

Determining optical absorption of colored dissolved organic matter in seawater with a liquid capillary waveguide

Abstract—Optical absorption spectra of 0.2- μm filtered seawater samples originating from diverse oceanic and coastal waters were measured with a long pathlength capillary waveguide; results were compared with those of three different laboratory spectrophotometers. The 0.5-m-long 550- μm (inside diameter) aqueous waveguide uses only 120 μl of filtered seawater, making it convenient for use in flow-through cells or when sample volumes are restricted. Source light propagates inside the capillary waveguide by total internal reflection because of the lower refractive index of the waveguide walls with respect to the aqueous core. The absorption coefficient of colored dissolved organic matter (CDOM) at 355 nm and S , the slope of the log-linearized CDOM absorption spectra, were determined for all samples. The CDOM absorption spectra measured by the capillary waveguide closely matched that measured by spectrophotometers for CDOM concentrations varying over an order of magnitude. The deviations between the absorption spectra obtained with the capillary waveguide and those obtained with the standard spectrophotometers increased with decreasing total absorption and with increase in wavelength, presumably because of the greater baseline offsets observed in the capillary waveguide. The offsets are due to differences in refractive indices between the seawater samples and the freshwater reference. With a suitable reference, the capillary waveguide will be very useful for monitoring surface seawater CDOM absorption semicontinuously.

Colored dissolved organic matter (CDOM) plays an important role in many oceanic processes. CDOM strongly absorbs light, particularly the biologically damaging ultraviolet (UV) B wavelengths (280–320 nm), thus protecting phytoplankton and other biota (Blough and Green 1995; Arrigo and Brown 1996). It can also reduce the photosynthetically active radiation available to phytoplankton, thus decreasing primary production. High CDOM absorption in the blue region of the spectrum can also degrade the accuracy of satellite-derived phytoplankton chlorophyll estimates (Yentsch 1983; Carder et al. 1991). Knowledge of CDOM distributions, the processes controlling CDOM, and its influence on optical properties are limited by the methods currently used for measurement. Spectrophotometers with 5- and 10-cm optical cells can measure CDOM absorption with sufficient sensitivity in the UV and visible wavebands for many coastal and shelf waters. However, in oligotrophic waters, the levels of CDOM absorption approach the detection limit of these instruments. Measuring CDOM absorption spectra in these waters requires long pathlength cells (Bricaud et al. 1981; Peacock et al. 1994) or sample concentration (Carder et al. 1989). These approaches are time consuming. Alternative methods have used fluorescence measurements to retrieve CDOM absorption coefficients (Hoge et al. 1993; Green and Blough 1994).

One way of increasing sensitivity in spectrophotometry is to increase the length of the cuvette. Usually this increase is

accompanied by an increase in sample volume. Various types of fluid-filled light waveguide capillary tubing that increase effective pathlength and reduce sample volume for spectral absorption measurements have been proposed (Dasgupta 1984; Tsunoda et al. 1989). To attain total reflection of source light inside a glass capillary cell, Fujiwara and Fuwa (1985) used carbon disulfide as a solvent in the spectrophotometric detection of iodine. Because carbon disulfide has a refractive index greater than that of the glass capillary cell, light from the source propagates inside the capillary cell via total reflection at the inner cell wall as in a solid optical fiber. Tsunoda et al. (1989) used the capillary glass–air interface as the total reflecting surface without any coating. Although in principle this apparatus can function as an effective fluid-filled waveguide, its performance will degrade if there is any contamination of its reflecting surface. Recently, the availability of an amorphous fluorocarbon material with a refractive index less than that of water has been used to create a practical aqueous waveguide (Liu, U.S. patent 5570447) for spectral absorption measurements of aqueous fluid samples, including seawater (D'Sa et al. 1998).

The World Precision Instruments capillary waveguide has the liquid forming the optical core contained by a rigid quartz capillary tubing that is coated by an amorphous polymer optical cladding with a refractive index less than that of an aqueous solution (Fig. 1). Source light that is axially introduced into the waveguide via an optical fiber is transmitted and constrained within the capillary cell by total internal reflection because of the higher refractive index of the seawater in relation to the cell wall. At the opposite end of the waveguide, a detection fiber conducts the light that is not absorbed by the aqueous medium to a fiber-optics-based spectrometer that uses a diffraction grating to disperse the transmitted light into a CCD detector array. The 0.5-m-long waveguide is coiled into a 10-cm-diameter coil and has standard ST fiber optic connectors that attach to external optical fibers. There is an inlet or outlet connection at each end of the waveguide for injecting filtered seawater samples or any other aqueous solution. A deuterium lamp was used as a light source for UV wavelengths, and a halogen lamp provided visible wavelengths. Using electronically controlled shutters, source light from either of the lamps was coupled into the waveguide using an optical fiber that was attached to the ST connector. The option of combining the UV and visible waveband spectra at a particular wavelength was provided through software. The performance of the capillary waveguide to measure CDOM absorption spectra of diverse water types was evaluated by comparing the absorption spectra of 0.2- μm -filtered seawater samples to that obtained with commercial spectrophotometers.

