Spectrophotometric Calibration of pH Electrodes in Seawater Using Purified m-Cresol Purple

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ABSTRACT: This work examines the use of purified meta-cresol purple (mCP) for direct spectrophotometric calibration of glass pH electrodes in seawater. The procedures used in this investigation allow for simple, inexpensive electrode calibrations over salinities of 20−40 and temperatures of 278.15−308.15 K without preparation of synthetic Tris seawater buffers. The optimal pH range is ~7.0−8.1. Spectrophotometric calibrations enable straightforward, quantitative distinctions between Nernstian and non-Nernstian electrode behavior. For the electrodes examined in this study, both types of behavior were observed. Furthermore, calibrations performed in natural seawater allow direct determination of the influence of salinity on electrode performance. The procedures developed in this study account for salinity-induced variations in liquid junction potentials that, if not taken into account, would create pH inconsistencies of 0.028 over a 10-unit change in salinity. Spectrophotometric calibration can also be used to expeditiously determine the intercept potential (i.e., the potential corresponding to pH 0) of an electrode that has reliably demonstrated Nernstian behavior. Titrations to ascertain Nernstian behavior and salinity effects can be undertaken relatively infrequently (~weekly to monthly). One-point determinations of intercept potential should be undertaken frequently (~daily) to monitor for stable electrode behavior and ensure accurate potentiometric pH determinations.

INTRODUCTION

Solution pH is considered to be a master descriptive variable for characterization of chemical, biological, and biogeochemical systems. Measurements of pH facilitate characterizations of acid/base equilibria, primary production/respiration, mineral saturation states, and the status of the carbon dioxide system, as well as trace metal speciation, fluxes, and bioavailability. In the context of the large changes in ocean chemistry[1−3] and biology[4,5] that are presently occurring due to increasing levels of atmospheric CO_2, pH measurement protocols are undergoing refinement to improve accuracy, precision, and simplicity.

Measurements of seawater pH are currently obtained both potentiometrically and spectrophotometrically. Spectrophotometric pH measurements are much more precise (±0.0004)[6] than potentiometric measurements (±0.003)[7,8] and are increasingly preferred for direct ocean monitoring, but potentiometric measurements are advantageous for many types of studies for which less precise measurements are adequate. As such, use of glass electrodes and other potentiometric pH devices[9] is likely to continue as a very common practice in laboratory and field investigations.

Although the accuracy of both potentiometric and spectrophotometric measurements is intimately related to calibration protocols, calibration procedures for the two methodologies are distinct in one critical aspect. Modern spectrophotometric pH measurements, which involve the use of indicator absorbance ratios and characterizations of the intrinsic molecular properties of purified substances, do not require periodic calibration.[10−13] In contrast, pH measurements with glass electrodes require frequent conjugate measurements

in standard solutions in order to ensure consistent measurement accuracy.[14]

For direct potentiometric pH measurements with a glass electrode,[14]

$$\text{pH}(X) = \text{pH}(S_B) - (E_X - E_B)/(R_G T \ln 10/F) \tag{1}$$

where pH(X) is the pH value of the sample and pH(S_B) is the pH of a standard buffer. E_X is the measured emf of the pH cell in the sample solution, and E_B is the emf of the cell developed in the buffer solution. T is the measurement temperature (Kelvin), and R_G and F are the gas and Faraday constants. At a standard measurement temperature of 298.15 K, the magnitude of $R_G T \ln 10/F$ (hereafter referred to as g) is 59.16 millivolts (mV). For high-quality measurements, it is imperative that the temperatures of the buffer and the sample solution be the same.

The Guide to Best Practices for Ocean Acidification Research and Data Reporting[15] describes two critically important limitations associated with potentiometric pH measurements obtained via eq 1. The primary standard buffer for measurement of seawater pH on the recommended total hydrogen ion concentration scale is based on 2-amino-2-methyl-1,3-propanediol (Tris) in synthetic seawater. The protocol for composing this buffer is sufficiently complex that "homemade" buffers (i.e., buffers constructed using the prescribed seawater buffer recipe[16]) should "...be calibrated against a primary standard

buffer whenever practical."[15] The best practices guide also states that, "ideally, the salinity of the buffer matches the salinity of the sample being tested." This concern would generally require the use of a number of Tris seawater buffers of different ionic strengths, formulated to match the likely range of sample salinities. This requirement can be especially demanding in studies of nearshore or estuarine waters.

