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An Automated Precise Winkler Titration for Determining Dissolved Oxygen on Board Ship

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An automated titration for the Winkler method is presented for measuring primary productivity in the ocean. The system is based on a microcomputer-controlled titration with potentiometric endpoint detection. By the use of 0.005-N sodium thiosulfate as a titrant and a program designed to shorten the time for measuring, the method achieves a precision of 0.04% coefficient of variation with a range of 0.01 to 0.10% for six replicates of samples at oxygen concentration of 70 to 250 μM. It takes about four to five minutes to measure one sample.

1. Introduction

Dissolved oxygen is central in studies on biological productivity and biochemical processes in the aquatic environments. Various methods are used for determining oxygen concentration. Because of the highest accuracy and precision, the iodometric Winkler method (Winkler, 1888) and its modifications have been widely used. Since the classical Winkler titration imposes a difficulty in visual determination of the endpoint of titration by means of the changes in the color of the starch-iodine complex, there have been attempts to apply instrumental detection of the endpoint to increase the precision of the method. Furthermore, automated titration techniques for the Winkler method have been developed in the last decade. Williams and Jenkinson (1982) proposed a system based on a photometric endpoint detector. Their system was applied successfully for measuring plankton primary productivity and community respiration in the oligotrophic ocean (Williams *et al*., 1983). Since the photometric endpoint detection can be affected by particles in the sample solution and bubbles generated during the measurement, Culberson and Huang (1987) proposed a system by using an amperometric endpoint detector. They reported that the precision of the method was 0.3 μM. Considering magnitudes of daily oxygen fluxes of plankton metabolism is in the order of 1 μM in the oligotrophic oceans, a better precision is required for productivity measurements.

Compared to the photometric and amperometric endpoint detection, little attention has been paid to the potentiometric detection for use in automated titration probably because the electrochemical equilibrium at the platinum indicator electrode is considered to be established slowly (Grasshoff, 1981) and it is difficult to detect iodine at low concentration of 10^{-6} N (Potter, 1957). However, since potentiometry is simple, convenient and generally precise, it seems worth while exploiting potentiality of the potentiometric endpoint detection for shipboard analysis.

We have developed an automated titrator using a potentiometric endpoint detection with a redox potential electrode. The system substantially consists of commercially available components which are controlled by an improved computer program which facilitates a short measurement

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time. Our primary concern is the use for plankton productivity on board ship. Using the automated system, we attain a high precision enough for measurements of plankton productivity.

2. Materials and Methods

2.1 Apparatus

The automated system is composed of five major components (Fig. 1): (1) the oxidation-reduction potential electrode (Toa, PTS-5011C) as an endpoint detector, connected with a pH/mV meter (Toa, HM-18ET or HM-70V), (2) a DC signal isolator (Watanabe, DCE 21R-1) which amplifies the electrode output, (3) a 12-bit analog to digital convertor (Canopus, Analog Pro 1) to interface the isolator with a microcomputer, (4) a microcomputer (NEC, PC9801) to control the titration, and (5) a piston burette (Metrohm, Dosimat 665) to feed sodium thiosulfate as titrant and to read volume of the titrant dispensed. The burette is fitted with a non-diffusive burette tip (Metrohm) which eliminates diffusion of thiosulfate into specimens. Although a combination of an Ag/AgCl reference and a platinum indicator electrode is also satisfactory in terms of responses, we prefer the composite electrode because of easy handling of the electrode and narrow neck of BOD bottles in which a whole titration is processed.

The Dosimat 665 is fitted with a 50-mL cylinder (Metrohm, E552), with which a minimum amount of titrant in a single stepwise feeding is 5 μL. Since we use lower concentration of sodium thiosulfate than 0.01 N as used in the usual analysis, we need the large capacity burette cylinder to enable titration of one sample without refill that is a source of errors. The burette was chosen because the Dosimat provides the largest cylinder capacity among commercially available products and offers remote-controllable operation with the computer. The control is made by serial transmission of command and volume data on titrant via an interface according to RS 232 C in half duplex procedure at baud rate of 600. The whole titration process is controlled by a program written in BASIC (Furuya and Harada, in preparation).

