Determination of the rate constants for the carbon dioxide to bicarbonate inter-conversion in pH-buffered seawater systems

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Abstract

Experimental setups to study modes of inorganic carbon acquisition and fixation rates by marine phytoplankton commonly make use of so-called disequilibrium techniques. The chemical or isotopic disequilibrium, either caused by phytoplankton cells taking up inorganic carbon or by a small disturbance of the isotopic equilibrium in the carbonate system, requires to account for the relatively slow chemical interconversion of carbon dioxide (CO2) to bicarbonate (HCO3−/CO32−) in seawater. Because in such experiments a constant pH is a prerequisite, pH buffers are generally used. However, a possible influence of such buffers on the kinetics of the carbonate system has hitherto not been investigated. Here, a model of the carbonate system in seawater is employed to show how pH buffers are operating. Furthermore, a new approach is presented to determine the rate constants, k+, and k−, for the conversion reaction of CO2 to HCO3− and vice versa, by means of membrane inlet mass spectrometry (MIMS). For the two pH buffers tested (HEPES and BICINE) it is shown that measured rate constants are in good agreement with calculated values for k+ and k− in a pH range of 7 to 8.5 and at temperatures from 10 to 25 °C.

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1. Introduction

In the last 200 years, starting with the industrial revolution, the ocean has taken up ~50% of the carbon dioxide (CO2) emitted by mankind’s consumption of fossil fuels. The projected doubling of current atmospheric CO2 around the year 2100 (Houghton et al., 1995) and its continuing oceanic uptake will give rise to a 60% increase in hydrogen ion concentration in the surface ocean (Sabine et al., 2004). This ocean acidification also involves a redistribution in the dissolved inorganic carbon (DIC) pool, increasing CO2 and bicarbonate (HCO3−) at the expense of carbonate ion (CO32−) concentrations. It has been shown, both in experimental and in modelling studies, that mechanisms and efficiencies of inorganic carbon acquisition by marine phytoplankton, which is responsible for about half of global net primary production (Field et al., 1998), are sensitive to the availability of CO2 in seawater (Burkhardt et al., 2001; Rost et al., 2003; Thoms...
et al., 2001). Moreover, these can differ greatly between various groups of phytoplankton species (Rost et al., 2003, in press). The consequences of future DIC redistribution for species composition and inorganic carbon fixation, however, are largely unknown. Assessment of these potential changes includes studies on mechanisms and efficiencies of inorganic carbon acquisition by different phytoplankton groups, making use of so-called disequilibrium techniques. While the mass spectrometric approach measures the disequilibrium caused by photosynthetic uptake of inorganic carbon (Badger et al., 1994), the 14C disequilibrium technique monitors inorganic carbon fixation upon a small disturbance in the isotopic equilibrium of the carbonate system (Cooper et al., 1969; Espie and Colman, 1986). Both methods require exact knowledge of the response of the carbonate system to the disequilibrium employed, i.e., the kinetic rate constants for the chemical interconversion between CO2 and HCO3−. Here we present a method for the exact determination of these rate constants by means of membrane inlet mass spectrometry (MIMS) and compare it with an approach described previously. Furthermore, adopting a model of the carbonate system in seawater, we explore the validity of the assumptions associated with these two approaches.

2. Methods

2.1. The experimental setup

All measurements were performed in artificial seawater following the recipe of Roy et al. (1993) without addition of DIC. The artificial seawater was divided into two batches to which BICINE or HEPES buffer were added yielding final concentrations of 50 μmol kg−1. Sub-samples were taken and their pH was adjusted at room temperature to values of approximately 7.0, 8.0 and 8.4 by addition of NaOH. Subsamples were then incubated at temperatures of 11, 17 and 26 °C. For a measurement 8 ml of a seawater sample was filled into a thermostated cuvette (set to the desired temperature), attached to a sectorfield multicolonlector mass spectrometer (Isoprime; GV Instruments, England) via a gas permeable membrane (0.01 mm PTFE) inlet system. The area for gas exchange via the inlet system was about 24 mm2 and was located at the bottom of the cuvette. The cuvette was closed without headspace to prevent significant gas exchange between atmosphere and water, and was equipped with a magnetic stirring rod to enhance mixing. Manipulations of the carbonate system in the cuvette were carried out via a tiny hole drilled into the stopper. Changes in concentrations of 12CO2 and 13CO2, the only species of dissolved inorganic carbon (DIC) which are measured directly by the membrane inlet mass spectrometer (MIMS), upon disturbance of the seawater carbonate system were monitored continuously with a resolution of 0.1 s (carbon species without 13C or 12C notation will refer to the sum of both). The MIMS was calibrated for [CO2] by injections of known amounts of NaH12CO3 (~ 99% 12C) and NaH13CO3 (~ 99% 13C) solutions (10 mmol kg−1) into the cuvette filled with 8 ml of 0.2 M HCl. As in such acidic solution DIC is only present as CO2 the measured CO2 recording can be directly converted into concentration. The CO2 baseline was determined by injection of 20 μl of 10 M NaOH. This increased seawater pH to values at which [CO2] is practically zero. As a single measurement did not exceed 10 min CO2 consumption by the mass spectrometer through the membrane inlet system was negligible.

Special care was taken in determining the pH in all buffered seawater solutions. Known amounts (40 μl) of a NaHCO3 solution (100 mmol kg−1) were added to these and subsequent equilibration in the CO2 signal was monitored. The [CO2] calibration of the MIMS was used to determine the DIC to CO2 ratio (RC) in equilibrium. Together with the dissociation constants of carbonic acid of Roy et al. (1993), the pH was calculated on the total scale (for details see Zeebe and Wolf-Gladrow (2001)). The pH of the seawater buffered with HEPES or BICINE remained rather constant (± 0.05 units) upon NaHCO3 addition which was checked independently with a pH meter.