An additional concern in the use of eq 1 is uncertainty in the inherent quality of the electrode. Does the pH cell exhibit theoretical Nernstian behavior? To answer this question using conventional calibration procedures, multiple seawater buffers over a pH range relevant to natural seawater would be required. Finally, even well-prepared buffers are susceptible to CO_2 uptake and other undesirable processes such as microbial activity. Over extended periods of time, uptake of CO_2 from laboratory atmospheres can result in considerable buffer acidification.[17,18]

Spectrophotometric dyes have been used to precisely measure pH[10,19,12,13] in a variety of natural systems: open ocean,[11] freshwater,[20] estuarine,[21] and brine.[22] Spectrophotometric measurements of pH typically have a reproducibility of 0.001−0.0004 for shipboard[19,11,23,24] and laboratory measurements and 0.0014−0.004 for in situ measurements.[25−28] Spectrophotometric indicators have also been used to improve the accuracy and precision of measurements of other inorganic carbon system parameters,[29] including total alkalinity,[30] partial pressure of CO_2,[31] and dissolved inorganic carbon.[32] Calibrated pH indicators (i.e., those for which the required molecular properties have been well characterized) can also be used to assess the compositional quality of buffers and directly assess changes in buffer quality through time.[17] As such, spectrophotometric pH measurements can be used to standardize "standard" buffers.[17] Recent work has demonstrated that indicator purification is required to eliminate systematic pH measurement errors (e.g., errors as large as 0.018 for unpurified meta-cresol purple).[33,34]

In this work we examine the use of spectrophotometric pH measurements as an alternative to the use of buffers for calibration of glass electrodes. A well-calibrated spectrophotometric indicator can be used to directly provide pH(X) in eq 1. Spectrophotometric pH measurements can then be used for electrode calibration via eq 1 written in the following form:

$$pH(X) = (E_0 - E_X)/g' \qquad (2)$$

Here pH(X) is the pH of the sample, E_X is the emf developed by the pH cell at the spectrophotometrically measured pH(X), E_0 is the electrode intercept potential (i.e., the potential corresponding to pH 0), and g' is the experimentally determined electrode slope. Ideally (i.e., for an electrode exhibiting Nernstian behavior at 25 °C), $g' = g = R_G T \ln10/F = 59.16$ mV. Through conjugate measurements of spectrophotometric pH(X) and potentiometric E_X, regressions of E_X against spectrophotometric pH(X) can be used to calibrate glass electrodes accurately without preparation of complex buffers: specifically, to (a) evaluate the extent to which electrode behavior is Nernstian (i.e., the extent to which g'/g deviates from a value of 1), (b) evaluate the behavior of E_0 through time, and (c) examine the relationship between E_0 and salinity.

■ MATERIALS AND METHODS

Spectrophotometric pH Measurements. Absorbance ratios of the protonated (HI^-) and unprotonated (I^{2-}) forms of sulfonephthalein indicator dyes are used to measure hydrogen ion concentrations ($[H^+]$) in solution based on the following equilibrium reaction:

$$HI^- \underset{}{\overset{K_2}{\rightleftharpoons}} H^+ + I^{2-} \qquad (3)$$

Meta-cresol purple (mCP) is commonly used for measurements of oceanic seawater pH over a range of 7.2−8.2. Following on the work of Yao et al.,[33] who described the effect of indicator impurities on spectrophotometric pH measurements, Liu et al.[34] demonstrated that purified mCP could be used to accurately and precisely determine seawater pH on the total hydrogen ion concentration scale (pH_T) over a wide range of temperature ($278.15 \leq T \leq 308.15$) and salinity ($20 \leq S \leq 40$):

$$pH_T = -\log(K_2^T e_2) + \log\left(\frac{R - e_1}{1 - R\frac{e_3}{e_2}}\right) \qquad (4)$$

with R being the ratio of absorbances measured at 578 nm (λ_2) and 434 nm (λ_1): $R = _{\lambda_2}A/_{\lambda_1}A$. The salinity and temperature dependence of $K_2^T e_2$ (eq 5) is given as

$$-\log(K_2^T e_2) = a + (b/T) + c\ln T - dT \qquad (5)$$

where

$$a = -246.64209 + 0.315971S + 2.8855 \times 10^{-4}S^2 \qquad (6)$$

$$b = 7229.23864 - 7.098137S - 0.057034S^2 \qquad (7)$$

$$c = 44.493382 - 0.052711S \qquad (8)$$

$$d = 0.0781344 \qquad (9)$$

and the temperature and salinity dependence of e_1 and e_3/e_2 are given by

$$e_1 = -0.007762 + 4.5174 \times 10^{-5}T \qquad (10)$$

$$e_3/e_2 = -0.020813 + 2.60262 \times 10^{-4}T + 1.0436 \times 10^{-4}(S - 35) \qquad (11)$$

