Buffers of known quality for the calibration of seawater pH measurements are not widely or commercially available. Although there exist published compositions for the 0.04 mol kg\textsuperscript{-1} equimolar buffer 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS)-TRIS \textsuperscript{+} in synthetic seawater, there are no explicit procedures that describe preparing this buffer to achieve a particular pH\textsubscript{T} with a known uncertainty. Such a procedure is described here which makes use of easily acquired laboratory equipment and techniques to produce a buffer with a pH\textsubscript{T} within 0.006 of the published pH\textsubscript{T} value originally assigned by DelValls and Dickson (1998), 8.094 at 25°C. Such a buffer will be suitable for the calibration of pH measurements expected to fulfill the “weather” uncertainty goal of the Global Ocean Acidification Observation Network of 0.02 in pH\textsubscript{T}, an uncertainty goal appropriate to “identify relative spatial patterns and short-term variation.”
although 0.01 would be more usual. This suggests that with proper calibration, glass electrodes are able to fulfil the “weather” uncertainty goal proposed by GOA-ON. However, certain requirements of the calibration standard are necessary for the pH measurement to be useful in CO2 system calculations. First, while glass electrodes measure the potential \(E\) of a solution, this measurement strictly depends on the unitless activity of hydrogen ions \(a(H^+)\) (Fig. 1). However, for seawater CO2 system calculations, it is the amount of hydrogen ion expressed in moles per kilogram of solution \([H^+]\) that is needed. \([H^+]\) and \(a(H^+)\) are related by the activity coefficient of \(H^+, \gamma(H^+)\), which is a function of solution ionic strength and composition. If \(\gamma(H^+)\) is the same in both the calibration standard \((S)\) and sample \((X)\), the measured pH can be interpreted as \([H^+]\) instead of \(a(H^+)\). In addition, the composition of the solution being measured also influences the potential of the glass electrode through the liquid junction that connects the external measured solution (either calibration standard or sample) with the internal reference electrode solution (Fig. 1). The potential across this junction \((E_J)\) will likely be different in the calibration standard and in the sample, and this “residual liquid junction potential” \((\Delta E_J)\) is not easily quantified (see, e.g., Buck et al. 2002 and citations therein). By calibrating the glass electrode in a standard with a similar ionic strength and composition to the sample, \(\Delta E_J\) can be minimized (Hansson 1973). Lastly, the expression used to convert the measured potential, \(E\), to a pH value assumes “Nernstian behavior” of the electrode, meaning a change of one unit in pH results in a potential change equal to the temperature-dependent “Nernst factor” \(k\) (Fig. 1). Ideal Nernstian behavior is unlikely for any given glass electrode system, and to account for this a “bracketing calibration” is often used. Bracketing means the electrode is calibrated with two or more standards of different pH values, where the expected sample pH value is between the highest and lowest calibration point (cf. Buck et al. 2002). The pH range in the ocean is fairly narrow, however, and the pH range of the global surface ocean is less than one unit (Takahashi et al. 2014). Provided the one-point calibration standard has a pH within the observed ocean pH range (~8), the error associated with likely non-Nernstian behavior will be small.

A preferred calibration standard for seawater pH \(pH_S\) measurements has become the buffer formed from the base species: 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS), and its conjugate acid: TRIS \(- H^+, \text{prepared in an ionic medium with a concentration similar to that of seawater (Hansson 1973; Ramette et al. 1977). At a temperature of } 25^\circ C \text{ and a salinity (S) of 35 the 0.04 mol kg-H}_2\text{O}^{-1} \text{ (or } 0.03827 \text{ mol kg-solution}^{-1} \text{) equi- molar buffer has a } pH \text{ of 8.094, which is within the observed open ocean pH range (DeValls and Dickson 1998; Olsen et al. 2016). An equimolar buffer implies that the buffering species, TRIS and TRIS \(- H^+\) are present in equal amounts. For such a buffer, the pH is determined by the acid dissociation constant \((pK^{'})\) of the buffering substance and the quotient of the appropriate activity coefficients (Eq. 1). While the thermodynamic pK\(^{'}\) is a function of temperature and pressure alone, the activity coefficient term is also a function of solution composition. TRIS, however, is an amine buffer, meaning that the activity coefficient term includes a singly-charged cation in both the numerator and the denominator (Eq. 1; Bates 1961):

\[
pH(\text{TRIS}) = pK^{'}(\text{TRIS} \cdot H^+) - \log\left(\frac{[\text{TRIS} \cdot H^+]}{[\text{TRIS}]}\right) - \log\left(\frac{\gamma(\text{TRIS} \cdot H^+)}{\gamma(\text{TRIS}) \cdot \gamma(H^+)}\right)
\]

Because of this, amine buffers offer the advantage of their pH not being very sensitive to changes in ionic strength and composition \((S)\) at any given temperature.

The assumption that is typically made when using a TRIS buffer is that the activity coefficient product in Eq. 1 is similar in value in the synthetic seawater (SSW; the ionic background) to what it would be the in natural seawater of the same nominal S. As a result, the activity coefficient product can be considered largely to be a function of S as well as temperature and pressure (a more detailed discussion of this can be found in Müller et al. 2018). A key consequence of this assumption

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**Fig. 1.** The use of a combination glass/reference electrode in a high-ionic strength solution.
when using such a buffer to calibrate the measurement of \([H^+]\) in seawater is that, if the calibration buffer and the measured sample differ significantly in \(S\), a systematic error will be introduced. Its magnitude is not well-defined as it results from two factors: the changes in activity coefficient with solution composition, and the change in the liquid junction potential \(E_J\) in Fig. 1 between the calibration buffer and the sample—again a result of the changing composition. This has been evaluated empirically by Butler et al. (1985) for a particular junction design and a change of \(\sim 5\) in \(S\) resulted in an error of \(\sim 0.01\) in \(pH\).

