Spectrophotometric pH measurements in the presence of dissolved organic matter and hydrogen sulfide

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Abstract

Spectrophotometric pH measurements were first introduced for oceanic environments and they facilitated the determination of the marine CO₂ system, including the direct observation of Ocean Acidification. Extended characterizations of the indicator dye m-Cresol purple over the past two decades enabled the application of this method to natural waters ranging from brines to freshwaters. However, the required determination of the dye’s dissociation constants and absorbance properties were exclusively performed in buffer solutions prepared with artificial seawater. Potential perturbations by substances that occur in natural waters, but are not included in the buffer solutions, have never been tested. Therefore, we studied the impact of elevated amounts of dissolved organic matter (DOM) and hydrogen sulfide (H₂S) on spectrophotometric pH measurements. We did not observe an impact on spectrophotometric pH measurements by H₂S concentrations up to 400 \( \mu \text{mol kg}^{-1} \), which reflect high levels such as those reported from the Black Sea. Likewise, natural DOM did not interfere with the spectrophotometric measurements at concentrations typical for oceanic environments and large estuarine systems. However, strongly colored river waters can cause spectral disturbances resulting in calculated pH values that are up to tenths of pH units too low. To circumvent such disturbances, we recommend using intense light sources, a shorter cuvette length or spectrophotometrically calibrated glass electrodes when performing spectrophotometric measurements under critical conditions.

The need to understand the oceans’ role in the global CO₂ system (Le Quéré et al. 2015) and the aim to track the ongoing process of Ocean Acidification (Doney et al. 2009) stimulated the development of high-quality pH measurement techniques. Spectrophotometric measurements have proven to be the most reliable method to determine seawater pH. The analytical procedure is based on the addition of a pH-sensitive indicator dye to the water sample. The calculation of pH from recorded absorbance spectra requires the knowledge of the dissociation constants and absorbance properties of the dye. More than two decades ago Clayton and Byrne (1993) first determined these properties of the dye m-Cresol purple (mCP) for oceanic measurements. The authors introduced the method stating that “the relative constancy of seawater composition throughout the world’s ocean basins, plus low concentrations of reactive trace elements, produce only small medium-induced changes in the physical-chemical characteristics of the indicator dyes.” Accordingly, the theoretically predicted Ocean Acidification processes could be directly observed by spectrophotometric pH measurements in open ocean surface waters (Byrne et al. 2010).

The rising awareness that understanding Coastal Acidification (Waldbusser and Salisbury 2014) requires more detailed knowledge about the processes affecting pH (Hofmann et al. 2011; Duarte et al. 2013), stimulated extended characterizations of mCP for the application first in brackish (Mosley et al. 2004) and most recently in freshwaters (Lai et al. 2016). Currently, spectrophotometric measurements are the most reliable method to measure pH across the entire spectrum from ground to ocean waters, achieving uncertainty levels below 0.01 pH units (Degrandpre et al. 2014; Dickson et al. 2015). However, the extended characterization of mCP was exclusively based on experiments performed in buffered artificial sea- or freshwater solutions. These solutions have a stable and defined pH, controlled by comparably high concentrations of a buffering substance like TRIS (DelValls and Dickson 1998), and contain the major salt components of seawater. However, for the broad spectrum of natural waters over which spectrophotometric pH measurement can in principle be applied, the requirement of “low concentrations
of reactive trace elements” originally expressed by Clayton and Byrne (1993) is not always fulfilled. In contrast, natural waters contain several substances that are not included in the artificial buffer solutions but potentially impact spectrophotometric pH measurements. Two important and widespread chemical components of natural waters are dissolved organic matter (DOM) and hydrogen sulfide (H$_2$S).

DOM occurs ubiquitously in natural waters typically with increasing concentrations from oceanic toward freshwater environments: open ocean DOM concentrations in terms of moles carbon per volume are typically $< 100$ μmol-C L$^{-1}$ (Nelson et al. 1998), whereas higher DOM concentrations were reported from large estuaries like the Baltic Sea (300–400 μmol-C L$^{-1}$, Kuliński et al. 2014) and the Chesapeake Bay (around 250 μmol-C L$^{-1}$ toward the freshwater end-member, Rochelle-Newall and Fisher 2002). The weighted worldwide river DOM concentration was estimated at 500 μmol-C L$^{-1}$ (Ertel et al. 1986), but many rivers like the Rio Negro in the Amazon basin ($\sim 1000$ μmol-C L$^{-1}$, Ertel et al. 1986) and the Suwannee river in Florida ($> 2000$ μmol-C L$^{-1}$, Averett et al. 1994) feature much higher concentrations.

In contrast to the ubiquitous presence of DOM, hydrogen sulfide occurs regionally in areas where the hydrographical conditions favor anoxic conditions. Examples include extended stratified basins like the Black Sea, the Baltic Sea, and the Caríaco basin with H$_2$S concentrations up to 400 μmol kg$^{-1}$ (Luther et al. 1991), 100 μmol kg$^{-1}$ (Fonselius and Valderrama 2003; Gustafsson et al. 2014), and 75 μmol kg$^{-1}$ (Scranton et al. 2001), respectively. Smaller estuarine systems like silled fjords with restricted water exchange can temporarily develop even higher H$_2$S concentrations. Apart from stagnant water masses, hydrogen sulfide can also be formed in coastal-reinforced oxygen minimum zones (e.g., anoxic events on the Peruvian and Namibian shelf), but typically at lower concentrations (Canfield 2006; Schunk et al. 2013).

