO}_2^*] = [\text{CO}_2] + [\text{H}_2\text{CO}_3].\)

\[
\text{TA} = [\text{HCO}_3^-]_T + 2[\text{CO}_3^{2-}]_T + [\text{B(OH)}_4^-]_T + [\text{OH}^-]_T + [\text{HPO}_4^{2-}]_T + 2[\text{PO}_4^{3-}]_T + [\text{SiO(OH)}_3^-]_T - [\text{H}^+]_T - [\text{H}_3\text{PO}_4^0] + [\text{organic bases}]\]

\[
\text{pH}_T = -\log[\text{H}^+]_T
\]

Brackets ([ \(\cdot\)\(_T\)] denote the total concentrations of each species. Carbonate alkalinity is given by \([\text{HCO}_3^-]_T + 2[\text{CO}_3^{2-}]_T\). The carbon dioxide fugacity of seawater is a quantitative measure of the equilibrium status of dissolved CO\(_2\) with respect to CO\(_2\) gas exchange with the atmosphere.
Seawater CO\textsubscript{2} is in exchange equilibrium with the atmosphere when the CO\textsubscript{2} fugacity of each phase is identical. Gas phase CO\textsubscript{2} fugacity is numerically quite similar to gas phase partial pressure, but f\textsubscript{CO\textsubscript{2}} accounts for the non-ideal behavior of the gas and p\textsubscript{CO\textsubscript{2}} does not.

Due to limited resources, investigators sometimes measure only two of the four parameters. Some researchers choose the (DIC, TA) pair because samples can be poisoned (thus halting photosynthesis and respiration), stored, and analyzed at a later time. Some choose (pH, DIC) because pH measurements are instantaneous and precise, and the DIC measurement is free of matrix effects (i.e., organics). Typical carbon system research cruises involve measurement of two, or possibly three, of the four parameters. Simultaneous measurement of all four is rare.

Investigators instead rely on the fact that the four parameters are related by thermodynamic models such that only two are required to calculate any of the others.\textsuperscript{15}

Equilibrium constants for these calculations include:

\[
K_0 = \frac{CO_2^*}{fCO_2}\quad \quad 4.4
\]

\[
K_1 = \frac{[HCO_3^-]_T[H^+]_T}{[CO_2^*]}\quad \quad 4.5
\]

\[
K_2 = \frac{[CO_3^{2-}]_T[H^+]_T}{[HCO_3^-]_T}\quad \quad 4.6
\]

Other relationships between measured and calculated parameters are described in Dickson.\textsuperscript{15}

Because carbonate ion concentrations [CO\textsubscript{3}\textsuperscript{2-}] \textsubscript{T} can be calculated from any two measured CO\textsubscript{2} system parameters, saturation states (Ω) for the polymorphs of calcium carbonate (e.g., calcite, aragonite) can be calculated as well:\textsuperscript{16}

\[
\Omega = \frac{[Ca^{2+}]_T[CO_3^{2-}]_T}{K_{sp}}\quad \quad 4.7
\]
where $[Ca^{2+}]_T$ is the total calcium concentration and $K'_{SP}$ is the apparent solubility product of the polymorph (a function of $S$, temperature $T$, and pressure). Where $\Omega < 1$, conditions are thermodynamically favorable for dissolution of solid calcium carbonate $CaCO_3(s)$. Where $\Omega > 1$, precipitation is favored and marine organisms require less energy to form and maintain $CaCO_3(s)$ shells or tests.

In the open ocean, carbonate saturation states are typically relatively high ($\Omega \geq 2$) at the sea surface, then decrease with depth ($\Omega \leq 1.0$). The three-dimensional horizon (saturation horizon (SH)) defined by the depths where $\Omega = 1$ serves as a common indicator or metric in CO$_2$ system studies. In recent years, the shoaling of the SH due to ocean acidification has been reported, and this change is expected to profoundly affect marine ecosystems.$^{3, 5, 6, 8, 17-19}$ Accurate mapping of saturation states in seawater is an imperative. Because $\Omega$ cannot be directly measured, a thorough understanding of the influences of measurement choices on calculated $\Omega$ is required.

The principal goal of this research is to assess, in light of recent analytical advances, the consequences of choosing different measurement pairs to calculate other parameters. Recent CO$_2$ system data are available from two extensive coastal cruises, one of which obtained measurements of all four carbon system parameters. This overdetermination allows for unique comparisons that are not possible when fewer parameters are measured. This work is the first investigation of the internal consistency of CO$_2$ system calculations involving pH measurements obtained using purified spectrophotometric indicator (i.e., improved pH accuracy). Our work especially focuses on the consequences of choosing different parameter pairs for the calculation of aragonite saturation states in seawater.
4.3 Methods

4.3.1 GOMECC-2 Cruise (2012)

The Gulf of Mexico East Coast Carbon-2 (GOMECC-2) cruise was a 24-day NOAA Ocean Acidification Program (OAP) cruise conducted from July 21st – August 13th 2012 aboard the R/V Ronald H. Brown (Figure 4.1). All four carbon system parameters (DIC, TA, pH, and $f$CO$_2$) were measured throughout the water column. Conductivity–temperature–depth (CTD) and nutrient data were also collected. The number of data points from GOMECC-2 that contained the full suite of carbon system, nutrient, and CTD data was 885.

Figure 4.1. GOMECC-2 cruise track, with Line 7 in yellow and Stations 53-56 of Line 5 in red.
4.3.2 W1108C Cruise (2011)

The West Coast Ocean Acidification (W1108C) cruise was a 28-day NOAA OAP cruise conducted in August 11th –September 3rd, 2011 aboard the R/V Wecoma (Figure 4.2). DIC, TA, and pH were measured. CTD and nutrient data were also collected. The number of W1108C samples used in this work is 1005.

Figure 4.2. W1108C cruise track.
4.3.3 CO₂ System Calculations

All of the CO₂ system analytical methods and quantitative relationships are described in Dickson.¹⁵ One exception is that the pH measurements were performed using purified meta cresol purple (mCP)²⁰ and the updated pH equations of Liu et al.²¹

Calculations were performed using the Excel macro CO2SYS version 2.1.²² Because pH was determined on the total hydrogen ion scale, the dissociation constants (K₁’ and K₂’) of Lueker et al.²³ were used unless otherwise noted. The value of K_{HSO₄} was taken from Dickson et al.,²⁴ and the total boron-to-salinity ratio was from Lee et al.²⁵ Pressure was 1 atm.

Spectrophotometric pH measurements were performed at 25°C and discrete fCO₂ measurements were performed at 20°C. The notation X(Y, Z) indicates that parameter X was calculated using the input (measured) parameters Y and Z. For example: pH(DIC, TA) refers to a pH value calculated using measurements of DIC and TA. The calculation of Ω_A(fCO₂, pH) at in situ conditions required an ancillary calculation of fCO₂(25°C) from DIC and fCO₂(20°C) so the two parameters had the same input temperature.

Residuals were expressed in terms of (a) differences between measured and calculated parameters: ΔX = measured X – calculated X and (b) differences between parameter values calculated from different measurement pairs. In some cases, it was redundant to calculate all possible combinations of calculation parameters. For example: The pH-DIC-TA system can be sufficiently studied via TA residuals (TA-TA(pH, DIC)) so there was no need to display DIC residuals ((DIC-DIC(pH, TA))).
4.4 Results and Discussion

4.4.1 Aragonite Saturation State

The GOMECC-2 data are particularly useful for internal consistency assessments because all four CO₂ system parameters were measured on this cruise. In situ aragonite saturation states (Ωₐ) were calculated from the following data pairs: (DIC, TA), (pH, DIC), (fCO₂, DIC), and (fCO₂, pH). The Ωₐ range observed on GOMECC-2 was 0.644 in deeper waters to 4.284 in surface waters.

Absolute residuals of Ωₐ (ΔΩₐ) calculated from different pairs of measurements were as large as 0.257 units (Table 4.1). These deviations originate from DIC and TA measurement imprecision that is magnified by use of the (DIC, TA) pair to calculate saturation states. See Appendix B for a discussion of impacts of measurement imprecision on calculated saturation states. When this pair is excluded from the comparisons, the maximum residual is only 0.061.

The large residuals produced by the (DIC, TA) pair is also reflected in the standard deviations (SDs) shown in Table 4.1. For calculations that do not involve this pair, SD values are markedly reduced.