Previously, similar experimental setups were used for estimation of the rate constants for the CO2 to HCO3− interconversion (e.g., Badger et al., 1994; Sülttemeyer et al., 1995). Basically, CO2 evolution was measured after injection of known amounts of a K2CO3 solution (100 mmol kg−1) into DIC free seawater medium. In the alkaline K2CO3 solution (pH ~11) DIC is only present as CO32− and HCO3− while CO2 is practically zero. Hence, injection of such solution will result in a net conversion of CO32− to HCO3− and finally to CO2 in any seawater sample buffered at a pH lower than that of the K2CO3 solution. From the initial slope of the CO2 evolution signal, monitored with the MIMS, the rate constants were then estimated. However, three assumptions regarding this procedure remain to be tested. First, the pH buffer is able to keep the pH constant on time scales of seconds recorded by the MIMS. Second, on these time scales the CO32− to CO2 conversion, so that the back-reaction can safely be
ignored. The first two assumptions are discussed in Sections 3.1, 3.2 and 3.4. As the third clearly poses a limitation a new method was developed which uses not only the initial slope but rather the entire CO₂ evolution curve, allowing explicitly for the back-reaction to take place. Practically, this is achieved by nonlinearly fitting a suitable equation to the measured CO₂ evolution curve leading to determination of the rate constants. However, these can also be assessed by adding known amounts of a solution in which DIC is mainly present in the form of CO₂. Such a solution was prepared by bubbling the artificial seawater described above with (H₂CO₃), constitutes less than 0.1

K pools in equilibrium predominantly present in three forms, CO₂(aq), HCO₃⁻ and CO₃²⁻. The fourth compound, true carbonic acid (H₂CO₃), constitutes less than 0.1% of the total dissolved inorganic carbon (DIC) and is therefore generally added to the CO₂(aq) pool, defining CO₂ as the sum of CO₂(aq) and H₂CO₃. Then, this system is characterized by two reactions. The first can be described as the relation between the CO₂ and HCO₃⁻ pools in equilibrium

\[ K_1^* = \frac{[H^+][HCO_3^-]}{[CO_2]} = \frac{k_+}{k_-} \] (1)

with \( K_1^* \) being the stoichiometric equilibrium constant and \( k_+ \) and \( k_- \) referring to the rate constants for the overall reaction of CO₂ to HCO₃⁻ and vice versa, respectively. The kinetics between these pools can then be described as

\[ H_2O + CO_2 \xrightleftharpoons[k_-]{k_+} HCO_3^- + H^+. \] (2)

This overall reaction comprises several reaction pathways for the CO₂ to HCO₃⁻ interconversion, given by Eqs. (17) and (18). Please note that in Eq. (17) the reaction pathway via H₂CO₃ is implicitly included (see Appendix). Rate constants are dependent on temperature and salinity, but the actual rate at which equilibrium will be restored after a disturbance in one pool also depends on pH (note the [H⁺] in Eq. (1)). The second reaction in the carbonate system, the one between HCO₃⁻ and CO₃²⁻, is virtually instantaneous compared to the reaction in Eq. (2), as it involves just protonation and deprotonation steps (see Section 3.4 for details).

The ratio of [DIC] to CO₂ in equilibrium ([CO₂]ₑq) as measured with the MIMS is defined as

\[ R_C = \frac{[DIC]}{[CO_2]_{eq}}. \] (3)

From Eq. (2) follows that changes in [CO₂] can be described as:

\[ \frac{d[CO_2]}{dt} = +k_-[H^+][HCO_3^-] - k_+[CO_2] \] (4)

this is a safe assumption on a time scale of seconds, as the HCO₃⁻ and CO₃²⁻ pools will be in equilibrium by the comparatively rapid reactions between them (see Section 3.4 for details). As [CO₂] is the only parameter which can be monitored online by the MIMS, ([DIC]–[CO₂]–[CO₃²⁻]) is substituted for [HCO₃⁻]. Now, the only unknown in a redistribution of the carbonate species is [CO₃²⁻]. Note that [DIC] is always conserved, even when the relative contribution of the three carbonate species changes. If the pH buffer (HEPES, or BICINE) is able to keep the pH constant (this assumption will be investigated with a model described in Section 2.4), [CO₃²⁻] can be described as a constant fraction \( f \) of [HCO₃⁻], yielding \([CO_3^{2-}] = f[HCO_3^-] \) (see 3.4 for details). Combining the last two equations gives

\[ [HCO_3^-] = \frac{[DIC] - [CO_2]}{(1 + f)}. \] (5)

By substituting [HCO₃⁻] from Eq. (5) into Eq. (4) it follows that

\[ \frac{d[CO_2]}{dt} = +k_-[H^+][DIC] - [CO_2] - k_+[CO_2]. \] (6)

Rearrangement with \( x=1/(1+f) \) yields

\[ \frac{d[CO_2]}{dt} = -\left(xk_-[H^+] + k_+[CO_2] + xk_-[H^+][DIC] \right). \] (7)

