

- 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

(I_3^- and I_2), absorbance can depend on their ratio in the sample, which makes the method somewhat more sensitive to temperature and added KI concentration than the titrimetric method. Furthermore, there are potential interferences by absorbance from other materials in the field. Ashton and Twinch (1985) used the spectrophotometric method in a hypertrophic system in South Africa and found little interference from chlorophyll-*a*. They suggested, however, that organic color or high turbidity, factors that they did not test, might have impeded the accuracy. Therefore we tested, both in the laboratory and in the field, the effects of turbidity and organic C on the accuracy and precision of the spectrophotometric method.

All samples (both spectrophotometric and titrimetric) were fixed with $MnSO_4$, alkaline iodide-azide solution, and acid according to the Winkler procedure (Golterman et al. 1978). We purchased prepared reagent solutions (Fisher, certified) and fixed-volume repipets (Labindustries; 0.1% reproducibility) to ensure precision. The DO concentration was obtained in parallel by titration with $Na_2S_2O_3$, as in the standard Winkler procedure (Wetzel and Likens 1991), and by spectrophotometer. The spectrophotometric determinations were made in 1-cm glass cuvettes at a wavelength of 430 nm on a Shimadzu UV-160. Although the absorbance peak for the I_3^- - I_2 couple is in the UV range (287.5 nm, Custer and Natelson 1949; Duval et al. 1974), we followed the procedures of Mackereth et al. (1978) and Ashton and Twinch (1985) and used the 430-nm wavelength. This modification obviated the need for a UV spectrophotometer and costly quartz cuvettes, which may not be available in some laboratories. We expressed the absorbance as 1,000 times the absorbance reading in a 1-cm cuvette.

Absorbance of I_3^- - I_2 —To look directly at the absorbance of the I_3^- - I_2 couple without interferences, we added known concentrations of $KH(IO_3)_2$ to deionized water in the presence of the same KI and H_2SO_4 used in the Winkler procedure. This generated a concentration series of the I_3^- - I_2 couple. In addition, we varied the amount of KI added to examine the effects of changing the proportion of I_3^- and I_2 on absorbance. Finally, we examined the effect of varying KI on absorbance for a series of replicate DO samples.

Correlative approach—We related the absorbance at 430 nm (Abs_{430}) in Winkler-fixed samples to the DO values obtained by titration over a large range of DO concentrations and environmental conditions. Samples were taken from a series of 20 lakes in Canada, seasonally at one site on the Hudson River, from one humic lake in Connecticut (Old Man McMullen Pond; Reche et al. 1999), and from a humic lake in Northern Michigan (Hummingbird Lake; He and Wright 1992). For the 20 Canadian lakes, samples were taken over depth by peristaltic pump into 300-ml BOD bottles and fixed in the field as in the usual Winkler procedure (Wetzel and Likens 1991). Each lake was visited three or four times, and five depths were sampled at each lake; the data set includes 200 samples. The lakes varied over a gradient of eutrophication, water hardness, algal biomass, and organic color. For a complete description of the lakes, see del Giorgio and Peters (1994). To obtain the Hudson River

samples, 60-ml bottles were filled at weekly intervals near Rhinecliff, New York, (river km 152) and fixed in the field. Additional samples were taken at another location (near Poughkeepsie, New York, river km 122) at less frequent intervals. In total, 104 samples were included in that data set. In the laboratory, each sample was analyzed by both spectrophotometric and titrimetric procedures.

Calibration against known oxygen concentrations—To generate a range of known DO concentrations, we manipulated samples from the Hudson River in the laboratory. We vigorously bubbled water with outside air (>4 h) at several temperatures to generate oxygen-saturated water, monitoring the DO with a YSI digital oxygen meter. By mixing known amounts of these saturated waters directly in BOD bottles we were able to produce known concentrations of DO. Saturated values of DO were calculated following Golterman et al. (1978). For each sample of known DO, we measured DO by titration and by Abs_{430} .

Interferences—We used a dual approach to investigating potential interferences. First, at a single DO concentration, we varied interference from turbidity, chlorophyll, or organic color to obtain an appropriate correction. Then, we tested this derived correction over a range of each interfering factor. The test consisted of comparing the spectrophotometric estimate of DO (including the correction) with the absolute concentration of DO (as generated above).

For turbidity, we collected suspended solids from the Hudson River by filtration through 0.2- μ m pore size Nuclepore filters to create concentrated turbidity. We added this material to produce final turbidities ranging from 2 to 200 nephelometry turbidity units (NTU). For chlorophyll interference, we added phytoplankton by filter-concentrating water from the Hudson River (using Whatman GF/D filters). We generated values from 0 to 127 μ g chlorophyll-*a* liter⁻¹. Organic color absorbs light at many wavelengths, including those near Abs_{430} , and can seriously interfere with measuring absorbance of the I_3^- - I_2 couple. For organic color, we compared the relationship between Abs_{430} and DO in highly colored humic waters with that relationship in clear water. Further, we collected leaves from the forest floor and leached these in deionized water. We filtered this leachate (using Whatman GF/F filters) and added it back to Hudson River water to create a range of organic color.