Table 4.1. Differences in situ aragonite saturation states, Ωₐ calculated from different parameter pairs (GOMECC-2)

<table>
<thead>
<tr>
<th>Residual (ΔΩₐ)</th>
<th>Mean</th>
<th>SD</th>
<th>Max</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ωₐ(DIC, TA) – Ωₐ(pH, DIC)</td>
<td>0.011</td>
<td>0.068</td>
<td>0.250</td>
<td>−0.245</td>
</tr>
<tr>
<td>Ωₐ(DIC, TA) – Ωₐ(fCO₂, DIC)</td>
<td>−0.014</td>
<td>0.066</td>
<td>0.233</td>
<td>−0.257</td>
</tr>
<tr>
<td>Ωₐ(fCO₂, DIC) – Ωₐ(pH, DIC)</td>
<td>0.025</td>
<td>0.013</td>
<td>0.058</td>
<td>−0.022</td>
</tr>
<tr>
<td>Ωₐ(fCO₂, pH) – Ωₐ(pH, DIC)</td>
<td>−0.027</td>
<td>0.014</td>
<td>0.027</td>
<td>−0.061</td>
</tr>
</tbody>
</table>
Consequences of the large scatter introduced by the (DIC, TA) measurement pair can be seen in ocean cross-sections of $\Delta \Omega_A$ (Figure 4.3). When the (DIC, TA) pair is used, absolute differences of $\geq 0.15$ are evident in offshore surface waters (Figure 4.3a–b). Differences of this magnitude can create erroneous impressions of temporal changes in saturation states when saturation states are examined over a period of decades via a series of oceanic measurement expeditions. When pairings that do not include (DIC, TA) are compared (Figure 4.3c–d), the scatter is greatly reduced with a small offset between saturation states calculated with different measurement pairs.

Figure 4.4 shows the same four sets of $\Delta \Omega_A$ residuals as a function of $\Omega_A$. With one exception (Figure 4.4d), linear fits of the residuals exhibited negative slopes; steepest slopes (indicative of lack of consistency as a function of $\Omega_A$) are seen for the two comparisons (Figure 4.4a–b) that included $\Omega_A$ (DIC, TA). Excluding (DIC, TA) as an input pair resulted in an approximately fivefold reduction in slope and intercept standard errors, plus greatly reduced residual sums of squares (Table 4.2). At the $\Omega_A$ value that defines the aragonite saturation horizon (ASH) in the oceans ($\Omega_A = 1$), the scatter introduced by the (DIC, TA) pair can create differences in calculated $\Omega_A$ on the order of $\pm 10\%$. For a discussion of W1108C aragonite saturation states see Appendix B. The selection of which two measurements to input for the calculation of $\Omega_A$ can therefore affect estimates of the depth of the ASH. For example, at four stations along Line 5 of the GOMECC-2 cruise (Figure 4.5), the difference in estimated ASH at a given station could be $>200$ m, depending on the choice of parameter pair. When the ($f$CO$_2$,DIC) and (pH, DIC) pairs are compared, the depth offset is fairly consistent at all three stations ($\sim 100$ m).
Figure 4.3. Cross-sections of in situ aragonite saturation state residuals $\Delta \Omega_A$ along a transect just south of Cape Cod (Line 7 of the GOMECC-2 cruise): (a) $\Omega_A$(DIC, TA)$-\Omega_A$(pH, DIC), (b) $\Omega_A$(DIC, TA)$-\Omega_A$(fCO$_2$, DIC), (c) $\Omega_A$(fCO$_2$, DIC) - $\Omega_A$(pH, DIC), (d) $\Omega_A$(fCO$_2$, pH) - $\Omega_A$(pH, DIC). The location of Line 7 is shown in Figure 4.1.
Figure 4.4. ΔΩ_A calculated by various measurement pairs (a) (DIC, TA) – (pH, DIC), (b) (DIC, TA) – (f CO_2, DIC), (c) (f CO_2, DIC) – (pH, DIC), and (d) (f CO_2, pH) – (pH, DIC). Red lines are linear regressions.

Table 4.2. Regression statistics for Figure 4.4.

<table>
<thead>
<tr>
<th>Residual (ΔΩ_A)</th>
<th>Slope</th>
<th>Intercept</th>
<th>Residual Sum of Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔΩ_A(DIC, TA) – ΔΩ_A(pH, DIC)</td>
<td>-0.0243</td>
<td>0.0697</td>
<td>3.511</td>
</tr>
<tr>
<td>ΔΩ_A(DIC, TA) – ΔΩ_A(f CO_2, DIC)</td>
<td>-0.0167</td>
<td>0.0270</td>
<td>3.532</td>
</tr>
<tr>
<td>ΔΩ_A(f CO_2, DIC) – ΔΩ_A(pH, DIC)</td>
<td>-0.0078</td>
<td>0.0436</td>
<td>0.092</td>
</tr>
<tr>
<td>ΔΩ_A(f CO_2, pH) – ΔΩ_A(pH, DIC)</td>
<td>0.0064</td>
<td>-0.0422</td>
<td>0.120</td>
</tr>
</tbody>
</table>
This magnitude of offset is consistent with the $\Delta \Omega_A$ values shown in Figure 4.4. Values of $\Omega_A$(pH, DIC) are systematically lower than $\Omega_A$(fCO$_2$, DIC), particularly at low $\Omega_A$ (Figure 4.4c). At low saturation states, $\Omega_A$(pH, DIC) for a given sample (and depth) is consistently ~0.03 units less than $\Omega_A$(fCO$_2$, DIC) (Figure 4.5). As a consequence, the estimated ASH(pH, DIC) for a given station (in this example) is consistently ~100 m higher in the water column (Figure 4.5).

Differences between $\Omega_A$(DIC, TA) and the other two options do not show the same fairly constant offset (Figures 4.4a–b and 4.5). As a result, $\Omega_A$(DIC, TA) for a given sample (depth) may be higher or lower than $\Omega_A$(pH, DIC) or $\Omega_A$(fCO$_2$, DIC) and the estimated depth of ASH(DIC, TA) may be higher or lower in the water column. This behavior could be explained by station-to-station variations in the accuracy of the TA measurements. Precise estimates of saturation depths and changes in saturation depths require that either pH or fCO$_2$ be used in the calculations.

The large $\Delta \Omega_A$ (~0.15) introduced by internal consistency issues (i.e., differences in calculated outcomes based on different input parameter pairs) seen in Figure 4.3a–b and 4.4a–b are significant in the context of carbon system models. While modeled biogeochemical projections are ideally based on direct ocean observations, saturation states cannot be directly measured. Instead, saturation states are calculated from measurements of two or more of the major CO$_2$ system parameters (DIC, TA, pH, and fCO$_2$), salinity, temperature, and pressure. Depending on the parameter choice for CO$_2$ system calculations, uncertainties in modeled saturation states can have magnitudes that are comparable to the saturation-state changes that are projected to occur over coming decades. For example, assuming a model surface water ($S = 35$, $T = 25^\circ C$, TA = 2400 $\mu$mol kg$^{-1}$) that is in equilibrium with an atmosphere of 400 ppm CO$_2$, an
atmospheric CO$_2$ increase of 2 ppm yr$^{-1}$ and an increase in surface ocean temperature of 0.02 degrees yr$^{-1}$ would generate a 0.15 unit change in $\Omega_A$ over 15 years.

Figure 4.5. Vertical profiles of aragonite saturation state and identification of depths of aragonite saturation horizons (where $\Omega_A = 1$) off the coast of North Carolina (GOMECC-2 cruise, Line 5, Stations 53-56).
4.4.2 Total Alkalinity

In order to examine the mechanisms that underlie observed differences in calculated saturation states we examined TA residuals (ΔTA = measured TA – calculated TA) for the GOMECC-2 and W1108C data sets (Figure 4.6). Figure 4.6a shows residuals where TA was calculated from pH and DIC. For GOMECC-2 waters of high alkalinity (TA ≥ 2290 µmol kg⁻¹), the internal consistency of the DIC, TA, and pH measurements was excellent, with a mean residual (± standard deviation) of 0.457 (±7.045) µmol kg⁻¹. However, for GOMECC-2 waters of low TA (TA < 2290 µmol kg⁻¹), calculated TA was lower than observed TA by ~6 µmol kg⁻¹. The mean residual was 6.294 (±6.947) µmol kg⁻¹. For high-TA W1108C waters, the mean residual was 5.795 (±3.448) µmol kg⁻¹; for low-TA W1108C waters the mean was 3.804 (±4.326) µmol kg⁻¹. Thus, for all waters collectively, calculated TA is lower than measured TA by ~4 µmol kg⁻¹ or more. Table 4.3 summarizes the residual statistics for both cruises.

