Ocean Sci. Discuss., 11, 2447–2459, 2014
www.ocean-sci-discuss.net/11/2447/2014/
doi:10.5194/osd-11-2447-2014

Ocean Science
Discussions
Open Access

Technical Note: How long can seawater oxygen samples be stored before titration?

M. Lankhorst[1], G. Chavez[1], S. H. Nam[2], and U. Send[1]

[1]Scripps Institution of Oceanography, 9500 Gilman Drive, La Jolla, CA 92093, USA
[2]Research Institute of Oceanography/School of Earth and Environmental Sciences, Seoul National University, Seoul 151-742, Republic of Korea

Received: 8 October 2014 – Accepted: 8 October 2014 – Published: 29 October 2014

Correspondence to: M. Lankhorst (mlankhorst@ucsd.edu)
and S. H. Nam (namsh@snu.ac.kr)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

The concentration of dissolved oxygen in seawater is routinely measured using a standardized titration method that involves analysis shortly after the water sample has been collected. However, none of the existing procedural documents are specific about how soon after collection the titration has to be done. Here, we report on a small number of samples where duplicates were collected and one batch was titrated within days after collection, while the other batch was stored for several weeks before titration. In addition, for a subset of the samples a third batch was taken that was stored like the others but with a particular chemical already added before storage. Comparison between the batches confirms that there is no significant difference between the ones that were stored and the ones that were analyzed sooner, indicating that a month-long storage period is acceptable. The implication of this is that such oxygen samples do not necessarily have to be analyzed while still on the ship; instead, it is possible to transport them ashore for analysis there.

1 Introduction

The method to determine the concentration of dissolved oxygen in seawater that is typically used in oceanography today has been described by Carpenter (1965b), and is in turn based on the method by Winkler (1888). Carpenter (1965a) reports that the accuracy of the method is 0.1 %. This would amount to 0.006 mL L^{-1} for the higher concentrations presented here, but this error estimate refers to the lab procedure alone and does not include the errors introduced by handling the samples prior to analysis, especially not drawing the samples on the ship. A more recent description of the method has been given by Dickson (1996) as part of the WOCE program (World Ocean Circulation Experiment), who demands the precision of the measurements by one operator to not exceed 0.5 µmol kg^{-1}, and the bias between different operators to not exceed 2.0 µmol kg^{-1}. These numbers correspond to 0.011 and 0.046 mL L^{-1}, respectively. The precision is understood as the repeatability of the measurement, and the inter-operator

OSD
11, 2447–2459, 2014

bias is essentially the absolute accuracy of the method including on-board procedures. An automated endpoint detection method for the titration can achieve even higher precision (0.15 µmol kg^{-1} or 0.003 mL L^{-1}, Langdon, 2010). Again, this number does not take into account sampling error from handling the water prior to analysis, nor is it an estimate of total accuracy or inter-operator bias.

The purpose of the oxygen water samples used for this study was to calibrate and validate autonomous electronic oxygen sensors that were either on a profiling CTD system or on a mooring. The instruments used were of two different types: one utilizing a polarographic membrane (Clark et al., 1953), the other an optode (Klimant et al., 1995). The polarographic membrane types were Sea-Bird Electronics (SBE) model 43, and the optodes were varying models, including model 4831, made by Aanderaa Data Instruments (AADI). The online specification sheets for the SBE 43 list the initial accuracy of the sensor as 2 % of the measurement value. SBE also offers an optode sensor, for which a manual is available (SBE-63 reference, 2014) that lists the accuracy as the greater of 2 % or 0.07 mL L^{-1}, presumably the same as the SBE 43. The specification sheet of the AADI 4831 (AADI-4831 reference, 2012) defines the accuracy of that optode as the greater of 5 % or 8 µmol kg^{-1} (0.19 mL L^{-1}), but some reports (A. Tengberg, personal communication, 2014; Methods section by Champenois and Borges, 2012) suggest that it is smaller and likely similar to the SBE numbers. These accuracy estimates of the sensors are somewhat larger than those reported for the oxygen titration method, implying that titration can indeed constrain the calibrations of the sensors (and not vice versa). This is especially true when sensor drift over time is added to the above accuracy estimates, which are understood as initial accuracies. These accuracies are included as green background shading in Fig. 1.

