Contributions of organic alkalinity to total alkalinity in coastal waters: A spectrophotometric approach

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Abstract

Contributions of organic alkalinity (Org-Alk) to total alkalinity (TA) were investigated in surface waters from three different coastal environments (estuary, urban, mangrove) and offshore sites in the Gulf of Mexico. Δ TA was calculated as the difference between directly measured TA, and TA calculated from total dissolved inorganic carbon (DIC) and pH. In low nutrient surface waters, Δ TA should be dominated by Org-Alk with minor contributions from inorganic nutrients (e.g., HPO_4^{2-} and $\text{SiO}(\text{OH})_3^-$). Average values of Δ TA were $0.1 \pm 5.0 \mu\text{mol kg}^{-1}$ at coastal sites outside the Mississippi-Atchafalaya River Estuary ($n = 17$), $33.6 \pm 18.0 \mu\text{mol kg}^{-1}$ in the Suwannee River Estuary ($n = 17$), $16.0 \pm 25.4 \mu\text{mol kg}^{-1}$ in the Tampa Bay, Caloosahatchee River, and Ten Thousand Islands area ($n = 55$), and $-1.0 \pm 4.9 \mu\text{mol kg}^{-1}$ in offshore waters ($n = 14$) in the northern Gulf of Mexico. In addition to Org-Alk assessments based on Δ TA, procedures were developed for direct spectrophotometric measurements of Org-Alk via titrations of samples that were purged of CO_2 . Two-step titrations of these DIC-free samples consisted of a first titration from pH 4.5 to 6.0 performed using bromocresol purple (BCP), and a second titration, from pH 6.0 to about 8, using cresol red (CR) as the indicator. By diluting all samples, including the offshore reference sample, to a common salinity (the lowest salinity of the coastal samples), borate alkalinity was presumed to be identical for all samples. Org-Alk values were calculated as differences between titration results obtained for coastal samples and the offshore reference sample and, through ancillary nutrient measurements, accounted for alkalinity contributions from silicate and phosphate. The direct titrations confirmed the existence of substantial Org-Alk in coastal samples. Spectrophotometric titration data were also used for model fitting in order to assess the dissociation constants (pK_i) of the organic acids. The pK_i of the organic acids were within the previously reported range for riverine fulvic acids.

Key words: organic alkalinity, spectrophotometric titrations, coastal waters, CO₂ system calculations

1. Introduction

Total alkalinity (TA), pH, dissolved inorganic carbon (DIC), and partial pressure ($p\text{CO}_2$) of carbon dioxide (CO_2) are four parameters used to describe the carbon dioxide system in seawater and freshwaters. Based on characterizations of relevant thermodynamic equilibria, measurements of any two of these parameters allow the other two to be calculated. TA is one of the most frequently measured parameters among these four because it is not affected by air/sea CO_2 exchange (Wolf-Gladrow et al., 2007). TA is defined as “the number of moles of hydrogen ion (H^+) equivalent to the excess of proton acceptors (bases formed from weak acids with dissociation constant $K \leq 10^{-4.5}$ at 25°C and zero ionic strength) over proton donors (acids with $K > 10^{-4.5}$) in one kilogram of sample” (Dickson, 1981). Hydrogen ion acceptors include bicarbonate and carbonate ions (C-Alk), borate ions (B-Alk), phosphate (P-Alk), silicate (Si-Alk), and organic (Org-Alk) species. With the definition given above, TA can be expressed as follows (Cai et al., 1998):

$$\begin{aligned}\text{TA} &= \text{C-Alk} + \text{B-Alk} + \text{P-Alk} + \text{Si-Alk} + \text{Org-Alk}... \\ &= [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + 2[\text{PO}_4^{3-}] + [\text{HPO}_4^{2-}] - [\text{H}_3\text{PO}_4] \\ &+ [\text{SiO}(\text{OH})_3^-] + \text{Org-Alk} + [\text{OH}^-] - [\text{H}^+]... \end{aligned} \quad (1)$$

State-of-the-art software packages for seawater CO_2 system calculations (e.g., CO2SYS) are commonly used to account for the contributions of C-Alk, B-Alk, P-Alk and Si-Alk, (Lewis et al., 1998), and contributions from organic species such as the bases of humic acids are usually assumed to be negligible when TA is used in carbonate system calculations. However, a number of studies have shown that TA contributions from dissolved organic matter (DOM) can be significant, especially in riverine and coastal waters. Cai et al. (1998) developed a potentiometric

titration method to directly determine the Org-Alk of estuarine waters. The method consisted of an initial open-cell potentiometric titration for determination of TA, and a subsequent titration that was performed after (a) CO₂ had been removed from the sample and (b) NaOH was used to return the sample to its initial pH. The study showed significant contributions of humic materials (20–120 µmol·kg⁻¹) to TA in riverine and low-salinity estuarine waters.

Org-Alk has also been measured indirectly as a difference (Δ TA) between TA measured by direct titration (TA_{meas}) and TA calculated (TA_{cal}) from observations of DIC and pH:

$$\Delta TA = TA_{\text{meas}} - TA_{\text{cal}} \quad (2)$$

It is important to note that Δ TA obtained from Eq.2 is equal to Org-Alk plus alkalinity contributions from P-Alk and Si-Alk. However, nutrient concentrations are usually low in surface waters. For example, the values of dissolved phosphorus and silica in surface waters were no more than 0.9 and 11 µmol kg⁻¹, respectively, at the Suwannee River plume (Bledsoe and Philips, 2000), and about 0.7 and 15 µmol kg⁻¹, respectively, in Tampa Bay (Karlen, 2014). At these levels, P-Alk and Si-Alk in combination only contributed about 1 µmol kg⁻¹ to TA, therefore the Δ TA values serve as a good indicator of Org-Alk. Although TA does not vary with changes in temperature, it should be noted that, due to the varying influences of temperature on dissociation constants, the relative proportions of the components of TA (i.e., C-Alk, B-Alk, Org-Alk, etc.) will, in general, be somewhat different at different temperatures. Because TA_{cal} is obtained from pH measured at 25.0 °C, it follows that C-Alk, B-Alk, and Org-Alk all have concentrations appropriate to 25.0 °C. As such, Δ TA calculated with Eq. 2 refers to values at 25.0 °C.