Seawater samples for this study were obtained from (1) a transect in the Middle Atlantic Bight (MAB) from the Delaware Bay southeast to the Gulf Stream, (2) the Gulf of

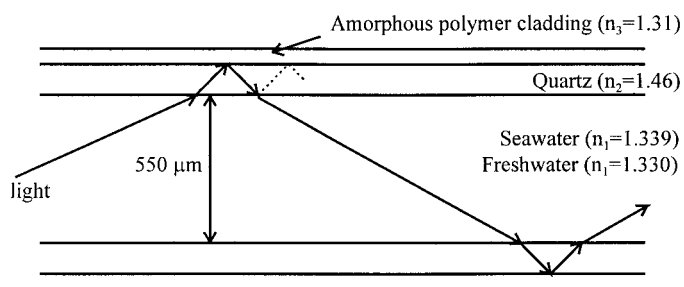


Fig. 1. Cross-sectional view of light transmission through the liquid capillary waveguide cell. The refractive index of seawater, quartz glass, and the amorphous polymer cladding are shown as n_1 – n_3 , respectively.

Maine, and (3) Florida Bay. Water samples were filtered through 0.2- μm filters (GF/F for Gulf Stream and Millipore Sterivex-GS cartridges for Gulf of Maine samples), collected in clean glass bottles, and refrigerated at 4°C in the laboratory. Prior to measurements, samples were refiltered through 0.2- μm nylon syringe filters (Blough et al. 1993). Samples were referenced to purified water from a Milli-Q system. Aliquots of the filtered samples were injected into the capillary waveguide using a 1-ml syringe. The absorption coefficient, $a(\lambda)$ (m^{-1}), was obtained from the relationship

$$a(\lambda) = 2.303A(\lambda)/l,$$

where $A(\lambda)$ is the absorbance and l is the cell pathlength in meters. For the capillary waveguide the effective pathlength (0.459 m) was determined using Beer's law on absorption measurements of an National Institute of Standards and Technology standard solution and compared with a calibrated spectrophotometer. The spectrophotometers all used 0.1-m cells. Seawater CDOM spectral absorption values generally decrease approximately exponentially throughout the near UV and visible wavebands (Bricaud et al. 1981; Blough et al. 1993). The spectral slopes, S (nm^{-1}), were calculated from plots of the natural log of the absorption coefficient vs. wavelength using the linear least-squares regression over the interval of 270–450 nm. The slope S provides a measure of change in CDOM absorption as a function of wavelength.

Previous studies have shown that CDOM absorption measurements that are referenced to pure freshwater and obtained using long pathlength cells are frequently affected by baseline offsets that appear as a small apparent optical density value in the range 700–800 nm. These discrepancies have been mainly attributed to the refractive index differences between the freshwater reference and seawater and not to scattering by small particles that may have passed through filters (Green and Blough 1994). This offset appears to be enhanced in the 0.5-m-long capillary waveguide. Absorption spectra were corrected for these offsets for both the waveguide and the spectrophotometers by subtracting the average apparent absorbance from 700 to 750 nm from each spectrum. For the MAB transect samples, the waveguide absorption spectra were compared with those of a Shimadzu UV-2401PC double-beam spectrophotometer in the spectral range 250–750 nm. For the Gulf of Maine samples, the waveguide measurements were compared with those of a

Bausch and Lomb Spectronic 2000 spectrophotometer, and the Florida Bay samples were compared with those of a Perkin Elmer Lambda-18 spectrophotometer.

