



The stability and reproducibility of Tris buffers in synthetic seawater

B.V. Nemzer, A.G. Dickson*

*Marine Physical Laboratory, Scripps Institution of Oceanography, University of California, San Diego,
9500 Gilman Drive, La Jolla, CA 92093-0244, USA*

Received 2 July 2004; received in revised form 22 December 2004; accepted 6 January 2005

Available online 21 April 2005

Abstract

The equimolar Tris buffer (0.04 mol/kg-H₂O Tris+0.04 mol/kg-H₂O Tris-HCl) prepared in synthetic seawater of salinity 35 has been shown to be stable when sealed in a borosilicate glass bottle with a greased ground-glass stopper (drift rate ≤ 0.0005 in pH per year). The error in pH of such buffers resulting from uncertainties in the preparation of such buffers is typically less than 0.002 in pH (relative to the results of DelValls and Dickson, 1998 [DelValls, T.A., Dickson, A.G., 1998. The pH of buffers based on 2-amino-2-hydroxymethyl-1,3-propanediol ('tris') in synthetic sea water. *Deep-Sea Research I*, 45, 1541–1554]).

© 2005 Elsevier B.V. All rights reserved.

Keywords: Artificial seawater; pH; Buffers; Accuracy; Stability

1. Introduction

Tris buffers—buffers based on 2-amino-2-hydroxymethyl-1,3-propanediol—have been widely used to define seawater pH. Early workers (Smith and Hood, 1964) used Tris buffers in natural seawater as secondary pH standards. Tris buffers in synthetic seawater are now used as primary buffers to define seawater pH scales (Hansson, 1973; Ramette et al.,

1977; Dickson, 1993; DelValls and Dickson, 1998) either directly for subsequent calibration of electrometric pH measurements (e.g., Whitfield et al., 1985; Millero et al., 1993; DOE, 1994), or indirectly by using them to define the pK^* of indicator dyes for seawater pH measurements (e.g., Clayton and Byrne, 1993; Zhang and Byrne, 1996; Nakaguchi et al., 2001).

If such buffers are to be used widely, it is desirable to understand their stability and also to estimate the likely uncertainty involved in preparing them. Here we report our experiences with such buffers, focusing on the 0.04 mol/kg-H₂O equimolar (Tris/Tris-HCl) buffer prepared in synthetic seawater of salinity 35.

* Corresponding author. Tel.: +1 858 822 2990; fax: +1 858 822 2919.

E-mail address: adickson@ucsd.edu (A.G. Dickson).

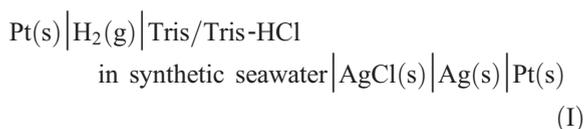
2. Experimental approach

2.1. Preparation of buffer solutions

Two batches of 20 kg each of buffer were prepared to the recipe given in Table 1. All the salts used (NaCl, Na₂SO₄, KCl, MgCl₂, CaCl₂) were analytical-grade reagents purified further by recrystallizing from water. The MgCl₂ and CaCl₂ were used as stock solutions that had been assayed by titration with silver nitrate with an accuracy of better than 0.1%. The HCl used was a stock solution prepared from double-distilled HCl and assayed coulometrically with an accuracy of better than 0.02%. The Tris used was ultra-pure THAM[®] (trihydroxyaminomethane) obtained from the Fisher Scientific Corporation. Once a batch of buffer had been prepared, it was transferred to a number of Corning 500-mL reagent bottles (#442332-7789 manufactured from Schott Duran[®] glass). These were sealed with Apiezon-L[®] grease and stored at room temperature in the dark until use.

2.2. Measurement of pH of buffer solutions

The pH was monitored using the cell



to measure the pH of a previously unopened bottle of buffer. The cells used for these analyses were of the all-glass type described by Bates (1965). The silver–silver chloride electrodes were of the thermal electrolytic type (Bates, 1973); the hydrogen electrodes were platinized in accordance with the recommendations of Feltham and Spiro (1971). The e.m.f. was measured using a Hewlett-Packard data acquisition system (HP3497A), temperature was monitored using a platinum resistance thermometer (Hart model 1502) and atmospheric pressure was measured using a digital quartz barometer (Paroscientific model 740-16B).

