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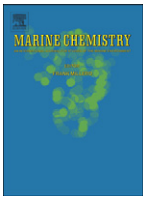

The effect of pressure on meta-Cresol Purple protonation and absorbance characteristics for spectrophotometric pH measurements in seawater

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ABSTRACT

The sulfonephthalein indicator meta-Cresol Purple (*m*CP) is well suited to direct spectrophotometric pH measurements throughout the oceanic water column. In prior work the molar absorbance and proton exchange characteristics of purified *m*CP were characterized over a wide range of salinities and temperatures, allowing use of the indicator over a broad range of oceanic conditions in the surface ocean. Modern spectrophotometric instrumentation allows direct in situ measurements of seawater pH at depths that create substantial pressure-dependent changes in the physical chemical behavior of *m*CP. In order to allow use of purified *m*CP for quantitative pH measurements over essentially the full range of ocean depths, prior calibrations of *m*CP for measurements of seawater pH were extended to include pressures up to 827 bar. Using purified *m*CP, seawater pH is measured using the equation

$$\mathrm{pH_T} = -\log(K_2e_2) + \log\left(\frac{R - e_1}{1 - R\cdot\frac{e_3}{e_2}}\right)$$

where

$$\begin{aligned}
e_1 &= \left\{-0.007762 + 4.5174\times10^{-5}\,T\right\} + \left(1.7\times10^{-6}\right)P\\
e_3/e_2 &= \left\{-0.020813 + 2.60262\times10^{-4}\,T + 1.0436\times10^{-4}(S\text{–}35)\right\} + \left(4.6\times10^{-6}\right)P\\
-\log(K_2e_2) &= \{\mathrm{a} + (\mathrm{b}/T) + \mathrm{c}\ln T - \mathrm{d}T\} - (0.05645/T)\times P
\end{aligned}$$

and

$$\begin{aligned}
\mathrm{a} &= 246.64209 + 0.315971S + 2.8855\times10^{-4}S^2\\
\mathrm{b} &= 7229.23864 - 7.098137S - 0.057034S^2\\
\mathrm{c} &= 44.493382 - 0.052711S\\
\mathrm{d} &= 0.0781344.
\end{aligned}$$

P and *T* are gauge pressure (bar) and Kelvin temperature, and the coefficients a, b and c are salinity dependent coefficients determined in previous work. The influence of pressure on the properties of mCP is sufficient to cause easily measureable effects at depths less than 100 m.

1. Introduction

Spectrophotometric pH measurements provide a reliable, molecularly based means of observing changes in the marine CO_2 system on short and long time scales — for example, diurnal cycles of photosynthesis and respiration (Liu et al., 2006) and decennial to millennial trends in ocean acidification (Byrne et al., 2010). Sulfonephthalein indicators have been used at sea for seawater pH observations for more than 20 years (Byrne and Breland, 1989; Clayton and Byrne, 1993; Millero et al., 1993). These shipboard measurements are generally made at constant temperature (e.g., 25 °C or 20 °C) whereby quantitative pH determinations require an understanding of only (a) the influence of salinity and temperature on the equilibrium ($HI^- \leftrightarrow L^{2-} + H^+$) between the highly colored acid (HI^-) and base (L^{2-}) forms of indicators and (b) the very small influences of salinity and temperature on the indicator's molar absorbance characteristics. However pressure also

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affects the position of the above equilibrium. Shipboard measurements have proven to be an important component of assessments of marine CO_2 system dynamics (e.g., Bellerby et al., 1995, 2002; Clayton et al., 1995; McElligott et al., 1998; Byrne et al., 1999; Tapp et al., 2000), but sensor networks that collect data at in situ conditions (i.e., salinity, temperature, and pressure) are needed to provide the greater spatial and temporal resolution required for comprehensive models of marine CO_2 system dynamics (Liu et al., 2006; Seidel et al., 2008; Byrne et al., 2010).

The recent work of Liu et al. (2011) provides a detailed characterization of the influence of salinity and temperature on pH measurements made with purified meta-Cresol Purple (*m*CP), an indicator that is suitable for the range of pH conditions encountered within the entire oceanic water column. The current manuscript builds on that work by examining the influence of pressure on pH measurements obtained with purified *m*CP. The combined work provides a model that allows pH measurements to be made in seawater for salinities between 20 and 40, temperatures between 5 and 35 °C, pressures between 1 and 827 bar, and the full range of pH encountered between the ocean surface (high pH) and oxygen minimum zones (low pH).

2. Methods

The model for pH measurements developed in this work follows from the Zhang and Byrne (1996) model developed for thymol blue (an indicator especially well suited for measurements of the relatively high pH encountered in the surface-ocean mixed layer). The methods used for determining the pressure dependence of *m*CP are similar to that of the thymol blue study of Hopkins et al. (2000). However the lower pH values of seawater at great depths is outside the optimum indicating range of thymol blue, making *m*CP a more suitable indicator for measuring the pH of the entire water column. In addition, this study assesses the pressure dependence of *m*CP equilibrium behavior over a range of temperatures, thereby extending the measurement protocol of Hopkins et al. (2000) that involved observations of pressure dependencies at a single temperature.