**Buffer preparation**

**Background**

Although a detailed method for the preparation of equimolar TRIS-\(H^+\) buffers in SSW has not been published, DelValls and Dickson (1998) presented a buffer solution composition (Table 1) to which they also assigned a \(pH_T\). Their method of buffer preparation is intended to produce buffers for analysis by a high-precision electrometric method, and if followed carefully ensures buffers with highly reproducible \(pH_T\) values (Nemzer and Dickson 2005; Pratt 2014; Müller et al. 2018). The uncertainty and purity goals associated with the various buffer components (Table 1, column 4) used by DelValls and Dickson are quite stringent, however, and preparing a buffer to this high level might not only be impractical for many research groups, but even unnecessary. To simplify the buffer preparation method, it is important to keep in mind the two key features required for the resulting TRIS buffer to have the expected \(pH_T\). This includes ensuring that the buffering species TRIS and TRIS - \(H^+\) are present in a 1-to-1 ratio, and that the SSW background has the same composition as used by DelValls and Dickson. While the buffer ratio has the largest effect on the \(pH_T\) of TRIS, the composition of the SSW ensures that the activity coefficient term and \(E_J\) are comparable between the calibration standard and the seawater sample. As noted earlier, this consistency in activity coefficients for calibration and measurement provides the basis of using the \(pH_T\) measurement in further calculations dealing with other acid–base systems, including the \(CO_2\) system. A discussion regarding to what extent the synthetic seawater needs to be “similar enough” to real seawater and its implication for relevant activity coefficients can be found elsewhere (Dickson et al. 2016; Pratt 2014).

The SSW chosen to represent natural seawater for this purpose is a simple mixture comprising the six major ions of seawater (\(Cl^-\), \(Na^+\), \(SO_4^{2-}\), \(Mg^{2+}\), \(Ca^{2+}\), and \(K^+\)). Minor components occurring in natural seawater, including acids and bases, have been replaced by an equivalent amount of one of the major ions of similar charge. Matching of ion charge helps to ensure that activity coefficients can be assumed the same in the SSW as in real seawater. The one exception to this is sulfate which reacts with hydrogen ion to form the hydrogen sulfate ion, and has proven hard to replace due to its relatively large amount and it being a double charged anion (Millero 1974). Instead of omitting sulfate from the SSW matrix, a \(pH\) scale intended for seawater use has been defined which implicitly includes the acid–base contribution of sulfate (Hansson 1973; Dickson 1993). This scale is known as the total hydrogen ion scale (\(pH_T\); eq. 2) and states that the \(pH_T\) of a solution is proportional to the free hydrogen ion amount content ([\(H^+\)\text{free}]). The factor relating \(pH_T\) to [\(H^+\text{free}\)] depends on the total sulfate amount content ([\(SO_4^{2-}\)]\text{total}) of the solution and the stoichiometric acid dissociation constant of hydrogen sulfate, \(K(\text{HSO}_4^-)\). Because sulfate is a conservative parameter in seawater, its total concentration [\(SO_4^{2-}\)]\text{total} can be estimated directly from \(S\). As long as the [\(SO_4^{2-}\)]\text{total} in the calibration standard corresponds to \(S\), the \(pH\) calibration can be made on the total \(H^+\) scale:

\[
 pH_T = -\log([H^+\text{free}] (1 + [SO_4^{2-}]_T/K(\text{HSO}_4^-))). \tag{2}
\]

Preparing TRIS buffers according to the published component uncertainties and purities (Table 1) is not necessary for the calibration of glass electrodes, nor for the majority of research concerning marine organisms and their physiological response to changing ocean acid–base chemistry. There is nevertheless a need for an explicit method of buffer preparation that is reproducible to a known uncertainty, using materials that are easily available to the majority of laboratories with basic chemical equipment. The key focus is to ensure that the buffer ratio is 1, and the SSW composition ensures activity coefficients that are consistent with other relevant seawater acid–base constants. The goal of this work is to describe a method for TRIS buffer preparation that will result in a buffer \(pH_T\) equivalent to the value assigned by DelValls and Dickson (1998). This buffer will be appropriate to calibrate \(pH_T\) measurements expected to fulfil the GOA-ON "weather" uncertainty goal of 0.02.