At $T = 298.15$ K and $S = 35$, eq 4 is given as

$$pH_T(25°C, S = 35) = 7.9881 + \log\left(\frac{R - 0.0046}{1 - 0.0503R}\right) \qquad (12)$$

Calibration Procedures. Electrode calibrations in seawater were performed using an HP 8453 spectrophotometer with an Orion pH meter (model 720A; 0.1 mV resolution) in the absolute millivolt mode (3 M NaCl filling solution). Unpurified mCP was obtained from MP Biomedicals LLC (Lot 1426K). A stock indicator solution (10 mM) was prepared by dissolving mCP, purified as described in Liu et al.,[34] in Milli-Q water. The R ratio ($_{578}A/_{434}A$) of the stock solution was adjusted to 1.6 by addition of NaOH and HCl. Salinity was measured for each sample using a YSI model 30 conductivity meter. Samples were housed in a customized open-top quartz cell obtained from NSG Precision Cells (10 cm path length). The cell volume was approximately 150 mL. Orion Ross pH electrodes (model 8102BN) were used for the potentiometric measurements, and each seawater sample was thermostatted (25 ± 0.05 °C) with a LAUDA-Brinkman water bath. Spectrophotometric absorbances were measured at 434 nm (λ_1) and 578 nm (λ_2); absorbance at 730 nm was used for baseline corrections.[34]

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Table 1. Summary of Least-Squares Electrode Calibrations in Seawater[a]

exp. number	S		fit from pH 7.0–8.1				fit from pH 6.0–8.1		
		n	experimental slope (g')	g'/g	intercept (E_0)	n	experimental slope (g')	g'/g	intercept (E_0)
Electrode 1									
1	34.2	4	59.32 (±0.11)	1.003	397.7 (±0.79)	9	59.29 (±0.08)	1.002	397.4 (±0.53)
2	34.2	5	59.23 (±0.33)	1.001	396.6 (±2.45)	10	59.37 (±0.20)	1.003	397.7 (±1.36)
3	34.2	4	60.13 (±0.66)	1.016	403.4 (±4.95)	7	59.39 (±0.24)	1.004	397.8 (±1.69)
4	34.2	4	59.31 (±0.41)	1.003	397.2 (±3.09)	9	59.76 (±0.13)	1.010	400.5 (±0.93)
5	34.2	4	58.27 (±0.52)	0.985	389.1 (±3.91)	8	58.75 (±0.24)	0.993	392.8 (±1.68)
6*	34.2	8	59.14 (±0.08)	1.000	396.9 (±0.61)	15	58.99 (±0.06)	0.997	395.8 (±0.40)
7*	36.1	7	59.61 (±0.06)	1.008	401.7 (±0.49)	14	60.01 (±0.06)	1.014	404.8 (±0.43)
8	36.0	6	59.26 (±0.05)	1.002	400.5 (±0.41)	12	59.69 (±0.13)	1.009	403.7 (±0.90)
9	36.0	6	59.33 (±0.31)	1.003	400.4 (±2.31)	11	59.60 (±0.10)	1.007	402.4 (±0.67)
10*	36.0	5	58.98 (±0.04)	0.997	398.0 (±0.31)	11	59.66 (±0.16)	1.009	403.1 (±1.10)
11*	36.0	5	59.32 (±0.05)	1.003	400.2 (±0.35)	11	59.59 (±0.04)	1.007	402.2 (±0.28)
12*	36.0	5	59.08 (±0.13)	0.999	398.1 (±1.00)	11	59.31 (±0.09)	1.002	399.8 (±0.61)
13*	36.0	5	59.25 (±0.13)	1.002	399.4 (±0.96)	10	59.40 (±0.11)	1.004	400.4 (±0.76)
14*	36.0	5	59.33 (±0.18)	1.003	399.9 (±1.37)	11	59.36 (±0.08)	1.003	400.1 (±0.56)
		mean	**59.25 (±0.39)**	**1.002**	**398.5 (±3.34)**		**59.44 (±0.32)**	**1.005**	**399.9 (±3.36)**
Electrode 2									
6*	34.2	8	59.21 (±0.06)	1.001	411.3 (±0.43)	15	59.04 (±0.08)	0.998	410.0 (±0.54)
7*	34.2	7	59.37 (±0.22)	1.004	413.0 (±1.67)	14	59.85 (±0.10)	1.012	416.6 (±0.72)
		mean	**59.29 (±0.12)**	**1.002**	**412.1 (±1.20)**		**59.44 (±0.57)**	**1.005**	**413.3 (±4.70)**
Electrode 3									
10*	36.0	5	57.55 (±0.40)	0.973	396.5 (±3.12)	11	59.44 (±0.24)	1.005	411.2 (±1.68)
11*	36.0	5	58.51 (±0.73)	0.989	403.8 (±5.60)	11	59.76 (±0.19)	1.010	413.5 (±1.31)
12*	36.0	5	58.00 (±0.24)	0.980	399.8 (±1.83)	11	58.93 (±0.17)	0.996	406.9 (±1.18)
13*	36.0	5	58.79 (±1.59)	0.994	404.3 (±12.3)	11	59.42 (±0.39)	1.004	409.2 (±2.74)
14*	36.0	5	58.93 (±0.44)	0.996	405.6 (±3.41)	11	58.29 (±0.54)	0.985	400.7 (±3.77)
		mean	**58.35 (±0.69)**	**0.986**	**402.0 (±3.76)**		**59.17 (±0.57)**	**1.000**	**408.3 (±4.88)**

