Preparation of 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS) pH buffers in synthetic seawater

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Keywords: Seawater pH, glass electrode, TRIS buffer

Abstract

Buffers of known quality for the calibration of seawater pH_T measurements are not widely or commercially available. Although there exist published compositions for the 0.04 mol $kg\text{-}H_2O^{-1}$ equimolar buffer 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS)-$TRIS{\cdot}H^+$ in synthetic seawater, there are no explicit procedures that describe preparing this buffer to achieve a particular pH_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_T within 0.006 of the published pH_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 fulfil the “weather” uncertainty goal of the Global Ocean Acidification Observation Network (GOA-ON) of 0.02 in pH_T, an uncertainty goal appropriate to “identify relative spatial patterns and short-term variation”.

Introduction

The observed decrease of surface ocean pH and accompanying changes in acid-base chemistry, resulting from the uptake of anthropogenic CO_2 in a process known as ocean acidification (OA), is of particular interest for many research groups and there is a broad interest in monitoring and understanding how marine organisms respond to changes in ocean pH. pH is one of the most commonly used parameters to describe seawater acid-base chemistry and is often used in conjunction with another parameter describing the CO_2 system so as to enable calculation of parameters such as the carbonate ion concentration, and hence the aragonite saturation state, which may be more relevant to particular organisms (Orr et al. 2005). However, for pH measurements to be appropriate for comparison both across time and space it is important that the data is of a known uncertainty. It is also important to ensure that the choice of calibration buffer produces a pH value that is consistent with the relevant acid-base constants used in such calculations. Presently, this includes that the buffer is based on a synthetic seawater recipe intended to ensure that activity coefficients of species in the buffer are similar to what they would be in real seawater of the same nominal salinity, and using a pH scale that is appropriate for seawater: the total hydrogen ion scale, pH_T (Dickson et al. 2016). Access to suitable seawater pH calibration buffers is therefore key and, ideally, these should either be easily available or simple to prepare reproducibly.

The Global Ocean Acidification Observation Network (GOA-ON; http://www.goa-on.org) has proposed a standard uncertainty goal for studying seawater pH of 0.02 in pH (Newton et al. 2015) – which will, when combined with measurement of another CO_2 parameter such as total alkalinity or total dissolved inorganic carbon (C_T), allow for calculation of carbonate ion concentration with a relative standard uncertainty of $\leq 10\%$. This level of uncertainty (the "weather goal") is intended to be sufficient to identify relative spatial patterns, and short-term variations while also supporting mechanistic interpretation of the response to, and impact on, local and immediate ocean acidification processes. In the coastal ocean, the weather uncertainty goal is particularly relevant as the observed changes are usually much larger than those observed in the open ocean (see, for example, fig. 2 in Hofmann et al. 2011). This uncertainty goal for pH measurements will also enable laboratory studies of physiological processes possibly affected by ocean acidification such as calcification or primary production, and will enable comparison of similar studies performed at different locations.

The uncertainty of a pH measurement necessarily includes both the uncertainty of the sample measurement process and the uncertainty associated with the calibration. The combination glass/reference cell ("glass electrode" hereafter) which uses potentiometry to measure pH, is perhaps the most widely used pH measurement technique and can resolve changes in pH of ~0.003 depending on electrode design and sample handling procedure (Dickson et al. 2007; Easley and Byrne 2012), 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 CO_2 system calculations. First, while glass electrodes measure the potential (E) of a solution, this measurement is proportional to the activity of hydrogen ions ($a(H^+)$, unitless) (fig. 1). To use the pH measurement in e.g., CO_2 system calculations it is the hydrogen ion amount content ($[H^+]$, mol kg-solution^{-1}) that is desirable. $a(H^+)$ and $[H^+]$ 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 the calibration standard (S) and sample (X), the measured pH can be interpreted as $[H^+]$ instead of $a(H^+)$. Second, the composition of the solution being measured also influences the potential of the 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" (ΔE_J) is not easily quantified (see, for example, 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, ΔE_J can be minimized. Lastly, the equation 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 (c.f. 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_T measurements has become the buffer formed from the base species: 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS), and its conjugate acid: $TRIS{\cdot}H^+$, prepared in an ionic medium with a composition similar to that of seawater (Hansson 1973; Ramette et al. 1977). At a temperature of 25 °C and a salinity (S) of 35 the 0.04 mol $kg\text{-}H_2O^{-1}$ equimolar buffer has a pH of 8.094, which is within the observed open ocean pH range (DelValls and Dickson 1998; Olsen et al. 2016). An equimolar buffer implies that the buffering species, TRIS and $TRIS{\cdot}H^+$ 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 at a particular temperature it offers the advantage of the pH not being very sensitive to changes in ionic strength and composition (S)

because the activity coefficient term includes a singly-charged cation in both the numerator and the denominator (eq. 1; Bates 1961).