**Table 1.** Composition of 0.04 mol kg\(^{-1}\)H\(_2\)O \text{equimolar TRIS-TRIS} - \(H^+\) buffers at a \(S\) of 35 by DelValls and Dickson

| Component | mol kg\(^{-1}\)H\(_2\)O | mol kg solution\(^{-1}\) | Impurity specification (<\(\pm\)) or relative uncertainty \((\pm)\) in component amount\(^*\) |
|-----------|-----------------|-----------------|-----------------------------------------------|
| HCl       | 0.04000         | 0.03827         | \(\pm\)0.02\%                               |
| TRIS      | 0.08000         | 0.07654         | \(\pm\)0.02\%                               |
| NaCl      | 0.38764         | 0.37089         | \(<\)0.1\%\(^*\)                           |
| Na\(_2\)SO\(_4\) | 0.02927     | 0.02801         | \(<\)0.1\%\(^*\)                           |
| KCl       | 0.01058         | 0.01012         | \(<\)0.1\%\(^*\)                           |
| Mg\(_2\)Cl\(_2\) | 0.05474     | 0.05237         | \(<\)0.1\%\(^*\)                           |
| CaCl\(_2\) | 0.01075         | 0.01029         | \(<\)0.1\%\(^*\)                           |

\(^*\)Reported values from DelValls and Dickson (1998).
\(^\dagger\)Recrystallized for purification, exact impurity not characterized.
\(^\ddagger\)Added as solutions, see Method.
Simplifying buffer preparation

The TRIS buffers used by DelValls and Dickson (1998) were prepared using highly purified and carefully characterized reagents. This included using doubly distilled and coulometrically standardized HCl, using TRIS from the National Institute of Science and Technology (NIST) of certified purity, and SSW salts that had been purified by recrystallization (dissolving in deionized water followed by reprecipitation by partially evaporating the solution). Furthermore, NaCl, Na₂SO₄, and KCl were dried thoroughly following recrystallization. The recrystallized MgCl₂ and CaCl₂ were prepared into solutions rather than dried salts, due to their highly hygroscopic nature which makes it difficult to know the exact amount of water in their crystal structure. These two solutions were subsequently calibrated by analyzing their chloride content through precipitation of AgCl from an addition of excess AgNO₃. All buffer components were weighed using a high-resolution (0.01 mg) balance and quantitatively transferred to the container. Finally, the buffer solution was brought to the desired total solution weight by adding deionized water.

To design a simplified method for preparing TRIS buffers, three areas of experiments were carried out. These included using a simple colorimetric acid–base titration to calibrate HCl directly against commercially available TRIS solid, thus ensuring a buffer ratio of 1, while avoiding having to use purified and carefully characterized TRIS and HCl. The buffer was further prepared to a total volume, eliminating the need for determining the weight of the final solution. Lastly, a combination of ionic interaction-modelling and simple experiments was used to investigate the sensitivity of the pH of the buffer to changes in the SSW matrix (∆pH/∆salt), changes that exceeded the likely errors that could occur during preparation of the synthetic seawater.

Methods

Calibrating the buffer ratio by titration

This method makes use of a simple colorimetric acid–base titration that is described in detail in Appendix A1, together with all the calculations involved. Briefly, ~1 g (recorded to a resolution of 0.1 mg) of TRIS was dissolved in approximately 80 g of deionized water, to which six drops of 0.1% methyl red indicator were added. The yellow-colored solution was titrated by weight with (approximately) 1 mol kg⁻¹ HCl using disposable transfer pipettes until a distinct pink color was reached. The weights of TRIS and HCl were corrected to mass (Schoonover and Jones 2002) and the amount content of the HCl solution, [HCl]ₚ, was calculated assuming the TRIS was 100% pure. We performed these titrations primarily using TRIS from Macron (LOT 61548), NIST (SRM723e), and Fisher Scientific (LOT 144607), while a small number of titrations were carried out with TRIS from Sigma Aldrich (LOT 11K5445) and MP Biomedicals (LOT Q4553) for additional comparisons.

Preparing the buffer

TRIS buffers were prepared in two ways, one set of more carefully prepared buffers (“primary buffers”) to assess the success of calibrating the buffer ratio by titration, and one set of less carefully prepared buffers (“prepared volumetrically”) to evaluate a simpler overall preparation approach. The SSW of the primary buffers were prepared using NaCl, Na₂SO₄, and KCl as dried salts, MgCl₂ and CaCl₂ as calibrated solutions, and the buffer solution was brought to a particular total mass. Buffers prepared volumetrically used salts that had not been dried, MgCl₂ and CaCl₂ solutions with manufacturer calibration, and the buffer solution was brought to a particular total volume rather than mass. For both kinds of buffer, the buffer ratio was calibrated as described in the section above. All salts used for the SSW conformed to the American Chemical Society reagent grade specification (ACS; Tyner and Francis 2017) and were used without further purification.

For the primary buffers, the salts were dried at 200°C for at least 4 h and cooled to room temperature in a desiccator prior to preparing the buffer. Solutions of MgCl₂ and CaCl₂ were prepared in our laboratory and calibrated by titration against standardized ~0.3 mol kg-solution⁻¹ AgNO₃ in the presence of a chromate/dichromate indicator as described in Vogel (1961), also known as a Mohr titration. The estimated relative standard uncertainty for this titration method is 0.5%. For the volumetrically prepared buffers, the manufacturer’s calibrations of ~1 mol kg⁻¹ MgCl₂ and CaCl₂ solutions were used. No certificate of analysis was provided for the MgCl₂ solution (beyond being sold as a “1 mol L⁻¹” solution), while the CaCl₂ had a calibrated concentration of 1.04 mol L⁻¹.