Results and discussion—Representative absorption spectra of 0.2- μm -filtered seawater samples obtained from diverse marine environments and measured with the capillary waveguide and the spectrophotometers are shown in Figs. 2a and 3a. The corresponding log-linearized spectra for the same samples are shown in Figs. 2b and 3b. In Fig. 2a, spectral absorption plots s4 and s27 are seawater samples from Delaware and Chesapeake Bays, and plots s7, s11, and s16 are from samples obtained from the shelf, the Gulf Stream edge, and the Gulf Stream in August 1997. The spectral plots generally correlated well for samples containing higher CDOM levels and at the UV wavebands for all the samples. For the Gulf Stream samples with low CDOM concentrations, the spectral differences increased with increasing wavelength. Values of $a(355)$ measured with the Shimadzu spectrophotometer ranged from 0.07 to 0.65 m^{-1} and increased toward the coast, whereas the waveguide values ranged from 0.18 to 0.72 m^{-1} (Table 1). At station 16, where CDOM level was low, the difference has been magnified by the baseline offset correction made for the waveguide measurements. Offset due to the effect of temperature on the sample absorption spectrum measured with the Shimadzu and the effect of refractive index offset caused by the waveguide combined to enhance the differences in the corrected spectra. Values of the spectral slope S also covaried closely but differences increased in the Gulf Stream samples with low CDOM. The log-linear plots (Fig. 2b) appear similar at lower wavebands and tend to diverge toward the visible. One of the factors that can be attributed to the increasing differences in the absorption coefficients and the spectral slope is the magnitude of the differences in the baseline correction near the visible.

Figure 3a shows the CDOM absorption plots (s6, s24) measured with a Bausch and Lomb (B&L) spectrophotometer for the Gulf of Maine and Georges Banks waters, and plots 1a, 1c, sw, and 1d correspond to Florida Bay samples that were analyzed with a Lambda-18 spectrophotometer. Figure 3b shows the corresponding log-linear absorption spectra. CDOM absorption plot s6 is from a surface sample from Georges Banks, and plot s24 corresponds to a Gulf of Maine sample obtained from 75 m. The different stations sampled showed very little variation in CDOM levels (Table 1). Values of $a(355)$ were relatively low, and measurement differences between the waveguide and the B&L spectrophotometer were small. Offsets observed between the spectra at 355 nm and at 440 nm are caused by the lamp change from tungsten to deuterium for the B&L and waveguide spectrophotometers, respectively. The log-linearized plots tended to diverge at increasing wavelength. The absorption spectra of the Florida Bay samples were relatively high, with $a(355)$ values lying between 0.7 and 3.0 m^{-1} , and the waveguide spectra were virtually identical to those of Perkin Elmer spectrophotometer. CDOM levels were highest in the inner Bay and decreased towards the outer Bay. The signal resolution of the spectra measured by the waveguide was low in the visible because only the UV lamp was used during

