UNITED NATIONS

EP

UNEP/MED WG.509/17

Mediterranean
Action Plan
Barcelona
Convention

25 August 2021
Original: English

Meeting of the MED POL Focal Points

Teleconference, 27-28 May and 6-7 October 2021

Agenda item 12: Harmonization and standardization of IMAP Pollution Cluster Monitoring

Monitoring Guidelines/Protocols for Determination of Hydrographic Chemical Parameters

For environmental and cost-saving reasons, this document is printed in a limited number. Delegates are kindly requested to bring their copies to meetings and not to request additional copies.

UNEP/MAP
Athens, 2021

Table of Contents

1. Introduction 1
2. Technical note for measuring dissolved oxygen 2
2.1. Protocol for sample preparation and analysis of dissolved oxygen in seawater by Winkler method 2
3. Technical note for measuring pH 10
3.1. Protocol for sample preparation and analysis of pH using a potentiometric method 11
3.2. Protocol for sample preparation and analysis of pH using spectrophotometric method 13

Annexes

Annex I: Dickson et al., 2007. SOP6a: Determination of the pH of sea water using a glass / reference electrode cell

Annex II : Dickson et al., 2007. SOP6b: Determination of the pH of sea water using the indicator dye m-cresol purple

Annex III: References

Note by the Secretariat

In line with the Programme of Work 2020-2021 adopted by COP21 the MED POL Programme has prepared the Monitoring Guidelines related to IMAP Common Indicators 13, 14, 17 and 20 for consideration of the Integrated Meeting of the Ecosystem Approach Correspondence Groups on Monitoring (December 2020), whilst the Monitoring Guidelines for Common Indicator 18, along with the Monitoring Guidelines related to data quality assurance and reporting are under finalization for consideration of the Meeting on CorMon on Pollution Monitoring planned to be held in April 2021.

These Monitoring Guidelines present coherent manuals to guide technical personnel of IMAP competent laboratories of the Contracting Parties for the implementation of the standardized and harmonized monitoring practices related to a specific IMAP Common Indicator (i.e. sampling, sample preservation and transportation, sample preparation and analysis, along with quality assurance and reporting of monitoring data). For the first time, these guidelines present a summary of the best available known practices employed in marine monitoring by bringing integrated comprehensive analytical practices that can be applied in order to ensure the representativeness and accuracy of the analytical results needed for generation of quality assured monitoring data.

The Monitoring Guidelines/Protocols build upon the knowledge and practices obtained over 40 years of MED POL monitoring implementation and recent publications, highlighting the current practices of the Contracting Parties' marine laboratories, as well as other Regional Seas Conventions and the EU. A thorough analysis of presently available practices of UNEP/MAP, UNEP and IAEA, as well the HELCOM, OSPAR and European Commission Joint Research Centre was undertaken in order to assist an innovative approach for preparation of the IMAP Monitoring Guidelines/Protocols.

In order to support national efforts, this Monitoring Guidelines for Determination of Hydrographic Chemical Parameters provide the two following Technical Notes: a) Technical Note for the measurement of dissolved oxygen which includes the Protocol for sample preparation and analysis of dissolved oxygen in seawater by Winkler method; and b) Technical Note for the measurement of the pH which includes the two following Protocols: i) Protocol for sample preparation and analysis of pH using a potentiometric method; and ii) Protocol for sample preparation and analysis of pH using a spectrophotometric method.

The Monitoring Guidelines/Protocols for IMAP Common Indicators 13 and 14, including the one related to Determination of Hydrographic Chemical Parameters, establish a sound ground for further regular update of monitoring practice for a purpose of successful IMAP implementation.

In accordance with the Conclusions and Recommendations of the Integrated Meetings of the Ecosystem Approach Correspondence Groups on IMAP Implementation (CORMONs) (Videoconference, 1-3 Dec. 2020), and in particular paragraph 22, this Meeting requested the Secretariat to amend the Monitoring Guidelines by addressing agreed technical proposals that were described in the Report of the Meeting in line with its agreement to proceed with submission of these documents to the Meeting of MEDPOL Focal Points. Given the Integrated Meetings of CORMONs did not provide any request for further changes in this Monitoring Guideline, it is submitted for consideration of the present Meeting of MEDPOL Focal Points in the format as it has been discussed and agreed by the Integrated Meetings of CORMONs.

List of Acronyms/Abbreviations

BOD	Biological Oxygen Demand
BODC	British Oceanographic Data Centre
CAS	CAS Registry Number, is a unique numerical identifier assigned by the Chemical Abstracts Service (CAS)
CI	Common Indicator
COP	Conference of the Parties
CORMON	Correspondence Group on Monitoring
EcAp	Ecosystem Approach
EO	Ecological Objective
EU	European Union
GES	Good Environmental Status
HELCOM	Baltic Marine Environment Protection Commission - Helsinki Commission
LOD	Limit of Detection
IMAP	Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria
INFO/RAC	Information and Communication Centre of the Barcelona Convention
IPTS-68	International Practical Temperature Scale of 1968
IRM	Internal Reference Material
ISO	International Standard Organization
ITS-90	International Temperature Scale of 1990
IUPAC	International Union of Pure and Applied Chemistry
MAP	Mediterranean Action Plan
MEDPOL	Programme for the Assessment and Control of Marine Pollution in the Mediterranean Sea
MSFD	Marine Strategy Framework Directive
NBS	National Bureau of Standards
OSPAR	Convention for the Protection of the Marine Environment for the North-East Atlantic
SI	International System of Units (SI, abbreviated from the French Système international (d'unités))
SCOR	Scientific Committee on Oceanic Research
SYKE	The Finnish Environment Institute (Suomen YmpäristöKEskus)
UNESCO	United Nation Educational Scientific and Cultural Organization
USAC	Unit Standard Atmospheric Concentration

1. Introduction

1. In this Monitoring Guidelines for Determination of Hydrographic Chemical Parameters the supporting chemical parameters dissolved oxygen and pH are elaborated. Dissolved oxygen (DO) is an essential component which determines the water quality and trophodynamics of an aquatic system. On the other hand, the pH today is important mainly due to the acidification process: when CO_2 is absorbed by seawater, a series of chemical reactions occur resulting in the increased concentration of hydrogen ions (pH). This process has far reaching implications for the ocean and the creatures that live there.

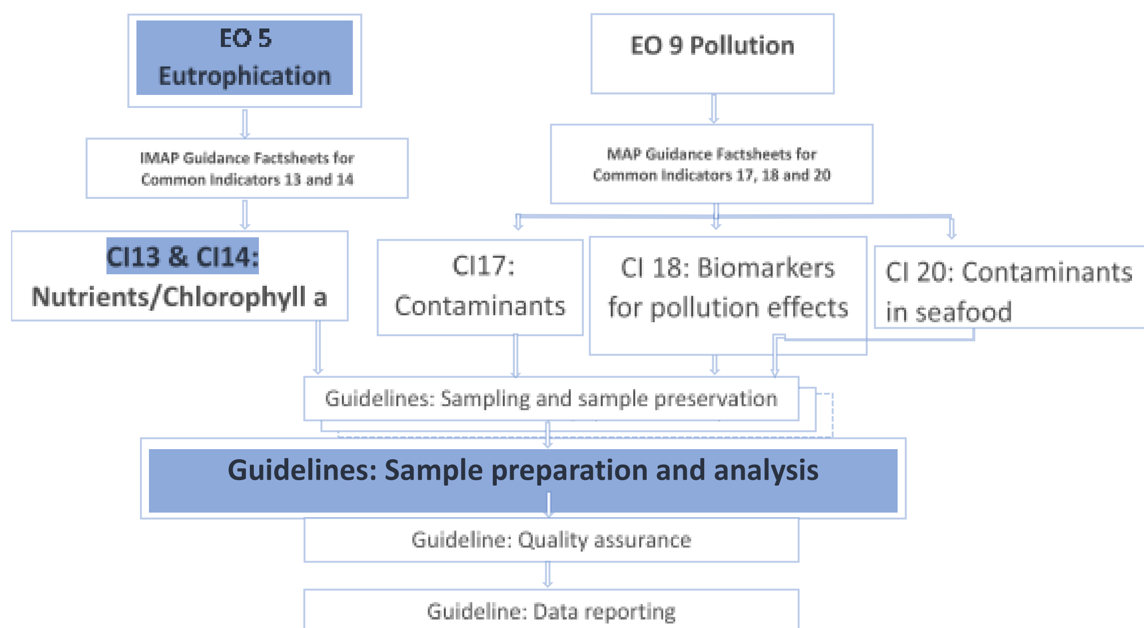
2. The IMAP Protocols elaborated within this Monitoring Guidelines for Determination of Hydrographic Chemical Parameters provide detail guidance on the necessary equipment, chemical reagents, analytical procedures along with appropriate methodologies for measurement of the core hydrography chemical supporting parameters, calculations, data transformation if necessary and identify weak points, including important specific notes and elaborated possible problems. However, they are not intended to be analytical training manuals, but guidelines for Mediterranean laboratories, which should be tested and accordingly modified, if need be, in order to validate their final results.