Subsequent to the investigations of Org-Alk by Cai et al. (1998), more recent descriptions of Org-Alk have included measurements of Org-Alk concentrations in both the natural environment and cultivation experiments. The influence of organic acids on the inorganic carbon system in a marine microalgae cultivation medium was observed by Rigobello-Masini and Masini (2001) using potentiometric pH measurements combined with DIC and TA estimations based on closed-cell titrations similar to those of Hernández-Ayón et al. (1999). With the same method, Hernandez-Ayon et al. (2007) reported potential contributions of organic bases to TA in both microalgae cultures (Org-Alk = 400–800 $\mu\text{mol}\cdot\text{kg}^{-1}$) and coastal waters (Org-Alk $\geq 50 \mu\text{mol}\cdot\text{kg}^{-1}$). Muller and Bleie (2008) subsequently used this method on samples from an *in-situ* mesocosm system in coastal waters. Although the Org-Alk values they obtained were relatively low (2–22 $\mu\text{mol kg}^{-1}$), their results showed reproducible trends of increasing Org-Alk over time. Using potentiometric measurements of pH and TA, and non-dispersive infrared (NDIR) measurements of DIC, Hunt et al. (2011) showed that non carbonate alkalinity (NC-Alk) represented a significant buffer component (21–100% of TA) of New England and New Brunswick rivers. With the same method, Hu et al. (2015) compared measured and calculated TA from two coastal cruises in the southern Yellow Sea during the green algae bloom that occurred in the summer of 2008. The average differences (measured minus calculated) for the two cruises were 1116 $\mu\text{mol kg}^{-1}$ and 1039 $\mu\text{mol kg}^{-1}$, respectively. Kuliński et al. (2014) used state-of-the-art techniques (spectrophotometric pH measurements, closed-cell potentiometric titrations of TA, and coulometric measurements of DIC) to study the influence of DOM on the acid-base system of the Baltic Sea. Their results showed Org-Alk in the range of 22–34 $\mu\text{mol}\cdot\text{kg}^{-1}$, corresponding to 1.5–3.5% of TA.

Efforts have also been made to characterize Org-Alk with modeling approaches. By using potentiometric titration data from Satilla River estuarine waters, Cai et al. (1998) studied organic anions in four functional groups (with pK_a values of 4.46, 6.64, 8.94, and ~ 10 , respectively). Using closed-cell potentiometric titration data, Muller and Bleie (2008) identified two classes of titratable organic species in coastal seawaters with pK_a values around 4.0 ± 0.2 and 9.1 ± 0.2 . Kuliński et al. (2014) and Ulfsbo et al. (2015) used a bulk dissociation constant of DOM, K_{DOM} , to represent all weakly acidic functional groups in DOM. By using the K_{DOM} obtained from Baltic Sea field data they formulated an equation to calculate Org-Alk with pH and dissolved organic carbon (DOC) data. Ulfsbo et al. (2015) also used a Humic-Pitzer chemical speciation modeling approach based on the Windermere Humic Aqueous Model (WHAM) VII, to explain the contribution of Org-Alk to TA observed by Kuliński et al. (2014).

The present study is the first to use spectrophotometric titrations for systematic quantification and characterization of organic bases that contribute to TA. Compared with conventional potentiometric titrations, spectrophotometric titrations have the advantages of quick response, high sensitivity, and freedom from liquid junction potential issues created by variations in ionic strength (Easley and Byrne, 2012). As the first step in this investigation, samples from estuaries, urban areas, mangroves, and offshore sites were collected for coulometric measurements of DIC and spectrophotometric measurements of pH and TA. These measurements were used for indirect assessments of Org-Alk based on ΔTA observations (Eq. 2). Subsequently, spectrophotometric titrations were performed on offshore reference and organic-rich coastal samples for direct quantification and characterization of the organic bases. Finally, the full pH range titration data ($4.5 \leq pH \leq 8$) were used for modeling the dissociation constants (pK_i) of the organic acids/bases.

2. Materials and Methods

2.1. Sample collection

Eighty-six surface samples with $S > 20$ were collected in Gulf of Mexico coastal waters (Fig. 1). Sampled areas included estuaries of the Mississippi-Atchafalaya River, the Suwannee River, the Caloosahatchee River, urban estuaries (Tampa Bay and Old Tampa Bay), and mangrove areas (Tampa Bay and Ten Thousand Islands). Surface and water column samples were taken from three offshore sites with bottom depths greater than 1000 m. Each sample was collected in a 300 ml borosilicate glass bottle (Wheaton Industries, Inc., USA), vacuum filtered using a 0.45 μm pore size MF-Millipore Membrane Filter (EMD Millipore, USA), and preserved with saturated HgCl_2 solution. The purpose of the filtration was to remove particles that could contribute to DIC and TA. Due to gas exchange during the filtration process, pH and DIC will change from their original values in the environment. TA is not affected by gas exchange during the filtration process and, due to the fact that DIC and pH measurements were both obtained from the same filtered sample, calculations of TA from DIC and pH are also unaffected by the filtration process.

2.2. Measurement of pH and DIC

Samples for measurements of pH and DIC were collected simultaneously from the same sample bottle. This procedure ensured that, although DIC and pH are altered by filtration, the coupled measurements could be directly used for calculation of the TA of each sample. Spectrophotometric pH (total scale) was measured at 25.0 $^{\circ}\text{C}$ with purified meta-cresol purple on an Agilent 8453 UV-Visible spectrophotometer (Agilent Technologies, USA) following the protocol described by Dickson et al. (2007) and Liu et al. (2011). DIC was measured using a

CM5014 CO₂ coulometer (UIC, Inc., USA) with a custom automatic acidification/purging system, following the protocol described by Dickson et al. (2007). The precisions of the pH and DIC measurements were determined as 0.001 and $\pm 0.1\%$ ($\pm 2 \mu\text{mol}\cdot\text{kg}^{-1}$), respectively.