From here on, the paths for describing the CO₂ evolution curve upon addition of a K₂CO₃ or CO₂ solution split up. First, K₂CO₃ addition is considered. As right after the injection of a K₂CO₃ solution the reaction involving \( k_- \) will be the dominant one, \( k_- \) is expressed in terms of \( k_- \) as described by equilibrium conditions in Eq. (1). Additionally, for convenience, the rate constant \( k_- \) is combined with [H⁺] giving \( k_- = k_-[H^+] \) (note...
that this is justified by the assumption that the pH is constant).

\[
\frac{k_+}{k^-} = \frac{[\text{HCO}_3^-]}{[\text{CO}_2]_{\text{eq}}} = \frac{z([\text{DIC}] - [\text{CO}_2]_{\text{eq}})}{[\text{CO}_2]_{\text{eq}}}
\]

\[
= z(R_C - 1) \tag{8}
\]

Substituting now \(k_+\) in Eq. (7) it follows that

\[
\frac{d[\text{CO}_2]}{dt} = -zR_C k_+^* [\text{CO}_2] + zk_+^* [\text{DIC}]. \tag{9}
\]

The general solution of the homogeneous version of Eq. (9) (i.e., \([\text{DIC}] = 0\)) is

\[
[\text{CO}_2](t) = A \exp(-zR_C k_+ t)
\]

where \(A\) is a constant to be determined from the initial conditions. One arbitrary solution of the non-homogeneous equation is (this can usually be found by assuming \([\text{CO}_2](t) = \text{constant}\):

\[
[\text{CO}_2](t) = \frac{1}{R_C} [\text{DIC}] \tag{10}
\]

Hence, the general solution of the non-homogeneous equation is:

\[
[\text{CO}_2](t) = A \exp(-zR_C k_+ t) + \frac{1}{R_C} [\text{DIC}] \tag{12}
\]

Now \(A\) has to be determined from the initial condition \([\text{CO}_2](t=0) = [\text{CO}_2]_0 = A + z[DIC]/zR_C\). And thus

\[
A = [\text{CO}_2]_0 - \frac{1}{R_C} [\text{DIC}] \tag{13}
\]

which leads to

\[
[\text{CO}_2](t) = \left([\text{CO}_2]_0 - \frac{1}{R_C} [\text{DIC}]\right) \exp(-zR_C k_+ t)
\]

\[
+ \frac{1}{R_C} [\text{DIC}] \tag{14}
\]

with \([\text{CO}_2]_0\) being the initial \([\text{CO}_2]\) prior to addition of a K\(_2\)CO\(_3\) solution. The constant \(R_C\) is determined with the MIMS as described above, and \(f\) can be calculated using the second dissociation constants of carbonic acid given by Roy et al. (1993). Then, Eq. (14) is fitted for \(k_+\) in a least square procedure to the observed CO\(_2\) evolution data upon K\(_2\)CO\(_3\) addition. From equilibrium conditions described in Eq. (1), \(k_+\) can then be calculated as

\[
k_+ = \frac{K^*_c k^*}{[\text{H}^+]} \tag{15}
\]

with \(k_- = k_*/[\text{H}^+]\).

In principle, Eq. (14) is equally suitable for CO\(_2\) addition. However, the first dominant reaction will be the conversion of CO\(_2\) to HCO\(_3^-\). Hence, \(k_+\) is substituted for \(k_-\) in Eq. (7). Further rearrangement and solving of the resulting differential equation gives

\[
[\text{CO}_2](t) = \left([\text{CO}_2]_0 - \frac{1}{R_C} [\text{DIC}]\right) \exp(-\gamma k_+ t)
\]

\[
+ \frac{1}{R_C} [\text{DIC}] \tag{16}
\]

with \(\gamma = R_C/(R_C - 1)\).

### 2.3. The fitting procedure

Fitting of Eqs. (14) and (16) to the observed CO\(_2\) evolution curve \((^{12}\text{CO}_2 + ^{13}\text{CO}_2)\) upon addition of a K\(_2\)CO\(_3\) or CO\(_2\) solution is achieved by a least square minimization using the Levenberg–Marquardt method (More´, 1977), yielding \(k_+\) or \(k_-\), respectively. There are, however, always two processes influencing the CO\(_2\) evolution curve in the first couple of seconds following a disturbance in the carbonate system, i.e., changes in the gas flow through the membrane and mixing of the DIC addition in the cuvette. This is accounted for by discarding the first couple of seconds and starting the fitting procedure from the inflection point (see Fig. 1).

As stated earlier, a crucial prerequisite for the fitting equations is that the pH of the seawater is kept constant by the pH buffer throughout the measurement. Therefore, in the next section a model of the carbonate system in seawater is described to test this assumption.

### 2.4. The model of the carbonate system in seawater

A model similar to that of Zeebe and Wolf-Gladrow (2001) was developed, including all important reactions of the carbonate system in seawater. Additionally, a parameterization for the kinetics of different pH-buffers was added. Boron species, important constituents in natural seawater, are excluded because they are not contained in the artificial seawater used. The following reactions are considered (the letter \(A\) denotes a proton acceptor, i.e., a pH buffer).