2.1. Theory

Sulfonephthalein indicators (H_2I) are used for spectrophotometric determinations of pH in natural waters by measuring the relative absorbances of the protonated (HI^-) and unprotonated (I^{2-}) forms of the indicator (Byrne, 1987; Byrne and Breland, 1989; Clayton and Byrne, 1993; Zhang, and Byrne, 1996; Yao and Byrne, 2001). The pH can be calculated using the equation

$$\mathrm{pH_T} = \mathrm{p}K_2 + \log\left(\frac{R - e_1}{e_2 - R \cdot e_3}\right) \tag{1}$$

where $\mathrm{p}K_2 = -\log K_2$, R is an absorbance ratio, and e_i are molar absorbance ratios (Byrne, 1987). In this work, $\mathrm{pH_T}$ is determined on the total hydrogen ion concentration scale (Liu et al., 2011). The second dissociation constant of H_2I is defined as

$$K_2 = \frac{[\mathrm{H}^+]_\mathrm{T}[\mathrm{I}^{2-}]}{[\mathrm{HI}^-]}. \tag{2}$$

For *m*-cresol purple the absorbance maxima for HI^- and I^{2-} occur at 434 and 578 nm respectively, and R in Eq. (1) is defined as the ratio of the absorbances

$$R = \frac{{}_{578}A}{{}_{434}A}. \tag{3}$$

The molar absorbance ratios, e_i, are ratios of the molar absorbance coefficients (ε) for the acid and base forms at 578 and 434 nm, and are given as

$$e_1 = \frac{{}_{578}\varepsilon_{\mathrm{HI}}}{{}_{434}\varepsilon_{\mathrm{HI}}}, \quad e_2 = \frac{{}_{578}\varepsilon_{\mathrm{I}}}{{}_{434}\varepsilon_{\mathrm{HI}}}, \quad e_3 = \frac{{}_{434}\varepsilon_{\mathrm{I}}}{{}_{434}\varepsilon_{\mathrm{HI}}}. \tag{4}$$

Eq. (1) can be written in an alternative form with fewer experimental parameters as

$$\mathrm{pH_T} = -\log(K_2 e_2) + \log\left(\frac{R - e_1}{1 - R \cdot \dfrac{e_3}{e_2}}\right). \tag{5}$$

While the physical meaning of the pK_a is now lost in going from Eq. (1) to Eq. (5), the singular intent of this study was to obtain an empirical relationship to measure pH, not to measure thermodynamic quantities. Liu et al. (2011) characterized *m*CP parameters as a function of temperature in kelvin (T) and salinity (S) at atmospheric pressure as:

$$-\log(K_2 e_2) = \mathrm{a} + (\mathrm{b}/T) + \mathrm{c}\ \ln T - \mathrm{d}T \tag{6}$$

$$e_1 = -0.007762 + 4.5174 \times 10^{-5}\ T \tag{7}$$

$$e_3/e_2 = -0.020813 + 2.60262 \times 10^{-4}\ T + 1.0436 \times 10^{-4}(S–35) \tag{8}$$

where

$$\begin{aligned}
\mathrm{a} &= -246.64209 + 0.315971S + 2.8855 \times 10^{-4}S^2 \\
\mathrm{b} &= 7229.23864 - 7.098137S - 0.057034S^2 \\
\mathrm{c} &= 44.493382 - 0.052711\mathrm{S} \\
\mathrm{d} &= 0.0781344.
\end{aligned}$$

2.2. Materials and instrumentation

All reagents were prepared from ACS certified chemicals. Deionized water was obtained from a Millipore Super Q system. Natural seawater came from the Gulf of Mexico at a depth of 10 m. Its salinity was measured as 36.7 with a Seabird Model 49 CTD; it was subsequently diluted to $S = 35.0$ with deionized water. The *m*-cresol purple (*m*CP) powder (Acros Organics) was purified by preparative HPLC according to the procedure of Liu et al. (2011), and the following analysis of the pressure characteristics of *m*CP apply only to this purified form. The concentration of the stock solution was 10 mM.

Absorbance measurements were made with a Hewlett Packard 8453 UV–visible spectrometer using its tungsten lamp with the deuterium lamp off. The HP 8453 has a linear array detector so the entire spectral range (400–800 nm) can be measured simultaneously. Absorbance was measured at 434 and 578 nm, wavelengths of maximum absorbance for the indicator acid and base species, and also at 730 nm, a non-absorbing wavelength for *m*CP, to monitor for baseline shifts. The absorbance peaks at 434 and 578 nm are broad and well-rounded, so small variations in slit width (or spectral bandwidth) from one spectrometer to another will have a negligible effect on the measured absorbances. For the same reason, if changes in pressure, temperature, or salinity were to cause subtle shifts in the wavelength maxima, there would be minimal change in absorbance.

The pressure system was constructed at the University of South Florida using parts from an Enerpac (Div. of Applied Power, Inc.) kit. The absorbance cell was pressurized using a hand pump (Model #11-400) with deionized water as the pressurizing fluid. Inside the absorbance cell, sample solutions were contained within a quartz constant diameter optical cell (le Noble and Schlott, 1976) approximately 5 cm in length. These cells have a variable pathlength because of pressure-induced compression. The properties of these cells are described by Byrne (1984). The Enerpac pressure gauge (Model #GP-15S) had a