Turbidity was measured using an HF Scientific model DRT 100B turbidimeter. Chlorophyll-*a* was measured fluorometrically according to Holm-Hansen and Riemann (1978) using a model 5000 Turner Designs fluorometer. Organic color was quantified by measuring absorbance at 440 nm (Cuthbert and del Giorgio 1995). The range in color was from 0.001 to 0.180 absorbance units (1-cm path-length cuvette).

Results: Absorbance of the I_3^- - I_2 couple—The I_3^- - I_2 couple could be quantified by spectrophotometry. Absorbance at 430 nm was a linear function of the amount of $KH(IO_3)_2$ added (or I_2 generated) from near 0 to about 1,700 μ eq liter⁻¹ and to absorbance levels approaching 2,000 (Fig. 1). Above this concentration, absorbance exceeded the useful range (Fig. 1). Inverting the linear relationship in Fig. 1 and ex-

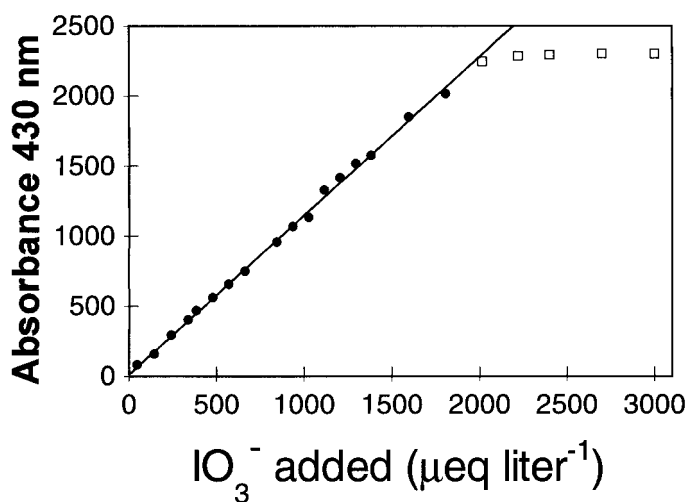


Fig. 1. Spectrophotometric absorbance of the I_3^- - I_2 couple. The concentration range was generated by varying $KH(IO_3)_2$ in the presence of KI and H_2SO_4 . Absorbance is expressed as 1,000 times the absorbance reading in a 1-cm cuvette at 430 nm. For the linear part of the curve, $R^2 = 0.99$.

pressing it in terms of DO, the relationship suggests that Winkler-fixed DO samples should be linearly related to absorbance up to nearly $14 \text{ mg O}_2 \text{ liter}^{-1}$ and the slope should be near $0.007 \text{ mg O}_2 \text{ liter}^{-1} \text{ absorbance-unit}^{-1}$.

At a single relatively low concentration of added $KH(IO_3)_2$ ($500 \mu\text{eq liter}^{-1}$) the absorbance from the I_3^- - I_2 couple varied slightly over broad changes in the KI concentration. Increasing the KI sixfold over what is normally used in the Winkler method increased absorbance from 545 to 553, a change of 1.5%. At a single concentration of DO ($9.7 \text{ mg liter}^{-1}$) in a water sample, we varied the volume of alkali-iodide reagent from 0.9 to 1.1 ml to simulate the effect that variance in the delivery of iodide would have on the final absorbance value. Under these conditions a 10% ($\sim 10\times$ our pipeting error) difference in alkali-iodide added resulted in a 1% difference in absorbance.

Results: Correlative approach—The absorbance at 430 nm of the I_3^- - I_2 couple in the Winkler-fixed samples was strongly correlated to titrimetric measurements of DO in both the Hudson River (Fig. 2a) and the Canadian lakes (Fig. 2b). Although the conditions of color, turbidity, and DO concentration ranges were quite different in the two data sets, we obtained almost identical slopes when we restricted the DO concentration to 4–12 $\text{mg O}_2 \text{ liter}^{-1}$. The linear model for the Hudson River samples was

$$\text{DO (mg liter}^{-1}\text{)} = 0.0093 \times \text{Abs}_{430} - 1.624$$

$$(R^2 = 0.95; n = 104; P < 0.0001).$$

(1)

The Canadian lakes covered a larger range of DO ($0.15\text{--}20 \text{ mg liter}^{-1}$) than did the Hudson ($5.5\text{--}8 \text{ mg liter}^{-1}$). As DO approached 13 mg liter^{-1} , absorbance became nonlinear, as we expected from previous studies (Ashton and Twinch 1985) and from the $KH(IO_3)_2$ results, although the correla-