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+ \tag{17}
\]

\[
\text{CO}_2 + \text{OH}^- \rightleftharpoons \text{HCO}_3^- \tag{18}
\]
It is noted that, what is referred to as $k_+$ or $k_-$ in Eq. (2) must not be confused with $k_{+1}$ or $k_{-1}$ in Eq. (17). The former are the rate constants for the overall reactions between the CO$_2$ and HCO$_3^-$ pools in this coupled chemical system. The values for the different reaction rate constants are given in Table 1. The set of differential equations therefore reads

\[
\frac{d[CO_2]}{dt} = (k_{-1}[H^+] + k_{-4}[OH^-])[CO_2] - (k_{+1} + k_{+4}[OH^-])[CO_2]
\]

\[
\frac{d[HCO_3^-]}{dt} = + (k_{+1} + k_{+4}[OH^-])[CO_2] - (k_{-1}[H^+] + k_{-4})[HCO_3^-] + \left(k_{+5}[H^+] + k_{+5}[OH^-]\right)[CO_3^{2-}] - \left(k_{-5}[H^+] + k_{-5}[OH^-]\right)[HCO_3^-]
\]

\[
\frac{d[CO_3^{2-}]}{dt} = + (k_{+5}[H^+] + k_{+5}[OH^-])[HCO_3^-] - \left(k_{+5}[H^+] + k_{-5}[OH^-]\right)[CO_3^{2-}]
\]

\[
\frac{d[H^+]}{dt} = + k_{+1}[CO_2] - k_{-1}[H^+][HCO_3^-] + k_{+5}[HCO_3^-] - k_{-5}[H^+][CO_3^{2-}] + k_{+6} + k_{-6}[H^+][OH^-] + k_{+6}[A^-][AH]
\]

\[
\frac{d[A^-]}{dt} = - k_{-6}[H^+][A^-]
\]
Table 1
Rate constants and their respective check values used in this study

<table>
<thead>
<tr>
<th>Rate constant</th>
<th>Check value $T=298.15$ K, $S=35$</th>
<th>Dependence on $T$ and $S$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{-4}$</td>
<td>$3.71 \times 10^{-2}$ s$^{-1}$</td>
<td>$\exp (1246.98 - 6.19 \times 10^4/T - 183.0 \ln (T))$</td>
<td>1</td>
</tr>
<tr>
<td>$k_{-1}$</td>
<td>$2.67 \times 10^5$ kg mol$^{-1}$ s$^{-1}$</td>
<td>$k_{-1}/K^*_1$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$k_{+4}$</td>
<td>$2.23 \times 10^3$ kg mol$^{-1}$ s$^{-1}$</td>
<td>$A_d \exp (-90.16663/(RT))/K^*_W$</td>
<td>Refitted from 1</td>
</tr>
<tr>
<td>$k_{+4}$</td>
<td>$9.71 \times 10^{-2}$ s$^{-1}$</td>
<td>$k_{+4}/K^*_W$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$k_{+53}$</td>
<td>$5.0 \times 10^{10}$ kg mol$^{-1}$ s$^{-1}$</td>
<td>None</td>
<td>Calculated</td>
</tr>
<tr>
<td>$k_{+5}$</td>
<td>$59.44$ s$^{-1}$</td>
<td>$k_{+5}^* \times K^*_2$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$k_{+2}$</td>
<td>$6.0 \times 10^9$ kg mol$^{-1}$ s$^{-1}$</td>
<td>None</td>
<td>Calculated</td>
</tr>
<tr>
<td>$k_{+2}$</td>
<td>$3.06 \times 10^5$ s$^{-1}$</td>
<td>$k_{+2}^* \times K^*_W$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$k_{+6}$</td>
<td>$1.40 \times 10^{-3}$ mol kg$^{-1}$ s$^{-1}$</td>
<td>None</td>
<td>Calculated</td>
</tr>
<tr>
<td>$k_{+6}$</td>
<td>$2.31 \times 10^{-10}$ kg mol$^{-1}$ s$^{-1}$</td>
<td>$k_{+6}^* \times K^*_W$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$k_{-4}$</td>
<td>$s^{-1}$</td>
<td>$k_{-4} \times 10^{-pK_a}$</td>
<td>Calculated</td>
</tr>
<tr>
<td>$k_{-1}$</td>
<td>kg mol$^{-1}$ s$^{-1}$</td>
<td>$k_{-1}/S$</td>
<td>Varied</td>
</tr>
</tbody>
</table>

Ref. 1 refers to the work of Johnson (1982), while Ref. 2 refers to the work of Eigen (1964) (see Zeebe and Wolf-Gladrow (2001), p. 105 for a detailed discussion). $k_{-4}$ has been refitted in this study, with $A_d = 499,002.24 \times \exp(4.2986 \times 10^{-4} S^2 + 5.75499 \times 10^{-5} S)$, with $S$ representing salinity because otherwise measured and calculated values for $k_1$ did not match well, especially at high temperature. $pK_d$ denotes the $pK$ value of the pH buffer used, i.e. HEPES: $pK = 7.94 - 0.014 \times (T - 273.15)$ and BICINE: $pK = 8.82 - 0.018 \times (T - 273.15)$ after Good et al. (1966), with $T$ representing temperature in Kelvin. These are values extrapolated to zero ionic strength (see Beynon and Easterby (1996) for details). In the calculations, however, they have been adjusted to the correct ionic strength with the Davies approximation (Davies, 1962). Rate constants for the pH buffer were varied by applying a factor $f_o$ to $k_{-4}$. $R$ denotes the universal gas constant of $8.31451$ J/mol, $K^*_W$ the equilibrium constant for the ion product of water calculated after DOE (1994), and $K^*_1$ and $K^*_2$ the first and second dissociation constants of carbonic acid calculated according to Roy et al. (1993). The corresponding reactions for the different rate constants are listed in Eqs. (17)–(22).

\[
\frac{d[OH^-]}{dt} = k_{-4}[HCO_3^-] - k_{+4}[OH^-][CO_2] - k_{+5}[OH^-][HCO_3^-] + k_{-5}[HCO_3^-][CO_2^-] + k_{+6} + k_{-6}[H^+][OH^-] \quad (27)
\]

\[
\frac{d[AH]}{dt} = k_{-a}[H^+][A^-] - k_{+a}[AH] \quad (28)
\]

\[
\frac{d[A^-]}{dt} = k_{+a}[AH] - k_{-a}[H^+][A^-]. \quad (29)
\]

This set of coupled differential equations was integrated numerically with the matlab ‘ode15s’ solver for ‘stiff’ problems (Shampine and Reichelt, 1997). These equations are called ‘stiff’ because the coupled system exhibits extremely different relaxation times (Zeebe et al., 1999).

