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Gaëlle Capitaine, Daniela Stoica, Thibaut Wagener, Paola Fiscaro. Production of a reference material for seawater pHT measurements by a National Metrology Institute. *Marine Chemistry*, 2023, 252, pp.104244. 10.1016/j.marchem.2023.104244 . hal-04155430

HAL Id: hal-04155430

<https://hal.science/hal-04155430>

Submitted on 7 Jul 2023

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Production of a reference material for seawater pH_T measurements by a National Metrology Institute

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Abstract

Measurements of seawater pH on the total scale, noted pH_T , contribute to the assessment of ocean acidification and its impact on the marine environment if a sufficient data quality is achieved. In order to achieve this, reference materials known as TRIS buffers (equimolar buffer of 2-Amino-2-(hydroxymethyl)-1,3-propanediol (TRIS) and TRIS hydrochloride (TRIS.HCl) in artificial seawater of salinity 35) have been developed and commercialised in the last decades. This paper describes, for the first time, the production of this reference material according to the ISO guide 35 and ISO standard 17034 guidelines that includes a thorough uncertainty budget evaluation carried out following the Guide to the expression of Uncertainty in Measurement (GUM). Characterization with Harned Cell at three temperatures as well as homogeneity and long-term stability assessments were performed and taken into account in the final uncertainty budget. The global standard uncertainty budget of the produced reference material for seawater pH_T measurements is of 0.0025, 0.0024 and 0.0022 at, respectively, 15°C, 25°C and 30°C for a shelf life of six months. The level of uncertainty obtained meets the requirements of the “climate” quality objective requested by the Global Ocean Acidification Observing Network (GOA-ON) for the monitoring of ocean acidification.

Keywords: Seawater, Ocean Acidification, pH on a total scale, Reference Material, Uncertainty budget, Stability study

1. Introduction

Since the beginning of the industrial revolution, the amount of carbon dioxide (CO_2) in the atmosphere has increased by 50% due to anthropogenic emission (NOAA, 2022). The ocean is a major carbon sink for the atmosphere by absorbing about 25% of these anthropogenic

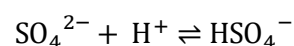
CO₂ emissions (Friedlingstein et al., 2022). However, the progressive absorption and dissolution of CO₂ in the ocean leads to the decline of seawater pH and changes in the carbonate chemistry. This phenomenon, generally described as Ocean Acidification, has direct impacts on marine biodiversity, mainly on the development of species with carbonate exoskeletons such as seashells, corals or pteropods (Berkowitz, 2014). Ocean acidification is a climatic phenomenon, that needs to be assessed over prolonged time scales (i.e. decades to centuries). The pH of the surface ocean has decreased by at least 0.1 compared to pre-industrial levels and the Intergovernmental Panel on Climate Change (IPCC) has estimated a potential decrease of around 0.4 units by 2100 (IPCC, 2021).

For this reason, ocean acidification is identified as one of the nine planetary boundaries (a concept that offers support to international governance systems in an objective of global sustainability (Rockström et al., 2009)). A need to gather pH measurements data as a global indicator is also required by different organisms as the World Meteorological Organization (WMO) and the Intergovernmental Oceanographic Commission (IOC) of the UNESCO (IOC-UNESCO, 2019; WMO, 2015). Making reliable observations is thus of major importance in decision making for mitigation of, and adaptation to, ocean acidification.

Among the four independently measurable parameters used to describe the oceanic carbonate system (pH, Total Alkalinity, partial pressure of carbon dioxide and Dissolved Inorganic Carbon), pH is, if properly measured, a powerful parameter to follow changes in ocean acidity. It also allows determining the carbonate system when associated to another independently measured parameter.

The pH scale, first described by Soren Peter Lauritz Sørensen in 1909, is defined by the International Union of Pure and Applied Chemistry (IUPAC) as a function of free hydrogen ion activity (Buck et al., 2002). However, the convention used to define chemical activity do not accurately estimate activity coefficients for solutions with ionic strengths above 0.1 mol/kg (Stoica et al., 2014). Natural seawater is a multielectrolyte solution with a high ionic strength, around 0.7 mol/kg for a salinity of 35. Therefore, marine chemists introduced an alternative concept to accurately measure and quantify changes in seawater acidity, a pH expressed on a total scale, noted pH_T (Dickson, 1993). This scale has been widely adopted and is now used for oceanic measurements.

The total pH scale is based on a reference state in a constant ionic medium. It considers amount contents (expressed in moles per kilogram of solution, noted mol/kg sol), of free hydrogen ions, [H⁺]_F, and hydrogen ions combined with sulphate ions, [HSO₄⁻], involved in the following equilibria, and defined according to equations 1.a and 1.b.



$$\text{pH}_T = -\log [\text{H}^+]_T \quad (1.a)$$

$$[\text{H}^+]_T = [\text{H}^+]_F (1 + S_T/K_S) \approx [\text{H}^+]_F + [\text{HSO}_4^-] \quad (1.b)$$

where S_T is the total sulphate amount content ($[\text{HSO}_4^-] + [\text{SO}_4^{2-}]$) and K_S is the dissociation constant of HSO_4^- (Dickson et al., 2007).

To be able to highlight significant trends in ocean acidification (i.e. change of 1% in the dissolved carbonate ion concentration), the Global Ocean Acidification Observing Network (GOA-ON) has fixed a data quality objective for the monitoring of ocean acidification, called the “climate” quality objective, for which pH_T measurement uncertainties must be less than 0.003 (GOA-ON, 2019). The aforementioned “climate” data quality objective of the GOA-ON differs from the “weather” quality objective, for which an uncertainty of only 0.02 in pH_T is required. Requirements for high data quality become even more stringent as data shared in international databases are collected over diverse spatial and temporal scales, from many sources and with a large variety of instruments (Pearlman et al., 2019).

The number and frequency of seawater pH measurements have increased significantly through the development of a highly-reproducible spectrophotometric measurement method (Byrne and Breland, 1989) together with the introduction of the concept of pH on a total scale. Although spectrophotometric analyses are readily implemented and straightforward, there are several aspects that can affect the quality of pH_T measurements (e.g. the purity of the indicator dye, the perturbation of adding dye on the pH_T of the sample, the temperature control, the performance characteristics of the instrument, etc.). Assessing the reliability of pH_T measurements requires the availability of appropriate reference materials. Reference materials with assigned value and quantified uncertainty play an important role in the implementation and maintenance of a robust quality assurance system and represent key elements in the traceability of measurement results. As such, they can be used for the spectrophotometric pH_T measurement method validation, the calibration of the indicator dye or the estimation of routine measurement uncertainty by following a top-down approach.

The metrological traceability chain of pH_T spectrophotometric measurements requires the reference material. In order to obtain the lowest uncertainty on routine pH_T measurements, it is mandatory to have previously obtained the lowest uncertainty on the reference material pH_T . A particular attention is thus being paid to the uncertainty estimates, which should consider the contribution of the homogeneity and stability of the reference material. The ISO Guide 35, “Reference materials – Guidance for characterization and assessment of homogeneity and stability” (“ISO Guide 35”, 2017) provides technical recommendations for the assessment of these components.

The commonly accepted reference material for seawater pH_T is an equimolar buffer of 2-Amino-2-(hydroxymethyl)-1,3-propanediol (TRIS) and TRIS hydrochloride (TRIS.HCl) in

artificial seawater of salinity 35 (Wolfe et al., 2021). The studied buffer is thus a solution at $b(\text{TRIS}) = b(\text{TRIS.HCl}) = 0.04 \text{ mol/kg H}_2\text{O}$ (where b expresses molality in moles per kilogram of solvent, i.e. water in this study, noted mol/kg H_2O); which equals a pH_T of 8.1 at 25°C (DelValls and Dickson, 1998; Müller et al., 2018; Pratt, 2014). This buffer is chosen as it has a pH_T similar to the one of surface seawater. This buffer can be characterized in terms of pH_T by potentiometric measurements made in an electrochemical cell without junction called a Harned cell. The Harned cell is the highest metrological standard for pH measurements, enabling to characterize standard buffer solution with a small standard uncertainty, typically around 0.002 (Buck et al., 2002).

The aim of this study is to describe the feasibility of a reference material for oceanic pH_T measurements produced by the Laboratoire National de Métrologie et d'Essais (LNE). This article will focus on the production of the TRIS buffer reference material, its characterisation using the Harned cell as well as homogeneity and long-term stability assessments. The uncertainty budget associated to the certified pH_T value will also be detailed. All these steps were conducted according to the ISO standard 17034, "General requirements for the competence of reference material producers" and ISO Guide 35 ("ISO 17034", 2016; "ISO Guide 35", 2017).

