Direct measurement of pCO₂ in cultures of marine phytoplankton: how good is the estimate from \( \text{pH}_{\text{NBS}} \) and single point titration of alkalinity?

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ABSTRACT: In physiological studies of marine phytoplankton in culture, the CO₂ equilibrium is typically calculated from measurement of \( \text{pH}_{\text{NBS}} \) (calibrated in dilute National Bureau of Standards buffers) and a single point titration of alkalinity (AT). This approach has widespread appeal because it is simple, inexpensive, and requires very low sample volumes. However, its continued application ignores advances in analytical and theoretical oceanic CO₂ chemistry that suggest fundamental flaws in the assumption \( \text{pH}_{\text{NBS}} = -\log[\text{H}^+] \) as a consequence of the high ionic strength of seawater and liquid residual junction errors in the electrode. Here we compare directly measured pCO₂ in sterile artificial seawater with calculated values, adopting the usual assumptions and commonly used equilibrium constants. Calculated values either overestimated (+15 to +23 % error) or underestimated (-9 to -11 % error) directly measured pCO₂ depending upon whether constants were derived, respectively, on the \( \text{pH}_{\text{NBS}} \) or 'seawater' (\( \text{pH}_{\text{sws}} \)) scales. With the currently accepted equilibrium constants on the 'total hydrogen ion' pH scale (\( \text{pH}_{\text{tot}} \)), and converting \( \text{pH}_{\text{NBS}} \) to \( \text{pH}_{\text{sws}} \) using an apparent activity coefficient \( f_H \) (optimum value 0.85) and then to \( \text{pH}_{\text{tot}} \), excellent agreement was achieved between calculated and measured pCO₂ both in sterile seawater and in cultures of the diatom Thalassiosira pseudonana. However, \( f_H \) is generally unknown and is specific to electrode and electrode condition, making calculated pCO₂ a rather nebulous concept. Without knowledge of \( f_H \), calculated pCO₂ had a total uncertainty of an order (-120 ppmv at atmospheric equilibrium 360 ppmv) similar to the variation in atmospheric pCO₂ between glacial periods and the present (-160 ppmv). This method therefore clearly lacks the resolution required to address the biogeochemical significance of key physiological questions. Future studies should measure pCO₂ directly, and then if required calculate CO₂ from pCO₂ and the solubility coefficient. Alternatively, since analytical precision of calculated pCO₂ was excellent, and accuracy is potentially good when \( f_H \) is known, we advocate improved interdisciplinary collaboration in order to improve this pH & \( A_T \) approach as a simple but effective tool for the study of marine phytoplankton physiology under controlled conditions.

KEY WORDS: Dissolved carbon dioxide · Marine phytoplankton · Cultures · pCO₂ · pH · Alkalinity · Total CO₂

INTRODUCTION

Almost a quarter of a century ago, Hansson (1973a) made the following simple statement: 'The pH-value of the seawater sample obtained by this procedure is not a measure of the concentration of \( \text{H}^+ \) or the activity of \( \text{H}^+ \)... it is just a value read on the pH-meter'. Hansson was referring to the calibration of pH electrodes in National Bureau of Standards (NBS) buffers and subsequent determination of pH of a sample of seawater. However, Hansson (1973a) was certainly not the first to voice such concerns (see Bates 1963, Spencer 1965). Consequently, within the marine chemistry community the use of pH as an observable in the calculation of the CO₂ equilibrium fell from favour for some time (Dickson 1993a). Subsequently, marine chemists devoted considerable efforts towards establishing meaningful scales for the determination and interpretation of pH of seawater (Dickson 1984, 1993a, b, Byrne & Breland 1989, Millero et al. 1993a) such that pH measurements are now approaching the resolution and accuracy required of a reliable observable of the CO₂ system.
In parallel with the improved understanding of seawater pH, major improvements have been made in recent years in the theoretical and analytical chemistry of the dissolved CO₂ equilibrium (see DOE 1994) such that estimation of the contribution of physical and chemical processes to the global carbon cycle is becoming relatively reliable. The role of biological processes remains less certain and is complicated by a number of contentious issues. For example, despite high concentrations (2100 μmol kg⁻¹) of total dissolved inorganic carbon (CT) in seawater, it has been proposed that the growth rate of marine phytoplankton might be limited by diffusional transport of free CO₂ in the boundary layer around the cell (Riebesell et al. 1993). It has also been suggested that ancient oceanic pCO₂ could be hindcast from the record of the stable isotope ¹³C in particulate organic carbon (POC) preserved in oceanic sediments (Rau et al. 1989, Freeman & Hayes 1992) since there is some evidence that bulk δ¹³C of phytoplankton could be a proxy for ambient pCO₂ during growth (Laws et al. 1995). A fundamental question which relates to both of these proposals is whether marine phytoplankton have direct access to the relatively large pool of dissolved bicarbonate (HCO₃⁻) in the ocean. Evidence for utilisation of HCO₃⁻ is extensive in carbon limited cultures (Burns & Beardall 1987, Merrett 1991, Raven & Johnston 1991, Colman & Rotatore 1995), but equivocal at contemporary ambient oceanic pCO₂ (e.g. Raven 1993). These questions are spawning an increasing number of controlled contemporary studies on the influence of uptake of inorganic carbon both on the dissolved CO₂ equilibrium and on isotopic composition of phytoplankton POC (e.g. see Fry 1996 for review). In order that information from these studies be meaningfully extrapolated to oceanic conditions, it is imperative to establish that analyses of the dissolved CO₂ system in culture are precise and accurate, and thus consistent with those made in the open ocean.

A fundamental problem with the dissolved CO₂ equilibrium is that neither the concentration of free carbon dioxide [CO₂] (square brackets refer to concentration), [CO₂] represent CO₂[H₂] + H₂CO₃ nor the concentration of bicarbonate [HCO₃⁻] can be directly measured, but must instead be derived indirectly from other measurable parameters. Assuming equilibrium conditions, it has long been accepted (e.g. Skirrow 1975) that all components of the CO₂ equilibrium can be calculated with knowledge of any 2 of the 4 measurable parameters CT, pCO₂, pH and total alkalinity (A_T). This procedure also requires temperature (T), salinity (S), solubility coefficient (K_w) for CO₂ (Weiss 1974), and equilibrium constants (summarized in DOE 1994) for carbonic acid (K₁ and K₂), boric acid (K₃), water (K_w) and minor bases. Alternatively, [CO₂] can be calculated more directly from measured pCO₂ and K_w (i.e. using Weiss 1974); this involves less uncertainty than the calculation described above. Recent improvements in both analytical and theoretical aspects of CO₂ chemistry (see DOE 1994) have considerably improved precision and accuracy of both measured and calculated values for pCO₂, as for other components of the dissolved CO₂ equilibrium.

