NOTES

Limnol. Oceanogr., 44(4), 1999, 1139–1141

Storage of marine particulate samples for light-absorption measurements

Abstract—Spectral absorption studies of marine particulates by spectrophotometry usually require the concentrating of material, and it is often desirable to store concentrated samples prior to analysis. The effects of various storage methods for particles collected on glass-fiber filters were examined using natural marine particles. Sensitivity to storage effects differed for particles collected from different environments, but results consistently indicated that refrigeration for more than a few hours and freezing at −10°C are inadequate for preserving absorption by phytoplankton pigments in the visible region of the spectrum. Storage at −80°C and at liquid-nitrogen temperatures was found to be satisfactory, with liquid nitrogen providing the most reliable results for the longest periods evaluated (up to 1 yr). Increases in absorption were observed at ultraviolet wavelengths under all storage conditions, which suggests that optical properties in this region of the spectrum cannot be reliably assessed unless samples are analyzed immediately after collection.

Spectral measurements of light absorption by marine particles are necessary in order to explain variability in the inherent and apparent optical properties of ocean waters, including ocean color, and to estimate the amount of solar energy potentially used for photosynthesis. While changes in the abundance and type of particles are often the dominant source of optical variability in upper ocean waters, the optical density of particles at natural concentrations is too low and the light scattering is too high to allow absorption coefficients to be adequately measured with conventional spectrophotometry. Yentsch (1962) introduced the approach that consists of concentrating and measuring the optical density of particles on optically diffuse glass-fiber filters. This approach enhances the measurable signal and allows light-scattering effects to be controlled. The method has been tested and modified (e.g., Kiefer and SooHoo 1982; Kishino et al. 1985; Mitchell 1990; Cleveland and Weidemann 1993) and, since it is inexpensive and simple, the quantitative filter technique (QFT) is now in common use by optical and biological oceanographers (e.g., Mitchell and Kiefer 1988; Babin et al. 1993; Bricaud et al. 1995; Sosik and Mitchell 1995).

A practical constraint on the QFT approach is that the samples are susceptible to degradation and must be handled carefully in order to avoid loss of light-absorption capability (e.g., Stramski 1990). Phytoplankton pigments in particular, and potentially other chromophores present in natural particles, are highly sensitive to exposure to light, heat, and oxygen. Filtered particles must be kept hydrated and are usually analyzed in a spectrophotometer within minutes of collection. However, there are always situations in which it would be desirable to store filtered samples prior to analysis. This note describes the effects of different methods used to freeze filtered samples for varying periods of time. The results demonstrate that for common types of particles and appropriate storage conditions, it is possible to keep samples for at least 1 yr without significant change in the light-absorption signals in the visible portion of the spectrum.

Methods—Three independent but similar experiments were conducted in order to test for the effects that the freezing of marine particles has on light-absorption properties. Surface-water samples were collected from the Scripps Institution of Oceanography pier (La Jolla, California) on 23 July and 24 August 1992 for experiment 1 (EXP I) and experiment 2 (EXP II), respectively; sampling for the third experiment (EXP III) was carried out from a small boat in Buzzards Bay near Woods Hole, Massachusetts, on 12 September 1997. Replicate volumes of water (350 ml for EXP I; 400 ml for EXP II and III) were filtered through GF/F glass-fiber filters (Whatman) as quickly as possible, six at one time. For EXP I and II, within ~1–2 min of completion of filtration, well-hydrated filters were placed particle-side up in sealed plastic petri dishes at −10°C until all sample filtration was complete, while in EXP III, samples were immediately transferred directly to the final storage condition.

For each experiment, the samples were divided into three groups and stored in three different ways—in sealed petri dishes in a standard household freezer at −10°C or in a −80°C freezer, or in Histo Prep tissue capsules (Fisher Scientific) in a dewar containing liquid nitrogen. During EXP III, an additional six filters were stored at 2°C in a standard refrigerator. Three remaining samples were filtered and immediately analyzed using a Perkin Elmer ultraviolet (UV)/visible dual-beam spectrophotometer (Lambda 6 for EXP I and II and Lambda 18 for EXP III). During EXP I and II, the ''initial'' sample filters were placed particle-side into the light beam over a quartz window, which was positioned against the detector side of the instrument sample compartment. During EXP III, filters were positioned at the entrance port to a 60-mm integrating sphere attachment. For EXP I and II, a blank wetted filter was positioned in the reference beam during all measurements, while for EXP III, nothing was placed in the reference beam. Beam-slit width was set to 4 nm, spectral scanning was conducted at 240 nm min^{-1}, and the instrument was zeroed with a blank wet filter in the sample position. Measurements were made within several minutes of the completion of filtration. In each case, the mean value of measured optical density between 740 and 750 nm was subtracted from the entire spectrum in order to minimize differences resulting from variations between fil-