Each type of buffer was prepared by first estimating the target weight of HCl solution that would be needed to prepare the desired quantity of buffer (either a particular weight of primary buffer, or a particular volume otherwise). A quantity of HCl solution approximating this target value was then weighed out. The desired weights of TRIS, the various SSW salts, and the total solution weight (primary buffers only), were each scaled to correspond to the weight of dispersed HCl so as to produce a buffer of the relative proportions shown in Table 1, and an effort was made to weigh out these desired amounts closely. The weights of HCl solution, TRIS, and the SSW salts, were recorded to a resolution of 0.1 mg. Deionized water was used to quantitatively transfer all
components into the buffer container and to dilute to the desired total buffer quantity. The total weight of the primary buffer solution was recorded using a high-capacity balance of 0.01 g resolution. A total of six buffers were prepared this way by one laboratory technician. Buffers prepared volumetrically were brought to a total volume of 1 liter using a volumetric flask and a knowledge of the density of the resulting buffer (removing the need for a high-capacity balance). A total of ten buffers were prepared this way by two different laboratory technicians, and this buffer preparation approach is described in detail in Appendix A2. Equations are provided that characterize the density of the resulting TRIS buffer, and that enable scaling of the desired weights of all components to the original dispensed weight of HCl.

Assessing effects of uncertainties in preparing the synthetic seawater

Errors can arise during the preparation of the buffer, and ACS grade chemicals are only provided with an upper limit of impurities. While water is likely the main impurity, access to a drying oven (or other means of reducing the level of this impurity) might not always be available. It is possible to estimate the likely implications on the buffer pH2 of small compositional changes resulting from weighing errors or water contamination. The approach used here was to perform calculations with a Pitzer-type ionic interaction model similar to those used by Waters and Miller (2013) and by Gallego-Urrea and Turner (2017). These calculations were carried out for us by Dr. Simon Clegg of the University of East Anglia, United Kingdom. The change in the molality (mol kg-H2O) of free hydrogen ion, m(H+) resulting from a 1% change in the total concentration of each of the various buffer components (Table 1) was calculated using a Pitzer model. The primary sensitivity was due to changes in the amounts of TRIS and of HCl, which in addition to affecting the buffer ratio (Eq. 1) have a comparatively minor effect on the relevant activity coefficients. In fact, for a 1% change in the buffer ratio, the buffer pH will change by about 0.004 pH units, due almost entirely to the change in buffer ratio. The next most significant change in m(H+) resulted from an error in the amount of NaCl where a 1% change resulted in a small change of < 0.001 in pH2. A 1% error in the amount of Na2SO4 results in a very small change in m(H+) (< 0.04%), but as can be seen from Eq. 2 the change in the sulfate ion concentration can have an additional effect when considering (as we do here) the total H+ concentration. We estimate the overall effect from a 1% error in amount of Na2SO4 to be ∼ 0.3%, or an error of a little more than 0.001 in pH2, using Eq. 2. For the other components, the largest effect is for a 1% change in the amount of MgCl2 which results in an estimated change of pH2 of 0.0001 (i.e., a negligible amount).

This Pitzer-modelling approach was supplemented with a simplistic experiment where six buffers were prepared with an identical buffer ratio but slightly different SSW compositions. These six buffers were prepared similarly to the primary buffers described above, with the exception that an HCl-TRIS mixture was prepared and divided into the six bottles before adding the remaining components (scaling them to the weight of HCl in the mixture). This ensured identical buffer ratio in the six buffers. While one of the six buffer solutions was prepared as a regular, “unaltered” TRIS buffer, the amount of one of the SSW salts: NaCl, Na2SO4, KCl, MgCl2, or CaCl2 was increased by approximately 15% in each of the remaining five bottles. This resulted in five different buffers which all had a different composition from that of a regular TRIS buffer, and a different composition from one another. This simplistic experiment was repeated a total of three times.

The unaltered and altered buffer solutions were subsequently examined spectrophotometrically at 25°C using the (purified) pH-sensitive dye meta-cresol purple (mCP) and the method described by Carter et al. (2013). The pH values of the unaltered and altered TRIS buffers were calculated based on the equations of Liu et al. (2011). It is, however, important to recognize that the changes in the ionic composition of the TRIS solution will also affect the activity coefficients of the mCP dye. This would result in a calculated pH value, obtained spectrophotometrically, that is likely not consistent with other relevant acid–base parameters in seawater. Thus, this calculated “pH” will not be identical to the actual pH of the buffer, and the pH values from these experiments are referred to here as pHspec.

Results and discussion

Using and acid–base titration to ensure the buffer ratio

For one batch of HCl, [HCl]titr was determined using Macron TRIS on five separate days, as the mean of ≥ 3 titrations each day, and on each day that particular value was used to prepare a batch of primary TRIS buffer. This particular batch of HCl had been standardized previously using coulometry, [HCl]coul, which enabled an estimate of the accuracy of the titration method (or, largely, of TRIS impurities). The relative percent difference between [HCl]titr and [HCl]coul was small for each of the five days, and within the relative standard deviation, 0.1%, of the titration method (Fig. 2a).

The resulting pH2 of the buffer, measured spectrophotometrically, was within 0.002 of the value originally assigned by DelValls and Dickson (1998) (Fig. 2b). Furthermore, the range of pH2 values was within the expected range resulting from the [HCl]titr calibration uncertainty, and within the uncertainty estimated for the spectrophotometric measurement technique itself (∼ 0.004; Müller and Rehder 2018). There appeared to be no correlation between the deviation in buffer pH2 from the assigned value (8.094) and %Δ[HCl].