3. This Monitoring Guidelines builds upon the UNEP/MAP Integrated Monitoring and Assessment Programme (IMAP) respectively IMAP Guidance Fact Sheets for IMAP Common Indicators 13 and 14 (UNEP/MAP, 2019)[1]; standardized protocols (UNEP/MAP, 2019a)[2] and Data Quality Assurance schemes (UNEP/MAP, 2019b)[3] in order to allow the comparability of the data and build of regional assessment schemes. They also take into account previous Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of MED POL (UNEP/MAP/MED POL, 2005)[4], however providing detail procedures that are of relevance for IMAP implementation. With the details of the protocols for hydrographic chemical parameters, the needs of the measurements both in offshore areas and in narrow coastal area are addressed.

4. In the Subchapters "Symbol, units and precision" at the end of each Protocol, for all parameters described in it, the symbol and unit suggested by the International System of Units (SI) are presented. The expected accuracy, precision and where possible the Limit of Detection (LOD) are also presented. A Method identifier is also presented as it is provided in the Library P01 of the British Oceanographic Data Centre (BODC) Parameter Usage Vocabulary respectively included in Data Dictionaries and Data Standards for eutrophication built in IMAP Pilot Info System.

5. The below flow diagram informs on the category of this Monitoring Guidelines related to determination of hydrographic chemical parameters within the structure of all Monitoring guidelines prepared for IMAP Common Indicators 13, 14, 17, 18 and 20.

[1] (UNEP/MAP, 2019), UNEP/MED WG.467/5. IMAP Guidance Factsheets: Update for Common Indicators 13, 14, 17, 18, 20 and 21: New proposal for candidate indicators 26 and 27.
[2] (UNEP/MAP, 2019a), UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution.
[3] (UNEP/MAP, 2019b), UNEP/MED WG.46710. Schemes for Quality Assurance and Control of Data related to Pollution
[4] UNEP/MAP/MED POL, 2005. Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of MED POL. MAP Technical Reports Series No. 163. UNEP/MAP, Athens, 46 pp.



Flow Diagram: Monitoring Guidelines for IMAP Ecological Objectives 5 and 9

2. Technical note for measuring dissolved oxygen

6. The concentration of dissolved oxygen (DO) present in sea water depends on physico-chemical factors that determine the solubility of the gas and on biological activities (photosynthesis and respiration). Knowing temperature and salinity of the water, it is possible to trace the concentration of the theoretical dissolved oxygen which does not consider the processes of organic production and consumption. The positive (over-saturation) or negative (under-saturation) imbalance between the experimentally obtained and theoretical oxygen concentration is often used as an estimate of the processes prevalent in the water column, i.e. the prevalence of oxygen production by photo-synthetic processes, or consumption by the processes of mineralization of organic debris. From a precise determination of the DO concentration it is therefore possible to estimate the net production and respiration of the planktonic community.

7. Under this Technical Note, the Monitoring Guidelines for Determination of Hydrographic Chemical Parameters elaborate the Protocol for sample preparation and analysis of dissolved oxygen in seawater by Winkler method.

2.1. Protocol for sample preparation and analysis of dissolved oxygen in seawater by Winkler method

8. The Winkler titration method for the determination is based on the method developed by Winkler in 1888 (Winkler 1888)[5]. The method has seen several modifications to encompass interferences, and the basic method today for the determination of oxygen concentration is the one prepared by Grasshoff (1983)[6]. It is an iodometric titration, in which the amount of oxygen in the sample is determined indirectly via iodine. It is the most precise and reliable titrimetric procedure for DO analysis.

9. *Briefly:* A divalent manganese solution is added followed by strong alkali to a water sample in a glass stoppered bottle. Any DO present in the sample rapidly oxidizes an equivalent amount of the

---

[5] Winkler, L.W., 1888. Die Bestimmung des in Wasser gelösten Sauerstoffen. Berichte der Deutschen Chemischen Gesellschaft, 21: 2843–2855.

[6] Grasshoff, K., 1983. Determination of oxygen. In: Grasshoff, K., Ehrhardt M., Kremling K. (eds), Methods of Seawater Analysis, Verlag Chemie, Weinheim: 61-72.

dispersed divalent manganous hydroxide precipitate to hydroxides. The sample is then acidified with H_2SO_4. In the presence of iodide ions in an acidic solution, the oxidized manganese reverts to the divalent state, with the liberation of iodine equivalent to the original DO content. The iodine is then titrated with sodium thiosulfate and starch as an indicator. For the analysis of field samples, DO analysis is best done in the field, as there is less chance for the sample to be altered by atmospheric equilibration, changes in temperature and chance of escape of gasses.

a. Sample preparation

a1. Equipment

10. The equipment for sample preparation of dissolved oxygen in seawater by Winkler method include:

i) Transparent plastic tube (e.g. Tygon) to be connected to the sampling bottle taps;
ii) 60-90 ml pyrex bottles, BOD type, with ground flute beak or rounded truncated cone cap. Each bottle must have been pre-calibrated for its closed cap volume with an accuracy of ± 0.1ml;
iii) Laboratory glassware;
iv) Dispenser, automatic micropipettes or polyethylene syringes with notches every 0.5 mL;
v) Insulated container, shielded from light;
vi) 100 mL volumetric flasks;
vii) 6 bottles for the determination of the reagent blank. These bottles must be selected from those with a known volume used for the withdrawal of oxygen, preferably so that they are two by two of equal volume (± 0.1 mL), and with a difference in volume between one pair and the next of 1 ± 0.1 mL.

a.2. Chemicals

11. The following reagents and chemicals are needed:

i) manganese chloride [$MnCl_2 \cdot 4H_2O$] or manganese sulphate [$MnSO_4 \cdot H_2O$];
ii) sodium hydroxide [NaOH] or potassium hydroxide [KOH];
iii) potassium iodide [KI].

a.3. Preparation of reagents

Solution of Mn_2^+ (R1)

12. 40 g of $MnC1_2 \cdot 4H_2O$ or 35 g of $MnSO_4 \cdot H_2O$ is dissolved in 80 ml of reagent grade water and adjusted to volume in a 100 mL flask. The reagent, if stored in a closed bottle and not inadvertently contaminated with R2 containing iodide, is stable indefinitely.

Alkaline solution of ion I^- (R2)

13. 20 g of sodium hydroxide or 30 g of potassium hydroxide is dissolved in 40 mL of reagent grade water. 60 g of potassium iodide is dissolved in 40 ml of reagent grade water. The two solutions are gradually mixed in a flask and adjusted to a final volume of 100 mL with H_2O. The solution should then be stored in a dark, well-capped plastic bottle. If it is not contaminated with R1 or with reducing or oxidizing agents, it is stable indefinitely.

a.4. Procedure

14. The sub-sampling of DO from the Niskin bottle, or similar, must be done quickly as the dissolved gas tends to balance itself with the atmosphere. This process will be further accelerated by the temperature difference existing between the sample and the environment.

15. For sub-sampling, the transparent plastic tube to the Niskin bottle, possibly with a diameter of no more than 5 mm and a length that can easily reach the bottom of the BOD bottles for sample collection is connected.

16. The bottles, previously cleaned from the residues of the previous samplings and analyzes, are rinsed with water from the sample to be analysed. To prevent the formation of a layer of supersaturated oxygen along the walls the bottles are not shacked vigorously.

17. The sample is allowed to flow into the bottle, checking that the filling tube is free of air bubbles and avoiding the bubbling of air in the sample. The sampling tube must touch the bottom of the bottle, which must be filled slowly by overflowing a quantity of water equal to at least half of its total volume.

18. The tube is slowly removed from the bottle, always letting the water flow, so that the bottle always remains full to the brim. Before adding the reagents, it is checked that no air bubbles are trapped in the bottle, otherwise the bottle is emptied, and the filling operation repeated.

19. In the case bottles for BOD of about 100 ml is used, 0.5 mL of R1 and 0.5 mL of R2 is added in rapid succession, using two automatic dispensers or two normal syringes equipped with a long and narrow needle in order to inject the reagents at least below the free surface of the sample, preferably at the bottom of the bottle. In the case bottles for BOD with a volume other than about 100 ml are used proportional volumes of R1 and R2 are added.

20. The cap is carefully inserted avoiding the formation of air bubbles between the cap and the liquid, letting excess water escape. The well capped bottle is shacked inverting it several times for at least 30 seconds.

21. The bottles are placed in a dark place at a temperature similar to that of sampling. After the precipitate settle for 2/3 of the volume the bottles are shacked again. To further limit the possibility of gas exchange with the environment, it is suggested to keeps the cap firmly pressed on the neck of the bottle, using for example rubber bands, adhesive tape, etc.

Sampling for the determination of the reagent blank

22. Pre-selected bottles for blanks are sampled from the same sampling bottle, preferably not from the one relating to the surface level.

23. One dose of each reagent is added to the lowest volume pair of bottles, two doses to the volume greater than 1 mL and three doses to the volume greater than 2 mL. This operation must be carried out at least once during a sampling day.

a.5. Sample storage

24. The fixed samples should be stored in the dark and at a temperature as close as possible to that of sampling and analysed within the day of sampling.

