INTERNATIONAL STANDARD

ISO 5667-26

First edition
2022-11

Water quality — Sampling —

Part 26:
Guidance on sampling for the parameters of the oceanic carbon dioxide system

Qualité de l'eau – Echantillonnage —

Partie 26: Lignes directrices pour l'échantillonnage d'eau de mer en vue de l'analyse des formes du carbone

Reference number
ISO 5667-26:2022(E)

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Published in Switzerland

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 6, *Sampling (general methods)*.

A list of all parts in the ISO 5667 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The ocean is currently absorbing about one quarter of the carbon dioxide that humans are emitting. When carbon dioxide combines with seawater, chemical reactions occur that reduce the seawater pH, hence the term ocean acidification. Acidification can affect many marine organisms, but especially those that build their shells and skeletons from calcium carbonate. Over the past few years, several high-profile reports have highlighted the urgent need to better understand the effects of changes in carbonate chemistry on marine organisms and ecosystems. Research in this field was limited to a few groups around the world until recently but the number of scientists involved in ocean acidification research has been rapidly rising over the past few years. The reliable characterization and manipulation of the carbonate system involves good analytical skills and measuring facilities and continuous monitoring of seawater chemistry in the field and during experimentation. The predictive power of field surveys and the robustness of results from experiments critically depend on proper sampling and experimental protocols.

The oceanic carbonate system can be described by measuring at least two parameters of the following four parameters, total dissolved inorganic carbon (C_{T}), total alkalinity (A_{T}), fugacity of carbon dioxide (f_{CO2}) and pH in seawater. This document describes how to collect and preserve discrete seawater samples, from a Niskin bottle or other water samplers, for the analysis of four measurable inorganic carbon parameters including: $C_{\mathrm{T},}$ $A_{\mathrm{T},}$ f_{CO2} and pH, according to highest standard levels accepted by global ocean carbon community.

NOTE This document is based on Reference [5].

Water quality — Sampling —

Part 26:
Guidance on sampling for the parameters of the oceanic carbon dioxide system

1 Scope

This document specifies how to collect discrete seawater samples, from a Niskin or other water sampler, that are suitable for the analysis of the four measurable inorganic carbon parameters: total dissolved inorganic carbon, total alkalinity, pH and CO_2 fugacity.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-14, *Water quality — Sampling — Part 14: Guidance on quality assurance and quality control of environmental water sampling and handling*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
total alkalinity
A_T
<sea water> number of moles of hydrogen ion equivalent to the excess of proton acceptors (bases formed from weak acids with a dissociation constant $K \leq 10^{-4,5}$ at 25 °C and zero ionic strength) over proton donors (acids with $K > 10^{-4,5}$) in 1 kg of sample

Note 1 to entry: The formula to determine A_T is:

$$A_T = \left[HCO_3^-\right] + 2\left[CO_3^{2-}\right] + \left[B(OH)_4^-\right] + \left[OH^-\right] + \left[HPO_4^{2-}\right] + 2\left[PO_4^{3-}\right] + \left[SiO(OH)_3^-\right] + [NH_3] + \left[HS^-\right] + \ldots$$
$$- \left[H^+\right]_F - \left[HSO_4^-\right] - [HF] - [H_3PO_4] - \ldots$$

Note 2 to entry: The brackets represent total concentrations of these constituents in solution, $[H^+]_F$ is the free concentration of hydrogen ion and the ellipses stand for additional minor acid or base species that are either unidentified or present in such small amounts that they can be safely neglected. In open ocean water, the concentrations of NH_3 and HS^- are typically so low that they can be neglected; they may, however, be significant in anoxic environments.

3.2
total dissolved inorganic carbon

C_T

dissolved inorganic carbon content of sea water is defined as

$$C_T = \left[CO_2^*\right] + \left[HCO_3^-\right] + \left[CO_3^{2-}\right]$$

where the brackets represent total concentrations of these constituents in solution (in µmol kg^{-1}) and $[CO_2^*]$ represents the total concentration of all unionized carbon dioxide, whether present as H_2CO_3 or as CO_2

3.3
fugacity

f

chemical potential of an individual component of a vapor phase

Note 1 to entry: The fugacity of carbon dioxide, f_{CO2}, is not the same as its partial pressure the product of mole fraction and total pressure, $x_{CO2} \cdot p$ but rather takes account of the non-ideal nature of the gas phase. The fugacity of gas such as CO_2 can be determined from knowledge of its formula of state:

$$f_{CO2} = x_{CO2} \cdot p.\exp\left[\frac{1}{RT}\int_0^p (V_{CO2} - RT\, p')\, dp'\right]$$

where

x_{CO2} is the mole fraction of CO_2;

p is the total pressure;

p' is the partial pressure of CO_2;

V_{CO2} is the volume of CO_2;

R is the molar gas constant which is 8.31446261815324 J K^{-1} mol^{-1};

T is the temperature in kelvin.