In open ocean surface waters, an overdetermination of the CO$_2$ system—by determining more than two out of the four measurable parameters alkalinity ($A_T$), dissolved inorganic carbon (DIC), pH, and CO$_2$ partial pressure (pCO$_2$)—allows researchers to evaluate the analytical performance by internal consistency checks (Byrne 2014). However, this evaluation becomes ambiguous in the presence of DOM and H$_2$S because both contribute to $A_T$ (Kuliński et al. 2014; Yang et al. 2015) and this contribution is not accounted for in the available software for CO$_2$-system calculations, e.g., CO$_2$sys (Pierrot et al. 2006). In addition, analytical techniques to determine the pCO$_2$ of discrete samples are not well developed and optical pCO$_2$ sensors are prone to poisoning by H$_2$S (Atamanchuk et al. 2014; Fritzsche et al. 2017). Consequently, $A_T$ and pCO$_2$ are often not accessible for an overdetermination of the CO$_2$ system. Under such conditions, it is even more important that the remaining parameters (DIC and pH) are determined with low uncertainty.

The impact of DOM and H$_2$S on the acid-base system of natural waters needs to be clearly distinguished from potential methodological interferences with spectrophotometric pH measurements. Such perturbations could for example be induced by spectral disturbances or molecular interactions with the dye and would lead to incorrect pH readings. In regard to H$_2$S and DOM, spectral disturbances need to be expected from chromophoric dissolved organic matter (CDOM), the fraction of DOM that absorbs light in the UV and visible spectrum (Bricaud et al. 1981; Green and Blough 1994; Stedmon et al. 2000). Concerning the characterization of mCP for freshwater conditions, it was already pointed out by Lai et al. (2016) that the effect of “colored dissolved organic matter on measurements needs to be more systematically studied in the future.” Furthermore, it was shown that DOM enhances the water solubility of nonionic organic solutes (Averett et al. 1994). Although mCP has different molecular properties than the organic solutes studied by Averett et al. (1994), comparable molecular interactions between DOM and mCP could alter the dissociation constants and/or extinction coefficients of the dye, both of which would cause erroneous pH readings. Likewise, molecular interactions with H$_2$S, or rather its dissociation products HS$^-$ and S$^{2-}$ that are present under typical pH conditions in seawater, cannot be excluded. In particular the formation of polysulfides (Kremling 1983) could alter the chemical properties of the seawater matrix and impact the dye properties through molecular interactions.

Keeping in mind the importance of high quality pH measurements for the characterization of natural waters and being aware of the risk of disturbances that spectrophotometric pH measurements face in waters that deviate from oceanic standard conditions, this study aims for a systematic evaluation of potential perturbations on mCP-based spectrophotometric pH measurements caused by DOM and H$_2$S, covering the entire concentration range encountered in natural waters.

**Materials and procedures**

**Approach**

DOM and H$_2$S impact the pH of natural waters by interacting with a complex set of other acid-base equilibrium reactions (see Introduction). Here we investigated only potential perturbations of spectrophotometric pH measurements, which we distinguished from actual pH effects of DOM and H$_2$S by the following two approaches:

- Buffer solutions prepared in artificial seawater (Dickson 1993; Millero et al. 1993) were used to investigate whether increasing amounts of DOM impact the pH value obtained from absorbance measurements with mCP. The advantage of using buffered solutions is a stable pH that remains unaffected even when adding relative small amounts of a DOM stock solution. However, the disadvantage of buffered solutions is that their chemical composition differs from
that of natural waters, which could change potential molecular interactions between DOM and the dye.

- Artificial seawater (ASW) solutions and natural water samples were used to study the impact of DOM and H2S on spectrophotometric pH measurements. As the pH of these weakly buffered solutions is sensitive to changes in the DOM/H2S content, this approach requires potentiometric comparison measurements and therefore relies on the assumption that the selected glass electrodes are not affected by the presence of DOM/H2S. In a first series of measurements the glass electrodes were calibrated against the spectrophotometric method (Easley and Byrne 2012). Thereafter, parallel potentiometric and spectrophotometric measurements were performed at increasing concentrations of the potentially perturbing substance. At each DOM/H2S level, the pH-dependence of the perturbation was investigated by manipulating the CO2 content of the solution.

### Spectrophotometric Measurements

The basic principles of spectrophotometric pH measurements were described extensively in the existing literature (Clayton and Byrne 1993; Mosley et al. 2004; Liu et al. 2011) and will only be briefly summarized here. The measurements are based on the addition of a pH-sensitive indicator dye to a water sample. The second dissociation constant \( pK_2 \) of the diprotic dye mCP is in the pH range typical for seawater and in this case the pH of the solution can be expressed as:

\[
\text{pH} = pK_2 + \log_{10} \left( \frac{[I^2^-]}{[HI^-]} \right) 
\]

where \([HI^-]\) and \([I^2^-]\) are the concentrations of the monoprotonated and deprotonated species of the indicator dye, respectively. The concentration ratio \([HI^-]/[I^2^-]\) is determined by absorbance \((A)\) measurements, which in general relate the light intensity recorded for the blank \(I_0\) to the light intensities recorded for the sample solution \(I\):

\[
A = -\log_{10} \left( \frac{I}{I_0} \right) 
\]

HI and \(I^2^-\) have two clearly distinguishable absorbance maxima at wavelength \(\lambda_1 = 434\) nm and \(\lambda_2 = 578\) nm, respectively (Clayton and Byrne 1993). However, the absorbance spectra of both indicator species overlap. Therefore, at both wavelength \(\lambda_1\) and \(\lambda_2\) the absorbance \(A_i\) needs to be expressed by the Lambert-Beer-law describing the additive absorbance contribution of both indicator species as:

\[
A_i = A_i(I^2^-) + A_i(HI^-) = (\varepsilon_i(HI^-) \times [HI^-]) + (\varepsilon_i(I^2^-) \times [I^2^-]) \times d 
\]

where \(\varepsilon_i(X)\) are the molar extinction coefficients of the indicator species \(X\) at wavelength \(\lambda_i\) and \(d\) is the cuvette length.

Illustrative absorption spectra of mCP at high, low, and intermediate pH are displayed in Fig. 1.