2.3. Measurement of TA

After sampling for pH and DIC, the remaining seawater in the sample bottle was used for a TA measurement. TA was measured using a single-point spectrophotometric titration method with bromocresol purple (BCP) (Yao and Byrne, 1998, Liu et al., 2015). Throughout each titration the pH of each sample was continuously monitored by measuring the absorbance ratio (R) of the basic/acidic forms of BCP as the titration was in progress. The custom automated titration system used in this work (Fig. 2) consisted of a USB-4000 fiber optic spectrometer (Ocean Optics, Inc., USA), a 665 Dosimat auto-titrator with stir plate (Metrohm AG, Switzerland), and a purging system with high-purity N₂ gas (Liu et al., 2015). The HCl (0.25 M) used for titrations was standardized with certified reference material (CRM). The CRM (Dickson et al., 2003) used in this work was provided by A.G. Dickson of Scripps Institution of Oceanography. Repeat measurements on CRM batch 109 showed a standard deviation of $\pm 0.29 \mu\text{mol}\cdot\text{kg}^{-1}$ ($n=7$). This is substantially better than the generally expected precision of $\pm 3 \mu\text{mol}\cdot\text{kg}^{-1}$ for TA measurements (Millero, 2007).

2.4. Carbonate system calculations

Measured pH (25.0 °C), DIC, and TA were input to the CO2SYS Excel macro version 2.1 (Pierrot et al., 2006) for carbonate system calculations, following the same procedure described by Patsavas et al. (2015). The calculation was performed on the total pH scale using the carbonate dissociation constants (K_1' and K_2') of Lueker et al. (2000). The HSO₄⁻

dissociation constant was taken from Dickson et al. (1990), and the B_T/S ratio was taken from Lee et al. (2010).

2.5. Full pH range titration for Org-Alk

Using an offshore sample as the reference, coastal samples with substantial ΔTA were selected for direct full-range pH titrations ($4.5 \leq \text{pH} \leq 8$) and characterization of Org-Alk. The automatic titration system described in section 2.3 was also used for the full-range pH titrations. These titrations were performed at room temperature ($\sim 25^\circ\text{C}$).

Fig.3 illustrates the full-range pH titration processes. Prior to each titration, samples were adjusted to a common salinity (in this case to the lowest salinity of all measured samples, 21.7) by dilution with deionized water. This procedure ensured that B_T was the same in all samples, and thereby that the borate alkalinities (**B-Alk**) for all samples were identical at a given pH. Two indicators (BCP and cresol red, CR) were used to cover the full pH range between pH 4.5 and 8. For BCP titrations, a 150 g seawater sample was first titrated with HCl following the TA protocol described above. After CO_2 was removed from the sample with a stream of N_2 gas, **the R ratio (R_1) and HCl volume (V_1) were used to calculate TA of this sample**. After that a 0.1 M NaOH (Fisher Scientific, USA) solution was added using a Gilmont burette to bring the R ratio to 2.5 (under an N_2 atmosphere). The solution was then titrated with HCl again under the N_2 atmosphere, bringing the R ratio to 0.045 and thereby covering the pH range between 4.5 and 6. A second 150 g sample of the same seawater was used for the subsequent CR titration. The same amount of HCl (**V_1**) that was used in the first (BCP) titration was added to the sample, and CO_2 in the sample was removed by N_2 purging. After taking the blank absorbance reading and adding 100 μL 10 mmol kg^{-1} CR, the 0.1 M NaOH solution was added to bring the R ratio to 2.5. Then

the solution was titrated with HCl to bring the R ratio to 0.045, covering the pH range of 6–8. Throughout the full-range pH titrations, readings of R ratios and corresponding volumes of added HCl (V_{HCl}) were taken continuously at an interval of 0.25 sec. The R ratios, along with the salinity and temperature, were then used to calculate pH_T (Yao and Byrne, 1998; Patsavas et al. 2013) and plot titration curves (pH_T versus V_{HCl}).

To examine the possible influence from the trace amounts of CO_3^{2-} in the NaOH titrant solution, 150 g of a 0.7 M NaCl solution was titrated with the procedure described above and then purged of CO_2 with a stream of N_2 . Subsequently, 1 ml of the 0.1 M NaOH solution was added under an N_2 atmosphere, and the DIC of this solution was measured using the coulometric method described above. The carbonate ion concentration of the 0.1 M NaOH solution was determined as 1.5 mM. Consequently, an addition of 100 – 150 μL of the 0.1 M NaOH solution to the 150 g sample solutions caused alkalinity additions (as CO_3^{2-}) to the sample solutions equivalent to about 2 – 3 $\mu\text{mol kg}^{-1}$. In view of the fact that the NaOH titrant is added to both the sample solution and the reference solution, this concentration is negligibly small.

For the samples used in the full range pH titrations, the phosphate and silicate concentrations were measured spectrophotometrically using the method described by Clescerl et al. (1999). Measured ΔTA values along with P-Alk and Si-Alk were used to calculate Org-Alk.

2.6. Characterization of Organic Anion contributions to TA

Assuming that the Org-Alk of the offshore reference sample was zero, by using the differences in the amounts of HCl (ΔV_{HCl}) observed for the reference and coastal full-range pH titrations, the Org-Alk of coastal samples were determined with Eq.3.

$$\text{Org-Alk} = \frac{\Delta V_{\text{HCl}} \times C_{\text{HCl}}}{m_{\text{sample}}} - \text{P-Alk} - \text{Si-Alk} = \frac{(V_{\text{HCl}}^{\text{Sample}} - V_{\text{HCl}}^{\text{Ref}}) \times C_{\text{HCl}}}{m_{\text{sample}}} - \text{P-Alk} - \text{Si-Alk} \quad (3)$$

In order to show the hydrogen ion neutralization capacity of organic anions at each pH point, $\Delta V_{\text{HCl}} - \text{pH}_T$ was plotted for each titration point. As the starting point in this analysis, to obtain the amount of added HCl at each pH point for both reference and sample, the $\text{pH}_T - V_{\text{HCl}}$ data were interpolated with an interval of 0.1 pH units. Subsequently, differences (ΔV_{HCl}) in the amounts of HCl used for the reference and the sample at each pH point were plotted against the pH_T . A positive ΔV_{HCl} at a given pH indicates that more HCl was added to reach that pH. This indicates in turn that, relative to the offshore reference, organic anions were contributing to TA. The shapes of the $\Delta V_{\text{HCl}} - \text{pH}_T$ curves reflect different compositions of organic anions in different samples.