Critical parameters as the TRIS buffer water mass fraction definition and pH_T measurements traceability are identified and discussed.

2. Materials and methods

2.1 Instrumentation

Harned Cell set-up

The characterization of the reference material is carried out with a junction-free cell, known as a Harned cell (Figure 1), a silver-silver chloride (Ag/AgCl) reference electrode (Figure 1.a) and a standard hydrogen electrode (Pt, H_2) (Figure 1.b). A Harned cell is used as it allows the humidity saturation of hydrogen before it enters the measurement compartment (Figure 1.c) and eliminates errors due to residual junction potentials thanks to a junction-free measurement compartment (Figure 1.d).

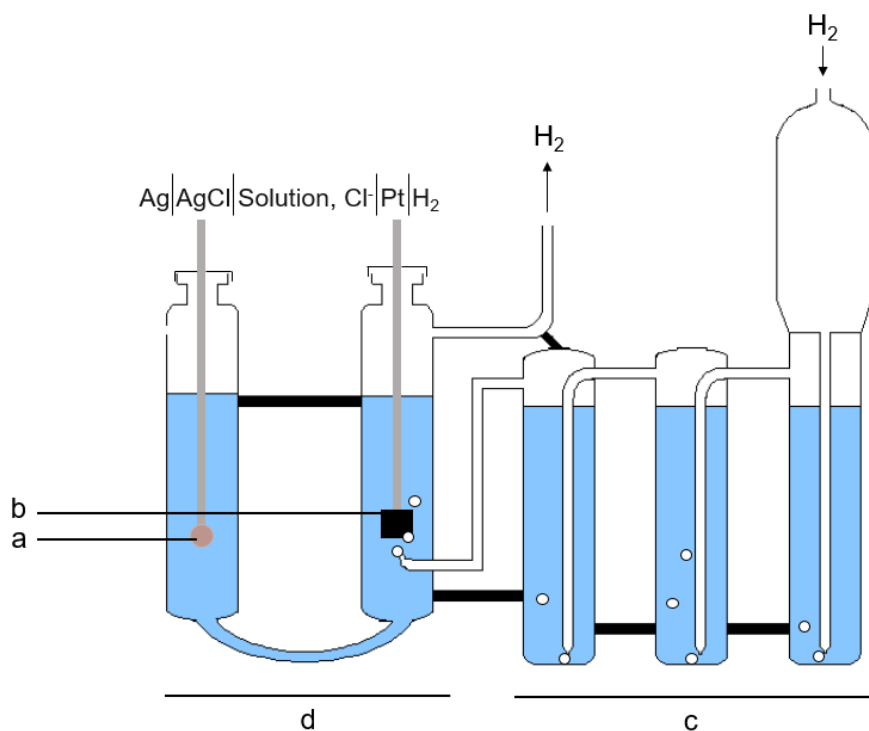


Figure 1. Harned cell set-up description. (a) silver-silver chloride electrode; (b) standard hydrogen electrode; (c) hydrogen humidity saturation system; (d) junction-free measurement compartment. Color is not mandatory.

The LNE has a quality system that follows the principles of the ISO standard 17025, “General requirements for the competence of testing and calibration laboratories” (“ISO 17025”, 2017). All measurements carried out for the present study comply with the requirements of the aforementioned ISO standard.

Each Harned cell measurement was carried out at 15°C, 25°C and 30 °C. The cells were placed in a TAMSON thermostatic bath regulated within $\pm 0.01^\circ\text{C}$ and connected to a recirculating chiller Agilent G3292A, allowing measurements to be carried out at a known, stable and reproducible temperature. Five calibrated 4-wire resistance Pt100 probes were placed at different locations in the bath to check for temperature stability and homogeneity. Potential difference between the two electrodes in the Harned cell was measured with an Agilent 34972A, a high input-impedance data acquisition system. It was recorded using the Agilent Benchlink Data Logger software along with the bath temperature measurement. Standard measurement uncertainties were respectively of $10\ \mu\text{V}$ and 0.01°C . The Agilent 34972A is equipped with two multiplexers allowing for six simultaneously potentiometric measurements. Atmospheric pressure was measured with a Druck DPI 740 sensor having a resolution of 1 Pa and an uncertainty of 100 Pa. Pressure was recorded with Acquisit, an LNE in-house software. All data (i.e. potential difference, temperature and pressure) were collected every 2 minutes. The pH_T calculations were carried out using data collected during

one hour of acquisition (i.e. 30 points), after achieving a stable potential signal (i.e. standard deviation below 20 μV).

The Pt,H₂ and Ag/AgCl electrodes were prepared at LNE according to the methods described by Bates (Bates, 1973) for primary pH measurements.

Spectrophotometric pH_T measurement set-up

The pH_T spectrophotometric measurements were carried out with an Agilent Cary 60 UV-vis spectrophotometer. The internal support of the spectrophotometer can accommodate a cylindrical cell of 10 cm path-length. Starra quartz cells, with a 10 cm optical path-length, two opening chimneys and PTFE plugs, were used.

The cells were pre-thermostated in a LAUDA ECO GOLD RE 2025 GN recirculating chiller. A Lauda Loop L100 circulation thermostat was also connected to the internal support by two heat-insulated tubes allowing the stabilisation and control of the temperature inside the cell. The two PTFE plugs of the spectrophotometry cell contain a calibrated temperature probe allowing for precise measurements of the temperature inside the cell during the pH measurement. Both the recirculating chiller and the circulation thermostat were set in order to obtain a temperature of $25.0 \pm 0.1^\circ\text{C}$ inside the cell.

The indicator dye used is a concentrated solution of purified meta cresol purple (2mmol/L) with a pH close to that of marine waters. It was prepared following the recommendations of Dickson et al. (2007). A micropipette with a volume of 50 μL -1000 μL was used to inject the indicator dye solution into the sample.

The Cary WinUV software was used for the acquisition of absorbances. The acquisition was carried out in the "Kinetics" mode (Version 5.1.3.1042), which allows the absorbances to be acquired at individual wavelengths. Absorbances were measured at the two wavelengths corresponding to the dye acid and basic forms, 434nm and 578nm, as well as at a non-absorbing wavelength, 730nm, to check for an eventual baseline shift. Three absorbance measurement steps were performed: the background acquisition in the sample itself, after adding 60 μL of dye and after realising a second addition of 60 μL of dye. The second dye addition allows the correction of the pH_T that could have been slightly modified from the dye addition in the sample.

The final pH_T value was calculated following the method described in Liu et al. (2011).

The linearity of the device was checked with LNE homemade optical density standards.

2.2 Reference Material preparation

Artificial seawater matrix

In order to simulate natural seawater, an artificial seawater matrix was chosen as defined by the International Association for the Physical Sciences of the Oceans (IAPSO) for a practical

salinity of 35 (Millero et al., 2008). For simplicity, the anions CO_3^{2-} , $\text{B}(\text{OH})_4^-$, OH^- , F^- and HCO_3^- were replaced by chloride ions and Sr^{2+} were replaced by calcium ions (Pratt, 2014). Moreover, as Br^- ions can damage silver-silver chloride electrodes (Sandifer, 1981), they were eliminated from the composition to allow the use of the potentiometric system for pH_T measurements.

Preparation procedure and Materials

Reference materials should be prepared in such a way that they are homogeneous and stable (i.e. have constant characteristics over a sufficiently long period). The production of the reference material included the gravimetric preparation from reagents easily weighable and of known composition together with the use of a protocol that enables to reproduce the composition between different batches.

The products used for the preparation of the TRIS buffer in the artificial seawater matrix are TRIS (*NIST Standard Reference Material 723e*), NaCl (*VWR chemicals, 99.945%*), KCl (*VWR chemicals, 99.928%*), Na_2SO_4 (*VWR chemicals, 99.994%*), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (*VWR chemicals*), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (*Merck*), HCl (*Slovak Institute of Metrology (SMU) Certified Reference Material, amount content of 1 mol/kg sol*) and Milli-Q water.

As calcium chloride and magnesium chloride are highly hygroscopic salts, they are dissolved independently in two separate solutions in order to facilitate weighing.

Great care was taken on the quality of the products used. To this respect, all the purchased salts and solutions used for the preparation of the artificial seawater were characterized in term of mass fraction based on halide content except for Na_2SO_4 for which mass fraction of anion content has been determined. The characterization was carried out by the Slovak Institute of Metrology (SMU) using coulometric assay. The Milli-Q water used to prepare the buffer was boiled then left overnight under Argon bubbling to get rid of oxygen and prevent bacterial development.