$$\mathrm{pH(TRIS)} = \mathrm{p}K^{\circ}(\mathrm{TRIS}\cdot\mathrm{H}^{+}) - \log\left(\frac{[\mathrm{TRIS}\cdot\mathrm{H}^{+}]}{[\mathrm{TRIS}]}\right) - \log\left(\frac{\gamma(\mathrm{TRIS}\cdot\mathrm{H}^{+})}{\gamma(\mathrm{TRIS})\cdot\gamma(\mathrm{H}^{+})}\right) \qquad (1)$$

The assumption that is typically made when using a TRIS buffer in synthetic seawater is that the activity coefficient product in eq. 1 is quite similar to its value in the base recipe for the synthetic seawater (the ionic medium), and that the activity coefficient term would be the same 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 when using such a buffer to calibrate the measurement of a hydrogen ion concentration 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 ~5 in S resulted in an error of ~0.01 in pH.

Buffer preparation

Background

Although a detailed method for the preparation of equimolar TRIS-TRIS·H^{+} buffers in synthetic seawater (SSW) has not been published, DelValls and Dickson (1998) presented a buffer solution composition (table 1) to which they also assigned a pH. Their buffer preparation method was intended to prepare buffers for analysis by a high-precision electrometric method and, if followed carefully, is highly reproducible (e.g., Müller et al. 2018; Nemzer and Dickson 2005; Pratt 2014). 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. This includes ensuring that the buffering species TRIS and $\text{TRIS}\cdot\text{H}^+$ 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 of TRIS, the composition of the SSW ensures that the activity coefficient term and E_J is 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 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 consisting of the six major ions in 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 ensures that activity coefficients can be assumed the same in the SSW as in real seawater. The one exception to this is sulfate, a weak base, which has proven hard to replace due to its large amount relative to the other major ions, 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 (Dickson 1993; Hansson 1973). This scale is known as the total hydrogen ion scale ($\mathrm{pH_T}$; eq. 2) and states that the $\mathrm{pH_T}$ of a solution is proportional to the free hydrogen ion amount content ($[\mathrm{H^+}]_{\mathrm{free}}$), where the constant of proportionality depends on the total sulfate amount content $[\mathrm{SO_4^{2-}}]_\mathrm{T}$, and the acid dissociation constant of bisulfate, $K(\mathrm{HSO_4^-})$. Because sulfate is a conservative parameter in seawater, its total concentration $[\mathrm{SO_4^{2-}}]_\mathrm{T}$ can be estimated directly from the *S*. Therefore, the total $\mathrm{H^+}$ scale will be a suitable pH scale for seawater measurements as long as the sulfate concentration of the solution being measured, calibration standard or sample, is proportional to *S*.

$$\mathrm{pH_T} = -\log\left(\left[\mathrm{H^+}\right]_{\mathrm{free}}\left(1+[\mathrm{SO_4^{2-}}]_\mathrm{T} / K(\mathrm{HSO_4^-})\right)\right) \tag{2}$$

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 provide a method for TRIS buffer preparation that will result in a buffer $\mathrm{pH_T}$ equivalent to the value assigned by DelValls and Dickson (1998). This buffer will be appropriate to calibrate pH measurements expected to fulfil the GOA-ON “weather” uncertainty goal of 0.02.

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 de-ionized water followed by re-precipitation by partially evaporating the solution). Furthermore, NaCl, Na_2SO_4 and KCl were dried thoroughly following re-crystallization. The recrystallized $MgCl_2$ and $CaCl_2$ 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 $AgNO_3$. They weighed all components using a high-resolution (0.01 mg) balance and quantitatively transferred them to the buffer container. Finally, the buffer solution was brought to a certain total solution weight by adding de-ionized 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 against commercially available TRIS solid which ensured 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 (ΔpH/Δsalt), changes exceeding 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 de-ionized 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 HCl ($c(\mathrm{HCl})_{\mathrm{titr}}$ in mol kg-$\mathrm{solution}^{-1}$) 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 small number of titrations were carried out with TRIS from Sigma Aldrich (LOT 11K5445) and MP Biomedicals (LOT Q4553) for additional comparison. The HCl was prepared by diluting 35–37 % ACS reagent grade HCl solution from Fisher Scientific.