[a]Asterisks (*) indicate experiments performed with two electrodes. The number of data points is indicated by n. Experimental slopes (g') and intercepts (E_0) have units of mV. All experiments were conducted at 25 (±0.05) °C. Experimental slopes (g') are compared to the ideal Nernst slope (g) of 59.16 mV at 25 °C.

For each spectrophotometric pH measurement, a blank (background) measurement was first taken with a seawater sample in the optical cell. Stock mCP solution was then mixed into the seawater such that the final working concentration of indicator ranged from 2.5×10^{-6} to 4.2×10^{-6} M. Electrode calibrations were performed by titrating seawater samples ($n = 7–15$) (Table 1) with a solution of 0.1 M HCl (Mallinckrodt) that had been adjusted to the nominal ionic strength of open ocean seawater (0.7 M) using NaCl (Sigma-Aldrich). Solutions were mixed using an overhead stirrer; complete mixing of cell contents required approximately 90 s. Absolute mV values were recorded along with mCP absorbances after the rate of change of electrode potential became 0.1 mV or less over a period of two minutes. Several experiments were performed using two electrodes simultaneously in the same seawater sample. In the time between experiments, all electrodes were conditioned by storage in seawater.

Calibration plots (millivolts vs pH_T) were generated for each experiment, and electrode slopes (g') and intercepts (E_0) were obtained using eq 2 in linear least-squares analyses. Seawater pH on the total H^+ scale (pH_T) was calculated using eq 4 as described by Liu et al.[34] (Glass pH electrodes respond directly to the activity (a_H) of free hydrogen ions in solution (pH = $-\log a_H$), but for a given temperature and salinity, there is a constant offset between the free hydrogen ion activity scale and the total hydrogen ion concentration scale.)

Salinity Effects on Liquid Junction Potentials. The effect of salinity changes on liquid junction potentials was examined using procedures similar to those outlined above. Electrode response was monitored using a thermostatted (25 ± 0.05 °C) seawater sample that was progressively diluted to lower salinities. Solution pH, determined spectrophotometrically, and electrode potentials were recorded as the solution was titrated with Milli-Q water to lower the salinity from an initial salinity, S^0, of 36.1 to approximately 29. Special care was taken to ensure temperature control because the overall volume of added titrant was large (~25 mL). Using eq 2 with $g' = g = 59.16$, values of E_0 were calculated at each salinity. Linear regressions were then performed to model the influence of salinity on E_0 (and therefore the liquid junction potential) of each electrode.

RESULTS AND DISCUSSION

Three electrodes were tested over a 3-month period to directly examine, spectrophotometrically, the consistency of glass electrode behavior in seawater. For each experiment, data were obtained over a pH range between 6.0 and 8.1. The influence of salinity on electrode behavior was also examined.