These contemporary analytical approaches to oceanic CO₂ chemistry do have a number of logistical limitations however; they are expensive, involve considerable technical expertise and require relatively large sample volumes. In CO₂-related studies in cultures of marine phytoplankton, the requirement to vary CT often dictates the use of artificial seawater, or seawater initially stripped of inorganic carbon, and so sample volume can become an important consideration. Thus high precision oceanic techniques have not always been appropriate (or even accessible) to studies involving routine, regular sampling from limited volume cultures of marine phytoplankton in artificial seawater. In consequence, calculated values for pCO₂, [CO₂] or [HCO₃⁻] in cultures have not benefitted from recent improvements, and generally continue to depend upon somewhat dated and rather questionable foundations, usually being derived from rather simple measurements of pH and A_T. To our knowledge, direct measurements of pCO₂ in culture have not been made, or are rare. Of more concern is the absence of published estimates for precision or accuracy of values for pCO₂ or [CO₂] calculated from these simple measurements. This pH and A_T approach is typically based upon some variation of the method of Strickland & Parsons (1972) where pH is determined using buffers calibrated on the NBS scale, and A_T is determined using a single simple point titration with dilute hydrochloric acid (i.e. based upon Anderson & Robinson 1946). Calculations either utilise simple tabulated values (e.g. Strickland & Parsons 1972) or can be improved by including T and S dependence of K₁ and K₂ (e.g. Edmond & Gieskes 1970, Mehrbach et al. 1973) derived on the pH_NBS scale. These calculations may or may not include corrections for boric acid dissociation...
(i.e. constants given by Edmond & Gieskes 1970), and rarely include correction for dissociation of water or minor bases (i.e. as described by DOE 1994). Attempts are sometimes made to 'improve' calculations by using more 'up-to-date' constants for \( K_1 \) or \( K_2 \) (i.e. those of Hansson 1973b, Dickson & Millero 1987, Goyet & Poisson 1989, Roy et al. 1993a) or \( K_0 \) (Hansson 1973b, Dickson 1990, Roy et al. 1993b) in combination with \( pH_{NBS} \) values. However, the above equilibrium constants were not in fact derived on the \( pH_{NBS} \) scale and are thus inappropriate for use with \( pH_{NBS} \) values.

The concept of \( pH_{NBS} \) measurements in seawater has been fundamentally criticized by marine chemists because of the assumed relationship:

\[
pH = -\log(a_H) = -\log[H^+]
\]

This relationship is purely notional, however, since \( a_H \) cannot in fact be measured (Dickson 1993a). Moreover, activity and concentration are related by an activity coefficient \( f_H \), such that:

\[
a_H = [H^+] \times f_H
\]

and because of the ionic strength of seawater, \( f_H \) does not approximate to unity, and thus \( a_H \) does not approximate to \([H^+]\). However, this equation is a gross oversimplification, as the problem is compounded by liquid residual junction errors, so that in fact \( f_H \) is electrode specific (Dickson 1993a) and can vary with the condition of the electrode (Perez & Fraga 1987). In order to circumvent these problems, a number of \( pH \) scales have been adopted for seawater work:

- The 'free' hydrogen ion scale \([H^+]_f = [H^+]\)
- The 'seawater' scale \([H^+]_{NBS} = [H^+] + [HSO_4^-] + [HF]\)
- The 'total' hydrogen ion scale \([H^+]_{TOT} = [H^+] + [HSO_4^-] + [HF]\)

Direct oceanic \( pH \) measurements now use potentiometric or spectrophotometric methods standardized with seawater buffers which are rigorously calibrated on the 'total' \([H^+]\) scale (Dickson 1993b, Millero et al. 1993a). The most robust contemporary approach for calculation of the \( CO_2 \) equilibrium is to use the \( pH_{TOT} \) scale in conjunction with the equilibrium constants of Roy et al. (1993a, b) derived on that scale (procedure summarized in DOE 1994). Measured \( C_T \), \( A_T \) and \( H^+ \) are input in units of moles per kilogram solution (seawater), i.e. mol kg\(_{sw}^{-1} \), with \( K_0 \) also derived on the mol kg\(_{sw}^{-1} \) scale (Weiss 1974).

Despite these fundamental questions regarding the validity of \( pH_{NBS} \), even now most culture studies continue to rely on the questionable assumption that \( pH_{NBS} = -\log[H^+]\). The complacency can be such that comparisons are often made of small differences in half saturation constants for growth (in terms of free \( CO_2 \), \( HCO_3^- \) or \( C_T \)) without any consideration of the uncertainties involved. Another problem is that questions relating to uptake of free \( CO_2 \) or \( HCO_3^- \) involve considerable exchange of information with the freshwater literature where uncertainties in \( pH_{NBS} \) and \( f_H \) may be less significant, or at least different, because of the lower ionic strength.

This study attempts to draw together the fields of marine \( CO_2 \) chemistry and phytoplankton physiology in order to examine the validity of calculations of \( pCO_2 \) or \( [CO_2] \) from simple measurements of \( pH_{NBS} \) and \( AT \). Indeed there are certain advantages and valid reasons to use the \( pH_{NBS} \) and \( AT \) method in terms of simplicity of measurements and low sample volume required. An important consideration, therefore, was whether a meaningful description of the \( CO_2 \) equilibrium could be extracted from this method despite its potential limitations. That is, could the problems with \( pH_{NBS} \) be reconciled, at least to some extent, by providing a 'working' value for \( f_H \) from which an estimation of \( pH_{TOT} \) could be derived? Measurements with the precision of oceanic analytical methods are not necessarily required, since changes in \( C_T \) and \( pCO_2 \) are relatively large in cultures, but some estimate of precision and accuracy is clearly overdue.

## MATERIALS AND METHODS

### General Experimental

Experiments were conducted in artificial seawater of salinity 30.5% (Harrison et al. 1980) bubbled with air prior to experiments to achieve equilibrium between \( C_T \) and the \( pCO_2 \) of laboratory air of 400 to 450 ppmv. Artificial seawater was supplemented with nitrate (549 μM), phosphate (22 μM) and silicate (106 μM); trace elements and vitamins were supplemented according to the original recipe (Harrison et al. 1980). In order to remove free \( CO_2 \) purely by physical/chemical diffusion and dehydration of \( HCO_3^- \) to \( CO_2 \), sterile artificial seawater was bubbled with \( N_2 \) gas.