The cells were thermostated in a water bath set to 298.15 K, and controlled to ± 0.005 K. All e.m.f. measurements were corrected to a standard pressure of

Table 1

Nominal composition of equimolar (0.04 mol/kg-H₂O Tris+0.04 mol/kg-H₂O Tris-HCl) Tris buffer in synthetic seawater of salinity 35

Component	Concentration (mol/kg-H ₂ O)	Concentration (mol/kg-soln)	Weight (g) in 1 kg solution ^a
NaCl	0.38762 ^b	0.37087	21.6884
Na ₂ SO ₄	0.02927	0.02801	3.9808
KCl	0.01058	0.01012	0.7551
MgCl ₂	0.05474	0.05238	–
CaCl ₂	0.01075	0.01029	–
Tris	0.08000	0.07654	9.2748
HCl	0.04000	0.03827	–

^a Weights in air at sea level (i.e., not corrected to mass). If a weight is not given, the component is added as the appropriate amount of a calibrated solution.

^b $m(\text{NaCl})=0.42762-0.04$ mol/kg-H₂O, i.e. replacing NaCl with HCl.

101.325 kPa (1 atm), and have been adjusted by subtracting the difference

$$\Delta E^\circ/V = E^\circ(298.15 \text{ K})/V - 0.22240 \quad (1)$$

from each e.m.f. reading so as to normalize the values for the standard potential of cell (I) to correspond to those of Bates and Bower (1954). E° (298.15 K) was measured regularly using cells containing HCl at a concentration of 0.01 mol/kg-H₂O and assuming that $\gamma_{\pm}(\text{HCl})=0.904$ (Bates et al., 1956, 1957; Dickson, 1987). Such calibration measurements were made with every set of measurements on these buffer solutions to minimize any uncertainty resulting from the preparation of the electrodes.

The e.m.f. of cell (I)–corrected to 1 atm fugacity of hydrogen—is given by the expression (DelValls and Dickson, 1998)

$$E = E^* - \left(\frac{RT}{F} \right) \ln \left(\frac{m(\text{H}^+)m(\text{Cl}^-)}{(m^\circ)^2} \right) \\ - \left(\frac{2RT}{F} \right) \ln \left(\frac{\gamma_{\pm}(\text{HCl})}{\gamma_{\pm}^{\text{tr}}(\text{HCl})} \right) \quad (2)$$

where

$$E^* = E^\circ - \left(\frac{2RT}{F} \right) \ln \gamma_{\pm}^{\text{tr}}(\text{HCl}) \\ + \left(\frac{RT}{F} \right) \ln \left(1 + \frac{T m(\text{SO}_4^{2-})}{K_m(\text{HSO}_4^-)} \right) \quad (3)$$

(Dickson, 1990), i.e., a standard potential referred to an ionic medium standard state, based on total hy-

Table 2

E.m.f. of cell (I) measured in equimolar (0.04 mol/kg-H₂O Tris+0.04 mol/kg-H₂O Tris-HCl) buffer in synthetic seawater of salinity 35 at 298.15 K

Analysis date	<i>E</i> /V			Mean $\langle E \rangle$ /V	Std. Dev. $s(E)$ /V
<i>Batch A (bottled July 13, 2000)</i>					
July 18, 2000	0.73849	0.73854	0.73853	0.73852	0.00003
August 1, 2000	0.73860	0.73861	0.73859	0.73860	0.00001
August 17, 2000	0.73852	0.73855	0.73852	0.73852	0.00002
September 25, 2000	0.73851	0.73853	0.73853	0.73853	0.00001
November 17, 2000	0.73854	0.73853	0.73855	0.73854	0.00001
February 2, 2001	0.73855	0.73858	0.73853	0.73855	0.00003
<i>Batch B (bottled June 19, 2001)</i>					
June 25, 2001	0.73849	0.73846	0.73846	0.73847	0.00002
July 19, 2001	0.73851	0.73849	0.73848	0.73849	0.00002
October 8, 2001	0.73852	0.73849	0.73848	0.73850	0.00002
May 6, 2002	0.73851	0.73849	0.73848	0.73849	0.00002
May 13, 2002	0.73851	0.73851	0.73853	0.73851	0.00001
May 19, 2002	0.73847	0.73854	0.73850	0.73853	0.00003
November 19, 2002	0.73857	0.73855	0.73854	0.73855	0.00002
February 10, 2003	0.73854	0.73854	0.73856	0.73858	0.00002
February 21, 2003	0.73854	0.73854	0.73855	0.73854	0.00001