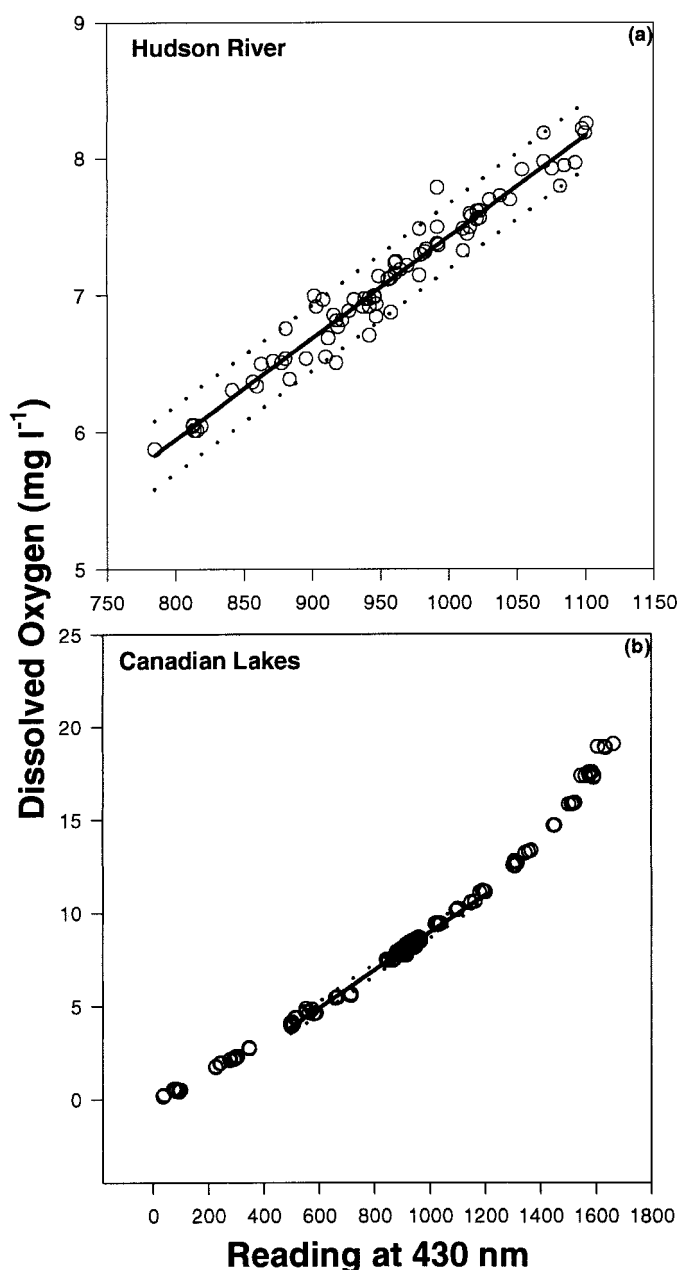


Fig. 2. Relationship between DO concentration (determined by Winkler titration) and absorbance at 430 nm of Winkler-fixed samples. The reading at 430 (A_{430}) is the actual absorbance in a 1-cm cuvette times 1,000 and is not corrected for any interference. (a) Hudson River samples. (b) Samples from multiple depths from 20 different Canadian lakes. The regression is shown for the working range of the spectrophotometric method (4–12 $\text{mg O}_2 \text{ liter}^{-1}$; values outside this range were excluded from the regression. Dotted line indicates 95% confidence limits.

tion with DO was very high over the entire range ($R^2 = 0.99$). Below about 4 mg liter^{-1} , the relationship also departed from linearity. Since the I_3^- - I_2 absorbance was linear at the low end (Fig. 1), this departure suggests that other factors affected light absorption in the low range. Note that the data in Fig. 2b are drawn from multiple depths of mul-

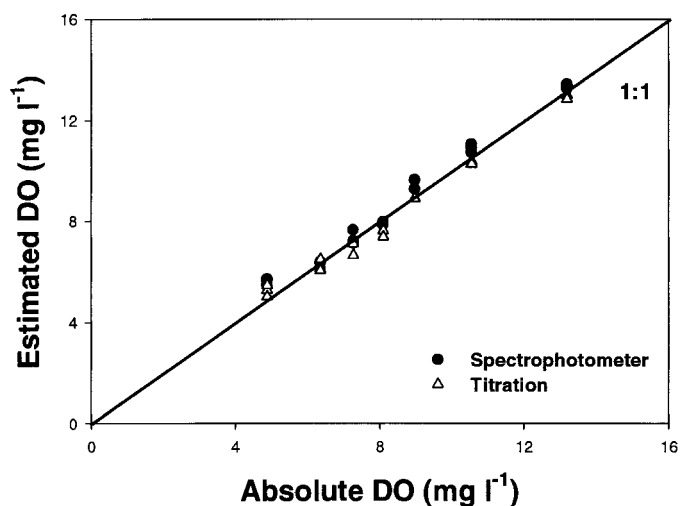


Fig. 3. Comparison of spectrophotometric (filled circles) and titrimetric (open triangles) estimates of DO, plotted against the absolute DO. Samples were filtered (Whatman GF/D) Hudson River water. Spectrophotometric data were converted to DO using the model shown in Fig. 2 (Eq. 1) without corrections for either color or turbidity.

multiple lakes, and constant interference would not be expected over the full DO range. Over the range 4–12 mg liter⁻¹, the relationship was linear and nearly identical to that in the Hudson.

$$\text{DO (mg liter}^{-1}\text{)} = 0.0101 \times \text{Abs}_{430} - 1.18$$

$$(R^2 = 0.99; n = 157; P < 0.0001).$$

(2)

For this DO range, no curvilinear model significantly improved the relationship between DO and Abs₄₃₀.