The TRIS buffer was prepared gravimetrically from stock solutions of the different components: NaCl, minor salts (KCl, CaCl_2 , MgCl_2), Na_2SO_4 , HCl and TRIS (Tables S1 and S2, Supplementary Material).

In order to maintain the same nominal ionic strength as the one of natural seawater, the 0.04 mol/kg H_2O of HCl added were subtracted from the targeted NaCl molality during TRIS buffer solution preparation (Dickson et al., 2007).

After being prepared, the 9 litres of buffer solution were bottled in 125 mL borosilicate-33 glass bottles with screw caps and sealed using Parafilm to minimize evaporation and gas exchanges with the atmosphere, especially for carbon dioxide. The bottles are filled to the brim with a peristaltic pump, which delivers the same volume in all bottles; the final volume contained in each bottle is of approximately 160 mL. Bottles were stored in the dark in a temperature and humidity controlled room.

2.3 Characterization method

The determination of the pH_T by the potentiometric method is well described in the literature (DelValls and Dickson, 1998; Millero et al., 1993; Müller et al., 2018; Pratt, 2014) and is obtained by equation 2.a.

$$pH_T = \frac{E-E^{o*}}{k} + \log(b_{Cl}) - \log(\omega_{H_2O}) \quad (2.a)$$

Where E is the potential difference corrected to a pressure of H₂ equal to 1 atm (V) (equation 2.b), E^{o*} the standard potential of the Ag/AgCl electrode in saline media (V), k the Nernstian term (equation 2.c), b_{Cl} the molality of chloride ions in the TRIS buffer solution (mol/kg H₂O) and ω_{H_2O} the water mass fraction (g/kg sol).

The term ω_{H_2O} in equation 2.a, representing the mass fraction of water in the buffer solution, enables to express the calculated pH_T value in amount content (i.e. mol/kg sol) and to align with spectrophotometric pH_T values.

$$E = E_{mes} - \frac{k}{2} \log\left(\frac{P-p_{vap}+0.4 \times \rho \times g \times h}{p^o}\right) \quad (2.b)$$

Where E_{mes} is the measured potential difference (V), P the measured ambient pressure (Pa), p_{vap} the saturation vapour pressure (Pa), ρ the density of the solution (kg/m³), g the standard gravity (m/s²), h the liquid height (m) and p^o the standard pressure (101 325Pa).

$$k = \frac{RT \ln(10)}{F} \quad (2.c)$$

Where F is the Faraday constant (C/mol), R the Gas constant (J/mol.K), and T the measured temperature (K).

Figure 2 shows the two Harned cell measurement steps allowing the calculation of the pH_T value. The determination of the terms in boxes in the equation in Figure 2 requires these two Harned cell measurements. Other terms are known with gravimetric information or temperature measurement. Each of the steps described in Figure 2 was performed at 15°C, 25°C and 30°C.

$$pH_T = \frac{E' - E^{\circ*}}{k} + \log(b_{Cl}) - \log(\omega_{H_2O})$$

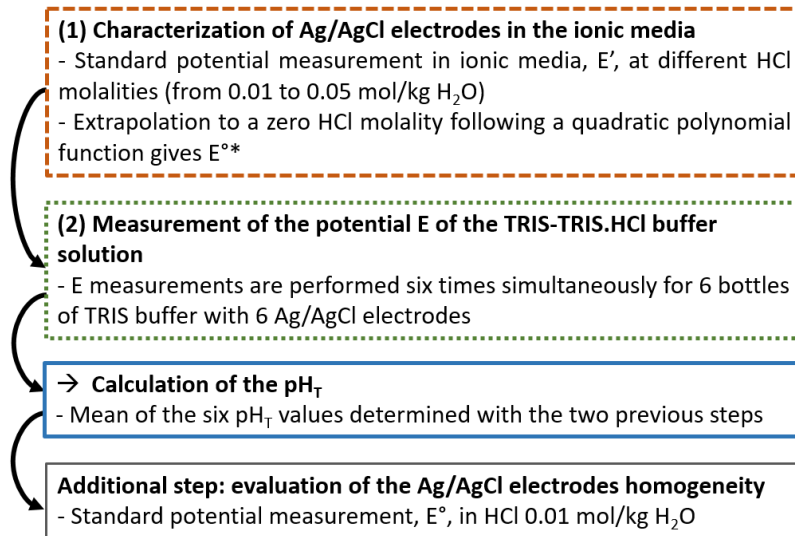


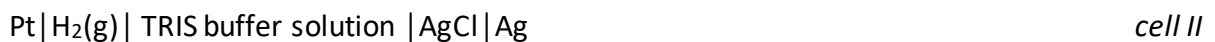
Figure 2. Harned cell measurements steps allowing the determination of the pH_T . Color should be used.

(1) The first step consists in the determination of the $E^{\circ*}$ term in equation 2.a. Potential measurements are performed with a batch of Ag/AgCl electrodes in solutions of HCl molalities varying between 0.01 and 0.05 mol/kg H_2O in the artificial seawater matrix (ASW), while conserving a constant ionic strength. The electrochemical cell can be written as:



These measurements allow for the determination of the standard potential of the Ag/AgCl electrodes in saline media for the studied chloride molalities, noted E' . The extrapolation of these standard potential values E' to an HCl molality of zero mol/kg H_2O following a quadratic polynomial function gives the $E^{\circ*}$ value (Figure 3).

(2) The second step is the determination of E in equation 2.a, measured in the TRIS buffer solution itself. The electrochemical cell can be written as:



This measurement was carried out six times simultaneously for six different bottles of TRIS buffer using the same batch of silver-silver chloride electrodes.

Based on measurements carried out with *cell I* and *cell II*, pH_T values are calculated using the equation 2.a. Terms b_{Cl} and ω_{H_2O} in equation 2.a are determined from the TRIS buffer gravimetric preparation while k is determined from constants and temperature measurements. The final pH_T value is the mean of the pH_T calculated from six distinct potentials E measured with *cell II*.

An additional step conducted with the Harned cell setup is the evaluation of the homogeneity within the batch of Ag/AgCl electrodes used (Figure 2). This step corresponds to the determination of their standard potential, noted E° , in a solution of HCl at a molality of 0.01 mol/kg H_2O . It was performed at the three studied temperatures. This step is important, as it will be included in the final uncertainty budget. Indeed, to be able to attribute the $E^{*\circ}$ value obtained by extrapolation with *cell I* to each of the six Ag/AgCl electrodes used, the discrepancy between these electrodes must be taken into account. To do so, the standard deviation between the E° values of the batch of six Ag/AgCl electrodes is included in the uncertainty budget of the $E^{*\circ}$ value.

In this study, ω_{H_2O} is calculated from the actual buffer composition ignoring the TRIS and considering the molality of HCl as part of the NaCl amount in order to represent a pure artificial seawater (equation 3). However, the definition given to this term has not yet reached a consensus and is thus discussed in more details in section 4.2.

$$\omega_{H_2O} = \frac{1000 - ((v_{HCl} + v_{NaCl}) \times M_{NaCl} + \sum (v_i \times M_i))}{1000} \quad (3)$$

Where ω_{H_2O} is the water mass fraction (g/kg sol) of pure artificial seawater, v the amount content of stock solutions that make up the pure artificial seawater composition (i.e. TRIS is removed and HCl is added to the NaCl amount) (mol/kg sol), M the molar mass (g/mol), i corresponds to the remaining salts (i.e. KCl, $MgCl_2$, $CaCl_2$, Na_2SO_4).

2.4 Homogeneity assessment

Homogeneity is one of the defining characteristics of reference materials and should include estimates for both within and between bottle homogeneity. Because of the amount of solution needed for one Harned cell measurement (i.e. 150mL, which is a bit less than the volume included in one bottle filled up to the brim), only the latter component was evaluated. For this purpose, six bottles were randomly selected across the entire batch, representing around 10% of the total number of produced bottles.

Harned cell measurements were carried out under repeatability conditions (i.e. same time, operator, environment conditions and protocol) and immediately after the preparation of the batch. Data acquired were used both for the homogeneity assessment and for the calculation of the pH_T value of the reference material described in section 2.3.

2.5 Stability assessment

According to the definition, reference materials should be sufficiently stable for their intended use, so that the end user can rely on the assigned value at any point within the period of validity. Stability study should evaluate long-term storage conditions, and consider transport conditions (“ISO 17034”, 2016; “ISO Guide 35”, 2017).