One striking feature of the TA residuals (Figure 4.6a) is clearly evident in waters for which TA < 2290 µmol kg⁻¹. For both cruises, the residuals in this low-TA range are almost all positive (rather than randomly distributed about the zero line). This distribution has an apparent dependence on salinity (Figure 4.6b)—i.e., samples with S < 35 exhibit mostly positive residuals. This generalization is observed over the full range of TA. Through alteration of the salinity-dependent terms of the pH computational algorithms, and through use of alternative equilibrium constants in CO₂ system calculations, it was found that no changes or combination of changes in calculational algorithms significantly improved the correspondence between directly measured and calculated parameters. One possible explanation might be high concentrations of organic bases in lower-S waters. The alkalinity contributions of these bases would be accounted for in TA measurements but not calculations of TA, and the result would be positive TA residuals, as
seen in Figures 4.6a–b. Both cruises were in coastal waters where high biological productivity and proximity to terrestrial runoff could have resulted in high organic base concentrations. In some coastal locations, organic bases have been reported to contribute as much as 50 µmol kg\(^{-1}\) to the total alkalinity.\(^{26}\) Wang et al.\(^{27}\) reported Congo River organic base concentrations that were \(~60\%\) of the total alkalinity. Near our GOMECC-2 Georgia transect, organic base concentrations were previously measured to be as high as 25 µmol kg\(^{-1}\) \((S = 27)\) to 110 µmol kg\(^{-1}\) \((S = 0.1)\) for the Satilla River estuary and 10 µmol kg\(^{-1}\) \((S = 31)\) to 40 µmol kg\(^{-1}\) \((S = 0.1)\) for the Altamaha River estuary.\(^{28}\) Assuming an organic base–to–DOC ratio of \((280 \text{ µmol-Alk \, L}^{-1})/(0.0023 \text{ µmol-C \, L}^{-1}) = 0.122 \text{ mol-Alk/mol-C}^{28}\) and an average inner-shelf water \((S < 35)\) DOC concentration of 110 µmol/L \((\text{July 2005, Jiang et al.}^{29})\), we derive a potentially available organic base of \(~13 \text{ µmol L}^{-1}\), sufficient for explaining the observed differences. Cai et al.\(^{28}\) characterized three proton-binding groups for DOC with \(pK_1 = 4.5\), \(pK_2 = 6.6\), and \(pK_3 = 8.9\). The \(pK_2\) and \(pK_3\) groups would fully contribute to the measured alkalinity while part of the \(pK_1\) site would likely not be fully titrated during our TA titration (pH 3.8 to 3.0).

Even minor adjustments to measured TA (to account for organic contributions) can profoundly affect the apparent residuals. For example, if a 0.18% correction (a subtraction of \(~4 \text{ µmol kg}^{-1}\)) is applied to measured TA for shelf-water samples, the distribution of TA residuals around zero (i.e., the internal consistency of pH, DIC, and TA) is substantially improved for both GOMECC-2 and W1108C (Figure 4.6c). The average offset from zero for all of the data shown in Figure 4.6c is <0.1 µmol kg\(^{-1}\).

Calculating TA from \(f\text{CO}_2\) and DIC (Table 4.3) yields an average low-TA residual of 3.442 (±6.692), which is slightly lower than the 6.294 (±6.947) µmol kg\(^{-1}\) average obtained using pH and DIC. Thus, TA(\(f\text{CO}_2, \text{DIC}\)) residuals also suggest the existence of organic bases in low-
alkalinity samples (i.e., where $S$ is also low). For high-TA samples, the mean TA($f$CO$_2$, DIC) residual is negative (~2.509 ±6.618 µmol kg$^{-1}$) and is larger than the TA(pH, DIC) residual (0.457 ±7.045 µmol kg$^{-1}$). Negative TA($f$CO$_2$, DIC) residuals, and in particular TA($f$CO$_2$, DIC) residuals that are lower than TA(pH DIC) residuals, might be in part attributable to a loss of CO$_2$ prior to or during measurement. Decreasing $f$CO$_2$ at a constant DIC causes calculated TA to increase. The range of offsets between high and low TA (~6 µmol kg$^{-1}$) is the same for either TA(pH, DIC) or TA($f$CO$_2$, DIC). On average, TA($f$CO$_2$ DIC) > TA(pH DIC) by ~3 µmol kg$^{-1}$ (Figure 4.6d).

![Diagram](image)

Figure 4.6. TA residuals as a function of measured TA (a) by cruise, (b) with salinity color bar, (c) with 0.18% TA correction applied to shelf-water samples (i.e., where $S < 35$), (d) binned averages, showing also residuals for TA($f$CO$_2$, DIC).
Table 4.3. Residuals for TA, pH, and $f$CO$_2$ ($\Delta =$ measured minus calculated).

<table>
<thead>
<tr>
<th>Residual ($\Delta$)</th>
<th>Data Set</th>
<th>Calculated Parameter</th>
<th>Range Viewed</th>
<th>Numerical Range Viewed</th>
<th>Residual Mean</th>
<th>Residual SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>GOMECC-2</td>
<td>TA(pH, DIC)</td>
<td>high</td>
<td>2290–2430</td>
<td>0.457</td>
<td>7.045</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TA(pH, DIC)</td>
<td>low</td>
<td>2100–2290</td>
<td>6.294</td>
<td>6.947</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TA(pH, DIC)</td>
<td>all</td>
<td>2100–2430</td>
<td>1.512</td>
<td>7.374</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TA($f$CO$_2$, DIC)</td>
<td>high</td>
<td>2290–2430</td>
<td>–2.509</td>
<td>6.618</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TA($f$CO$_2$, DIC)</td>
<td>low</td>
<td>2100–2290</td>
<td>3.442</td>
<td>6.692</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TA($f$CO$_2$, DIC)</td>
<td>all</td>
<td>2100–2430</td>
<td>–1.433</td>
<td>7.013</td>
</tr>
<tr>
<td>W1108C</td>
<td></td>
<td>TA(pH, DIC)</td>
<td>high</td>
<td>2290–2430</td>
<td>5.795</td>
<td>3.448</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TA(pH, DIC)</td>
<td>low</td>
<td>2100–2290</td>
<td>3.804</td>
<td>4.326</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TA(pH, DIC)</td>
<td>all</td>
<td>2100–2430</td>
<td>4.176</td>
<td>4.246</td>
</tr>
</tbody>
</table>

4.4.3 Comparison of Field and Laboratory Measurements

In the laboratory study of Lueker et al.,$^{23}$ differences between measured and calculated $f$CO$_2$ values (calculated from DIC and TA) were determined over a range of temperatures. For $f$CO$_2 < 500 \mu$atm, Lueker et al.$^{23}$ observed good agreement between calculated and measured values ($0.07 \pm 0.50 \%$). For $f$CO$_2 > 500 \mu$atm, they obtained positive residuals that were on average equal to $3.35 (\pm 1.22)\%$. For the highest $f$CO$_2$ values (1300–1700 \muatm), the average difference was $1.3 (\pm 2.4)\%$. The apparent $f$CO$_2$ dependence of the residuals could not be explained by the authors, but the variability seen in replicate measurements at high $f$CO$_2$, even in a highly controlled study, indicates the challenges of achieving internal consistency for high-$f$CO$_2$ (low-pH) waters with a low buffer capacity. To the extent that the $f$CO$_2$, DIC, and TA measurements of Lueker et al.$^{23}$ are accurate, it could be concluded that the thermodynamic model ($K'_1$ and $K'_2$, etc.) used in that work includes systematic errors.