Results: Calibration against known DO concentrations—In the laboratory and using water from the Hudson River, we generated a range in DO from 4.88 to 13.19 mg liter⁻¹. Using Eq. 1, we calculated DO based on the spectrophotometric readings for these samples. The correlation between calculated and absolute DO was close to unity and there was no statistically significant difference between titration and spectrophotometric results (Mann-Whitney rank-sum test, $P = 0.481$, Fig. 3). The linear model relating spectrophotometric procedure and absolute DO on the experimental data from filtered samples from the Hudson River was expressed in Eq. 3:

$$\text{DO (mg liter}^{-1}\text{)} = 0.0093 \times \text{Abs}_{430} - 1.808$$

$$(R^2 = 0.98; n = 84; P < 0.0001).$$

(3)

Interferences: Turbidity—We experimentally altered the turbidity between 2 and 200 NTU at a single oxygen concentration (9.52 mg liter⁻¹) and examined the effect by comparing the absorbances at 430 and 750 nm (Fig. 4). The incremental increase in turbidity created a linear increase in the absorbance read at both 430 and 750 nm. Since the I₃-I₂

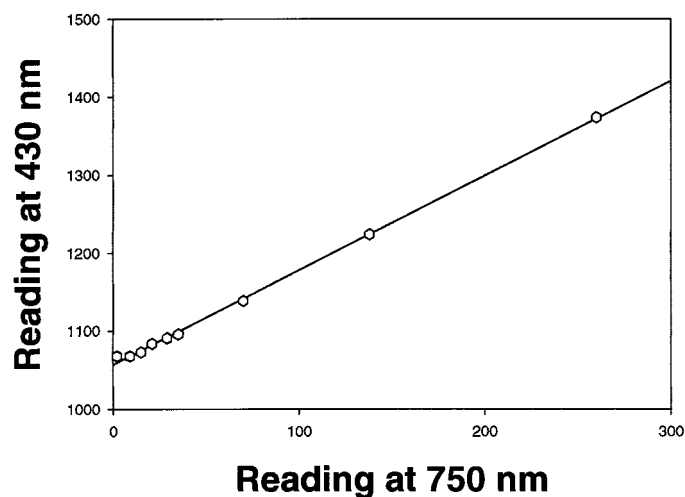


Fig. 4. Effect of turbidity on A₄₃₀ in Winkler-fixed samples. At one level of DO (9.52 mg liter⁻¹), turbidity was varied from 2 to 200 NTU. Plot shows change in A₄₃₀ against A₇₅₀ (a measure of turbidity). The slope of this plot (1.21) was used as the correction factor to estimate DO in turbid samples.

couple has little absorbance at 750 nm and suspended material has peak absorbance there, we used a plot of absorbance at 430 nm (for a single DO value) versus the absorbance at 750 nm to experimentally determine a reasonable correction factor (CF) for turbidity. Thus, the corrected absorbance (CA_{turbidity}) due to the I₃-I₂ couple alone is equal to Abs₄₃₀ - CF × Abs₇₅₀, where CF is the slope of the plot in Fig. 4 (1.21). Thus, for every unit increase in absorbance at 750 nm due to turbidity, there is 1.2 times the absorbance at 430 nm. We then used the corrected absorbance in Eq. 3 to calculate DO.

We tested this approach by varying both oxygen and turbidity (Fig. 5). At three turbidity levels (0, 25, and 80 NTU) and for DO ranging from 5 to 13 mg liter⁻¹, there was no statistically significant difference between estimated DO from the spectrophotometric and titrimetric approaches (Mann-Whitney rank-sum test, $P = 0.528$), and both reproduced the actual oxygen level without bias. Thus, although suspended matter does increase the Abs₄₃₀, these data show that this interference is easily correctable by also reading at 750 nm. In most cases, the needed correction will be negligible.

Interferences: Chlorophyll—At two levels of chlorophyll (12 and 127 μg Chl-*a* liter⁻¹ as final concentrations), we compared the estimate of DO using the turbidity correction with the absolute DO. At 12 μg Chl-*a* liter⁻¹, the Abs₇₅₀ in a 1-cm cuvette was 0.011. In this case, without the turbidity correction, the DO concentration would have been increased by 0.14 mg liter⁻¹. At 127 μg Chl-*a* liter⁻¹, the Abs₇₅₀ in a 1-cm cuvette was 0.168. Without the turbidity correction, in this case the DO concentration would be increased by 1.88 mg liter⁻¹. At both chlorophyll levels the turbidity correction reproduced the actual DO concentration.

