CDOM Fluorometer Calibration

Equipment Required (*Refer to Appendix)

- 1 Litre volumetric flask*
- 2 Litre container, non-reflective.
- 2 Litres of high grade, ultra-pure deionised water
- 2 Litres of 0.05M Sulphuric Acid
- Quinine sulphate dihydrate
- Precision weighing scales (0.001/thousandth gram accuracy) and small weigh dish/boat*
- Magnetic stirrer and bean*
- High accuracy pipette for measuring 1ml of fluid*
- Support Clamp (6kg capacity) – Please ensure it can accommodate the circumference of your Proteus and weight whilst remaining vertical.

Ensure all fluid vessels are acid cleaned to remove traces of naturally occurring amino acids and all precautions taken to avoid the contamination of equipment during the process of making the stock solution and during the sensor calibration process.

Making CDOM Stock Solution

1. Accurately weigh out 0.2g of Quinine Sulphate Dihydrate Powder.
2. Add the Quinine Sulphate powder to an empty and clean 1Ltr Volumetric flask.

TIP: Rinse the Quinine Sulphate Dihydrate powder from the boat and inner surfaces of the flask neck with sulphuric acid, ensuring all powder enters the main bulb of the flask.

3. Accurately fill the flask to the 1Ltr mark with 0.05M Sulphuric Acid.
4. Place the flask on the magnetic stirrer. Carefully add the stirring bean and switch on the stirrer to fully dissolve the powder in the acid. This may take up to 20mins.

TIP: Add the bean after the flask has been filled with water in stage 3 to account for displacement.

This 1Ltr of stock solution has a Quinine Sulphate concentration of 200mg/l or 200,000 ppb.

Where: 100mg (0.1g) QS powder in 1Ltr = 100mg/l or 100,000ppb

Therefore: 200mg (0.2g) QS powder in 1Ltr = 200mg/l or 200,000ppb

This Stock solution should be kept refrigerated in an air-tight sealed container and can expect to last 4 weeks

Making smaller volumes requires small amounts of powder which will significantly increase the risk of error in the final concentration value.

Cleaning and Preparation of the Proteus

New Units

If the Proteus is new, rinsing sensors with weak acid and deionised water will be sufficient before calibration.

Dirty Units

IMPORTANT NOTES.
DO NOT MANUALLY ROTATE THE WIPER ARM AT ANY POINT. THIS WILL PERMENENTLY DAMAGE THE SENSOR.
DO NOT USE A BRUSH ON, OR WIPE THE WINDOW/FACE OF OPTICAL SENSORS.
ENSURE THE SCREW CAP IS FITTED TO PROTECT THE 6 PIN CONNECTOR.

Cleaning and Preperation of the Proteus

Rinse the sensors under running water.

Remove the wiper fitted to the Turbidity sensor by undoing the small grub screw with the Allen key provided. The rubber blade and nylon brush should be replaced if needed. Refit

Ensure the bodies of ALL sensors are clean and all crevices between the sensors are free from dirt and deposits. Mild detergent and warm water can be used on sensors with a soft toothbrush and cotton buds. Rinse under running water regularly. Once the loose debris are rinsed off, the optical sensor windows can be lightly wiped with a clean cotton bud or optical cloth in one direction across the surface.

Using a squeezy bottle helps direct water between sensors to remove debris.

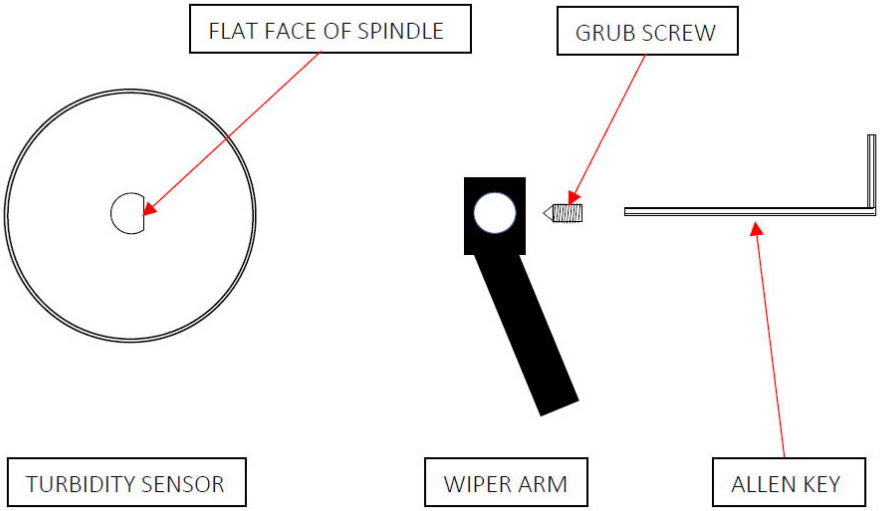
Only wipe sensor faces after the unit has been cleaned to avoid dirt scratching the surface.

