Modified Winkler Method (MWM):
Primary Method for Calibration of Dissolved Oxygen (DO) Sensors

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Abstract

A primary method has been developed by the Chemistry Department (DMQ) of Costa Rican Metrology Laboratory (LACOMET) for the calibration of dissolved oxygen (DO) sensors using a Modified Winkler Method (MWM). This calibration service was developed in order to satisfy the metrological needs of the region related to traceability in DO measurements in bodies of water and environmental samples. The experimental procedure is based on two subsystems (1) Oxygen Saturation System and (2) Amperometric Titration System. First subsystem focuses on water saturated sample preparation under controlled laboratory conditions for subsample recollection and instrument calibration; for the second subsystem, the fixed DO content present in each subsample is quantified by the semi-automated MWM. The MWM was exhaustively validated in the performance characteristics of precision, trueness, and measurement uncertainty. In addition, the LACOMET participated in a regional comparison in order to demonstrate the comparability of results between National Metrology Institutes (NMI). Finally, the results presented in this work demonstrate that the DMQ-LACOMET has a traceable method to perform the calibration of DO sensors with a measurement uncertainty as low as 0.10 mg/L (k=2). This new service pretends to support the metrological needs in Costa Rican industry and testing laboratories, as well as the regional comparability in the calibration of DO measurement sensors.

1. Introduction

Dissolved oxygen (DO) content in bodies of water is an analytical measurement performed on a daily basis by testing laboratories to assess the quality of natural waters, marine waters, and wastewater (Wetzel & Likens, 2000; Helm, I., et al, 2018). The DO concentration allows identifying if a body of water has the optimal conditions to preserve the microbial biodiversity, aquatic plants, zooplankton and algae (Zhao, Fan, & Zhao, 2021). The absence of DO in water can produce an increase in the concentration of pollutants and the emergence of anoxic areas that are uninhabitable for most living beings (Helm, I., et al, 2012). High DO content is an indicator of water that could be rapidly purified and its presence helps the degradation of some organic pollutants in water bodies, for example, cleaning products (Wei, Y., et al, 2019). The measurement of DO in water is used in ready biodegradability test for organic substances (OECD, 2006); therefore, the reliability and metrological traceability of biodegradability results are directly related with the reliability of the method used to measure the DO content in water.

Winkler method (WM) is a standardized method to determine DO, and is usually used as a reference because it is a primary method. However, it is tedious because requires a lot of time, it is not possible to obtain real time results, and typically uses highly polluting reagents (Näykki et al. 2013; Wei, Y., et al, 2019). Several authors have focused on improving this "classic method" experimental process and minimizing its limitations (Novič, M., et al, 1988; Helm, I., et al, 2009; Shriwastav, A., et al, 2010; Helm, I., et al, 2012; Wei, Y., et al, 2019).

Actually, in most of the cases, sensor technologies for DO measurement are preferred for its capability to provide real time results, high sensitivity, portability and easy to use. Nevertheless, despite the described advantages, in-situ interlaboratory comparisons have not yield good results and sensors could be not as robust as is believed. These newer technologies are easy to use but could sacrifice the reliability and even measurement comparability (Näykki et al. 2013).

The main limitation of DO sensors may be its fragility and susceptibility to deviations, especially when they are routinely exposed to unfavorable conditions or drastically mishandled (Näykki et al. 2013; Wei, Y., et al, 2019). The metrological disadvantages of DO sensors can be reduced with preventive maintenance (recommended by manufacturer) and a regular metrological control program (MCP). The identification (and adjust needed) of systematic errors caused by performance degradation can be estimated by calibration, because the results expressed as an error or a correction, with their respective measurement uncertainty, can be used to correct the known systematic errors of a specific measurement system (JCGM 200, 2012). Worldwide, the DO sensor calibration service is not usually provided by National Metrology Institutes (INM's) and the availability of reference materials as quality control is limited to solutions with "zero DO content".

All previous information denotes a very important metrological need in the field of Chemical Metrology for the calibration of DO sensors; therefore, the Scientific Group of Electrochemistry (GC-EQ in Spanish) from Chemical Metrology Division (DMQ in Spanish) has been dedicated to implementing, optimizing, and validating the semiautomatic primary method for the calibration of OD measurement sensors. The results described in the present work allow the NMI of Costa Rica to support the OD measurement carry out by testing laboratories through a new calibration service with metrological traceability.