25. Theoretically, the fixed samples could be kept for a longer time if there were no gaseous diffusion through the closures of the corks which unfortunately occurs, albeit to different degrees, in all bottles. In order to reduce this phenomenon, it is customary to keep the bottles, well closed, completely immersed in water of the same origin temperature as the sample.

b. Analytical procedure

b.1. Equipment in the laboratory

26. The equipment for analysis of dissolved oxygen in seawater by Winkler method includes:

i) 1 L class A volumetric flasks
ii) 1 mL or 5 mL glass or piston microburette
iii) 5 Pyrex bottles of the same type as those used for sampling
iv) 0.500 mL precision micropipette; 0.200 mL micropipette
v) Fluorescent lamp with opaque screen or diffuser
vi) Cold magnetic stirrer
vii) Magnetic stirrers
viii) 2 automatic dispensers or micropipettes or polyethylene syringes with notches every 0.5 mL (for oxygen reagents)
ix) 1 mL dispenser (for concentrated sulfuric acid).

27. An alternative to the micro burette is:

i) Potentiometric titrator;
ii) Combined redox platinum electrode, semi-micro.

b.2. Chemicals

28. The following reagents and chemicals are needed:

i) Sodium thiosulfate [$Na_2S2O_3 \cdot 5H_2O$];
ii) Potassium iodate [KIO_3], possibly ultrapure;
iii) Sodium chloride [NaCI];
iv) Chloroform [$CHC1_3$] or sodium-azide [NaN_3];
v) Soluble starch;
vi) Concentrated sulfuric acid, analytical grade [H_2SO_4].

b.3. Preparation of reagents

Thiosulfate solution ~ 0.1 mol L^{-1} (or ~ 0.1 M)

29. 24.82 g of $Na_2S_2O_3 \cdot 5H_2O$ are dissolved in 800 ml of reagent grade H_2O in a 1 L volumetric flask and adjusted to the mark. Few drops of chloroform or sodium azide are added as a stabilizer.

30. The solution should be stored in a dark glass bottle. Since thiosulfate is involved in numerous redox reactions, the solution is relatively unstable and therefore must be standardized against the potassium iodate solution before and after use. It is possible to use pre-stabilized commercial vials of thiosulfate solution of known titre.

KIO_3 standard solution 0.01667 mol L^{-1} (or 0.01667 M)

31. About 5 g of iodate are dried in an oven at 110 °C for at least an hour and cooled in the dryer or taken directly from a stock that was once dried and stored cold in a dryer in the presence of a strong dehydrator. Exactly 3.567 g are weighted and them dissolved quantitatively in 800 mL of reagent grade water in a 1 L volumetric flask (class A). The solution is adjusted exactly to volume at a temperature around the flask calibration (usually 20-25 ° C). Commercial iodized standard vials are also available.

32. The solution must then be stored in tightly capped dark glass bottles, kept away from the sun and opened for the shortest time possible only for sampling. Under these conditions the standard solution is to be considered stable for at least one year.

Stabilized colloidal starch solution (starch weld)

33. A saturated solution of sodium chloride by dissolving approximately 350 g of it in 1 L of distilled water in a beaker is prepared. 10 g of soluble starch in the saturated sodium chloride solution is hot dissolved.

The solution should be kept in a dark bottle and can be used until it becomes cloudy and flocculates.

b.4. Preparation of standard solutions

34. 5 BOD bottles are filled to 3/4 of the volume at least with sea water or with distilled water and 0.5 mL of concentrated sulfuric acid, 0.5 ml of R2 and 0.5 mL of reagent R1 are added to each one in succession using the same dispensers used to "fix" the samples. It is preferable to carry out these operations under continuous stirring, allowing the complete mixing of each reagent before adding the next. The bottles can then be capped and stored in the dark until the iodate standard solution is added;

35. Exactly 10.00 mL of standard KIO_3 solution to each bottle using an automatic pipette are added;

36. The bottle is shacked a few seconds and placed in the dark for about 1 minute to allow the reaction of iodate dismutation to take place by producing molecular iodine. The standards are then titrated with the thiosulfate solution as indicated below for the samples.

b.5. Analysis of samples

Dissolution of the precipitate

37. The bottle number and its volume are recorded;

38. The cap is gently removed from the bottle containing the precipitate and placed on the switched off magnetic stirrer;

39. A magnetic stir bar is quickly inserted into the bottle trying to lift as little precipitate as possible, add 0.5 mL of concentrated sulfuric acid with a dispenser, the stirrer is started by adjusting its speed in order to avoid the formation of vortices and turbulence;

40. When the complete dissolution of the precipitate (the solution becomes a clear yellowish colour due to the presence of iodine) is achieved, as sun as possible the titration with sodium thiosulfate is performed.

Titration

41. The tip of the burette containing the thiosulfate solution is immersed in the bottle containing the sample or standard;

42. At the beginning the thiosulfate solution is added rapidly, and then the flow is slowed when the yellow colour of the sample clears and, importantly, and it is stopped before the total disappearance of the yellow colour.

43. When the solution is almost colourless, the lamp is turned on and about 0.2 mL of starch solution is added (an intense purple colour appears), the addition of thiosulfate is resumed slowly until the blue colour almost disappears.

44. After few seconds, when viewed transparently against diffuse fluorescent light, a faint dispersed colour like a cloud is displayed in the bottle. The titration is proceeded very slowly until the complete disappearance of the colour, the end point (EP) of the titration. The volume of added thiosulfate is recorded.

45. If an automatic titrator with combined redox / platinum electrode is used, the titration program must show a decrease in the titrant flow near the EP which will correspond to the inflection point of the titration curve.

c. Calculations

c.1. Standardization of thiosulfate (C_{tio})

46. The prepared KIO_3 standards are titrated with the ~ 0.1 M thiosulfate solution (see "Preparation of reagents").

47. The molar titre C_{tio} of the thiosulfate solution is:

$$c_{tio} = 6 * (V_{KIO3} * c_{KIO3}) / V_{tio}$$

where

c_{tio} = exact molar concentration (M) of the $Na_2S_2O_3 \cdot 5H_2O$ solution

V_{KIO3} = volume in mL of injected KIO_3 standard (see "Preparation of standard solutions")

c_{KIO3} = molar concentration (0.01667 M) of the KIO3 standard used

V_{tio} = volume in mL of thiosulfate required to titrate the standard

48. The mean and standard deviation of V_{tio} in the replicates must be calculated and any value that differ by more than two standard deviations from the mean discarded. The mean and standard deviation of V_{tio} which will be used in the calculation of c_{tio} must be recalculated with the new values.

c.2. Determination of the reagent blank

49. The 3 pairs of bottles dedicated to the determination of the blank must be titrated and the volume of thiosulfate used noted. The concentration of DO (see "Calculation of dissolved oxygen concentration") must be calculated as if the blank (c_{bl}) were zero. The slope of the correlation line between the concentrations of DO thus obtained and the volume of R1 + R2 added corresponds to the blank of the reagents (c_{bl}).

50. A simpler way would be to calculate the difference between the average values for each pair of bottles and the next, but given the considerable variability in the differences this method is to be used in the alternative. This procedure allows the determination of the reagent blank, not that of the sample blank, i.e. the presence in the sample of interfering chemical substances (e.g. iodate) capable of producing elemental iodine in the acidified solution. If the desired level of accuracy required it, the blank should also be measured for each sample, according to the procedure suggested by Tijssen and van Bennekom (1989)[7].

[7] Tijssen S.B., Van Bennekom A.J., 1989. High precision determination of dissolved oxygen. ICES C.M. 1989/c:6, Annex C: 11-12.

c.3. Calculation of the micro-molar concentration (M or µmol L^{-1}) of dissolved oxygen

51. The following equitation applies for calculation of the micro-molar concentration (M or µmol L^{-1}) of dissolved oxygen:

$$c(O_2)/\mu mol\ L^{-1} = [(c_{tio} \cdot V_{tio}) / (4 \cdot (Y - y)) \cdot 10^6] - c_{bl}$$

where:

c_{tio} = exact molar concentration of the $Na_2S_2O_3 \cdot 5H_2O$ solution, from the standardization procedure

V_{tio} = volume in mL of thiosulfate required to titrate the unknown sample

c_{bl} = reagent blank (see reagent blank determination)

Y = volume in mL of the specific BOD bottle used for each sample

y = total volume, in mL, of reagents R1 + R2 added to each sample bottle (in the case shown, 1 mL)

c.4. Transformations :

52. The transformation of units needs to follow below provided scheme:

Unit A	Unit B	Transformation factor
mg/L	mL/L	0.7
mL/L	mg/L	1.429
µmol/L	mL/L	11.196
mL/L	µmol/L	0.0893
mg/L	µmol/L	0.06251
µmol/L	mg/L	15.997

d. Dissolved oxygen expressed as a percentage of the saturation value

53. The calculation of the percentage of the saturation value can be made only by knowing the value of the oxygen solubility in the sea water sample that has been analysed. It is known that the solubility of a gas in a liquid depends not only on the properties of the solvent (composition and temperature), but also on the partial pressure exerted on the solution by the gas in question (Henry's law). The solubility value therefore corresponds to the amount of oxygen that would dissolve in water in conditions of equilibrium between the surface layer of the sea and the atmosphere above.