The full range pH titration data were also used for modeling the dissociation constants (pK_i) of organic anions in the samples. In this study, the model (Eq. 4) developed by Cai et al. (1998) was used for this purpose.

$$\begin{aligned} V_0 \sum_i \frac{X_{iT}}{1 + \frac{[\text{H}^+]_T}{K_i}} + V_0 \frac{B_T}{1 + \frac{[\text{H}^+]_T}{K_B}} + V_0 \frac{P_T}{1 + \frac{[\text{H}^+]_T}{K_P}} + V_0 \frac{\text{Si}_T}{1 + \frac{[\text{H}^+]_T}{K_{\text{Si}}}} - (V_0 + v) \left([\text{H}^+]_T - \frac{K'_w}{[\text{H}^+]_T} \right) \\ - v C_{\text{HCl}} + V_0 \frac{K'_w}{C_H^0} = 0 \end{aligned} \quad (4)$$

where K_i , K_B , K_P , and K_{Si} are the dissociation constants of organic acids (X_i), boric acid, phosphate (as H_2PO_4^-) and silicic acid (Millero, 1995), K'_w is the ion product of seawater, X_{iT} , B_T , P_T , and Si_T are the total concentrations of X_i , borate, phosphate, and silicate, v is the volume of the HCl solution, V_0 is the volume of the sample that was titrated with base, C_{HCl} is the

concentration of the HCl titrant solution, and C_H^0 is the hydrogen ion concentration after the sample was titrated with base.

With the $[H^+]_T$, v , and X_i values obtained from the full-range pH titrations, parametric fits of $\frac{K'_w}{C_H^0}$ and K_i were performed progressively with the Matlab® Curve Fitting Toolbox (MathWorks, Inc., USA). In this case, data from the first titration (BCP as indicator, pH 4.5–6) was first used to determine $\frac{K'_w}{C_H^0}$ and K_1 , and then K_1 was fixed so that $\frac{K'_w}{C_H^0}$ and K_2 could be determined with the data from the second titration (i.e., with CR, pH 6–8). It is important to note that the $\frac{K'_w}{C_H^0}$ term is slightly different in each titration due to the fact that the hydrogen ion concentration (C_H^0) varies slightly after the sample was titrated with base.

3. Results and Discussion

3.1. Org-Alk distributions along the Gulf Coast

No significant ΔTA ($0.1 \pm 5.0 \mu\text{mol kg}^{-1}$, $n = 17$) was observed at sample sites adjacent to the Mississippi-Atchafalaya River Estuary, with values ranging from -9.7 to $7.9 \mu\text{mol kg}^{-1}$ (Table 1). ΔTA values ranged from -8.7 to $7.7 \mu\text{mol kg}^{-1}$ for offshore sites in the northern and northeastern Gulf of Mexico, showing near-zero ΔTA values ($-1.0 \pm 4.9 \mu\text{mol kg}^{-1}$, $n = 14$) in offshore waters (Table 1). For samples obtained from the depth profiles at the offshore sites, nutrient data from a nearby station (27.582 N, 89.998 W, Wanninkhof et al., 2014) were used to correct the ΔTA values for nutrient alkalinity. The corrected results are shown in parentheses in Table 1.

High ΔTA values ($33.6 \pm 18.0 \mu\text{mol kg}^{-1}$, $n = 17$) were found at the Suwannee River Estuary, with values ranging from -12.6 to $75.0 \mu\text{mol kg}^{-1}$ (Table 2). Samples from urban areas of Tampa Bay evidenced substantial ΔTA ($16.0 \pm 25.4 \mu\text{mol kg}^{-1}$, $n = 55$), with values as large as $89.8 \mu\text{mol kg}^{-1}$ (Table 2).

Based on estimated TA_{meas} uncertainties of $3 \mu\text{mol kg}^{-1}$ (1σ), and 1σ uncertainties in TA_{cal} (based on pH-DIC) equal to $2.7 \mu\text{mol kg}^{-1}$ (Millero, 2007), the calculated 1σ uncertainty of ΔTA (σ_z) is $4.0 \mu\text{mol kg}^{-1}$ (*i. e.*, $\sigma_z = \sqrt{\sigma_x^2 + \sigma_y^2}$). As such, for two samples with identical alkalinities and $\text{Org-Alk} = 0$, it follows that ΔTA would be $0.0 \pm 4.0 \mu\text{mol kg}^{-1}$ ($\pm 1\sigma$). Thus, in cases where $\text{Org-Alk} + \text{P-Alk} + \text{Si-Alk} = 0$, it is expected that an appreciable fraction of ΔTA observations would have negative values (and positive values) simply due to imprecisions in DIC, TA and pH measurements. Given that the 3σ ΔTA imprecision is estimated as $12 \mu\text{mol kg}^{-1}$, and the number of samples measured in this work is 86, it is expected that ΔTA observations more negative than $-12 \mu\text{mol kg}^{-1}$ would not be observed in this study. Observations of ΔTA in the northern and northeastern Gulf of Mexico (Table 1) showed no value more negative than $-9.7 \mu\text{mol kg}^{-1}$. Observations in the Suwannee River estuary showed one value as low as $-12.6 \mu\text{mol kg}^{-1}$, and Tampa Bay had only one ΔTA observation more negative than $-8.9 \mu\text{mol kg}^{-1}$. Thus, for ΔTA values calculated with Eq. 2, observations of negative ΔTA residuals are generally consistent with 1σ ΔTA uncertainties equal to $\pm 4 \mu\text{mol kg}^{-1}$. In contrast, of the four ΔTA observations obtained in the Caloosahatchee River and the Ten Thousand Islands area, two had ΔTA values of $-16.3 \mu\text{mol kg}^{-1}$ and $-17.1 \mu\text{mol kg}^{-1}$. These values are somewhat larger than the theoretically expected 3σ uncertainties ($\pm 12 \mu\text{mol kg}^{-1}$) for ΔTA calculations. The origin of the unusual ΔTA values in the Caloosahatchee River and the Ten Thousand Islands area cannot be identified at this time.

3.2. Full range spectrophotometric pH titrations

Table 3 shows the results of full pH range titrations on reference and coastal samples, along with auxiliary data, and Fig. 4 shows examples of full pH range titration curves and calculations of Org-Alk from full-range pH titration data.

Titration curves (pH_T vs V_{HCl}) for the offshore reference (PCB_2) and two coastal samples (COQ-N and Bayboro) are given in Fig. 4a (pH 6–8) and Fig. 4b (pH 4.5–6). The figures show that more acid is required to titrate samples with higher Org-Alk. Based on the intercepts of the titration curves on the V_{HCl} axis (solid circles in Fig. 4a and 4b), the overall differences in the amounts of HCl ($\Delta V_{\text{HCl}} = \Delta V_{\text{HCl}}^{\text{CR}} + \Delta V_{\text{HCl}}^{\text{BCP}}$) used for reference and coastal sample titrations can be determined. These summed ΔV_{HCl} values were used in Eq. 3 to calculate the Org-Alk results shown as Org-Alk² in Table 3.