2. Methodology

This section pretends to detailly describe the necessary materials, equipment involved, and experimental procedure used for the development of the present manuscript.

2.1. Materials

Type I (resistivity 18.2 MΩ·cm) water was used in all operations. Produced every day prior to measurements using the following equipment: ELGA LabWater, PURELAB Flex 3.

Reagents involved in this work were:

- Potassium iodate (KIO_3), maker: Merck, purity: ≥ 99.99% m/m (metrological traceability).
- Potassium Iodide (KI), maker: Mallinckrodt, purity: ≥ 99.5% m/m.
- Sodium Thiosulfate ($Na_2S_2O_3$), maker: J.T. Baker, purity: ≥ 99.5% m/m.
- Manganese sulfate monohydrate ($MnSO_4 \cdot H_2O$), maker: J.T. Baker, purity: ≥ 99.5% m/m.
- Potassium hydroxide (KOH), maker: J.T. Baker, purity: ≥ 87.2% m/m.
- Sulfuric Acid (H_2SO_4), Concentrated 98% w/v, Reagent Grade.

Additional material immersed in this work:

- 2 glass syringes of 250 μL.
- 1 glass syringe of 5.0 mL.
- 1 plastic syringe of 5.0 mL.
- 3 glass impingers.
- Sample containers: 10 mL glass flasks.
- Saturation container: 5.0 L stainless steel.
- Titration vessels: 30 mL beaker (same quantity as sample containers).
- 1,0 mL micropipette.
- Additional communication and measurement cables.

2.2. Equipment

The equipment described below constitutes the global measurement system, and it can be classified into two subsystems:

Oxygen Saturation System (Subsystem 1):

- Environment meter (Pressure, temperature, and humidity), maker: Vaisala, model: PTU303.
- Flowmeter, maker: Perkin Elmer, model: 1000.
- Digital stirrer, maker: IKA, model: EURO-ST 60 C S001.
- Precision bath, maker: Fluke, model: 7015.
- Digital thermometer, maker: Thomas Scientific, model: Traceable 9337U17.

Amperometric Titration System (Subsystem 2):

- Voltage generator, maker: NA model: NA (self-made).
- Analytical balance, maker: Mettler Toledo, model: XPE205.
- Digital multimeter (mV), maker: Fluke, model: 289.
- Digital multimeter (μA), maker: Keithley, model: 2002.
- Magnetic stirrer, maker: Scinics CO. LTD. model: MC303.
- Micro-double platinum electrode, maker: SI Analytics, model: KF 1150.

2.3. Experimental procedure

2.3.1. Oxygen Saturation System

This section describes the necessary subsystem to prepare the water saturated sample, which consists in saturating an airflow (clean of oil and particles) with a humidity $> 90\%$ RH, and then using it to saturate and stabilize the DO present into the saturation container which is immersed in a precision bath (20.00 ± 0.02 °C). Figure 1 represents the system used by the DMQ-LACOMET for this purpose.

The DO measuring instruments subject to calibration were immersed into saturation container unit until a stable DO measurement is achieved ($\pm$ 0.02 mg/L). The instruments results were compared with the MWM result and the theoretical Benson & Krause (B&K) equation (ISO 5814: 2012). The DO content was determined with the MWM using at least 7 subsamples, these were taken by immersing the sample containers into the saturation container (avoiding air bubbles entrance). In these subsamples, the OD was fixed directly by the simultaneous addition (to the container bottom) of 200 µL $MnSO_4$ (2.1 mol/L) and 200 µL of KI (2.1 mol/L)/KOH (8 0.7 mol/L) solution. This addition replaced a similar sample volume. Immediately after reagent adding, the sample container was capped and mixed, nevertheless, during this process it is essential to avoid the bubbles entrance because the concentration of oxygen in the air is at least 30 times higher than the concentration of oxygen present in a DO saturated water sample (Helm, I., et al, 2012).