All experiments were conducted at 15°C, and those with phytoplankton used the marine diatom *Thalassiosira pseudonana* (NEPCC #58). Experiments were carried out in culture flasks effectively closed to minimize \( CO_2 \) exchange. Cultures were grown in about 1.5 l of medium in 2 l (nominal) Pyrex flat-bottomed round flasks leaving an initial headspace of about 850 ml. Experiments without phytoplankton used exactly the same medium and experimental set-up. Flasks were sealed with a solid silicone stopper; spongy silicone stoppers were shown to allow a 2-fold higher leakage of \( CO_2 \) into the flask. Three outlet ports were provided through the stopper by means of glass and Tygon tubing, and 2 of these were sealed on the outside end with self-sealing push-fit connectors to prevent gas exchange into the culture flask. One tube
extended down to a frit in the culture medium, whilst the second one simply extended into the headspace. By means of a short length of Tygon tubing (low CO₂ permeability; Cole Parmer), 2 push-fit connectors and an external peristaltic pump (Masterflex), the headspace could then be recirculated through the medium in a closed system with no invasion of outside air (other than the contents of the Tygon tubing prior to pumping — about 1 ml). This allowed equilibration of pCO₂ between medium and headspace prior to pCO₂ analysis. The third outlet port extended down into the medium on the inside, and was connected on the outside to a syringe with a short length of Tygon tubing. This allowed samples to be taken from the culture for pH, A₇ and C₇, but the Tygon tubing did not come into contact with culture medium except during such sampling. Using the syringe, 25 to 30 ml was removed for immediate pHₙbs analysis, of which 20 ml was then pipetted into a separate vessel for determination of A₇, and where necessary 1 ml pipetted off for determination of C₇. Sampling in this manner clearly allowed a small amount of air into the flask after sampling, due to equilibration of atmospheric pressure. However, although this obviously caused a slight increase in pCO₂ and decrease in pHₙbs, this was subsequently removed during subsequent bubbling with N₂ or growth of T. pseudonana. Since equilibrium was always established before sampling, this invasion did not result in any errors either in measured or calculated parameters of the CO₂ system. Mass balance considerations suggest that in any event the total invasion of atmospheric CO₂ into the flask to replace the volume of all of the samples removed would have increased C₇ by about 2 μmol kg⁻¹, or only about 0.1%.

**Measurement of pHₙbs.** pHₙbs was measured using a Corning 350 pH meter with a Corning ‘3 in 1’ combination ATC probe (ATC = automatic temperature compensation), reading to 0.001 pH units. This allowed pH to be measured immediately after sampling, the ATC probe correcting for the difference in temperature between the sample (17 to 20°C) and the buffer calibration values (calibrated at 25°C). The meter and probe were calibrated with fresh NBS buffers (Sigma) at 4.00, 7.00 and 10.00 immediately prior to each set of triplicate measurements. After each 3-point calibration, the slope as a percentage of the ideal nernst slope (59.16 mV per unit pH) was usually between 99 and 100% and always over 98%. If the slope fell below 98% the probe was re-calibrated. Calibration was conducted at room temperature (~20 to 25°C), however, the ATC probe automatically corrects for small differences in buffer calibration temperature. For example, if the meter is calibrated with buffer at 23°C, then a small adjustment is automatically made for the 2°C difference between pH of the buffer at 25°C and that at 23°C. pH measurements of the samples themselves were also corrected manually for the small increase in temperature between the in situ flask (15°C) and the pH determination (~17 to 20°C) using the temperature reading from the ATC probe at the moment of pH determination. This increase influences the CO₂ equilibrium and must be corrected using the appropriate equation pH(t₂) = pH(t₁) + 0.0114 (t₁ − t₂) where t₁ and t₂ are the sample and in situ (incubation) temperatures respectively (Gieskes 1969, Grasshoff et al. 1983, Parsons et al. 1984). Alternatively the final calculated pCO₂ could have been corrected for temperature according to the equations of Copin-Montegut (1988).

These corrections ensured that pHₙbs measurements were of the highest possible quality; in fact, the average standard deviation of a large number of triplicate measurements of pHₙbs was about 0.005 after correction to in situ temperature. This represents a precision (% 1 σ) in [H⁺] of about 1.1%.

**Measurement of A₇.** A₇ was determined according to a reduced volume version of the Strickland & Parsons (1972) method which was taken from the original method of Anderson & Robinson (1946). 20 ml of medium from the pHₙbs determination was pipetted into a small beaker with 5 ml of 0.01 N HCl (±1%, Fisher Scientific) and the pH was determined immediately (to 0.001 units) after careful calibration (see above). From this pHₙbs measurement, A₇ was calculated according to the calculation given by Strickland & Parsons (1972). Average standard deviation of a large number of triplicate measurements of A₇ was typically about 6 μmol kg⁻¹ representing a precision (% 1 σ) of about 0.3%. Accuracy of A₇ was more difficult to define, since true calibration standards have only very recently become available (see footnotes to Table 1).

**Measurement of pCO₂ and [CO₂].** pCO₂ was measured directly using an ADC 225 Mk 3 infra-red gas analyser (IRGA). The headspace in the culture flask was recirculated through the medium using an external pump, as described above, for about 20 min until equilibration was reached between medium and headspace (stable pCO₂ reading). This time was checked independently and agrees well with that established for standard pCO₂ methods (e.g. DOE 1994, Purdie & Finch 1994, Robertson et al. 1994). The push-fit connectors from the external pump were then disconnected from those on top of the flask and replaced with push-fits to short lengths of Tygon tubing leading to the IRGA. The internal pump of the IRGA passed the headspace gas through the analysis cell and back into the flask via the tube and frit (i.e. a closed system passing through the IRGA). The IRGA was calibrated at 0 ppmv using N₂ gas, and at 500 ppmv using a calibrated CO₂ in air mixture (Praxair). The tubing and the IRGA cell and pump were flushed with N₂ prior to each
analysis, and subsequent pCO₂ measurements were corrected for the small dilution caused by the presence of N₂ in the tubing and analysis cell (typically <5%). Since the resolution of the analogue instrument was about 10 ppmv (5 ppmv at best), the relatively small correction for atmospheric pressure (to convert ppmv to μatm) was not undertaken. pCO₂ values were reported as ppmv in moist air (as recommended by DOE 1994). The instrument is not sensitive to water vapour interference, but care was taken to ensure a temperature differential between the instrument and the culture medium to prevent condensation inside the analysis cell. Since [CO₂] cannot be directly measured, 'measured [CO₂]' was in fact calculated from measured pCO₂ and the solubility coefficient K₀. This was the only available estimate for [CO₂] which was independent of pHNBs uncertainty. The standard deviation of triplicate measurements of pCO₂ was about 10 ppmv and was largely determined by the limited resolution of the analogue scale on the instrument. This represents a precision (% 1 σ) of about 3% at atmospheric equilibrium.

**Measurement of CT.** When required, direct measurement of CT was made by injecting 1 ml subsamples of culture medium through a septum into a 1 l flask flushed with N₂. 0.5 ml phosphoric acid (1 M) was then injected into the flask and the gas passed in a closed loop through the IRGA (i.e. returned to the flask) to measure liberated CO₂. The method was calibrated against NaCO₃ standards. The standard deviation of triplicates was about 30 μmol kg⁻¹, and as with pCO₂, this was largely determined by the resolution of the analogue scale on the instrument. This represents a precision (% 1 σ) of around 1.5%.