Interferences: Organic color—At one oxygen level (10.7 mg liter⁻¹), leaf leachate predictably increased Abs₄₃₀ in the

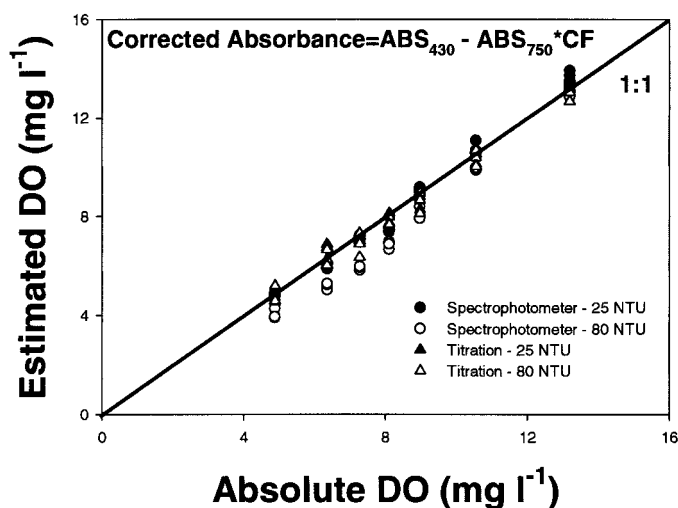


Fig. 5. Comparison of titrimetric (triangles) and spectrophotometric (circles) measures of DO against the absolute value of DO at two levels of turbidity (25 NTU, filled symbols; 80 NTU, open symbols). Samples were filtered (GFD/Whatman) Hudson River water with added turbidity. The spectrophotometric data were converted to DO using the model shown in Fig. 3, using the slope shown in Fig. 4 as a correction factor. The line is the 1:1 line.

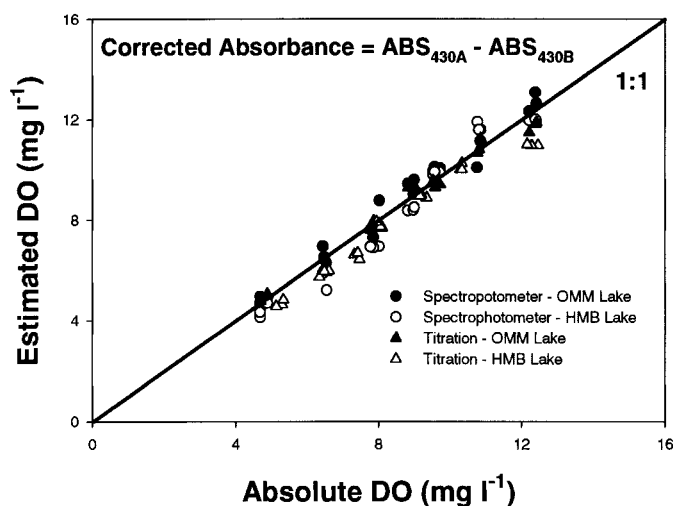


Fig. 6. Comparison of titrimetric (triangles) and spectrophotometric (circles) measures of DO against the absolute value of DO for water from two highly colored humic lakes (Old Man McMullen, filled symbols; Hummingbird Bog, open symbols). Spectrophotometric data were converted to DO using the model shown in Fig. 3 (Eq. 3) and corrected for color. 430A: absorbance at 430 nm in Winkler-fixed sample; 430B: absorbance at 430 nm of "NO MnSO₄" sample (all Winkler reagents except MnSO₄).

Winkler-fixed sample. Although this organic color presents a potential for interference, our data suggest that the effect will be quite minor in all but the most strongly colored environments. At this level of DO we found a change in Abs₄₃₀ of about 10% over our color gradient. Our gradient, however, was much more strongly colored than most natural waters. Very dark bog waters would have an Abs₄₄₀ of 0.9 in a 10-cm cell or 0.09 in a 1-cm cell. At these levels, the effect is less than a few percent of the I₃⁻-I₂ absorbance. An increment of 8 color units (1-cm cell), for example, results in 0.02 mg liter⁻¹ in DO obtained by spectrophotometric reading.

At very high color, a correction does need to be made. To correct for organic color we read Abs₄₃₀ in a range (over both color and DO) of samples that included all of the Winkler reagents and in replicates that included all the reagents except MnSO₄. In the absence of MnSO₄, no iodine is formed and Abs₄₃₀ is caused largely by the organic color present. The organic color in the presence of these reagents (Abs_{430-b}), especially the H₂SO₄, will frequently be different than ambient water color (Abs₄₃₀), so it is a better measure of the effect of color on the DO measurement. In this case, corrected absorbance (CA_{color}) was equal to Abs₄₃₀ - Abs_{430-b}, where Abs₄₃₀ is the absorbance of the sample with all of the Winkler reagents and Abs_{430-b} is the absorbance without MnSO₄.