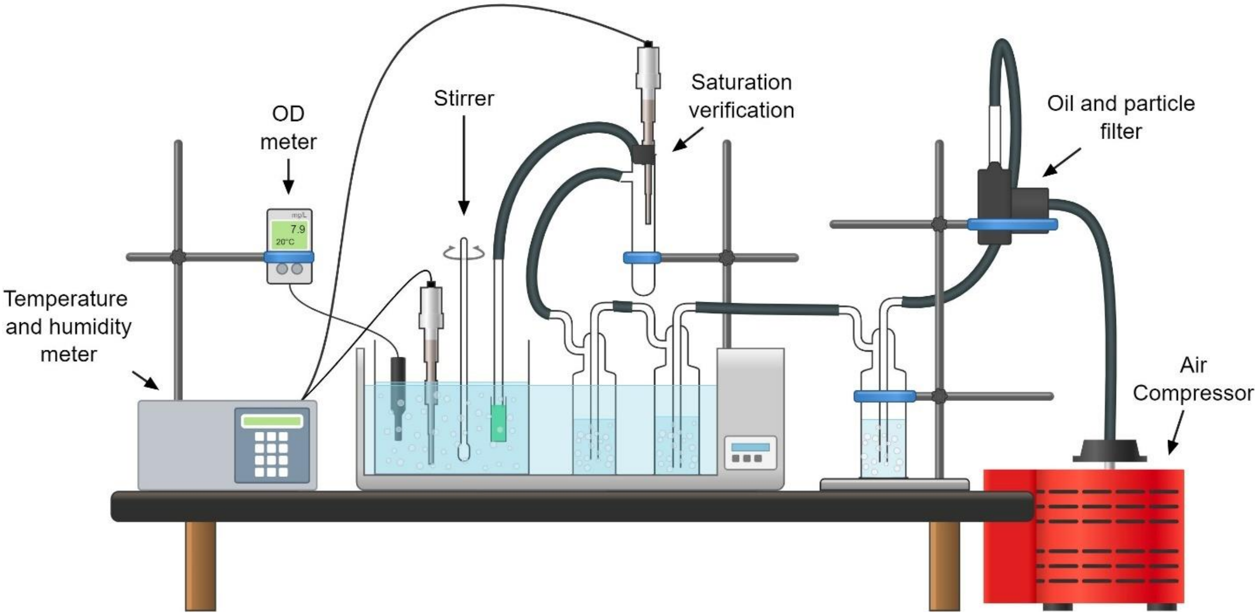

Figure 1. Diagram of the Oxygen Saturation System developed by DMQ-LACOMET according to the work of (Helm, I., et al, 2012; Helm, I., et al, 2018).

According to Wei, Y., et al, 2019, there are multiple reaction process subsequent to reagent addition. The first step caused the visible formation of a precipitate due to the interaction of manganese with the basic medium, as described by reaction Eq. 1.

$$4MnSO_4 + 8NaOH \rightarrow 4Mn(OH)_2 \downarrow + 4Na_2SO_4 \qquad Ec. 1$$

The Mn(OH)$_2$ produced is highly unstable and rapidly combines with the DO to produce a second (more stable and darker color) precipitate of permanganic acid, corresponding to Eq. 2 and 3. The oxygen precipitation can be clearly identified by the appearance of a yellowish/orange solid that settles to the bottom over approximately 45 ± 15 min (Helm, I., et al, 2012; Wei, Y., et al, 2019).

$$2Mn(OH)_2 + O_2 \rightarrow 2H_2MnO_3 \qquad Ec. 2$$

$$2H_2MnO_3 + 2Mn(OH)_3 \rightarrow 2MnMnO_3 \downarrow + 4H_2O \qquad Ec. 3$$

If after sedimentation time are any bubbles present on samples containers these samples were discarded. If not, the precipitate was redissolved following Eq. 4, which causes the Mn^{3+} to be immediately reduced by the presence of excess I^-, and finally derivates in I_2 formation (Eq. 4) (Wei, Y., et al, 2019).

$$2MnMnO_3 \downarrow + 4H_2SO_4 + HI \rightarrow 4MnSO_4 + 2I_2 + 6H_2O \qquad Ec. 4$$

2.3.2. Amperometric Titration System

The molecular oxygen content originally dissolved in the known sample volume is directly related to the final content of molecular iodine (responsible for the yellowish color). For titration, each sample was transferred to individual titration container and gravimetric titration (with a solution of $S_2O_3^{-2}$ 0.0015 mol/L) was performed accordingly to the chemical reactions of the classical WM.