Experimentally Determined Electrode Slopes. Table 1 shows the results of two types of analyses. In the first case, least-squares slopes and intercepts were obtained within the optimal pH range of mCP ($7.0 \leq pH_T \leq 8.1$). In the second case, least-squares fits included all data obtained in the

spectrophotometric−potentiometric titrations above pH 6 (6.0 ≤ pH_T ≤ 8.1). All fits exhibited r^2 values greater than 0.999. Within the uncertainties calculated for the least-squares slopes (Table 1), electrodes 1 and 2 exhibited excellent accord with theoretical Nernstian behavior. For electrode 1, the average slopes obtained for analyses between pH 7.0 and 8.1 ($\overline{g}'$ = 59.25 ± 0.39) and for analyses between pH 6.0 and 8.1 ($\overline{g}'$ = 59.44 ± 0.32) were in excellent agreement. For electrode 2, the average slope between pH 7.0 and 8.1 was $\overline{g}'$ = 59.29 (±0.12); for pH between 6.0 and 8.1, the average slope was $\overline{g}'$ = 59.44 (±0.57). Given that pH values lower than 7.0 are below the optimal indicating range for mCP, the accord obtained between the 7.0 ≤ pH ≤ 8.1 results and the 6.0 ≤ pH ≤ 8.1 results is remarkably good. Only two experiments were performed using electrode 2 because it was subsequently broken during use on another project.

The results shown for experiments 10 through 14 were obtained with electrodes 1 and 3 simultaneously immersed in the same sample of seawater. For these five experiments, the slopes obtained for electrode 3 between pH 7.0 and 8.1 ($\overline{g}'$ = 58.35 ± 0.69) were in each case substantially lower than those obtained for electrode 1 ($\overline{g}'$ = 59.19 ± 0.15). In addition, the uncertainties for the slopes obtained with electrode 3 over that pH range are much larger than the uncertainties for electrode 1 (Table 1). These observations demonstrate that the potentials obtained with electrode 3 were substantially more erratic than those obtained with electrode 1, as will be discussed further below. In contrast with the results obtained for analyses between pH 7.0 and 8.1, the average slope obtained for electrode 3 over the larger pH range 6.0 to 8.1 ($\overline{g}'$ = 59.17 ± 0.57) corresponds closely with expected Nernstian behavior.

Figure 1a shows the residuals observed in the least-squares fits that produced the slope and intercept results for 7.0 ≤ pH ≤ 8.1 (Table 1). For electrode 1 and electrode 2 combined, 87 of the 88 residuals fall within ±0.5 mV. For electrode 3, only 21 of 25 residuals were within ±0.5 mV. Figure 1b shows residuals obtained for the fits between pH 6.0 and 8.1. The erratic behavior of electrode 3 relative to electrodes 1 and 2 is clearly visible. This comparatively poor performance was apparently a precursor to the spontaneous demise of electrode 3. With no known triggering event, a crack developed in the body of the electrode and it ceased to function in a Nernstian manner. Electrodes 1 and 4 were used for all subsequent measurements.

Electrode Intercept Potentials. Given the excellent correspondence between the experimentally determined electrode slopes and the ideal Nernst slope (Table 1), eq 2 was used to determine electrode intercept potentials (i.e., calibration intercepts, E_0) with $\overline{g}'$ = g = 59.16 mV and the spectrophotometric−potentiometric data obtained for pH ranges of 7.0 −8.1 and 6.0 −8.1. The E_0 results obtained for all three electrodes (Table 2) are in excellent agreement; with one exception (Experiment 14, electrode 3), E_0 differences (ΔE_0) were always within ±0.5 mV. In 17 of 21 comparisons, ΔE_0 was ±0.3 mV, corresponding to agreement within 0.005 in pH. These results indicate that spectrophotometric electrode calibrations performed within the optimal indicating range of mCP (7.0 ≤ pH ≤ 8.1) provide an excellent account of electrode response well outside the normal range of seawater pH (i.e., at pH_T well below 7). Nevertheless, as the uncertainties shown for the E_0 values within the optimal indicating range (most being less than 0.1 mV) are generally significantly smaller than those obtained over the wider pH range, it is recommended that electrode calibrations obtained
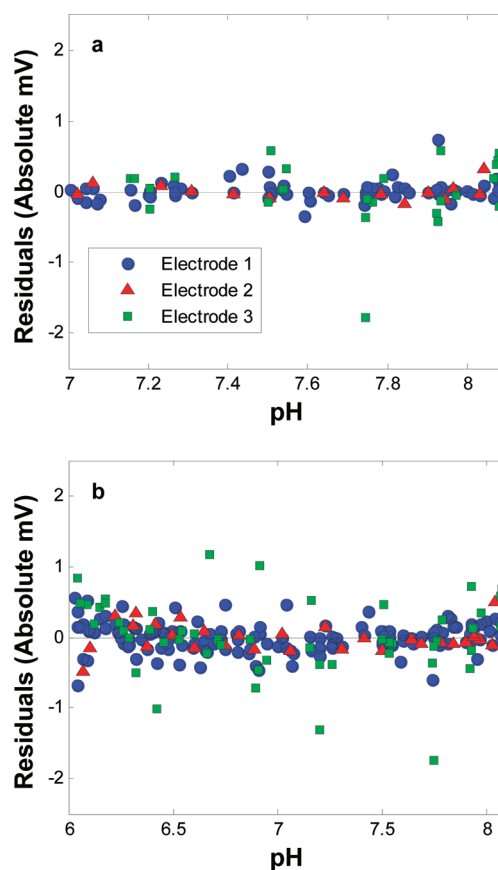