All values have been corrected to $p^\circ=101.325$ kPa (1 atm) and $E^0=0.22240$ V.

drogen ion concentration, $m(\text{H}^+)$, rather than free hydrogen ion concentration, $^Fm(\text{H}^+)$, such that

$$m(\text{H}^+) = ^Fm(\text{H}^+) \left(1 + \frac{^Tm(\text{SO}_4^{2-})}{K_m(\text{HSO}_4^-)} \right); \quad (4)$$

$\gamma_{\pm}^{\text{tr}}(\text{HCl})$ is the trace activity coefficient of HCl in synthetic seawater (i.e., the limiting value of $\gamma_{\pm}^{\text{tr}}(\text{HCl})$ as the composition approaches that of pure synthetic

seawater) and $K_m(\text{HSO}_4^-)$ is the corresponding ionic medium dissociation constant for HSO_4^- .

The pH assigned to the buffer solution is then given by the expression (DelValls and Dickson, 1998)

$$\text{pH}_m = \frac{E - E^*}{RT \ln 10 / F} + \log \left(\frac{m(\text{Cl}^-)}{m^\circ} \right) \approx - \log \left(\frac{m(\text{H}^+)}{m^\circ} \right), \quad (5)$$

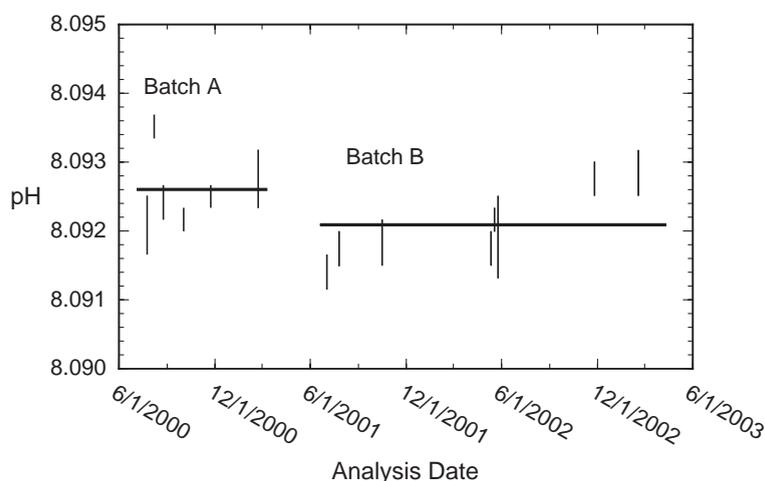


Fig. 1. pH values (mol/kg-soln) for batch A and batch B measured at 298.15 K and plotted against analysis date. The solid symbol represents the mean of data from a particular bottle, the vertical line the range. The horizontal lines are the mean of all the data for that batch.