Using the turbidity CF and Eq. 3, we found nearly perfect agreement between the spectrophotometric estimates and the absolute values of DO over a range from 5 to 13 mg liter⁻¹ in water from the two humic lakes (Fig. 6). The spectrophotometric estimate was also in excellent agreement with the titrimetric measurements, and there was no statistically significant difference between the results from the two procedures (*t*-test, *P* = 0.416).

Over a wide range of DO concentrations in both natural and experimentally manipulated samples, we found a tight correlation between Abs₄₃₀ of Winkler-fixed samples and the titrimetric estimates of DO. For DO between 4 and 12 mg liter⁻¹, this relationship could be modeled as linear. Further, Abs₄₃₀ was well correlated to the known concentration of DO in samples that were air-saturated at varying temperatures. Turbidity, chlorophyll, and organic color affect the relationship between Abs₄₃₀ and DO, but these effects are generally minor and correctable. Probably because of changes in the amount of I₂ present in the I₃⁻-I₂ couple (which is dependent on the concentration of iodide), the absorbance was somewhat dependent on the amount of alkali-iodide reagent that was initially added. To obtain good results it is necessary to deliver this reagent in a reproducible way and to read all samples at the same temperature.

Discussion: Turbidity—Most waters have turbidity less than ~25 NTU. The turbidity in the Hudson River, for instance, ranges from 5 to 30 NTU (rarely reaches higher values) and averages about 16 NTU. At 25 NTU, the Abs₇₅₀ in a 1-cm cuvette was 0.024 ± 0.003 (3.4% of the lowest Abs₄₃₀, 0.736). Without making the correction described above and using Eq. 4, this would increase the estimate of DO by only 0.27 mg liter⁻¹ (average of complete DO range obtained using 25 NTU, SD = 0.03). Thus, at reasonably high DO values the turbidity effect, even without correction, is quite small for most systems. The same turbidity correction worked for both inorganic suspended material and for phytoplankton particles. Because this correction involves only the reading of an additional wavelength on the spectrophotometer, it does not slow down the analysis significantly.

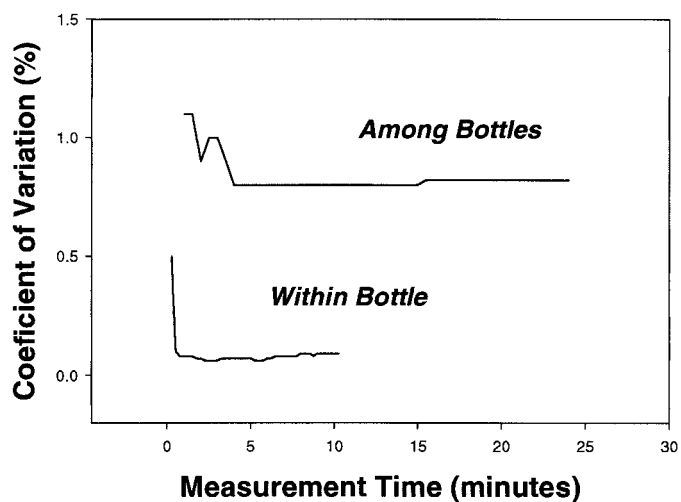


Fig. 7. The relationship between analysis time and the coefficient of variation ($100\% \times \text{SD}/\text{mean}$) for spectrophotometric methods. Lines are shown for this relationship within a single bottle (“Within Bottle”) and for readings taken among different bottles (“Among Bottles”).

Discussion: Color—Our study suggests that color high enough to significantly interfere with the spectrophotometric DO method is uncommon. In a large survey of lakes in the Eastern United States, only 2% of the 1,742 lakes studied exceeded color of 6 units (e.g., 0.006 absorbance units in a 1-cm cell; Linthurst et al. 1986). For higher color, the correction we tested adequately deals with the interference. Unfortunately, this color correction requires additional work and time, since a second sample needs to be fixed with all of the reagents except MnSO_4 . On the other hand, if the color in a given system is reasonably stable over time, it may be possible to test this interference only occasionally. We suggest using a color correction especially for experimental designs involving comparison across systems. Depending on the organic substrate, both titration and spectrophotometric procedures can generate inaccurate results.

Precision and effort—The major advantages of the spectrophotometric method are its ease, speed, and low cost. From a single 60-ml BOD bottle one can easily measure at least 15 replicates in 5 min. The within-bottle coefficient of variation, or CV ($100\% \times \text{SD}/\text{mean}$) is a declining function of time and decreases to $<0.08\%$ after about 10 min (48 readings; Fig. 7). In practice, our laboratory usually measures four readings from each bottle. From a large data set on bacterial respiration (Roland and Cole 1999), the mean CV for quadruplicate measurements from 684 bottles was 0.08%. The variance among bottles, which includes the errors in filling, fixing, and handling, are, of course, somewhat larger. Using 60-ml bottles, we were able to achieve a CV of about 0.8% after 10 min of effort (Fig. 7). Using the titrimetric procedure on the same samples, we still had a CV above 1% after 100 min of effort. Given enough analytical time, the titrimetric method can be more precise, with CVs near 0.03–0.1% reported by various investigators (e.g., Car-

ignan et al. 1998), but this high precision requires a great deal of time.