54. To determine it, reference is made to a sample in thermodynamic equilibrium with a gaseous mixture of composition equal to the standard atmosphere, at the pressure of a standard atmosphere (mole fraction of oxygen = 0.20946) and saturated with water vapor. Depending on whether the oxygen concentration is related to the unit of mass or volume of the solvent, two concentration values are obtained, called USAC (acronym for "Unit Standard Atmospheric Concentration). These values are represented by the symbols C_0^i and C_0^* according to the symbology introduced by Benson and Krause (1980[8], 1984[9]). These quantities have recently been recalculated on the basis of a more rigorous procedure introduced by the authors themselves and subsequently recommended by

[8] Benson B.B., Krause D. Jr., 1980. The concentration and isotopic fractionation of gases in freshwater in equilibrium with atmosphere. Limnol. Oceanogr., 25: 662-671.
[9] Benson B.B., D. Krause, Jr., 1984.The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with atmosphere. Limnol. Oceanogr., 29: 620-632.

UNESCO (Millero, 1986)[10] to replace the values contained in the UNESCO oceanographic tables (1973)[11] which were based on the Weiss algorithms (1970)[12].

55. The equation is the product of numerical interpolations of data obtained from equations that more rigorously calculate the needed quantities. Furthermore, it should be noted that the equation is based on the practical temperature scale of 1968 (IPTS-68) and therefore, if values measured on the basis of the ITS-90 scale are used, the appropriate conversions must be applied.

d.1. Calculations

56. The following equitation applies for calculation of Dissolved oxygen expressed as a percentage of the saturation value:

$$\varphi(O_2/O_2') = 100 \cdot c(O_2)/C_0$$

where

$$\ln C_0 = a_0 + a_1/T + a_2/T^2 + a_3T^3 + a_4/T^4 - S \cdot (b_0 + b_1/T + b_2/T^2)$$

57. In the equation C_0 corresponds to the concentration of the theoretical DO C_0^i and C_0^* reported per unit of volume. The constants to be inserted in the equation are:

$a_0 =$ -135.90205
$a_1 =$ $1.575701 \cdot 10^5$
$a_2 =$ $-6.642308 \cdot 10^7$
$a_3 =$ $1.243800 \cdot 10^{10}$
$a_4 =$ $-8.621949 \cdot 10^{11}$
$b_0 =$ 0.017674
$b_1 =$ -10.754
$b_2 =$ 2140.7

e. Important notes

58. When fixing the samples contact between the reagents R1 and R2 must be avoided.

59. During manual titration, the same criterion for identifying the titration EP for both standards and samples must be used, best avoiding changing operator.

60. The titration must be performed quickly, decreasing the flow of thiosulfate only in the vicinity of the titration EP, in order to minimize errors due to the photo-oxidation of the iodide and the reduction of iodine by the starch.

f. Possible problems

61. A problem that usually occurs is the formation of bubbles in the bottle containing the sample; to prevent this phenomenon, the bottles must be washed with detergents and rinsed thoroughly.

62. Sometimes an air bubble is formed under the cap of the bottle containing the sample already fixed; in this case, the possible existence of an error due to the excess, however not quantifiable, of the amount of dissolved oxygen must be considered and noted.

---

[10] Millero F.J., 1986. Solubility of oxygen in seawater. UNESCO Technical Papers in Marine Science, 50: 13- 17.
[11] UNESCO. 1973. International oceanographic tables, Vol. 2. NIO-UNESCO, Paris.
[12] Weiss, R.F., 1970. The solubility of nitrogen, oxygen and argon in water and seawater. Deep-Sea Research, 17: 721–735.

g. Symbol, units and precision

63. For the parameters described in this protocol, the symbols and units suggested by the International System of Units (SI), as well as the expected accuracy, along with the Method identifiers as provided in the Library P01 of BODC Parameter Usage Vocabulary are provided as follows:

Concentration of dissolved oxygen

Symbol: $c(O_2)$ **Unit:** µmol L^{-1}

Precision: 0.1

Method identifier: SDN:P01::**DOXYWITX** Concentration of oxygen {O2 CAS 7782-44-7} per unit volume of the water body [dissolved plus reactive particulate phase] by Winkler titration

Saturation of Dissolved Oxygen

Symbol: $\varphi(O_2/O_2')$ **Unit:** % *(percent)*

Precision: 0.1

Method identifier: SDN:P01::**OXYSBW01** Saturation of oxygen {O2 CAS 7782-44-7} in the water body [dissolved plus reactive particulate phase] by Winkler titration and computation from concentration using Benson and Krause algorithm

3. Technical note for measuring pH

64. Since ocean acidification is a growing concern, monitoring of pH is necessary for studies of acidification and its effects on the carbonate buffer system. As many important biological processes are likely to be affected by rapid changes in pH, it is important to include accurate determination of pH among monitoring parameters.

65. pH is operationally defined, and several pH scales are used in environmental monitoring. The NBS (National Bureau of Standards) scale is suitable for waters of low ionic strength and used for freshwater monitoring. The total hydrogen ion scale is often used for pH determinations in oceanic waters.

66. pH is also used in marine environmental monitoring as a co-factor in measurements of primary production.

67. Two different principles for pH measurement are available, based on potentiometric and spectrophotometric detection. Potentiometric detection has the advantages of being fast and simple and requires no advanced or expensive equipment. Buffers used for calibration should ideally have an ionic strength matching that of the samples, which is challenging when an area with a large salinity gradient is monitored. Several pH meters, electrodes and buffers are commercially available.

68. Spectrophotometric detection is more accurate, has a higher precision, but requires expensive equipment. It is widely used in measurements under oceanic conditions, but less in estuarine waters. Since commercial applications for the spectrophotometric methods are not widely used; users must assemble instruments and software for data processing. Methods based on spectrophotometric detection are therefore not yet recommended for monitoring purposes.

69. Therefore, under this Technical Note, the Monitoring Guidelines for Determination of Hydrographic Chemical Parameters elaborates the two following Protocols: i) the Protocol for sample preparation and analysis of pH using a potentiometric method; and ii) the Protocol for sample preparation and analysis of pH using a spectrophotometric method.

3.1. Protocol for sample preparation and analysis of pH using a potentiometric method

70. pH is measured using a glass/combined electrode. The total hydrogen ion scale should be used. Temperature is measured and recorded both during pH measurement and at sampling depth.

71. Subsamples for pH should be drawn from sampler bottles as early as possible (after samples for oxygen and hydrogen sulphide, but before samples for nutrients and salinity) to avoid gas exchange between water and air. Samples should be collected in gas-tight bottles. Bottles should be rinsed thoroughly with sample water before filling. Bottles are filled with a laminar flow of sample water, allowing 2-3 bottle volumes to overflow before capping. Bottles should be completely filled, leaving no headspace. Avoid trapping bubbles of air when capping bottles. Samples should preferably be analysed as soon as possible directly after sampling.

72. Determination of pH using a glass electrode is described in ISO 10523[13].

73. Temperature must be monitored and controlled during calibration of instrument and analysis, preferably by use of a tempered water bath. Make sure temperature of buffers and samples is constant (±1 °C) during the process. To maintain constant temperature, select a bath temperature slightly above ambient temperature (for normal room temperature, set bath temperature to 25 °C – in a cooler environment 20 °C may have to be used). pH analysis can also be made in + 15 °C in a cooling bath which has been shown to produce comparable results.

74. pH meter should be calibrated daily when in use. Manufacturer's instructions for a 2-point calibration (pH 7 and pH 9 are recommended) are followed. NBS buffers for calibration is used. Attention to expiry dates of buffers has to be paid.

75. Electrode and temperature probe must be rinsed with deionized water and wiped between buffers/samples.

76. Electrode must be allowed to equilibrate in sample water for 15 minutes before first measurement. The best is if equilibrium is reached for each sample before recording a reading.

77. Open-cell measurements allow gas exchange between sample and air during the time of measurements. Closed-cell measurements eliminate the interferences.

78. Manufacturer's instructions must be followed for handling and storage of electrodes. Electrodes may require cleaning and conditioning when exposed to samples from intense plankton blooms. Anoxic water containing high concentration of hydrogen sulphide may shorten the life of electrodes.

79. A correction for in situ pH (Gieskes, 1969)[14] is sometimes applied. A better option is to report measured pH, temperature from pH measurement and in situ temperature.

80. pH values from potentiometric detection should be reported with two decimals. Temperature from measurement and sampling depth should also be reported.

81. Information on which pH scale is used must be included in metadata.

---

[13] ISO 10523: Water quality – Determination of pH.

[14] Gieskes, J. M., 1969. Effects of temperature on the pH of seawater. Limnology and Oceanography Vol 14 Issue 5, p 679-685.