A water sample is typically taken with a rosette water sampling system at depth and then brought onto the deck of the ship. At that time, the water is transferred into an Erlenmeyer flask, and certain chemicals are added (manganese chloride, sodium iodide in sodium hydroxide). These flasks are stored at room temperature in the dark, and the stoppers are made airtight by filling the flask brims with deionized water, following the

OSD
11, 2447–2459, 2014

recommendations by Langdon (2010). Standard practice is to proceed with the titration of the samples within a few days, but no sooner than a few hours after sampling to allow the samples to equilibrate in temperature to the lab environment. The aforementioned procedural documents are not specific about just how long this storage period could be. Langdon (2010) suggests between 1.5 h and "many days", provided that the airtight seal is not breached. When we asked experts from several institutions that had been involved in WOCE, the responses varied dramatically: that the samples could not sit at all for extended periods of time, or that the samples could very well be stored for several weeks. In addition, one advice was that the samples would possibly be more stable in storage if a particular chemical (sulfuric acid) from the titration process were already added to the sample prior to storage. The uncertainty in these responses motivated the present study.

2 Data and methods

The data used here come from two cruises on research ships in the northeastern Pacific Ocean: one dataset was collected on the vessel *New Horizon* during cruise NH1409 in the California Current region in April/May 2014, the other one on the vessel *Melville* during cruise MV1404 en route to/from Ocean Station Papa (see Sect. 4.2.3 of Cronin et al., 2012) in June 2014. In all cases, the analysis method by Carpenter (1965b) was used to determine the oxygen concentrations in the samples, following these steps:

- Collect water samples and transfer to Erlenmeyer flasks
- Add "pickling" reagents (manganese chloride, sodium iodide in sodium hydroxide)
- Storage of the samples at room temperature in a dark location, keeping the flask brims filled with deionized water to maintain an airtight seal
- Add sulfuric acid
- Titration using sodium thiosulfate

OSD
11, 2447–2459, 2014

Repeat samples were taken with different storage durations, to judge the effect of that storage. Each one of these repeat samples was obtained from a separate rosette water bottle, such that each one was the first water taken from the rosette bottle. This eliminates contamination by air in a growing headspace in the rosette bottle, which would otherwise adversely affect the later samples. However, it does add an element of environmental variability if there are gradients in the water between the rosette bottles.

2.1 New Horizon cruise NH1409

Repeat oxygen samples from New Horizon cruise NH1409 were collected in three batches for inter-comparison: batch 1 was titrated about a week after collection of the samples and serves as the reference, i.e. is considered to be the batch that was processed "immediately". Batch 2 was stored for about one month in a dark box at room temperature before titration. Batch 3 was treated exactly like batch 2, except that a short time after sampling (circa 30 to 60 min) and before storage, the sample bottles were re-opened and sulfuric acid, a chemical that would otherwise be added just prior to the titration process, was added. Then, the bottles were re-sealed and returned to storage. The process of adding this acid is awkward, because it exposes the samples to air, spills a small amount of the sample, and is likely to trap at least a small air bubble in the sample when re-sealing. All oxygen samples from New Horizon cruise NH1409 were analyzed on shore, i.e. none were titrated on the ship. The titrations of all three batches were done by the Oceanographic Data Facility (ODF) at Scripps Institution of Oceanography.

The raw data are listed in Table 1 and plotted in Fig. 1. Table 3 shows statistics about the differences between the repeat batches. Three outliers are not included in the statistics. For the differences between batches 2 and 1, the mean and SD amount to -0.02 and $0.03\,\mathrm{mL\,L^{-1}}$, respectively. For batches 3 and 1, these values are -0.03 and $0.03\,\mathrm{mL\,L^{-1}}$.

