Statistical Comparison of Multiple Methods for the Determination of Dissolved Oxygen Levels in Natural Waters

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Received November 3, 2001. Accepted February 27, 2002

Abstract: The following experiment reinforces students' working knowledge of statistics by utilizing the *t* test to compare the results of two independent methods for the determination of dissolved oxygen (DO). In this experiment students utilize a dissolved oxygen probe to determine the levels of DO in natural waters at two sampling locations while obtaining samples of water from the laboratory for analysis using the classic Winkler titration. The importance of using proper sampling methods and techniques to obtain representative samples is a large focus of the prelaboratory discussion and is continually stressed during fieldwork. After analyzing the water samples by the DO meter and the Winkler titration, students pool the class data and are asked to determine if the two methods for dissolved oxygen agree at each sampling location. The students are then asked to determine if the DO levels at the different sampling locations are statistically different or not. The students are asked to consider why their results agree or differ from the theoretical value they calculate using Henry's law.

Introduction

The purpose of the analytical chemistry laboratory is to produce effective practitioners in the art of chemical analysis. This entails a wide scope of skills and knowledge, including, but not limited to sampling, statistics, and methods of analysis. Accordingly, the experimental aspect of analytical courses should incorporate these same skills. Granted, many times it is more efficient to focus on a single issue during a laboratory experiment. This is especially true for students new to the analytical laboratory; however, it is prudent after a number of introductory experiments to integrate those previously learned skills into an advanced experiment in order to definitively illustrate the wide spectrum of skills they are expected to master.

A number of previously published articles that illustrate a single concept or aspect of the analytical process were used as information sources in the development of this experiment. The importance of properly teaching sampling and sampling statistics was recently discussed by Vitt and Engstrom [1]. There are a number of publications that discuss statistical evaluation of experimental data [2–5], Henry's law [6], and the Winkler titration for dissolved oxygen determination [7–9]. An excellent paper recently published in this journal discussed the importance of field environmental-analytical chemistry experiences in the quantitative analysis course [10]; however, few laboratory exercises that statistically compare two independent methods of analysis for a single analyte can be found. The pedagogical advantage of this experiment is that students must address the problems and concerns of determining dissolved oxygen levels in natural waters and statistically compare two methods commonly used to measure this variable.

Experimental

The prelaboratory assignment consists of a handout that details calculating the theoretical value of dissolved oxygen in water according to Henry's law. The students complete this portion of the experiment to familiarize themselves with the concept of the diffusion of gas into a liquid and what are the typical values of dissolved oxygen. The students are also presented with eqs 1 through 5 that are the basis of the Winkler titration.

$$4Mn^{2+} + O_2 + 8OH^{-} + 2H_2O \rightleftharpoons 4\,Mn(OH)_3(s) \quad (1)$$

$$2Mn(OH)_3(s) + 3H_2SO_4 \rightleftharpoons 2Mn^{3+} + 3SO_4^{2-} + 3H_2O \quad (2)$$

$$2Mn^{3+} + 2I^{-} \rightleftharpoons 2Mn^{2+} + I_2 \quad (3)$$

$$I_2 + I^{-} \rightleftharpoons I_3^{-} \quad (4)$$

$$I_3^{-} + 2S_2O_3^{2-} \rightleftharpoons 3I^{-} + S_4O_6^{2-} \quad (5)$$

The students then answer a series of questions designed to illustrate that the dissolved oxygen levels may vary as a function of collection location, sampling depth, and sampling method.

The laboratory portion is completed over two laboratory periods that last three hours each. The first laboratory session is devoted to a more in-depth discussion of the biological, chemical, and physical factors that affect dissolved oxygen levels. These include photosynthesis, decomposition, pH, and temperature. The laboratory discussion includes the requirement that our samples come from homogeneous populations and that all samples have an equal probability of being sampled. There are several precautions required to collect water samples for later analysis of DO, namely, the use of Biological Oxygen Demand (BOD) bottles to eliminate ambient air above the sample, flushing the sample bottle, and treating the sample with manganous sulfate to fix the dissolved oxygen according to eq 1. The procedures that will be used to analyze the data are discussed, namely, the data must follow a normal distribution and the different variations of the *t* test.