82. A detailed analytical protocol for the analysis of pH using a potentiometric method (Dickson et al, 2007)[15] is presented in the Annex I. (Guide to best practices for ocean CO_2 measurements, Ch: 4. Recommended standard operating procedure, SOP6a: Determination of the pH of sea water using a glass/reference electrode cell).

a. Quality control

83. Laboratories carrying out analyses of pH should have established a quality management system according to ISO 17025[16].

84. Data for samples for estimation of measurement uncertainty (repeated measurements from a sample, multiple subsamples from different samplers closed at same depth).

85. An internal reference material (IRM) should be analysed daily.

86. It is strongly recommended that all laboratories participate in interlaboratory comparisons and proficiency testing programs, to provide external verification of laboratory performance. Proficiency testing for pH in environmental waters are provided by e.g. SYKE. More proficiency testing schemes are listed at www.eptis.bam.de.

87. Validation of the adopted method needs to be performed on the relevant matrix and concentration range e.g. by taking part regularly at intercomparison studies or proficiency testing schemes.

88. Measurement uncertainty should be estimated using ISO 11352[17].

89. Estimation should be based on within - laboratory reproducibility, IRM, and, if available, data from proficiency testing and CRM.

b. Symbol, units and precision

90. For the parameter described in this protocol, the symbol and unit suggested by the International System of Units (SI), as well as the expected accuracy, along with a Method identifier as provided in the Library P01 of BODC Parameter Usage Vocabulary are provided as follows:

Symbol: *pH* **Unit:**

Precision: ± 0,003 **Accuracy:** -

Method identifier: SDN:P01:: **PHXXPR01** pH per unit volume of the water body by pH electrode

[15] Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to Best Practices for Ocean CO2 Measurements. PICES Special Publication 3, 191 pp.
[16] ISO 17025: General requirements for the competence of testing and calibration laboratories.
[17] ISO 11352: Water quality – Estimation of measurement uncertainty based on validation and quality control data.

3.2. Protocol for sample preparation and analysis of pH using spectrophotometric method

91. Recently, the spectrophotometric method of measuring the pH value of seawater has been proposed, which consists in measuring the visible absorption of a coloured pH indicator added to the seawater sample. The measurement is precise, sensitive, and theoretically free from the need for calibrations (of calibration lines), but the instrumentation is more expensive and the analysis speed lower than the potentiometric method (Dickson 1993)[18]. As précised before, this method is therefore not yet recommended for monitoring purposes. To compare the pH values obtained by this method with the potentiometric ones, it must be referred to the total hydrogen ion concentration pH scale.

92. A detailed analytical protocol for the analysis of pH using spectrophotometric method recommended by the International Scientific Community (IOC and SCOR) collected in Dickson et al., 2007 is presented in the Annex II. (Guide to best practices for ocean CO_2 measurements, Ch: 4. Recommended standard operating procedure, SOP6b: Determination of the pH of sea water using the indicator dye *m*-cresol purple).

a. Symbol, units and precision

93. For the parameter described in this protocol, the symbol and unit suggested by the International System of Units (SI), as well as the expected accuracy, along with a Method identifier as provided in the Library P01 of BODC Parameter Usage Vocabulary are provided as follows:

Symbol: *pH* **Unit:**

Precision: 0.001 **Accuracy:** ± 0.005

Method identifier: SDN:P01:: **PHXXSP01** pH per unit volume of the water body by spectrophotometry

[18] Dickson, A.G.,1993. The measurement of sea water pH. Mar. Chem., 44: 131-142.

Annex I

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.), 2007. Ch: 4. Recommended standard operating procedure, SOP6a: Determination of the pH of sea water using a glass/reference electrode cell. In: Guide to best practices for ocean CO_2 measurements. PICES Special Publication 3, 7 pp.

SOP 6a

Determination of the pH of sea water using a glass / reference electrode cell

1. Scope and field of application

This procedure describes a method for the potentiometric determination of the pH of sea water on the total hydrogen ion concentration pH scale. The total hydrogen ion concentration, $[H^+]$, is expressed as moles per kilogram of sea water.

2. Definition

The total hydrogen ion concentration of sea water includes the contribution of the medium ion sulfate and is defined as

$$\begin{aligned}[H^+] &= [H^+]_F(1 + S_T / K_S) \\ &\approx [H^+]_F + [HSO_4^-]\end{aligned} \quad (1)$$

where $[H^+]_F$ is the *free* concentration of hydrogen ion in sea water, S_T is the total sulfate concentration $([HSO_4^-]+[SO_4^{2-}])$ and K_S is the acid dissociation constant for HSO_4^-. The pH is then defined as the negative of the base 10 logarithm of the hydrogen ion concentration:

$$\text{pH} = -\log_{10}\left(\frac{[H^+]}{\text{mol kg-soln}^{-1}}\right). \quad (2)$$

3. Principle

Values of pH are determined experimentally from sequential measurements of the e.m.f. (E) of the cell

in a standard buffer (S) of known (defined) pH and in the sea water sample (X).

The operational pH is defined by the expression

$$\mathrm{pH(X)} = \mathrm{pH(S)} + \frac{E_\mathrm{S} - E_\mathrm{X}}{RT \ln 10 / F} \ . \tag{3}$$

Residual liquid junction error is minimized by matching the composition of the standard buffer to the sea water sample, *i.e.*, by making the buffer up in synthetic sea water.

Values of pH(S) have been assigned to various standard buffers in synthetic sea water. These are based on careful laboratory measurements made using cells without liquid junction.

4. Apparatus

4.1 pH cell

A combination glass/reference electrode is typically the most convenient cell to use; however, measurement quality can often be improved by using separate glass and reference electrodes.

4.2 Voltmeter with high input impedance

The e.m.f. of the glass/reference electrode cell can be measured with a pH meter or other voltmeter with a high input impedance ($>10^{13}\ \Omega$). If a pH meter with a sensitivity of ± 0.1 mV is used to measure the e.m.f., the sensitivity in determining the pH is ± 0.002 pH units. The use of a 5½ digit voltmeter with a high input impedance[1] can improve the sensitivity to better than ± 0.001 pH units. (The accuracy of the measurement is dependent upon the reliability of the assignment of pH(S) values to the calibration buffers used.)

4.3 Closed measurement container

It is necessary to measure the pH on a sample that has not had the opportunity to exchange CO_2 with the atmosphere so as to ensure reliable pH results.

4.4 Thermometer (accurate to ± 0.05°C)

The temperature should be known or controlled to within 0.1°C during the measurement.

---

[1] An external circuit based on a high input impedance operational amplifier (*e.g.*, an FET electrometer amplifier) configured as a voltage follower (unity gain amplifier) can be used to achieve this.

5. Reagents

5.1 Synthetic sea water

- Reagent grade NaCl (dried in an oven at 110°C),
- Reagent grade Na_2SO_4 (dried in an oven at 110°C),
- Reagent grade KCl (dried in an oven at 110°C),
- Calibrated solution of reagent grade $MgCl_2$,[2]
- Calibrated solution of reagent grade $CaCl_2$,[2]
- Deionized water.

5.2 Buffer substances

- Calibrated solution of HCl prepared from redistilled reagent grade HCl. Its concentration should be known to within 0.1%[3].
- 2-amino-2-hydroxymethyl-1,3-propanediol ("TRIS"), crushed and dried in a desiccator at room temperature over phosphorus (V) oxide before use.
- 2-aminopyridine, recrystallized from a benzene–petroleum ether mixture, crushed and dried in a desiccator at room temperature over phosphorus (V) oxide before use.

6. Sampling

It is essential that the samples analyzed are collected, poisoned, and stored according to the procedures detailed in SOP 1. Care should be taken to avoid the exchange of CO_2 with the atmosphere both during sampling and during subsequent manipulation.

7. Procedure

7.1 Preparation of buffers in synthetic sea water

The compositions of a TRIS/HCl buffer and of a 2-aminopyridine/HCl buffer in synthetic sea water with a salinity of 35 are given in Table 1. This recipe is based on synthetic sea water (see Chapter 5, Table 4) in which 0.04 mol/kg-H_2O of NaCl has been replaced with HCl, and contains a total of 0.08 mol/kg-H_2O of the desired base. The simplest way to prepare this buffer accurately is first to weigh out the hydrochloric acid and then to scale the amounts of the other constituents to match the exact amount of HCl that was weighed out. Such buffers can be stored for a number of weeks, in a sealed, almost full, container.

---

[2] Solutions of $MgCl_2$ and $CaCl_2$ can be analyzed either by measuring the density of the stock solution, by titrating with a calibrated silver nitrate solution (*e.g.*, using K_2CrO_4 as an indicator) or by gravimetric precipitation of chloride.

[3] Solutions of HCl can be analyzed accurately by coulometric titration, by a careful titration against a standard base (*e.g.*, TRIS—NIST 723) or by gravimetric precipitation of chloride.

Table 1 Composition of a buffer solution for pH in synthetic sea water of salinity 35 (weights based on 1000 g of H_2O).