**Calculation of pCO₂, [CO₂] and [H⁺]TOT from other measurables.** Calculated pCO₂ was determined from pHNBs and AT by several alternative approaches:

1. Simple tabulated values from the conversions of Strickland & Parsons (1972).
2. Spreadsheet calculation typifying the approach usually used for cultures of marine phytoplankton. This included T and S dependence of K₁ and K₂ of Mehrbach et al. (1973), Edmond & Gieskes (1970), Hansson (1973b), and Goyet & Poisson (1989), and the T and S dependence of K₀ on the mol⁻¹ scales (Weiss 1974). AT was converted to carbonate alkalinity (Aₐ) using total boron (Bₐ) estimated from salinity (Uppström 1974) and the T and S dependence of boron acid dissociation (Edmond & Gieskes 1970). pCO₂ and [CO₂] were calculated from [H⁺] and Aₐ according to the standard equations of the CO₂ equilibrium (Skirrow 1975, DOE 1994). Aₐ was converted to carbonate alkalinity (Aₐ) using total boron (Bₐ) estimated from salinity (Uppström 1974) and the T and S dependence of boron acid dissociation (Edmond & Gieskes 1970). pCO₂ and [CO₂] were calculated from [H⁺] and Aₐ according to the standard equations of the CO₂ equilibrium (Skirrow 1975, DOE 1994). This meant that the 4 approaches differed only in the estimates of K₁ and K₂.
3. A more refined spreadsheet was constructed utilizing all of the currently accepted constants available (DOE 1994). All equations necessary have been converted to common scales of pHₘᵦ and mol kg⁻¹ by DOE (1994). T and S dependence of K₁, K₂, K₈ and K₉ are given by DOE (1994) adapted from Roy et al. (1993a, b) and of K₈ from Weiss (1974) on the mol kg⁻¹ scale. Measured AT was converted to mol kg⁻¹ using the seawater density routine of Millero & Poisson (1981) (also given by DOE 1994). An improved estimate of Bₐ was taken directly from Harrison et al. (1990) in order to calculate Aₐ from Aₐ using the T and S dependence of K₈ (DOE 1994). pHNBs was converted to pHₘᵦ (Butler et al. 1985, Whitfield et al. 1985, Dickson & Millero 1987) using pHₘᵦ = pHNBs + log₁₀[H⁺] and as with pCO₂, was largely determined by the limited resolution of the analogue scale on the instrument. This represents a precision (% 1 σ) of around 3% at atmospheric equilibrium.
and 106 μmol kg⁻¹ respectively, using dissociation constants K₁P, K₂P, & K₃P, and K₅Si (DOE 1994). This gave contributions of P and Si to A₇ of about 23 and 3 μmol kg⁻¹ respectively at pH around 8, representing about 1% of A₇. This does not cause an error in A₇ because this measures all contributions to alkalinity. However, there could be a small resulting error in calculated pCO₂ because A₇ must be corrected to A₅C for minor bases in order to calculate the CO₂ equilibrium; an error of 1% in A₇ results in an error in calculated pCO₂ of 1% (Dickson & Riley 1978). Although P₅ and Si₅ decrease during growth of a culture, these were non-limiting and so would not decrease to zero. Calculations suggest that the increase in pH and subsequent dissociation of P₅ and Si₅ would offset the decrease in concentration so that the 1% contribution to A₇ is approximately maintained throughout the culture.

**RESULTS**

The simplest calculation of pCO₂ from pH₅BS & A₇ is to use the tables of Strickland & Parsons (1972), and the relationship between calculated and directly measured pCO₂ using this approach is shown in Fig. 1. The right hand side of the y-axis shows the range of free CO₂ concentration equivalent to the range of pCO₂, for the given temperature and salinity of the experiment (i.e. calculated according to Weiss 1974). For such a simple approach, measured and calculated pCO₂ are in remarkable agreement with the 1:1 relationship; however, this does not validate the use of the Strickland & Parsons (1972) tables because of problems with the electrode specificity of pH₅BS measurements (see Fig. 5 and 'Discussion').

The Strickland & Parsons (1972) tables are also limited in their ability to calculate low pCO₂ values encountered during the late stage of a culture, and the approach also lacks the precision required of many contemporary questions regarding dissolved CO₂ in cultures. Improvement upon these tabulated values required a breakdown of the theoretical basis behind the calculations, and the Strickland & Parsons (1972) tables do not give this in detail. In order to formulate a calculation which could resolve small changes in pH, and thus pCO₂ or [CO₂], and one which could handle very low values of pCO₂ at pH values beyond the range of the Strickland & Parsons (1972) tables, the calculations for pCO₂, [CO₂] & C₇ from pH & A₇ (Skirrow 1975) have been utilized by many authors. Here we adopted this approach using several alternative estimates of the equilibrium constants K₁ and K₂ commonly utilized: those of Edmond & Gieskes (1970) and Mehrbach et al. (1973), both derived on the pH₅BS scale and commonly used with pH₅BS measurements, and the constants of Hansson (1973b) and Goyet & Poisson (1989), both derived on the pH₅WS scale.

The same 6 pairs of pH₅BS & A₇ measurements as shown in Fig. 1 were treated with the 4 different calculation routines described in the 'Materials and methods' (Fig. 2). The 4 routines used the same equations and assumptions, but varied the constants for K₁ and K₂. The constants of Mehrbach et al. (1973) and Edmond & Gieskes (1970) gave calculated values for pCO₂ which were similar, but with an error of the order of +15 to +23% of the directly measured values. In contrast, the constants of Hansson (1973b) and Goyet & Poisson (1989) gave calculated values with an error of the order of -9 to -11% of measured values.

To optimize the calculation in accordance with potentially the most precise and accurate constants available, pH₅BS was converted to pH₅T using f₇ as outlined in the 'Materials and methods' section, in order to utilize the constants derived on the pH₅T scale (see DOE 1994; constants adapted from Roy et al. 1993a, b). The calculation was performed with a choice of 3 values for f₇ of 1, 0.85, and 0.75. The value f₇ = 1 corresponds to the situation where activity and concentration are equal, and as would be expected calculated pCO₂ corresponded approximately with that derived using the Goyet & Poisson (1989) constants (Fig. 2). However, this calculated value was then
Fig. 2. Comparison of directly measured pCO₂ with values calculated from the same pairs of data as shown in Fig. 1. pCO₂ was calculated from measurements of pH₁₀ aty₂ using units of mol⁻¹ per kg seawater (mol kg⁻¹sw), the equilibrium constants of Mehrbach et al. (1973), Edmond & Gieskes (1970), Hansson (1973b) and Goyet & Poisson (1989), and assuming pH = -log a₂ = -log [H⁺]. Solid lines represent least squares regression fits to data (all r² > 0.99, all p < 0.001). Error bars smaller than symbol size; for analytical precision and accuracy see Table 1.