After combining the Eqs. 1 and 3 and with algebraic manipulation, the pH of the solution can be expressed as:

\[
\text{pH} = pK_2 + \log_{10} \left( \frac{R - e_1}{e_2 - e_3 \times R} \right) 
\]

where \(R = A_{578}/A_{434}\) is the ratio of the absorbance measured at the two peak wavelengths and \(e_1 = e_{578}(\text{HI}^-)/e_{434}(\text{HI}^-)\), \(e_2 = e_{578}(I^2^-)/e_{434}(I^2^-)\), and \(e_3 = e_{434}(I^2^-)/e_{434}(\text{HI}^-)\) are the molar absorptivity ratios (Clayton and Byrne 1993).

In this study, pH values were calculated on the total scale based on the \(pK_2\), \(e_1\), \(e_2\), and \(e_3\) values reported by Mosley et al. (2004). This bears minor uncertainties in the calculated absolute pH values because the coefficients were determined with nonpurified mCP, whereas all experiments reported here were performed with mCP purified according to Liu et al. (2011) and kindly provided by the lab of Robert H. Byrne, Univ. of South Florida. However, error propagation analysis revealed that uncertainties in the coefficients do not significantly alter relative pH changes, which are reported in this study and interpreted as perturbations by DOM and H2S. The stock solution of the purified dye was prepared by dissolving 0.08 g of the purified dye in 100 mL of deionized water. For better dissolution and pH adjustment, the stock solution was sonicated and 2.75 mL of 0.1 M NaOH were added.

The majority of spectrophotometric pH measurements were executed with an instrumental set-up as described by Carter et al. (2013), which consists of an Agilent 8453 diode array spectrophotometer (Santa Clara, U.S.) and a 10-cm, cylindrical, jacketed, flow-through cuvette (custom made by Hellma Analytics, Müllheim, Germany). The mantle of the cuvette was permanently flushed with water from a Julabo...
F30 heating circulator (Seelbach, Germany), and the temperature (25 ± 0.05°C) was controlled with a Burster Kelvimat 4306 + needle probe 42905 (Gernsbach, Germany) inserted into the water stream just behind the cuvette. The cuvette was filled/emptied with a computer-controlled syringe pump (Supporting Information Fig. S1a). The performance of the instrument was routinely verified by running the self-test of the instrument (covering wavelength accuracy and reproducibility through deuterium lines test, as well as noise-, baseline flatness-, and stability tests) and measuring TRIS buffer solutions and CRM standards provided by the lab of Andrew G. Dickson, Univ. of California (deviation < 0.005 pH units from assigned value).

The routine measurement sequence as reported by Carter et al. (2013) determines the mCP absorption spectra from two intensity spectra (Eq. 2): the sample (I_D) and the sample with mCP added (I). For the purpose of this study we also aimed at recording the DOM self-absorption of the spiked ASW solutions against a clean blank. Accordingly, for each measurement sequence intensity spectra were recorded for three types of solutions: blank solution (subscript: ASW), spiked solution (subscript: DOM), and spiked solution with dye addition (subscript: mCP). From those three intensity spectra two absorption spectra were derived, which we refer to as:

1. The self-absorption spectra were derived from the spiked solution (I_DOM) vs. the blank solution (I_DASW).

2. The mCP-absorption spectra were derived from the spiked solution with mCP (I_mCP) vs. the spiked sample (I_DOM). This second step corresponds to the routine measurement procedure and was used to calculate the spectrophotometric pH of the solution according to Eq. 4.

The mCP-absorption spectra in the second step were taken in replicates of six from a single filling of the cuvette, except for the first series of measurements performed in TRIS buffered solutions.

A critical point for the examination of potential spectral disturbances is the light source used for the absorbance measurements. The Agilent spectrometer is equipped with a tungsten- and a deuterium lamp that can be run independently. The intensity spectra of only the tungsten lamp and the spectra of both lamps operating in parallel are displayed in the Supporting Information (Fig. S2). The tungsten lamp emits light in the visible range, with higher intensities at 578 nm (~ 60,000 counts) than at 434 nm (~ 10,000 counts). The deuterium lamp primarily emits light in the UV range. Operating both lamps in parallel increases the intensity of the emitted light slightly at 578 nm (~ 90,000 counts) and more than doubles the intensity at 434 nm (~ 30,000 counts). Carter et al. (2013) performed their experiments with both lamps operating together. However, to avoid the degradation of the dye or organic matter, the authors recommended operating the instrument with the deuterium lamp off and compensating for the added noise by averaging six successively recorded spectra. As the change in the light source intensity could impact the characteristics of spectral disturbances by DOM, both approaches were tested in this study (deuterium lamp on and no averaging vs. deuterium lamp off and averaging six spectra).

In addition to the experiments with the spectrophotometric pH measurement setup (Carter et al. 2013) described above, we performed comparison measurement with a CONTROS HydroFIA® pH measurements system currently under development within the framework of the EU BONUS project PINBAL (see Acknowledgments). The system is a continued development of the flow injection analysis (FIA) approach described by Aßmann et al. (2011). The overall measurement principle of the instrument is based on the injection of a mCP stock solution into a continuous sample flow. After the injection of the dye, a concentration peak passes through the cuvette. The backward flank of the dilution curve is continuously recorded, resulting in more than 100 absorbance spectra per single pH measurement. The pH-perturbation caused by the dye addition can be corrected by extrapolating the pH values calculated from each spectrum to zero absorbance at the isosbestic wavelength, which is directly related to the dye concentration. The absorbance measurements are performed with a VIS broadband LED as light source and a CCD spectrometer as detector (see Aßmann et al. 2011 for details). The HydroFIA® operates with a 1-cm cuvette, and a nonpurified indicator dye stock solution provided by Kongsberg Maritime Contros GmbH.

**DOM experiments**

Equimolar ($n = 0.04$ mol kg$^{-1}$) buffer solutions of 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS, Amresco, Solon, U.S.), 2-aminopyridine (AMP, Acros Organics, New Jersey, US), and 2-amino-2-methyl-1,3-propanediol (BIS, Alfa Aesar, Karlsruhe, Germany) were prepared in an artificial seawater (ASW) matrix with a salinity of 7. Furthermore, an ASW solution with a carbonate alkalinity (1650 $\mu$mol kg$^{-1}$) and salinity (7) typical for Central Baltic Sea surface water was produced. All solutions were produced with the ionic composition as recommended by Dickson et al. (2007) and the salts used were of analytical grade.