For direct comparison with the $f$CO$_2$ residuals reported by Lueker et al.$^{23}$ we calculated GOMECC-2 $f$CO$_2$ residuals as percentages (Figure 4.7a, Table 4.4). The average of the $f$CO$_2$ –
$f\text{CO}_2$(DIC, TA) residuals is $-0.58$ ($\pm 3.45$)%. Thus the overall internal consistency of the GOMECC-2 DIC-TA-$f\text{CO}_2$ measurements is excellent, even without any consideration of (i.e., correction for) TA contributions from organic bases. For waters where measured $f\text{CO}_2 < 500$ µatm, the average residual was $-1.03$ ($\pm 2.65$)%; where $f\text{CO}_2 > 500$ µatm, the average residual was $-0.17$ ($\pm 4.02$)%.

While our DIC, TA, and $f\text{CO}_2$ field measurements show good internal consistency, the results using DIC, TA, and pH show better agreement with the laboratory relationships between DIC, TA, and $f\text{CO}_2$ observed by Lueker et al.$^{23}$ (see below).

Figure 4.7. (a) $f\text{CO}_2$ residuals calculated as $[f\text{CO}_2 - f\text{CO}_2$(DIC, TA)$] \times 100/f\text{CO}_2$. (b) $f\text{CO}_2$ residuals calculated as $[f\text{CO}_2$(pH, DIC) $- f\text{CO}_2$(DIC, TA)$] \times 100/f\text{CO}_2$(pH, DIC).

We also examined the correspondence between the cruise-based DIC-TA-$f\text{CO}_2$(pH, DIC) and laboratory-based DIC-TA-$f\text{CO}_2$ relationships of Lueker et al.$^{23}$—this time using the pH$_T$ values measured at sea to obtain calculated $f\text{CO}_2$ values. Specifically, we looked at the differences between $f\text{CO}_2$(pH, DIC) and $f\text{CO}_2$(DIC, TA). For waters where $f\text{CO}_2$(pH, DIC)$ < 500$ µatm, the calculated mean residuals were $-0.45$ ($\pm 2.66$) % (GOMECC-2) and $0.77$ ($\pm 2.45$) % (W1108C), similar to what was reported in Lueker et al.$^{23}$ (i.e., $0.07 \pm 0.5$ %) but with
substantially higher standard deviations seen in the field data. For waters where \( f_{CO_2}(pH, DIC) > 500 \text{ µatm} \), the calculated mean residuals were 2.23 (±3.94)% (GOMECC-2) and 3.04 (±2.71)% (W1108C). These results are quite similar to those reported by Lueker et al.\textsuperscript{23} (i.e., 3.35 ±1.22 %). As such, \( f_{CO_2} \) predictions involving field-measured pH and DIC are consistent with the laboratory-based relationships between \( f_{CO_2} \), DIC, and TA determined by Lueker et al.\textsuperscript{23} These observations may point to a potential issue with the measured \( f_{CO_2} \) values from GOMECC-2, with negative residuals suggesting a possible loss of CO\(_2\) before or during the \( f_{CO_2} \) measurements. It is also possible that these findings indicate that both the GOMECC-2 pH measurements and the Lueker et al.\textsuperscript{23} \( f_{CO_2} \) measurements are problematic. Improvements in field measurements of pH and \( f_{CO_2} \) (e.g., high precision) have made it possible to discern these subtle trends and inconsistencies, but it must also be emphasized that the observed inconsistencies are generally within the statistical uncertainty created by the DIC-TA pairing (Table 4.4).

Table 4.4. Comparison of \( f_{CO_2} \) residuals (as %) from Lueker et al. (2000) with GOMECC-2 (\( (f_{CO_2} - f_{CO_2}(DIC, TA)) \)) and W1108C and GOMECC-2 (\( (f_{CO_2}(pH, DIC) - f_{CO_2}(DIC, TA)) \)).

<table>
<thead>
<tr>
<th>Data</th>
<th>Numerical Range Viewed</th>
<th>Calculation</th>
<th>%Residual Mean</th>
<th>%Residual SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOMECC-2</td>
<td>&gt;500 µatm</td>
<td>( f_{CO_2} - f_{CO_2}(DIC, TA) )</td>
<td>-0.17</td>
<td>4.02</td>
</tr>
<tr>
<td>Lueker</td>
<td>&gt;500 µatm</td>
<td>( f_{CO_2} - f_{CO_2}(DIC, TA) )</td>
<td>3.35</td>
<td>1.22</td>
</tr>
<tr>
<td>GOMECC-2</td>
<td>&gt;500 µatm</td>
<td>( f_{CO_2}(pH, DIC) - f_{CO_2}(DIC, TA) )</td>
<td>2.23</td>
<td>3.94</td>
</tr>
<tr>
<td>W1108C</td>
<td>&gt;500 µatm</td>
<td>( f_{CO_2}(pH, DIC) - f_{CO_2}(DIC, TA) )</td>
<td>3.04</td>
<td>2.71</td>
</tr>
<tr>
<td>GOMECC-2</td>
<td>&lt;500 µatm</td>
<td>( f_{CO_2} - f_{CO_2}(DIC, TA) )</td>
<td>-1.03</td>
<td>2.65</td>
</tr>
<tr>
<td>Lueker</td>
<td>&lt;500 µatm</td>
<td>( f_{CO_2} - f_{CO_2}(DIC, TA) )</td>
<td>0.07</td>
<td>0.50</td>
</tr>
<tr>
<td>GOMECC-2</td>
<td>&lt;500 µatm</td>
<td>( f_{CO_2}(pH, DIC) - f_{CO_2}(DIC, TA) )</td>
<td>-0.45</td>
<td>2.66</td>
</tr>
<tr>
<td>W1108C</td>
<td>&lt;500 µatm</td>
<td>( f_{CO_2}(pH, DIC) - f_{CO_2}(DIC, TA) )</td>
<td>0.77</td>
<td>2.45</td>
</tr>
<tr>
<td>GOMECC-2</td>
<td>all</td>
<td>( f_{CO_2} - f_{CO_2}(DIC, TA) )</td>
<td>-0.58</td>
<td>3.45</td>
</tr>
</tbody>
</table>
4.4.4 Influence of Dissociation Constants

Differences between measured vs. calculated values of \( f_{CO_2} \) have been observed in other investigations of high \( f_{CO_2} \) waters,\textsuperscript{30-32} but the trends and magnitudes of the differences differed for different campaigns and depended on the dissociation constants that were used for the calculations. To examine the effect of choosing one published set of dissociation constants over another, we calculated residuals for \( f_{CO_2} - f_{CO_2}(pH, DIC) \) using the GOMECC-2 data and a variety of dissociation constants (Figure 4.8).

![Figure 4.8. Comparison of GOMECC-2 \( f_{CO_2} - f_{CO_2}(pH, DIC) \) residuals calculated using different dissociation constants.](image)

The residuals calculated from the Dickson and Millero\textsuperscript{33} and Mojica Prieto and Millero\textsuperscript{34} constants showed very little difference relative to residuals calculated from Lueker et al.\textsuperscript{23} The constants of Millero et al.\textsuperscript{35} and Millero\textsuperscript{36} resulted in the most negative residuals. Results obtained using the algorithm of Millero et al.\textsuperscript{31} exhibited the most distinctive distribution of residuals by being the only choice that produced positive residuals. Overall, possible improvements in residuals were indicated only for the constants of Millero et al.\textsuperscript{31} This choice,
however, produced improved residuals at high CO$_2$ fugacity but worse residuals at low CO$_2$
fugacity (especially when expressed as percentages). The results shown in Figure 4.8 provide no
evidence that improved thermodynamic consistency would be achieved by an alternative to the
dissociation constants Lueker et al.$^{23}$ The clear trend in $f$CO$_2$ residuals ($f$CO$_2$ – $f$CO$_2$(pH, DIC))
at high $f$CO$_2$ (Figure 4.8) might be attributable to an experimental artifact such as CO$_2$ loss
during analysis of $f$CO$_2$ or incomplete equilibration. These possibilities should be investigated in
future experiments conducted under controlled laboratory conditions.