Table 3 shows that organic alkalinity determined with the full pH titration method (Org-Alk²) is in good agreement with values calculated (Org-Alk¹) from pH, DIC, TA, and nutrient measurements. The Org-Alk values in Table 3 range from -0.2 to 26.4 $\mu\text{mol kg}^{-1}$ (in diluted samples). The last two columns show the Org-Alk in the original samples (corrected for dilution). The comparisons in Table 3 indicate that the direct spectrophotometric titrations developed in this work can provide precise assessments of Org-Alk in coastal areas.

Fig. 5a and Fig. 5b show high resolution relationships between ΔV_{HCl} and pH_T . Titrations start at $\text{pH} \sim 8$, with $\Delta V_{\text{HCl}} = 0$. Subsequently ΔV_{HCl} increases as organic anions are neutralized by added hydrogen ions. For the Bayboro sample, organic anion concentrations were highest within the pK range of 4.5–6, while for COQ-N sample, organic anion contributions to TA were similar in the two titration ranges (4.5–6 and 6–8). Moreover, from the shape of the $\Delta V_{\text{HCl}} - \text{pH}_T$

curves, the speciation of organic anions in the different samples can be roughly compared. For example, Bayboro and COQ-N samples had similar $\Delta V_{\text{HCl}} - \text{pH}_T$ trends for the 5.9–7.0 pH range, which indicates that they might have similar organic anions. In contrast, $\Delta V_{\text{HCl}} - \text{pH}_T$ trends for Bayboro and COQ-N were distinct below pH 5.9 or above pH 7.0, which indicates that contributions to TA **might be** from different species of organic anions.

3.3. Model Characterization of Organic Anions

Dissociation constants (pK_i) of organic anions from model fitting of COQ-N and Bayboro samples are shown in Table 4. Based on the pH range of each titration (BCP and CR), the K_1 and K_2 boundary conditions were specified as $(10^{-7}, 10^{-4})$ and $(10^{-8}, 10^{-6})$, respectively.

The riverine fulvic acid studies of Paxeus and Wedborg (1985) showed six titratable groups with pK_i of 2.66 (Group 1), 4.21 (Group 2), 5.35 (Group 3), 6.65 (Group 4), 8.11 (Group 5), and 9.54 (Group 6). Since sample pH ranged from 4.5 to 8.1 during the TA titrations, only Group 2 (partially) and Group 3–5 in Paxeus and Wedborg's work contributed substantially to the TA. The pK_i of the two groups from our full pH range titration data are all confined within this range, with $\text{pK}_1 = 5.31$ for COQ-N and 5.45 for the Bayboro sample, and $\text{pK}_2 = 7.05$ for COQ-N and 7.32 for Bayboro. Therefore it is reasonable that Group I in this study is a combination of Paxeus and Wedborg's Groups 2 and 3, and our Group II is a combination of their Groups 4 and 5. In either case, our work supports the contention that organic anions of fulvic acids contributed substantially to our Org-Alk observations.

4. Implications

As a conservative variable which is independent of temperature and pressure, TA is one of the most frequently measured CO_2 system parameters, and is often used for calculations of

other parameters. Table 5 shows an example of the influence of Org-Alk on CO₂ system calculations. The initial conditions of a hypothetical seawater sample were defined as shown in Table 5a. With nutrient concentrations equal to zero, measured TA (TA_{meas}) is the sum of Org-Alk and C-Alk+B-Alk+[OH⁻]. In Table 5b, we compare the results of CO₂ system calculations with and without consideration of Org-Alk. With the DIC-TA as the input pair in CO2SYS calculations, if a seawater sample has an Org-Alk concentration of 20 μmol kg⁻¹ that is not taken into account, subsequent pH and *p*CO₂ calculations lead to a 0.040 unit overestimation of pH and a 61.9 μatm underestimation of *p*CO₂. With pH-TA as the input pair, if a seawater sample has an Org-Alk value of 20 μmol kg⁻¹ that is not explicitly accounted for, DIC will be overestimated by 18.9 μmol kg⁻¹ and *p*CO₂ will be overestimated by 5.7 μatm.

With the same initial conditions shown in Table 5, the influence of increasing levels of Org-Alk on CO2SYS calculations of *p*CO₂ are shown in Fig. 6. These results show that, when Org-Alk in samples is not explicitly taken into account, use of DIC-TA as the input pair for *p*CO₂ calculations causes much larger misestimation than in the case when pH-TA is used as the input pair. Org-Alk contributions to TA can cause errors in *p*CO₂ (calculated from DIC-TA) that are very large compared with the typical precision of direct *p*CO₂ measurement (0.3 – 0.4 ppm, Feely et al., 1998), and errors that are even substantial compared to the average diurnal *p*CO₂ change in Tampa Bay (140 μatm, Yates et al., 2007). Therefore, for rigorous CO₂ system calculations in coastal waters, it is important to consider the contributions of organic anions to TA. For culture and mesocosm experiments in marine biological research, which are usually carried out at high nutrient levels, with substantial amounts of organic matter production, misestimates of *p*CO₂ due to Org-Alk can be especially important (Koeve and Oschlies, 2012, Hagens et al., 2015).

Although ΔTA from TA_{meas} and TA_{cal} can be used to estimate Org-Alk, our study shows the limitation of this indirect approach. Based on the most negative value of ΔTA obtained in this work ($-18.8 \mu\text{mol kg}^{-1}$), ΔTA values between the range of $(-20, 20)$ were used in a histogram analysis to evaluate the potential errors in estimation of Org-Alk using ΔTA . Analysis of this dataset passed the normality test (Shapiro-Wilk) with a W-Statistic of 0.980, and P value of 0.368. With the mean $= -1.1 \mu\text{mol kg}^{-1}$ and $\sigma = 7.6$, a conservative estimate is that ΔTA values within the range of $(-7.6, 7.6)$ may be a result of measurement uncertainties or operational errors. As such, positive ΔTA values of $8 \mu\text{mol kg}^{-1}$ or smaller are not strongly indicative of Org-Alk contributions to TA. On the other hand, ΔTA values substantially larger than ~ 8 ($N = 28$ in this study) provide substantial evidence for Org-Alk contributions to TA. Because uncertainties in evaluations of Org-Alk via ΔTA analyses are relatively large, direct measurement of Org-Alk (i.e., full range spectrophotometric pH titrations), with uncertainties much smaller than $8 \mu\text{mol kg}^{-1}$, are highly advisable for use in coastal systems where Org-Alk concentrations are suspected to be significant.