Well-characterized humic (HA) and fulvic acid (FA) extracts from the Suwannee River (International Humic Substances Society (IHSS), catalogue numbers: 2S101H and 2S101F) were used to produce high concentrated DOM stock solutions. Hundred milligrams of each acid extract was dissolved in 50 mL of the ASW solution described above, and the pH was adjusted to 8 with the addition of 0.1 M NaOH solution. Both stock solutions were sonicated for 15 min and subsequently filtered through 0.2 $\mu$m polyethersulfone filters (Sartorius, Göttingen, Germany) to ensure the absence of particles and colloids. The stock solution DOM concentration was diluted and analyzed with a HTCO Shimadzu TOC analyzer (Sugimura and Suzuki...
with CO2 by dissolving 20 mL of pure CO2 gas in 40 mL of pH/C24

Subsamples (110 mL) of three buffer solutions (AMP, TRIS, /C15
analyzed independently:

- Subsamples (110 mL) of three buffer solutions (AMP, TRIS, and BIS) were spiked with the HA and FA stock solutions to achieve HA concentrations of 0, 500, 1000, and 1500 μmol-C L⁻¹ and FA concentrations of 0, 500, 1500, and 2500 μmol-C L⁻¹ (see Supporting Information Fig. S1b for a series of spiked buffer solutions). The pH of the buffered solutions did not change by the addition of the DOM stock solutions, which was verified by potentiometric measurements.
- As an alternative approach, ASW solution subsamples (1 L) were spiked with the HA stock solution to achieve HA concentrations of 0, 300, and 1300 μmol-C L⁻¹. This HA addition changes the pH of the solution. Therefore, the impact of DOM on spectrophotometric measurements was traced by comparisons measurements with glass electrodes, which are described below.

For the DOM experiments in ASW, a glass electrode (Sentix 980, WTW, Weilheim, Germany) was calibrated with comparison measurements using the spectrophotometric method as proposed by Easley and Byrne (2012). Potentiometric and spectrophotometric measurements were performed in parallel with non-spiked ASW water stirred in a double-walled, temperature-controlled, gastight glass container, which is in the following referred to as the mini ocean (Supporting Information Fig. S1a). The sample compartment of the mini ocean had a volume of 1 L that could be accessed from three sealable openings. In contrast to Easley and Byrne (2012), we achieved different pH levels for the calibration by manipulating the DIC content of the ASW solution, rather than by adding hydrochloric acid. This procedure lets the alkalinity of the solution remain unaffected and mimics the natural processes of CO2 addition and removal. The DIC manipulation was achieved by flushing with nitrogen gas prior to the calibration run (highest pH ~ 8.7), followed by the successive addition (6–8 steps) of a CO2-enriched subsample of the same ASW solution (lowest pH ~ 6.5). The added subsample was enriched beforehand with CO2 by dissolving 20 mL of pure CO2 gas in 40 mL of the solution in a syringe. For the established range of pH levels, the relation between the electromotive force (emf) reading from the glass electrode and the spectrophotometric pH was analyzed by linear regression analysis. The subsequent spike experiments were performed on the same day and exactly like the calibration run described above, except that the mini ocean container was filled with the DOM-spiked ASW solutions. The previously obtained linear regression coefficients were used to convert emf readings of the glass electrode to pH units, which were finally compared to the spectrophotometric pH readings.

Hydrogen sulfide experiments

Hydrogen sulfide spike experiments were performed in natural seawater sampled from Landsort Deep (salinity = 11, depth = 110 m) in the Baltic Sea, which regularly becomes anoxic during stagnation periods (Fonselius and Valderrama 2003). The water sample contained H2S on the day of sampling but was oxidized during transport to the laboratory. The water sample was filtered through 0.2 μm polyethersulfone filters prior to the experiment and filled into the mini ocean container described above. A H2S-resistant glass electrode with a split ring diaphragm (Sentix HWS, WTW, Weilheim, Germany) was calibrated in the Landsort Deep water as described above for the DOM experiments. The initial flushing with nitrogen gas ensured that the solution was fully deoxygenated. After calibration of the glass electrode, the DIC content of the sample was adjusted to achieve a pH of around 7, which reflects the typical pH level present when the Landsort Deep becomes anoxic during a stagnation period (SMHI, monitoring data). This solution was then spiked stepwise with a highly concentrated H2S stock solution (‘H2S’ in the following represents the sum of H2S, HS⁻, and of S²⁻). This stock solution was produced by dissolving 0.57 g sodium sulfide nonahydrate (Na2S·9H2O) in 100 mL deoxygenated Landsort Deep water. The pH of the H2S stock solution was adjusted to 7 by adding a CO2-enriched subsample. This CO2-enrichment of the H2S stock solution reduced pH changes during the spike experiment and reflected the natural process of sulfate reduction coupled to organic matter oxidation and CO2 production. The H2S concentration of the solution was measured with the standard procedure of methylene blue formation (Fonselius 1962) after each stepwise addition of the H2S stock solution. Spectrophotometric and potentiometric pH measurements were performed in parallel to the sampling for H2S measurements, which was followed by the immediate addition of reagents.

Assessment

DOM self-absorption

For a wide range of concentration levels, self-absorption spectra of humic (HA) and fulvic acids (FA) dissolved in buffered artificial seawater (ASW) solutions were recorded against the non-spiked solution as a blank (see Material and Methods: “Approach” and “Spectrophotometric measurements”). Figure 2 shows illustrative self-absorption spectra of TRIS buffer solution spiked with HA at concentrations of 0–1500 μmol-C L⁻¹ and with the deuterium lamp (UV light) off. In general, the self-absorption spectra of DOM reveal a typical exponential increase of the absorbance toward lower wavelength. With respect to the subsequently recorded mCP-absorption spectra (Fig. 1), this implies that the peak absorbance of the monoprotonated species of the indicator (HI⁻) at 434 nm, is more likely to be affected by spectral disturbances than the peak absorbance of the deprotonated species.