Constituent	Moles	Weight (g)[a]
NaCl	0.38762[b]	22.6446
KCl	0.01058	0.7884
$MgCl_2$	0.05474	–
$CaCl_2$	0.01075	–
Na_2SO_4	0.02927	4.1563
HCl	0.04000	–
One of:		
2-amino-2-hydroxymethyl-1,3-propanediol (TRIS)	0.08000	9.6837
2-aminopyridine	0.08000	7.5231
Total weight of solution containing:		
2-amino-2-hydroxymethyl-1,3-propanediol (TRIS)	–	1044.09
2-aminopyridine	–	1041.93

[a] Weight in air at sea level (*i.e.*, not corrected to mass). If a weight is not given, the component is added as the appropriate amount of a calibrated solution.
[b] $m(\text{NaCl}) = 0.42762 - 0.04$ mol/kg-H_2O, *i.e.*, replacing NaCl with HCl.

To compute the composition for a buffer with a salinity different from 35[4], first compute the composition of the basic artificial sea water—containing no base or HCl and with the full amount of NaCl—corresponding to the new salinity, S:

$$m_S = m_{35} \times \frac{27.5695S}{1000 - 1.0019S}, \quad (4)$$

then adjust the m(NaCl) down by 0.04 mol/kg-H_2O and add 0.08 mol/kg-H_2O of base.

7.2 Confirm response of pH cell[5]

Before a pH cell (a glass electrode/reference electrode pair) is used to measure pH, it should be tested to ensure that it is performing properly, *i.e.*, that it has an ideal Nernst response.

Bring both buffers (TRIS and 2-aminopyridine) to the same known temperature (*e.g.*, 25°C). Measure and record the e.m.f. of the pH cell in each buffer. The difference in the e.m.f. is used to check the response of the pH cell (see

[4] The magnitude of the error involved in using a salinity 35 buffer for most oceanic measurements (*i.e.*, in the salinity range 33–37) is probably less than 0.005 in pH. For a more complete discussion of this error see Whitfield *et al.* (1985) and Butler *et al.* (1985).

[5] Some investigators make use of the titration curve obtained from titrating a sodium chloride solution with HCl (see SOP 3) to confirm that the electrode pair has the theoretical response (RT/F). However, the value of the slope and the value of $E°$ obtained by fitting experimental results in this fashion are highly correlated and thus not particularly reliable. It is better to verify the response of the electrode pair used with suitable buffers, as is done here.

section 8.1). If the response is not theoretical (within the experimental uncertainty), the electrodes should be rejected. E.m.f. readings obtained with a well-behaved pH cell should be stable with time (drift < 0.05 mV $\min^{-1}$)[6].

7.3 Measurement of pH

Bring the TRIS buffer and the sea water samples to be measured to the same known temperature (*e.g.*, 25°C). The e.m.f. of the pH cell is then measured, first in the TRIS buffer (E_S) and then in the sea water sample (E_X). Care should be taken to minimize any exposure of the sea water samples to the atmosphere so as to limit loss or gain of CO_2.

8. Calculation and expression of results

8.1 Calculation of response of pH cell

The defined pH values of the two buffers recommended for use in this procedure are:

2-amino-2-hydroxy-1,3-propanediol (TRIS)

$$\begin{aligned}\mathrm{pH(S)} = {} & (11911.08 - 18.2499S - 0.039336S^2)\frac{1}{T/\mathrm{K}} \\ & - 366.27059 + 0.53993607S + 0.00016329S^2 \\ & + (64.52243 - 0.084041S)\ln(T/\mathrm{K}) - 0.11149858(T/\mathrm{K}). \end{aligned} \tag{5}$$

2-aminopyridine (AMP)

$$\begin{aligned}\mathrm{pH(S)} = {} & (111.35 + 5.44875S)\frac{1}{T/\mathrm{K}} + 41.6775 - 0.015683S \\ & - 6.20815\ \ln(T/\mathrm{K}) - \log_{10}(1 - 0.00106S). \end{aligned} \tag{6}$$

The electrode response, *s*, can then be calculated:

$$s = \frac{E_{\mathrm{AMP}} - E_{\mathrm{TRIS}}}{\mathrm{pH(S)}_{\mathrm{TRIS}} - \mathrm{pH(S)}_{\mathrm{AMP}}} \tag{7}$$

and compared with the ideal Nernst value: $RT \ln 10/F$. If it is more than about 0.3% different, the pH cell should be replaced.

8.2 Calculation of pH

Values of pH are calculated from the expression

$$\mathrm{pH(X)} = \mathrm{pH(S)} + \frac{E_S - E_X}{RT\ln 10/F} \tag{8}$$

---

[6] This is an upper limit to the acceptable level of drift. An ideal level is as much as an order of magnitude lower, *e.g.*, < 0.3 mV h^{-1}.

where pH(S), the pH of TRIS buffer (Table 1) on the total hydrogen ion scale (expressed in mol kg-soln^{-1}) is given by equation (5).

8.3 Example calculation

Input data:

$$
\begin{aligned}
t &= 25°\text{C } (\textit{i.e.}, T = 298.15 \text{ K}), \\
S &= 35, \\
E_{\text{TRIS}} &= -0.0724 \text{ V}, \\
E_{\text{AMP}} &= 0.0049 \text{ V}, \\
E_{\text{X}} &= -0.0670 \text{ V}.
\end{aligned}
$$

Hence

$$
\begin{aligned}
\text{pH(S)}_{\text{TRIS}} &= 8.0936, \\
\text{pH(S)}_{\text{AMP}} &= 6.7866, \\
RT \ln 10/F &= 0.05916 \text{ V/pH unit.}
\end{aligned}
$$

Thus

$$s = \frac{0.0049 - (-0.0724)}{8.0936 - 6.7866} = 0.05914 \text{ V / pH unit}$$

and, using equation (8),

$$\text{pH(X)} = 8.0936 + \frac{-0.0724 - (-0.0670)}{0.05916} = 8.0023 .$$

9. Quality assurance

9.1 For general principles of analytical quality control see Chapter 3

9.2 Specific applications of analytical quality control

9.2.1 Ideality of pH cell behavior

The measured electrode response, s, should be compared with ideal Nernst behavior on a regular basis (see section 7.2). If the value is more than 0.3% from theoretical, try cleaning the glass electrode of surface deposits and measuring again. If the discrepancy persists, the electrode should be replaced.

The value of E_S in TRIS buffer, when measured at a constant temperature (*e.g.*, 25°C), should remain stable to within a few mV. A sudden change in E_S is indicative of potential problems. Similarly, the e.m.f. of a well-behaved pH cell immersed in a thermostated buffer should be stable (drift < 0.05 mV min^{-1}).

9.2.2 Precision

A precision of 0.003 pH units (1 SD) is possible with care. Plot the results of duplicate analyses on a range control chart (SOP 22).

9.2.3 Bias

The bias of potentiometric pH measurements depends on the care with which the buffer was prepared, especially with regard to the ratio between the TRIS and the HCl, and on the accuracy with which the values of pH(S) were originally assigned. This latter value has been estimated as being within 0.004 pH units.

10. Bibliography

Bates, R.G. and Erickson, W.P. 1986. Thermodynamics of the dissociation of 2-aminopyridinium ion in synthetic seawater and a standard for pH in marine systems. *J. Soltn. Chem.* **15**: 891–901.

Butler, R.A., Covington, A.K. and Whitfield, M. 1985. The determination of pH in estuarine waters. II: Practical considerations. *Oceanol. Acta* **8**: 433–439.

DelValls, T.A. and Dickson, A.G. 1998. The pH of buffers based on 2-amino-2-hydroxymethyl-1,3-propanediol ('tris') in synthetic sea water. *Deep-Sea Res.* **45**: 1541–1554.

Dickson, A.G. 1993. pH buffers for sea water media based on the total hydrogen ion concentration scale. *Deep-Sea Res.* **40**: 107–118.

Dickson, A.G. 1993. The measurement of sea water pH. *Mar. Chem.* **44**: 131–142.

Millero, F.J., Zhang, J.-Z., Fiol, S., Sotolongo, S., Roy, R.N., Lee, K. and Mane, S. 1993. The use of buffers to measure the pH of sea water. *Mar. Chem.* **44**: 143–152.

Nemzer, B.V. and Dickson, A.G. 2004. The stability and reproducibility of Tris buffers in synthetic seawater. *Mar. Chem.* **96**: 237–242.

Ramette, R.W., Culberson, C.H. and Bates, R.G. 1977. Acid-base properties of tris(hydroxymethyl)aminomethane (tris) buffers in seawater from 5 to 40°C. *Anal. Chem.* **49**: 867–870.

Whitfield, M., Butler, R.A. and Covington, A.K. 1985. The determination of pH in estuarine waters. I. Definition of pH scales and the selection of buffers. *Oceanol. Acta* **8**: 423–432.

Annex II

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.), 2007. Ch: 4. Recommended standard operating procedure, SOP6b: Determination of the pH of sea water using the indicator dyem-cresol purple. In: Guide to best practices for ocean CO_2 measurements. PICES Special Publication 3, 7 pp.