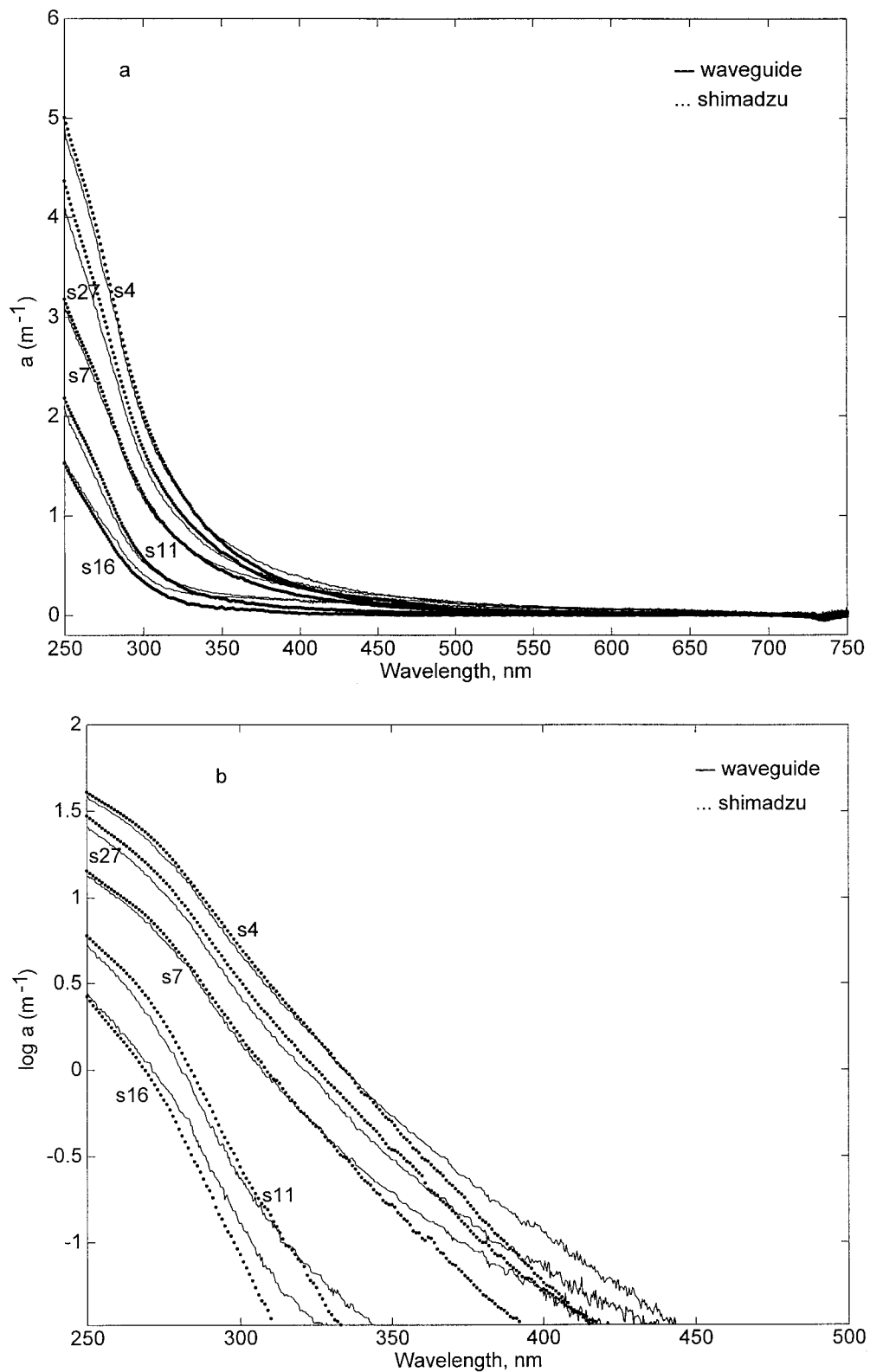


Fig. 2. Seawater samples from a Middle Atlantic Bight transect. (a) Optical absorption spectra measured by the waveguide and by the Shimadzu spectrophotometer. (b) Spectra plotted as the natural logarithm of the absorption coefficient (m^{-1}) vs. wavelength.