The gravimetric titration is carried out by using a syringe to titrant addition. The syringe is weighted at the beginning of the titration and again at the end, this difference represents titrant mass. Endpoint detection was possible through the micro doble platinum electrode applying a voltage of 100 mV with a voltage generator (Helm, I., et al, 2012). Figure 2 shows a diagram of the semiautomatic Amperometric Titration System implemented by the DMQ-LACOMET to gravimetric titration of each sample based on the work of (Helm, I., et al, 2012; Helm, I., et al, 2018).

The Modified Winkler Method (MWM) includes the following modifications: 1. Replacement of the $K_2Cr_2O_7$ salt (highly contaminant) by a certified KIO_3 salt with metrological traceability, 2. Pre-titration by adding 80% – 90% of the total expected volume to be consumed by the sample (to minimize iodine volatilization), 3. Gravimetric titration of sodium thiosulfate and sample (OD content), 4. Semiautomatic amperometric determination of endpoint titration, and, 5. Exhaustive evaluation of measurement uncertainty sources and incorporation of their respective value (Helm, I., et al, 2012).

A drop of the used syringe weighs 0,0103 ± 0,0005 g. This is a small quantity compared to a buret drop, which is why the gravimetric titration beats volumetric titrations, besides its lower uncertainty.

Amperometric endpoint detection is also better than the traditional starch indicator because the endpoint value ranged in different days from 0.02 to 0.05 μA (but was constant within a day), and this variation is consistent with reagent blank signal.

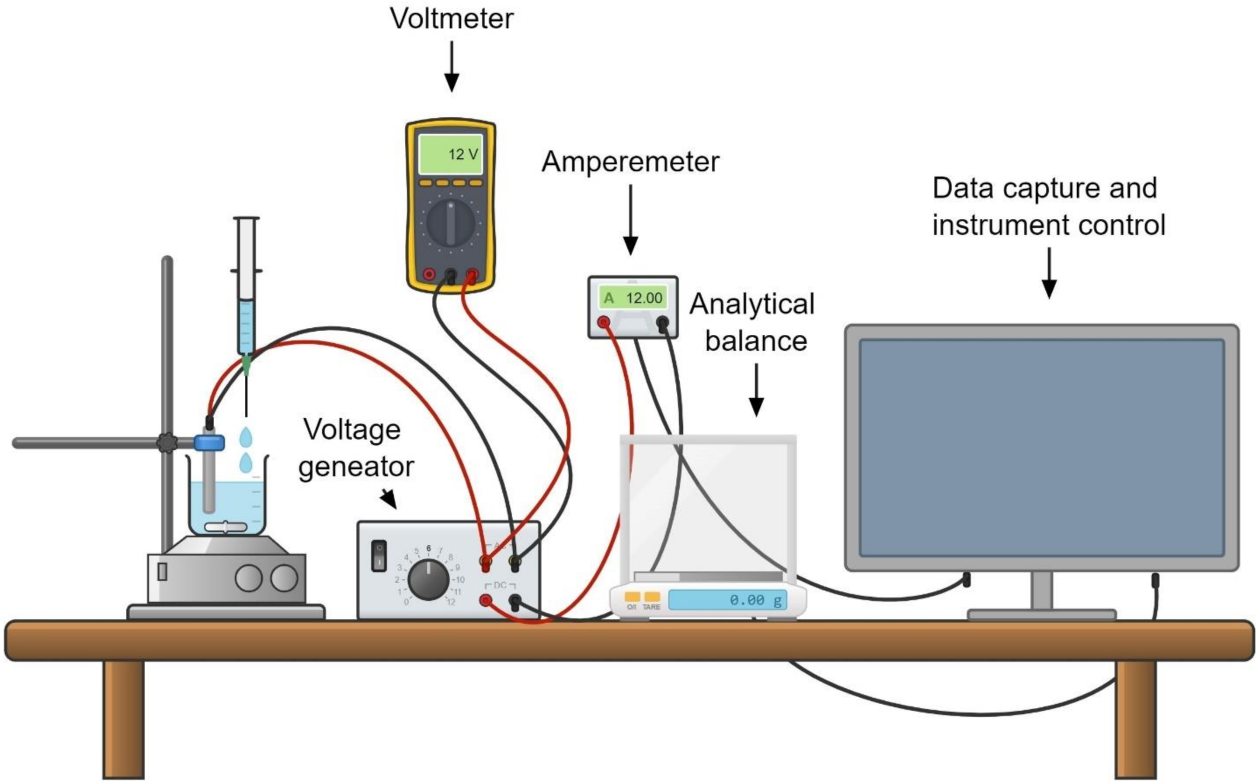

Figure 2. Diagram of the Amperometric Titration System developed by DMQ-LACOMET for MWM titration, according to the work of (Helm, I., et al, 2012; Helm, I., et al, 2018).