SOP 6b

Determination of the pH of sea water using the indicator dye *m*-cresol purple

1. Scope and field of application

This procedure describes a method for the spectrophotometric determination of the pH of sea water on the total hydrogen ion concentration pH scale. The total hydrogen ion concentration, $[H^+]$, is expressed as moles per kilogram of sea water.

2. Definition

The total hydrogen ion concentration of sea water includes the contribution of the medium ion sulfate and is defined as

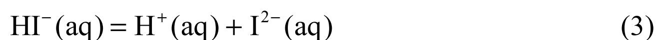
$$\begin{aligned}[\mathrm{H}^+] &= [\mathrm{H}^+]_\mathrm{F}(1 + S_\mathrm{T} / K_\mathrm{S}) \\ &\approx [\mathrm{H}^+]_\mathrm{F} + [\mathrm{HSO}_4^-] \end{aligned} \quad (1)$$

where $[H^+]_F$ is the *free* concentration of hydrogen ion in sea water, S_T is the total sulfate concentration $([\mathrm{HSO}_4^-] + [\mathrm{SO}_4^{2-}])$ and K_S is the acid dissociation constant for HSO_4^-. The pH is then defined as the negative of the base 10 logarithm of the hydrogen ion concentration:

$$\mathrm{pH} = -\log_{10}\left(\frac{[\mathrm{H}^+]}{\mathrm{mol\ kg\text{-}soln}^{-1}}\right). \quad (2)$$

3. Principle

The values of pH are determined by adding an indicator dye to sea water. For the sulfonephthalein indicators such as *m*-cresol purple, the reaction of interest at sea water pH is the second dissociation



$$\mathrm{HI}^-(\mathrm{aq}) = \mathrm{H}^+(\mathrm{aq}) + \mathrm{I}^{2-}(\mathrm{aq}) \quad (3)$$

where I represents the indicator dye, which is present at a low level in a sea water sample. The total hydrogen ion concentration of the sample can then be determined:

$$\mathrm{pH} = \mathrm{p}K(\mathrm{HI}^-) + \log_{10}\frac{[\mathrm{I}^{2-}]}{[\mathrm{HI}^-]}. \tag{4}$$

The principle of this approach uses the fact that the different forms of the indicator have substantially different absorption spectra. Thus the information contained in the composite spectrum can be used to estimate $[\mathrm{I}^{2-}]/[\mathrm{HI}^-]$.

At an individual wavelength, λ, the measured absorbance in a cell with a path length, l, is given by the Beer–Lambert law as

$$\frac{A_\lambda}{l} = \varepsilon_\lambda(\mathrm{HI}^-)[\mathrm{HI}^-] + \varepsilon_\lambda(\mathrm{I}^{2-})[\mathrm{I}^{2-}] + B_\lambda + e \tag{5}$$

where B_λ corresponds to the background absorbance of the sample and e is an error term due to instrumental noise. Provided that the values of the extinction coefficients: $\varepsilon_\lambda(\mathrm{HI}^-)$ and $\varepsilon_\lambda(\mathrm{HI}^{2-})$ have been measured as a function of wavelength, absorbance measurements made at two or more wavelengths can be used to estimate the ratio $[\mathrm{I}^{2-}]/[\mathrm{HI}^-]$.

In the case that only two wavelengths are used, and provided that the background can be eliminated effectively by a subtractive procedure, (5) can be rearranged to give (assuming no instrumental error)

$$\frac{[\mathrm{I}^{2-}]}{[\mathrm{HI}^-]} = \frac{A_1/A_2 - \varepsilon_1(\mathrm{HI}^-)/\varepsilon_2(\mathrm{HI}^-)}{\varepsilon_1(\mathrm{I}^{2-})/\varepsilon_2(\mathrm{HI}^-) - (A_1/A_2)\varepsilon_2(\mathrm{I}^{2-})/\varepsilon_2(\mathrm{HI}^-)} \tag{6}$$

where the numbers 1 and 2 refer to the wavelengths chosen. For the best sensitivity, the wavelengths corresponding to the absorbance maxima of the base (I^{2-}) and acid (HI^-) forms, respectively, are used. The various terms ε are the extinction coefficients of the specified species at wavelengths 1 and 2, respectively.

4. Apparatus

4.1 Flexible drawing tube

Approximately 40 cm long, sized to fit snugly over cell port. Silicone rubber is suitable for this (see Footnote 1 in SOP 1).

4.2 Spectrophotometric cells

These should be made of optical glass with a 10 cm path-length, two ports and polytetrafluoroethylene (Teflon®) stoppers. A sufficient number of cells are needed to collect all the samples that will be analyzed from a particular cast (see section 6).

4.3 Micropipette

A micropipette is used to add the dye to the cell. It should be of ~ 0.1 cm^3 capacity with a narrow Teflon® tube attached to act as a nozzle.

4.4 High-quality spectrophotometer

For work of the highest sensitivity and precision, a double-beam spectrophotometer is desirable. However, good results can be obtained with a high-quality single-beam instrument.

4.5 Temperature-control system for spectrophotometer cell

Commercially manufactured, thermostated spectrophotometer compartments that can accommodate 10 cm cells are rarely available and one will probably have to be custom-made. The temperature should be regulated to within 0.1°C.

4.6 System to warm samples to measurement temperature

Although it is possible to warm up the cells containing samples in Ziploc® bags in a thermostat bath, this is inconvenient. It is much better to build a custom-made thermostated compartment that can hold approximately 12 cells at once without getting them wet.

4.7 Thermostat bath (± 0.05°C)

A thermostat bath is used to regulate the temperature of the cell compartment and the temperature of the system described in section 4.6.

5. Reagents

5.1 Solution of *m*-cresol purple

A concentrated (at least 2 mmol dm^{-3}) dye solution of known pH adjusted to be in the range 7.9 ± 0.1 pH units—chosen to match pH measurements from an oceanic profile—is required; this implies that for *m*-cresol purple $A_1/A_2 \approx 1.6$.[1]

6. Sampling

Draw the sample—using the drawing tube—directly from the Niskin bottle (or other water sampler) into the optical cell. After flushing with several hundred cm^3 of sea water—a flushing time of 15–20 seconds—seal the cell with the Teflon® caps ensuring that there is no headspace. Since the pH samples must be analyzed immediately, there is no long-term storage or preservation protocol. However, while awaiting analysis, store the samples in the dark at room temperature.

[1] The absorbance ratio of a concentrated dye solution can be measured using a cell with a short path length (0.5 mm).

7. Procedure

7.1 Warm sample cell to 25.0°C (± 0.1°C)

This is done by placing a number of cells in a thermostated compartment (see section 4.6) for a few hours.

7.2 Measure absorbances for the cell + sea water

Clean and dry the exterior of the cell; place the cell in the thermostated sample compartment of the spectrophotometer. Measure and record the absorbances at three wavelengths: a non-absorbing wavelength (730 nm for *m*-cresol purple) and at the wavelengths corresponding to the absorption maxima of the base (I^{2-}) and acid (HI^{-}) forms of the dye respectively (578 and 434 nm).

7.3 Inject dye into cell

Remove one of the cell caps, add approximately 0.05–0.1 cm^3 of concentrated dye (~2 mmol dm^{-3}) to the sample, replace the cap and shake the cell to mix the sea water and dye. The amount of dye required is that which will produce absorbance values of between 0.4 and 1.0 at each of the two absorbance peaks.

7.4 Measure absorbances of cell + sea water + dye

Return the cell to the spectrophotometer and again measure the absorbances at the three wavelengths used in section 7.2. Cells should be positioned to maintain consistent alignment(s) between baseline and indicator absorbance measurements.

8. Calculation and expression of results

8.1 Correction of measured absorbances

At each of the three wavelengths, subtract the absorbances measured for the background measurement (without dye) from the corresponding absorbances measured for the system containing dye.

In addition, the absorbance measured at a non-absorbing wavelength is used to monitor and correct for any baseline shift due to error in repositioning the cell, instrumental shifts, *etc.*[2]. This assumes that the magnitude of any observed baseline shift is identical across the visible spectrum. To do this, subtract the measured shift from the background-corrected absorbances at wavelengths 1 and 2 to obtain the final corrected absorbance value at each wavelength.

These final absorbance values, corrected for background absorbances *and* any observed baseline shifts, are used to calculate A_1/A_2, the absorbance ratio which describes the extent of protonation of the dye.

[2] The difference between the baseline absorbance (sea water only) and the absorbance of the sample + dye at 730 nm should be no greater than ± 0.001; if this value is exceeded, the cell should be removed and the optical windows cleaned before the absorbances are measured again.

8.2 Calculation of the pH of the sea water + dye

The pH of the sea water and dye in the cell is computed from

$$\mathrm{pH} = \mathrm{p}K_2 + \log_{10}\left(\frac{A_1/A_2 - \varepsilon_1(\mathrm{HI}^-)/\varepsilon_2(\mathrm{HI}^-)}{\varepsilon_1(\mathrm{I}^{2-})/\varepsilon_2(\mathrm{HI}^-) - (A_1/A_2)\varepsilon_2(\mathrm{I}^{2-})/\varepsilon_2(\mathrm{HI}^-)}\right) \tag{7}$$

where pK_2 is the acid dissociation constant for the species HI^- (expressed on the total hydrogen ion concentration scale in mol kg-soln^{-1}), and A_1 and A_2 are the corrected absorbances measured at the wavelengths corresponding to the absorbance maxima of the base and acid forms, respectively. The various extinction coefficient terms ε correspond to values measured for the specified species at wavelengths 1 and 2, respectively (Table 1).