Because this method assumes that the TRIS is 100% pure, any impurities in the TRIS (see Discussion), would result in an inaccurate amount content for the HCl (while still ensuring that an accurate buffer ratio of 1 can be obtained). To evaluate the accuracy of this titration approach we standardized one batch of HCl using coulometry ($c(\mathrm{HCl})_{\mathrm{coul}}$), as described in the appendix of Dickson et al. (2003), and compared this to the amount content determined by titration, $c(\mathrm{HCl})_{\mathrm{titr}}$.

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, Na_2SO_4, and KCl as dried salts, $MgCl_2$ and $CaCl_2$ as calibrated solutions, and the buffer solution was brought to a particular total mass. Buffers prepared volumetrically used un-dried salts, $MgCl_2$ and $CaCl_2$ solutions with manufacturer calibration, and the buffer solution was brought to a particular total volume instead of mass. The buffer ratio was calibrated as described in the section above, for both kinds of buffer. 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 hours and cooled to room-temperature in a desiccator prior to preparing the buffer. Solutions of $MgCl_2$ and $CaCl_2$ were prepared in our laboratory and calibrated by titration against standardized ~0.3 mol kg-solution^{-1} $AgNO_3$ 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 calibration of ~1 mol kg^{-1} $MgCl_2$ and $CaCl_2$ solutions were used. No certificate of analysis was provided for the $MgCl_2$ solution (beyond being sold as a “1 mol L^{-1}” solution), while the $CaCl_2$ had a calibrated concentration of 1.04 mol L^{-1}.

Each type of buffer was prepared by first adding a weighed amount of HCl, after which the desired weights of TRIS, the SSW salts, and the total solution weight (primary buffers only), were each scaled to the weight of dispensed HCl to produce a buffer of the relative proportions shown in table 1. The weights of HCl, followed by TRIS, and the SSW salts, were recorded to a resolution of 0.1 mg. De-ionized water was used to quantitatively transfer all components into the buffer container. 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 instead brought to a total volume using a volumetric flask and knowledge of the resulting density of the buffer (removing the need for a low-resolution, 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 include the density of the resulting TRIS buffer, and scaling of the desired weights of all components to the 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 pH 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 Millero (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-H_2O^{-1}) 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 not only can have an effect on the relevant activity coefficients, but can also change the buffer ratio in eq. 1 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 pH change of <0.001 in pH. A 1% error in the amount of Na_2SO_4 results only 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 hydrogen ion concentration. We estimate the overall effect from this error in Na_2SO_4 to be ~0.3 %, or an error of a little more than 0.001 in pH_T, using eq. 2. For the other components, the largest effect is for a 1 % change in the amount of $MgCl_2$ which results in an estimated change of pH 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 compositions of the SSW. 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 assured 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 the SSW salts: NaCl, Na_2SO_4, KCl, $MgCl_2$, or $CaCl_2$ 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 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 not consistent with other relevant acid-base parameters in seawater.

Thus, this calculated "pH" will not be identical to the actual pH_T of the buffer and the pH values from these experiments are referred to here as pH_{spec}.

Results and discussion

Using and acid-base titration to ensure the buffer ratio

For one particular batch of HCl, $c(HCl)_{titr}$ 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 TRIS buffer. This particular batch of HCl had been standardized previously using coulometry, $c(HCl)_{coul}$, which enabled an estimate of the accuracy of the titration method (or, essentially, TRIS impurities). The relative percent difference between $c(HCl)_{titr}$ and $c(HCl)_{coul}$, was small for each of the five days, and within the relative standard deviation, 0.1 %, of the titration method (fig. 2a).

The resulting pH_T 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 pH_T values found spectrophotometrically was within the range resulting from this $c(HCl)$ calibration uncertainty, and the mean of all pH_T values was 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 pH_T from the assigned value (8.094) and $\%\Delta c(HCl)$.