It should be noted that to apply the results of four measurable inorganic carbon parameters to calculate carbonate chemistry of seawater, the in-situ temperature, pressure, salinity as well as phosphate and silicate concentration of the seawater may need to be measured.

3.4
total hydrogen ion concentration

total hydrogen ion concentration of sea water is defined as:

$$\left[H^+\right] = \left[H^+\right]_F (1 + S_T / K_S)$$

$$\approx \left[H^+\right]_F + \left[HSO_4^-\right]$$

where

$[H^+]_F$ is the free concentration of hydrogen ion in sea water;

S_T is the total sulfate concentration ($[HSO_4^-]+[SO_4^{2-}]$);

K_S is the acid dissociation constant for HSO_4^-

3.5

pH

negative of the base 10 logarithm of the hydrogen ion concentration:

$$\mathrm{pH} = -\log_{10}\left[\mathrm{H}^{+}\right]$$

where $\left[\mathrm{H}^{+}\right]$ is the hydrogen ion concentration, expressed in expressed in mol kg-soln $^{-1}$

4 Principle

For the analysis of A_T, C_T, pH and f_{CO2} in seawater, samples are collected in clean glass containers in a manner designed to minimize gas exchange with the atmosphere.

NOTE CO_2 exchange affects the various carbon parameters to differing degrees ranging from the very sensitive CO_2 fugacity, f_{CO2}, to alkalinity which is not affected by gas exchange.

If the sample is treated with a mercuric chloride solution to prevent biological activity, this occurs prior to the container being closed to prevent exchange of carbon dioxide or water vapour with the atmosphere.

5 Apparatus

5.1 General

The sample containers are somewhat different depending on which parameter is being collected, but the basic concept is similar for the four possible inorganic carbon samples. In general, a flexible plastic drawing tube, a clean glass sample container with stoppers, a container and dispenser for the mercuric chloride solution (if it is being used) and a sampling log to record when and where each of the samples were collected.

5.2 Drawing tube

Tygon® tubing1) is normally used to transfer the sample from the Niskin water sampler to the sample container (5.3); however, if dissolved organic carbon samples are being collected from the same Niskins, then it may be necessary to use silicone tubing to prevent contamination from the Tygon®. The drawing tube can be pre-treated by soaking in clean seawater for at least one day. This minimizes the amount of bubble formation in the tube when drawing a sample.

5.3 Sample container

5.3.1 General

Sample containers depends on the parameter being measured, volume of sample required for analysis, length of anticipated storage and collection method. Important considerations in bottle choice include volume, leaching of bottle material, gas permeability, opening size, neck size, and sealing[1]. Ideally, sample containers should be prepared by cleaning in a 1 M HCl bath for approximately 24 h, followed by rinsing for approximately 24 h in Milli-Q water (18,2 MΩ cm^{-1} resistivity). However, care shall be taken to remove all residual acid during rinsing. Then, containars should be wrapped in aluminium foil and placed in a 450 °C muffle furnace for 4 h to remove organic carbon.

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1) Tygon® tubing is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

5.3.2 Samples for f_{CO2} measurement

Typically, the f_{CO2} samples are analysed directly from a sample container. Borosilicate glass should be used along with a cap that is impermeable to gas exchange (e.g. 500 cm^3 volumetric flasks that have been pre-calibrated for a documented volume and sealed with screw caps that have internal plastic conical liners). f_{CO2} samples should be collected with a headspace approximately 1 % of the bottle volume. This is done to prevent possible breakage due to the expansion of the sample if its temperature increases. Required sample volume depends on instrument and analysis. Systems for measuring discrete f_{CO2} samples are not common and are often custom made in research laboratories. More commonly, f_{CO2} measurements are taken using a flow-through system which present a separate set of protocols to be followed to ensure that a representative environmental sample is measured[1].

5.3.3 Samples for C_T and A_T measurement

For C_T and A_T, high quality borosilicate glass bottles, such as Schott Duran2) (l.c.e. $32 \times 10^{-7} K^{-1}$), are recommended for both temporary and longer-term storage. The bottles should be sealed by using greased ground glass stoppers held in place with some form of positive closure, or in some alternate gas-tight fashion (e.g. some groups use screw-cap bottles with apparent success, but this method has not been thoroughly tested and should not be used if samples are stored for long extended periods). Storing samples in soda lime glass is not recommended because it can cause significant increases in alkalinity concentrations as seawater can leach sodium and other compounds from the glass over time (see Reference [7]). Alkalinity samples are less sensitive to gas exchange than C_T samples so it is less critical but still advisable to collect them in gas impermeable containers.