In an incubation experiment, the ability to detect DO changes depends on the confidence limits around the mean estimate. The confidence limit depends on both the CV among bottles and the number of bottles one is willing to analyze. Assuming a typical surface-water DO value of $9.5 \text{ mg O}_2 \text{ liter}^{-1}$ and a CV (among bottles) of 0.8% for both the initial and final values, we could resolve a change of $100 \mu\text{g O}_2 \text{ liter}^{-1}$ ($3.1 \mu\text{mol liter}^{-1}$) with $n = 4$ bottles using the spectrophotometric method. To detect a change of $50 \mu\text{g O}_2 \text{ liter}^{-1}$ would require 7 bottles. The analytical time would be a few minutes, which would allow us to confidently measure DO consumption rates as low as $20 \mu\text{g O}_2 \text{ liter}^{-1} \text{ h}^{-1}$ in an incubation of $<6 \text{ h}$. This degree of resolution makes the present method useful in all but extremely oligotrophic waters. It approaches the resolution obtained by automatic potentiometric titrations (Graneli and Graneli 1991; Sondergaard and Theil-Nielsen 1997) and very careful applications of manual titrations (Carignan et al. 1998). We suspect that by using 300-ml BOD bottles and the filling and reagent delivery procedures of Carignan et al. (1998), the resolution of the spectrophotometric method could be even better than we obtained.

The modification of the spectrophotometric DO method is useful over a fairly wide range of oxygen concentrations. We urge precaution, however, above $12 \text{ mg O}_2 \text{ liter}^{-1}$. It appears that at high DO, the relationship between spectrophotometric and titrimetric measurement becomes nonlinear. This effect is likely due to high absorbance at these DO levels, as we saw in the $\text{KH}(\text{IO}_3)_2$ addition experiment (Fig. 1).

The general model relating spectrophotometric readings at 430 nm and DO obtained from our experimental data (corrected absorbances for turbidity and color experiments) is this:

$$\text{DO (mg liter}^{-1}\text{)} = 0.0081 \cdot \text{CA} - 0.410$$

$$(R^2 = 0.96; n = 276; P < 0.0001),$$

$$(4)$$

where CA is the corrected absorbance for either turbidity or color. This equation is not far from that obtained by measuring the A_{430} for I_3^- – I_2 couple directly (e.g., Fig. 1). The discrepancy is due almost entirely to the differences in the intercept. We believe that Eq. 4 can be applied generally for DO values between about 4 and 12 mg liter^{-1} , but we suggest that calibration curves be tested in each system against titration. Further, our model may not be applied at very low DO. As DO becomes smaller, the intercept in the general model becomes relatively more significant and may need to be better evaluated as well.

Fabio Roland¹
Nina F. Caraco
Jonathan J. Cole

Institute of Ecosystem Studies
Box AB, Millbrook, New York 12545

Paul del Giorgio

Horn Point Environmental Laboratory, CEES,
University of Maryland, Cambridge, Maryland 21613