The gravimetric titration implemented by the DMQ was semiautomated using the NI LabView programming language. This upgrade provided major time savings and effective workflow through the control of amperemeter and analytical balance, easily identify the endpoint titration, and enabled the real time data visualization from anywhere with internet connection using the Grafana® software.

3. Results and discussion

Figure 3 summarizes the validation results found across 4 independent experiments. This graph evidences implemented method good performance as overlapped results, even taking into consideration error bars (as expanded uncertainty) as described. No significant differences were found between the OD concentration according to the MWM results (blue) and B&K equation results (orange) (adopted by ISO 5814: 2012). Besides, performance evaluation through normalized error (E_N) was used to assess the accuracy of the MWM implemented with the B&K value as reference value (ISO/IEC 17043).

The described B&K equation is used to estimate the theoretical OD concentration in water in equilibrium taking into consideration experimental parameters such as sample temperature, humidity in the saturated airflow and atmospheric pressure at room laboratory; the uncertainty estimation associated with the B&K value considers contribution from oversaturation/undersaturation, atmospheric CO_2, bath temperature stability, among others (Benson & Krause, 1980). Also, this value from B&K equation can be used for sensors calibration, nevertheless, usually grants higher uncertainty than primary MWM.

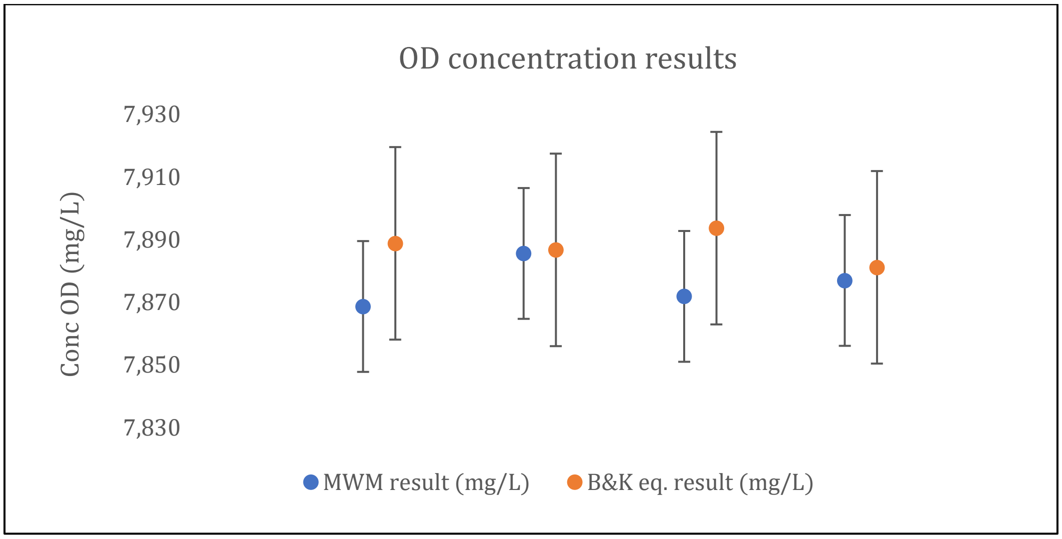

Figure 3. Results of DO concentration for MWM result (blue) in contrast to the theoretical B&K equation result (orange). Error bars shown as expanded uncertainty.

Table 1 displays the percent error and the E_N for each experiment. The operating parameters for this determination were optimized to ensure reproducibility and maximum resources use. From the analysis of optimization tests results, it was possible to determine the necessity to include a third in-series impinger (Figure 1) in the Oxygen Saturation to significantly reduce the stabilization time in half (from 2 h to 1 h).