Improved to give excellent agreement between calculated and measured pCO₂ when f_H was ‘tuned’ to an optimum value of around 0.85 (Fig. 3). Using the value for f_H = 0.75, taken approximately as a ‘typical’ value for the given T and S of these experiments (e.g. Mehrbach et al. 1973, Perez & Fraga 1987), the calculated pCO₂ again became a significant overestimate, approximating the estimate of pCO₂ derived using the constants of Mehrbach et al. (1973).

Clearly this method optimized the value for f_H as the one giving the best fit between calculated and measured pCO₂, and so could have been simply fortuitous rather than representing a realistic value for f_H. Taking a rather different approach, this value of f_H was checked independently. The calculation for pCO₂ from pH_TO_T & A_T was modified so that pH_TO_T could be calculated either from pCO₂ & A_T, from pCO₂ & C_T or from C_T & A_T (e.g. Skirrow 1975, DOE 1994). These 3 pairs of measurables are independent of f_H and so were utilized to check the value of pH_TO_T converted from pH_NS using f_H = 0.85 (i.e. ‘measured’ pH_TO_T). Fig. 4 confirmed that pH_TO_T calculated from pH_NS using f_H = 0.85 indeed gave excellent agreement with values for pH_TO_T calculated independently from the other measurables.

However, when this approach was repeated this ‘optimum’ value for f_H varied with the same electrode over time, consistent with the observation that liquid residual junction errors are electrode specific and vary.

Fig. 3. Comparison of directly measured pCO₂ with values calculated from the same pairs of data as shown in Figs. 1 & 2. pCO₂ was calculated from measurements of pH_NS & A_T using units of mol per kg seawater (mol kg⁻¹sw), the equilibrium constants of Dixon & Goyet (1994) and converting pH_NS to pH_TO_T using a range of values for the apparent activity coefficient f_H of 0.75, 0.85 and 1.0. Right hand y-axis represents the range of concentration of free CO₂ (μmol kg⁻¹sw) equivalent to the range of pCO₂ at the given temperature and salinity of the experiment (using Weiss 1974). Solid lines represent least squares regression fits to data (all r² > 0.99, all p < 0.001). Error bars smaller than symbol size, for analytical precision and uncertainty see Table 1.

Fig. 4. Comparison of ‘measured’ pH_TO_T with pH_TO_T calculated from pairs of other measurables of the CO₂ equilibrium from the experiment shown in Figs. 1, 2 & 3. The pairs of measurables A_T & pCO₂, C_T & pCO₂, and A_T & C_T are independent of f_H. ‘Measured’ pH_TO_T was in fact calculated from directly measured pH_NS using f_H = 0.85. Error bars represent analytical uncertainty (± 1 σ) of calculated pH_TO_T derived according to Dickson & Riley (1978) for the 3 sets of measurable pairs (see Table 1 for details). Solid line represents least squares regression fit through all data (r² = 0.96, p < 0.001).
with age of electrode (Whitfield et al. 1985, Perez & Fraga 1987, Dickson 1993a). A simple experiment confirmed this; a new identical electrode was bought when the original electrode was about 15 mo old. The response of the 2 electrodes was compared instantaneously using the twin channels of the pH meter. The old and new electrodes were simultaneously calibrated in separate beakers of NBS buffers of pH 4.00, 7.00 and 10.00, and then placed back in buffers of pH 7.00. The data logger was then switched on and the 2 electrodes placed into 2 separate beakers of air-equilibrated artificial seawater for about 15 min, before being placed back into buffer of pH 7.00. The responses of the 2 electrodes (Fig. 5) suggest that despite calibrating identically in pH 7.00 buffer, both before and after placement in seawater, the electrodes gave quite different values for $pH_{\text{NBS}}$ of the seawater sample. Also of note is the time taken for the $pH_{\text{NBS}}$ reading to stabilize after placing in seawater; Strickland & Parsons (1972) recommended waiting for 5 min, but we would clearly recommend at least 10 min (Fig. 5). For routine measurements, a more effective solution was employed: the electrode was placed in artificial seawater after calibration, so that when the samples were ready, the probe had already stabilized in the seawater for at least 15 min. This both eliminated stabilization time and, because of this, reduced contact time of the sample with the atmosphere to about 1 min, suggesting that atmospheric exchange of CO$_2$ during measurement of pH was negligible.

The same approach as the experiment in Fig. 3 was repeated using cultures of the marine diatom Thalassiosira pseudonana, and confirmed that this worked equally well when removal of $C_\text{T}$ was effected by a marine phytoplankter (Fig. 6) rather than by bubbling with N$_2$. With the same assumed value of $f_{\text{H}} = 0.85$, excellent agreement was achieved between calculated and measured pCO$_2$ (Fig. 6). However, there were some minor discrepancies between calculated and measured values, particularly after inoculation and also towards stationary phase.

**DISCUSSION**

In the ensuing discussion, reference to pCO$_2$ can equally be directed to [CO$_2$] as at constant temperature this is proportional to pCO$_2$ through the solubility coefficient $K_a$ (Weiss 1974). Predicted pCO$_2$ derived from the tables of Strickland & Parsons (1972) gave surpris-
ingly good agreement with the measured values (Fig. 1) when compared to the poor agreement observed between calculated and measured values (Fig. 2) using the constants of Edmond & Gieskes (1970) and Mehrbach et al. (1973). It is conceivable that the Strickland & Parsons (1972) approach was based upon some sort of empirical correction factor, thus explaining the observed good agreement. However, when Fig. 5 was also considered, it became clear that the degree of agreement observed (Fig. 1) was in fact electrode dependent, and that another electrode could equally have given poor agreement using the Strickland & Parsons (1972) tables. Electrode dependent uncertainty in measured pHNWs (Fig. 5) was of the order of 0.17 units, and could even be greater under certain circumstances. In terms of accuracy, therefore, this surely negates the use of the Strickland & Parsons (1972) tables for calculation of pCO2. In any event, this approach suffers from the limited range and lack of precision of the tabulated pH values.

Calculated pCO2 using the constants of Edmond & Gieskes (1970) and Mehrbach et al. (1973) was of the order of a 15 to 23% overestimate of measured pCO2 (Fig. 2), an error well in excess of typical analytical precision and estimated uncertainty (Table 1). Calculated pCO2 was significantly changed, but in terms of accuracy, barely improved by utilizing the constants of Hansson (1973b) and Goyet & Poisson (1989), which resulted in underestimates in calculated pCO2 of the order of 9 to 11%, again in excess of analytical precision and estimated uncertainty (Table 1). The differences between calculated and measured pCO2 using any of the equilibrium constants demonstrates invalidity of this approach from pHNWs and AT when assuming pH = \(-\log[H^+}\). Although the ‘within method’ precision (i.e. using one set of constants) was good (see Table 1), the precision ‘between methods’ using the range of constants was very poor, as indeed was the potential accuracy (Fig. 2). The calculated pCO2 derived using the constants of Mehrbach et al. (1973) was about 34% higher than that derived using the constants of Goyet & Poisson (1989). However, when the constants of Mehrbach et al. (1973) are adjusted to the pHNWs scale, it has been shown that they agree well with those of Hansson (1973b) and Goyet & Poisson (1989) derived on that scale (Dickson & Millero 1987, Goyet & Poisson 1989).