Table 1 Extinction coefficient ratios for *m*-cresol purple.

$\varepsilon_1(\mathrm{HI}^-)/\varepsilon_2(\mathrm{HI}^-)$	0.00691
$\varepsilon_1(\mathrm{I}^{2-})/\varepsilon_2(\mathrm{HI}^-)$	2.2220
$\varepsilon_2(\mathrm{I}^{2-})/\varepsilon_2(\mathrm{HI}^-)$	0.1331

$\lambda_1 = 578$ nm; $\lambda_2 = 434$ nm.

The equilibrium constant K_2 is a function of salinity and temperature and has been determined by careful laboratory measurements[3]. For *m*-cresol purple,

$$\mathrm{p}K_2 = \frac{1245.69}{(T/\mathrm{K})} + 3.8275 + 0.00211(35 - S) \tag{8}$$

where $293 \le T/\mathrm{K} \le 303$ and $30 \le S \le 37$.

8.3 Correction for pH change resulting from addition of the dye

The addition of indicator dye to the sea water sample will perturb the pH (another acid–base system has been added!). Although care is taken to minimize this (by adjusting the dye solution pH), it is desirable to correct for the addition of dye to obtain the best pH measurements.

Although, in principle, the pH perturbation could be calculated from a knowledge of the equilibrium chemistry of the sample and the dye, it is simpler to evaluate the magnitude of the correction empirically. A pair of additions of dye is made to each of a series of sea water samples with different pHs, and the change in the measured ratio (A_1/A_2) with the second addition of indicator solution is determined as a function of the measured value (A_1/A_2) determined after the first addition of dye using a least-squares procedure (SOP 23):

$$\frac{\Delta(A_1/A_2)}{V} = a + b(A_1/A_2) \tag{9}$$

[3] Although DelValls and Dickson (1998) have suggested that this pK_2 may be in error because of an error in calibrating TRIS buffer, it seems that there may be a compensating error that largely mitigates the proposed correction. The pK_2 given here is that from Clayton and Byrne (1993).

where V is the volume of dye added at each addition. The final, corrected, absorbance ratio is

$$(A_1 / A_2)_{corr} = (A_1 / A_2) - V[a + b(A_1 / A_2)] . \qquad (10)$$

8.4 Example calculation

$$t = 25°C,$$
$$S = 35,$$
$$pK_2 = 8.0056,$$

and for indicator stock solution with $A_1/A_2 = 1.6$,

$$.\frac{\Delta(A_1 / A_2)}{V} = 0.125 - 0.147(A_1 / A_2) ..$$

Measured absorbances:

Sea water: $A_{434} = 0.02433$; $A_{578} = 0.01936$; $A_{730} = 0.08365$
Dye + sea water: $A_{434} = 0.45123$; $A_{578} = 0.84574$; $A_{730} = 0.08298$

After addition of dye,

$$A_1 / A_2 = \frac{0.84574 - 0.01936 - (0.08298 - 0.08365)}{0.45123 - 0.02433 - (0.08298 - 0.08365)} = 1.93430.$$

Corrected to zero dye addition ($V = 0.08$ cm^3),

$$\begin{aligned}(A_1 / A_2)_{corr} &= 1.93430 - 0.08[0.125 - 0.147(1.93430)] \\ &= 1.94705 \qquad (11)\end{aligned}$$

and thus

$$pH = 8.0056 + \log_{10}\left(\frac{1.94705 - 0.00691}{2.2220 - 1.94705 \times 0.1331}\right) = 8.0005.$$

9. Quality assurance

9.1 For general principles of analytical quality control see Chapter 3

9.2 Specific applications of analytical quality control

9.2.1 Spectrophotometer performance

The spectrophotometric performance of the instrument used can be confirmed using reference materials that are available from the U.S. National Institute for Standards and Technology (NIST). SRM 2034 is a holmium oxide solution in a sealed cuvette that allows the wavelength accuracy of the spectrophotometer to be determined; SRM 930d is a set of absorbance filters that allows the

absorbance measurement accuracy to be verified. Property control charts of these measurements should be maintained, and the spectrophotometer adjusted if it goes out of tolerance. (Nevertheless, the procedure detailed here is fairly insensitive to minor changes in spectrophotometer performance.)

A more important concern is that the spectrometer must have a high stability. This can be confirmed by making a series of repeated measurements on a system of constant absorbance (*e.g.*, SRM 930d or a thermostated buffer solution containing indicator dye) and computing the standard deviation at the wavelengths of interest.

9.2.2 Precision

A precision of better than 0.001 pH units (1 SD) is possible with care—particularly in regard to the sample handling. The results of duplicate analyses should be plotted on a range control chart (SOP 22).

9.2.3 Bias

The bias of spectrophotometric pH measurements depends on the accuracy with which the various extinction coefficient ratios were determined, and on the accuracy of the values assigned to the values of pK_2. A significant advantage of spectrophotometric measurements is that, if more accurate information becomes available for these parameters at a later time, the pH results obtained can be adjusted without any degradation in precision provided that the original data are retained. At present, the likely bias is estimated to be less than 0.005 pH units.

10. Bibliography

Byrne, R.H. and Breland, J.A. 1989. High precision multiwavelength pH determinations in seawater using cresol red. *Deep-Sea Res.* **36**: 803–810.

Byrne, R.H., Robert-Baldo, G., Thompson, S.W. and Chen, C.T.A. 1988. Seawater pH measurements: an at-sea comparison of spectrophotometric and potentiometric methods. *Deep-Sea Res.* **35**: 1405–1410.

Clayton, T.D. and Byrne, R.H. 1993. Spectrophotometric seawater pH measurements: total hydrogen ion concentration scale calibration of m-cresol purple and at-sea results. *Deep-Sea Res.* **40**: 2115–2129.

DelValls, T.A. and Dickson, A.G. 1998. The pH of buffers based on 2-amino-2-hydroxymethyl-1,3-propanediol ('tris') in synthetic sea water. *Deep-Sea Res.* **45**: 1541–1554.

Dickson, A.G. 1993. The measurement of sea water pH. *Marine Chem.* **44**: 131–142.

Annex III
References

References

UNEP/MAP/MED POL, 2005. Sampling and Analysis Techniques for the Eutrophication Monitoring Strategy of MED POL. MAP Technical Reports Series No. 163. UNEP/MAP, Athens, 46 pp.

UNEP/MAP, 2019. UNEP/MED WG.467/5. IMAP Guidance Factsheets: Update for Common Indicators 13, 14, 17, 18, 20 and 21: New proposal for candidate indicators 26 and 27.

UNEP/MAP, 2019a. UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution.

UNEP/MAP, 2019b. UNEP/MED WG.463/10. Schemes for Quality Assurance and Control of Data related to Pollution.

Dissolved oxygen

Benson B.B., Krause D. Jr., 1980. The concentration and isotopic fractionation of gases in freshwater in equilibrium with atmosphere. Limnol. Oceanogr., 25: 662-671.

Benson B.B., D. Krause, Jr., 1984.The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with atmosphere. Limnol. Oceanogr., 29: 620-632.

Grasshoff, K., 1983. - Determination of oxygen. In: Grasshoff, K., Ehrhardt M., Kremling K. (eds), Methods of Seawater Analysis, Verlag Chemie, Weinheim: 61-72.

Millero F.J., 1986. Solubility of oxygen in seawater. UNESCO Technical Papers in Marine Science, 50: 13- 17.

Tijssen S.B., Van Bennekom A.J., 1989. High precision determination of dissolved oxygen. ICES C.M. 1989/c:6, Annex C: 11-12.

UNESCO. 1973. International oceanographic tables, Vol. 2. NIO-UNESCO, Paris.

Weiss, R.F., 1970. The solubility of nitrogen, oxygen and argon in water and seawater. Deep-Sea Research, 17: 721–735.

Winkler, L.W., 1888. Die Bestimmung des in Wasser gelösten Sauerstoffen. Berichte der Deutschen Chemischen Gesellschaft, 21: 2843–2855.

pH

Dickson, A.G.,1993. The measurement of sea water pH. Mar. Chem., 44: 131-142.

Dickson, A.G., Sabine, C.L. and Christian, J.R. (Eds.) 2007. Guide to Best Practices for Ocean CO2 Measurements. PICES Special Publication 3, 191 pp.

Gieskes, J. M., 1969. Effects of temperature on the pH of seawater. Limnology and Oceanography Vol 14 Issue 5, p 679-685.

ISO 10523: Water quality – Determination of pH.

ISO 11352: Water quality – Estimation of measurement uncertainty based on validation and quality control data.

ISO 17025: General requirements for the competence of testing and calibration laboratories.