2.2 Procedure

The titration protocol substantially follows the manual titration. There are three stages in the titration protocol, each called initial, absolute and differential modes in sequence (Fig. 2) after

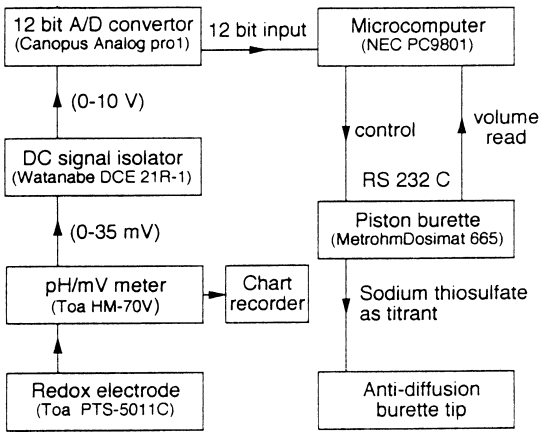

Fig. 1. Diagram of an automated potentiometric titrator.

Williams and Jenkinson (1982). First, in the initial mode, approximately 50 to 60% of a rough estimate of the final volume of titrant is dispensed continuously. Once information on sample and BOD bottle number has been entered via keyboard, the program begins. The program requests input of the rough estimate. With the input of the estimate the titrant is fed at a rate of 2.5 mL s^{-1}. The rough estimate is arbitrarily decided according to nature of samples. When the oxygen content of a sample is difficult to make the rough estimate, a conservative value is preferable to avoid over-feeding of the titrant. In this mode, the redox potential does not change much compared to the later modes. For surface water of the Kuroshio, the redox potential of the acidified fixed sample is around 720 mV, which decreased to around 685 mV during the initial mode.

Next, in the absolute mode, the titrant is dispensed by a series of pulse addition. With a pulse

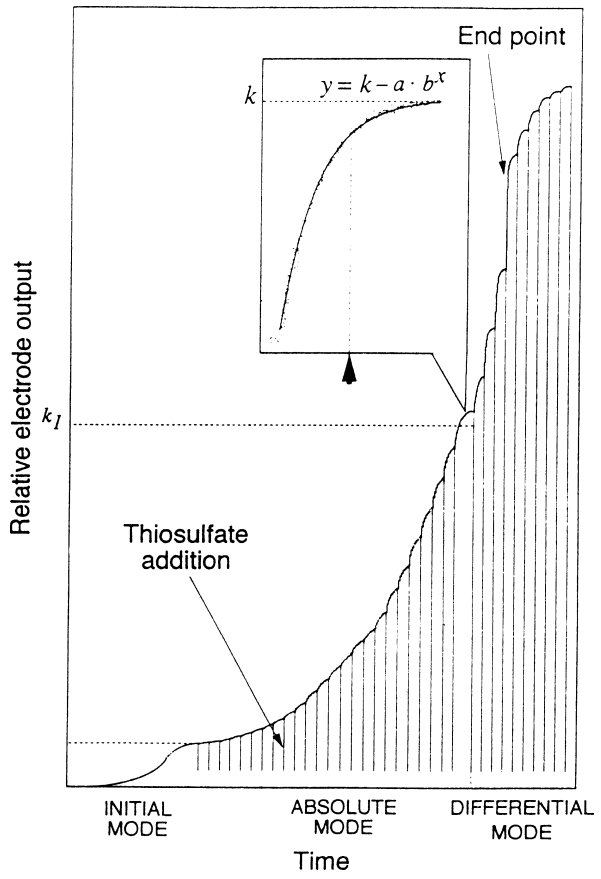

Fig. 2. Schematic change in electrode output during the titration. A series of vertical bars indicate successive addition of thiosulfate. Titration protocol changes from the absolute mode to differential mode when the output reaches a predetermined value, k_1. The largest shift of the output during the differential mode is taken as an endpoint. An inset gives an example of a time course of the electrode output in an actual sample after an addition of thiosulfate. Using 576 successive electrode readings taken from two seconds after a pulse addition through the serial port, the microcomputer estimates a plateau value k by the curve fitting to a formula shown here. Then next feeding is prompted at the time indicated by an arrow in the inset.