5.3.4 Samples for pH measurement

Samples for pH are typically analysed directly from the sample containers. For spectrophotometric pH measurements[2], the samples are collected directly into 10 cm path-length optical cells and sealed with polytetrafluoroethylene (Polytetrafluoroethylene (PTFE)®3)) caps ensuring that there is no headspace. In the case of applying wet chemical flow spectrophotometric systems for determination of pH (e.g. CONTROS HydroFIA®4)), custom Schott Duran borosilicate glass bottles that is impermeable to gas exchange can be used for collection of samples[3]. Samples collected for analysis using the potentiometric method shall be collected with minimal headspace and the volume should be sufficient to allow the electrode to be immersed. Due to gas exchange driven pH changes and ion consumption by the electrode, a larger volume of sample collected potentially gives more stable and precise readings[1]. The headspace should be entirely avoided when filling the sample container.

5.4 Mercury dispenser

Any appropriately sized Eppendorf pipette can be used to add the mercuric chloride solution; it can be more convenient to use a repipetter that can be mounted near the sample collection area. All equipments should be properly labelled for safety.

6 Reagent: Mercuric chloride solution, $HgCl_2$

Samples are collected for f_{CO2}, C_T and A_T, and in some cases, should be preserved with a mercuric chloride solution to stop biological activity from altering the carbon distributions in the sample container before analysis. A typical solution is saturated mercuric chloride in deionized water. However, saturated solutions have been known to clog the pipette in very cold weather, so some laboratory examiners use

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2) Schott Duran borosilicate glass bottle is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

3) Polytetrafluoroethylene (PTFE)® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

4) CONTROS HydroFIA® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

twice the volume of a 50 % saturated solution. Standard volumes are used for saturated solutions are 0,05 % to 0,02 % of the total sample volume.

7 Procedure

7.1 General

Collection of water at sea from the Niskin bottle (or other sampler) shall be done immediately after opening the sampler and before much other water has been removed from it. This is necessary to minimize the exchange of CO_2 with the air space in the sampler which affects all carbon parameters except A_T. Other gas samples (e.g. He, CFCs, O_2) have faster exchange rates than CO_2 and are usually sampled before carbon, but it is desirable that the carbon samples be collected before the Niskin bottle is half empty and within 10 min of it being first opened. The recommended sampling order for inorganic carbon parameters is f_{CO2}, pH, C_T, then, A_T.

7.2 Filling procedure

7.2.1 Rinse the sample bottle

If the bottle is not already clean, rinse it twice with 30 cm^3 to 50 cm^3 of sample to remove any traces of a previous sample.

7.2.2 Fill the sample bottle

Fill the bottle smoothly from the bottom using a drawing tube which extends from the Niskin drain to the bottom of the glass sample bottle. For f_{CO2}, pH and C_T, it is critical to remove any bubbles from the draw tube before filling. Overflow the water by at least a half, and preferably by a full, bottle volume.

NOTE The amount of overflow water can be estimated by measuring how long it takes to fill a sample bottle and allowing the water to flow for a period of 1,5 times.

Samples containing high biological and inorganic particulate matter can need filtration before preservation. Filtering, however, can have a significant effect on carbonate measurements for f_{CO2}, pH, and C_T due to pressure changes and turbulence in most filtering apparatus. Benchtop and syringe filtering using glass fibre filters should not be used because their use will lead to atmospheric exchanges and, thus, alter the carbonate chemistry. Reference [4] describes a method using a peristaltic pump in conjunction with a membrane filter to remove phytoplankton and $CaCO_3$ particles from a sample without altering the carbonate chemistry of the sample.

7.2.3 Adjust the headspace

A headspace of 1 % of the bottle volume is left to allow for water expansion (see Annex A for this procedure), i.e. 2,5 cm^3 for a 250 cm^3 bottle. This can be achieved by pinching off the draw tube before removing it from the sample bottle or removing excess water using a plastic pipette with a bulb. pH samples should not have a headspace.

7.2.4 Prevent biological activity in the sample

A saturated mercuric chloride solution (in deionized water) is the standard preservative used for seawater carbonate system sampling. Immediate preservation is advised in SOPs, but there is no clear guidance on how quickly samples degrade after collection and what exactly constitutes immediate preservation (i.e. delays less than seconds, minutes, or hours) which is largely related to the relative productivity of the waters[1]. Water samples with high levels of nutrients and organic matter probably require more immediate fixing. Freezing is not an acceptable method of preserving samples. In any case, it has been suggested that a well-preserved sample in a high-quality sealed borosilicate glass bottle has a shelf life of at least one year[1]. The f_{CO2} and C_T samples should be preserved with mercuric chloride solution at the time of sampling. The A_T samples have historically been preserved as well, but tests have suggested that preserving can possibly not be required if open ocean samples are kept in the dark at room temperature and are analysed within 12 h. Samples for pH are typically not preserved. In general, there is no accepted method for the preservation of pH samples, so many investigators proposed direct in situ measurement or immediate analysis of collected samples (i.e. within seconds or minutes)[1].