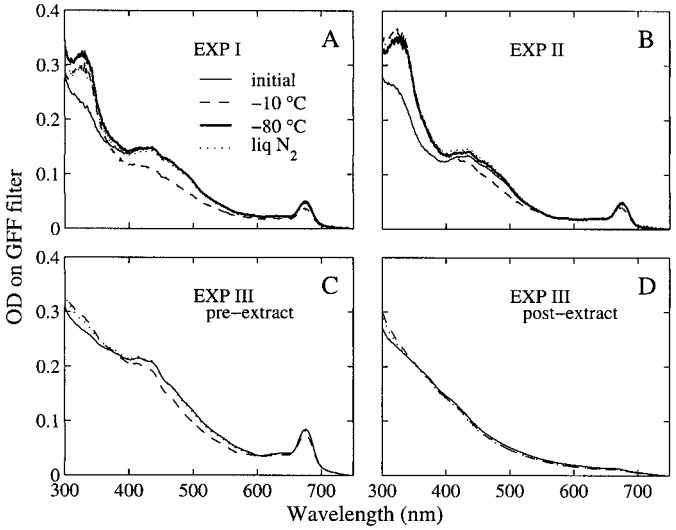

Fig. 1. Example absorption spectra for initial unfrozen particulate samples and samples stored for 3 months during each of the experiments. Results are shown for samples stored at −10°C, −80°C, and in liquid nitrogen. Each spectrum represents the mean optical density of duplicate (triplicate for the initial) samples analyzed on GF/F glass-fiber filters. (A) EXP I, (B) EXP II, (C) EXP III before extraction, and (D) EXP III after extraction. The legend indicated in (A) applies to all panels [except that −80°C samples are missing in (C) and (D)].

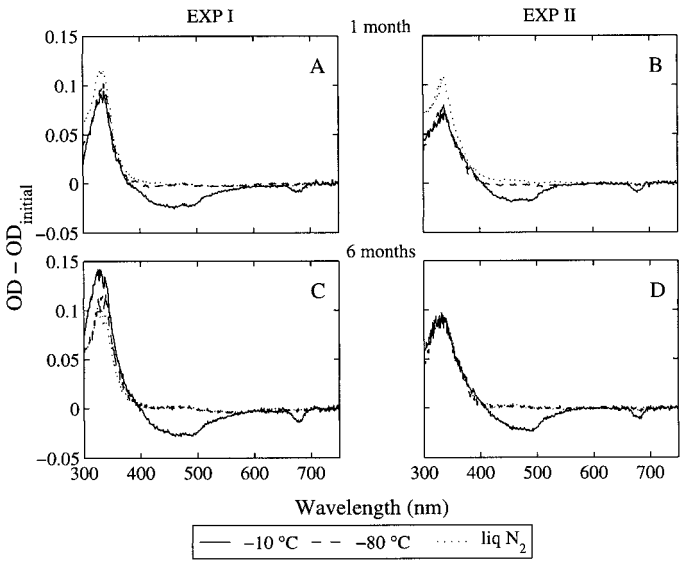

Fig. 2. Difference spectra relative to the initial unfrozen result for the 1- and 6-month time points and for each of the storage conditions examined in EXP I (panels A and C) and EXP II (panels B and D). Samples frozen for 3, 9 (EXP I only), and 12 months were also measured, with results similar to those found in samples frozen for 6 months (except for −80°C in one case, see text for details).

ters, but no corrections were applied to account for pathlength amplification effects (as in Mitchell 1990) or to adjust for concentration factors.

During EXP III, following each of the spectrophotometric analyses, sample filters were returned to the filtration funnels and covered with methanol for 20–30 min to extract pigments (Kishino et al. 1985). After drawing the methanol through the filter and rehydrating it with filtered seawater, the residual optical density was measured with the same procedure used prior to extraction.