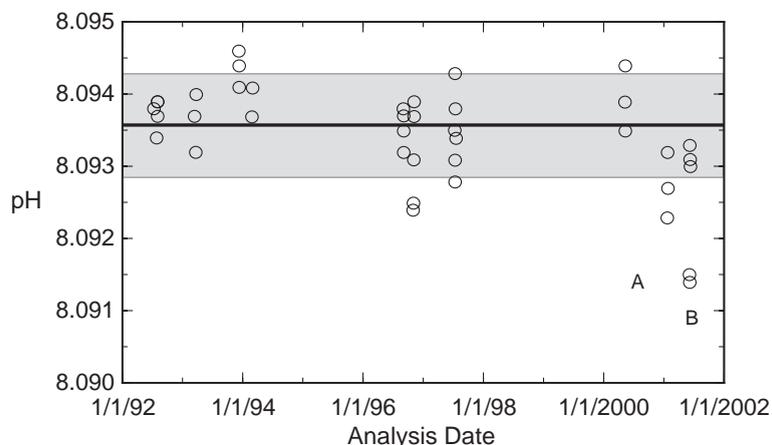


Fig. 2. pH values (mol/kg-soln) at 298.15 K determined using cell (I) on 16 batches of equimolar (0.04 mol/kg-H₂O Tris+0.04 mol/kg-H₂O Tris-HCl) buffer in synthetic seawater of salinity 35 prepared in our laboratory since 1992. (Note: typically 3 measurements are made on each batch—see, for example, Table 1). The initial measurements on Batch A and Batch B are shown here as filled circles. The horizontal line corresponds to the pH value recommended by DelValls and Dickson (1998) in their Eq. (18), the gray band is ± 1 standard error on this equation.

which neglects the activity coefficient term in Eq. (2). pH_m is defined on the total hydrogen ion scale in mol/kg-H₂O. The value (at $S=35$) in mol/kg-soln,

$$\text{pH} = \text{pH}_m + 0.0164. \quad (6)$$

3. Results

The e.m.f. measurements on the two batches of buffer are presented in Table 2. Batch A was monitored for 7 months, Batch B for 20 months. The results—converted to pH—are plotted in Fig. 1.

In addition, Fig. 2 shows the results of all measurements in our laboratory on 16 independent preparations of this buffer (including batches A and B) over the period 1992–2002.

4. Discussion

The buffer concentration used here (0.04 mol/kg-H₂O Tris+0.4 mol/kg-H₂O Tris-H⁺ in $S=35$ synthetic seawater) is one that has been recommended for use in seawater media (Ramette et al., 1977; Dickson, 1993; DOE, 1994; DelValls and Dickson, 1998). This is because its pH corresponds approximately to surface ocean pH (8.1) and also the relatively high

concentration of buffer substances is thought to ensure good stability and reproducibility.

An examination of Fig. 1 suggests that such Tris buffer solutions in synthetic seawater are fairly stable for at least a period of a year or so after bottling. The outlying set of measurements made on August 1, 2000 had a low measured E° value—relative to the data of July 18 or August 17—resulting in the data being adjusted upwards by about 0.0012 pH units relative to the other measurements shown. If this data point is disregarded, it appears that a small upward drift is occurring with time in both Batch A and Batch B, however this drift is only about 0.0005 pH units per year.

Fig. 2 illustrates the range of pH values found from making up this buffer a number of times according to the recipe in Table 1 but using different batches of chemicals. Most of these buffer solutions were made up in small quantities 250–500 g, with the exception of Batches A and B; these were prepared in 20 kg quantities. The total range of the measurements shown in Fig. 2 is 0.0034 in pH. This almost certainly is the result of small errors in weighing, and small differences in the purity of the Tris used. Weighing errors are probably more significant in the preparation of Batches A and B. As it was impractical to weigh the resulting 20 kg solution (our largest capacity balance can only weigh 6 kg), its weight was calculated from

the sum of the weights of the various constituents, including a number of separate additions of water.

Thus, any error in preparing an equimolar buffer ratio (Tris/Tris-H⁺) will have a consequent effect on the pH of the resulting solution; an error of 0.23% in this ratio will result in an error of 0.001 in pH. In our laboratory we prepare this buffer ratio by mixing an accurately known HCl stock solution with a weighed amount of Tris. For the work of DelValls and Dickson (1998), and for many of the earlier small-volume studies represented by the open circles in Fig. 2, we used SRM 723a or SRM 723b, i.e., Tris of known purity, assayed by the U.S. National Institute of Standards and Technology. For the larger volume studies reported here we used a commercially available ultra-pure Tris whose purity is less well characterized. In particular, it may be contaminated with water (see, e.g., Koch et al., 1975); the specifications for the Tris we used permit up to 0.5% of such contamination, and—based on information on the Fisher Scientific web-site—a water content of 0.1–0.2% seems fairly typical for various lots of this chemical. A reduction of 0.15% in the total Tris concentration would result in an error of 0.3% in the buffer ratio and a reduction of 0.0013 in pH. This is probably the principal reason for the apparently low pH observed for these large batches—filled circles in Fig. 2.