Figure 1. Residuals for the electrode calibrations depicted in Table 1. (a) Calibrations between pH 7.0 and 8.1. (b) Calibrations between pH 6.0 and 8.1.

using the spectrophotometric procedures outlined in this work not be extended to values substantially less than 7.0.

Influence of Salinity on Liquid Junction Potentials. Four experiments were performed to determine the influence of salinity on the E_0 values (and therefore the liquid junction potentials) of electrode 1 and electrode 4. The electrode behaviors depicted in Table 3 and Figure 2 demonstrate that the responses of the two electrodes to changing salinity were nearly indistinguishable. Four experiments with electrode 1 provided an average change in electrode potential (E_0) per unit change in salinity equal to 0.169 (±0.006) mV. Four experiments with electrode 4 yielded an average change in E_0 per unit change in salinity equal to 0.163 (±0.004) mV. The observations shown in Figure 2a indicate that potentiometric pH measurements obtained over a range of salinity can be calculated using eq 2 rewritten as follows:

$$pH(X) = [E_0^0 - (\Delta E_0/\Delta S)(S^0 - S) - E_X]/g \qquad (13)$$

where E_0^0 is an electrode potential corresponding to salinity S^0 (i.e., the value of E_0 obtained in an electrode calibration at initial salinity S^0); E_X is a directly measured electrode potential at any salinity S; and $\Delta E_0/\Delta S$ is the observed change in E_0 for a one-unit change in salinity. Note that when calibrations and measurements are performed at constant salinity ($S^0 = S$), eq 2 and eq 13 have the same form and $E_0^0 = E_0$. Figure 2b and c shows the results of pH comparisons for the data depicted in Figure 2a: pH_T(spectrophotometric) − pH_T(potentiometric) as a function of S. Due to the salinity dependence of E_0, the residuals for pH_T(potentiometric) calculated using eq 2

Table 2. Electrode Intercepts (E_0) Calculated by Assuming $g' = g = 59.16$ mV for Electrodes 1, 2, and 3. E_0 has Units of mV

| | | fit for pH 7.0−8.1 | fit for pH 6.0−8.1 | |
| | | intercept (E_0) | intercept (E_0) | |
exp. number	date			ΔE_0
Electrode 1				
1	3/30/2011	396.41 (±0.04)	396.48 (±0.05)	−0.07
2	3/31/2011	396.06 (±0.06)	396.23 (±0.11)	−0.17
3	4/1/2011	396.14 (±0.26)	396.17 (±0.14)	−0.04
4	4/7/2011	396.01 (±0.05)	396.34 (±0.15)	−0.33
5	4/23/2011	395.73 (±0.15)	395.63 (±0.14)	+0.10
6*	5/4/2011	397.09 (±0.02)	397.00 (±0.04)	+0.09
7*	6/17/2011	398.31 (±0.06)	398.78 (±0.15)	−0.47
8	6/21/2011	399.71 (±0.02)	399.99 (±0.12)	−0.28
9	6/27/2011	399.13 (±0.08)	399.36 (±0.10)	−0.23
10*	6/28/2011	399.36 (±0.03)	399.61 (±0.13)	−0.26
11*	6/29/2011	399.98 (±0.02)	399.23 (±0.08)	−0.25
12*	6/30/2011	398.74 (±0.04)	398.80 (±0.07)	−0.06
13*	7/1/2011	398.68 (±0.03)	398.79 (±0.08)	−0.11
14*	7/2/2011	398.63 (±0.04)	398.77 (±0.06)	−0.14
mean		**397.78 (±0.39)**	**397.94 (±0.41)**	**−0.16**
Electrode 2				
6*	4/23/2011	410.90 (±0.02)	410.84 (±0.05)	+0.06
7*	5/4/2011	411.35 (±0.07)	411.76 (±0.13)	−0.41
mean		**411.12 (±0.23)**	**411.30 (±0.46)**	**−0.18**
Electrode 3				
10*	6/28/2011	408.96 (±0.28)	409.22 (±0.16)	−0.27
11*	6/29/2011	408.80 (±0.20)	409.23 (±0.17)	−0.43
12*	6/30/2011	408.76 (±0.19)	408.54 (±0.14)	+0.22
13*	7/1/2011	407.16 (±0.45)	407.33 (±0.26)	−0.17
14*	7/2/2011	407.37 (±0.13)	406.77 (±0.40)	+0.60
mean		**408.21 (±0.39)**	**408.22 (±0.50)**	**−0.01**