References

- AMINOT, A. 1988. Precision and accuracy of dissolved oxygen measurements; a comment on the paper by Oudot et al. 1988: Precise shipboard determination of oxygen (Winkler procedure) for productivity studies with a commercial system. *Limnol. Oceanogr.* **33**: 1646–1648.
- ASHTON, P. J., AND A. J. TWINCH. 1985. An assessment of rapid and convenient spectrophotometric adaptation of the Winkler procedure for determination of dissolved oxygen in fresh waters. *J. Limnol. Soc. So. Afr.* **11**: 62–65.
- ATWOOD, D. K., W. K. KINARD, M. J. BARCELONA, AND E. C. JOHNSON. 1977. Comparison of polarographic electrode and Winkler titration determinations of dissolved oxygen in oceanographic samples. *Deep-Sea Res.* **24**: 311–313.
- BROENKOW, W. W., AND J. D. CLINE. 1969. Colorimetric determinations of dissolved oxygen at low concentrations. *Limnol. Oceanogr.* **14**: 450–454.
- BRYAN, J. R., J. P. RILEY, AND P. J. L. WILLIAMS. 1976. A Winkler procedure for making precise measurements of oxygen concentration for productivity and related studies. *J. Exp. Mar. Biol. Ecol.* **21**: 191–197.
- CARIGNAN, R., A. M. BLAIN, AND C. VIS. 1998. Measurement of primary production and community respiration in oligotrophic lakes using the Winkler method. *Can. J. Fish. Aquat. Sci.* **55**: 1078–1084.
- CARPENTER, J. H. 1965. The accuracy of the Winkler method for dissolved oxygen analysis. *Limnol. Oceanogr.* **10**: 135–140.
- CARRIT, D. E., AND J. H. CARPENTER. 1966. Comparison and evaluation of currently employed modifications of the Winkler method for determining dissolved oxygen in seawater; a NASCO report. *J. Mar. Res.* **24**: 286–318.
- CUSTER, J. J., AND S. NATELSON. 1949. Spectrophotometric determination of microquantities of iodine. *Anal. Chem.* **21**: 1005–1009.
- CUTHBERT, I. D., AND P. DEL GIORGIO. 1995. Toward a standard method of measuring color in freshwater. *Limnol. Oceanogr.* **37**: 134–148.
- DEL GIORGIO, P., AND R. PETERS. 1994. Patterns in planktonic P:R ratios in lakes: Influence of lake trophy and dissolved organic carbon. *Limnol. Oceanogr.* **39**: 772–787.
- DUVAL, W. S., P. J. BROCKINGTON, M. S. VON MELVILLE, AND G. H. GEEN. 1974. Spectrophotometric determination of dissolved oxygen concentration in water. *J. Fish. Res. Board Can.* **31**: 1529–1530.
- GOLTERMAN, H. L., R. S. CLYMO, AND M. A. M. OHNSTAD. 1978. IBP handbook no. 8: Methods for physical and chemical analysis of fresh waters, 2nd ed. Blackwell Scientific.
- GRANELI, W., AND E. GRANELI. 1991. Automatic potentiometric determination of dissolved oxygen. *Mar. Biol.* **108**: 341–348.
- HARTWIG, E. O., AND J. A. MICHAEL. 1978. A sensitive photoelectric Winkler titrator for respiration measurements. *Environ. Sci. Technol.* **12**: 712–715.
- HE, X., AND R. A. WRIGHT. 1992. An experimental study of piscivore-planktivore interactions: Populations and community responses to predation. *Can. J. Fish. Aquat. Sci.* **49**: 1176–1183.
- HOLM-HANSEN, O., AND B. RIEMANN. 1978. Chlorophyll-*a* determination: Improvement in methodology. *Oikos* **30**: 438–447.
- LINTHURST, R. A., D. H. LANDERS, J. M. EILER, D. F. BRAUKE, W. S. OLAJON, E. P. MEIER, AND R. E. CROW. 1986. Characteristics of lakes of the Eastern United States. U.S. Environmental Protection Agency, pub. no. EPA 600/4–86/007A.
- MACKERETH, F. J. H., J. HERON, AND J. F. TALLING. 1978. Water analysis: Some revised methods for limnologists. Freshwater Biological Association scientific publications No. 36. Titus Wilson.
- RECHE, I., M. L. PACE, AND J. J. COLE. 1999. Regulation of photobleaching of dissolved organic matter in lake ecosystems. *Biogeochemistry.* **44**: 259–280.
- REYNOLDS, C. S. 1972. Growth, gas vacuolation and buoyancy in a natural population of a planktonic blue-green alga. *Freshwater Biol.* **2**: 87–106.
- REYNOLDS, J. F. 1969. Comparison studies of Winkler vs. oxygen sensor. *J. Water Pollut. Control Fed.* **41**: 2002–2009.
- ROLAND, E., AND J. J. COLE. 1999. Prediction of bacterial growth efficiency in a riverine ecosystem. *Verh. Int. Verein. Limnol.* In press.
- SONDERGAARD, M., AND J. THEIL-NIELSEN. 1997. Bacterial growth efficiency in lakewater cultures. *Aquat. Microbiol. Ecol.* **12**: 115–122.
- WETZEL, R. G., AND G. E. LIKENS. 1991. *Limnological analysis*. Springer-Verlag.
- WILLIAMS, P. J. L., AND N. W. JENKINSON. 1982. A transportable microprocessor-controlled precise Winkler titration suitable for field station and shipboard use. *Limnol. Oceanogr.* **27**: 576–584.
- AND D. A. PURDIE. 1991. In vitro and in situ derived rates of gross production, net community production and respiration of oxygen in the oligotrophic subtropical gyre of the North Pacific Ocean. *Deep-Sea Res.* **38**: 891–910.
- WILCOCK, R. J., C. D. STEVENSON, AND C. A. ROBERTS. 1981. An interlaboratory study of dissolved oxygen in water. *Water Res.* **15**: 321–325.
- WINKLER, L. W. 1888. Die Bestimmung des im Wasser gelosten Sauerstoffes. *Chem. Ber.* **27**: 2843–2855.

Acknowledgments

This is a contribution of the Institute of Ecosystem Studies. Financial support was provided in part by the Hudson River Foundation, the National Science Foundation, and CNPq Brazil.

¹ Also affiliated with the Institute of Biological Sciences, Biology Department/UFJF, Juiz de Fora, MG, Brazil, 36036-330.

Received: 2 January 1998

Accepted: 26 January 1999

Amended: 9 March 1999