4.5 Implications

An examination of the internal thermodynamic consistency of CO$_2$ system measurements
obtained on two extensive coastal cruises indicates that redundant high-quality measurements
(i.e., three or more parameters among DIC, TA, pH, and $f$CO$_2$) are essential. Only two measured
parameters are required to calculate all others, but three or more measured parameters are
required to resolve consistency issues. Furthermore, since different subsets of the four measured
parameters produce different outcomes for derived parameters, evaluations based on all four
measurable parameters are especially useful for optimizing CO$_2$ system characterizations:

(A) The calculation of in situ aragonite saturation states (which cannot be directly
measured at present) is sensitive to which pair of measured CO$_2$ system parameters is selected as
input for the calculation. Near the saturation horizon (where $\Omega_A = 1$), differences in $\Omega_A$
calculated according to different pairings can be on the order of $\pm10\%$. Associated uncertainties
in the estimated depth of the saturation horizon can be 100 m or more in coastal deep waters.
This uncertainty is exacerbated when the slope of saturation state as a function of depth is large.
Over a period of two decades the open ocean ASH has reportedly shoaled 25–155 m in the
Pacific Ocean,$^{18}$ and 16–124 m in the Indian Ocean.$^{37}$ Since the onset of the Industrial
Revolution, it has been reported to have shoaled an estimated 100–200m in the Arabian Sea and Bay of Bengal, and 80–150 m in the high-latitude Atlantic Ocean. The large range of these estimates for ASH shoaling can be narrowed in the future through use of parameter pairings other than (DIC, TA). Precise estimates of saturation depths require that either pH or $f$CO$_2$ be used in the calculations. Developing methods to directly measure in situ aragonite saturation states (i.e., evaluation of accuracy) is essential.

(B) Measurements of the (DIC, TA) pair are common because commercial instrumentation is available and samples can be conveniently preserved. However, the use of this pair in CO$_2$ system calculations adversely affects precision and introduces the potential for adverse impacts on accuracy. Calculations involving TA as an input parameter include the assumption that all contributors to alkalinity in a sample are known and have well characterized equilibrium behavior. In the presence of substantial organic base concentrations this expectation is rarely satisfied. Techniques to directly determine the concentrations and protonation characteristics of organic bases in seawater need to be developed.

It should be noted that in the presence of high organic base concentrations with unknown total concentrations and protonation characteristics, the (DIC, TA) pair can become substantially unreliable for calculations of $f$CO$_2$, pH, and $\Omega_A$. This problem could become especially significant for nearshore waters, such as along the US East or West coasts or in the Arctic Ocean, where large organic-rich riverine inputs result in especially high levels of dissolved organics. In these cases, we recommend choosing the (pH, DIC) or ($f$CO$_2$, DIC) pair. Ideally, as many parameters as possible should be measured.

(C) Laboratory observations of DIC–TA–$f$CO$_2$ relationships are inconsistent with DIC–TA–$f$CO$_2$ relationships observed during the GOMECC-2 expedition. For high-fugacity
waters, the field results show better internal agreement than the laboratory results. Comparisons between the work of Lueker et al.\textsuperscript{23} and the $f$CO$_2$-pH-DIC-TA relationships observed in the GOMECC-2 data show a small bias in the $f$CO$_2$ measurements. In view of the importance of $f$CO$_2$ measurements in assessments of ocean acidification and the incongruities observed during the GOMECC-2 expedition, it would be useful to continue to compare the characteristics of seagoing measurement systems with the characteristics of identical systems in a controlled laboratory environment.

4.6 Acknowledgements

This work was supported by a U.S. National Science Foundation award (OCE-1220110) and also a NOAA Ocean Acidification Program award (NA11OAR4320199). Partial support for M. Patsavas was provided by the USF Robert M. Garrels Memorial Fellowship and the USF William and Elsie Knight Endowed Fellowship for Marine Science. Advice and insightful comments from Dr. T. Clayton are greatly appreciated. We would like to thank Bo Yang, Wei-Jun Huang, Andrew Joesoef, Xuewu Liu, Regina A. Easley, Yong-Rae Kim and Xiaoling Ding for performing cruise measurements, and Kathleen M. Watson for producing the cruise track images.

4.7 References


CHAPTER FIVE:

PROCEDURES FOR DIRECT SPECTROPHOTOMETRIC MEASUREMENTS OF CARBONATE ION CONCENTRATIONS:
MEASUREMENTS IN GULF OF MEXICO AND EAST COAST US WATERS

5.1 Abstract

Refined procedures were developed for direct determination of carbonate ion concentrations in seawater using spectrophotometric measurements of Pb(II) absorbances in the ultraviolet. Carbonate ion concentrations were calculated from pH and dissolved inorganic carbon (DIC) observations obtained on a large NOAA ocean acidification cruise (GOMECC-2: Gulf of Mexico and East Coast Carbon). These calculated carbonate concentrations, in conjunction with Pb(II) absorbance measurements obtained using the same seawater samples, were used to (a) examine the influence of carbonate on Pb(II) absorbance behavior in the ultraviolet and (b) refine a previous model for direct spectrophotometric determination of carbonate ion concentrations in seawater. The precision of spectrophotometric carbonate measurements is affected by the concentration of the Pb(II) titrant that is added to seawater samples. Doubling the concentration of the titrant improved precision relative to previous published UV procedures but required formulation of a correction for changes in carbonate ion
concentration caused by the titrant addition. Minor changes in published computational algorithms for the spectrophotometric method produced carbonate ion values in good agreement with values calculated from the pH and DIC pair. The new algorithms were tested on three research cruises in the Gulf of Mexico.

5.2 Introduction

Documentation of the chemical changes that accompany ocean acidification is necessary for understanding the long-term changes in biogeochemical processes that are caused by anthropogenic influences on the global carbon cycle. Changes in seawater chemistry that accompany ocean acidification notably include decreases in carbonate ion concentrations. Such changes are likely to substantially influence the life cycles of calcareous organisms.

Carbonate ion concentrations are calculated from any two of the four major CO$_2$ system parameters: pH, fugacity (fCO$_2$), dissolved inorganic carbon (DIC), or total alkalinity (TA). Byrne and Yao pointed out that carbonate ion concentrations ([CO$_3^{2-}$]$_T$) can be directly measured via spectrophotometric observations of lead carbonate complexation in seawater. Spectrophotometric carbonate ion determinations are analogous to pH measurements and are equally rapid and robust. Spectrophotometric methods are advantageous due to the simplicity of the measurements, and the comparatively low cost of the required instrumentation. Easley et al. demonstrated that minor changes to preexisting computational algorithms could result in improved agreement between spectrophotometrically measured [CO$_3^{2-}$]$_T$ and [CO$_3^{2-}$]$_T$ calculated from pH and DIC. In this work we present procedures that improve upon the original spectrophotometric methods of Byrne and Yao and the methods of Easley et al. A more concentrated titrant provides increased free lead concentrations and improved signal to noise ratios. The original algorithm of Byrne and Yao is modified using field data obtained in the
Gulf of Mexico and east coast waters of the United States. The accuracy of the updated algorithm is then evaluated using data obtained on three separate cruises in the Gulf of Mexico.

5.2.1 Analytical Background

Direct measurements of $[\text{CO}_3^{2-}]_T$ in seawater can be obtained via observations of lead carbonate complexation spectra in the ultraviolet. Ratios ($R$) of absorbances ($A$) at two wavelengths (234 and 250 nm) are used in the following algorithm to calculate $[\text{CO}_3^{2-}]_T$:

$$ R = \frac{250 A}{234 A} = \frac{250 \varepsilon_{\text{Pb}} + 250 \varepsilon_{\text{PbCO}_3, \text{CO}_3} \beta_i [\text{CO}_3^{2-}]_T}{234 \varepsilon_{\text{Pb}} + 234 \varepsilon_{\text{PbCO}_3, \text{CO}_3} \beta_i [\text{CO}_3^{2-}]_T} $$  \hspace{1cm} (5.1)

which can be rearranged as:

$$ -\log[\text{CO}_3^{2-}]_T = \log \left( \frac{\text{CO}_3 \beta_i}{e_2} \right) + \log \left( \frac{R - e_1}{1 - R \frac{e_1}{e_2}} \right) $$  \hspace{1cm} (5.2)

The parameters $\log(\beta_i/e_2)$, $e_1$, and $e_3/e_2$ are dependent on salinity, and are expressed as follows:

$$ e_1 = \frac{250 \varepsilon_{\text{PbCO}_3}}{234 \varepsilon_{\text{PbCO}_3}}, e_2 = \frac{250 \varepsilon_{\text{Pb}}}{234 \varepsilon_{\text{PbCO}_3}}, e_3 = \frac{234 \varepsilon_{\text{Pb}}}{250 \varepsilon_{\text{Pb}}} $$  \hspace{1cm} (5.3)

and the stability constant for formation of PbCO$_3^0$ is given as\(^8\)
\[ \text{log}(\beta_1/e_2) = -6.0 + 0.33 \times \frac{[\text{PbCO}_3^2]}{[\text{Pb}^{2+}]_T[\text{CO}_3^{2-}]_T} \]

where \([\text{Pb}^{2+}]_T\) represents the total concentration of all non-carbonate lead species (i.e. \(\text{Pb}^{2+}\), \(\text{PbCl}_x\)).