For the feasibility study presented in this paper, only long-term storage was evaluated. The reference material was monitored by Harned cell measurements four and eight months after the preparation and characterization date.

The stability is assessed based on the acidity function value measured at 25°C, described by equation 4. This limits the complexity of the experimental protocol (i.e. E^* is not redetermined, although E° is), while offering the accuracy needed to highlight an eventual instability.

$$a.f. = \frac{E_{mes} - E^\circ}{k} + \log(b_{Cl}) - \frac{1}{2} \log\left(\frac{P - p_{vap} + 0.4 \times \rho \times g \times h}{p^\circ}\right) \quad (4)$$

Where a.f. is the acidity function.

To evaluate the stability one must highlight with a statistical Student test (or t test) whether there is a significant trend in the evolution of the material or not (“ISO Guide 35”, 2017).

This test is based on the determination of the slope, noted b_1 , of the regression line of the acidity function values as a function of time (Figure S1, Supplementary Material). It computes t_0 , defined as being the ratio of the slope on its standard deviation, noted sb_1 , and compares it to the threshold value t_α in the Student's table with $n-2$ degrees of freedom (for this study, $n=11$) at a 95% confidence level (“ISO Guide 35”, 2017; Linsinger et al., 2001).

The spectrophotometric pH_T measurement method was used as a complementary tool to assess the stability over time of the reference material. Measurements were realized with instrumentation information given in section 2.1 and with the method detailed in the ISO standard 18191, which is equivalent to the Standard Operating Procedure 6b of Dickson and co-workers (Dickson et al., 2007; “ISO 18191:2018”, 2018). Spectrophotometric measurements were performed up to ten months after the reference material preparation (Figure S2, Supplementary Material).

2.6 Global uncertainty budget

The global standard uncertainty budget of the reference material pH_T takes into account the TRIS buffer preparation and characterization carried out with the Harned cell system (u_{charac}), the homogeneity (u_{hom}) and the stability over time (u_{stab}) of the material and is thus assessed from equation 5 (“ISO Guide 35”, 2017).

$$u_{MR} = \sqrt{u_{charac}^2 + u_{hom}^2 + u_{stability}^2} \quad (5)$$

Uncertainty budgets are generally expressed by expanded uncertainties (*JCGM 100:2008*, 2008). This requires the introduction of a coverage factor, which provides an interval corresponding to a particular level of confidence. The level of confidence often chosen is of 95 percent, corresponding to a coverage factor of 2. The expanded uncertainty is obtained by multiplying the combined standard uncertainty by the chosen coverage factor.

Preparation and characterization: u_{charac}

The term u_{charac} corresponds to the standard uncertainty obtained during preparation and characterization of the reference material. The pH_T is determined with the Harned cell system from equation 2.a. u_{charac} is assessed based on this equation using the law of uncertainty propagation (Shi et al., 2021) following the Guide to the expression of Uncertainty in Measurements recommendations (JCGM 100:2008, 2008).

In order to assess the standard uncertainties of each term in equation 2.a, allowing a determination of u_{charac} , several steps are followed.

The uncertainties relative to the water mass fraction, ω_{H_2O} and the chloride ion molality in the buffer solution, b_{Cl} , are obtained from the reference material gravimetric preparation. It includes weighing scales calibration and uncertainties of the molar masses, which are taken from IUPAC (Meija et al., 2016).

The temperature, pressure and density uncertainties are known from the device calibration certificates. Other terms in the Nernstian term, k , and in the equation allowing the correction of the measured potential to a hydrogen pressure of 1 atm (equations 2.b and 2.c), are taken from the literature or can be neglected (Pratt, 2014).

The uncertainty relative to the measured potential, E_{mes} , includes the uncertainty given by the calibration and the maximum standard deviation obtained between the potentials acquired during one hour for each of the six simultaneous measurements.

The same methodology (i.e. following the law of uncertainty propagation) is applied to establish the uncertainty of the standard potential E' of Ag/AgCl electrodes in saline media for a chosen chloride molality. The uncertainty evaluation of E' is calculated only for a chloride molality of 0.01 mol/kg H_2O as it is the lowest molality and thus the one with the largest uncertainty on the gravimetric preparation.

Finally, the $E^{\circ*}$ term uncertainty is given by equation 6.

$$u_{E^{\circ*}} = \sqrt{(u_{extrapolation})^2 + (u_{SE^{\circ}})^2 + (u_{E'})^2} \quad (6)$$

Where $u_{extrapolation}$ is the uncertainty relative to the extrapolation to a zero chloride molality of E' values, $u_{SE^{\circ}}$ the standard deviation between standard potentials (E°) of the six Ag/AgCl electrodes used and $u_{E'}$ the uncertainty relative to E' term described above.

The standard uncertainties of each term of equation 2.a were carefully assessed and described which allow the final calculation of u_{charac} .

The detailed assessment of the preparation and characterization uncertainty budget is given in the Supplementary Material. All standard uncertainties values calculated for the assessment of u_{charac} are given in Table S3 of the Supplementary Material.

Homogeneity: u_{hom}

The procedure for the assessment of the reference material homogeneity is described in section 2.4. The standard uncertainty due to in-between bottle heterogeneity was calculated with equation 7.

$$u_{hom} = \frac{s^2}{N} \quad (7)$$

Where s^2 is the standard deviation between the six pH_T values obtained from characterization and N the number of bottles analysed.

Stability: u_{stab}

If no significant trend is established by the t test described in section 2.5 then the stability uncertainty contributing to the overall uncertainty budget is calculated with equation 8, as described in the ISO Guide 35 ("ISO Guide 35", 2017).

$$u_{stab} = s_{b1} \cdot t \quad (8)$$

Where s_{b1} corresponds to the standard deviation of the slope and t to the time.

A material for which a significant trend is established by the t test can be suitable for certification if the degradation rate is included in the uncertainty budget. In that case, the stability uncertainty must take into account both the estimates of the degradation and the slope uncertainty (equation 9). The estimate of the degradation corresponds to the real degradation during half the chosen shelf life (Linsinger et al., 2001).

As the trend is considered linear, the degradation estimate is converted to an uncertainty using a rectangular law ("ISO Guide 35", 2017).

$$u_{stab} = \sqrt{\left(\frac{b1 \cdot t}{\sqrt{3}}\right)^2 + (s_{b1} \cdot t)^2} \quad (9)$$

Where t corresponds to the material shelf life.

3. Results

Table 1 summarizes the pH_T values for the reference material obtained with the Harned cell measurement method at 15°C, 25°C and 30°C. The pH_T values in Table 1 are the mean of the six values measured with *cell II* at each temperature. Table 1 also gives the Ag/AgCl electrodes standard potential in saline media values (E^{o*}) obtained from the *cell I* measurements at each of the three temperatures. Figure 3 illustrate the method applied to obtain this value at 25°C (described in section 2.3).

Temperature (°C)	Characterization values	
	$E^{\circ*}$ (V)	pH_T
15	0.24947	8.4136
25	0.24611	8.0915
30	0.24441	7.9375

Table 1. Results of the reference material pH_T values characterization at 15°C, 25°C and 30°C obtained with the Harned cell measurement method.

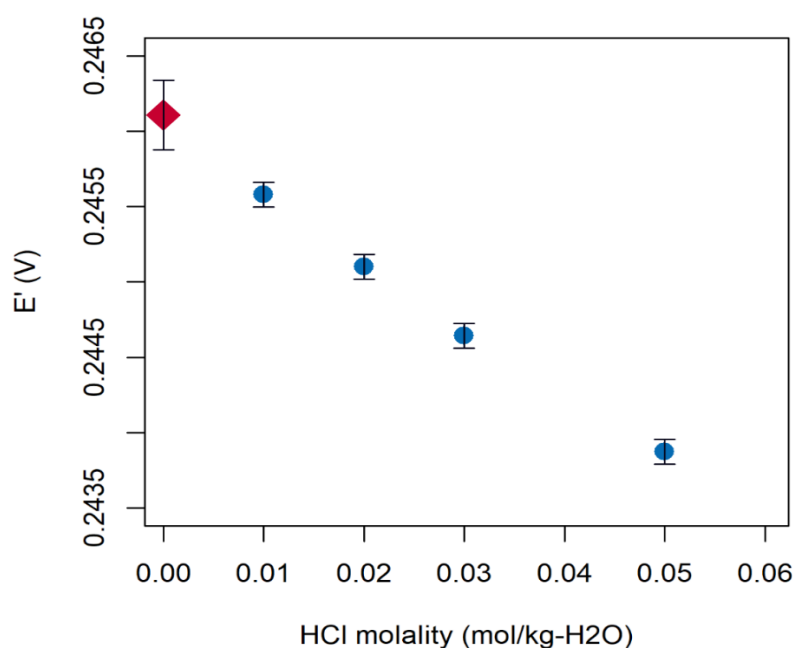


Figure 3. Determination of $E^{\circ*}$ value at 25°C. Where ● represents E' (V) of the four solutions with an HCl molality of respectively 0.01, 0.02, 0.03 and 0.05 mol/kg H₂O and ◆ represents the $E^{\circ*}$ value obtained from E' values as function of HCl molality extrapolated to an HCl molality equal to zero, with their respective expanded uncertainties (i.e. with a coverage factor of 2). Color is not mandatory.