Calibrating the HCl solution against TRIS solid will likely not yield the true $c(HCl)$ because TRIS crystals can have varying levels of water occluded in their crystal structure (Koch et al. 1975). This will act to over-estimate $c(HCl)$ relative to the true value, as our approach assumes the TRIS is 100 % pure. The $c(HCl)_{titr}$ is however appropriate for use to prepare the TRIS buffer (e.g., 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, e.g., 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 phosphorus pentoxide, in vacuum is preferred, most laboratories do not have easy access to this kind of drying equipment. It is therefore more practical to use TRIS "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 $\mathrm{pH_T}$ of the buffer appreciably (see e.g., fig. 1 and 2b in DelValls and Dickson 1998).

It should be pointed out that the synthetic seawater composition used here is slightly different from that of "pure" synthetic seawater because the addition of HCl (and TRIS) and its effect on the ionic strength is compensated for by reducing the amount of NaCl. Thus, while the estimated $c(\mathrm{HCl})_{\mathrm{titr}}$ might achieve the correct buffer ratio, if it is higher or lower than the true amount content, the amount of NaCl will also be in error by the same amount and of opposite sign. This error is likely much smaller than the likely uncertainty introduced by using reagent-grade NaCl (≤ 1 % impurity; see "Preparing synthetic seawater" below).

The relative purity of TRIS from various commercial manufacturers is suggested in fig. 3, which shows $\%\Delta c(\mathrm{HCl})$ for a single batch of HCl which was 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 both for with and without drying and further homogenization. As such, NIST SRM723e TRIS would be appropriate when it is necessary to accurately determine c(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 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 L buffer (~10 g). In terms of calibrating $c(\mathrm{HCl})_{\mathrm{titr}}$, this would result in an increased standard deviation, which is perhaps the case when comparing e.g., $c(\mathrm{HCl})_{\mathrm{titr}}$ of TRIS from Macron versus from NIST in fig 3. Although the titration data using TRIS from other commercial suppliers than Macron is limited, there appears to be little difference between the various commercially available sources of TRIS with the exception of MP Biomedicals. Whether the significantly lower $c(\mathrm{HCl})_{\mathrm{titr}}$ 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, is hard to ascertain.

Preparing synthetic seawater

There was a measurable increase in $\mathrm{pH}_{\mathrm{spec}}$ ($\Delta\mathrm{pH}_{\mathrm{spec}}$) between the unaltered TRIS buffers and those altered with 15 % of any single salt of the SSW matrix. $\Delta\mathrm{pH}_{\mathrm{spec}}$ correlated to the total amount of salts in solution (fig. 4; table 1), in other words, $\Delta\mathrm{pH}_{\mathrm{spec}}$ seemed to be largely a function of change in the ionic strength of the buffer. $\Delta\mathrm{pH}_{\mathrm{spec}}$ 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 per the change in ionic strength their respective salts caused, compared to the salts that only contained monovalent ions. This was likely caused by stronger interactions of the divalent ions with TRIS, TRIS·H$^+$, and with the spectrophotometric dye mCP.

This simplistic view on sensitivity of the buffer pH to changes in the background ionic composition, supported by Pitzer modelling, suggests NaCl and KCl can be added to synthetic seawater as dried salts of 99 % purity or higher, without changing the buffer pH by more than ~0.0005 (table 2). $MgCl_2$ and $CaCl_2$ solutions used for this particular experiment were calibrated to ±0.5 % in our laboratory. Our results suggest that they can be used successfully as the commercially available 1 mol L^{-1} (provided these are calibrated to ±2 % or better from the manufacturer) solutions, producing an accumulated buffer pH uncertainty of less than 0.0005 (table 2). It is possible to add $MgCl_2$ and $CaCl_2$ directly as solids ($MgCl_2 \times {\sim}6H_2O$ and $CaCl_2 \times {\sim}2H_2O$) provided their exact level of hydration is known. However, the number of H_2O 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_2SO_4 did not have a large effect on observed pH_{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 one percent at a constant $[H^+]_{free}$ changes pH_T by nearly 0.001 unit (using eq. 2), much larger than the observed ΔpH_{spec} from an increase of the total amount of Na_2SO_4 by 1 % (fig. 4). Nevertheless, carefully adding Na_2SO_4 as a dried salt of 99 % purity or better should not cause an error in pH_T of more than 0.001.