Calibrating the HCl solution against TRIS solid will likely not yield the true [HCl] because TRIS crystals can have varying levels of water occluded in their crystal structure (Koch et al. 1975). This will act to overestimate [HCl] relative to the true value, as our approach assumes the TRIS is 100% pure. This value of [HCl] is however appropriate for use to prepare the
TRIS buffer (Fig. 2b), as the presence of a (small but unknown amount of) water impurity in the TRIS solid will be accounted for in this calibration. This implies that it is important to treat the TRIS similarly prior to HCl calibration and to preparing the buffer, for example, it should either be dried for both purposes or not at all. Thoroughly drying TRIS can be difficult and requires careful homogenization of the crystals. Furthermore, drying at high temperatures can decompose the molecule and while drying the salt over a hygroscopic substance, such as phosphorous pentoxide, in vacuum is preferred, most laboratories do not have easy access to this approach. It is therefore more practical to use TRIS solid “as is” without any further treatment. Any additional water added to the buffer solution in this way, (< 2% of the total weight of TRIS according to the reagent grade specification), will decrease the total buffer amount by < 2%, and the S of the resulting solution by < 0.02%. Neither of these effects will change the pH_t of the buffer appreciably (see, e.g., figs. 1, 2b in DelValls and Dickson 1998).

It should be pointed out that the SSW composition used here is slightly different from that of “pure” SSW. The addition of HCl increases the ionic strength of the solution, and this effect is compensated for by reducing the amount of NaCl. Thus, while the calibrated [HCl]_titr might achieve the this effect is compensated for by reducing the amount of NaCl. The calibration of HCl increases the ionic strength of the solution, and while drying the salt over a hygroscopic substance, such as phosphorous pentoxide, in vacuum is preferred, most laboratories do not have easy access to this approach. It is therefore more practical to use TRIS solid “as is” without any further treatment. Any additional water added to the buffer solution in this way, (< 2% of the total weight of TRIS according to the reagent grade specification), will decrease the total buffer amount by < 2%, and the S of the resulting solution by < 0.02%. Neither of these effects will change the pH_t of the buffer appreciably (see, e.g., figs. 1, 2b in DelValls and Dickson 1998).

The relative purity of TRIS from various commercial manufacturers is suggested in Fig. 3, which shows %Δ[HCl] for a single batch of HCl calibrated against TRIS from five different manufacturers. Four of these, Macron, Fisher Scientific, Sigma Aldrich and MP Biomedicals, were all of “reagent grade,” reported a water content of 2% or less, and were used “as is.” TRIS from NIST is far more homogenized than the other commercial sources, and it is sold with a certificate of purity for both with and without drying and further homogenization. As such, NIST SRM723e TRIS would be appropriate when it is necessary to accurately determine [HCl]. However, the high level of purity does not increase the quality of the buffer for the purposes described herein. NIST SRM723e is also more than 30 times as expensive as TRIS from the other commercially available sources.

A potential drawback to the less expensive sources of TRIS is increased crystal heterogeneity, where the weights used for titration (~1 g) may not necessarily be representative for the average water impurity of the amount of salt used for a 1 liter buffer (~10 g). In terms of calibrating [HCl]_titr, this would result in an increased standard deviation, which is perhaps the case when comparing, for example, [HCl]_titr of TRIS from Macron versus from NIST in Fig 3. Although the titration data using TRIS from other commercial suppliers than Macron are limited, there appears to be little difference between the various commercially available sources of TRIS with the exception of MP Biomedicals. It is hard to ascertain whether the significantly lower [HCl]_titr determined using TRIS from this source is due to the crystals being more heterogeneous, an overall higher amount of impurities, or just an artefact of the limited number of titrations performed.

Preparing synthetic seawater

There was a measurable increase in pH_{spec} (ΔpH_{spec}) between the unaltered TRIS buffers and those altered with 15% of any single salt of the SSW matrix. ΔpH_{spec} correlated to the total amount of salts in solution (Fig. 4; Table 1), in other words, ΔpH_{spec} seemed to be largely a function of change in the ionic strength of the buffer. ΔpH_{spec} did not, however, scale linearly to the change in ionic strength. The change caused by salts containing divalent ions (Mg^{2+}, Ca^{2+}, and SO_{4}^{2-}) was larger than might be expected from the change in ionic strength their respective salts caused, compared to the

Fig. 2. (a) Mean %Δ[HCl] = 100 · ([HCl]_{titr} - [HCl]_{samp}) / [HCl]_{samp} %, (n ≥ 3) for the five different days of measuring [HCl]_{titr} while the bars represent one relative standard deviation of the titration technique and not for each individual set of titrations. (b) Mean pH_t (n ≥ 4) of each of the five TRIS buffers, the bars represent one standard deviation of each set of the pH_t measurements and the black drawn line indicates the value 8.094.

Fig. 3. The mean %Δ[HCl] and one relative standard deviation for each [HCl]_{titr} determined. The suppliers were: Macron (▲), Fisher Scientific (●), NIST SRM723e (■), Sigma Aldrich (♦), and MP Biomedicals (▼). Number of titrations are indicated in the parentheses in the legend.
salts that only contained monovalent ions. This was likely caused by stronger interactions of the divalent ions with TRIS, TRIS \( \cdot \) H\(^+\), and with the spectrophotometric dye mCP.