The \(e_3/e_2\) ratio is obtained in solutions with low pH where no influence from \(\text{CO}_3^{2-}\) complexation is observed\(^{12}\) and is directly measured as a quotient of Pb(II) absorbances \((e_3/e_2 = 234\text{A}/250\text{A})\). The \(e_1\) and \(\text{log}(\beta_1/e_2)\) parameters are determined through iterative analyses of Pb(II) spectra over a range of carbonate ion concentrations.

5.3 Methods

5.3.1 GOMECC-2 Cruise Measurements

The Gulf of Mexico East Coast Carbon (GOMECC-2) study was performed over a period of 23 days on board the NOAA Ship *Ronald H. Brown* in the Gulf of Mexico and along the east coast of the United States (Figure 5.1). During GOMECC-2, spectrophotometric \([\text{CO}_3^{2-}]_T\), pH, DIC, TA, \(f\text{CO}_2\), nutrients, salinity, temperature, and depth were measured. Measurement procedures and thermodynamic relationships for these parameters (excluding spectrophotometric \([\text{CO}_3^{2-}]_T\)) are given in Dickson.\(^{18}\) The pH indicator used on GOMECC-2 was purified meta-Cresol Purple (mCP).\(^{19-21}\) GOMECC-2 data sets that did not contain the full suite of CO\(_2\) parameters (pH, DIC, TA, \(f\text{CO}_2\)) were not used in this work, resulting in a sample set that contained 885 observations of pH, DIC, TA and \(f\text{CO}_2\).

5.3.2 Spectrophotometric \([\text{CO}_3^{2-}]_T\) Field Measurements

Direct spectrophotometric measurements of \([\text{CO}_3^{2-}]_T\) were performed using procedures similar to those outlined in Byrne and Yao\(^{12}\) and Easley et al.\(^{17}\) Sample seawater was collected in
10 cm quartz spectrophotometer optical cells without contact with the atmosphere. The cells were capped with Teflon stoppers to prevent air exchange. The cells were warmed in a custom aluminum heat-exchanger attached to a recirculating water bath maintained at 25 ± 0.01°C. After the samples were equilibrated to 25°C, each individual cell was transferred to a custom water-jacketed cell holder of an Agilent 8453 UV-Vis Spectrophotometer. A reference spectrum was recorded, and then 20 µL of 0.022M lead perchlorate (PbClO₄) was added to the sample cell (final concentration: 1.52x10⁻⁵ mol kg⁻¹) and six absorbance spectra were recorded and averaged. It should be noted that both Byrne and Yao¹² and Easley et al.¹⁷ used lead chloride as the titrant. In this study, lead perchlorate (PbClO₄) (Fisher Scientific, 99.99% purity), which is more soluble than lead chloride, was used as the titrant, doubling the lead concentrations of the analyzed seawater samples.

5.3.3 Parameterization Procedure

CO₂ system calculations were made using CO2SYS version 2.1.²² Because spectrophotometric pH is determined on the total hydrogen ion scale,¹⁹ the dissociation constants of Lueker et al.²³ were used. The other selected parameters and relationships included K_{HSO₄} from Dickson et al.²⁴ and the total boron to salinity ratio from Lee et al.²⁵ After obtaining calculated [CO₃²⁻]ₜ values from DIC and pH, Eq. 5.2 was fit to the calculated [CO₃²⁻]ₜ values with SigmaPlot software. The best fit parameters in this analysis were e₁ and log(β₁/e₂), and the e₃/e₂ parameterization was taken directly from Byrne and Yao¹². Both fitted parameters were expressed as quadratic functions of salinity at 25°C.

5.3.4 Verification of Results through Independent Tests in the Gulf of Mexico

Three Gulf of Mexico (GOM) cruise data sets were used to independently test the new [CO₃²⁻]ₜ measurement algorithm. The datasets used in this work were obtained in February
(N=44), May (N=49), and August (N=25) of 2013 (Figure 5.1). Carbonate ion concentrations ([CO$_3^{2-}$]$_T$), pH, and DIC were measured and calculated with procedures that were identical to those used during the GOMECC-2 cruise.

Figure 5.1. GOMECC-2 cruise track in green. February, May, August 2013 GOM repeat cruise tracks are shown in red.

5.3.5 CO$_2$ System Perturbation

The addition of lead in seawater causes a perturbation of each sample’s CO$_2$ system due to complexation of added lead by carbonate ions present in seawater. This perturbation is
accounted for using a theoretical model based on initial parameters, TA = 2250 µmol kg⁻¹ and S=35, over a range of pH. The complexation of lead and carbonate is represented as:

\[
Pb^{2+} + CO_3^{2-} \rightleftharpoons PbCO_3^0
\]  

5.5

The total lead in solution (Pb_T) is known by volumetric addition and is a summation of the carbonate and non-carbonate species of lead:

\[
Pb_T = [Pb^{2+}]_T + [PbCO_3^0]
\]  

5.6

Equations 5.4, 5.5, and 5.6 can be rearranged to describe the concentration of PbCO₃ in solution:

\[
[PbCO_3^0] = \frac{Pb_T \cdot \beta [CO_3^{2-}]_T}{1 + \beta [CO_3^{2-}]_T}
\]  

5.7

The total alkalinity (TA) is given as:

\[
TA = [HCO_3^-]_T + 2[CO_3^{2-}]_T + [B(OH)_4^-]_T + [OH^-]_T + [HPO_4^{2-}]_T + 2[PO_4^{3-}]_T + [SiO(OH)_3^-]_T - [H^+]_T - [H_3PO_4^0]
\]  

5.8

and the dissolved inorganic carbon (DIC) is given as:

\[
DIC = [CO_2^*] + [HCO_3^-]_T + [CO_3^{2-}]_T
\]  

5.9
where $[\text{CO}_2^*] = [\text{CO}_2] + [\text{H}_2\text{CO}_3]$.

In the presence of lead, TA and DIC can be written in terms of contributions exclusive of PbCO$_3$ (i.e., TA' and DIC') plus the direct contributions from PbCO$_3$:

$$TA' = TA - 2[\text{PbCO}_3^0]$$

$$DIC' = DIC - [\text{PbCO}_3^0]$$

Initial $[\text{CO}_3^{2-}]_T$ and DIC values were calculated assuming TA=2250 $\mu$mol kg$^{-1}$ and a range of pH values between 7.0 to 8.5 (all at $S=35$ and $T=25^\circ$C). The PbCO$_3^0$ concentration ([PbCO$_3^0$]) was calculated via Eq. 5.7 using the PbCO$_3^0$ formation constant ($\text{CO}_3^\beta_1$) from Byrne and Yao$^{12}$ ($\log(\text{CO}_3^\beta_1) = 4.106$ at $S=35$). The total lead concentration was 1.52x10$^{-5}$ mol kg$^{-1}$ (identical to the final concentration of lead in seawater during spectrophotometric carbonate ion measurements). TA’ and DIC’ were then determined from Eqs. 5.10 and 5.11. The TA’ and DIC’ were subsequently entered in CO2SYS as the starting parameters in order to refine the carbonate ion concentration ([CO$_3^{2-}]_T$). The calculations of [PbCO$_3^0$], TA' and DIC' were repeated iteratively until TA' and DIC' stabilized within 10$^{-12}$ mol kg$^{-1}$. The change in the carbonate ion concentration caused by the addition of lead was determined over a range of carbonate ion concentrations, and corresponding changes in $R$-ratios were calculated from Eq. 5.2 using the parameterization of Byrne and Yao.$^{12}$ The absorbance ratio perturbation relationship obtained using this procedure (see Results) was applied to all measured absorbance ratios to convert each measured absorbance ratio to a value that would have been obtained at Pb(II) concentrations too low to create carbonate system perturbations.
5.4 Results and Discussion

5.4.1 Perturbation Systematics

The equation that describes the titrant-induced perturbation of the $R$-ratio is given as:

$$\log(R_p - R_i) = -17.6664 R_p^2 + 19.8995 R_p - 7.7324$$

where $R_p$ is the perturbed (measured) absorbance ratio, and $R_i$ is the initial $R$-ratio before the system was perturbed (Figure 5.2). The $R$-ratios obtained during field measurements ($R_p$) must be converted to conjugate $R_i$ values for calculation of carbonate ion concentrations. Users can input the field-measured $R_p$ into Eq. 5.12 and solve for $R_i$. Altering the initial TA ($2150 \leq TA \leq 2450 \, \mu$mol kg$^{-1}$) and $S$ ($20 \leq S \leq 40$) had an effect on the resulting perturbation equation smaller than the absorbance ratio measurement precision ($\pm 0.001$). As such, the theoretical perturbation model at $TA=2250$ and $S=35$ is sufficient for $[\text{CO}_3^{2-}]_T$ measurements in typical seawater at $25^\circ C$ and $20 \leq S \leq 40$.