Table 1. Experimental concentrations (MWM results) and theoretical concentrations (B&K equation results) for DO and their respective relative and normalized error (E_N).

Day	MWM result (mg/L)	B&K eq. result (mg/L)	Error (%)	E_N
1	7,869 ± 0,038	7,889 ± 0,061	-0,26	-0,56
2	7,886 ± 0,042	7,887 ± 0,061	-0,01	-0,03
3	7,872 ± 0,036	7,894 ± 0,061	-0,28	-0,61
4	7,877 ± 0,040	7,881 ± 0,061	-0,05	-0,11

Results shown with their respective expanded uncertainty.

After method optimization tests, the analytical validation was focused on the following performance characteristics: precision, trueness, and measurement uncertainty. The main results of the validation of the method were:

I. **Precision**: Estimated from 4 independent tests; the expected variability between same day replicates (0.19%) and variability between different days replicates (0.25%) are quantified. The contribution of precision uncertainties source (repeatability) was of 0.31%.

II. **Trueness**: Estimated from 4 independent tests; the bias is quantified from the experimental results obtained with the MWM and the result of the B&K equation (adopted by ISO 5814: 2012) calculated according to each day measurement conditions. The evaluation of this parameter was performed by using the normalized error criteria (E_N) using $-1 \leq E_N \leq 1$. The values from bias evaluation are summarized in Table 1.

III. **Measurement uncertainty**: Exhaustively estimated assessing the influence of all possible contributions of uncertainty associated with systematic errors and random errors.

The expanded uncertainty value obtained during the validation was between 0.036 mg/L to 0.050 mg/L (k=2). In all cases the experimental uncertainty was lower than the theoretical value corresponding to ISO 5814: 2012 (0.061 mg/L) (k=2).

The major sources of uncertainty were the following:

Contribution of 81 % according to the standard concentration ($S_2O_3^{2-}$) estimated from the titration with KIO_3 as metrological traceability.

Contribution of 12 % according to method repeatability.

Contribution of 6 % according to introduction of parasitic oxygen through the stopper and the flask junction.

Contribution of 1 % according to parasitic oxygen from the reagents (saturated and unsaturated).

Contributions related to parasitic oxygen introduction were verified accordingly to the reference (Helm, I., et al, 2012; Helm, I., et al, 2018).

Oxygen introduction due to gaps between the flask junction and its stopper is accounted as 6 % of measurement global uncertainty. Its exact value was adopted from Helm's work.

Oxygen introduction due to reagent addition depends on atmospheric pressure, however, for present work, 2 tests with reagent saturation and 2 without reagent saturation were made. No differences were found.

This primary method for the calibration of DO measurement sensors developed by the DMQ was subjected to a regional comparison achieve as a consequence of the financing and support of the German Federal Ministry for Economic Cooperation and Development (BMZ, in German) through the Institute of Metrology of Germany (PTB, in German) and within the project called "Accurate measurements of Dissolved Oxygen, Phosphorus and Chlorophyll in several aquatic environments for the correct assessment of Biodiversity monitoring". NMIs from the economies of Uruguay, Peru, Argentina, Nicaragua, Bolivia, Ecuador, Estonia, and Costa Rica participated in this regional comparison. The LACOMET recently issued its measurement results and is currently awaiting the final report. Nevertheless, the data presented by DMQ in Figure 3 and Table 1 completely demonstrate the method fitness for the calibration of DO sensors in water.

4. Conclusions

The reliability and precise determination of DO with calibrated sensors are very important to assess the quality of natural water, marine water, and residual waters, as well as to provide confidence to the biodegradability test methods in organic substances that use the DO parameter as an indicator of biological capacity degradation of organic substances.

DO instruments, especially those used for field measurements, must be subjected to a rigorous metrological control program to ensure the quality and reliability of the results.

This work results demonstrate that the DMQ has an appropriate system for the calibration of DO sensors with a measurement uncertainty of less than 0.10 mg/L (k=2), by producing a water saturated sample and quantifying the OD with the gravimetric MWM.

The LACOMET, through the GC-EQ, has developed a primary semiautomatic calibration method that will solve the metrological needs of Costa Rican testing laboratories and industry, as well as the regional needs in the calibration of DO sensors.

5. References

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