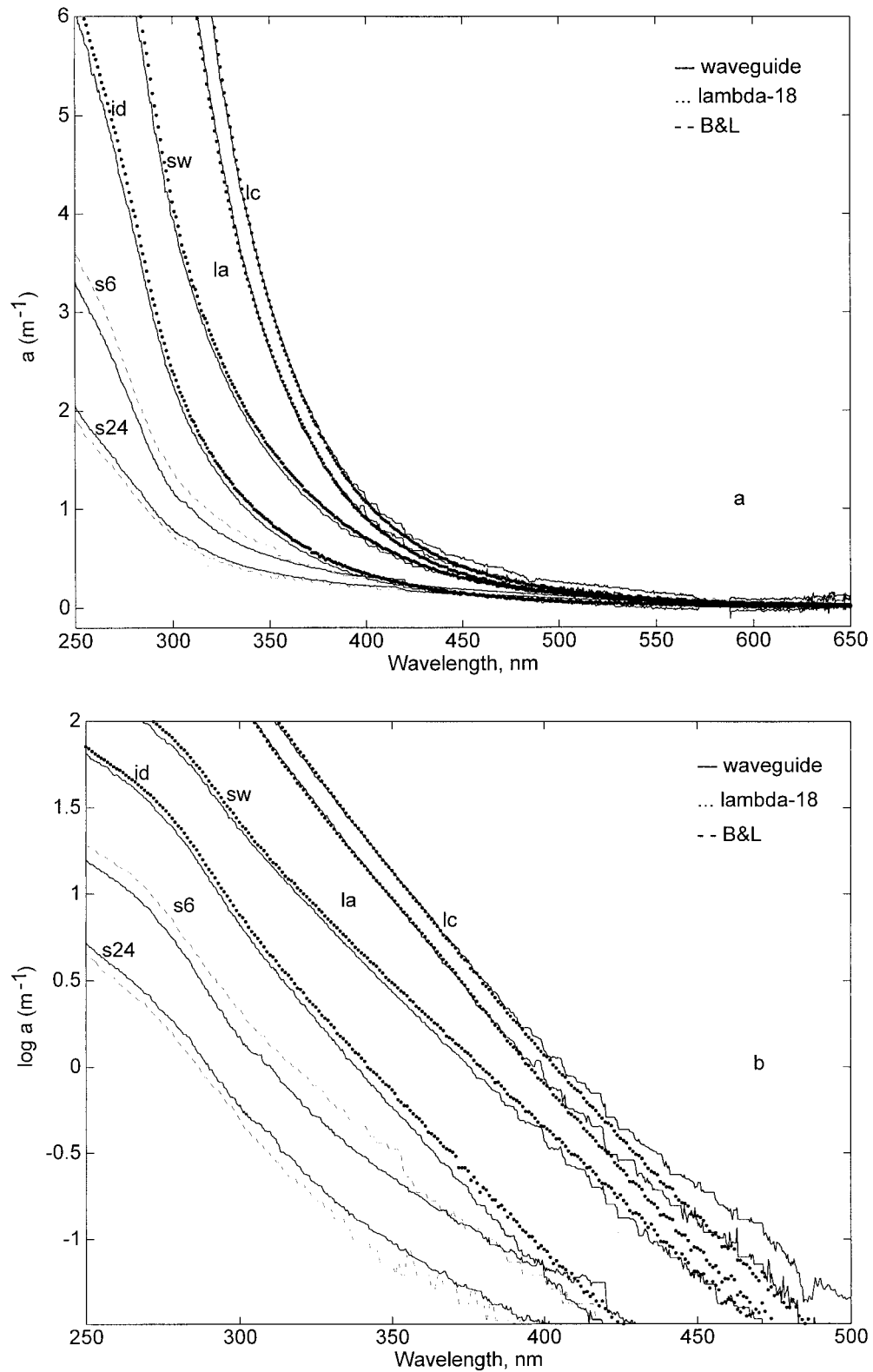


Fig. 3. Filtered Florida Bay seawater samples (la, lc, sw, id) and Gulf of Maine and Georges Banks samples (s6, s24). (a) Optical absorption spectra measured by the waveguide and the Perkin Elmer Lambda-18 spectrophotometer (la, lc, sw, id) and by a Bausch and Lomb (B&L) spectrophotometer (s6, s24). (b) Corresponding log-linearized optical absorption spectra.

Table 1. Optical absorption parameters $a(355)$ and S of 0.2- μm -filtered seawater samples measured by the waveguide and the spectrophotometers.

Station No.	Depth (m)	N lat, W long (decimal)	Waveguide		Spectrophotometers	
			$a(355)$ (m^{-1})	S ($\times 10^3 \text{ nm}^{-1}$)	$a(355)$ (m^{-1})	S ($\times 10^3 \text{ nm}^{-1}$)
MAB transect						
4	11.6	38.594, 74.776	0.72	0.016	0.65	0.019
27	9.5	37.001, 76.050	0.56	0.015	0.58	0.016
7	36	38.364, 74.464	0.46	0.014	0.41	0.017
11	surface	37.926, 73.931	0.21	0.012	0.14	0.019
14	surface	37.119, 72.925	0.34	0.014	0.32	0.017
16	surface	36.401, 72.001	0.18	0.010	0.07	0.025
Florida Bay						
FA	surface	24.863, 80.936	1.36	0.016	1.18	0.018
SW	surface	24.877, 81.104	1.42	0.017	1.47	0.017
ID	surface	24.986, 81.011	0.72	0.019	0.77	0.019
LA	surface	24.933, 80.716	2.39	0.021	2.35	0.022
LC	surface	25.033, 80.716	2.81	0.020	2.72	0.022
Gulf of Maine/Georges Banks						
3	2	40.893, 68.067	0.49	0.014	0.59	0.014
6	2	40.597, 67.592	0.50	0.013	0.52	0.017
9	2	41.429, 67.129	0.53	0.013	0.46	0.017
12	2	42.323, 65.911	0.43	0.013	0.35	0.017
19a	2	44.380, 66.684	0.37	0.013	0.30	0.016
19b	75	44.380, 66.684	0.35	0.013	0.32	0.012
24a	2	42.621, 67.504	0.38	0.013	0.32	0.013
24b	75	42.621, 67.504	0.34	0.012	0.34	0.012

the measurements. The overall shapes of the log-linear plots for the two instruments are similar; however, there is a divergence with increasing wavelength.

The correlations between absorption coefficients at 355 nm measured by the waveguide and the spectrophotometers are shown in Fig. 4. For the Florida Bay samples (high $a(355)$ values) most of the points fell close to the 1:1 line. The MAB transect samples showed a small increase in scatter from coastal to offshore waters due to decreasing CDOM concentrations. Most of the $a(355)$ values for the Gulf of Maine and Georges Bank samples fell within the range of 0.2–0.6 m^{-1} , with a few points deviating from the line.