![Figure 5.2. Modeled perturbation in absorbance ratio caused by addition of lead to seawater](image-url)
5.4.2 New Lead Carbonate Formation Constant and Molar Absorptivity Ratios

The log($\beta_1/e_2$) and $e_1$ relationships obtained via the iterative use of GOMECC-2 carbonate data in equations 5.2 and 5.3 produced the following relationships:

$$\log(CO_3 \beta_1 / e_2) = 5.507074 - 0.041259S + 0.000180S^2 \quad 5.13$$

$$e_1 = 0.311907 - 0.002396S + 0.000080S^2 \quad 5.14$$

with:

$$e_3 / e_2 = 3.061 - 0.0873S + 0.000936S^2 \quad 5.15$$

and it is noted that Eq. 5.15 is identical to Eq. 23 of Byrne and Yao.\textsuperscript{12}

Use of equations 5.13, 5.14 and 5.15 in the procedure that was used to produce equation 5.12 led to insignificant differences in the perturbation equation. Consequently, equations 5.12 through 5.15 are internally consistent. Figure 5.3 shows that observed residuals ($\Delta[CO_3^{2-}]_T = \text{calculated } [CO_3^{2-}]_T - \text{measured } [CO_3^{2-}]_T$) for the entire GOMECC-2 data set are centered about zero for the whole range of carbonate ion concentrations encountered in the study.

The work of Easley et al.\textsuperscript{17} produced estimates of $e_3/e_2$ that differed from those of Byrne and Yao\textsuperscript{12}. Since $e_3/e_2$ is well defined experimentally through simple observations of absorbance ratios in solutions with low pH, and ancillary experiments have shown that the $e_3/e_2$ values of Byrne and Yao\textsuperscript{12} are quite reproducible, use of the $e_3/e_2$ from Byrne and Yao\textsuperscript{12} is preferred relative to the $e_3/e_2$ parameterizations of Easley et al.\textsuperscript{17} Subsequently, the present study showed that only minor changes in $e_1$ and log($\beta_1/e_2$) are required to obtain agreement with conventional calculations of $[CO_3^{2-}]_T$ from DIC and pH. Finally, although investigations of lead complexation in synthetic solutions\textsuperscript{26} suggest that high concentrations of $[CO_3^{2-}]_T$ in seawater at high pH might necessitate an expansion of the Pb(II) absorbance model to include the second carbonate
complex of lead ($\text{Pb}($CO$_3$)$_2^2$), the results obtained in the present work indicate that a single carbonate species model was sufficient. The $r^2$ of the spectrophotometrically determined $[\text{CO}_3^{2-}]_T$ vs. calculated $[\text{CO}_3^{2-}]_T$ from DIC and pH was 0.999.

Figure 5.3. GOMECC-2 residuals fit.

5.4.3 Parameter Comparisons

Figure 5.4 shows each of the parameters in Eq. 5.2 obtained in this work and in previous studies. Results are plotted over the salinity range encountered during GOMECC-2. Each of the parameters obtained by Easley et al.\textsuperscript{17} is offset from the corresponding result of Byrne and Yao\textsuperscript{12} by an additive constant. In other words, the slopes and curvature of the plots of $e_1$, log($\beta_1/e_2$) and
are identical in the models of Easley et al.\textsuperscript{17} and Byrne and Yao\textsuperscript{12}. In the present work, \(e_1\) and \(\log(\beta_1/e_2)\) are fully optimized the using high quality GOMECC-2 field data, without altering the directly determined Byrne and Yao\textsuperscript{12} values of \(e_3/e_2\).

### 5.4.4 Field Tests of the New Model

Three Gulf of Mexico cruise datasets (February, May, and August, 2013) were used to test the algorithm developed from the GOMECC-2 data. Measurements of spectrophotometric pH (with purified mCP), along with DIC, and spectrophotometric \([\text{CO}_3^{2-}]_T\) allowed calculation of carbonate residuals (\(\Delta[\text{CO}_3^{2-}]_T = \text{calculated} \ [\text{CO}_3^{2-}]_T - \text{measured} \ [\text{CO}_3^{2-}]_T\)). The resulting residuals for these cruises are shown in Figure 5.5.

The mean and standard deviation of the residuals were -0.638 ± 5.230 µmol kg\(^{-1}\) (February), -1.246 ± 2.964 µmol kg\(^{-1}\) (May), and -0.782 ± 8.450 µmol kg\(^{-1}\) (August). For the entire dataset, the mean and standard deviation of the residuals were -0.921 ± 5.327 µmol kg\(^{-1}\).

Residuals were fit with linear regressions and had near zero slopes and small intercepts. The new algorithm produced \([\text{CO}_3^{2-}]_T\) values that are consistent with \([\text{CO}_3^{2-}]_T\) values calculated from pH and DIC for three cruises, and are independent of the dataset used to obtain Eqs 5.13 – 5.15.

### 5.5 Implications

The algorithms presented in this work allow for direct spectrophotometric determinations of \([\text{CO}_3^{2-}]_T\) that are in good agreement with calculated \([\text{CO}_3^{2-}]_T\) from the pH and DIC pair. Doubling the concentration of the lead perchlorate reagent improved the signal to noise ratio and therefore improved precision. A correction was introduced to account for the perturbation caused by addition of lead to the system. The simple nature of spectrophotometric pH and \([\text{CO}_3^{2-}]_T\) measurements allows for rapid and inexpensive assessments of the entire CO\(_2\) system.
Figure 5.4. (a) $e_1$, (b) $e_3/e_2$, and (c) $\log(\beta_1/e_2)$ vs. salinity calculated from Byrne and Yao\textsuperscript{12} (black dots), Easley et al.\textsuperscript{17} (green triangles), and this work (red dots).
Figure 5.5. Carbonate ion residual vs. calculated carbonate ion concentrations for February, May, and August 2013 Gulf of Mexico cruises calculated via the new algorithm presented in this work.

For example: One cruise track line of GOMECC-2 consisted of 10 stations each with 24 samples. If calculations of the CO$_2$ system were made with the DIC and TA pair, it would have required two people working for 20 hours to analyze the DIC and TA samples (~5 min per sample). In contrast, two people could easily perform the pH and [CO$_3^{2-}$]$_T$ measurements (~2 min per sample) in 8 hours.

Both pH and [CO$_3^{2-}$]$_T$ measurements can be obtained using relatively inexpensive spectrophotometers and uncomplicated methods. Use of this pair to calculate the other CO$_2$ system parameters could be beneficial when resources and time are limited.

Because the GOMECC-2 dataset is limited to coastal Gulf of Mexico and waters of the US east coast, future work will include measurements in other areas to ensure wider applicability.
of the new algorithm. To date no other carbon cruise dataset contains absorbance ratios that were obtained with lead perchlorate as the titrant. Because the $[\text{CO}_3^{2-}]_T$ measurement method is simple and fast, a natural extension of this work is development of an autonomous in-situ $[\text{CO}_3^{2-}]_T$ sensor. Autonomous platforms that support indicator-based spectrophotometric measurements at visible wavelengths (e.g. pH, DIC, $\text{fCO}_2$)\(^{16,27}\) can be modified to perform measurements in the UV.