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I$^2$ at 578 nm. A complete overview of all self-absorption spectra recorded for three buffer solutions, AMP (pH 6.7), TRIS (pH 8.1), and BIS (pH 8.8), each separately spiked with HA and FA, and with the deuterium lamp on/off is given in the Supporting Information (Fig. S3).

The DOM self-absorbance at $\lambda = 434$ nm is linearly correlated to the concentration of dissolved humic- or fulvic acids (Fig. 3). However, at the same DOM concentration (in units of moles carbon per volume) the absorbance of humic acids is roughly twice as strong as the absorbance of fulvic acids. In addition, the DOM self-absorbance slightly decreases with decreasing pH at a constant HA or FA concentration (Fig. 3). The measured DOM self-absorption is independent of the deuterium lamp being on or off, except when the self-absorbance is > 1.5 (HA at 1500 $\mu$mol-C L$^{-1}$). In this case, the measured DOM self-absorption is slightly higher with the deuterium lamp off (Light: vis), which is an indication of the spectrometers tendency to overestimate the absorbance when the light intensity at the detector is low.

**Impact of high DOM concentrations on spectrophotometric pH measurements**

After the addition of the indicator dye, the mCP-absorption spectra (measured against the same DOM-spiked solution without mCP as blank) reveal disturbances in the low wavelength range, which impact the absorbance reading at $\lambda = 434$ nm. The spectral disturbances increase with increasing HA and FA concentration and are considerably less pronounced with the deuterium lamp (UV light) on. Disturbed mCP-absorption spectra are shown in Fig. 4 for TRIS buffer solutions spiked with humic acids. The presented spectra are normalized to the undisturbed absorbance at 578 nm ($A_{578}$) according to the following equation:

$$A_{\lambda,\text{norm}} = \frac{A_{\lambda}}{A_{578}} \times A_{578}$$

where $A_{\lambda,\text{norm}}$ is the normalized absorbance at wavelength $\lambda$, $A_{\lambda}$ is the absorbance at wavelength $\lambda$ as measured, and $A_{578}$ is the mean absorbance at 578 nm of all displayed spectra. This normalization procedure eliminates slight spectral differences caused by variations in the amount of dye added, but does not change the absolute absorbance significantly.

Figure 4 shows that the mCP-absorption spectra are not disturbed at wavelength > 500 nm. However, at $\lambda = 434$ nm the absorbance of the HI$^-$ species of the indicator is increasingly overestimated toward higher HA concentrations. With the deuterium lamp off (Fig. 4, right panel), the normalized absorbance at $\lambda = 434$ nm increases from 0.61 at zero DOM to 0.77 at a DOM concentration of 1500 $\mu$mol-C L$^{-1}$. In contrast, the disturbance is reduced with the deuterium lamp on and the normalized absorbance does not exceed 0.64 (Fig. 4, left panel). It should be noted that the observed disturbances appear when very low amounts of light reach the detector, e.g., at $\lambda = 434$ nm and a HA concentration of 1000 $\mu$mol-C L$^{-1}$ the DOM self-absorption amounts to 1.2 absorption units (Fig. 2) to which 0.7 absorption units are added by the mCP-absorption (Fig. 4). Thus, the observed disturbances occur under conditions known as the performance limits of the Agilent spectrometer (~ 1.3 absorption units, pers. comm. R.H. Byrne). The overestimation of the absorbance at $\lambda = 434$ nm was also observed in the other buffer solutions (AMP, TRIS, BIS) and when the solutions were spiked with fulvic acid. A complete overview of all mCP-absorption spectra is given in the Supporting Information (Fig. S4). The clear dependence of the spectral disturbance on the light source (vis vs. UVvis) indicates that this spectral disturbance might reflect instrument-specific limitations, rather than being caused by direct interactions between the dye and DOM.
The mCP-absorption spectra (Fig. 4) show a disturbance maximum in the low wavelength range. The disturbance maxima shift toward higher wavelength with increasing self-absorbance of the solution (DOM concentration) and with the deuterium lamp off. This indicates that the disturbance maxima are not regular absorption peaks, but rather spectral artefacts that mark the wavelengths at which an overestimation of the absorption signal (associated to low light intensity at the detector) turns into noise. The pH error is not directly affected by this change of the disturbance maximum position, as only the absorbance measured at $\lambda = 434 \text{ nm}$ is included in the pH calculation.

As a consequence of the overestimated HI$^+$ absorbance at $\lambda = 434 \text{ nm}$, the absorbance ratios ($R$) and the resulting pH values calculated from the disturbed spectra (Fig. 4) are underestimated. Figure 5 presents the pH values calculated from the disturbed absorbance spectra as a function of the self-absorbance of the solution at $\lambda = 434 \text{ nm}$ before adding the indicator dye. The true pH value of the solutions corresponds to the pH measured at zero self-absorbance. The addition of the DOM stock solution does not change the pH significantly ($< 0.005 \text{ pH units}$), which was controlled by potentiometric measurements. For spectrophotometric pH measurements in solutions with a DOM self-absorbance at $\lambda = 434 \text{ nm}$ of around 0.3, no significant deviation ($< 0.002 \text{ pH units}$) between the spectrophotometrically obtained pH and the true pH was observed (Fig. 4). In contrast, when the self-absorbance of the solutions is in the range 1.0–1.5 the calculated pH values in the range 1.0–1.5 the calculated pH values are $> 0.01 \text{ pH units}$ below the true value, even with the deuterium lamp on. Without the UV light source, the deviations increase to levels $> 0.1 \text{ pH units}$. The deviations appear to be more pronounced toward high (BIS buffer, pH 8.8) and low (AMP buffer, pH 6.7) pH of the solution, compared to the optimum working range of mCP around pH 8.1 (TRIS buffer). The observed pH error correlates well with the self-absorbance of the spiked solutions, independent of the nature of the DOM (HA vs. FA). This is a further indication that the disturbances are instrument-specific and arise when low light levels reach the detector, rather than being caused by a direct interaction between the dye and the DOM.