The spectral slope parameter S allows prediction of CDOM absorption coefficients at visible wavelengths from measurements in the UV and also may be used to characterize water types (Vodacek et al. 1997). Differences in S values were inversely related to CDOM. S increased from coastal to offshore waters: from 0.019 to 0.025 nm^{-1} as measured with the Shimadzu spectrophotometer and from 0.016 to 0.01 nm^{-1} as measured with the waveguide. The measurement differences between the waveguide and the spectrophotometer can be attributed mainly to the different baseline corrections that were applied to the absorption spectral curves. The magnitude of the baseline corrections was always larger for the waveguide ($\sim 0.14 \text{ m}^{-1}$) than for the spectrophotometers ($\leq 0.10 \text{ m}^{-1}$). As a result, there was a shift in the curvature of the plots that skewed S towards lower values. Until the baseline offset problem is resolved for the capillary waveguide, measurements of S with this instrument in very weakly absorbing waters will be problem-

atic. We are currently evaluating waveguide measurements by matching refractive indices of samples and a reference.

Conclusion—The waveguide can determine CDOM spectral absorption with reasonable accuracy in diverse water types ($a(355)$ in our samples ranged from <0.1 to $>3.0 \text{ m}^{-1}$). Most of the differences in the CDOM absorption spectra obtained with the waveguide and the spectrophotometers are due to differences in baseline corrections. The higher baseline offset required for the waveguide is presumably due to refractive index differences between the seawater sample and the freshwater reference. The higher refractive index of the seawater ($n_1 = 1.3393$, for salinity = 34.8%) in comparison to freshwater ($n_2 = 1.3330$) apparently increases the signal that is collected by the detection fiber in visible wavelengths. A procedure that may minimize this effect would be to use seawater samples obtained from oceanic regions with very low CDOM and subjected to UV treatment to destroy any residual CDOM as the reference. Alternatively, UV-treated synthetic seawater having a refractive index matched to the sample could also be used.

The waveguide was interfaced to a low-cost CCD fiber-optics-based spectrometer; the long pathlength of the waveguide compensates for the lower detector sensitivity. Waveguides of various lengths could be used in water types containing different CDOM levels to optimize performance. An important advantage of the waveguide in field measurements of CDOM spectral absorption is the very small sample volume required. This should allow the technique to be readily adapted to continuous flow-through monitoring of absorption coefficients of filtered seawater.