The other potentially significant source of error—particularly when preparing a large batch in the way we did—is in weighing the various medium salts and particularly the water added. If this error is small (<0.5% in the salinity), then the effect on the actual pH of the buffer will be small (<0.0004), however a significant error in measuring pH using cell I will result because of the consequent error in the estimation of $m(\text{Cl}^-)$ in Eq. (5). Here again, an error of 0.23% will result in a pH error of 0.001.

The mean (8.0932) and standard deviation (0.0009) of the 46 measurements shown in Fig. 2 are similar to the pH value proposed by DelValls and Dickson (1998) based on their measurements alone (8.0936 ± 0.0007 ; Eq. (18) on p. 1546 of their paper). Note that the DelValls and Dickson data are also included in this figure (1996–1997).

Thus it is clear that Tris buffers with the composition given in Table 1 can be prepared reliably in synthetic seawater, and that they are stable for a period of a year or so (Table 2; Fig. 1). If the assigned pH value is not

verified using a hydrogen electrode cell (cell I), the likely uncertainty (95% confidence) in the pH value of the buffer prepared is about ± 0.0018 (Fig. 2).

However, it is important to note that the pH defined by Eqs. (5) and (6) does not correspond exactly to the negative logarithm of the total hydrogen ion concentration, rather

$$-\log\left(\frac{m^*(\text{H}^+)}{m^\circ}\right) = \text{pH}_m - 2\log\left(\frac{\gamma_{\pm}(\text{HCl})}{\gamma_{\pm}^{\text{tr}}(\text{HCl})}\right). \quad (7)$$

The magnitude of the activity coefficient term is not known, but is thought to be quite small (0.002–0.003 in pH)—see also the discussion in Bates and Macaskill (1975). The measurements of Ramette et al. (1977) and of DelValls and Dickson (1998) show that halving the buffer concentration (while maintaining an equimolar buffer ratio) results in a change of the potential of cell I of less than 0.08 mV (<0.0015 in pH).

To minimize this problem, recent work in our laboratory, aimed at calibrating the pH indicator dye *m*-cresol purple to measure total hydrogen ion concentrations in seawater media, uses seawater buffers with differing amounts of Tris—but each calibrated separately using cell (I). The ionic medium dissociation constant of the indicator dye (at a particular salinity and temperature) is obtained by extrapolating measured values to the intercept in pure synthetic seawater (zero Tris concentration). As the concentration of dye used when making pH measurements is always very low, this approach may be the best way to define a true hydrogen ion concentration scale in seawater media that corresponds to the pH scale used in the definition of acid–base dissociations constants in seawater.

Acknowledgements

We would like to thank George Anderson for a variety of help with this project. This work was supported by the U.S. National Science Foundation through grants OCE9819007 and OCE0137287.

References

- Bates, R.G. (Ed.), 1965. *Electrochemical Analysis: Studies of Acids, Bases, and Salts by Emf Conductance, Optical and Kinetic Methods*. July 1964 to June 1965. N.B.S. Technical Note, vol. 271.