Approximately 1 and 3 months after the start of each experiment, duplicate samples were removed from each of the storage conditions, defrosted, and analyzed spectrophotometrically, using the same procedure as for the initial samples. Both long- and short-term effects were also evaluated, with 6-, 9-, and 12-month storage periods during EXP I and II and with 2-h, 24-h, and 1-week periods in EXP III. For each storage condition, the spectra presented represent the mean of duplicate samples. Standard deviations for replicates were comparable for initial unfrozen samples (mean = 3.0%, range = 0.8–5.4% at 440 nm) and treatments (e.g., liquid-nitrogen storage, mean = 1.9%, range = <0.1–3.7% at 440 nm) and were smaller than differences observed as a result of storage under suboptimal conditions (see below).

Results and discussion—Results from the first two particle-storage experiments showed similar trends (Fig. 1). At wavelengths longer than 400 nm, samples were stable for at least 6–12 months when stored well hydrated at −80°C or in liquid nitrogen (Fig. 2). For liquid nitrogen, standard deviations between frozen and stored samples were similar to those for replicates (mean = 2.6%, range = <0.1–11% at 440 nm). After the longest storage periods, samples held at −80°C were occasionally observed to be desiccated (e.g., 12 months in EXP I) and then exhibited some loss of absorption at visible wavelengths. When stored in the freezer compartment of a standard household refrigerator (−10°C), however, there were consistent decreases in the measured optical density on the filters after only 1 month of storage. Little further decrease in signal for samples stored at −10°C was observed in subsequent months for up to 1 yr. In all cases, regardless of storage method, optical densities at wavelengths less than 400 nm increased relative to the initial observations, with a peak evident near 325 nm (Fig. 1). This change was observed after 1 month of storage and did not increase significantly in later months (Fig. 2).

The absorption losses in samples stored at −10°C appear to reflect decreases in absorption by phytoplankton pigments: they occur both near 675 nm, which reflects chlorophyll *a* (Chl *a*) loss, and at blue–green wavelengths, probably because of the degradation of Chl *a* and of some accessory pigments (Fig. 2). These losses represent approximately 20–40% of the total signal at blue-to-green wavelengths and in the red absorption peak.

The approximately 50% increase in absorption at UV wavelengths under all storage conditions examined in EXP I and II was unexpected and cannot be easily explained based on the available information. During investigation of short-term (<1 h) sample-handling effects for phytoplankton cultures on GF/F filters, Stramski (1990) observed small increases in absorption below ~440 nm, increases that were associated with pigment degradation. In contrast, the increases in UV absorption observed here cannot be ascribed to degradation of pigments which otherwise absorb at visible wave-

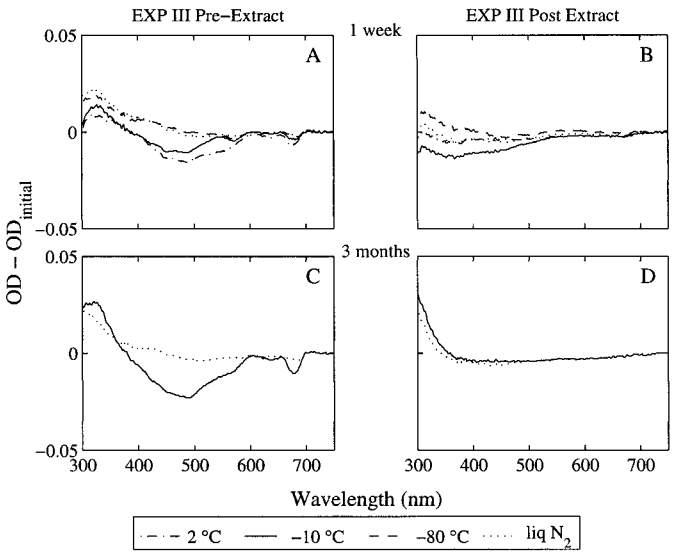

Fig. 3. Pre- and postextract results for EXP III storage times of 1 week (panels A and B) and 3 months (panels C and D), presented as difference spectra relative to the initial measurements on unfrozen samples. Samples were stored for up to 1 week in a standard refrigerator (2°C) and were frozen for up to 3 months in a standard freezer (−10°C) and at liquid-nitrogen temperatures. Samples stored for 2 h showed very little difference from initial results, 24-h values were intermediate between the 2-h and 1-week results, and 1-month results were similar to those at 3 months. Note difference in scale from Fig. 2.

lengths, since no losses in visible absorption were evident in samples stored at −80°C or at liquid-nitrogen temperatures. While further work is required to explain this observation, these results indicate that it is not possible to reliably assess the absorption of UV light by marine particles after they have been stored using any of the methods examined here. It may be possible to reliably freeze samples for periods shorter than 1 month, but this hypothesis requires further testing to confirm.