3. pH-buffered seawater systems

Relaxation times of the carbonate system in pH-buffered seawater upon disturbance depends on the type and amplitude of the disturbance and most importantly on the kinetics of the pH buffer. However, rate constants for the protonation and deprotonation of widely used pH buffers are not available, and a general assumption is that the two reactions are almost instantaneous or at least sufficiently fast compared to other reactions that they can be ignored. In the following considerations, the rate constants for the pH buffer (i.e., its speed) were chosen to be about 100 times slower than the comparatively rapid $CO_3^{2-}$ to $HCO_3^-$ interconversion.

3.1. $K_2CO_3$ versus $CO_2$ addition

In Fig. 2 the response of the carbonate system upon addition of a $K_2CO_3$ solution to low-DIC (5 $\mu$mol kg$^{-1}$), pH-buffered seawater is shown (see caption of Fig. 2 for details). The amount of solution added was chosen to result in a 500 $\mu$mol kg$^{-1}$ increase in DIC in the seawater sample. This setup resembles those previously used to determine $k_-$ from the initial slope of $CO_2$ evolution (e.g., Badger et al., 1994; Sültemeyer et al., 1995). The re-equilibration in the carbonate system following the addition of a $K_2CO_3$ solution includes three characteristic time scales.

3.1.1. Time scale $10^{-10}$ to $10^{-5}$ s

Injection of a highly alkaline $K_2CO_3$ solution (pH ~11) into seawater of pH 8 significantly increases the OH$^-$ concentration as illustrated by the drop in pOH, i.e., the negative common logarithm of [OH$^-$] in analogy to pH (Fig. 2E). This decrease in pOH leads to an immediate increase in pH (Fig. 2F) as the OH$^-$ added consumes the H$^+$ present. Also shortly after injection of the $K_2CO_3$ solution, the conversion of the
added CO$_3^{2-}$ to HCO$_3^-$ begins (Fig. 2B,C). In this reaction CO$_3^{2-}$ combines with water giving HCO$_3^-$ and OH$^-$ (compare Eq. (20)) leading to a concomitant decrease in pOH (Fig. 2E). Why this reaction is dominating rather than the competing one, in which CO$_3^{2-}$ combines with H$^+$ (Eq. (19)), can easily be understood by comparing the relevant terms, $k_-^{\text{OH}^-}$ and $k_+^{H^+}[H^+]$, in Eq. (25). As $k_-^{\text{OH}^-}$ is about seven orders of magnitude larger than $k_+^{H^+}[H^+]$, HCO$_3^-$ is formed almost entirely by the combination of CO$_3^{2-}$ with water. The decrease in pOH is not a mirror image of the increase in pH, indicating that the ion product of water (Eq. (21)) is not constant on this time scale. The reason is that the conversion of CO$_3^{2-}$ to HCO$_3^-$ is slightly faster than the combination reaction of H$^+$ and OH$^-$. Hence, the increase in pH lags behind the decrease in pOH. Furthermore, the protonated form of the pH buffer is already starting to release protons to compensate for the loss of H$^+$, dampening the increase in pH. This initial pH-buffering, however, is not visible in [AH] (Fig. 2D) as the amount of H$^+$ released by the protonated form of the buffer is about six orders of magnitude lower than the buffer’s concentration (note that [H$^+$] is in the nanomolar and [AH] in millimolar range). A temporary pH plateau of about 8.35 is reached after about 10$^{-6}$ s. The height of this plateau depends on the rate constants of the pH buffer chosen. Increasing the rate constants increases the speed of the buffer and the temporary plateau will be closer to the final equilibrium value of about pH 8.0.

3.1.2. Time scale $10^{-5}$ to $10^{-1}$ s

The comparatively massive proton release by the protonated form of the buffer (Fig. 2D) yields to both, an increase of pOH (due to protonation of OH$^-$) and a decrease of pH (Fig. 2E,F). Hence, a second phase of CO$_3^2$ to HCO$_3^-$ conversion is initiated (Fig. 2B,C). A temporary quasi steady-state between all these pools is established after about $10^{-2}$ s (Fig. 2B–F).
3.1.3. Time scale larger than $10^{-1}$ s

It is by now that the $\text{HCO}_3^-$ and $\text{CO}_2$ pools start to re-equilibrate (Fig. 2A,B), owing to the slow interconversion rate. Again, the protonated form of the pH-buffer re-delivers the protons consumed by the conversion of $\text{HCO}_3^-$ to $\text{CO}_2$ (Fig. 2D). However, as the pH-buffer was assumed orders of magnitude faster than the $\text{HCO}_3^-$ to $\text{CO}_2$ conversion and the amount of protons released is small compared to those released due to the conversion of $\text{CO}_3^{2-}$ to $\text{HCO}_3^-$, the pH and the pOH stay constant during this last phase of re-equilibration.