This simplistic view on buffer pH\(_T\) sensitivity to changes in the background ionic composition suggests NaCl and KCl can be added to the SSW as salts of 99% purity or higher, without changing the buffer pH\(_T\) by more than \( \approx 0.0005 \) (Table 2), a view that is supported by the Pitzer modelling. MgCl\(_2\) and CaCl\(_2\) solutions used for this particular experiment were calibrated to \( \pm 0.5\% \) in our laboratory. Our results suggest that they can be used successfully as the commercially available 1 mol L\(^{-1}\) solutions (provided these are calibrated to \( \pm 2\% \) or better from the manufacturer), producing an accumulated buffer pH\(_T\) uncertainty of less than 0.0005 (Table 2). While it is possible to add MgCl\(_2\) and CaCl\(_2\) directly as solids (MgCl\(_2\)×6H\(_2\)O and CaCl\(_2\)×2H\(_2\)O), their exact level of hydration would need to be known. The number of H\(_2\)O in their crystal structure can vary significantly depending on the environmental conditions in the laboratory where the salts are stored, and determining the level of hydration would involve additional analysis.

While increasing the amount of Na\(_2\)SO\(_4\) did not have a large effect on observed pH\(_{\text{spec}}\), a 1% change in [SO\(_4^{2-}\)]\(_T\) has implications for the use of the pH\(_T\) scale. Increasing [SO\(_4^{2-}\)]\(_T\) by 1% at a constant [H\(^+\)]\(_T\)free changes pH\(_T\) by nearly 0.001 unit (using Eq. 2), which is much larger than the observed \( \Delta \text{pH}_{\text{spec}} \) from such an increase in the amount of Na\(_2\)SO\(_4\) (Fig. 4). Nevertheless, carefully adding Na\(_2\)SO\(_4\) as a salt of 99% purity or better should not cause an error in pH\(_T\) of more than 0.001.

As was pointed out earlier, this simplistic experiment necessarily illustrates that the change in observed pH is not only caused by the actual buffer pH changing as a consequence of the extra salt, but also because the mCP dye behaves differently in the altered ionic background. The difference between \( \Delta \text{pH}_{\text{spec}} \) and the \( \Delta \text{pH} \) implied by the Pitzer model is particularly large for MgCl\(_2\) and it is believed that the base form of mCP, a doubly charged anion, interacts more strongly with the divalent Mg\(^{2+}\) compared to the other SSW ions. Despite the slightly different results from the two approaches, the likely error in a normal buffer preparation will be small.

### Table 2. Suggested specifications of reagents for the preparation of 1 liter* TRIS buffers at an S of 35, and contributions to buffer pH\(_T\) uncertainties from the various components.

| Component       | Weight of component (g) | Tolerable impurity (<) or uncertainty (±) in relative component amount | Contribution to buffer pH\(_T\) uncertainty |
|-----------------|-------------------------|---------------------------------------------------------------------|---------------------------------------------|
| HCl (1 mol \( \text{kg-solution}^{-1}\)) | 39.270\(^a\)            | ±0.1%                                                              | 0.002                                        |
| TRIS            | 9.517\(^b\)             | ≤2%                                                                |                                             |
| NaCl            | 22.254                  | <1%                                                                | 0.0005                                      |
| Na\(_2\)SO\(_4\) | 4.085                   | <1%                                                                | 0.001                                       |
| KCl             | 0.775                   | <1%                                                                | ~0                                           |
| MgCl\(_2\) (1 mol L\(^{-1}\)) | 58.862                  | ±1.5%                                                              | 0.0004                                      |
| CaCl\(_2\) (1 mol L\(^{-1}\)) | 11.726                  | ±1.5%                                                              | 0.0001                                      |
| Deionized water | “Fill to line”           | ±1%                                                                | 0.001                                       |
|                  |                         | Accumulated maximum uncertainty relative to 8.094                  | 0.005                                       |

\(^a\)Assumes that volume is calibrated (and measured) at 20°C.
\(^b\)Weights of HCl and TRIS subject to the measured [HCl]\(_T\).
\(^c\)Includes error in weighing HCl to the desired amount, and implications of subsequent scaling amounts of remaining components.

An altered ionic composition may also affect measurements made with glass electrodes, although it is a less sensitive measurement than the spectrophotometric pH method. The pH of the experimental buffer with the largest change to its ionic composition (+15% NaCl) was measured and compared to the unaltered buffer using a glass electrode in our laboratory. This observed “\( \Delta \text{pH} \)” was 0.01, which was the reported resolution of the glass electrode pH meter. Therefore, no other altered solutions were tested in this way as this indicated small errors in the SSW matrix are unlikely to produce a measurable difference when using a glass electrode.

### Reproducibility in preparing TRIS buffers

Volumetrically prepared TRIS buffers were analyzed over the course of a couple of weeks, where several but not all buffers were analyzed on the same day. The ten buffers agreed very well with one another and their mean pH\(_T\), measured spectrophotometrically, was 8.088 ± 0.001 (mean ± one SD; \( n = 43 \)) showing that our method is highly reproducible. Alongside these were also measured the pH\(_T\) of four batches of primary TRIS buffers, where the mean pH\(_T\) was 8.089 ± 0.001 (\( n = 50 \)). It should be noted here that the expected pH\(_T\) of TRIS is 8.094, as determined by DeValls and Dickson (1998). This discrepancy of 0.005–0.006, if real, has many potential sources including the spectrophotometric measurement itself. To confirm that our buffers were consistent with historical Harned cell measurements made in our laboratory, we made a small number of additional measurements on a subset of the TRIS batches. Two batches of primary buffers and one volumetrically prepared batch were...
analyzed in a spectrophotometric cell whose values for TRIS pH₇₃ measurements had been previously cross-checked using the Harned cell. The average pH₇₃ for all three batches using this cell was 8.092 (± 0.001; n = 16), which is in good agreement with the value published by DelValls and Dickson.