Table 1. Summary of analytical precision and estimated uncertainty in measured and calculated parameters. Errors calculated using precision (% 1 σ) of measured parameters and the derivatives presented by Dickson & Riley (1978)

<table>
<thead>
<tr>
<th>Analytical precision (% 1 σ) of measured parameter</th>
<th>Derived parameter</th>
<th>Measured pair</th>
<th>Combined error (% 1 σ) of derived parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>0.3 (0.6)</td>
<td>0.5</td>
<td>2.3</td>
</tr>
<tr>
<td>1.5</td>
<td>0.3 (0.6)</td>
<td>0.5</td>
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<td>1.5</td>
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<tr>
<td>1.5</td>
<td>0.3 (0.6)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*a* Precision of derived parameter uses the combined errors of the measured pair only (Dickson & Riley 1978)

*b* Uncertainty of derived parameter takes into account errors in the measured pair and in the equilibrium constants (Dickson & Riley 1978)

1. First value represents % 1 σ of triplicates of pH and is used for combined precision. Value in parentheses used for combined uncertainty and represents the uncertainty in pH of 0.01 resulting from resolution of buffer calibration.

2. First value represents % 1 σ of triplicates of AT and is used for combined precision. Value in parentheses used for combined uncertainty and represents analytical uncertainty of AT based upon uncertainties in the correction of AT to AC using [H+] and K_B (see Dickson & Riley 1978). The real accuracy of the single point AT titration has been difficult to establish because ‘true’ calibration standards for AT have only just become available (Andrew Dickson pers. comm.). Preliminary comparisons (unpubl.) with potentiometric titrations indicate accuracy of better than 1% (in agreement with value of 0.6% quoted above from Dickson & Riley 1978), assuming of course that the potentiometric titration was reasonably accurate.

3. Taken from Dickson & Riley (1978)

4. Represents pCO2 as calculated for Fig. 2

5. Taken directly from Roy et al. (1993a). These constants were selected for the DOE (1994) handbook on the basis that they were the most precise values published, and because they were derived by a method giving potentially the most accurate results (Andrew Dickson per. comm.). This however does not necessarily ensure that they are the most accurate, and the likely range of error between all sets of published constants is about 0.015 in pK1 and 0.03 in pK2, or about 2.5% and 3.3% respectively (Andrew Dickson pers. comm.)

6. Represents pCO2 as calculated for Fig. 3 but uncertainties resulting from fH not included here — see text.
We conclude therefore, for reasons discussed later, that it is the misuse of constants derived both on the pHNBS scale (Edmond & Gieskes 1970. Mehrbach et al. 1973) and on the pHsws scale (Hansson 1973b, Goyet & Poisson 1989), rather than significant errors in the constants themselves, that results in the observed poor agreement between, and accuracy of, calculated pCO₂ values (Fig. 2). The scale of the imprecision and inaccuracy due to misuse of constants was approximately an order of magnitude greater than the analytical precision and uncertainty of the method itself.

In order to rectify this poor agreement and inaccuracy, the next calculation (Fig. 3) included the constants and equations recommended by DOE (1994), as outlined in the methods section. Assuming f_H = 1, calculated pCO₂ (Fig. 3) approximated the underestimate given using the Goyet & Poisson (1989) constants (Fig. 2). This was to be expected as there is little difference between the Goyet & Poisson (1989) constants derived for the pHsws scale and the constants of DOE (1994) derived for the pH_TOE scale. Forcing f_H < 1 resulted in an increase in calculated pCO₂ up to an optimum point (f_H = 0.85) at which excellent agreement was achieved. Higher values of calculated pCO₂ were forced by using f_H = 0.75 so that the total uncertainty resulting from variation in f_H was of the same order as that resulting from choice of equilibrium constants (Fig. 2), and again was approximately an order of magnitude greater than the analytical precision and uncertainty (Table 1). The optimum f_H = 0.85 was further confirmed by calculating pH_TOE from pairs of other measurables which were independent of f_H (Fig. 4). The f_H = 0.85 value also worked well for cultures of the marine diatom Thalassiosira pseudonana, although, for reasons unknown, some deviation between calculated and measured pCO₂ occurred during stationary phase and just after inoculation (Fig. 6). Although the value of f_H = 0.85 was applicable for the particular electrode and time of application, f_H varied between electrodes (Fig. 5) and probably with age and condition of electrode (see also Perez & Fraga 1987). f_H is also known to be T and S dependent (Butler et al. 1985, Whitfield et al. 1985) and so this optimum f_H = 0.85 is specific to the electrode, its condition, and our experimental conditions of S = 30.5%, and T = 15°C. Optimum f_H was higher than typical values in the literature (e.g. Mehrbach et al. 1973, Butler et al. 1985, Whitfield et al. 1985, Perez & Fraga 1987), but in our study the electrode was relatively new and experimental conditions were slightly different. Indeed, the f_H value required to optimize agreement appeared to decrease with age of this electrode (authors’ pers. obs.) and the value of f_H = 0.85 cannot be extrapolated to other situations. In retrospect, we derived f_H perhaps a little too pragmatically in this study; f_H derived in this manner could be influenced by any factors causing small errors in both calculated and measured pCO₂, and so may not represent the exact ‘true’ value for the given electrode and experimental conditions. We did however confirm f_H independently (Fig. 4) and therefore feel that the value is reasonably representative. Moreover, the purpose of this study was more to highlight inadequacies in the typical pHNBS & pH calculation, and in our understanding and interpretation of pH measurements in seawater, rather than to provide absolute values of f_H, or information on variation of f_H under different conditions.

