On the storage of dissolved inorganic phosphate, nitrate and reactive silicate in Atlantic Ocean water samples

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Abstract

Two unfiltered ocean water samples collected from two different depths (100 m and 2000 m) in the North Atlantic were examined for the effects of storage on nutrients. A total of 300 subsamples were stored in 60 cm^3 polypropylene bottles for a maximum time of 109 days and tested for the effects at −18 °C and 4 °C (with and without the addition of mercury(II)chloride). For phosphate and nitrate, samples stored acceptably only with the freezing technique, with maximum losses in the range of 5–7 %. The silicate samples stored well for all techniques, with no significant variations of the averages during the time of storage. For all three nutrients, however, the variance of subsamples increased considerably with the length of storage time.

Kurzfassung

Zur Lagerfähigkeit von gelöstem Phosphat, Nitrat und reaktivem Silikat in ozeanischen Wasserproben

Die Lagerfähigkeit unfiltrierter ozeanischer Nährstoffproben wurden an zwei Wasserproben aus 100 m und 2000 m Tiefe des Nordatlantiks untersucht. Insgesamt sind 300 Einzelproben, die in 60-cm^3-Polypropylen-Flaschen abgefüllt wurden, für max. 109 Tage bei unterschiedlichen Temperaturen gelagert worden, und zwar bei −18 °C und bei 4 °C (mit und ohne Zugabe von Quecksilber(II)chlorid). Für Phosphat und Nitrat erwies sich die Lagerung bei −18 °C als einzig zufriedenstellende Methode, mit max. Verlusten von etwa 5–7 %. Die Mittelwerte der Silikat-Proben zeigten dagegen während des gesamten Versuches bei keiner Konservierungsmethode signifikante Abweichungen. Bei allen Nährstoffen trat jedoch mit zunehmender Lagerzeit eine deutliche Streuung der Einzelwerte auf.

Introduction

There is little doubt that immediate determination of orthophosphate, nitrate and reactive silicate is the most reliable procedure for seawater samples. This is because nutrients are involved in metabolic processes that may take place rather rapidly. Sometimes, however, when the analysis must be postponed due to rough weather, shortage of personnel, or laboratory space problems, it is necessary to use a sample storage procedure.

The literature on the preservation of samples is voluminous but often very contradictory. It also contains only a few systematic studies, where samples have been stored over several months. Furthermore, most investigations were performed with coastal and

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estuarine waters, and were filtered before the storage tests (see e.g. RILEY 1975; GRASSHOFF et al. 1983; and the literature cited therein). This study presents the data from two unfiltered open-ocean samples (North Atlantic) which had been subjected to three different storage procedures: namely, freezing at −18 °C and storage at 4 °C with and without the addition of mercury(II)chloride. The aim of the investigation was to determine the effects of the different storage procedures on open-ocean samples with respect to accuracy (compared to the on board determination) and precision or scatter; and to study whether the used container material (polypropylene) was suitable for nutrient storage over a longer period of time.

Materials and methods

The two master samples from the North Atlantic were collected at 48° 46.0′ N, 10° 54.9′ W (100 m) and 47° 30.4′ N, 30° 49.9′ W (2000 m) during a cruise of the RV "Meteor" in July/August 1984. Each sample was collected in a 30 dm^3 "Go-Flo" Niskin sampler. A total of 150 unfiltered subsamples were drawn off into 60 cm^3 screw cap polypropylene bottles (Nalgene type No. 2105–0002) which had been originally stored in a 0.1 % v/v HCl solution (after previous cleaning in a detergent solution with subsequent rinsing). Before subsampling, the bottles were rinsed three times with the sample solution. One set of the subsamples (50) was spiked with mercury(II)chloride (10 mg $HgCl_2$ dm^{-3}; higher mercury (II) contents, from our experience, may lead to erratic nitrate measurements if copperized cadmium reductors are used) before analysis of all the subsamples, which was performed within about 18 h of collection (considered as on board or control data). Immediately after completion of the measurements, 50 subsamples had been frozen in upright position at −18 °C, while 100 subsamples (50 being spiked with $HgCl_2$) were stored at 4 °C in the refrigerator. At the shore laboratory, the same subsamples were analysed after three time intervals with a maximum storage time of 109 days and a thaw time for the frozen samples of about 15 h.

The nutrient analysis was performed according to the procedures described by GRASSHOFF et al. (1983) and by means of an automated system (AKEA components). The standards, prepared in artificial or surface seawaters and compared with the Sagami standards, were used for calibration several times each day.

Results and discussion

The results are summarized in Fig. 1 to 3 which illustrate the accuracy and precision of stored samples as a function of time and applied procedure. Figures 4a–4c show the effect of storage time (for the freezing technique) on the sample variability, estimated as coefficient of variation ($s/x \cdot 100$; where x is the arithmetic mean and s the standard deviation).