Table 3. Salinity Dependence of Intercept Potentials for Electrodes 1 and 4[a]

| | electrode 1 | | electrode 4 | |
exp. number	E_0^0	$\Delta E_0/\Delta S$	E_0^0	$\Delta E_0/\Delta S$
1	391.26 (±0.56)	0.180 (±0.016)	407.10 (±0.45)	0.172 (±0.013)
2	391.35 (±0.21)	0.176 (±0.006)	407.48 (±0.10)	0.163 (±0.003)
3	391.64 (±0.19)	0.167 (±0.006)	407.42 (±0.25)	0.163 (±0.007)
4	392.15 (±0.14)	0.153 (±0.004)	407.86 (±0.12)	0.152 (±0.004)
mean	**391.60 (±0.20)**	**0.169 (±0.006)**	**407.47 (±0.16)**	**0.163 (±0.004)**

[a] E_0 values at each salinity were calculated using eq 2 with $g' = g = 59.16$ mV. E_0^0 has units of mV.

become increasingly large as salinity decreases from the initial salinity of $S^0 \sim 36.1$ (Figure 2b). In contrast, the residuals calculated using eq 13 (taking into account the effect of S on liquid junction potential) show excellent accord between pH values calculated spectrophotometrically and those determined potentiometrically (Figure 2c).

These results demonstrate that spectrophotometric procedures, which can be used to sensitively measure the intercept potential, E_0, over a range of salinities, can thereby quantify the influence of salinity on the liquid junction potential. If not accounted for, salinity-induced variations in liquid junction potential would create pH inconsistencies of 0.028 over a ten-unit change in salinity, such as could be encountered in estuarine studies.[35]

Implications. Spectrophotometric indicator dyes are being increasingly used to measure the pH of seawater and other aqueous media in natural and laboratory environments. For many applications, however, glass pH electrodes will continue to play an important role. One example might be experiments in which the pH of a growth medium is monitored

continuously or frequently. If an experimental protocol is such that aliquots cannot be repeatedly drawn off for dye addition and spectrophotometric pH measurement, then immersion of a well-calibrated glass electrode in the medium would be the method of choice.

Direct spectrophotometric calibration of glass electrodes in seawater of known pH rather than in synthetic buffers of putative pH provides a convenient, inexpensive approach to the essential practice of routinely monitoring electrode behavior. The addition of acid or pure water to seawater is a straightforward operation, as is spectrophotometric pH measurement. Fresh or stored natural seawater can be used. Spectrophotometric pH can be measured using a standard benchtop spectrophotometer with a thermostatted sample cell. The calibration temperature should be the same as the temperature at which the electrode will be used. The use of purified spectrophotometric pH indicator[34] is essential.

