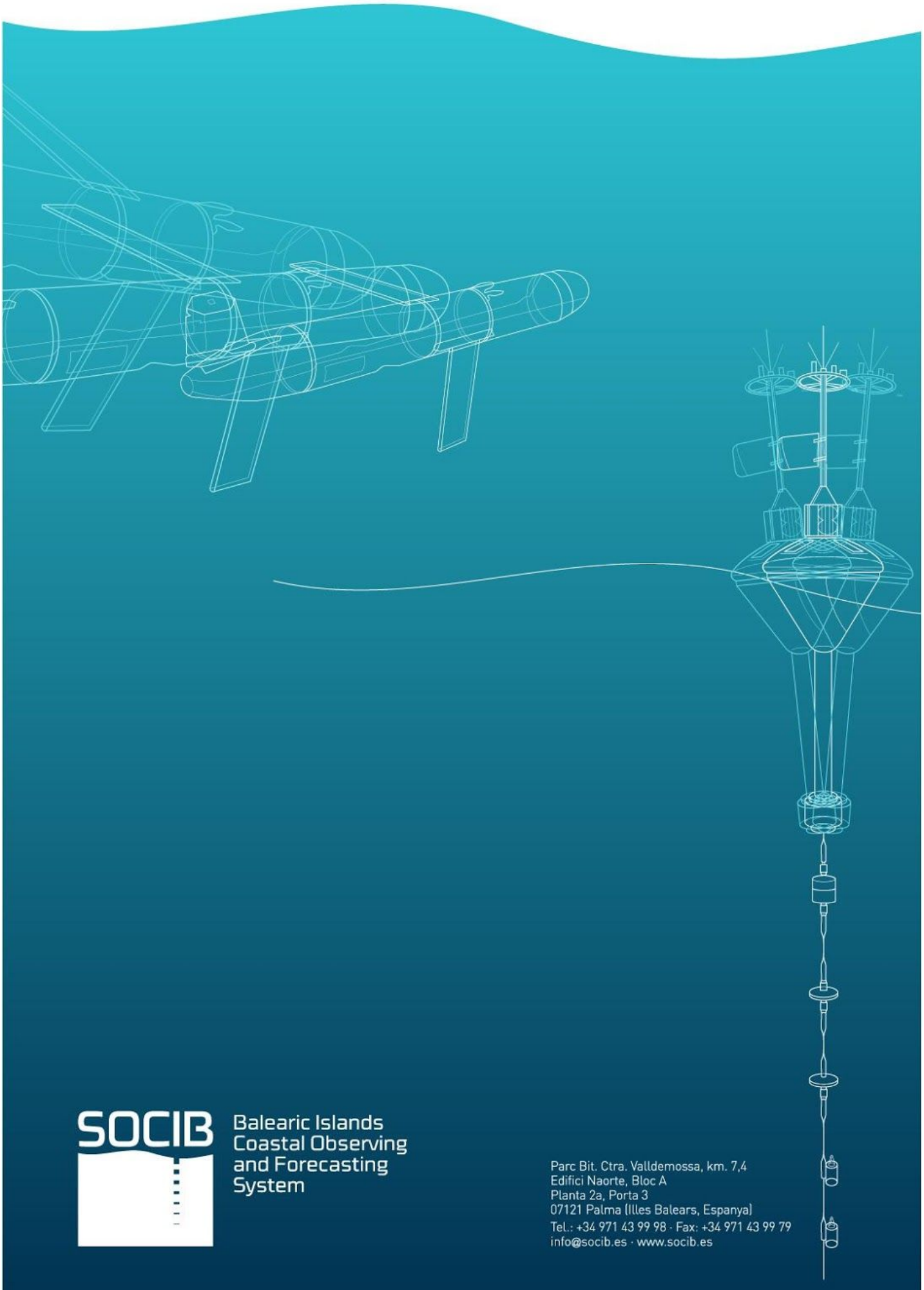

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Created	Updated	Changes description	Author	Supervision
November 2014	January 2016		A. Massanet/ E. Alou Font	J. Allen
	January 2018	Changed the oxygen units in the final results from umol/kg to umol/l	F. Iuculano	
	April 2018	Added detailed pictures of the lab instrument and the material used for the analysis. Added point number 2 in the Titration procedure. Added the caution notes at the end of the document.	L. Díaz/A. Cabornero	E. Alou
	January 2019	Added requirement of 5 blanks with similar numbers	E. Alou Font	