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Table 1. Statistical Analysis of Pooled Class Data for Fall 2000

Parameter	Inside Plume Location		Outside Plume Location	
	DO Meter (mg DO L^{-1})	Titration (mg DO L^{-1})	DO Meter (mg DO L^{-1})	Titration (mg DO L^{-1})
Average	10.33	10.12	10.56	11.43
Standard deviation	0.23	0.55	1.40	0.67
Agreement between DO methods at 95% CL	t_{calc}=1.056, t_{table} . 2.131 ˆNo Difference		t_{calc}= 1.682, t_{table} . 2.131 ˆNo Difference	
Agreement between DO Meter data at 95% CL	t_{calc}=0.486, t_{table} . 2.131 ˆNo Difference			
Agreement between titration data at 95% CL	t_{calc}=4.534, t_{table} . 2.131 ˆThere is a difference			
Agreement with Henry's law: 10.44 mg DO L^{-1} at 95% CL (16 EC , P_{O2} = 0.21 atm)	t_{calc} = 1.435 t_{table} . 2.262 ˆNo difference	t_{calc} = 1.745 t_{table} . 2.262 ˆNo difference	t_{calc} = 0.257 t_{table} . 2.262 ˆNo difference	t_{calc} = 4.433 t_{table} . 2.262 ˆThere is a difference

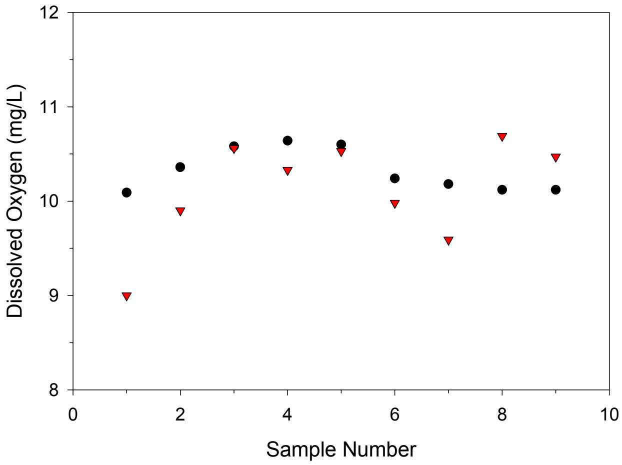

Figure 1. Dissolved oxygen levels determined for each sample by the Winkler Method (–) and by a dissolved oxygen probe (C) for samples collected within the discharge plume of a waste water treatment facility located on the Fox River in DePere, WI.

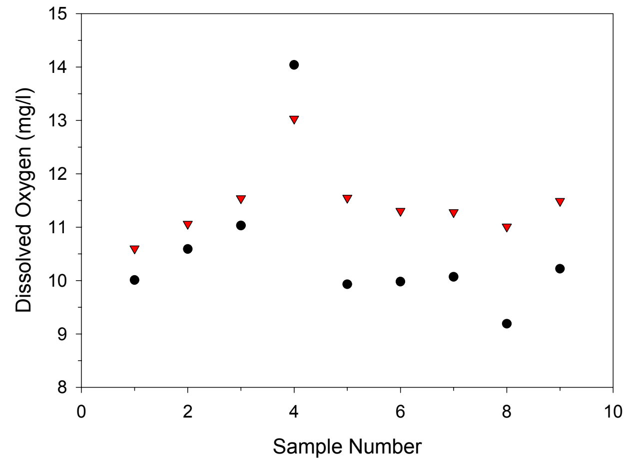

Figure 2. Dissolved oxygen levels determined for each sample by the Winkler Method (–) and by a dissolved oxygen probe (C) for samples collected outside of the discharge plume from a waste water treatment facility located on the Fox River in DePere, WI.