The DOM spike-experiments performed in unbuffered artificial seawater (Fig. 6, corresponding spectra in Supporting Information Fig. S6) reveal very similar deviations in the spectrophotometric pH values as observed in the buffered solutions (Fig. 5, corresponding spectra in Supporting Information Fig. S4). The calibration of the glass electrode against the spectrophotometric method revealed an almost exact linear relationship between the emf signal and spectrophotometric pH (deviations $< 0.01 \text{ pH units}$ from the linear regression are represented by the blue symbols and line in Fig. 6). At a humic acid concentration of 300 $\mu$mol-C L$^{-1}$, which corresponds to a self-absorbance of the solution between 0.32 and 0.38, no significant deviation between the spectrophotometric and potentiometric pH exists, regardless whether the deuterium lamp was running or not. At a humic acid concentration of 1300 $\mu$mol-C L$^{-1}$ (corresponding to a self-absorbance between 1.38 and 1.67) and with the deuterium lamp off, the deviations between the spectrophotometric and potentiometric pH reading are in the order of 0.06–0.17 pH units. Running the deuterium lamp reduces the
deviations significantly to levels below 0.03 pH units. The deviations increase slightly toward low pH at the highest DOM concentration and with the deuterium lamp off. The agreement of the pH deviations observed in strongly buffered solutions (Fig. 5) in which the ionic composition is dominated by the buffer substance and in artificial seawater solutions (Fig. 6) containing only carbonate alkalinity, again indicates that the disturbances are presumably related to technical aspects of the absorbance measurement, rather than being caused by dye-DOM interactions, as the latter would not necessarily be independent of the matrix.

In both experimental approaches (buffer vs. ASW solution), we observed significantly higher pH-errors at high DOM concentrations with the deuterium lamp off. However, Carter et al. (2013) reported that running the deuterium lamp decreases the pH by 0.00025 pH units per absorbance measurement (exposure to UV radiation) at a sample pH of 7.95 and attributed this to the degradation of the dye or organic matter contained in the sample. We observed pH changes in the same order of magnitude when doing repeated measurements (Supporting Information Fig. S10). These pH changes are more pronounced at high pH, but rather independent from the amount of DOM in the sample. However, the pH change caused by a single exposure to the UV beam is well below 0.001 pH units and therefore

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**Fig. 5.** Spectrophotometric pH values of buffered artificial seawater solutions spiked with variable amounts of humic (HA) and fulvic (FA) acids that cause the self-absorbance (yellowish color) of the solution. The true pH of the strongly buffered solutions TRIS, AMP, and BIS did not change from the value at zero self-absorbance by the addition of DOM, which was verified by potentiometric measurements. Lower pH values toward higher self-absorbance of the solution are caused by the spectral disturbances displayed in Fig. 4 and Supporting Information Fig. S4. Separate smooth curves are fitted to the measurements performed with the deuterium lamp turned off/on (Light vis/UVvis).

**Fig. 6.** Deviations between spectrophotometric and potentiometric pH in artificial seawater solutions spiked with three different levels of humic acids. Only for the highest DOM concentration a smooth curve is fitted separately for the measurements performed with the deuterium lamp turned off (dashed line) and on (solid line).
significantly lower than the potential pH error associated to measurements in strongly self-absorbing solutions when relying only on the tungsten lamp. We therefore recommend to run the deuterium lamps when analyzing strongly colored samples (see Recommendations section).

In contrast to the absorption spectra obtained with the Agilent 8453 spectrometer and a 10-cm cuvette (Fig. 4, Supporting Information S3, S5), the absorption spectra measured with the CONTROS HydroFIA pH system (1-cm cuvette) do not show a significant disturbance (Fig. 7a). Only a slight increase of the noise in the low wavelength range was observed in the presence of DOM. However, the high concentration of DOM does not impact the extrapolated pH values significantly (deviation < 0.001 pH unit, Fig. 7b). This can be attributed to the shorter cuvette length and proportionally higher indicator concentrations, which are directly related to the ratio of DOM self-absorbance to mCP-absorbance. The disadvantage of a high indicator concentration is the increased pH perturbation induced by the indicator addition (Chierici et al. 1999), which is especially critical for low alkalinity samples (Hammer et al. 2014). For oceanic waters and low dye concentrations, this perturbation is commonly corrected by adding a second aliquot of dye and linearly extrapolating to zero dye concentration (Carter et al. 2013). However, this approach was not yet tested for more challenging conditions (high dye concentrations, low-alkalinity samples).

Fig. 7. HydroFIA mCP-absorption spectra (a) and pH extrapolation plots (b) obtained from measurements of TRIS buffers (blue lines and symbols) and TRIS buffers spiked with humic acid extracts (HA) to achieve a DOM concentration of 1500 μmol-C L⁻¹ (red lines and symbols, same solutions as analyzed in Fig. 5). Squares at the left indicate the pH values extrapolated to zero dye concentration.
samples). Measurements performed with a FIA (flow injection analysis) approach can help to investigate this problem, as they allow extrapolating the pH to zero indicator concentration based on a high number of recorded spectra (Aßmann et al. 2011 and Fig. 7b).