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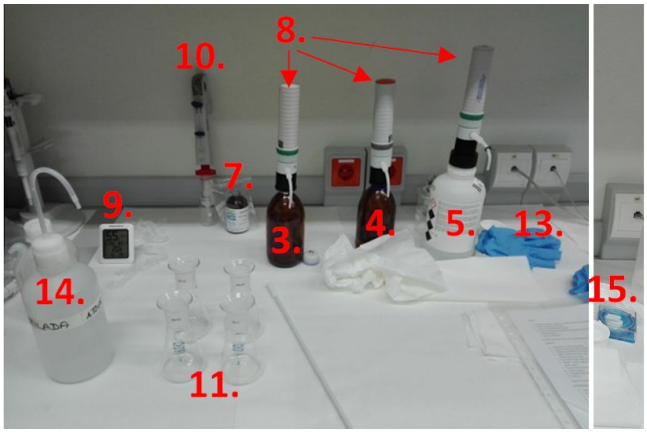
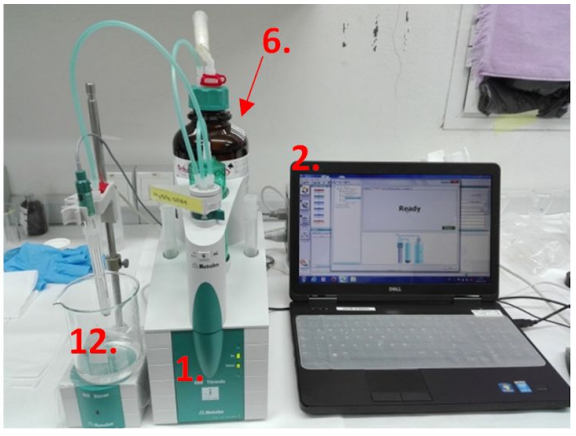
DETERMINATION OF DISSOLVED OXYGEN IN *in situ* WATER SAMPLES BY WINKLER TITRATION

In situ water samples for dissolved oxygen determination are analyzed after a period of at least 6 to 8 h and within 48 h after sampling.

Material

1. Titrator Metrohm 888 instrument.
2. Laptop with the Tiamo 2.4 software (Metrohom AG, 2013 Switzerland).
3. Reagent 1 (R_1): Manganous chloride, $MnCl_2$ (3M).
4. Reagent 2 (R_2): Sodium iodide/Sodium hydroxide, NaI (4M)/NaOH (8M).
5. Reagent 3 (R_3): Sulfuric acid, H_2SO_4 (5M).
6. Sodium thiosulfate, $Na_2S_2O_3$ (0.03N).
7. Potassium iodide, KIO_3 (0.01N).
8. R_1, R_2, R_3 reagent dispensers.
9. Thermometer.
10. Repeater pipette and tips.
11. Winkler flasks.
12. Glass beakers (50 ml and 400 ml).
13. Gloves.
14. Plastic wash bottle filled with distilled water.
15. Magnetic stir bars.





Titration procedure (Metrohm 888 titrator)

1. Plug the titrator Metrohm 888 instrument and connect the USB port to the laptop.

2. Access the program through the user “Equipos”

3. Open the program Tiamo.

4. Open the electrode’s cap.

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5. Select in the Tiamo program Manual/dosing device/prepare. This allows the burette to be filled with thiosulfate and purge bubbles. Please check there are no bubbles in the system before starting the analysis (repeat this process if necessary).

6. Rinse the electrode with distilled water.

7. Activate the system by doing either a reagent blank determination (see below) or a sample with distilled water. In this case, add 1 ml of R_1 and 1 ml of R_2, stir and measure the sample following the protocol for titration of samples (see below).

8. Rinse the electrode and repeat a few times until the measurements are stable.

Reagent blank determination

1.Select the O_2 blank method.

2. Fill in the file with the batch, flask number and laboratory temperature.

3. Gently shake the KIO_3 and transfer a small volume to a small glass beaker. Note the KIO_3 batch number and the date is opened.