OSD
11, 2447–2459, 2014

2.2 Melville cruise MV1404

Repeat oxygen samples from Melville cruise MV1404 were collected in two batches to analyze the effect of storage on the samples: batch 1 was titrated on board the ship a day after sample collection, and is considered to be the reference that was titrated "immediately". The operator on the ship was from Scripps Institution of Oceanography, but not affiliated with ODF, and used titration equipment that was rented from the Marine Chemistry Laboratory, School of Oceanography, University of Washington. Batch 2 was stored for about two weeks and titrated by ODF ashore. Therefore, the difference between the two batches will contain an element of inter-operator bias, in addition to whatever effect the storage may have had. The raw data from the two batches are listed in Table 2 and plotted in Fig. 1. The mean and SD of the differences between batches 2 and 1, as given in Table 3 and excluding one outlier, amount to 0.004 and 0.007 $\mathrm{mL\,L^{-1}}$.

3 Interpretation and conclusions

Judging the mean and SD values of the differences between repeat batches of oxygen samples, all samples were well suited for their purpose, which was calibration and validation of electronic sensors. This is best seen in Fig. 1, where all samples except for a few outliers fall within the green shading, which shows the target accuracies of the electronic sensors as specified by the manufacturers.

The results from the Melville cruise match particularly well, even though they were obtained by different operators. The agreement is substantially better than the targets for absolute accuracy put forth by Dickson (1996), shown in the introduction here, and both the mean and the scatter are in fact so close to zero that they are smaller than the target for same-operator precision (Dickson, 1996).

The results from the New Horizon cruise show somewhat larger differences, but the mean and SD numbers are still smaller than what Dickson (1996) lists as an acceptable

OSD
11, 2447–2459, 2014

bias between different operators. One possible explanation for the larger scatter in the New Horizon comparisons, compared to the Melville data, is that the data tended to be collected at shallower depths (see pressure data in Tables 1 and 2) where environmental gradients are greater and introduce additional variability, which has nothing to do with the analysis method. The slightly negative means in the New Horizon repeat batches, when compared against the reference batch, seem to be more likely an inter-operator bias rather than an effect of the sample storage. If anything, one would expect the effect of storage and contamination of the samples to be most visible in those samples that have low oxygen concentration and to result in a positive bias, because exposure to the atmosphere would increase the oxygen content. However, the discrepancies appear to be smaller in the samples with low concentrations, and the overall bias is negative. Batches 2 and 3 from the New Horizon data were processed by the same person on the same day, but batch 1 was processed on a different day. Therefore, a slight discrepancy in the day-to-day instrument setup can explain the existence of a small mean bias, and one possible candidate is the setting of the titration "standard" value. This standard determines the strength of the titration solution, and effectively comes into the equations for oxygen concentration as a multiplicative factor (Carpenter, 1965b). Changes in the standard would indeed show greater effect on those samples with greater oxygen concentration, as seem to occur here.

In conclusion, storage of the oxygen samples for up to three weeks prior to titration had no effect on the results that exceeded the target accuracies of the published methodology. Storage conditions were at room temperature, shielded from light, and such that the bottle brims were kept filled with deionized water to make a tight seal. Adding the sulfuric acid to the samples prior to storage did not improve the outcome, but bears an inherent risk of ruining the sample in the process. These results were obtained using a relatively small number of samples from two expeditions only. It remains to be seen if the results remain valid for a larger number of realizations, and for samples from other locations than the subpolar and subtropical North Pacific. If they do, it seems perfectly acceptable to store the samples after they are collected and treated

OSD
11, 2447–2459, 2014

with the first set of chemicals on the ship (manganese chloride and sodium iodide in sodium hydroxide), and only do the titrations on land after the cruise has commenced, or to titrate all samples in a single session towards the end of the cruise rather than doing them in daily batches.

Acknowledgements. This paper and the NH1309 cruise were funded by a grant from the National Oceanic and Atmospheric Administration, US Department of Commerce, under grant NA10OAR4320156. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies.

The MV1404 cruise was part of the Ocean Observatories Initiative (OOI). Funding for the OOI is provided by the National Science Foundation through a Cooperative Agreement with the Consortium for Ocean Leadership. The OOI Program Implementing Organizations are funded through sub-awards from the Consortium for Ocean Leadership.

SungHyun Nam was partly supported by Research Resettlement Fund for the new faculty of Seoul National University and partly by Ministry of Oceans and Fisheries, Korea through "East Asian Seas Time-series (EAST-I)".