Robustness of spectrophotometric pH measurements to hydrogen sulfide

Potential perturbations of spectrophotometric pH measurements by the presence of hydrogen sulfide (H$_2$S) were tested through comparison measurements with H$_2$S-resistant glass electrodes in a natural water sample. Applying linear regression analysis to the 5-point spectrophotometric calibration (see Materials and Procedures) of the glass electrode revealed only minor deviations (< 0.005 pH units) from a strict linear relationship (red points in Fig. 8b). The subsequent spiking of the sample with up to 400 $\mu$mol kg$^{-1}$ H$_2$S (including HS$^-$ and S$_2^-$) increased the pH of the solution from initially 6.9 to slightly above 7.3 (Fig. 8a), which indicates that the H$_2$S stock solution had a higher pH than the sample solution in the mini ocean container despite the CO$_2$ enrichment. However, the addition of H$_2$S did not cause deviations between the spectrophotometrically and the potentiometrically determined pH larger than 0.005 pH units (blue points in Fig. 8b). Furthermore, no trend of the pH deviations as a function of H$_2$S concentration was observed. Repeated measurements of the pH from a single filling of the cuvette resulted in a decrease of the pH in the range of 0.00055–0.0007 pH units per measurement (Supporting Information Fig. S7). This is higher than the pH change that Carter et al. (2013) attributed to the degradation of the dye by UV light. Currently, it cannot be determined, whether this observation is related to the degradation of the dye or whether the pH decrease is related to a potential oxidation of H$_2$S, which would correspond to a loss of alkalinity. The mCP-absorption spectra did not show any significant disturbances (Supporting Information, Fig. S8).

Discussion

Dissolved organic matter perturbations

Significant spectrophotometric pH measurement errors were observed at the highest DOM concentrations (> 1000
Fig. 9. Recalculated deviations between spectrophotometric and potentiometric pH in artificial seawater solutions spiked with three different levels of humic acids. The spectrophotometric pH values were calculated from the modified \( R^* = A_{578}/A_{488} \) (colored symbols and lines), instead of the classical \( R = A_{578}/A_{434} \) (gray symbols and lines, reproduced from Fig. 6 for comparison). This avoids using the absorbance measurement disturbed by the strong self-absorbance of the solution at \( \lambda = 434 \) nm (Fig. 4).

\( \mu \text{mol-C L}^{-1} \) for measurements with an Agilent 8453 spectrophotometer (Carter et al. 2013) equipped with a 10-cm cuvette. Determined pH values are tenths of pH units too low with the deuterium lamp off, and hundredths of pH units too low with the deuterium lamp on, which increases the light intensity at low wavelengths. Strong evidence was found that these deviations are caused by spectral disturbances related to the instrument performance, rather than molecular interactions that impact the dissociation or absorbance behavior of the dye. The following observations support this conclusion: First, the pH deviations correlate well to the self-absorbance of the solution at \( \lambda = 434 \) nm (Fig. 5). Second, the deviations are very similar in strongly buffered solutions (Fig. 5) and in artificial seawater solutions that contain only carbonate alkalinity (Fig. 6). Third, the light source (deuterium lamp on/off) clearly impacts the pH deviations. Fourth: No deviations were observed when performing measurements with the CONTROS HydroFIA® pH measurements system, which has a short cuvette length and a proportionally higher dye concentration (Fig. 7).

The underlying problem for the pH errors observed with the Agilent 8453 spectrophotometer seems to be an overestimation of the absorbance when the light intensity at the detector is low (Figs. 3, 4). This overestimation impacts only the absorbance reading at \( \lambda = 434 \) nm in the mCP-absorption spectra, because the DOM self-absorbance is much more pronounced at this wavelength than at \( \lambda = 578 \) nm (Fig. 2). Since only the mCP-absorbance at \( \lambda = 434 \) nm is overestimated the pH readings are too low. A low light intensity threshold cannot be determined because this quantity is not accessible from the instrument. Likewise, relating our findings to recommended operation ranges in terms of absorption units, which refer to measurements against a colorless blank, is not meaningful, because the measurements presented here were made against colored DOM blank solutions.

In order to confirm the hypothesis that the observed pH errors are attributed to an overestimation of the absorbance reading at \( \lambda = 434 \) nm and that significant molecular DOM-mCP interactions, which would alter the dissociation constant and/or the absorptivity ratios of mCP, can be ruled out, an alternative pH calculation was applied to the mCP-absorption spectra. To avoid relying on the absorbance of the HI− peak at \( \lambda = 434 \) nm, an alternative absorbance ratio \( R^* = A_{578}/A_{488} \) was used, where the HI− absorbance is replaced by the absorbance at the isosbestic wavelength \( \lambda = 488 \) nm. The latter is linearly related to the indicator concentration, irrespective of the solution pH. Therefore, \( R^* \) requires the exact same results as Fig. 6, except that the spectrophotometric pH was calculated from \( R^* \) instead of \( R \). The pH disturbance even at the highest DOM concentration is eliminated. Likewise, recalculating the pH of the spiked buffer solutions (Fig. 5) with \( R^* \) eliminates the pH disturbances (Supporting Information Fig. S12). As expected, toward high pH, where the absorbance at \( \lambda = 578 \) nm increases, pH values calculated from \( R^* \) reveal to be less precise. However, the absence of significant directional pH errors when applying \( R^* \) supports the finding that (i) no spectral disturbances impact the absorbance readings at \( \lambda = 488 \) nm and 578 nm and (ii) molecular interactions between mCP and DOM do not significantly alter the dissociation constant or absorption properties of the dye.

Exploring the reasons for the overestimation of the absorbance at low light intensities is beyond the scope of this study. However, it should be noted that this overestimation occurs when the combined DOM- and mCP-absorbance reaches the limits of the application range of the Agilent 8453 spectrometer. Other instruments will presumably show similar effects, which, however, might differ in strength and direction. In this respect, we consider our results instrument-specific and a procedure to correct the observed errors cannot be introduced. However, the described spectral perturbations, occurring primarily in the low wavelength range, are related to the patterns of the DOM self-absorption spectra and should thus be universal in nature.