addition, the electrode output increases stepwise. At each pulse, the electrode output is referred to a predetermined value. Once the output reaches the value, the procedure is repeated with a renewed set of a smaller constant volume of titrant and a smaller electrode-output reference value than those in the preceded stage. All these sets of titrant volume and reference value are predetermined in the laboratory, stored in the memory and called up. For the Kuroshio and subtropical open waters, we use six pulse volumes; 1, 0.5, 0.1, 0.05, 0.025 and 0.01 mL, each corresponding to the electrode-output reference values of 655, 631, 609, 599, 583, and 565 mV, respectively. Throughout this mode, each pulse feeding of the titrant is made before the electrode output reaches a plateau. The plateau is estimated from time course of the electrode output using an exponential function (Fig. 2):

$$y = k - a \cdot b^x \tag{1}$$

where x is time after a single feeding, y is the electrode output, k is estimated plateau value, a and b are empirical constants that vary according to titration setup, that is, volume of a BOD bottle, rotation rate of a magnetic stirrer within the BOD bottle, volume of added titrant and relative positions of the tip of the burette and the electrode. These constants a and b are predetermined in the preliminary experiments and stored in the memory. Different sets of a and b are needed, each corresponding to each pulse volume of titrant listed above. The program estimates k by the curve-fitting to the above formula using 576 successive readings taken from two seconds after a pulse feeding of titrant. The period of two seconds is for mixing time of the added titrant and the sample solution. Using the calculated k, next feeding is prompted without the output reaching a plateau to shorten the titration time.

The absolute mode is continued until an electrode output reaches the provisional value, for which we use 565 mV. Then the rest of the titration is made in the differential mode. During the mode a 5-μL titrant is dispensed every six seconds. The largest increase of electrode output on a single addition is taken as an endpoint. The titration continues for five additional feedings of the titrant after reaching the endpoint to ensure the endpoint, which is found around 535 mV.

2.3 Reagents and fixation of oxygen

Preparation of chemical reagents, fixation of oxygen, standardization of working solution of sodium thiosulfate, and acidification of the sample were made following Carpenter (1965b). Reagent blanks were corrected also after Carpenter (1965b). The CSK standard solution of KIO_3 was used for the standardization of sodium thiosulfate solution. Precise dispensers with repeatability of <0.1% coefficient of variation (CV)were used for the addition of alkaline iodide and manganese (II) chloride into the 200–220-mL BOD bottles. The titration was performed within the BOD bottles without transferring the whole sample to a titration vessel, as the transfer is a major source of errors due to volatilization of iodide (Carpenter, 1965a).

Volumetry of BOD bottles was made at 20°C down to two decimal places in mL after Culberson (1991). This procedure was repeated at least five times, and mean bottle volume was obtained.

2.4 Comparison of visual starch and potentiometric endpoint indications

The sensitivities of endpoint indication by starch and the potentiometric methods were compared using a pottasium iodate solution and a seawater sample. Two 10.00-mL portions of 0.0100-N potassium iodate (CSK standard solution, Wako Chemicals) were taken with a

calibrated automatic dispenser (ADT-1, Toa Electronics) and to each were added 0.3 g of potassium iodide and diluted in 90-mL portions of distilled water with 10-N sulfuric acid to adjust pH of the solution to be 2.0. For the starch endpoint detection 0.5 mL of 1% (w/v) starch solution was added to one portion. The seawater sample taken from the surface of the Kuroshio was introduced into 200- to 220-mL BOD bottles and filled after overflow by at least one bottle volume and the dissolved oxygen was fixed. The potassium iodate solution and the seawater were titrated with 0.004967-N sodium thiosulfate, and added amount of the titrant was compared.

2.5 Seawater samples and analysis

Field experiments were conducted during the three cruises of T/S Seisui Maru in the Philippine Sea in summer and a K93-03 cruise of R/V Kaiyo in the East China Sea in October 1993. Oxygen content was also analyzed ashore laboratory for seawater taken from Ise Bay and mesocosms settled over bottom sediments in an eutrophicated embayment in Gokasho Bay in July 1993. Seawater samples were taken into the BOD bottles and fixed.

The oxygen content in a part of East China Sea samples and all samples of Gokasho Bay was determined manually without the computer control by a practiced operator using the same apparatus and protocol described above. In the absolute mode, successive pulse addition of the titrant was made before the output reached plateaus, based on the electrode output on a digital chart recorder. This is referred as the potentiometric manual titration here.

3. Results and Discussion

3.1 Effect of the titrant concentration

Reproducibility of the oxygen determination depended on the concentration of sodium thiosulfate (Fig. 3). In our system, 0.005 to 0.0067 N of sodium thiosulfate gave lowest CV. Based on this low variance, we used 0.005 to 0.0067 N of sodium thiosulfate in the present study. In the differential mode, the endpoint was determined by a series of a 5-μL addition of titrant. With 0.005-N thiosulfate, each addition contains 0.025 μ normality of thiosulfate that is equivalent to 0.00625 μmole O_2. Since we used about 200-mL BOD bottles, 0.03 μM (0.00625 multiplied by 5) is considered to be lower limit of the precision, provided that other sources of errors are not taken into account.