References

AADI-4831 reference: Oxygen Optode 4831/4831F, Aanderaa Data Instruments AS, Bergen, Norway, specification sheet, document number D403, 2012. 2449, 2459

Carpenter, J. H.: The accuracy of the Winkler method for dissolved oxygen analysis, Limnol. Oceanogr., 10, 135–140, doi:10.4319/lo.1965.10.1.0135, 1965a. 2448

Carpenter, J. H.: The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method, Limnol. Oceanogr., 10, 141–143, doi:10.4319/lo.1965.10.1.0141, 1965b. 2448, 2450, 2453

Champenois, W. and Borges, A. V.: Seasonal and interannual variations of community metabolism rates of a Posidonia oceanica seagrass, Limnol. Oceanogr., 57, 347–361, 2012. 2449

Clark Jr., L. C., Wolf, R., Granger, D., and Taylor, Z.: Continuous recording of blood oxygen tensions by polarography, J. Appl. Physiol., 6, 189–193, 1953. 2449

OSD

11, 2447–2459, 2014

Cronin, M. F., Weller, R. A., Lampitt, R. S., and Send, U.: Ocean reference stations, in: Earth Observation, InTech, ISBN: 978-953-307-973-8, available at: http://www.intechopen.com/books/earth-observation/ocean-reference-stations (last access: 23 October 2014), 2012. 2450

Dickson, A.: Determination of dissolved oxygen in sea water by Winkler titration, available at: http://cchdo.ucsd.edu/manuals/pdf/91_1/dickson2.pdf (last access: 23 October 2014), 1996. 2448, 2452, 2459

Klimant, I., Meyer, V., and Kühl, M.: Fiber-optic oxygen microsensors, a new tool in aquatic biology, Limnol. Oceanogr., 40, 1159–1165, 1995. 2449

Langdon, C.: Determination of dissolved oxygen in seawater by Winkler titration using the amperometric technique, in: The GO-SHIP Repeat Hydrography Manual: A Collection of Expert Reports and Guidelines, edited by: Hood, E. M., Sabine, C. L., and Sloyan, B. M., IOCCP Report Number 14, ICPO Publication Series Number 134, available at: http://www.go-ship.org/HydroMan.html (last access: 23 October 2014), 2010. 2449, 2450

SBE-63 reference: SBE 63 User's Manual, Sea-Bird Electronics, Inc, Bellevue, WA, USA, version #008, 2014. 2449, 2459

Winkler, L. W.: Die Bestimmung des im Wasser gelösten Sauerstoffes, Ber. Dtsch. Chem. Ges., 21, 2843–2854, 1888. 2448

OSD
11, 2447–2459, 2014

Table 1. Water sample data of dissolved oxygen (DO) in seawater from the New Horizon cruise NH1409. The three batches are described in the text, and the data are shown in Fig. 1. Values that are considered outliers are given in parentheses, and the asterisk* symbol denotes bottles where anomalies were noted during the analysis.

	Batch NH 1 Titrated 6 May 2014 (Reference)	Batch NH 2 Titrated 30 May 2014 (Stored)	Batch NH 3 Titrated 30 May 2014 (Stored with Acid)
Sample Date Pressure [dbar]	Bottle No. DO [mL L^{-1}]	Bottle No. DO [mL L^{-1}]	Bottle No. DO [mL L^{-1}]
28 Apr 2014 577	1609 0.541	1750 (0.261)	1658* (0.936)
28 Apr 2014 458	1660 0.413	1163 0.399	878* 0.428
28 Apr 2014 345	1312 0.656	1790 0.651	1702* 0.669
28 Apr 2014 11	1311 5.861	1759 5.889	1837* 5.825
29 Apr 2014 46	1525 5.906	1666 5.828	1729 5.824
29 Apr 2014 23	1132 5.835	1122 5.826	1852 (5.456)
30 Apr 2014 72	1033 4.302	1733 4.266	1710 4.281
30 Apr 2014 16	726 5.926	1786 5.913	1744 5.893
30 Apr 2014 3	1638 5.956	1336 5.938	1654 5.909

* Known Problems:
Bottle 1658: big bubble in sample, forgot stirbar while titrating, value high,
Bottles 878 and 1702: big bubbles in sample,
Bottle 1837: added acid twice.