- Bates, R.G., 1973. Determination of pH, Theory and Practice. Wiley-Interscience, New York. 479 pp.
- Bates, R.G., Bower, V.E., 1954. Standard potential of the silver–silver–chloride electrode from 0° to 95 °C and the thermodynamic properties of dilute hydrochloric acid solutions. *Journal of Research of the National Bureau of Standards (United States)* 53, 283–290.
- Bates, R.G., Macaskill, J.B., 1975. Acid–base measurements in seawater. In: Gibb, T.R.P. (Ed.), *Analytical Methods in Oceanography*. A.C.S. Advances in Chemistry. American Chemical Society, Washington, DC, pp. 110–123.
- Bates, R.G., Guggenheim, E.A., Harned, H.S., Ives, D.J.G., Janz, G.J., Monk, C.B., Robinson, R.A., Stokes, R.H., Wynne-Jones, W.F.K., 1956. Standard electrode potential of the silver, silver chloride electrode. *Journal of Chemical Physics* 25, 361.
- Bates, R.G., Guggenheim, E.A., Harned, H.S., Ives, D.J.G., Janz, G.J., Monk, C.B., Prue, J.E., Robinson, R.A., Stokes, R.H., Wynne-Jones, W.F.K., 1957. Erratum: standard electrode potential of the silver, silver chloride electrode. *Journal of Chemical Physics* 26, 222.
- Clayton, T.D., Byrne, R.H., 1993. Spectrophotometric seawater pH measurements: total hydrogen ion concentration scale calibration of *m*-cresol purple and at-sea results. *Deep-Sea Research I* 40, 2115–2129.
- DelValls, T.A., Dickson, A.G., 1998. The pH of buffers based on 2-amino-2-hydroxymethyl-1,3-propanediol ('tris') in synthetic sea water. *Deep-Sea Research I* 45, 1541–1554.
- Dickson, A.G., 1987. Standardisation of the $(\text{AgCl} + \frac{1}{2}\text{H}_2 = \text{Ag} + \text{HCl})$ cell from 273.15 to 318.15 K. *Journal of Chemical Thermodynamics* 19, 993–1000.
- Dickson, A.G., 1990. Standard potential of the $(\text{AgCl}(\text{s}) + \frac{1}{2}\text{H}_2(\text{g}) = \text{Ag}(\text{s}) + \text{HCl}(\text{aq}))$ cell and the dissociation constant of bisulfate ion in synthetic sea water from 273.15 to 318.15 K. *Journal of Chemical Thermodynamics* 22, 113–127.
- Dickson, A.G., 1993. pH buffers for sea water media based on the total hydrogen ion concentration scale. *Deep-Sea Research* 40, 107–118.
- DOE, 1994. In: Dickson, A.G., Goyet, C. (Eds.), *Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water*. Version 2. ORNL/CDIAC-74.
- Feltham, A.M., Spiro, M., 1971. Platinized platinum electrodes. *Chemical Reviews* 71, 177–193.
- Hansson, I., 1973. A new set of pH scales and standard buffers for sea water. *Deep-Sea Research* 20, 479–491.
- Koch, W.F., Biggs, D.L., Diehl, H., 1975. Tris(hydroxymethyl)aminomethane—a primary standard? *Talanta* 22, 637–640.
- Millero, F.J., Zhang, J.-Z., Fiol, S., Sotolongo, S., Roy, R.N., Lee, K., Mane, S., 1993. The use of buffers to measure the pH of seawater. *Marine Chemistry* 44, 143–152.
- Nakaguchi, Y., Nemzer, B.V., Dickson, A.G., 2001. The calibration of indicator dyes for sea water pH measurements. 6th International Carbon Dioxide Conference, 2001, Sendai, Japan, pp. 631–634.
- Ramette, R.W., Culbertson, C.H., Bates, R.G., 1977. Acid–base properties of tris(hydroxymethyl)aminomethane (tris) buffers in seawater from 5 to 40 °C. *Analytical Chemistry* 49, 867–870.
- Smith Jr., W.H., Hood, D.W., 1964. pH measurement in the ocean: a sea water secondary buffer system. In: Y. Miyake, Y., Koyama, T. (Eds), *Recent Researches in the Fields of Hydrosphere, Atmosphere and Nuclear Geochemistry*. Published by Editorial Committee for Sugawara Volume, distributed by Maruzen Company, Ltd, Tokyo, Japan, pp. 185–202.
- Whitfield, M., Butler, R.A., Covington, A.K., 1985. The determination of pH in estuarine waters: I. Definition of pH scales and the selection of buffers. *Oceanologica Acta* 8, 423–432.
- Zhang, H., Byrne, R.H., 1996. Spectrophotometric pH measurements of surface seawater at in-situ conditions: absorbance and protonation behavior of thymol blue. *Marine Chemistry* 52, 17–25.