4. Add distilled water in a clean flask (below the mark) and introduce a magnet.

5. Add 1 ml KIO_3 using the repeater pipette and stir. Add 1 ml R_3 and stir. Add 1 ml R_2 and stir. Add 1 ml R_1 and stir.

6. Make up the volume until 110 ml (until the mark) adding distilled water.

7. Select Start in the program to start the titration to the endpoint.

8. When the measurement has ended (the program shows the message "sending data to data base"), add 1 ml of KIO_3 to the same flask and titrate a second time.

9. Rinse the electrode and repeat the process a few times until the measurements are stable.

* It is very important that the temperature of both the laboratory and the reagents remain constant during the analyses.

Standardization of thiosulfate

1. Select the O_2 method.

2. Fill in the file with the batch and flask numbers and laboratory temperature.

3. In a clean flask add distilled water (below the mark) and add a magnet.

4. Add 5 ml KIO_3 using the repeater pipette and stir. Add 1 ml R_3 and stir. Add 1 ml R_2 and stir. Add 1 ml R_1, and stir.

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5. Make up the volume to 110 ml using distilled water (up to the mark).

6. Select start for standardization.

7. Rinse the electrode and repeat a few times until the measurements are stable.

Titration of samples

1. Select O_2 method.

2. Write the batch, station, Niskin bottle and flask numbers and the temperature of sampling.

3. Note the temperature in the laboratory in the oxygen logbook.

4. Carefully remove the stopper and add a magnet.

5. Add 1 ml R_3 and stir until the precipitate is dissolved.

6. Titrate the sample to the endpoint (selecting start).

7. Rinse the electrode with distilled water and repeat the process with the next sample.

After the analysis

Once the analysis is completed close the electrode's cap and store it. Rinse all the material three times with tap water and once with distilled water. Let all the material dry.

Calculations and expression of the results

For the final calculations to obtain the dissolved oxygen concentration (µmol/l) of the *in situ* water samples follow the procedure as in Langdon 2010.

References

Carpenter JH (1965) The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. Limnology and Oceanography 10:141–143.

Langdon C (2010) Determination of dissolved oxygen in seawater by Winkler titration using the amperometric technique. In: Sloyan B.M., Sabine C. (Eds). GO_SHIP repeat hydrography manual: A Collection of Expert Reports and guidelines. IOC/ IOCCP. Paris.

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⚠ CAUTION NOTES:

For the analysis:

- **Check the temperature.** Before the analysis, is necessary and very important to check that we have a stable temperature in the lab.
- **Check the USB port.** We are having problems with the USB connection (we are using the right and behind port, 11/04/2018).
- **Check the connection of the Titrando.** With the movement on board is possible that the upper part would disconnected from the bottom part of the analyzer.
- **"Equipos" user.** If we access the tiamo program from another user or open the program with two users at same time we will have problems the Titrando wouldn't recognize either user.
- **Feed the R_1, R_2, R_3 reagent dispensers (1) and check that the measurement for the required 1 ml is ok, use a graduated cryovial (2).**

1.  2. 

- **KiO3.** If we don't have enough KIO3 during the analyses, we can open another, but we have to note the new #batch/lote code number it in the logbook.
- **Stirrer.** By default the stirrer sometimes is set up at the 8 speed level, it is better to be at 4 to secure both the electrode and the sample during the analysis.
- **Tiamo 2.4 program (the software of the instrument).** In the current file used for the analyses (RUN screen), all cells MUST be filled and completed with the requested information (e.g. User, Remark, Batch etc.), otherwise the analysis won't start or record. Check the past analyses (database) to review how the form needs to be filled in if you need to do so.

After the analysis:

- **Blanks.** We consider the run with Blanks to be good when we have at least five consecutive values that are the same xxx (check in the "mlOK" column).
- **Standardization.** We consider that the standardization run is good when we have at least three consecutive values that are the same and around xxx

Cleaning:

- **Clean the dispensers** with two different bottles. One for the R1 and R2 and other one for R3. Both bottles have to be filled with distilled water. It is advisable to fill the bottle for clean R1 and R2 repeatedly times.
- **Clean the titrando** changing the sodium thiosulfate to Milli-Q water. After that, the procedure is "Manual/dosing device/prepare".

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