The authors utilized a boat to obtain samples; however, it should be noted that this experiment can be applied to any natural water system, and the sampling can be accomplished from shore. The first group collected samples at a depth of 2 m from a location estimated to be in the discharge plume of a sewage treatment plant located on the Fox River in De Pere, WI. The second class obtained samples approximately 400 m outside of the discharge plume, also at a sampling depth of 2 m. The dissolved oxygen probe reading is taken at the same depth and concomitantly with collection of the water sample. The dissolved oxygen in the sample is determined during the next laboratory period using the Winkler titration. Excellent references for this portion of the experiment are provided by Stagg [8] and in the Standard Methods for the Examination of Water and Wastewater [9]. Experimental instructions are provided in the supplementary laboratory documentation; however, one should note that the provided method is the azide modification, which is used to minimize interference by nitrate ions.

The results from the DO meter and the titration are gathered by the instructor and the pooled class data is handed out to the students for analysis. The students are next assigned the task of calculating the average and standard deviation of the data for each DO method at each sampling location. Students are asked to evaluate the raw data and to determine if any data point should be considered an outlier by use of the *Q* test. Next, the students utilize several versions of the *t* test as shown in eqs 6 and 7 to evaluate the group data. [11]. The variation of the *t* test shown in eq 6 is for comparing independent measurements of the same variable and the student must determine whether the two DO methods agree at the 95% confidence level.

$$t_{calc} = \frac{\left|\overline{X}_1 - \overline{X}_2\right|}{s_{pooled}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \tag{6}$$

$$t_{calc} = \frac{\left|\text{Known Value} - \overline{X}\right|}{s} \sqrt{n} \tag{7}$$

The students are asked to choose one of the DO methods and determine if there is a difference in reported DO levels between the two sampling locations. Next, students use the *t* test shown in eq 7 to determine if the experimental values for DO agree with the theoretical value calculated using Henry's law in their prelaboratory assignment. The assumption is made that the theoretical value is the same as the known value, and implications of this assumption are discussed.

The final task the students are asked to accomplish is to think about the results they obtained and to determine what conclusions they can tentatively make about the dissolved oxygen levels. It is stressed that the results gathered, while informative, are only a fraction of the data that would be required in order to properly study the patterns of DO in the river and the effects of the discharge plume. The students are also asked to reflect back over their procedure and to make note of any possible sources of determinate errors that may have been introduced during the procedure. The authors believe that this reflection is as valuable as performing a flawless titration because students need to learn to critically evaluate their own technique.

Results & Discussion

Table 1 includes a summary of the data analysis obtained by the Fall 2000 Quantitative Analysis class. Figure 1 is the class data collected from the "inside plume" collection site while the data in Figure 2 is from the "outside plume" collection site. The data collected in Figure 2 provided an unexpected opportunity to discuss the *Q* test for the rejection of "bad" data. If either the DO meter readings or the titration data had been the lone method for the determination of dissolved oxygen, a strong case for the rejection of the outlier datum could be made by calculating the corresponding *Q* value; however, when both methods show the sample truly had an elevated dissolved oxygen content, one must retain that datum in the set. This facilitated a discussion on the subjective nature of evaluating "bad" data points and that the *Q* test is merely a tool for the analytical chemist and not an absolute rule one must blindly obey.

Conclusions

Anecdotal comments from students during the experiment and on anonymous course evaluations indicate that students appreciated the holistic approach of this laboratory experiment. The instructor has noted a sense of ownership of this experiment by the students that is not necessarily as pronounced in those laboratories where a student is given a sample in a vial for them to simply analyze in triplicate and report the average and standard deviation. It was also found that the students reported a greater appreciation for the critical steps needed during sampling and sample preparation.

Acknowledgments. The authors would like to acknowledge the Fall 1999 and 2000 Quantitative Analysis students at St. Norbert College for their assistance in developing this laboratory.

Suporting Materials. Three supporting files are available. A Chemicals list (http://dx.doi.org/ 10.1007/s00897020560b); student pre-lab and laboratory instuctions (http://dx.doi.org/ 10.1007/s00897020560c); and a water sampling primer (http://dx.doi.org/10.1007/ s00897020560d).

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