Table 2 gives the results of the statistical t test described in section 2.5 allowing the determination of the material stability. Given the Harned cell measurement method (i.e. acidity function measurements as described in section 2.5), since t_0 value is higher than t_α , it can be concluded that the slope of the linear regression is statistically significant. These results highlight a clear trend showing an instability of the material. The same test applied to the data acquired by the pH_T spectrophotometric measurement method give the same

conclusion. The reference material can still be certified by taking into account the instability of the material in the uncertainty budget, using equation 9.

Statistical t test parameters	Instrumentation	
	Harned cell ^a	Spectrophotometer ^b
Slope (b1)	-4.7E-04	-9.1E-04
Slope standard deviation (Sb1)	1.7E-04	2.4E-04
t ₀ (b1 / Sb1)	2.80	3.78
t _α (Student n-2)	2.26	2.36

Table 2. Results of the statistical t test for stability assessment of the reference material using the Harned cell and Spectrophotometric measurement methods.

^a The stability assessment performed with the Harned cell is based on acidity function measurements.

^b The stability assessment performed with the spectrophotometer is based on pH_T measurements.

The global standard uncertainty budget of the pH_T values given in Table 3 is calculated from equation 5. The methodology employed to assess each component of equation 5 is described in section 2.6. The stability uncertainty is given for a chosen shelf life of six months, which allows having a global standard uncertainty on the reference material pH_T value of maximum 0.0025, corresponding to an expanded uncertainty budget of 0.005 (i.e. with a coverage factor of 2).

Temperature (°C)	Global standard uncertainty budget			
	u charac	u hom	u stab	u global
15	2.09E-03	9.94E-05	1.44E-03	2.54E-03
25	1.95E-03	1.28E-04	1.31E-03	2.35E-03
30	1.76E-03	1.45E-04	1.28E-03	2.18E-03

Table 3. Detailed standard uncertainty budget of the reference material pH_T values obtained at 15°C, 25°C and 30°C.

Figure 4.a shows the part of each uncertainty component into the overall budget at 25°C. The same pattern is obtained for the two other studied temperatures. The main source of uncertainty is the TRIS buffer preparation and characterization performed with the Harned cell (u_{charac}), which mostly arises from the determination of Ag/AgCl electrodes standard potential in saline media ($E^{\circ*}$) (Figure 4.b).

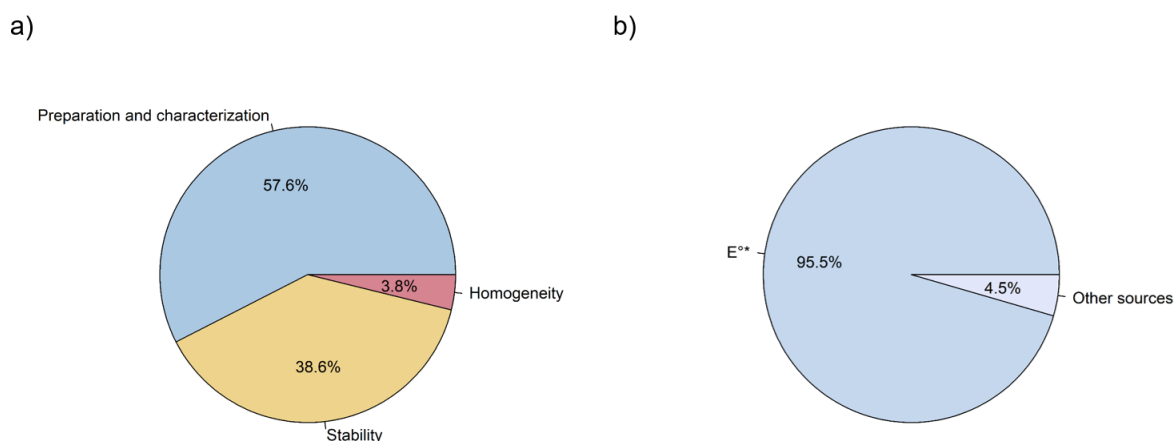


Figure 4. Uncertainty sources contribution rate to the uncertainty budget of the reference material pH_T at 25°C. a) Preparation and characterization, homogeneity and stability sources contribution to the global uncertainty budget of the reference material pH_T at 25°C. b) Uncertainty sources contribution to the preparation and characterization uncertainty term (u_{charac}) at 25°C. Color should be used.

4. Discussion

4.1. Comparison with existing reference materials

The pH_T values obtained with the Harned cell (Table 1) are consistent with literature values (DelValls and Dickson, 1998; Müller et al., 2018; Papadimitriou et al., 2016; Pratt, 2014), within the expanded uncertainty. Since September 2021, two other batches of TRIS buffer reference material were produced at LNE and characterized with Harned cells. The mean pH_T value is of 8.0941 with a standard deviation between the three batches of 0.0032. This demonstrates the reproducibility of the production of TRIS buffers.

Characterization of the reference material was performed at 15°C, 25°C and 30°C. The temperature coefficient of the produced reference material pH_T is of $-0.032/^\circ\text{C}$. It is in agreement with the temperature coefficient obtained from the literature for similar TRIS buffers (DelValls and Dickson, 1998; Müller et al., 2018) and for the same range of temperatures (i.e. between 15°C and 30°C). The temperature coefficient of a natural seawater in the same range of temperatures is two times lower, around $-0.015/^\circ\text{C}$ (Hunter, 1998). The temperature control is thus even more important when measuring the pH_T of a buffer solution compared to that of natural seawater samples.

4.2. Water mass fraction definition

The water mass fraction of the TRIS buffer, ω_{H_2O} , allowing to convert pH_T expressed in molality (mol/kg H₂O) to amount content (mol/kg sol), was first described by Dickson (1993) as being function of the salinity, using equation 10.

$$\omega_{H_2O} = 1 - 0.00106 \times S \quad (10)$$

Where S corresponds to the nominal salinity of the buffer solution.

The S coefficient in the above expression depends on the composition of the solution being considered, as it expresses the ratio between the total mass, in kg, of salts dissolved in 1 kg of artificial seawater and its nominal salinity (i.e. 35). This equation refers to the composition of a seawater solution. It doesn't refer to the actual water mass fraction of the TRIS buffer, it doesn't take into account the TRIS and TRIS.HCl contribution, but only the five dissolved salts (i.e. NaCl, KCl, MgCl₂, CaCl₂ and Na₂SO₄).

Clegg et al. (2022) recently published a similar conversion equation also based on nominal salinity (equation 11) which has been calculated using the same approach (equation A.2 of Clegg et al., 2022). It is based on the artificial seawater composition given by Dickson (1990).

$$\omega_{H_2O} = 1 - 0.00100198 \times S \quad (11)$$

Pratt (2014) and Müller et al. (2018) proposed that considering the actual water mass fraction of the buffer would be more appropriate (i.e. taking into account the TRIS and TRIS.HCl contribution). Nevertheless, to be in agreement with the final purpose of the reference material, that is to say a Quality Assurance and Quality Control (QA/QC) approach for pH_T measurements on natural seawater sample, we believe that ω_{H_2O} must refer to a pure seawater matrix (i.e. without TRIS and TRIS.HCl).