The full-range spectrophotometric pH titration method presented in this work allows for direct quantification of organic anion contributions to TA. Org-Alk obtained from full-range pH titrations showed good agreement with results from indirect measurements. Results from numerical modeling of titration data show substantial variations in both Org-Alk concentrations and pK_i values from two coastal samples in relatively proximate locations. As such, it appears that the distributions and equilibrium characteristics of organic anions in coastal waters can be quite complex.

CO_2 system calculations involving the DIC-TA pair in the presence of Org-Alk require characterizations of both Org-Alk concentrations and the equilibrium behavior of organic

acids/bases that contribute to Org-Alk (Byrne, 2014). Without characterizations of organic acid dissociation characteristics as a function of salinity, temperature and, in some cases, pressure, it is not possible to rigorously include organic acids/bases in CO₂ system calculations. Calculations based on the DIC-TA pair, including contributions from Org-Alk, require knowledge of the equilibrium behavior of all relevant acid-base equilibria at in situ conditions. In the absence of such information, rigorous descriptions of CO₂ systems that contain substantial organics (calculations of $p\text{CO}_2$ and carbonate saturation states for example) must rely on measurements of DIC plus either in situ pH or in situ CO₂ fugacity (Byrne, 2014). Rigorous inclusion of Org-Alk in CO₂ system calculations is a formidable undertaking. Our study suggests that both Org-Alk concentrations and the equilibrium behavior of the organic acid-base pairs that contribute to Org-Alk behavior can be quite heterogeneous in the environment. It is therefore useful to directly observe Org-Alk concentrations using, for instance, the procedures described in the present study, and assess whether uncertainties introduced by the presence of Org-Alk are within acceptable limits for in situ predictions.

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Fig.1. Map of sampling sites along the Gulf of Mexico

Fig.2. Automated titration system

Fig. 3. Process for full pH range titration of Org-Alk

Fig. 4. Titration curves and the process to determine Org-Alk. ΔV_{HCl} (i.e., $\Delta V_{\text{HCl}}^{\text{CR}} + \Delta V_{\text{HCl}}^{\text{BCP}}$) obtained from CR and BCP titrations were used to calculate organic alkalinity using Eq.3.

Fig. 5. ΔV_{HCl} versus pH_T relationships for COQ-N and Bayboro samples. ΔV_{HCl} is the difference of HCl used for the reference and the sample at each pH point. The slight mismatch in ΔV_{HCl} for the two titrations at $\text{pH}=6$ is likely due to either uncertainties from interpolation of raw titration data, or slightly different titration conditions for the BCP and CR titrations.

Fig. 6. Influence of Org-Alk on $p\text{CO}_2$ calculations using CO2SYS. Initial conditions: $t = 25^\circ\text{C}$, $p = 0$ dbar, $[\text{Si}] = 0$, $[\text{P}] = 0$. TA in this figure refers to the sum of (a) total alkalinity calculated from pH and DIC plus (b) Org-Alk contributions between zero and 100 $\mu\text{mol kg}^{-1}$.

Table 1 Sample Information and Δ TA data for Northern and Northeastern Gulf of Mexico. All samples were collected from the surface unless otherwise noted.

Sample NO	Sample Name	Type	Lat	Lon	S	pH _T 25°C	DIC $\mu\text{mol kg}^{-1}$	TA _{meas} $\mu\text{mol kg}^{-1}$	TA _{cal} $\mu\text{mol kg}^{-1}$	Δ TA $\mu\text{mol kg}^{-1}$
<i>Northern Gulf of Mexico (outside Mississippi-Atchafalaya River Estuary)</i>										
1	13-10	Estuary	28.223	-90.460	35.59	8.062	2040.1	2360.1	2364.0	-3.9
2	13-11	Estuary	28.311	-90.471	35.80	8.058	2059.6	2385.3	2384.4	0.9
3	13-12	Estuary	28.420	-90.489	36.02	8.053	2070.4	2388.3	2394.6	-6.3
4	13-3	Estuary	28.846	-90.533	22.95	8.252	1874.8	2190.8	2183.9	6.9
5	13-4	Estuary	28.772	-90.529	24.04	8.223	1893.8	2198.7	2198.0	0.7
6	13-5	Estuary	28.684	-90.516	30.82	8.082	1995.6	2278.2	2286.5	-8.3
7	13-7	Estuary	28.542	-90.500	35.93	8.047	2070.7	2386.5	2390.2	-3.7
8	13-8	Estuary	28.475	-90.489	35.91	8.046	2069.1	2385.7	2387.7	-2.0
9	13-6	Estuary	28.617	-90.505	34.79	8.047	2058.4	2362.8	2367.1	-4.3
10	UW1	Estuary	28.976	-90.558	31.73	8.023	2066.4	2325.6	2335.3	-9.7
11	UW2	Estuary	28.950	-90.551	32.05	8.003	2068.9	2333.3	2328.8	4.5
12	UW3	Estuary	28.847	-90.538	32.67	8.033	2054.7	2328.2	2336.3	-8.1
13	UW4	Estuary	28.738	-90.522	33.30	8.042	2037.4	2336.3	2328.4	7.9
14	UW5	Estuary	28.661	-90.510	34.42	8.052	2040.2	2342.7	2347.4	-4.7
15	UW6	Estuary	28.525	-90.490	35.40	8.065	2044.2	2372.2	2368.5	3.6
16	UW7	Estuary	28.383	-90.468	35.79	8.075	2044.5	2384.1	2379.2	4.9
17	UW8	Estuary	28.300	-90.454	35.97	8.044	2048.7	2369.6	2364.2	5.4
18	13-9	Offshore	27.861	-90.475	35.60	8.066	2064.5	2386.6	2393.5	-6.9
19	GMT14	Offshore	27.529	-90.343	36.08	8.084	2052.9	2393.5	2397.6	-4.2
20	CTD_1000m	Offshore	27.537	-90.344	34.96	7.685	2194.0	2331.9	2321.7	10.2 (7.7)
21	CTD_25m	Offshore	27.537	-90.344	36.26	8.063	2071.5	2401.4	2405.1	-3.7 (-3.7)
22	CTD_Surface	Offshore	27.537	-90.344	35.98	8.061	2068.2	2395.4	2397.3	-1.9 (-1.9)
23	CTD_700m	Offshore	27.537	-90.344	34.92	7.598	2217.0	2313.9	2310.7	3.2 (0.4)
24	CTD_480m	Offshore	27.537	-90.344	35.13	7.615	2214.4	2317.5	2315.5	2.1 (-0.5)
25	CTD_400m	Offshore	27.537	-90.344	35.30	7.655	2198.8	2325.5	2315.8	9.7 (7.2)
26	CTD_300m	Offshore	27.537	-90.344	35.60	7.706	2197.6	2336.5	2337.2	-0.7 (-2.5)
27	CTD_200m	Offshore	27.537	-90.344	36.17	7.807	2179.0	2365.7	2367.4	-1.7 (-3.1)
28	CTD_100m	Offshore	27.537	-90.344	36.40	7.989	2113.2	2398.9	2402.2	-3.3 (-3.3)
29	CTD_75m	Offshore	27.537	-90.344	36.31	8.017	2094.9	2399.6	2399.8	-0.2 (-0.2)
30	CTD_50m	Offshore	27.537	-90.344	36.21	8.054	2066.4	2398.2	2392.7	5.5 (5.5)
<i>Northeastern Gulf of Mexico</i>										
31	PCB	Offshore	29.114	-87.265	34.00	8.119	2132.6	2484.7	2493.3	-8.7