OSD
11, 2447–2459, 2014

Table 2. Water sample data of dissolved oxygen (DO) in seawater from the Melville cruise MV1404. The two batches are described in the text, and the data are shown in Fig. 1. Values that are considered outliers are given in parentheses, and the asterisk* symbol denotes bottles where anomalies were noted during the analysis.

	Batch MV 1 Titrated 20 Jun 2014 (Reference)	Batch MV 2 Titrated 3 Jul 2014 (Stored)
Sample Date Pressure [dbar]	Bottle No. DO [mL L^{-1}]	Bottle No. DO [mL L^{-1}]
19 Jun 2014 3492	218 2.873	123 2.890
19 Jun 2014 2903	228 2.381	125 2.383
19 Jun 2014 2000	231 1.203	124 1.206
19 Jun 2014 1117	220 0.416	126 0.420
19 Jun 2014 740	264 0.480	122 0.477
19 Jun 2014 495	256 0.743	121 0.749
19 Jun 2014 65	240* 6.753	132* (2.862)
19 Jun 2014 18	225A 6.624	131 6.621

* Known Problems:
Bottles 132 and 240: suspect temperature in rosette bottles.

OSD
11, 2447–2459, 2014

Table 3. Statistics of differences between repeat batches of water samples. All values denote dissolved oxygen concentration in mL L^{-1}. The underlying data are shown in Fig. 1 and Tables 1 and 2, and exclude four values marked as outliers therein.

No. of Obs.	NH 2 – NH 1 8	NH 3 – NH 1 7	MV 2 – MV 1 7
Median	−0.015	−0.033	0.003
Mean	−0.018	−0.027	0.004
RMS	0.034	0.042	0.007
SD	0.030	0.034	0.007

OSD
11, 2447–2459, 2014

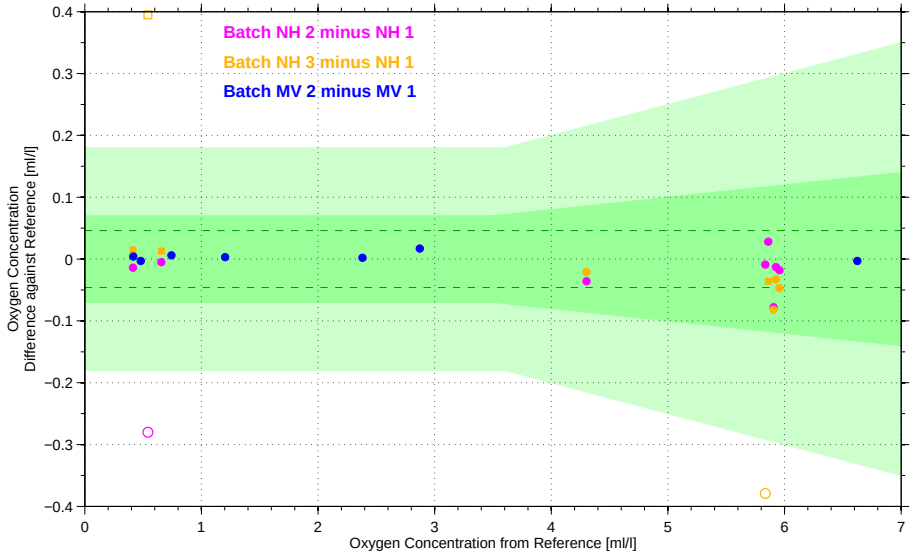

Figure 1. Differences in oxygen concentration determined from the batches that were stored minus the ones that were not. Colors denote the different batches. Data are from Tables 1 and 2. "Good" samples are shown as solid circles. Hollow symbols are outliers that are not used in the statistics. One additional outlier is off the scale and not shown. Square symbols denote samples for which the analyst has noticed anomalies. The green shading indicates accuracy limits of different electronic sensors (AADI-4831 reference, 2012; SBE-63 reference, 2014). Additional dark green dashed lines show the bias requirement from Dickson (1996).

OSD
11, 2447–2459, 2014