One of the objectives of the present study was to provide a thorough uncertainty estimate of the ω_{H_2O} term used for the pH_T calculation of the produced reference material. To that end, we calculated our own equation for water mass fraction calculation following Dickson's and Clegg's approach (equation 12, equivalent to equation 3). It is based on our buffer preparation (Table S2, Supplementary Material), while ignoring the TRIS component and considering the molality of HCl as being part of the NaCl amount in order to represent a pure artificial seawater composition (i.e. composition of Millero et al. (2008), simplified as described in section 2.2). This method enables us to derive an accurate uncertainty estimate on the water mass fraction of the buffer based on gravimetric information.

$$\omega_{H_2O} = 1 - 0.00099334 \times S \quad (12)$$

The applied equation to estimate ω_{H_2O} , (equation 12) compared to the equations of Dickson (1993) and Clegg et al. (2022) (i.e. equations 10 and 11, respectively) gives discrepancies of respectively 0.0023 and 0.0003 in terms of water mass fraction, which results in discrepancies in term of pH_T of respectively 0.0011 and 0.0001. The maximum discrepancy is

within the uncertainty of the characterization, which demonstrates that the applied equation is in accordance with the one described in the two aforementioned papers (Clegg et al., 2022; Dickson, 1993).

Consequently, it allows us to be consistent with most studies associated to Harned cell measurements in TRIS buffers (DelValls and Dickson, 1998; Müller et al., 2018; Papadimitriou et al., 2016) and to the related studies made on the characterisation of the indicator dye (Liu et al., 2011; Loucaides et al., 2017; Müller and Rehder, 2018). This is also an essential requirement to use the Harned cell measurements for the establishment of the metrological traceability of the spectrophotometric pH_T measurement results.

For comparison, pH_T values calculated with the water mass fraction defined on the actual buffer composition as described in Pratt (2014), (i.e. with TRIS and TRIS.HCl) are 0.0038 higher than the ones given in Table 1, calculated with equation 12. This discrepancy is not negligible, meaning that a consensus must be found to achieve consistency and comparability.

4.3. Uncertainty budget

Figure 4 shows that the main contribution to the global uncertainty budget is the E^{**} term. Among the uncertainty sources that are included in this term (equation 6), the ones coming from the extrapolation to a zero chloride molality ($u_{extrapolation}$) and from the determination of the standard potential at a given molality ($u_{E^{\circ}}$) are small. In contrast, the standard deviation of the Ag/AgCl electrodes standard potential ($u_{SE^{\circ}}$) accounts for about 80% of the E^{**} term uncertainty.

The standard deviation of Ag/AgCl electrodes standard potentials determined with the Harned cell in a solution of HCl at a molality of 0.01mol/kg H_2O are of 100 μV , 103 μV and 93 μV at respectively 15°C, 25°C and 30°C. The E° values obtained are in accordance with literature values (Bates and Bower, 1954).

A new batch of silver-silver chloride electrodes was produced at LNE in November 2021. A first analysis of their standard potential gave a standard deviation of 20 μV between the six closest electrodes. A second standard deviation measurement was made after these electrodes were used three times in an artificial seawater matrix, which gave a standard deviation of 40 μV . Pratt (2014) observed a decrease of the standard potentials after exposure of Ag/AgCl electrodes to a seawater matrix and hypothesized that bromide ion impurities in the salts used for the preparation can affect the electrodes (Pratt, 2014). This could explain the more important standard deviation obtained on the older batch used for this study, especially if the electrodes have not all been exposed to artificial seawater the same amount of time in the past. A selection of Ag/AgCl electrodes with close standard potential (i.e. below 100 μV) ahead of the characterization of the reference material could help improve the global uncertainty.

4.4. Stability over time

Stability assessment of the material is based on acidity function measurements performed up to 8 months with Harned cells (Figure S1, Supplementary Material). These measurements using the Harned cells remain complex and time-consuming, which explains the limited number of points covering the time period. Spectrophotometric measurements are easier and less time-consuming, but the quality of spectrophotometric measurements is difficult to evaluate because of missing metrological traceability and thorough uncertainty budget. Nevertheless, an estimate of the stability as a source of uncertainty to the global budget doesn't require an estimate of the single measurement uncertainty. Therefore, spectrophotometric measurements were performed up to ten months after the buffer preparation as an additional support for the stability study, allowing to have more quantitative information (Figure S2, Supplementary Material). The method is known to be highly reproducible. Some individual uncertainty sources (e.g., instrument, indicator dye, operator), could be cancelled out, offering perspective of achieving small uncertainties on pH_T variations over time.

The significant decrease of pH_T over time observed with the Harned cell measurements is confirmed with the spectrophotometric pH_T measurements (Table 2). Spectrophotometric pH_T measurements made over 10 months gave a slope of -0.00091 pH_T unit per month with a standard deviation of 0.00024 while acidity function measurements made with the Harned cell gave a slope of -0.00047 in acidity function unit per month with a standard deviation of 0.00017 for the stability assessment. The spectrophotometric measurement seems suitable for the evaluation of a material stability over time, but respective slope standard deviations demonstrate that evaluating the stability on the acidity function measured with the Harned cell system is more precise than by spectrophotometry. Moreover, as the $E^{\circ*}$ term accounts for almost all the preparation and characterization uncertainty (Figure 4.b), it makes sense to conduct the stability study on the acidity function instead of pH_T , as it does not include the $E^{\circ*}$ term (equation 4).

Nemzer and Dickson (2005) established a stability of more than a year with potentiometric measurements for TRIS buffer sealed in borosilicate glass bottles with a greased ground-glass stopper, with a slow drift of $+0.0005$ in term of pH_T per year, compared to -0.0056 in this study. The instability of the material produced at LNE may come from bacterial development in the buffer (that could use TRIS as a source of carbon), or, more likely, by the absorption of atmospheric CO_2 coming from an eventual lack of airtightness of the bottles used. In comparison, Wolfe and co-workers observed a similar drift of -0.0058 in term of pH_T per year for TRIS buffer stored in bags, that has been attributed to a CO_2 infiltration and/or microbial respiration (Wolfe et al., 2021).

To prevent bacterial development without using mercury-based additives, the bottles might be autoclaved once filled with the reference material. Mercuric chloride is still widely used to poison natural seawater sample, especially for alkalinity and pH measurements, but after

the United Nations Minamata Convention held in Japan in 2017, it became a worldwide priority to restrict the use of all mercury compounds (UN Environment Programme, 2019). A preliminary test shown non-significant impact of autoclaving a buffer bottle on the pH_T compared to another bottle of the same batch, as the pH_T discrepancy between the two bottles was lower than 0.0022. Nevertheless, before implementation, this method should be the subject of thorough feasibility study to ensure autoclaving doesn't affect the pH_T of the buffer.

To prevent CO_2 absorption, the bottles should be sealed with greased ground-glass stoppers as described in Nemzer and Dickson (2005), by a crimping system or by a glass-sealed vial instead of screw cap bottles with polypropylene stoppers, which could be permeable to air.

4.5. pH_T measurements traceability

Up to date, seawater pH_T measurements lack of established metrological traceability to the International System of Units (SI), which requires that every source of uncertainty is rigorously identified and quantified (Dickson et al., 2016). The pH_T measured with Harned cell is obtained by means of assumptions that up to recently, have not been scrupulously investigated (Clegg et al., 2022; Dickson, 1993). The pH_T measured with equation 2.a differs from the conventional thermodynamic definition of pH_T of around 0.0045 for the studied TRIS buffer. The main contribution to this discrepancy comes from the chloride ion activity coefficient that is neglected here. However, for a pure artificial seawater (i.e. $b(\text{TRIS}) = 0$ mol/kg H_2O), discrepancy is only of about 0.001 (Clegg et al., 2022).

This points out the complexity of the concept of pH on a total scale. Nevertheless, despite the hypothesis that have been made, it is reasonable to state that the described pH_T measurement method applied for the characterization of the reference material is able to provide highly consistent and precise measurement results. If pH_T measurement traceability to the SI is not achievable, a traceability established to a common procedure, which here is the Harned cell measurement method, could be adopted. This, however, requires the demonstration of the equivalence of the measurement procedures of the National Metrology Institutes via the participation to inter-laboratory comparisons at the International Bureau of Weights and Measures (BIPM) level.

5. Conclusion

The present study describes the preparation, characterization and uncertainty assessment method for equimolar TRIS and TRIS.HCl reference material production used for seawater pH_T measurements. Detailed homogeneity and long-term stability studies are presented for the first time for this type of reference material. The Harned cell measurements performed on the TRIS buffer allows us to estimate precisely its pH_T value, but also to thoroughly assess uncertainties coming from preparation and characterization of the buffer performed with

the Harned cell, homogeneity and stability of the material. This complete evaluation of the reference material allows the establishment of a global uncertainty budget according to the ISO 17034 and ISO Guide 35 standards.