The investigated DOM concentration range was designed to cover the concentrations reported from natural waters. (see
Introduction). However, it was found that interferences with spectrophotometric pH measurements are rather related to the self-absorbance of the sample than to the DOM concentration. In addition, the absorbance per carbon unit of natural DOM is typically lower than that of the isolated humic and fulvic acids we used (Alberts and Takács 2004). Thus, the following assessment of potential pH errors that might be associated to the analysis of natural samples refers to estimated self-absorbance properties of various waters rather than to reported DOM concentrations. Based on the typically exponential dependence of DOM-absorbance on wavelength (Fig. 1; Bricaud et al. 1981; Coble 2007), we calculated the self-absorbance at $\lambda = 434$ nm of various natural waters from the reported absorbance coefficients at other wavelength and converted it to a 10-cm cuvette length as used for our experiments (details in the Supporting Information Table S9). Accordingly, open-ocean waters have very low self-absorbance ($< 0.005$) at $\lambda = 434$ nm (Bricaud et al. 1981; Nelson et al. 1998). The Baltic Sea, a semi-enclosed brackish water system, has higher amounts of DOM (Bricaud et al. 1981), resulting in self-absorbancess at $\lambda = 434$ nm ranging from 0.01 to 0.03 (Ferrari et al. 1996) and IOW, unpubl. data). Similar levels were reported from the Chesapeake Bay (Rochelle-Newall and Fisher 2002). Relating this to the observed pH deviations, spectrophotometric pH measurements should work without restrictions at the DOM self-absorbance levels typical for ocean waters and large estuaries. The self-absorbance at $\lambda = 434$ nm of rivers entering the Baltic Sea are typically below 0.1 (Ferrari et al. 1996; Stedmon et al. 2000; IOW, unpubl. data). Even under these conditions reliable spectrophotometric pH measurements should be possible with errors $< 0.01$ pH units (Figs. 4, 5). However, river waters can also have significantly higher self-absorbances, for example Rio Orinoco with self-absorbancess at $\lambda = 434$ nm around 0.4 in the main river and above 1 in its minor tributaries (Battin 1998), as well as the Suwannee River with self-absorbancess around 0.5 (Yacobi et al. 2003). Even higher levels can be encountered in pore or ground waters (own observations). In the case of such high self-absorbance levels the water samples appear colored to the human eye. Under such conditions, the measurement quality can be improved by following the instructions given in the section Recommendations.

Robustness of spectrophotometric pH measurement to hydrogen sulfide

Over a wide range of H$_2$S concentrations from 0 $\mu$mol kg$^{-1}$ to 400 $\mu$mol kg$^{-1}$ the deviations between spectrophotometric and potentiometric pH measurements were below 0.005 pH units, which is within the expected uncertainty range for this kind of comparison measurement (Easley and Byrne 2012). The investigated H$_2$S range covers the naturally occurring concentrations in highly euchiinic environments such as the Cariaco Basin, the Baltic Sea, and the Black Sea (see Introduction). Only local extreme values, as they may emerge in fjords or salt marshes, are not covered by our study. Since there is no trend in the observed pH deviations we conclude that spectrophotometric pH measurements are not impacted by the presence of H$_2$S, which is further supported by the spectra that show the expected shape (Supporting Information Fig. S8).

Recommendations

For the vast majority of natural DOM concentrations, as typical for ocean waters and large estuaries, no perturbations of spectrophotometric pH measurements were detected. Therefore, neither re-evaluation of previous measurements, nor modifications of the method for future investigations are required under these conditions.

However, in strongly colored river waters we recommend examining the mCP-absorption spectra for any sign of disturbances. In addition, the self-absorbance of the sample at $\lambda = 434$ nm should be measured, as performed in this study. If it exceeds 0.3 absorbance units (threshold identified for an Agilent 8453 with 10-cm cuvette, potentially different for other instrumentation), we propose the following actions to improve the quality of the measurements:

1. The light intensity should be high at both wavelengths. For measurements with an Agilent 8453 spectrophotometer this can be achieved by running the deuterium lamp.

2. A shorter cuvette length and proportionally higher indicator concentrations help to reduce the spectral disturbances caused by DOM, as both decrease the ratio of solution self-absorbance to mCP-absorbance. However, at higher indicator concentrations the pH perturbation induced by the indicator addition is more pronounced, especially for samples with low alkalinitities (Hammer et al. 2014). Measurement systems with a FIA (flow injection analysis) approach could help to circumvent this problem, as they allow to extrapolate to zero indicator concentration (Åssmann et al. 2011).

3. When direct spectrophotometric pH measurements are impossible due to very strong DOM-absorbance, glass electrodes that are carefully calibrated against the spectrophotometric method can in principle be used to obtain accurate values on the total scale (Easley and Byrne 2012). However, a rigorous control of experimental conditions (e.g., temperature, stirring, and gas exchange) is critical.

For a wide range of naturally occurring H$_2$S concentrations of up to 400 $\mu$mol kg$^{-1}$, our results do not indicate interferences with spectrophotometric pH measurements. Accordingly, there is no need for a change of the method in this respect, nor is a re-evaluation of previous data required. Nevertheless, H$_2$S containing samples need to be handled with care to avoid gas exchange with the atmosphere, which could
impact the sample pH by two processes: (i) Oxygen uptake could cause the oxidation of H$_2$S components, which corresponds to an alkalinity loss, and thus a decrease in pH. (ii) H$_2$S containing samples are typically characterized by high pCO$_2$ and an outgassing of CO$_2$ leads to an increase in pH. Though both effects will partially cancel out, the net effects will still cause erroneous pH measurements.

**Conclusion**

Spectrophotometric pH measurements with the indicator dye mCP work robustly and produce correct pH values over a wide range of DOM and H$_2$S concentrations as typical for the vast majority of marine and brackish waters. We did not observe any impact on the characteristics of mCP due to molecular interactions with DOM or H$_2$S. However, strongly colored, self-absorbing river, ground or pore waters can impact the optical method. We propose three recommendations (strong light source, shorter cuvette length and spectrophotometrically calibrated glass electrodes) that allow to apply spectrophotometric measurements even under such challenging conditions. We conclude that spectrophotometric pH measurements in the presence of DOM and H$_2$S are a valuable method to support a full characterization of the CO$_2$ system in aqueous solutions, which is especially important when the carbonate alkalinity is not accessible due to organic alkalinity contributions by significant amounts of DOM.

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Conflict of Interest

None declared.

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