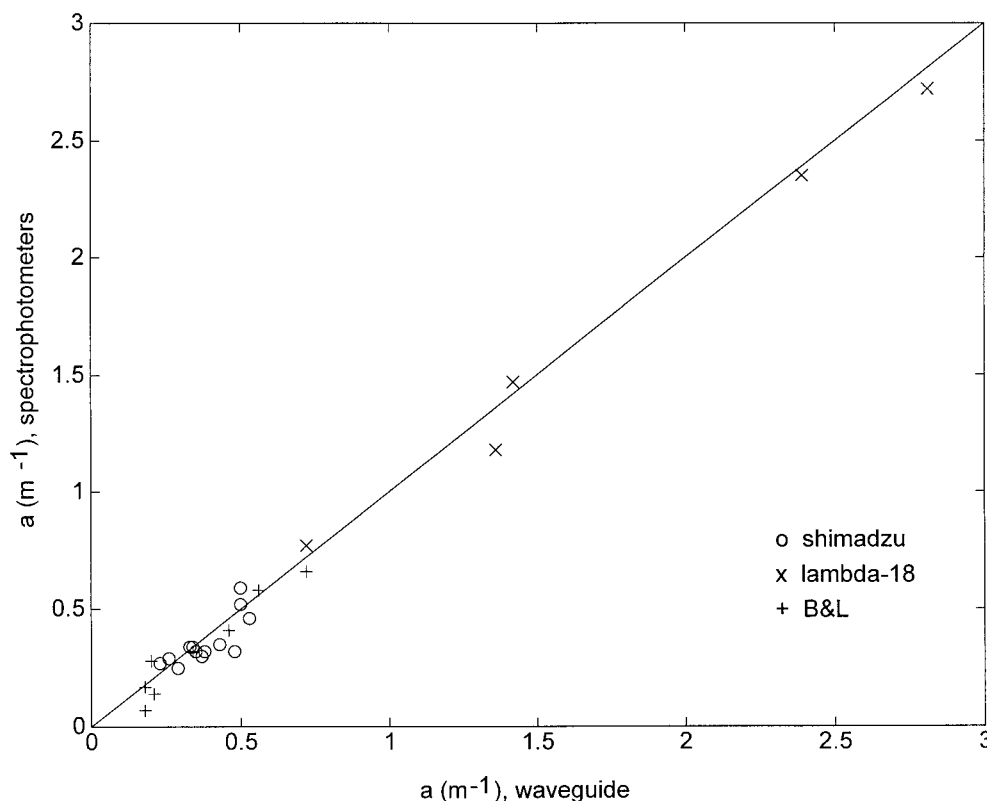


Fig. 4. Comparison between $a(355)$ measured by the waveguide and the three spectrophotometers: (1) Shimadzu for the MAB transect, (2) Bausch and Lomb for the Gulf of Maine and Georges Banks, and (3) Lambda-18 for the Florida Bay. Line is 1:1 correspondence.

Eurico J. D'Sa *References*

Office of Research and Applications
National Oceanic and Atmospheric Administration
NESDIS-E/RA3, 5200 Auth Road
Camp Springs, Maryland 20746

Robert G. Steward

Department of Marine Science
University of South Florida
St. Petersburg, Florida 33701

Anthony Vodacek
Neil V. Blough

Department of Chemistry and Biochemistry
University of Maryland
College Park, Maryland 20742

Dave Phinney

Bigelow Laboratory for Ocean Sciences
West Boothbay Harbor, Maine 04575

Acknowledgments

We thank Harry Fein of World Precision Instruments, Sarasota, for use of the waveguide spectrometer and Chris Brown for helpful suggestions. E.J.D. was supported by a National Research Council-NOAA Resident Research Associateship. Work at the University of South Florida was supported by NOAA Coastal Ocean Program and

- ARRIGO, K. R., AND C. W. BROWN. 1996. Impact of chromophoric dissolved organic matter on UV inhibition of primary productivity in the sea. *Mar. Ecol. Prog. Ser.* **140**: 207-216.
- BLOUGH, N. V., AND S. A. GREEN. 1995. Spectroscopic characterization and remote sensing of non-living organic matter, p. 23-45. *In* R. G. Zepp and C. Sonntag [eds.], *The role of non-living organic matter in the earth's carbon cycle*. Wiley.
- , O. C. ZAFIRIOU, AND J. BONILA. 1993. Optical absorption spectra of waters from the Orinoco River outflow: Terrestrial input of colored organic matter to the Caribbean. *J. Geophys. Res.* **98**: 2271-2278.
- BRICAUD, A., A. MOREL, AND L. PRIEUR. 1981. Absorption by dissolved organic matter of the sea (yellow substance) in the UV and visible domains. *Limnol. Oceanogr.* **26**: 43-53.
- CARDER, K. L., S. K. HAWES, K. A. BAKER, R. C. SMITH, R. G. STEWARD, AND B. G. MITCHELL. 1991. Reflectance model for quantifying chlorophyll *a* in the presence of productivity degradation products. *J. Geophys. Res.* **9**: 20599-20611.
- , R. G. STEWARD, G. R. HARVEY, AND P. B. ORTNER. 1989. Marine humic and fulvic acids: Their effects on remote sensing of ocean chlorophyll. *Limnol. Oceanogr.* **34**: 68-81.
- DASGUPTA, P. K. 1984. Multipath cells for extending dynamic range

NASA Earth Observing System. N.V.B. was supported by the Office of Naval Research contract N00014-95-1020. D.P. acknowledges support from NASA grant NAG5-6579 and NOAA grant NA67EC0362.

- of optical absorbance measurements. *Anal. Chem.* **56**: 1401–1403.
- D'SA, E. J., G. J. KIRKPATRICK, AND S. Y. LIU. 1998. Application of a long path-length aqueous capillary waveguide for seawater absorption spectral measurements. *Eos Trans. Am. Geophys. Union* **76**: 34.
- FUJIWARA, K., AND K. FUWA. 1985. Liquid core optical fiber total reflection cell as a colorimetric detector for flow injection analysis. *Anal. Chem.* **57**: 1012–1016.
- GREEN, S. A., AND N. V. BLOUGH. 1994. Optical absorption and fluorescence properties of chromophoric dissolved organic matter in natural waters. *Limnol. Oceanogr.* **39**: 1903–1916.
- HOGUE, F. E., A. VODACEK, AND N. V. BLOUGH. 1993. Inherent optical properties of the ocean: Retrieval of the absorption coefficient of chromophoric dissolved organic matter from fluorescence measurements. *Limnol. Oceanogr.* **38**: 1394–1402.
- PEACOCK, T. G., K. L. CARDER, P. G. COBLE, Z. P. LEE, AND S. K. HAWES. 1994. Long-path spectrometer for measuring gelbstoff absorption in clear waters. *Eos Trans. Am. Geophys. Union* **75**: 22.
- TSUNODA, K., A. NOMURA, J. YAMADA, AND S. NISHI. 1989. The possibility of signal enhancement in liquid absorption spectrometry with a long capillary cell utilizing successive total reflection at the outer cell surface. *Appl. Spectrosc.* **43**: 49–55.
- VODACEK, A., N. V. BLOUGH, M. D. DE GRANDPRE, E. T. PELTZER, AND R. K. NELSON. 1997. Seasonal variation of CDOM and DOC in the Middle Atlantic Bight: Terrestrial inputs and photooxidation. *Limnol. Oceanogr.* **42**: 674–686.
- YENTSCH, C. S. 1983. Remote sensing of biological substances, p. 263–297. *In* A. P. Cracknell [ed.], *Remote sensing applications in marine science and technology*. Reidel.