As was pointed out in the description of the methods, this simplistic experiment necessarily illustrates the change in pH not only from the actual pH of the buffer changing from the addition of extra salt, but also due to the changing behavior of the mCP dye caused by a changed ionic composition and strength. It is believed that changing the extent of complexation of the base form of mCP (a doubly charged anion) with changes in the divalent Mg^{2+} concentration may be responsible for the apparent increased sensitivity to this ion compared to that implied by the Pitzer model. Still, the likely error in a normal buffer preparation would be small.

An altered ionic composition could further 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 "ΔpH" 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 smaller errors in the SSW matrix are highly 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 standard deviation; 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 DelValls 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, using a spectrophotometric cell whose value for TRIS pH_T measurements had been previously confirmed via the Harned cell.

Three batches of TRIS were analyzed this way: two primary buffers and one batch that had been volumetrically prepared. The average pH_T for all three batches was 8.092 (±0.001), 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 added in excess, you cannot straightforwardly prepare the buffer to a larger 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 S that is slightly higher than 35. As mentioned previously, the pH of a TRIS buffer is quite insensitive to changes in S, and from a S of 25 to 35 the change in $pK(\text{TRIS}\cdot\text{H}^+)$ is a little less than 0.02 at 25 °C (Bates and Hetzer 1961; DelValls and Dickson 1998). Provided the weight of HCl deviated less than 1 % from what would be required for a 1 L solution, the final error in the S of the sample would not be more than 1 %. This, in turn, should cause an error of less than 0.001 in the pH of the buffer solution according to equation 18 of DelValls and Dickson (1998). Any small errors caused by preparing the buffer to a certain volume are therefore largely outweighed by the benefit of only needing one high-resolution analytical balance and not an additional high-capacity balance.

Modification of the buffer for use with external reference electrodes (e.g., SeaFET)

While the buffer prepared according to the method proposed above is largely intended for the calibration of glass electrodes and similar pH sensors incorporating a liquid junction, it may also be suitable for other seawater pH_T measurements as long as its limitations are recognized. In particular, the use of the SeaFET™ sensor is becoming more widespread and integrated in sensor packages such as the SeapHOx (Bresnahan et al. 2014). Unlike the glass electrode pH cell, the

SeaFET sensor utilizes a solid-state H^+ electrode, a reference electrode with a gel-filled junction, and an additional external chloride-sensitive reference electrode (Martz et al. 2010) which is also sensitive to bromide ion concentration. For those interested in calibrating such a sensor with TRIS buffers it will be necessary to add an appropriate amount of NaBr or KBr to their buffer during preparation. For 1 L of TRIS buffer 0.103 g of KBr is needed, which has a trivial effect on the ionic strength.

Storage of TRIS solutions

Nemzer and Dickson (2005) monitored TRIS buffers stored in borosilicate bottles sealed with greased ground glass stoppers over several years, showing that these buffers experienced less than 0.0005 drift in pH per year. What exactly causes this drift is unknown, although preliminary analysis in our laboratory suggest that TRIS buffers do absorb some CO_2 from the atmosphere. Buffers analyzed for C_T within two months of preparation and bottling (in greased borosilicate bottles) had a C_T of 30–60 µmol kg^{-1}. This would increase the buffer ratio term in eq. 2 by ~0.2 % and thus lower the pH_T by ~0.001. Part of this buffer C_T is likely caused by de-ionized water being in equilibrium with lab atmosphere. For example – at a mole ratio of 1000 ppm CO_2 in lab air, the C_T of de-ionized water will be approximately 40 µmol kg^{-1} which is consistent with the lower C_T values measured in the buffers. The level of C_T 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 buffer stored in such a container will almost certainly take up CO_2 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 $\mathrm{pH_T}$ 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 $\mathrm{pH_T}$ is more than sufficient for seawater pH measurements that are expected to fulfil 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.

Acknowledgements

The authors are grateful for the help of Lauren Briggs in preparing several TRIS batches, and George C. Anderson for coulometric analysis of the HCl. The authors would also like to thank Dr. Simon Clegg for his work with the Pitzer modelling of TRIS in synthetic seawater. Comments from two anonymous reviewers greatly enhanced the manuscript. This work was supported by the US National Science Foundation Grant OCE-1657799.

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Appendix

Appendix 1 Estimation of the amount content of an HCl solution 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 mL 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. A1 c and 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. A1 b). 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. A2 a). 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, 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. A2 b).

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 $c(\mathrm{HCl})_{\mathrm{titr}}$.