Preparing the buffer by total volume rather than total weight removes some flexibility. If the first component added (i.e., HCl) is slightly wrong, you cannot straightforwardly prepare the buffer to a scaled volume without gaining some uncertainty. Because the provided calculations will scale the weight of the remaining components to the added weight of HCl, the buffer might be prepared to a scaled volume without gaining some uncertainty. This is consistent with the lower Cᵢ values measured in the buffers. The level of Cᵢ in TRIS buffers over time will further depend on the amount of time the buffer has been exposed air and could vary depending on the headspace of the storage container. Most plastic containers (e.g., low- or high-density polyethylene) are permeable to gases, suggesting that TRIS buffers stored in such a container will almost certainly take up CO₂ from the atmosphere over time.

**Conclusion**

With access to standard laboratory equipment, including a balance readable to ± 0.1 mg, it is possible to prepare TRIS buffers in synthetic seawater to a pH₇₃ that has an uncertainty of 0.006 relative to the expected value of 8.094 at 25 °C (DelValls and Dickson 1998). The proposed colorimetric acid-base titration technique used to calibrate the HCl directly to the TRIS allows for significant savings on the materials used to prepare the buffer. This level of uncertainty in the buffer pH₇₃ is more than sufficient for seawater pH measurements that are expected to fulfill the GOA-ON weather uncertainty goal of 0.02 in pH, and the buffer ionic composition provides consistency with various acid–base equilibrium constants appropriate for seawater.

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Appendix 1

Estimation of the amount content of HCl solutions by titration against TRIS, using a colorimetric end-point determination

Materials

Magnetic stirrer and magnetic stir bar; 250 mL pyrex low-form beaker(s), small beaker (e.g., 50 mL) to hold pipettes during weighing, disposable Samco™ Narrow Stem Transfer Pipettes of 4.5 and 15.3 mL capacity, Tygon® tubing of 3/32" inner diameter, analytical balance (0.1 mg resolution), white paper and light source. A list of suggested materials and their catalogue numbers can be found in Appendix 3.

Chemicals

1.0 mol L⁻¹ HCl, TRIS solid, 0.1% methyl red indicator in alcoholic solution. A list of suggested chemicals and their catalogue numbers can be found in Appendix 3.
Modification of disposable pipettes

The tip of the 4.5 mL disposable pipette is modified to deliver a smaller drop size by carefully melting and stretching the tip over an ethanol flame, so it is able to deliver a drop size of less than 0.01 g (Fig. A1c,d). A cap for the 15.3 mL disposable pipette is prepared by tying a knot in the Tygon® tubing and cutting it to an appropriate length (Fig. A1b). Over the course of several hours, evaporation from the modified-tip 4.5 mL pipette is minimal, and a cap is not necessary.

Titration

a. One gram of TRIS solid is weighed into a 250 mL beaker, deionized water is added to a total solution weight of about 80 g, and a magnetic stir bar placed in the solution.
b. Six drops of indicator are added, turning the solution yellow (Fig. A2a). The 250 mL beaker is placed on a magnetic stirrer with a white paper as a background and a light shining down directly on the beaker (this allows for easier perception of the color change).
c. The two disposable pipettes are filled with the ~1.0 mol L⁻¹ HCl solution, the 15.3 mL pipette is capped, and they are both placed in a small beaker which is then weighed to ± 0.1 mg.
d. HCl is added, as the solution is stirring, from the 15.3 mL pipette until the solution shows a hint of pink that persist for less than a couple of seconds. At this point, the larger pipette is again capped and returned to the small beaker.
e. HCl is then added slowly using the modified 4.5 mL pipette until one drop changes the solution from an orange-pink color to a distinct pink color (Fig. A2b).
f. The beaker with the two pipettes is again weighed, and the difference from the first weighing equals the weight of HCl added. For a 1.0 mol L⁻¹ solution of HCl and 1 g of tris, the weight of HCl used should be close to 8 g.
g. Make sure to practice this method until you feel confident you can identify the appropriate color change. At this point, proceed to use the method for calibrating [HCl]titr.

Calculations

The amount content (in mol kg-solution⁻¹) of HCl is calculated based on the mass (m) in g of TRIS and HCl used in the titration. Their weights (w), in g, are corrected to mass by applying an air buoyancy correction as shown in Eq. A1, where the densities (ρ) of TRIS and HCl are 1.33 g cm⁻³ and 1.02 g cm⁻³, respectively.

\[
m_{\text{g}} = w_{\text{g}} \times (1 + 0.0012 \times (1/\rho - 1/8)) \tag{A1}
\]

The amount content of HCl, based on this titration technique, [HCl]titr, is then calculated according to Eq. A2, using the molar mass (M) of TRIS of 121.14 g mol⁻¹.

\[
[HCl]_{\text{titr}} = \frac{m_{\text{TRIS}} \times (\text{g mol}^{-1})}{M_{\text{TRIS}} \times (\text{g mol}^{-1})} \times \frac{1000 \text{ (g kg}^{-1})}{m_{\text{HCl, solution}} \times (\text{g})} \tag{A2}
\]

A spreadsheet implementation of this calculation is included as a supplement ("Preparing TRIS buffers.xlsx," sheet #1 Calibrating HCl and TRIS).
Appendix 2
Preparation of 1 liter TRIS buffer in synthetic seawater

Materials

Clean volumetric flask (1 liter), funnel, analytical balance (± 0.1 mg), seven glass beakers (< 250 mL) or weighing dishes/pouring boats, three disposable Samco™ Narrow Stem Transfer Pipettes of 15.3 mL capacity, two spatulas, magnetic stir bar, and stir plate. A list of suggested materials and their catalogue numbers can be found in Appendix 3.