Sensors can be soaked in a mild acid solution overnight (pH4). The screw on calibration cup should be used for this. Rinse the sensors under running water.

Ensure the Turbidity sensor wiper arm is refitted before calibration. Grub screw should mate with the flat face of spindle. Light pressure should be applied and the grub screw secured.

DO NOT over tighten and ensure the arm is level.

TURBIDTY SENSOR WIPER ARM



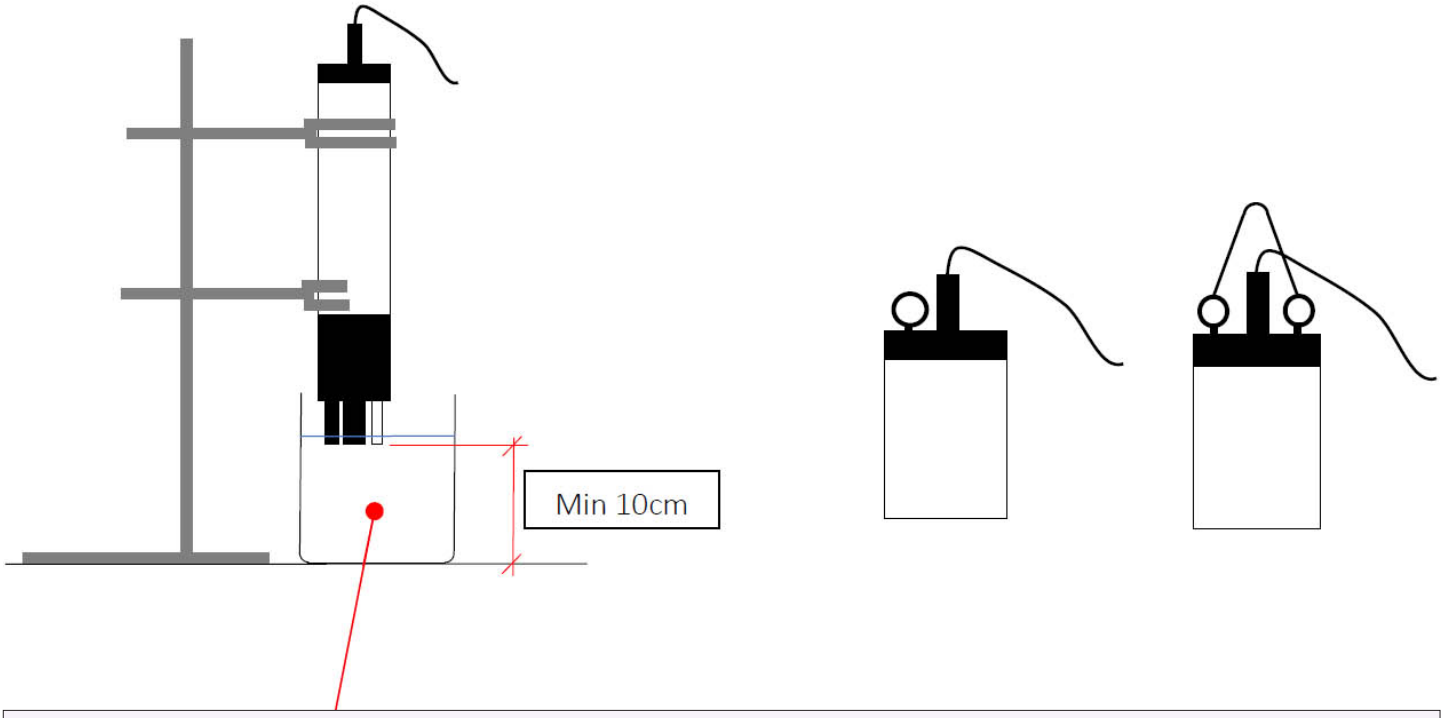
CDOM calibration is a Two Point process which creates a focal range within the CDOM fluorometer between two values for linear regression analysis.