Calculations

The amount content (c, 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 equations A1, where the densities (ρ) of TRIS and HCl are 1.33 g cm^{-3} and 1.02 g cm^{-3}, respectively.

$$m\ (\mathrm{g}) = w\ (\mathrm{g}) \cdot \left(1 + 0.0012 \cdot \left(1/\rho\ - 1/8\right)\right) \tag{A1}$$

The amount content of HCl, based on this titration technique ($c(\mathrm{HCl})_{\mathrm{titr}}$) is then calculated according to equation A2, using the molar mass (M) of TRIS of 121.14 g mol^{-1}.

$$\begin{gathered} n_{\mathrm{HCl}} = n_{\mathrm{TRIS}} \\ c(\mathrm{HCl}) \cdot m_{\mathrm{HCl,\,solution}} = \frac{m_{\mathrm{TRIS}}}{M_{\mathrm{TRIS}}} \\ c(\mathrm{HCl})_{\mathrm{titr}}\ (\text{mol kg-solution}^{-1}) = \frac{m_{\mathrm{TRIS}}\ (\mathrm{g})}{M_{\mathrm{TRIS}}\ (\mathrm{g\ mol}^{-1})} \cdot \frac{1000\ (\mathrm{g\ kg}^{-1})}{m_{\mathrm{HCl,\,solution}}\ (\mathrm{g})} \end{gathered} \tag{A2}$$

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 L TRIS buffer in synthetic seawater

Materials

1 L clean volumetric flask, 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, Na_2SO_4, KCl, 1.0 mol L^{-1} $MgCl_2$, 1.0 mol L^{-1} $CaCl_2$. A list of suggested chemicals and their catalogue numbers can be found in Appendix 3.

The desired weight of HCl ($w(\mathrm{HCl})_{\mathrm{desired}}$) depends on $c(\mathrm{HCl})_{\mathrm{titr}}$ as determined in Appendix 1, and is calculated based on equation A3, where $c(\mathrm{HCl})_{\mathrm{buffer\ solution}}$ is the target HCl (and thus $\mathrm{TRIS{\cdot}H^+}$) amount content (0.03827 mol kg-solution^{-1}, equivalent to 0.04 mol kg-H_2O^{-1}) in the buffer and $w(\text{buffer solution})_{\mathrm{desired}}$ the weight of 1 L of the buffer solution at 20 °C.

$$w(\mathrm{HCl})_{\mathrm{desired}}\ (\mathrm{g}) \approx w(\text{buffer solution})_{\mathrm{desired}}\ (\mathrm{g}) \cdot \frac{c(\mathrm{HCl})_{\text{buffer solution}}(\text{mol kg-solution}^{-1})}{c(\mathrm{HCl})_{\mathrm{titr}}(\text{mol kg-solution}^{-1})} \qquad (\mathrm{A3})^{1}$$

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[1] While 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 included in the calculation.

Mixing the buffer

a) *Weight out HCl*: Place the funnel in the neck of the volumetric flask and start by weighing out HCl in a beaker (or weighing boat) to within 0.3 g or better of the desired weight from table A1, where a disposable transfer pipette can be used to adjust the final weight. Record the dispensed weight of HCl and transfer the HCl quantitatively into the volumetric flask by rinsing the weighing vessel directly into the flask with de-ionized water (~100 mL).

b) *Scale desired weights to the dispensed weight of HCl*: Calculate to what proportion the dispensed HCl weight ($w(\mathrm{HCl})_{\mathrm{dispensed}}$) is different from the weight in table A1 ($w(\mathrm{HCl})_{\mathrm{desired}}$). To ensure the ratio of moles between all components remain the same, this factor is multiplied with the desired weights ($w(\mathrm{X})_{\mathrm{desired}}$) of the remaining components to re-calculate a target weight of each ($w(\mathrm{X})_{\mathrm{target}}$) as shown in equation A4. As long as HCl is added to within 0.3 g of the desired weight (for a 1 L buffer), and the remaining components are added in proportion to that, the resulting 1 L buffer will have a S of < 0.3 units different than the desired S of 35.

$$w(\mathrm{X})_{\mathrm{target}}\ (\mathrm{g}) = w(\mathrm{X})_{\mathrm{desired}}\ (\mathrm{g}) \cdot \frac{w(\mathrm{HCl})_{\mathrm{dispensed}}\ (\mathrm{g})}{w(\mathrm{HCl})_{\mathrm{desired}}\ (\mathrm{g})} \qquad \text{(A4)}$$

c) *Weigh out the remaining components*: weigh out the target weights, $w(\mathrm{X})_{\mathrm{target}}$, 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 ~100 mL de-ionized water per rinse.

d) *Add water and mix*: Once all components have been added, rinse the funnel into the flask with de-ionized water and fill the flask to a few centimeters below the 1 L 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 de-ionized water (using e.g., a transfer pipette) until the bottom of the solution meniscus is level with the etched 1-L 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_2SO_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.