Spectrophotometric electrode calibrations encompass three types of activities: evaluation of electrode response (slope, g') relative to theoretical Nernstian behavior, evaluation of the
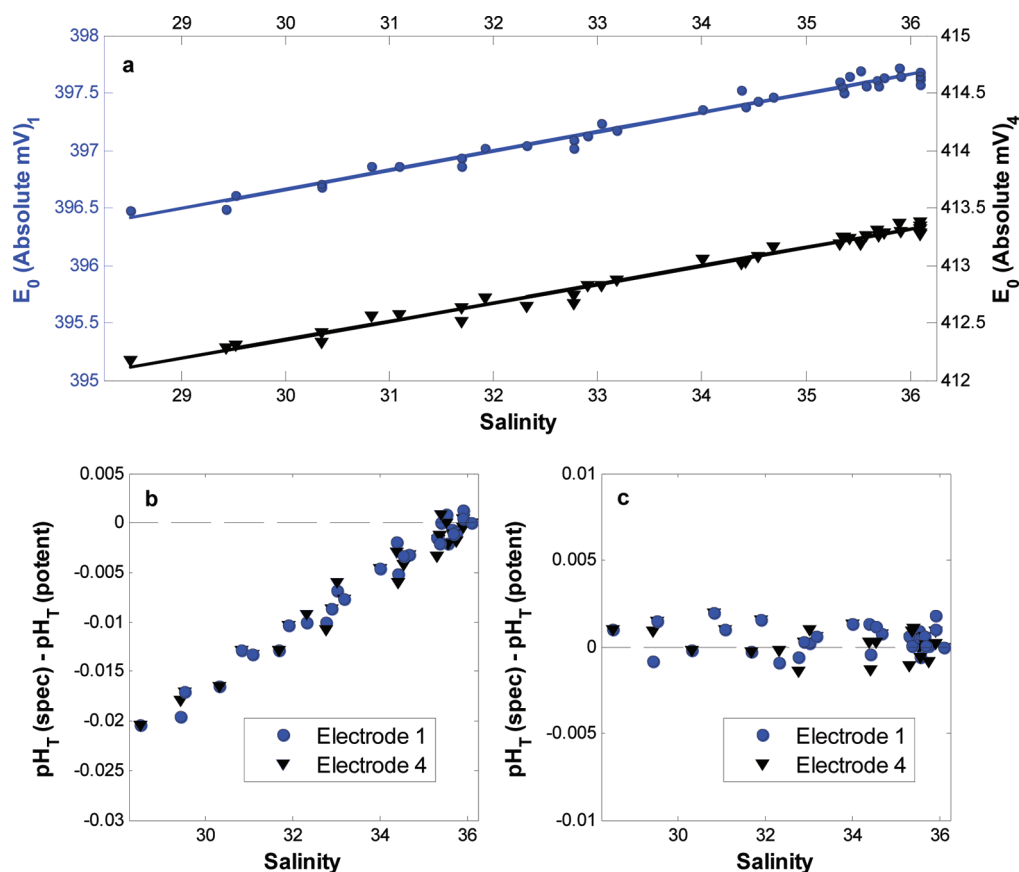
Figure 2. (a) Change in E_0 with changing salinity. (b) Residuals obtained via eq 2 calculations of potentiometric pH (i.e., neglecting consideration of salinity effects on liquid junction potential). (c) Residuals obtained via eq 13 calculations of potentiometric pH (i.e., taking into account salinity effects on liquid junction potential). Calculations of residuals via eq 13 used the experiment-specific E_0^0 values shown in Table 3 and the average $\Delta E_0 / \Delta S$ result obtained for both electrodes ($\Delta E_0 / \Delta S = 0.164$).

salinity dependence of electrode response, and evaluation of the electrode intercept potential (E_0). Assessments of Nernstian behavior (g'/g) can be conducted relatively infrequently (~weekly to monthly). Acid is added stepwise to seawater, and the resulting pH is measured spectrophometrically after each addition. The exact molarity of the acid and the volumes (or masses) of the solutions need not be known precisely, but care must be taken not to dilute the seawater. Assessments of the salinity dependence of electrode behavior are required only for electrodes that are subjected to wide ranges of salinity (e.g., as in estuarine studies). Pure water is added stepwise to seawater, and the resulting pH is measured spectrophometrically after each addition. Our observations indicate that an electrode's $\Delta E_0 / \Delta S$ behavior (eq 13) is quite consistent for extended periods of time. As such, this type of calibration can also be performed infrequently (~weekly to monthly). Evaluations of electrode intercept potential (E_0 in eq 2), on the other hand, generally need to be performed immediately prior to an electrode's use for pH measurements (e.g., daily). For an electrode that has reliably demonstrated Nernstian behavior, one-point E_0 calibrations are sufficient. A small amount of indicator solution is added to a seawater sample, and the pH is then measured spectrophometrically. Intercept electrode potentials can occasionally be consistent within ±0.1 mV for several days. Our experience indicates that errant electrode behavior is typically heralded by an uncharacteristically rapid change in the electrode's intercept potential. Such

changes signal that re-evaluation of the electrode's Nernstian behavior is in order.

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Notes

The authors declare no competing financial interest.

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