The values employed are

Ultra-Pure De-ionised water is used for First Calibration point with a CDOM value of 0ppb (Zero)

1ml of Quinine Sulphate stock solution in 2l of Sulphuric Acid is for your second calibration point of 100ppb.

1. Accurately measure 2ltrs of Ultra-pure deionised water and pour into a non-reflective container.
2. Using a clamp stand or similar method, secure and set up the Proteus with the sensors submerged in the deionised water to a depth of approx. 10-20mm (not critical), ensuring a minimum blanking distance of 75mm from the sensor windows to the bottom of the container.



2 Litres of Ultra-Pure De-ionised water used for the First Calibration Point with '0' (Zero) Tryptophan value

Partial submersion of the sensors helps to avoid unnecessary contamination of the water from any surface deposits that may be present on the sensors and increases fluorometer blanking distance.

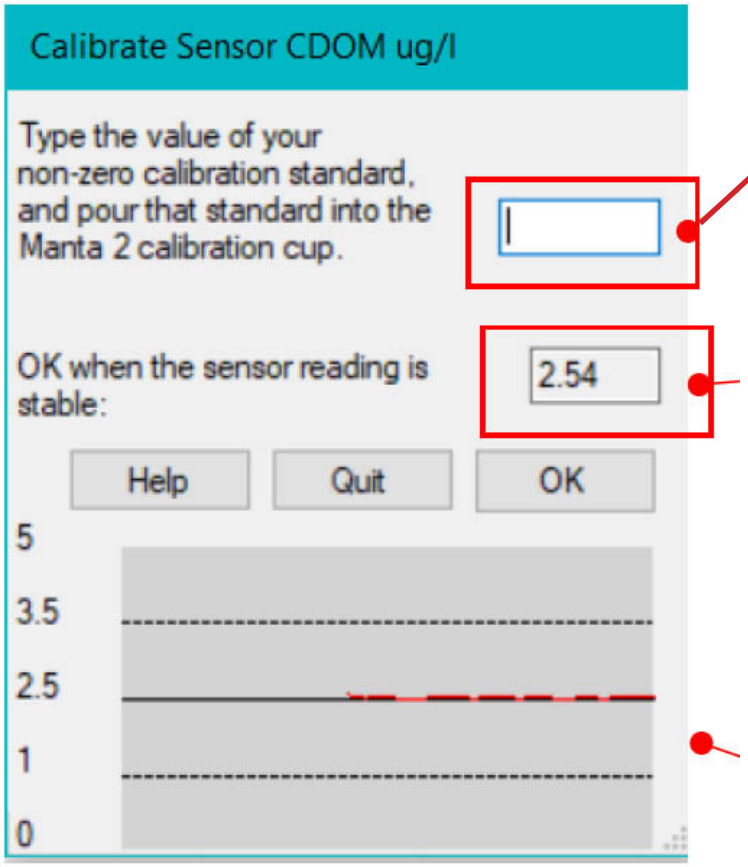
Locate the CDOM sensor and position it centrally to the container to create adequate edge distance. Ensure the Proteus is also perfectly vertical. Any tilt will affect the calibration.

After ensuring the sensor is adequately submerged with correct blanking distance, all light must be blocked out.

This can be done using a dark, non-reflective material (opposite). Avoid the use of plastic coverings as some plastics fluoresce. Ensure all lights are turned off.

Do not allow dust or fibres from any coverings to enter the water.

3. Connect the Proteus to the computer and open the software.
4. From the drop down menu, select 'sensor and parameter list' and check [ ] the boxes for 'CDOM ppb' and 'CDOM TempCorr' so they both appear in the main scrolling page.
5. From the menu, select 'Calibrate' and 'CDOM ppb' from the drop-down list.
6. Enter '0' (zero) for the First calibration point when in de-ionised water, (ignoring software prompt for nonzero as described above).