Table 2 Sample Information and Δ TA data for Eastern Gulf of Mexico surface waters

Sample NO	Sample Name	Type	Lat	Lon	S	pH _T 25°C	DIC $\mu\text{mol kg}^{-1}$	TA _{meas} $\mu\text{mol kg}^{-1}$	TA _{cal} $\mu\text{mol kg}^{-1}$	Δ TA $\mu\text{mol kg}^{-1}$
<i>Suwannee River Estuary</i>										
1	Swa1_10	Estuary	29.308	-83.325	32.03	7.962	2101.0	2372.0	2339.5	32.4
2	Swa1_11	Estuary	29.308	-83.293	30.47	7.963	2154.0	2400.1	2385.6	14.5
3	Swa1_12	Estuary	29.308	-83.262	29.99	7.964	2157.6	2424.2	2386.6	37.6
4	Swa1_13	Estuary	29.308	-83.231	28.76	7.959	2174.0	2451.0	2392.5	58.5
5	Swa1_9	Estuary	29.309	-83.364	32.30	7.939	2107.6	2350.6	2335.6	15.0
6	Swa1-5	Estuary	29.252	-83.305	32.58	7.728	1151.9	1233.2	1245.9	-12.6
7	Swa2_1	Estuary	29.307	-83.201	21.78	7.937	2436.5	2627.1	2609.0	18.1
8	Swa2_10	Estuary	29.308	-83.364	31.58	7.963	2120.4	2355.0	2357.9	-2.9
9	Swa2_3	Estuary	29.266	-83.243	26.00	7.972	2236.9	2475.4	2445.6	29.7
10	Swa2_4	Estuary	29.239	-83.240	26.70	7.963	2216.8	2460.6	2424.8	35.8
11	Swa2_5	Estuary	29.229	-83.281	28.44	7.914	2197.9	2414.8	2392.6	22.1
12	Swa2_6	Estuary	29.222	-83.320	31.10	7.889	2104.5	2373.8	2298.8	75.0
13	Swa2-2	Estuary	29.286	-83.222	26.70	7.812	2401.8	2586.4	2548.3	38.1
14	Swa3-13	Estuary	29.308	-83.262	22.24	7.724	1806.7	1909.6	1879.9	29.7
15	Swa3-3	Estuary	29.239	-83.240	24.81	7.774	1885.3	2029.0	1987.6	41.4
16	Swa3-5	Estuary	29.274	-83.363	30.06	7.855	2017.9	2218.5	2184.5	34.0
17	Swa3-9	Estuary	29.308	-83.325	27.03	7.841	1945.1	2109.2	2085.1	24.1
<i>Tampa Bay Area</i>										
18	Clam Bayou	Mangrove	27.739	-82.691	23.50	7.624	2460.6	2512.1	2521.0	-8.9
19	Coquina Key	Mangrove	27.735	-82.640	28.50	7.786	2324.4	2475.6	2466.9	8.7
20	Maximo	Mangrove	27.710	-82.684	31.50	8.082	2208.0	2572.9	2526.4	46.5
21	Point Pinellas	Mangrove	27.704	-82.641	29.50	8.028	2221.5	2483.1	2488.7	-5.6
22	Veteran	Mangrove	27.800	-82.770	32.50	7.332	2491.8	2500.2	2485.8	14.4
23	Alafia 1	Estuary	27.855	-82.394	20.16	7.868	2233.1	2353.1	2356.5	-3.4
24	Bayboro1	Urban	27.759	-82.633	25.00	7.744	2240.0	2434.0	2344.2	89.8
25	FWC_1	Urban	28.020	-82.675	21.53	7.856	2272.6	2401.4	2401.1	0.2
26	FWC_10	Urban	27.772	-82.579	26.54	7.877	2285.8	2458.4	2455.3	3.1
27	FWC_2	Urban	27.972	-82.673	23.06	7.914	2297.5	2458.2	2461.1	-2.9
28	FWC_3	Urban	27.980	-82.620	23.79	7.905	2296.8	2464.4	2461.0	3.4
29	FWC_4	Urban	27.949	-82.561	24.44	7.890	2330.0	2488.8	2493.9	-5.0
30	FWC_5	Urban	27.957	-82.622	23.53	7.919	2295.2	2464.1	2464.3	-0.2
31	FWC_6	Urban	29.308	-83.325	27.03	7.889	2266.7	2449.5	2443.6	5.9
32	FWC_7	Urban	27.906	-82.605	24.30	7.863	2313.5	2457.6	2463.8	-6.1
33	FWC_8	Urban	27.924	-82.550	24.69	7.868	2279.9	2438.6	2433.2	5.4
34	FWC_9	Urban	27.861	-82.589	25.41	7.902	2304.9	2460.5	2479.3	-18.8
35	JFWC_1	Urban	28.020	-82.675	23.31	8.091	2176.9	2459.6	2423.2	36.4
36	JFWC_2	Urban	27.972	-82.673	23.95	8.061	2199.2	2441.4	2436.0	5.5
37	JFWC_3	Urban	27.980	-82.620	24.85	7.923	2260.1	2436.7	2437.9	-1.3
38	JFWC_4	Urban	27.949	-82.561	25.41	7.966	2271.9	2475.1	2475.2	-0.1
39	JFWC_5	Urban	27.957	-82.622	24.39	7.950	2245.2	2457.3	2431.6	25.7
40	JFWC_6	Urban	27.942	-82.675	23.85	8.206	2127.7	2451.3	2445.3	6.0
41	JFWC_7	Urban	27.906	-82.605	25.25	7.937	2273.4	2462.4	2461.5	0.9
42	JFWC_8	Urban	27.924	-82.550	26.42	7.910	2242.2	2418.3	2423.6	-5.3
43	JFWC_9	Urban	27.861	-82.589	27.03	7.877	2260.1	2424.9	2431.2	-6.2
44	OTB_1	Urban	28.020	-82.675	20.00	7.880	2163.9	2373.0	2288.1	84.9
45	OTB_10	Urban	27.772	-82.579	25.92	7.953	2163.2	2390.4	2356.9	33.5
46	OTB_3	Urban	27.980	-82.620	20.86	7.988	2209.9	2415.0	2386.3	28.7
47	OTB_5	Urban	27.957	-82.622	20.93	7.988	2170.5	2375.4	2345.3	30.1
48	OTB_6	Urban	27.942	-82.675	20.41	7.939	2172.3	2364.4	2322.4	42.0
49	OTB-2	Urban	27.972	-82.673	20.10	7.996	2150.7	2370.0	2321.8	48.3
50	FAU_1	Urban	27.622	-82.687	32.69	7.832	2307.4	2486.8	2494.8	-8.1
51	FAU_4	Urban	27.639	-82.647	29.07	7.828	2335.9	2493.2	2500.9	-7.7
<i>Caloosahatchee River Estuary</i>										
52	Caloo1	Estuary	26.459	-82.034	35.57	8.073	2142.5	2468.1	2485.2	-17.1
53	Caloo2	Estuary	26.523	-81.999	32.82	8.016	2145.6	2418.1	2426.0	-7.9
<i>Ten Thousand Islands Area</i>										
54	TTL_1	Mangrove	25.941	-81.586	37.24	7.414	3721.0	3754.1	3761.7	-7.6
55	TTL_2	Mangrove	25.912	-81.530	38.28	7.691	3085.4	3250.5	3266.8	-16.3