The data in Fig. 2 became much clearer in the light of considerations of f_H (Fig. 3). The differences between values derived using the constants of Mehrbach et al. (1973) and those derived using Edmond & Gieskes (1970) probably resulted from smaller scale differences in f_H between the 2 studies. In contrast, the values obtained using the constants of Hansson (1973b) and those of Goyet & Poisson (1989) were in good agreement, reflecting the independence of the pHsws scale constants from consideration of f_H. The significantly greater differences in calculated pCO₂ (Fig. 2) observed between those based upon constants derived on the pHNBS scale and those derived on the pHsws Scale do not reflect major differences in K₁ and K₂, but are more due to the fact that these constants were derived on different pH scales. Dickson & Millero (1987) converted the constants of Mehrbach et al. (1973) to the pHNBS scale by dividing K₁ and K₂ by the f_H values presented by Mehrbach et al. (1973), and then found good agreement between those of Mehrbach et al. (1973) and those of Hansson (1973b). Ironically, although derived on the pHNBS scale, the constants of Mehrbach et al. (1973) are not valid with pHNBS measurements, such as those reported in the literature or measured in our study, unless the f_H values coincide, or unless the constants and pH values are converted to some common scale. The difference between calculated and measured pCO₂ (Fig. 2) using constants of Mehrbach et al. (1973) simply reflects the difference in f_H = 0.75 obtained in that study (value taken for similar conditions to ours) and our value of f_H = 0.85. If the constants of Mehrbach et al. (1973) had been adjusted to the pHNBS scale using f_H = 0.75, and our pHNBS measurements adjusted to the pHsws scale using f_H = 0.85, then good agreement should have resulted. This explains why the calculated pCO₂ with f_H = 0.75 (Fig. 3) is in reasonable agreement with the value calculated (Fig. 2) using the constants of Mehrbach et al. (1973). Whichever approach is used, without knowledge of f_H, comparisons must be specific to the experimental conditions and electrode, and calculation of accurate values for pCO₂ or [CO₂] is highly unlikely.
We conclude that even the constants of Mehrbach et al. (1973), derived on the pHNBS scale, cannot be used with pHNBS measurements in order to calculate pCO₂ or [CO₂]. Using such a combination, accurate values could only be calculated fortuitously in the unlikely event that the \( f_m \) value for the electrode employed coincided with that of Mehrbach et al. (1973). Neither can we recommend the use of the constants of Hansson (1973b) or Goyet & Poisson (1989) with pHNBS values, as this clearly gives inaccurate values; these 2 latter sets of constants were derived on the pH₅₅₅ scale and are clearly inappropriate for use with pHNBS measurements unless \( f_m \) is known (Fig. 3).

Most of the uncertainty arose therefore from choice and misuse of equilibrium constants (Fig. 2) and uncertainties in pHNBS and \( f_m \) [Fig. 3]; analytical precision and uncertainties appeared to be negligible (see Table 1) in the context of these major uncertainties. The precision of the single point AT titration (see Strickland & Parsons 1972) is obviously poorer than that of potentiometric methods, but our value of about 0.3% (Table 1; see also Dickson & Riley 1978) is by no means excessive. Accuracy of this AT method is harder to evaluate, and inaccuracies can arise from variations in acid strength (if not monitored) resulting from volatility, errors in electrode calibration and several other factors. However, since true AT standards have only just become available (see footnotes to Table 1), the question of accuracy is a problem common to most AT analyses. In any event, if uncertainty in AT is indeed better than 1% (see Table 1), then this will contribute negligibly to the major uncertainties in calculated pCO₂ resulting from the pHNBS measurement. Errors in AT of the order of 1% only result in errors in calculated pCO₂ of around 1% (Dickson & Riley 1978). AT does not change with removal of free CO₂ and so is not a major variable during growth of non-calcifying cultures of phytoplankton, other than the relatively small change which results from assimilation of nitrogen species (Brewer & Goldman 1976); because of this, the analytical uncertainties in AT will not be relatively ‘amplified’ as a culture grows because AT is not significantly reduced.

Measurement of pH after calibration in NBBS buffers (and assumption of \( pH = -\log[H^+] \)) has become so acceptable a practice in physiological studies of marine phytoplankton that details of pH measurements and calibrations are often omitted in publications (e.g. Merrett 1991, Colman & Rotatore 1995, Rotatore et al. 1995, Israel & González 1996, Korb et al. 1997). In the present study, we made very careful pH measurements, and achieved an excellent precision (1 σ) of about 0.005 units for triplicate pH measurements (after temperature corrections). This was in part achieved by allowing sufficient time for stabilization of the probe (Fig. 5; also see Strickland & Parsons 1972) in seawater after calibration, but prior to measurement of samples. It was also in part achieved by very careful temperature corrections using the ATC probe. This allowed correction of pH from the temperature at which samples were measured, back to the pH at \textit{in situ} temperature, and also allowed correction for small differences between the temperature at which buffer calibrations were made and the stated temperature at which buffer calibrations are in fact valid (25°C). The majority of studies do not describe such attention to detail in calibration and measurement of pH, and we would suggest that because of this, the real uncertainty in calculated pCO₂ or [CO₂] ‘between studies’ is even greater than that suggested by Figs. 2 & 3. For example, not taking into account the time required for stabilization in high ionic strength medium (Fig. 5) could add an additional error in calculated pCO₂ of 3 to 10%.

The application of calculations of the CO₂ equilibrium derived from pHNBS measurements are extensive in the field of phytoplankton physiology. The constants of Mehrbach et al. (1973) and others, such as Riley & Chester (1971), on the pHNBS scale are frequently used (e.g. Dong et al. 1993, Thompson & Calvert 1994, Colman & Rotatore 1995, Laws et al. 1997), and some have used the constants of Goyet & Poisson (1989) in combination with pHNBS (e.g. Merrett et al. 1996). Other studies such as Riebesell et al. (1993) cite the approach of Grasshoff et al. (1983), without specifying which of the 2 sets of constants described by Grasshoff (i.e. Mehrbach et al. 1973 and Hansson 1973b) were actually employed, an omission which according to our Fig. 2 is quite significant. One of the few physiological studies not to use pHNBS was that of Laws et al. (1995), who calculated the CO₂ equilibrium from CT & AT and thus avoided the uncertainties inherent with pH. We should point out that care is required when calculating the CO₂ equilibrium from these pH independent measurable pairs, such as CT & AT. If pH itself were to be calculated, then the relevant pH scale on which this is based should be specified, and this will depend upon the constants chosen. In the case of Laws et al. (1995),


The constants of Roy et al. (1993a) were used, and so if pH had been calculated, it would be a pH\(_{TOT}\) value and not directly comparable with pH\(_{NBS}\) values commonly quoted in the physiological literature.

pH is specified in the models of Rau et al. (1996) and Riebesell et al. (1993), seemingly with the implicit assumption pH = −log[H\(^+\)]. These models show much promise in the understanding of uptake of \(\text{CO}_2\) and isotope discrimination. However, before experimentalists begin to provide validation data for such models, some consensus and clarification is clearly required on meaningful definitions and applications of seawater pH measurements. Uncertainties in pH\(_{NBS}\) measurements not only influence the calculation of \(\text{pCO}_2\) and \([\text{CO}_3^-]\), but also velocities of reactions derived from pH dependent rate constants for hydration and dehydration of free \(\text{CO}_2\) and \(\text{HCO}_3^-\) (Johnson 1982). The combination of pH dependent concentrations with pH dependent rate constants suggests that the uncertainties in the final derived reaction velocities could be greater than acknowledged by physiological studies which utilize these velocities (e.g. Burns & Beardall 1987, Merrett 1991, Colman & Rotatore 1995, Israel & González 1996, Korb et al. 1997, Laws et al. 1997).