5.6 Acknowledgements

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5.7 References


CHAPTER SIX:

FUTURE WORK

6.1 Indicator Impurities

Chapter two of this dissertation provides procedures for purifying meta Cresol Purple (mCP) and Cresol Red (CR) on a bulk scale. These indicators are suitable for obtaining surface to deep pH profiles in seawater. Procedures have not yet been developed for purification of other indicators that are used for routine spectrophotometric carbon system measurements. Indicators yet to be purified include bromocresol green that is used for total alkalinity (TA) measurements, bromocresol purple that is used for both total alkalinity and dissolved inorganic carbon (DIC) measurements, and phenol red that is used for carbon dioxide fugacity measurements. A useful extension of the work described in chapter two would be purification of other routinely used indicators and characterization of their physical-chemical properties (following the procedures outlined in chapters two and three).

In order to obtain accurate in-situ measurements, the effects of pressure on indicator absorption characteristics needs to be studied. To date such characterizations include only the thymol blue and mCP indicator dyes.
6.2 Internal Consistency

Chapter four involves internal consistency analyses for carbon system measurements obtained on two large ocean acidification cruises. One unique aspect of this work is that the carbon system datasets included spectrophotometric pH measurements that were obtained with purified mCP – no other internal consistency study has involved pH measurements that were obtained with molecularly-characterized purified indicators. Because overall internal consistency was not observed, it can be concluded that overdetermination of the carbon system (i.e., measuring three or more parameters) should be routinely undertaken in order to address the causes of thermodynamic inconsistency in CO$_2$ system analyses. Thermodynamic comparisons should include both high quality laboratory measurements and high quality field measurements.

Quantifying the contributions of organic bases to total alkalinity determinations is necessary for CO$_2$ system calculations that involve total alkalinity as an input parameter.$^8$ This is likely to be important for measurements of coastal seawater.$^{9,10}$

To date, the accuracy of spectrophotometric pH measurements is tied to the accuracy of TRIS buffered synthetic seawater (mCP is tied to TRIS$^3$ and CR is tied to mCP$^2$). The gold-standard of pH measurements is obtained via a hydrogen electrode. Testing the accuracy of the spectrophotometric pH measurements via paired observations of pH obtained with purified indicators and a hydrogen electrode would remove uncertainties introduced by the TRIS buffer preparation.

DIC, TA, and carbon dioxide fugacity samples are routinely poisoned with mercuric chloride to halt photosynthesis and respiration,$^{11}$ while pH samples are generally not poisoned, but are measured promptly.$^3,12$ The effects of and effectiveness of mercuric chloride additions to samples should be investigated, especially for biologically productive waters.
6.3 Carbonate Ion Measurements

Direct spectrophotometric measurements of carbonate ion concentrations ([CO$_3^{2-}$]$_T$) are tied to the accuracy of measured pH and DIC. Only four cruise datasets, all in the same region, are currently available where pH was measured using purified mCP and [CO$_3^{2-}$]$_T$ was measured using lead perchlorate as the titrant. Ideally, the computational algorithms for converting field-measured Pb(II) absorbance ratios to carbonate ion concentrations should be linked to [CO$_3^{2-}$]$_T$ calculated from pH and DIC with datasets obtained in many oceanographic regions. It is important to demonstrate that the computational algorithms that allowed successful calculation of [CO$_3^{2-}$]$_T$ from UV spectra of Pb(II) in the GOM and along the US east coast, are universally applicable.

Saturation state is calculated from calcium and carbonate ion concentrations.$^{13}$ Calcium is estimated from salinity,$^{14}$ and recent advances allow for carbonate ion concentrations to be directly measured via UV spectroscopy.$^{15,16}$ Development of an in-situ carbonate ion sensor (and therefore saturation state sensor) is underway. The temperature dependences of all parameters used in [CO$_3^{2-}$]$_T$ computational algorithms will need to be determined in order to obtain in-situ measurements of saturation states.

6.4 References


APPENDIX A:

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APPENDIX B:

SUPPLEMENTAL SATURATION STATE CALCULATIONS

Figure B.1 shows $\Delta \Omega_A$ calculated by (DIC, TA) – (pH, DIC) for W1108C. The black line is the linear regression. Regression statistics are shown in Table B.1. The W1108C residuals have a smaller slope (-0.0074) compared to the GOMECC-2 residuals calculated in the same manner (-0.0243). The residual sum of squares, a reflection of scatter, is improved by a factor of three on W1108C compared to GOMECC-2. The improved slope and residual sum of squares indicates better internal consistency for the W1108C dataset vs. GOMECC-2 and suggests a discrepancy in measurement ability between research groups on two NOAA ocean acidification cruises. Figure B.2 shows the same W1108C residual $\Omega_A$ as in Figure B.1 overlaid on the GOMECC-2 residual $\Omega_A$ from Figure 4.4a. The higher amount of scatter in the GOMECC-2 $\Omega_A$ (standard deviation: ±0.0608) compared to the W1108C residual $\Omega_A$ (standard deviation: ±0.0308) is clearly visible when presented this way.
Figure B.1. $\Delta \Omega_A$ calculated by (DIC, TA) – (pH, DIC) for W1108C. Black line is linear regression. Supplement to Figure 4.4 of dissertation.

Figure B.2. $\Delta \Omega_A$ calculated by (DIC, TA) – (pH, DIC) for GOMECC-2 (black dots) and W1108C (red dots).
Table B.1. Regression statistics for Figure B.1 and Figure 4.4a.

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<td>GOMECC-2 Ω*(DIC, TA) –</td>
<td>–0.0243</td>
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<td>(Figure 4.4a)</td>
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<tr>
<td>W1108C Ω*(DIC, TA) –</td>
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<td>0.002</td>
<td>0.931</td>
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<tr>
<td>(Figure B.1)</td>
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Figure B.3 shows how measurement imprecision affects calculated saturation states. To construct the Figure B.3, saturation state was calculated using various parameter pairs at constant salinity and temperature (S=35, T=25°C). The value of one parameter pair was increased by typical measurement precision over a range of pH, TA, fCO2 or DIC: pH (+0.001), TA (+4 µmol kg⁻¹), fCO2 (+5 µatm), DIC (+3 µmol kg⁻¹). For pH, TA, and fCO2, DIC was held constant at 2000 µmol kg⁻¹, and for DIC, TA was held constant at 2200 µmol kg⁻¹. Saturation state residuals were calculated by: Ω*(pH, DIC) – Ω*(pH+0.001, DIC), Ω*(TA, DIC) – Ω*(TA+4, DIC), Ω*(fCO2, DIC) – Ω*(fCO2+5, DIC), or Ω*(TA, DIC) – Ω*(TA, DIC+3). At the saturation horizon (Ω=1), the largest residual (≈ 0.03) is created from TA imprecision. Figure B.3 does not account for combinations of imprecision (e.g. only one parameter’s imprecision is investigated at a time). At a given saturation state, the magnitude of the residual could be as large as the summation of the residuals found for each parameter. In other words, at the saturation horizon, if DIC imprecision...
causes a 0.02 residual and TA imprecision causes a 0.03 residual, then the combined residual would be 0.05, assuming the errors are acting in the same direction. The magnitude of the residuals caused by typical measurement imprecision of the (DIC, TA) pair does not account for the larger magnitude observed in Figures 4.4a,b (Figure 4.4a,b maximum $\Omega$ residual $\approx 0.2$ at $\Omega=1$). At the saturation horizon, the pH and $f$CO$_2$ imprecision could impact calculated saturation states by approximately $\pm 0.002$ and $\pm 0.003$, respectively. As such, for the most precise calculations, either pH or $f$CO$_2$ should be used as an input parameter.

Figure B.3. $\Omega_A$ residual vs. $\Omega_A$, where $\Omega_A$ Residual = $\Omega$(pH, DIC) – $\Omega$(pH+0.001, DIC), $\Omega$(TA, DIC) – $\Omega$(TA+4, DIC), $\Omega$(fCO$_2$, DIC) – $\Omega$(fCO$_2$+5, DIC), or $\Omega$(TA, DIC) – $\Omega$(TA, DIC+3). Note the y-axis on Figure B.3 is the same as Figure 4.4 for intercomparison.