The total variability in the data (S_{tot}) may be expressed as:

$$S_{tot} = S_{anal} + S_{bl} + S_{stor} ,$$

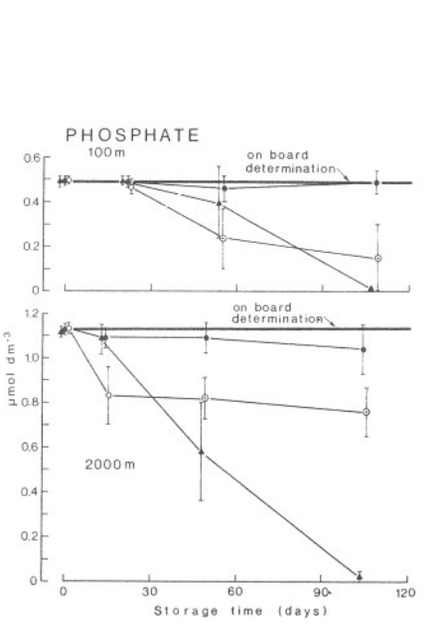
where (1) S_{anal} is the normally reported (analytical) precision based on a set of sequentially analyzed replicates; (2) S_{bl} is the variation caused by analysis on different days, i.e. effects of recalibration, different blanks etc.; and (3) S_{stor} is the variation resulting from the storage procedure. The analytical precision, S_{anal}, has been estimated from unstored subsamples (150) and is considered as constant during the time of experiment. The contribution of S_{bl} to S_{tot} has been proved as negligible in our experiment, very probably because of the unchanged operator and applied analytical procedures. Any change of the total variability with time denotes, therefore, variations which should result from the storage procedures.

Prior to statistical treatment, we removed a number of "maverick" data points (between 4 and 7 out of 150 samples), already identified during the on board measurements and probably caused by inadequate subsampling and "dirty" bottles. Although recognizing the somewhat dubious procedure, we decided to exclude these subsamples from further statistics only if they are outside the mean ±3 st.dev. calculated for the remaining values.

Phosphate

The results in Fig. 1 and 4a illustrate a rather clear tendency for both subsample series. With exception of the frozen 100 m samples, all storage procedures revealed a steady decrease of the orthophosphate mean concentration with time. However, while the refrigerator-stored subsamples (with and without $HgCl_2$ addition) show considerably lower concentrations after relatively short storage times (with losses of approx. 50 % after 60 d), the frozen subsamples deviate by a max. of only 7 % from the on board control measurements. A sample t-test proved, however, that these differences are significant for the frozen subsample averages ($p < 0.01$; KAISER and SPECKER 1956).

The second major result is the general increase of the total variability of subsamples with increasing storage time. This is demonstrated in Fig. 4a showing the coefficient of variation (in %) as a function of time. The indicated scatter for the unstored subsamples on board is ±5.2 and ±2.1 % (at concentration levels of 0.5 and 1.1 µmol dm^{-3}, respectively) which is very similar to the analytical precision reported by KOROLEFF (1983a). This agreement demonstrates also the homogeneity of the original subsamples. We conclude, therefore,



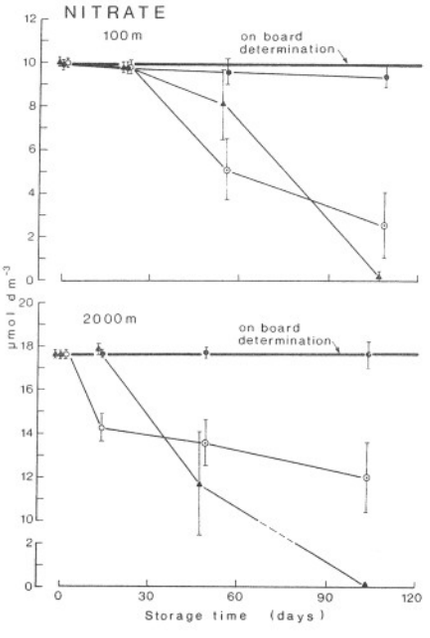


Fig. 1 (left). Phosphate concentration (x ± 1 st.dev.) in stored samples as a function of time. The indicated reference value is the average of approximately 150 on board determinations. The symbols refer to the samples stored at −18 °C (● − ●); 4 °C (○ − ○); 4 °C and frozen after first time-interval (⊙ − ⊙); 4 °C with 10 mg dm^{-3} $HgCl_2$ (▲ − ▲).

Fig. 2 (right). Nitrate concentration (x ± 1 st.dev.) in stored samples as a function of time. For symbols see legend to Fig. 1.

that the storage time must account for most of the increasing scatter, which results in a total coefficient of variation (for the freezing technique) of 10–12 % at the final stage of our experiment (Fig. 4a).

A similar effect was reported by MACDONALD and MCLAUGHLIN (1982), who found for frozen orthophosphate samples a c.v. of about 6 % after a storage time of 5 months. The cause of this scatter (and loss) is uncertain. It seems unlikely that (at −18 °C and in the darkness) the mechanism involves chemical adsorption of orthophosphate ions on the container walls or a phosphorus uptake by primary production. The only reasonable explanation to us, although not proved in our experiments, appears to be that the phosphate is taken up during the freezing (and thawing) period by proliferating microorganisms which attach themselves to the walls of the containers (see also RILEY 1975). This mechanism also seems responsible for the observed losses in the preserved and untreated subsamples, which originally were both stored at 4 °C, but showed for the 2000 m sample rather different concentrations after the first time interval (see Fig. 1). The somewhat different behavior of the 100 m sample is difficult to explain but may be caused by the various organisms or lower nutrient concentrations prevailing in the upper waters.