If mercuric chloride is added to preserve the sample; the recommended minimum amount is about 0,02 % by volume of a saturated aqueous solution. Thus, to preserve a 250 cm^3 sample requires 0,05 cm^3 (50 µl) of saturated mercuric chloride (or 0,10 cm^3 of a 50 % saturated solution). Maximum amount is 0,1 % by volume of a saturated aqueous solution, or a smaller percentage than measurement precision of C_T and f_CO2.

In some cases (e.g. in the countries that use of mercuric chloride is avoided), the samples should be analysed quickly (i.e. within a few hours) and preservation of properly handled samples can be unnecessary[1]. Proper handling of samples can include keeping them cool and dark after collection to slow down biological activity. Storing samples in a refrigerator[5] or a cooler with ice or cold packs is recommended.

7.2.5 Close the bottle

Seal the bottle carefully to ensure that it remains gas-tight. To form an airtight seal, the stoppers should be greased. Apiezon® L grease[5)] has been found suitable for this purpose; other greases can also be suitable. Care should be taken not to transfer the grease onto the Niskin bottle as this can interfere with other analyses. If it is to be sealed using a greased ground glass stopper, first wipe the excess water from the ground glass in the bottle neck, then insert the stopper completely, and finally twist the stopper to squeeze the air out of the grease to make a good seal.

7.2.6 Secure the lid

Use a rubber band or other positive closure and then invert the bottle several times to disperse the mercuric chloride solution thoroughly.

7.3 Sample storage

The samples should be stored in a cool, dark, location (preferably refrigerated but not frozen) until use (see Reference [6] for preservation and handling of water samples). It has been suggested that a well-preserved sample for C_T and A_T in a high-quality sealed borosilicate glass bottle has a shelf life of at least one year[1]. Some studies suggest that polypropylene (PP) or high-density polyethylene (HDPE) bottles can be used for A_T sample storage and small volume borosilicate glass vials with screw caps can be used for C_T sample storage for a period of at least 1,5 months[7]. To ensure data quality, we recommend that A_T and C_T measurements should be completed as soon as possible when using this kind of storage methods.

7.4 Sample documentation

The following information shall be recorded in the sampling logbook at the time of sampling:

- time and date when taken;
- full name of person who took sample;
- location: an unambiguous designation of the station, cast and bottle number from which the sample was taken;
- container designation: a number or alphanumeric symbol unique to the sample container;
- comments: additional information such as conditions when sampling, problems with sample collection, etc.

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5) Apiezon® L grease is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

8 Assurance and quality control

Quality control measures the quality requirement of a process and uses techniques to correct any deviation from a process. All material coming into contact with the sample should be subject to quality control to ensure that there is no contamination and/or loss of analytes. These quality controls are to be expected when acquiring equipment or when modifying/adapting it.

Some duplicate sampling shall be used, both from the same sampler (e.g. Niskin bottle) and, if possible, from two samplers tripped together at the same depth, to assess the quality of the sampling procedures in accordance with ISO 5667-14. Comparing measurements with a certified reference material, intercalibration/comparing measurements between different laboratories performing the same analysis and the other techniques should be included in the QA/QC section of the corresponding SOP of each parameter (when available or organized for the parameter).

Annex A
(informative)

Determination of the size of a headspace in the sample bottle

The volume of the headspace is chosen so as to leave room for expansion of the sea water on warming, while being sufficiently small to minimize the amount of gas exchange between the headspace and the bulk of the sample. The closure system shall be adequate to retain the pressure exerted by the expansion.

The apparent change in the volume of a fixed mass of sea water can be calculated by allowing for the change in the density of the sea water and the expansion of the glass container. The total change over the temperature range 0 °C to 40 °C is about 1 %. (The effect of expansion on the volume of the borosilicate glass bottle is only 0,04 % over this range.) One third of this expansion occurs on heating the water from 0 °C to 20 °C, the remaining two thirds on heating it from 20 °C to 40 °C.

The pressure in the headspace of a container heated from a temperature t_1 to t_2 can be estimated, allowing for the following:

- the expansion of the sea water in the bottle,
- the change in solubility of gases such as N_2, O_2, Ar,
- the thermal expansion of the gas phase, and
- the change in the vapour pressure of H_2O in the gas phase.

The initial headspace ratio, *r*, is defined as:

$$r = \frac{V_{\text{headspace}}}{V_{\text{sea water}}} \tag{A.1}$$

where

$V_{\text{headspace}}$ is the volume of the headspace;

$V_{\text{sea water}}$ is the volume of the sea water.