The t test, used to assess the stability over time of the material, shows a significant trend, which indicates an instability of the material. Considering this, the global expanded uncertainty (i.e. with a coverage factor of 2) of the reference material is of 0.005 for a material shelf life of six months. This gives a standard uncertainty budget of 0.0025, which meets the data quality objective of GOA-ON for the monitoring of ocean acidification (i.e. GOA-ON “climate” quality objective). The produced reference material is thus suitable for oceanographic needs and can assist marine scientists in their effort to guarantee the quality of their results.

The definition of the water mass fraction to be employed in the calculation of the pH_T of the reference material and, at a higher extent, the traceability of seawater pH_T measurements, are two key points that need to be discussed within the metrological community for oceanic measurements and on which a consensus must be found. This would allow offering a solid support to oceanographers in order to maintain the quality of their seawater pH_T measurements.

Author contributions

Gaëlle Capitaine: Conceptualization, Methodology, Investigation, Validation, Formal Analysis, Writing - Original Draft. **Daniela Stoica:** Conceptualization, Methodology, Validation, Writing - Review & Editing, Funding acquisition. **Thibaut Wagener:** Writing - Review & Editing, Supervision. **Paola Fiscaro:** Writing - Review & Editing, Supervision, Funding acquisition.

Acknowledgments

The authors acknowledge Simon Clegg for the discussions on the water mass fraction definition. The authors also acknowledge Andrew Dickson and an anonymous reviewer for their valuable inputs to the paper. The authors also acknowledge the editor and the associate editor, Alfonso Mucci, for the handling of the manuscript and the valuable inputs.

Funding

This work was supported by the Joint Research Project EMPIR 20NRM06 SApHTIES, which received funding from the EMPIR programme co-financed by the Participating States and from the European Union’s Horizon 2020 research and innovation programme. Gaëlle Capitaine was supported by a CIFRE scholarship provided by ANRT (Association Nationale de la Recherche et de la Technologie).

Glossary of Symbols

E_{mes}	Measured potential between Standard Hydrogen electrode and silver-silver-chloride electrode (V)
E	E_{mes} corrected to a pressure of H ₂ equal to 1 atm (V)
E°	Standard potential of silver-silver chloride electrode in HCl 0.01 mol/kg H ₂ O (V)
$E^{*\circ}$	Standard potential of silver-silver chloride electrode in saline media (V)
E'	Potential of silver-silver chloride electrode in saline media at a given chloride molality (>0 mol/kg H ₂ O) (V)
k	Nernstian term: $\frac{RT \ln(10)}{nF}$ (equation 2.c)
F	Faraday constant (C/mol)
R	Gas constant (J/molK)
T	Temperature (K)
p_{H_2}	Hydrogen pressure (Pa) : $\frac{P - p_{vap} + 0.4 \times \rho \times g \times h}{p^\circ}$
P	Measured Pressure (Pa)
p_{vap}	Saturation vapour pressure (Pa)
ρ	Density of the solution (kg/m ³)
g	Standard gravity (m/s ²)
h	Liquid height in the Hydrogen electrode compartment (m)
p°	Standard pressure (101 325 Pa)
b	Molality, expressed as moles per kilogram of water (mol/kg H ₂ O)
ν	Amount content, expressed as moles per kilogram of solution (mol/kg sol)
ω_{H_2O}	Water mass fraction, expressed as grams per kilogram of solution (g/kg sol)
a.f.	Acidity function
u	Standard uncertainty
b_1	Slope of degradation for stability assessment
s	Standard deviation
t	Lifetime of the reference material, in months
N	Number of analysis for homogeneity assessment

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Supplementary Material

Detailed TRIS buffer preparation

Table S1. Stock solutions molalities (mol/kg H₂O) used for the TRIS buffer preparation

		<i>b</i> (mol/kg H₂O)
Minor salts	KCl	0.054511
	CaCl₂	0.055392
	MgCl₂	0.282045
HCl		1.047969
NaCl		2.137023
Na₂SO₄		0.410475
TRIS		0.827402

Table S2. Detailed information from gravimetric data of the TRIS buffer preparation

	Stock solution weighed (g)	ν (mol/kg sol)	<i>b</i> (mol/kg H₂O)	Target <i>b</i> (mol/kg H₂O)	Delta <i>b</i> (mol/kg H₂O)
HCl	363.6014	0.038272	0.040000	0.040000	0.000000
NaCl	1871.7003	0.370789	0.387529	0.387531	-0.000002
KCl	1846.8833	0.010123	0.010580	0.010580	0.000000
Na₂SO₄	692.2908	0.028000	0.029264	0.029264	0.000000
CaCl₂	1846.8833	0.010287	0.010751	0.010751	0.000000
MgCl₂	1846.8833	0.052377	0.054742	0.054742	0.000000
TRIS	976.0824	0.076544	0.080000	0.080000	0.000000
Final buffer solution mass (g)	9589.7545				

Stability assessment

Figure S1. Linear regression of the acidity function measured with Harned cell at 25°C as a function of time for the t test stability assessment. To each acidity function value is associated its expanded uncertainty with a coverage factor of 2. Color doesn't have to be used.

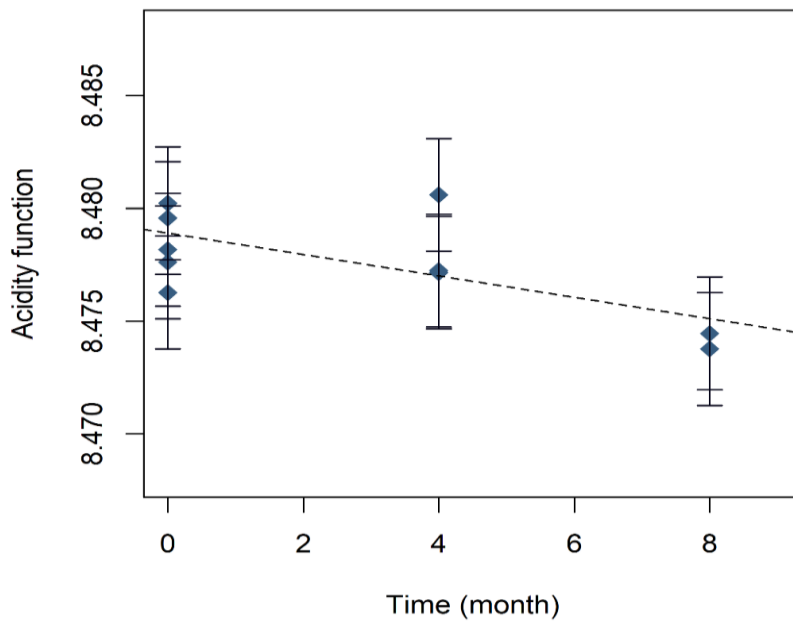
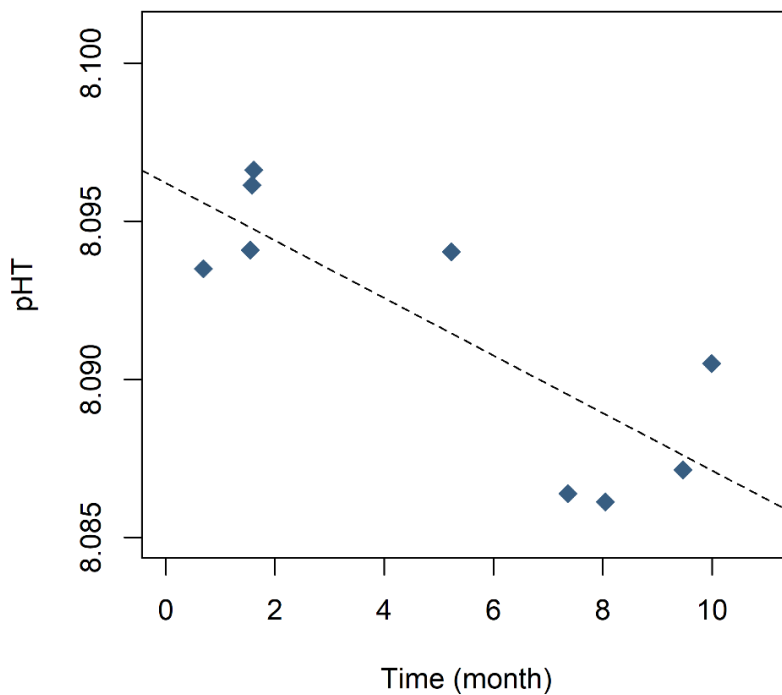


Figure S2. Linear regression of the pH_T measured by spectrophotometry at 25°C in function of time for the t test stability assessment. Color doesn't have to be used.



Preparation and characterization uncertainty evaluation

u_{charac} is assessed from equation 2.a using the law of uncertainty propagation.