In this configuration (see caption of Fig. 2 for details), the pH-buffer was able to keep the pH constant on a time scale of seconds (Fig. 2L), which is a prerequisite for the fitting procedure. However, it is obvious that this depends on the actual rate constants assumed for the protonation/deprotonation reactions of the buffer (i.e., its speed). When adding a $\text{K}_2\text{CO}_3$ solution to a seawater system as described above, the pH buffer has to release far more protons consumed in the rapid conversion of $\text{CO}_3^{2-}$ to $\text{HCO}_3^-$ than from the slow reaction of $\text{HCO}_3^-$ to $\text{CO}_2$.

This is opposite to addition of a $\text{CO}_2$ solution (Fig. 3), where the pH buffer has to accept more protons from the slow reaction. Again, there are three characteristic time scales for the re-equilibration.

3.1.4. Time scale $10^{-10}$ to $10^{-3}$ s

Injection of a $\text{CO}_2$ solution, yielding a final DIC concentration of 500 $\mu\text{mol kg}^{-1}$, in the buffered seawater (the preparation of such solution is described in Section 2.1), causes an initial drop in pH (Fig. 3F) as such a solution is highly acidic (pH $\approx$3.8). Almost instantly the unprotonated form of the buffer starts to accept the $\text{H}^+$ added, leading to an increase in pH and $[\text{AH}]$, the protonated form of the buffer (Fig. 3D). Please note that the total increase in pH from about 5.5 to 8.0 corresponds to a decrease in $[\text{H}^+]$ of about 3 $\mu\text{mol kg}^{-1}$. This is difficult to detect in the simultaneous increase in $[\text{AH}]$ as, again, the buffer concentration is about four orders of magnitude higher than the amount of protons accepted. pH reaches a temporary plateau of about 8.0 already before $10^{-5}$ s. The time required to achieve this pH which is close to equilibrium conditions crucially...
depends on the rate constants of the buffer, i.e., its speed. Already shortly after the initial increase in pH there is an increase in pOH. This is partly caused by the combination of HCO$_3^-$ with OH$^-$ yielding CO$_3^{2-}$ (Eq. (20)) because the injected CO$_2$ solution contains a small amount of HCO$_3^-$ (~2.5 µmol kg$^{-1}$). Hence, part of the HCO$_3^-$ (~0.2 µmol kg$^{-1}$) added convert to CO$_3^{2-}$ resulting in the increase in pOH. Also responsible for the increase in pOH is the combination of the H$^+$ added with the OH$^-$ present. However, the increase in pOH is not as pronounced as the increase in pH, indicating that the ion product of water on this time scale is not constant. This can be easily understood when comparing the equations competing for the H$^+$ added which are the formation of water with OH$^-$ (Eq. (21)) and the combination with the unprotonated form of the buffer A$^-$ (Eq. (22)). The relevant terms for these reactions given in Eq. (26) are $k_{-a}[OH^-]$ and $k_{-a}[A^-]$, respectively (note that [H$^+$] is the same for both of them). As $k_{-a}[A^-]$, is about 200 times larger than $k_{-a}[OH^-]$ the increase in pH is dominated by the protonation of the buffer and not by OH$^-$ consumption. Hence, the increase in pH is hardly reflected in pOH.

3.1.5. Time scale $10^{-5}$ to $10^{-1}$ s

The imbalance between the OH$^-$ and H$^+$ pools start to re-equilibrate and the pOH drops to its initial value whereas the pH is kept constant by the buffer. Again, a temporary quasi steady-state between all these pools is established after about $10^{-2}$ s (Fig. 3B–F).

3.1.6. Time scale larger than $10^{-1}$ s

The slow conversion reaction of CO$_2$ to HCO$_3^-$ is then initiated at about $10^{-1}$ s leading to a slight drop in pH and a concomitant increase in pOH compared to conditions prior to injection. And again, in this configuration (see caption of Fig. 3 for details) the buffer has been able to keep the pH very close to equilibrium values (Fig. 3L). Basically, if the protonation reaction of the buffer is faster than the conversion of CO$_2$ to HCO$_3^-$ it can keep up with the release of protons by this process.

3.2. Influence of the pH buffer kinetics on changes in pH

As outlined above, a prerequisite for fitting of the CO$_2$ evolution curve upon addition of a K$_2$CO$_3$ or CO$_2$ solution (Figs. 2G and 3G, respectively) is that the pH can be considered constant. The rate constants for the protonation and deprotonation of certain pH buffers, however, are not known. Therefore, their influence on pH, following the addition of a K$_2$CO$_3$ or CO$_2$ solution, was investigated by varying the rate constants of the pH buffer in the seawater carbonate system model. More specifically, the time was determined after which the pH reached a constant value. For that, a critical threshold was introduced of 0.05 pH units which was the maximum pH drift from steady state conditions after injection of a K$_2$CO$_3$ or CO$_2$ solution, observed in all measurements. Calculated times to reach that threshold depend to a small degree on whether K$_2$CO$_3$ or CO$_2$ is added (Fig. 4A/B), but mostly on the pH of the seawater. The differences in pK values between HEPES and BICINE are negligible for the following considerations. The time to reach constant pH values is faster the lower the pH is regardless whether K$_2$CO$_3$ (Fig. 4A) or CO$_2$ (Fig. 4B) is added. When adding K$_2$CO$_3$ the protonated form of the pH buffer (AH) releases protons to compensate for the loss of H$^+$ consumed by the conversion of CO$_3^{2-}$ to HCO$_3^-$. Hence, at low pH the initial concentration of AH is higher compared to high pH and the re-equilibration is faster. On the other hand, when adding CO$_2$ the unprotonated form of the buffer accepts the protons generated by the conversion of CO$_2$ to HCO$_3^-$. Hence at low pH, this protonation is enhanced by the increased [H$^+$].