Chemicals

1.0 mol L⁻¹ HCl, TRIS solid, NaCl, Na₂SO₄, KCl, 1.0 mol L⁻¹ MgCl₂, 1.0 mol L⁻¹ CaCl₂. A list of suggested chemicals and their catalogue numbers can be found in Appendix 3.

The desired weight of HCl (w(HCl)desired) depends on [HCl]titr as determined in Appendix 1, and is calculated based on Eq. A3, where [HCl]buffer solution is the target HCl (and thus TRIS - H⁺) amount content of 0.03827 mol kg-solution⁻¹ (equivalent to 0.04 mol kg-H₂O⁻¹) in the buffer and w(buffer solution)desired the weight of 1 liter of the buffer solution at 20°C.¹

\[
\frac{w(HCl)_{desired}(g)}{w(buffer\ solution)_{desired}(g)} \approx \frac{[HCl]_{buffer\ solution}}{[HCl]_{titr}} \left( \text{mol kg-solution}^{-1} \right) \tag{A3}
\]

Mixing the buffer

a. Weigh out HCl: Place the funnel in the neck of the volumetric flask and start by weighing out HCl into a beaker (or weighing boat) to within 0.3 g or better of the desired weight from Table A1, using a disposable transfer pipette to adjust the final weight. Record the dispensed weight of HCl and transfer the HCl quantitatively into the volumetric flask with deionized water (~ 100 mL).

b. Scale desired weights to the dispensed weight of HCl: Calculate by what proportion the dispensed HCl weight (w(HCl)dispensed) is different from the weight in Table A1 (w(HCl)desired). To ensure the ratio of moles between all components remain the same, this factor is used to adjust the desired weights (w(X)desired) of the remaining components to recalculate a target weight of each (w(X)target) as shown in Eq. A4. As long as HCl is added to within 0.3 g of the desired weight (for a 1 liter buffer), and the remaining components are added in proportion to that, the resulting 1 liter buffer will have a S of < 0.3 units different than the desired S of 35.

¹While this equation should strictly be in terms of mass (m), and not weight (in air), the ratio of the air buoyancy correction term for the buffer solution and the HCl solution approximately equals one and can be omitted. In the supplementary spreadsheet, however, this air buoyancy correction is explicitly included in the calculation.
\[ w(X)_{\text{target}}(g) = w(X)_{\text{desired}}(g) \frac{w(\text{HCl})_{\text{dispensed}}(g)}{w(\text{HCl})_{\text{desired}}(g)} \quad (A4) \]

c. **Weigh out the remaining components**: Weigh out the target weights, \( w(X)_{\text{target}} \), of the remaining components in individual beakers or weighing boats (disposable transfer pipettes can be used to adjust the final weights of the MgCl\(_2\) and CaCl\(_2\) solutions), and transfer each of these quantitatively into the volumetric flask by rinsing each individual weighing vessel into the flask, using \( \sim 100 \text{ mL} \) deionized water per rinse.

d. **Add water and mix**: Once all components have been added, rinse the funnel into the flask with deionized water and fill the flask to a few centimeters below the 1-liter mark. Replace the flask stopper and mix by hand by inverting the bottle a few times to dissolve the majority of the salts. This will increase the density and thus decrease the volume slightly. Fill the flask carefully with deionized water (using, e.g., a transfer pipette) until the bottom of the solution meniscus is level with the etched 1-liter mark on the bottle. Place a stir bar in the flask, replace the stopper, and set to stir for at least four hours.

A spreadsheet implementation of the calculations involved is included as a supplement (“Preparing TRIS buffers.xlsx,” sheet #2 Mixing buffer).

**Appendix 3**

**Suggested list of chemicals and materials, and their respective catalogue numbers**

**Chemicals**

- 1.0 mol L\(^{-1}\) HCl: Fisher Scientific catalogue number (FS#) 60-007-56.
- TRIS solid: FS# T395-100.
- NaCl: FS# S271-500.
- Na\(_2\)SO\(_4\): FS# S421-500.
- KCl: FS# P217-500.
- 1.0 mol L\(^{-1}\) MgCl\(_2\): FS# 50-751-7456/Amresco E525-500ml.
- 1 mol L\(^{-1}\) CaCl\(_2\): FS# 50-751-7510/Amresco E605-500ml.
- 0.1 % methyl red indicator in alcoholic solution: RICCA catalogue number 5045-4.

**Materials**

- Weighing dishes: FS# 08-732-113.
- Disposable Samco™ Narrow Stem Transfer Pipettes of 4.5 mL capacity: FS# 13-711-34/ThermoFisher Scientific 251PK.
- Disposable Samco™ Narrow Stem Transfer Pipettes of 15.3 mL capacity: FS# 13-711-36/ThermoFisher Scientific 252PK.
- Tygon® tubing of 3/32" inner diameter: Fisher Scientific catalogue number FS# 14-171-130/Saint Gobain ADF00004.

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