Figure captions

Figure 1 The use of a combination glass/reference electrode in a high-ionic strength solution

Figure 2 a) shows mean $\%\Delta c(\mathrm{HCl}) = 100\cdot\ (c(\mathrm{HCl})_{\mathrm{titr}} - c(\mathrm{HCl})_{\mathrm{coul}})/c(\mathrm{HCl})_{\mathrm{coul}}$ %, ($n \geq 3$) for the five different days of measuring $c(\mathrm{HCl})_{\mathrm{titr}}$ while the bars represents one relative standard deviation of the titration technique and not for each individual set of titrations. Panel b) shows the mean $\mathrm{pH_T}$ ($n \geq 4$) of each of the five TRIS buffers, the bars represent one standard deviation of each set of the $\mathrm{pH_T}$ measurements and the black drawn line indicates the value 8.094.

Figure 3 The mean $\%\Delta c(\mathrm{HCl})$ and one relative standard deviation for each $c(\mathrm{HCl})_{\mathrm{titr}}$ 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.

Figure 4 Estimated increase in $\mathrm{pH_{spec}}$ ($\Delta\mathrm{pH_{spec}}$) for the experimental buffers caused by the addition of 1 % extra of the salts NaCl (▲), $MgCl_2$ (●), Na_2SO_4 (■), $CaCl_2$ (◆), or KCl (△). $\Delta\mathrm{pH_{spec}}$ was estimated by the observed change in $\mathrm{pH_{spec}}$ from adding 15 % extra salt and scaled to 1 %, to better represent likely preparation errors.

Figure A 1 Pipettes used for titration, including a) A 15.3 mL pipette with b) Tygon®-tubing cap, c) modified tip of a d) 4.5 mL pipette.

Figure A 2 Color of sample after a) addition of indicator and after b) reaching the titration endpoint.

Table 1 Composition of 0.04 mol kg-H_2O^{-1} equimolar TRIS–TRIS·H^+ buffers at a *S* of 35 by DelValls and Dickson

Component	mol kg-H_2O^{-1}	mol kg-solution^{-1}	Impurity specification (<) or relative uncertainty (±) in component amount [a]
HCl	0.04000	0.03827	±0.02 %
TRIS	0.08000	0.07654	±0.02 %
NaCl	0.38764	0.37089	< 0.1 % [b]
Na_2SO_4	0.02927	0.02801	< 0.1 % [b]
KCl	0.01058	0.01012	< 0.1 % [b]
$MgCl_2$	0.05474	0.05237	< 0.1 % [b,c]
$CaCl_2$	0.01075	0.01029	< 0.1 % [b,c]

[a] Reported values from DelValls and Dickson (1998); [b] Recrystallized for purification, exact impurity unclear; [c] Added as solutions, see Method.

Table 2 Suggested specifications of reagents for the preparation of 1 L[a] TRIS buffers at a *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 kg-solution^{-1})	39.270[b]	±0.1 %	0.002
TRIS	9.517[b]	≤ 2 %	
NaCl	22.254	< 1 %	0.0005
Na_2SO_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
De-ionized water	"Fill to line"	±1 %[c]	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 $c(\text{HCl})_{\text{titr}}$; [c] Includes error in weighing HCl to the desired amount, and implications of subsequent scaling amounts of remaining components.

Table A 1 Desired weights to prepare a 1 L of 0.04 mol kg-H_2O^{-1} equimolar TRIS-TRIS·H^+ buffer at a *S* of 35

Component	Weight of component (g)
HCl	39.270/*c*(HCl)$_{\text{titr}}$
TRIS	9.517
NaCl	22.254
Na_2SO_4	4.085
KCl	0.775
$MgCl_2$ (1.0 mol L^{-1})	58.862
$CaCl_2$ (1.0 mol L^{-1})	11.726
De-ionized water	“Fill to line”