It should be emphasized that the problems of 'between study' imprecision and inaccuracy by no means necessarily invalidate the conclusions of studies which have calculated \(\text{pCO}_2\) and \([\text{CO}_3^-]\) using pH\(_{NBS}\) as a measurable. However, problems will materialize when comparisons are made between studies of calculated concentrations, or when critical 'threshold' concentrations or partial pressures derived in the laboratory are extrapolated to the ocean. The variation in estimated \(\text{pCO}_2\) from a single pH\(_{NBS}\) and \(\text{AT}\) pair is around 120 ppmv (Figs. 2 & 3) at atmospheric equilibrium of 360 ppmv, which is almost the order of the variation in \(\text{pCO}_2\) (around 160 ppmv) between glacial periods and the present day (Barnola et al. 1987). Clearly, in its present form, the pH\(_{NBS}\) & \(\text{AT}\) calculation lacks the resolution required to address the biogeochemical significance of key physiological processes in marine phytoplankton, and how these might have varied with changing oceanic conditions over geological time.

It has been suggested that it is twice as accurate to directly measure \(\text{pCO}_2\) as to calculate it (Dickson & Riley 1978). This is probably true using the highest precision IRGA instruments currently available. Our measurements were limited in precision (about 10 ppmv) by the resolution of the analogue instrument employed, and in accuracy by this resolution and by the lack of a correction for atmospheric pressure to convert ppmv to \(\mu\text{atm}\) (error of about ± 5 to 10 \(\mu\text{atm}\)). Thus, estimated accuracy of our measured \(\text{pCO}_2\) was of the same order as that of our calculated values, which had a total analytical uncertainty (ignoring \(f_H\) problems) of about 3 to 4% (−10 to 15 ppmv at 360 ppmv; Fig. 2, Table 1). When the errors regarding equilibrium constants and \(f_H\) were also considered (Figs. 2 & 3), total uncertainty of calculated \(\text{pCO}_2\) was around an order of magnitude greater (around 120 ppmv at 360 ppmv) and so calculation of \(\text{pCO}_2\) or \([\text{CO}_3^-]\) from pH\(_{NBS}\) as a measurable can be recommended no longer without consideration of \(f_H\).

Highly accurate and precise methods for analysis of the \(\text{CO}_2\) equilibrium in the oceans are now available, methods such as spectrophotometric and potentiometric pH analyses (Byrne & Breiland 1989, Dickson & Goyet 1994), potentiometric titration of \(\text{AT}\) (Millero et al. 1993b, DOE 1994), and coulometric determination of \(\text{CO}_3^-\) (e.g. Johnson et al. 1993, DOE 1994) and \(\text{pCO}_2\) using gas chromatography or infra-red gas analysis (Wanninkhof & Thoning 1993, DOE 1994). However, many physiological studies do not have access to the equipment required for such analyses, which also need considerable technical and theoretical expertise. Moreover, relatively large sample volumes are required, which can compromise studies using small volume cultures in artificial seawater. Changes in the \(\text{CO}_2\) equilibrium in nutrient supplemented cultures tend to be relatively large, and so methods of the highest precision and accuracy may not be as necessary as for oceanic measurements. One of the aims of this study was to determine whether a working value for \(f_H\) could be recommended so that a modification of the relatively simple pH\(_{NBS}\) & \(\text{AT}\) method could be retained for use in culture studies, but this is clearly impossible given the electrode specificity of \(f_H\). However, if direct measurements of \(f_H\) are made, and the true accuracy of the single point \(\text{AT}\) titration determined, then the continued use of the pH & \(\text{AT}\) method is possible, and would clearly be desirable in terms of working in cultures of phytoplankton. Indeed the analytical precision of triplicate pH\(_{NBS}\), \(\text{AT}\) and calculated \(\text{pCO}_2\) were excellent (Table 1), estimated inaccuracy of calculated \(\text{pCO}_2\) resulted from the choice and misuse of equilibrium constants, and from invalid assumptions regarding pH = −log[H\(^+\)].

Clearly, there could now be much confusion on how best to approach the calculation of the \(\text{CO}_2\) equilibrium in cultures of marine phytoplankton, and more work will be needed on this question before a clear strategy can be suggested. In the meantime we propose a number of interim recommendations:

1. \(\text{pCO}_2\) should preferably be measured directly, and if \([\text{CO}_3^-]\) is required then this can be calculated from \(\text{pCO}_2\) and the solubility coefficient \(K_s\). Direct measurement of \(\text{pCO}_2\) together with \(\text{AT}\) or \(\text{CT}\) would allow calculation of the equilibrium without the problems inherent to measurement of pH\(_{NBS}\). However, some caution will be required under the low \(\text{pCO}_2\) con-
ditions potentially encountered during the late stage of cultures (~10 ppmv, D.W.C. pers. obs.). Here pCO₂ is of a similar order both to the resolution of measurements, and to the corrections for water vapour and atmospheric pressure; under these conditions, analytical accuracy is clearly of paramount importance.

(2) Any use of pH_{NBS} as a measurable in calculations of the CO₂ equilibrium must take into consideration \( f_{H} \) and this should be independently measured. For example, \( f_{H} \) can be determined from changes in pH upon additions of acid beyond the equivalence point in the titration of A_T (e.g. Culberson et al. 1970, Mehrbach et al. 1973, Perez & Fraga 1987).

(3) An alternative to the use of pH_{NBS} is to calibrate electrodes directly using ‘total hydrogen ion scale’ seawater buffers (see Dickson 1993b, Millero et al. 1993a, DOE 1994). This avoids consideration of \( f_{H} \) and allows direct use of pH_{TOT} measurements with the equilibrium constants derived on that scale (DOE 1994). However, it must be accepted that systematic uncertainties may still remain even in these constants (see footnotes to Table 1).

(4) If single point titration of A_T is to be used in combination with either of the above pH measurements, then it must be accurately measured, preferably by calibration against A_T standards now available. An advantage of using A_T is that the measurement is very simple, and samples do not have to be protected from the atmosphere prior to titration; exchange of free CO₂ does not influence A_T. One disadvantage of using A_T is that there are many acid-base contributions to A_T, such as boric, phosphoric and silicic acids, and these must be corrected for to give A_C which is used in CO₂ equilibrium calculations (see ‘Materials and methods’). If artificial buffers are added in excess to the seawater, as in some recipes, then the A_T measurement cannot be used, as the artificial buffer becomes the major contributor to A_T and thus would require a major correction to give A_C. Direct measurement of C_T is an alternative option in combination with either of the above pH determinations; C_T is directly input into calculations thus avoiding the above complications with A_T.

Whatever approach is adopted, it is clear that major improvements in precision and accuracy are required in this field, and that these will only be realised through improved communication and collaboration between the fields of marine chemistry and phytoplankton physiology.

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