Nitrate

The results on nitrate show in principle the same trends as for orthophosphate (Fig. 2 and 4b). The storage at 4 °C had a significant effect for both master samples. After the final time interval (ca. 3.5 month storage time), all mercury(II)chloride spiked samples showed nitrate concentration below our detection limit (d.l.) of 0.1 μmol dm^{-3}, probably indicating an inadequate preservation against the ongoing biological activites at that storage temperature. The unpreserved samples, however, being frozen after the first storage interval (ca. 20 days) showed, similar to phosphate, a much slower concentration decrease. The technique of freezing did cause slight, but significant losses ($p < 0.001$) for the 100 m subsamples only. The averages after the second and third time interval are lower by about 3 and 5 %, respectively, in comparison to the on board determinations (Fig. 2).

As in the case of orthophosphate, an increasing sample variance has been noticed with the length of storage time. For the frozen samples, the total variation increased from a c.v. of 1 % during the on board measurements, which is close to the analytical precision reported by GRASSHOFF (1983), to about 3 and 6 % (at concentration levels of about 10 and 17.5 μmol dm^{-3}, respectively) after a 3.5 month storage interval.

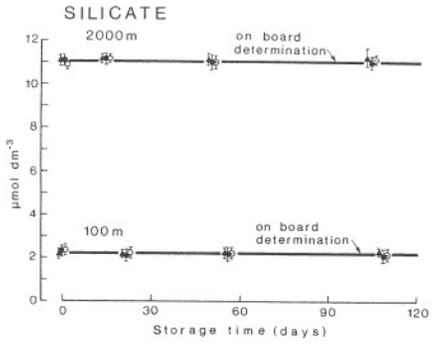


Fig. 3. Silicate concentration (x ± 1 st.dev.) in stored samples as a function of time. For symbols see legend to Fig. 1

Silicate

Figures 3 and 4c summarize the data obtained for silicate determinations. Unlike phosphate and nitrate, the reactive silicate concentrations are neither effected by the storage technique nor by the storage time. All averages (18) from the stored subsamples accurately reflect the on board determinations within the analytical precision. The subsample variability generally increased with storage time, which is similar to the phosphate and nitrate findings. The c.v. change in the 2000 m sample (11 µmol dm^{-3} range), however, was only of minor importance as illustrated for the freezing technique in Fig. 4c. The determined c.v. of about 2 % is in the reported range of the analytical precision (KOROLEFF 1983b). The scatter in the 100 m subsamples, the concentration of which was at the lower end of the oceanic silicate values (2.3 µmol dm^{-3}), increased by a factor of about 3 during the time of storage (Fig. 4c), corresponding to an increase of the standard deviation from 0.1 to about 0.3 µmol dm^{-3}.

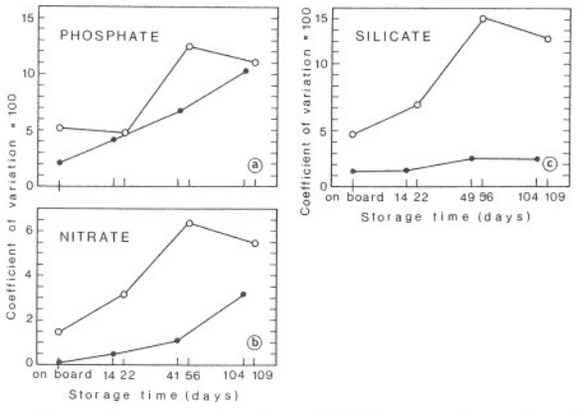


Fig. 4. The effect of sample storage time (at −18 °C) on sample variance for phosphate (a), nitrate (b), and silicate (c) determinations. The symbols refer to 100 m sample (○−○) and 2000 m sample (●−●)

The problem of polymer formation as an effect of freezing was not noticed during our experiments, very probably because of the ocean salinity range and the applied thawing time of our samples (15 h). It has been reported, however, that low salinity samples may show significant losses of silicate (in the range of 5–25 %) even after a 24 h thaw time (KOBAYASHI 1967; MACDONALD and MCLAUGHLIN 1982). This effect has to be considered, especially when coastal or estuarine samples should be stored by freezing technique.

Conclusions

This study demonstrates that, under the applied conditions and with polypropylene subsample bottles, freezing is the most suitable method when oceanic nutrient samples are to be stored for more than 2 weeks. Overall, however, freezing results in a slight decrease in phosphate and nitrate with time and also an increase in the variance of the samples. The causes are uncertain, but have to be attributed to biological activities during the pre-frozen and thawing state of the subsamples. A quick-freezing of the samples on board may offer considerable advantages and should be proved by further experiments. The storage of silicate causes less problems, but may be more complicated for frozen less saline waters.

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