The high concentration over 0.01 N yielded significantly higher CV (F-test, $p < 0.05$). This suggests that the concentration of the titrant is so high that the amount of the titrant fed in a single addition is too large to resolve endpoint well. This probably reduces the reproducibility. There is also another advantage in the use of a diluted titrant. We checked leakage of the titrant from the end of the tip into the sample by the use of Rhodamin B added in the titrant. Even without burette dosage, fluorescence was detectable in the sample as determined by fluorometry, indicating occurrence of the leakage in spite of the use of the anti-diffusion burette tip. A normal burette tip without the membrane resulted in a more intense fluorescence, suggesting more leakage than the anti-diffusion tip. Thus, low concentration of the titrant can reduce the erroneous effect of the leakage, especially in the automated titration with pulsing addition of the titrant.

3.2 Comparison of visual starch and potentiometric endpoint indications

The starch endpoint occurred before the potentiometric endpoint (Table 1). The difference in the endpoint between the two methods was significant (t-test at $p < 0.005$). Similar comparisons were repeated five times using surface waters of the Philippine Sea, and the endpoint

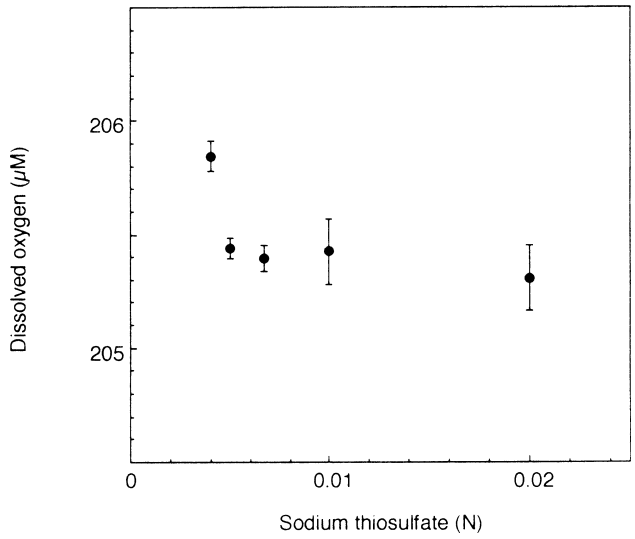

Fig. 3. Relationship between concentration of sodium thiosulfate and dissolved oxygen determination. Error bars denote one standard deviation for six replicates.

Table 1. Comparison of starch and potentiometric endpoints. 10 mL of 0.01-N KIO_3 and surface water of Kuroshio are titrated with 0.004967-N sodium thiosulfate. For the seawater titrant volume was normalized for 100-mL seawater. *n*: number of replicates.

Sample	Starch endpoint		Potentiometric endpoint	
	mean added titrant (mL)	CV (%)	mean added titrant (mL)	CV (%)
KIO_3 ($n = 5$)	19.900*[1]	0.2531*[2]	20.134*[1]	0.0111*[2]
Seawater ($n = 5$)	16.406*[3]	0.2381*[4]	16.522*[3]	0.0373*[4]

*[1], *[2], *[3], *[4]: significant at $p < 0.005$.

bias occurred consistently; more feeding of titrant was necessary in the potentiometric titration than in the starch method by 0.7 to 0.9% of titrant volume of the latter method. This difference in the endpoints is in agreement with the fact that the amperometric endpoint detection requires more feeding of titrant than the starch method (Bradbury and Hambly, 1952; Knowles and Lowden, 1953; Culberson and Huang, 1987; Culberson *et al*., 1991). Bradbury and Hambly (1952) concluded that the visual endpoint with starch is significantly different from the equivalence points detected by amperometry. However, the difference in the endpoint among the detection methods does not necessarily yield difference in oxygen concentration as long as the titrant is calibrated by the same detection method as used for samples. In the comparison shown in Table 1, we found no significant difference in oxygen concentration between the two methods.

The precision of the potentiometric endpoint detection in terms of CV was significantly higher than the starch method (Table 1) (F test at $p < 0.005$). Since precision is the primary

requirement for biological production studies, the obtained CV for the surface seawater is satisfactorily small for our purpose.