It seems that neither K$_2$CO$_3$ nor CO$_2$ addition bears any advantages for measuring the carbon dioxide to bicarbonate interconversion rates. However, the time to reach constant pH values is not only bound to buffer kinetics but also to the amplitude of the disturbance. Basically, the lower the amount of K$_2$CO$_3$ or CO$_2$ addition, the lower is the disequilibrium in [H$^+$] the buffer has to cope with. Injecting a small amount of CO$_2$ and measuring its disappearance into the HCO$_3^-$ and CO$_3^{2-}$ pools is possible, while the same amount injected as K$_2$CO$_3$ will result in almost no detectable change in [CO$_2$] (note that in seawater, with a pH ranging from 7–9, the dominant DIC form is HCO$_3^-$ while the MIMS is only able to detect CO$_2$ and the two other DIC species have to be deduced). This is illustrated in Figs. 3A and 2A, where the change in [CO$_2$] due to a 500 µmol kg$^{-1}$ addition of CO$_2$ is about 500 µmol kg$^{-1}$, while the addition of 500 µmol kg$^{-1}$ of K$_2$CO$_3$ results in a change in [CO$_2$] of only about 4 µmol kg$^{-1}$. Therefore, it is feasible to work with much lower additions of CO$_2$ compared to K$_2$CO$_3$, and the influence of the buffer kinetics on the time to reach constant pH values is becoming less critical (when adding 15 µmol kg$^{-1}$ of CO$_2$ instead of 500 µmol kg$^{-1}$ (compare Fig. 4A) the pH is constant from the first second, regardless of seawater pH and buffer kinetics). Hence, the experiments were carried
out with small amounts of CO$_2$ injected into seawater buffered at different pH values at certain temperatures, and the resulting decrease in [CO$_2$] was monitored and fitted with Eq. (16) (Fig. 1).

3.3. The rate constants for CO$_2$ to HCO$_3^-$ interconversion at different temperatures and pH

The $k_+$ values resulting from fitting the CO$_2$ evolution upon adding small amounts of CO$_2$ (yielding a final concentration of about 15 $\mu$mol kg$^{-1}$) to seawater buffered at pH values ranging from 7 to 9 and temperatures of 11, 16 and 26 $^\circ$C are shown in Fig. 5. Also shown are calculated $k_+$ values from the second part of Eq. (23) with $k_+ = k_{+1} + k_{+4}[OH^-]$, the combination of reactions (17) and (18) (see Appendix for details). Because application of $k_{+4}$ values, determined previously (Zeebe and Wolf-Gladrow, 2001) by fitting the original data from Johnson (1982), did not match the measurements (especially at 26 $^\circ$C), $k_{+4}$ has been refitted in this study (see Table 1 for details). Measured and calculated $k_+$ values are in good agreement in the pH range from 7 to 8.5, regardless whether HEPES or BICINE was used as pH buffer. This clearly indicates that both buffers are able to keep the pH constant under the experimental conditions tested. Above a pH of 8.5, however, correlation between measured and calculated values becomes less clear. One reason is that the error in pH determination with the MIMS increases with rising pH, because the contribution of CO$_2$ to DIC decreases. Hence, after additions of known amounts of NaHCO$_3$ to determine the DIC/CO$_2$ ratio, the change in [CO$_2$] will get close to the noise in the CO$_2$ signal detected by the MIMS. Under these circumstances the pH cannot be calculated reliably anymore.

Furthermore, $k_+$ increases with rising temperature and pH (Fig. 5). High pH values correspond to increased [OH$^-$] and therefore the reaction of CO$_2$ + OH$^- \rightarrow$ HCO$_3^-$ is faster compared to low pH and $k_+$ is larger. Strictly speaking, the CO$_2$ evolution is characterized not by $k_+$ alone, but by $\gamma k_+$ (see Eq. (16)). But at pH values above 7, $\gamma = R/(R - 1)$ is almost equal to one and can be neglected (also compare solid line in Fig. 5 with Fig. 6A) From equilibrium conditions described in Eq. (1), it follows that $k_+ = k_+ / K^*_{1}$.

From Fig. 1 it becomes evident that interpretation of initial CO$_2$ slopes recorded by the MIMS is difficult. This is due to the fact that in the first couple of seconds, following a disturbance, homogeneous mixing in the cuvette and changing gas fluxes through the membrane...
mask the kinetics in the carbonate system. Thus, the initial slope of the CO₂ evolution curve (defined as the maximum slope) is always shallower than the slope that would be observed in an ideal setup. This can only be accounted for by fitting the CO₂ signal from the inflection point to a suitable equation, which explicitly incorporates the forward and backward reactions between the CO₂ and HCO₃⁻/CO₃²⁻ pools. Hence, determination of \( k_+ \) from the initial slope as proposed previously underestimates \( k_+ \) (and therefore \( k_+ + \)). We estimate this error

Fig. 5. Graphical illustration of the \( k_+ \) values determined with the fitting procedure shown in Fig. 1 and described in Section 2.3, measured by additions of \( \sim 15 \mu \text{mol kg}^{-1} \) CO₂ at different temperatures and seawater pH (total scale). Squares denote seawater buffered with 50 mM HEPES and circles seawater with 50 mM BICINE. The lines depict calculated \( k_+ \) with \( k_+ = k_{+1} + k_{+4}[\text{OH}^-] \) (see Table 1 for details regarding the rate constants) at temperatures of 11 (dotted), 17 (dashed) and 26 °C (solid line).