Table 3 Sample information and results of full range pH titrations. Org-Alk¹ was calculated using Eq. 2 with the measured pH, DIC, TA, and nutrient data. Org-Alk² was determined using Eq. 3 with the full range pH titration data. Units for DIC, P, Si, TA, and Org-Alk are $\mu\text{mol kg}^{-1}$.

Sample	Location	<i>S</i>	<i>S</i>	pH _T 25°C	DIC	P	Si	TA _{mea}	TA _{cal}	Org-Alk ¹	Org-Alk ²	Org-Alk ¹	Org-Alk ²
		Original										In diluted samples	
Offshore Reference													
PCB_2	Gulf of Mexico	35.2	21.7	8.119	1508.9	1.76	16.80	1699.7	1701.2	-1.2	-	-1.9	-
Coastal Samples													
PC	Panama City, FL	32.2	21.7	8.169	1651.4	1.71	12.51	1880.9	1880.4	0.5	-0.2	0.7	-0.3
TB	Tampa Bay, FL	21.7	21.7	8.013	2104.5	0.97	6.85	2299.0	2293.6	5.4	8.5	5.4	8.5
COQ-S	Tampa Bay, FL	28.1	21.7	7.850	1945.0	1.07	13.83	2073.2	2060.7	12.5	11.5	16.1	14.9
COQ-N	Tampa Bay, FL	28.0	21.7	8.187	1775.1	1.17	14.43	2038.0	2025.0	13.0	10.9	16.8	14.0
Bayboro	Tampa Bay, FL	33.0	21.7	7.905	2082.9	0.99	7.02	2251.6	2224.6	27.0	26.4	41.0	40.2

Table 4 Boundary conditions and model fitting results using full range pH titration data of diluted samples

Sample	Boundary Conditions ^a		Model Output		pK ₁	pK ₂
	K ₁	K ₂	K ₁	K ₂		
COQ-N	(10 ⁻⁷ , 10 ⁻⁴)	(10 ⁻⁸ , 10 ⁻⁶)	4.85×10 ⁻⁶	8.89×10 ⁻⁸	5.31	7.05
Bayboro	(10 ⁻⁷ , 10 ⁻⁴)	(10 ⁻⁸ , 10 ⁻⁶)	3.55×10 ⁻⁶	4.75×10 ⁻⁸	5.45	7.32

^a Boundary condition for $\frac{K'_w}{C_H^0}$ was set to (0, 2×10⁻⁴)

Table 5 Influence of Org-Alk on CO₂ system calculations

<i>a. Initial Conditions</i>					
$S = 28, t = 25^{\circ}\text{C}, p = 0, [\text{P}] = 0, [\text{Si}] = 0$					
pH	DIC $\mu\text{mol kg}^{-1}$	Org-Alk $\mu\text{mol kg}^{-1}$	C-Alk+B-Alk+[OH ⁻] $\mu\text{mol kg}^{-1}$	TA _{meas} $\mu\text{mol kg}^{-1}$	pCO ₂ μatm
7.900	2200.0	20.0	2384.9	2404.9	657.5
<i>b. CO2SYS Calculation</i>					
Input		Output			
DIC $\mu\text{mol kg}^{-1}$	TA $\mu\text{mol kg}^{-1}$	pH	ΔpH	pCO ₂ μatm	ΔpCO_2 μatm
2200.0	2384.9	7.900	—	657.5	—
2200.0	2404.9	7.940	0.040	595.6	-61.9
pH	TA $\mu\text{mol kg}^{-1}$	DIC $\mu\text{mol kg}^{-1}$	ΔDIC $\mu\text{mol kg}^{-1}$	pCO ₂ μatm	ΔpCO_2 μatm
7.900	2384.9	2200.0	—	657.5	—
7.900	2404.9	2218.9	18.9	663.2	5.7