Calibration of Turner 10AU-005-CE fluorometer for measuring chlorophyll *a*

Production Summary

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History of Procedure

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Introduction

When a molecule absorbs light from one wavelength and responds by emitting light at a lower frequency it fluoresces. Fluorescence compliments absorption spectrometry offering greater flexibility, stability and sensitivity. Chlorophyll *a* and phaeopigments are essential factors in monitoring the health of the marine ecosystem.
Chlorophyll can be extracted into an organic solvent such as acetone, determined by measurements of optical density or fluorescence, and used as a measure of phytoplankton biomass. Such extracts contain a mixture of pigments. Those with extinction or fluorescence spectra different from chlorophyll *a* can be eliminated by a careful choice of measurement wavelength. An acidification step distinguishes between the primary pigments and their breakdown products, the pheopigments, with similar wavelengths of peak extinction or fluorescence.
Chlorophyll is labile and will break down in sunlight and fluorescent light.

Scope

This SOP describes the procedure for calibration of a Turner 10AU fluorometer for the determination of chlorophyll *a*. It does not include the collection, preparation and extraction phase or analysis of samples. (SOP EQ-BGC-006 & SOP EQ-BGC-006A). It does not include the preparation of 90% acetone or 1.2 molar hydrochloric acid.

Training

Operator must have been trained in the basics of absorption spectrometry and be familiar with Appendix 11.3 “Measurement of chlorophyll”, “Estuarine and brackish-water sciences association handbook”, chapter “Biological surveys of estuaries and coasts”. It is advisable for the operator to read this procedure thoroughly before starting the process. Some of the information below is brief and you will need to refer to the manual for full details. All page references refer to the large Turner Designs manual. (A4 black folder) unless otherwise stated. Run through the first few samples with an experienced operator.
Operator must be thoroughly trained in GLP and have read COSHH/LOW/EQBGC/NG008.

Apparatus

1. Turner 10AU Field fluorometer
2. 90% Acetone
3. Socorex 10ml dispenser
4. Chlorophyll a stock solution prepared in 90% acetone from: 1mg Chlorophyll *a* free of Chlorophyll *b* from Anacystis nidulans (Sigma part no. C-6144).
5. 4 x 100ml dark volumetric flask
6. 25 x 25ml dark volumetric flask

7. 100-1000µl adjustable pipette
8. 10-100µl adjustable pipette
9. 1-10µl adjustable pipette
10. Cecil scanning spectrophotometer
11. 2 x clean quartz 1 cm cells
12. 50 ml of 8% (1.2 molar) hydrochloric acid.

Procedure

Wear latex gloves for this procedure to avoid sample contamination. Manipulation of standards and the calibration must be carried out in the absence of fluorescent lighting to avoid degradation of standards.

Preparation of chlorophyll *a* standards

1. Prepare the stock solution of chlorophyll and analyse according to SOP EQ-BGC-006.
2. Prepare a 1 in 10 dilution of the stock to produce a primary standard (approximately 1000µg/l) and analyse according to SOP EQ-BGC-006. Enter the results of analysis into the lower half of the first sheet of *QC00n* created when carrying out analysis according to SOP EQ-BGC-006B.
3. Prepare a series of standards from the primary standard with which to calibrate the fluorometer. The standards required are detailed on the second page of *QC00n*. The volume of primary standard required to make each standard will be calculated automatically in the spreadsheet using the results of the analysis of the primary standard input on the first page.

Setting instrument basic operating level

Follow instructions given in Appendix 6 of the manual. The basic operating level should not need to be altered unless there has been a significant change in the sensitivity of the instrument which affects the range of sample concentrations which may be measured. Use a 40µg/l standard to set the basic operating level.

Calibrating the instrument

Switch on the fluorometer and allow at least 10 minutes for it to warm up. Determine the fluorescence response of each of the calibration standards as below.

1. Print out a copy of the second page of *QC00n*, containing the standards table.
2. After rinsing and cleaning glass cuvette fill with 90% acetone and place in fluorometer.
3. Set the range to "low".

4. Press the asterisk key which will enable the Discrete Sample Averaging sequence. This will average the readings over a defined period and allows each sample to be read after the same amount of time has passed. When the asterisk key is pressed the words “delay”, “average” and “done” will appear on the screen. When “done” appears, the readings have been completed and this is the result to be written down.
5. Make a note of the blank reading.
6. Repeat steps 4 and 5 on the medium and high ranges.
7. Add **3 drops** of 8% HCl to the cuvette whilst in the Turner and repeat steps 4 to 6.
8. Pour a few drops of the first standard carefully into the glass cuvette, rinse and empty. Fill cuvette to between two thirds and three quarters full, clean tube walls with a soft tissue and place in Turner.
9. Select appropriate scale to determine reading and note on logsheet. (if the sample is over- or under- range the instrument will display a warning message in the window). Press the asterisk key and note down the result on the logsheet when the word “done” appears at the top of the screen. This is **fo**.
10. Add 3 drops of 8% HCl directly to the cuvette in the fluorometer. Press the asterisk key and note down the result on the logsheet when the word “done” appears at the top of the screen. This is **fa**. It is important to use the same range as used in step 9.
11. Repeat steps 8 to 10 for all the standards to be analysed.
12. Enter the results into the second page of *QC00n*. The spreadsheet will automatically calculate the values of Hf and Kf for each of the low, medium and high ranges. These values are needed for the determination of chlorophyll in samples using the Turner fluorometer.
13. Enter the results into a new sheet in *QC011.xls* and onto the bottom of the existing data in *QC012.xls*.
14. From *QC011.xls* verify that the fluorometer is linear over each of the three ranges.
15. Once linearity has been verified, update the values of Hf and Kf in the chlorophyll sheet in the *Datatemp.xls* spreadsheet in the *Templates* folder of the nutrient$ directory.