Received: 31 August 1998

Accepted: 13 December 1998

Amended: 8 February 1999

Limnol. Oceanogr., 44(4), 1999, 1148–1154

© 1999, by the American Society of Limnology and Oceanography, Inc.

Rapid and precise determination of dissolved oxygen by spectrophotometry: Evaluation of interference from color and turbidity

Abstract—Several researchers have proposed spectrophotometric modifications of the Winkler titrimetric method for measuring dissolved oxygen (DO). These modifications, although simple, are not widely used because of concern about accuracy, calibration, and possible sources of interference. Here we show, using natural samples from lakes and rivers as well as samples manipulated in the laboratory, that the spectrophotometric method can provide accurate and very precise measurements of DO over a wide range of concentrations (4 to ~13 mg O₂ liter⁻¹). Further, interference from dissolved organic carbon (color) and turbidity are minor. We propose corrections for both color and turbidity, where necessary, that can be easily incorporated into the measurement design. Because of the speed and simplicity of the spectrophotometric method, it is easy to replicate measurements and thereby increase precision without greatly increasing analytical time. In 10 min of effort, we were able to achieve a coefficient of variation (CV) within one bottle of 0.09%, or 0.8% among different bottles. With $n = 7$ bottles, one can easily distinguish changes in DO of 0.05 mg liter⁻¹ with this method, which makes it useful for metabolic studies in many environments. To achieve a comparable CV by conventional titration would require about 100 min of effort.

The concentration of dissolved oxygen (DO) in aquatic systems, as well as the rate of its production and removal by metabolic and chemical processes, has proved to be a useful measurement across most branches of aquatic science. A number of different approaches have been devised to measure DO, including various kinds of electrodes (Reynolds 1969; Atwood et al. 1977; Wilcock et al. 1981), but most researchers needing high accuracy or precision still rely on the titrimetric method of Winkler (Winkler 1888; Aminot

1988; Carignan et al. 1998). Although it is very precise when replicated, this analysis is time-consuming. Also, in systems that are turbid or highly colored with organic matter, the end point is sometimes difficult to visualize. The use of a potentiometric end point for the titration overcame many of these problems (Carpenter 1965; Carrit and Carpenter 1966) and made possible automatic versions of the original Winkler titration (Bryan et al. 1976; Hartwig and Michael 1978; Williams and Jenkinson 1982; Graneli and Graneli 1991; Williams and Purdie 1991). The drawback of automatic titration for many researchers is the cost of the specialized equipment. Further, although the titration is itself automatic, it is still time-consuming, requiring some 3–5 min per sample.

In the Winkler DO method, tri-iodide is ultimately formed in proportion to the DO present. In an equilibrium that is dependent on both temperature and the concentration of KI (added in excess), tri-iodide dissociates into molecular iodine and iodide. In the titrimetric method, the I₃⁻–I₂ pool is measured by reducing it to NaI by the addition of sodium thio-sulfate. The spectrophotometric method makes use of the color of the I₃⁻–I₂ couple. The color of the resulting solution is quantified in a spectrophotometer, which can analyze it at several wavelengths (Custer and Natelson 1949).

A spectrophotometric modification of the Winkler DO method was first proposed by Broenkow and Cline (1969) for use at low DO levels. Since then, modifications of the spectrophotometric method have been used by a variety of researchers (Reynolds 1972; Duval et al. 1974; Ashton and Twinch 1985), but the method has not been standardized or widely tested in the field. There are several potential disadvantages to the spectrophotometric approach. Because one is simultaneously measuring light absorption by two species