Fig. 6. Comparison of \( \gamma k_+ \) (dots), with \( k_+ \) calculated as for Fig. 5, and the eigenvalues \( \lambda \) of the carbonate system (solid line) described in Section 3.4 at 25 °C at a salinity of 35 between pH 7 and 9. A compares \( \gamma k_+ \) with \( \lambda \) while B illustrates the inverse of these values, which are the relaxation times \( \tau \) of the carbonate system upon disturbance.
to be up to 50% depending on temperature, pH and MIMS setup.

3.4. Theoretical remarks on $k = k_+ + k_{\text{aq}}[OH^-]$

It can be shown that the conversion of CO$_2$ to HCO$_3^-$ on time scales monitored by the MIMS (i.e., seconds) is given by the slowest process, which is characterized by the relaxation time $\tau$:

$$\tau = \frac{1}{\lambda}$$

(30)

$$\lambda = -\frac{1}{2}(k_+ + k_- + \bar{k}_+ + \bar{k}_-) + \frac{1}{2} \sqrt{(k_+ + k_- - \bar{k}_+ - \bar{k}_-)^2 + 4k_-\bar{k}_-}$$

(31)

with $k_+ = k_{-1}[H^+] + k_{-4}$, $k_- = k_{+1} + k_{+4}[OH^-]$, $\bar{k}_+ = k_{H_3^+} + k_{OH_5^+}$, and $\bar{k}_- = k_{H_5^+}[H^+] + k_{OH_5^+}$. Because of the rapid interconversion of HCO$_3^-$ and CO$_2^2-$, the exact solution for $[CO_2(t)]$ at constant pH can excellently be approximated by Eq. (16), with

$$\tau \approx \frac{1}{\gamma k_+}$$

(32)

as shown in Fig. 6B.

In summary, if the pH buffer is able to keep the pH constant upon disturbance in the carbonate system, the rate constants for the CO$_2$ to HCO$_3^-$ interconversion, $k_+$ and $k_-$, can be calculated as $k_+ = k_{+1} + k_{+4}[OH^-]$ and $k_- = k_+ K_H^+$ (see Table 1 for details on the rate constants).

4. Summary and conclusions

Mass spectrometric and $^{14}$C disequilibrium techniques are widely used to assess modes and efficiencies of inorganic carbon acquisition in marine phytoplankton (Badger et al., 1994; Espie and Colman, 1986). These methods rely on the exact knowledge of the rate constants for the CO$_2$ to HCO$_3^-$ interconversion reaction, which depend on pH, temperature and salinity. In this study, a method is presented for measuring these rate constants, known as $k_+$ and $k_-$, by means of membrane inlet mass spectrometry (MIMS). For the two pH buffers tested (HEPES and BICINE) it was shown that measured rate constants are in good agreement with calculated values for $k_+$ and $k_-$ in a pH range of 7 to 8.5 and at temperatures from 10 to 25 °C. Moreover, it was shown that the method proposed previously to determine the CO$_2$ to HCO$_3^-$ interconversion rate constants tends to significantly underestimate them. Therefore, it is recommended that in future applications $k_+$ and $k_-$ are measured or calculated as described above.

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Appendix A

The reaction scheme for the hydration of carbon dioxide can be formulated as (cf. Eigen et al. (1961))

$$\text{(I)} \quad \text{CO}_2(\text{aq}) + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3$$

(II)

$$\text{HCO}_3^- + \text{H}^+$$

(III)

in which aqueous carbon dioxide (CO$_2$(aq)) is either hydrated in the transition of (I) to (II) or via H$_2$CO$_3$ in the transition of (I) to (II) to (III). The overall hydration and dehydration reaction as measured by Johnson (1982) is then given as

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

(A - 1)

with CO$_2$ denoting the sum of CO$_2$(aq) and H$_2$CO$_3$, and $k_{+1}$ and $k_{-1}$ being the effective rate constants. As the reaction between carbonic acid and bicarbonate (II) is diffusion-controlled, it is practically instantaneous and equilibrium can be assumed as

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

(A - 2)

with $K_{H^+\text{CO}_3}$ being the acidity constant of true carbonic acid. Hence, $k_{+1}$ and $k_{-1}$ of the overall hydration/dehydration reaction are given by

$$k_{+1} = k_{+1}^* + k_{+2}$$

(A - 3)

and

$$k_{-1} = k_{-1}^* + \frac{k_{-2}}{K_{H^+\text{CO}_3}^*}$$

(A - 4)
While the hydration/dehydration reaction is dominant at low pH, at high pH the reaction via hydroxylation is favored as

\[ \text{CO}_2 + \text{OH}^- \xrightleftharpoons{k_{+4}} \text{HCO}_3^- \quad (A - 5) \]

The combination of the hydration/dehydration and the hydroxylation reaction gives the overall interconversion reaction of \( \text{CO}_2 \) to \( \text{HCO}_3^- \) and vice versa as

\[ \text{H}_2\text{O} + \text{CO}_2 \xrightleftharpoons{k_+} \text{HCO}_3^- + \text{H}^+ \quad (A - 6) \]

with

\[ k_+ = k_{+4} + k_{+4}[\text{OH}^-] \quad (A - 7) \]

and

\[ k_- = k_-[\text{H}^+] + k_{-4} \quad (A - 8) \]

Note that the overall reaction (Eq. (A-6)) must not be confused with that given Eq. (A-1) as it incorporates also the hydroxylation pathway.

References