Amount content of each stock solution

The first step, needed to assess uncertainties introduced by b_{Cl} and ω_{H2O} , is to evaluate the standard uncertainties corresponding to the amount content of each stock solution.

Following is an example for the NaCl stock solution using equation S1:

$$v_{NaCl} = \frac{m_{NaCl} \times 1000}{M_{NaCl} \times m_{total}} \quad (S1)$$

Where v_{NaCl} is the amount content of the NaCl stock solution (mol/kg sol), m_{NaCl} the mass of NaCl salt weighed (g) with the purity and buoyancy factor, introduced by the Archimede's principle, taken into account, m_{total} the total mass of NaCl stock solution (g) with buoyancy factor taken into account and M_{NaCl} the molar mass of NaCl (g/mol).

As the masses of NaCl and total stock solution are obtained from a subtraction (e.g. $m_{NaCl} = m_{NaCl+ bottle} - m_{emptybottle}$); standard uncertainty of masses are calculated from equation S2.

$$x_m = \sqrt{(x_{i_{m1}})^2 - (x_{i_{m2}})^2} \quad (S2)$$

All uncertainties assigned to molar masses, M_i , are taken from IUPAC (Meija et al., 2016).

Standard uncertainties of the amount content of each stock solution is given in table S3.

The amount content of the stock solution of HCl purchased from SMU has a standard uncertainty of 5.50E-05, given by the Slovak Metrology Institute certificate.

Water mass fraction: ω_{H2O}

The second step evaluates the standard uncertainty of the water mass fraction in the buffer solution, given by equation S3:

$$\omega_{H2O} = \frac{1000 - ((v_{HCl} + v_{NaCl}) \times M_{NaCl} + \sum(v_i \times M_i))}{1000} \quad (S3)$$

Where ω_{H2O} is the water mass fraction (g/kg sol) of pure artificial seawater, v the amount content of stock solutions that make up the pure artificial seawater composition (i.e. TRIS is removed and HCl is added to the NaCl amount) (mol/kg sol), M the molar masses (g/mol), i corresponds to the remaining salts (i.e. KCl, MgCl₂, CaCl₂, Na₂SO₄).

The standard uncertainties of v_i and M_i are detailed in the step above.

ω_{H2O} standard uncertainty is given in table S3.

Chloride ion molality in the buffer solution: b_{Cl}

To evaluate the standard uncertainty corresponding to the chloride ion molality of the buffer solution, equation S4 is used:

$$b_{Cl} = \sum \left(\frac{v_i \times m_i}{m_{buffer} \times \omega_{H_2O}} \right) \quad (S4)$$

Where b_{Cl} corresponds to the molality of chloride ions in the buffer solution (mol/kg H₂O), v_i the amount content of NaCl, minor salts and HCl stock solutions (mol/kg sol), m_i the mass of corresponding stock solution weighed (g) and m_{buffer} the total mass of reference material produced (g).

The standard uncertainties of v_i , ω_{H_2O} and b_i are detailed in the steps above.

b_{Cl} standard uncertainty is given in table S3.

Nernstian term: k

The Nernstian term k is defined in equation 2.c, with the corresponding standard uncertainty of each term given in table S3.

F and R uncertainties are taken from (Pratt, 2014), T uncertainty is taken from the temperature probe calibration.

Hydrogen pressure: pH_2

The hydrogen pressure, defined by equation S5, is required to calculate the pH_T (equation 2.b) and the E' terms allowing the determination of E^{o*} (equation S6).

$$pH_2 = \frac{P - p_{vap} + 0.4 \times \rho \times g \times h}{p^o} \quad (S5)$$

Where P is the ambient measured pressure (Pa), p_{vap} the saturation vapour pressure (Pa), ρ the density of the solution (kg/m³), g the standard gravity (m/s²), h the liquid height (m) and p^o the standard pressure (101 325 Pa).

The uncertainty corresponding to P is obtained from the probe calibration.

Uncertainties of P , ρ and h are given in table S3. Uncertainties relative to other terms can be neglected.

Standard potential of Ag/AgCl in saline media at a chosen Cl molality: E'

E' is calculated with the equation S6.

$$E' = E_{mes} + k \log (b_{Cl^-} \times b_{HCl}) - \frac{k}{2} (\log pH_2) \quad (S6)$$

Where E' is the standard potential of AgAgCl electrode in saline media for a given chloride molality (V), E_{mes} the measured potential (V), k the Nernstian term, b_{HCl} and b_{Cl-} are respectively the HCl and total chloride ion molalities (mol/kg H₂O) and pH_2 the hydrogen pressure (Pa).

E_{mes} uncertainty is defined by equation S7.

$$u_{E_{mes}} = \sqrt{(u_{calibration})^2 + (u_{maxSD})^2} \quad (S7)$$

Where $u_{calibration}$ corresponds to the uncertainty given by the calibration and u_{maxSD} to the maximum standard deviation obtained between the potentials acquired for each of E' solutions analysed.

The uncertainties of b_{HCl} and b_{Cl-} are calculated for the 0.01 mol/kg H₂O HCl molality, as it is the less concentrated solution, thus the one with the biggest uncertainty in b_{HCl} and b_{Cl-} .

The uncertainty of the b_{Cl-} -term is defined with the same method as described above for the b_{Cl-} of the buffer uncertainty evaluation.

The b_{HCl} uncertainty is calculated using equation S8.

$$b_{HCl} = \frac{m_{HCl} \times \nu_{HCl}}{m_{total} \times \omega_{H_2O}} \quad (S8)$$

Where b_{HCl} is the HCl molality of the studied solution (mol/kg H₂O), ν_{HCl} the HCl amount content given by SMU (mol/kg sol), m_{HCl} the mass of HCl weighed (g), m_{total} the total mass of solution and ω_{H_2O} the water mass fraction of this solution (g/kg sol).

ω_{H_2O} uncertainty is defined with the same method as above but for the E' solution with a 0.01 mol/kg H₂O HCl molality and by taking into account the actual composition of this solution.

Table S3 reports the uncertainties of the different terms in equation S6 and the assessed standard uncertainty for E' .

Standard potential of AgAgCl in saline media: E^{o*}

E^{o*} is obtained from the extrapolation to zero chloride molality of E' values.

The uncertainty of E^{o*} is defined by equation 5.

The assessed standard uncertainty for E^{o*} is given in table S3.

U_{charac}

The standard uncertainties of each terms of equation 2a have been carefully assessed and described which allow the final calculation of u_{charac} .

The standard uncertainty introduced during preparation and characterisation of the reference material, u_{charac} is given in table S3 for each studied temperature.

Table S3. Detailed standard uncertainties of each source contributing to the preparation and characterization uncertainty, U_{charac} .

Equations terms	Temperature (°C)	Unit	Standard uncertainty
Molality of each stock solution			
ν_{NaCl}		mol/kg sol	4.51E-04
ν_{minor}		mol/kg sol	3.89E-04
$\nu_{Na_2SO_4}$		mol/kg sol	5.63E-04
ν_{TRIS}		mol/kg sol	6.21E-04
ν_{HCl}		mol/kg sol	5.50E-05
Water mass fraction			
ω_{H_2O}		g/kg sol	9.25E-06
Chloride ion molality in the buffer solution			
b_{Cl}		mol/kg H ₂ O	1.78E-04
Nernstian term			
R		J/mol.K	1.50E-05
T		K	1.10E-02
F		C/mol	8.30E-03
Hydrogen Pressure			
P		Pa	1.00E+02
ρ		kg/m ³	2.00E-02
h		m	5.00E-03
Standard potential of AgAgCl in saline media at a chosen 0.01m HCl			
ω_{H_2O}		g/kg sol	1.12E-05
b_H		mol/kg H ₂ O	1.43E-05
b_{Cl}		mol/kg H ₂ O	1.87E-04
E'	15	V	3.93E-05
E'	25	V	4.09E-05
E'	30	V	4.21E-05
Standard potential of AgAgCl in saline media			
Extrapolation		V	4.71E-05
SD_E°	15	V	9.99E-05
E°*		V	1.17E-04
Extrapolation		V	2.08E-05
SD_E°	25	V	1.03E-04
E°*		V	1.13E-04
Extrapolation		V	8.25E-06
SD_E°	30	V	9.33E-05
E°*		V	1.03E-04
Overall characterisation and preparation uncertainty budget			
u_{charac}	15	V	2.09E-03

<i>u_{charac}</i>	25	V	1.95E-03
<i>u_{charac}</i>	30	V	1.76E-03