Shorter time scales were examined in EXP III, specifically so that I could address whether the UV absorption increase occurs rapidly upon freezing. However, only a small increase in UV absorption was observed for these samples, even after 3 months of storage (Fig. 1). Since substantial effects were observed after 1 month during EXP I and II (Fig. 2), which used water collected from a completely different environment than in EXP III, these results suggest that storage effects may depend on the nature of the particles present. It will require substantial further work, including detailed characterization of particle properties, to address whether variations in storage effects can be predicted on a practical basis.

In addition to the difference in UV effects, EXP III samples were also not as severely affected in the visible region of the spectrum when compared with the earlier experiments (Fig. 3). Decreases at blue–green wavelengths after 1 month of storage at −10°C were 10–20% of the initial optical density, with no additional loss apparent at 3 months. Comparison of results before and after extraction confirms the conclusion that the losses in absorption occur in the methanol-extractable phytoplankton pigments.

Based on the results of these experiments, storage of filtered particulate samples at liquid-nitrogen temperatures emerges as the most reliable method for long-term preservation of absorption at visible wavelengths. Storage at −80°C is also effective, as long as the filters remain well hydrated, which may not be possible to guarantee for periods as long as 1 yr. Mean loss in absorption between 400 and 700 nm was not observed to exceed 25%, even for samples stored at −10°C, but losses at specific blue-to-green wavelengths were as much as 40%; consequently, this method is unsatisfactory. For wavelengths between 300 and 400 nm, it was not possible to preserve the original absorption properties for some particle types using any of the storage methods examined. If reliable estimates of absorption at UV wavelengths are required, samples must be analyzed immediately after filtration.

Heidi M. Sosik

Biology Department
Woods Hole Oceanographic Institution
Woods Hole, Massachusetts 02543

References

BABIN, M, J.-C. THERRIAULT, L. LEGENDRE, AND A. CONDAL. 1993. Variations in the specific absorption coefficient for natural phytoplankton assemblages: Impact on estimates of primary production. Limnol. Oceanogr. **38:** 154–177.

BRICAUD, A., M. BABIN, AND C. HERVE. 1995. Variability in the chlorophyll-specific absorption coefficients of natural phytoplankton: Analysis and parameterization. J. Geophys. Res. **100:** 13321–13332.

CLEVELAND, J. S., AND A. D. WEIDEMANN. 1993. Quantifying absorption by aquatic particles: A multiple scattering correction for glass-fiber filters. Limnol. Oceanogr. **38:** 1321–1327.

KIEFER, D. A., AND J. B. SOOHOO. 1982. Spectral absorption by marine particles of coastal waters of Baja California. Limnol. Oceanogr. **27:** 492–499.

KISHINO, M., N. TAKAHASHI, N. OKAMI, AND S. ICHIMURA. 1985. Estimation of the spectral absorption coefficients of phytoplankton in the sea. Bull. Mar. Sci. **37:** 634–642.

MITCHELL, B. G. 1990. Algorithms for determining the absorption coefficient of aquatic particulates using the quantitative filter technique (QFT), p. 137–148. *In* R. Spinrad [ed.], Ocean Optics X. Proc. SPIE (Society of Photo-Optical Engineers) 1302.

———, AND D. A. KIEFER. 1988. Variability in pigment specific particulate fluorescence and absorption spectra in the northeastern Pacific Ocean. Deep-Sea Res. **35:** 665–689.

SOSIK, H. M., AND B. G. MITCHELL. 1995. Light absorption by phytoplankton, photosynthetic pigments, and detritus in the California current system. Deep-Sea Res. **42:** 1717–1748.

STRAMSKI, D. 1990. Artifacts in measuring absorption spectra of phytoplankton collected on a filter. Limnol. Oceanogr. **35:** 1804–1809.

YENTSCH, C. S. 1962. Measurement of visible light absorption by particulate matter in the ocean. Limnol. Oceanogr. **7:** 207–217.

Received: 16 November 1998
Accepted: 18 February 1999

Acknowledgments

This work was supported in part by ONR grant N00014-95-1-0333. The author is indebted to Kenia Whitehead and Anne Canaday for laboratory assistance, to B. Greg Mitchell for valuable discussions, and to Michele DuRand and Rob Olson for comments on the manuscript. This is Woods Hole Oceanographic Institute contribution 9877.