3.3 Seawater analysis

Overall the titration procedure achieved a standard deviation of 0.026 to 0.191 μM (mean 0.096 μM) in measurements of dissolved oxygen concentration ranging from 70.91 to 250.01 μM (Fig. 4). The potentiometric manual titration yielded the standard deviation ranging from 0.040 to 0.161 μM (mean 0.103 μM). The automated titration attained the same precision as the potentiometric manual titration in the range 0.026 to 0.191 μM (mean 0.087 μM). The standard deviation of 0.1 μM of oxygen indicates that the automated method is suitable for measuring plankton primary production and community respiration in the open ocean, where daily oxygen change is generally in the order of one to several μmole per liter. The precision of the method expressed as CV is in the range of 0.01 to 0.10% with a mean of 0.049%. This precision is equivalent to or a little better than that of the automated photometric titration whose CV is in the range of 0.03–0.1% (Williams and Jenkinson, 1982).

Rough weather tended to produce high standard deviation on board ship analysis: the high standard deviation above 0.14 μM in the Philippine Sea and the East China Sea were obtained under rough sea conditions due to a low pressure (Fig. 4). High standard deviation was also observed during the mesocosm experiment, where one batch was intensely aerated to examine degradation of organic materials (Yamaguchi and Washio, unpublished). The aerated water showed significantly higher CV (F-test at $p < 0.01$) in the oxygen determination than non-aerated

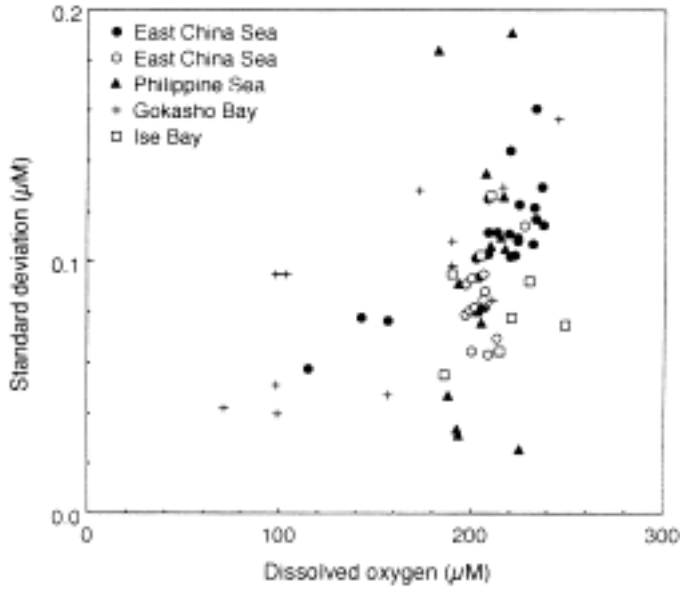

Fig. 4. Dissolved oxygen concentration and its standard deviation of seawater samples analyzed on shipboard (open and closed circle: East China Sea, closed triangle: Philippine Sea) and in land laboratory (open square: Ise Bay, cross: Gokasho Bay). The analysis was made for six replicate measurements in the East China Sea, Ise Bay and the Philippine Sea, and five replicates in Gokasho Bay. Samples of Gokasho Bay and a part of samples from the East China Sea (open circle) were analyzed manually without computer control.

Table 2. Comparison of variations in oxygen contents of aerated and non-aerated waters during a mesocosm experiment.

Sample	Mean oxygen contents (μM)	Average of standard deviation (μM)	Average of coefficient of variation (%)
Aerated water (n = 6)	241.27*[1]	0.214*[2]	0.089*[3]
Non-aerated water (n = 12)	154.41*[1]	0.090*[2]	0.060*[3]

*[1], *[2], *[3]: significant at $p < 0.01$.

water in the control mesocosm (Table 2). Although the high standard deviation during the rough sea conditions is not ascribed to either errors in the titration procedure, or sampling errors due to bad sea conditions, the observation made during the mesocosm experiment suggests the high standard deviation is a manifestation of variance of oxygen content itself among the BOD bottles.

We applied the potentiometric endpoint detection in very turbid waters near the mouth of Changjiang River, where the extinction coefficient of the photosynthetically available radiation exceeded 0.65 m^{-1} in February 1993. While samples were rich in small particles, in our measurements we observed high precision of mean CV of 0.079%, although turbid waters can produce low reproducibility in the precise photometry and in amperometry (Culberson and Huang, 1987). Thus, applicability to wide range of waters and simplicity are major advantages of the potentiometric titration. The improvement of our titration protocol in estimating k in the absolute mode shortens the measurement time. With this procedure, analysis time per bottle is about four to five minutes. A well-experienced operator can attain a shorter measurement time using the same titration protocol without the computer control. For a more rapid titration, maximization of magnetic stirrer speed control and rates of titrant feeding seems to be important.

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