Enter the '0' (first point) or temp corrected CDOM value (second point) here.

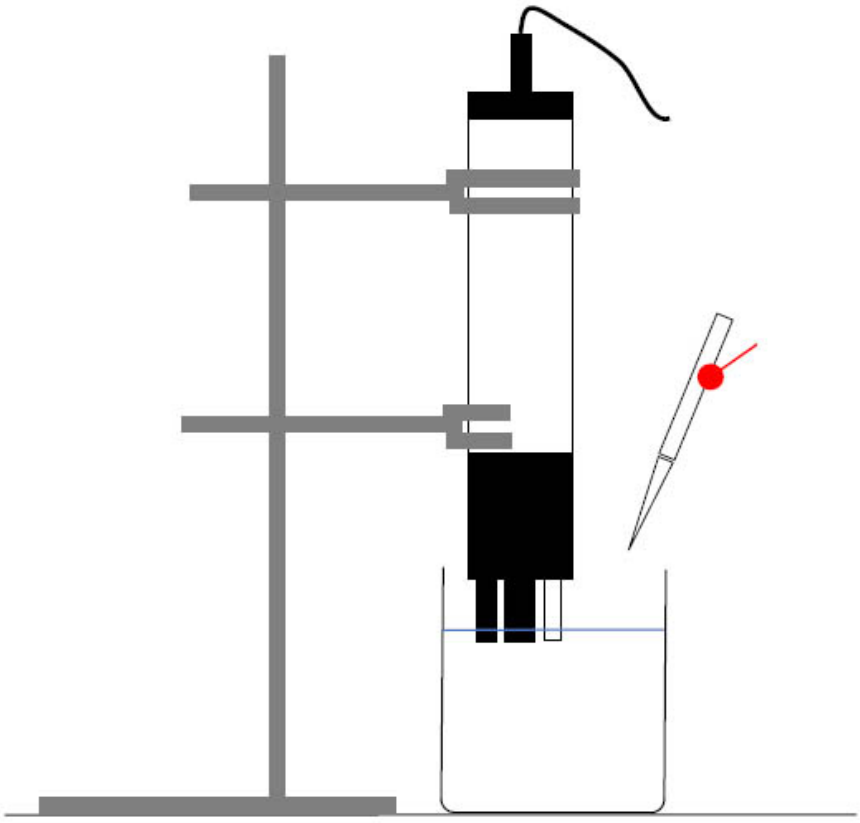
This reading is the reading the sensor is currently reading and not representative of accuracy until calibration is completed. When stable select 'OK'. This reading will fluctuate a few points. Quality of water and process is critical for a good calibration.

Allow line graph to travel full length of graph.

Select 'OK' when stable. A window will pop up briefly showing calibration successful, and a new window will open for the second calibration point.
DO NOT close any windows.

NOTE ABOUT SOFTWARE: The software will prompt for the first calibration point to be a non-zero value. The order in which a two-point calibration is conducted within the software is not critical. However, All sensors are initially calibrated in the order LOW to HIGH. It is recommended that this order is continued. Varying the order can force a sensor into error.

7. Allow line graph to travel full length of graph and when stable select 'OK'. This reading will fluctuate a few points. Quality of water and process is critical for a good calibration.
8. Click continue to calibrate a second point.
9. Replace the deionised water with precisely 2l of 0.05M sulphuric acid. After disposing of the deionised water, rinse the beaker with a small amount of sulphuric acid prior to adding in the 2l.
10. Using a pipette, add 1ml of stock solution (200,000ppb) to the 2 litres of sulphuric acid. This creates a CDOM solution with value of 100ppb. The container may need to be carefully agitated to mix.



Adding 1ml of the Stock solution creates a solution with a value of 100 ppb.

This solution is used for the second calibration point.

11. Use the excel spreadsheet calculator provided to obtain the Temperature Corrected value' of the water. Enter the value of the water (now 100ppb) and the temperature obtained in stage 5.

(1) These are our recommended buffer standard values. If you are using a different value standard, e.g. 500 ppb, make sure you adjust the below to reflect this.

(2) Enter the temperature of the solution in this column. Either use the temperature provided by the Proteus or a digital thermometer.

This is a fixed parameter. Do not change.

This is a fixed parameter. Do not change.

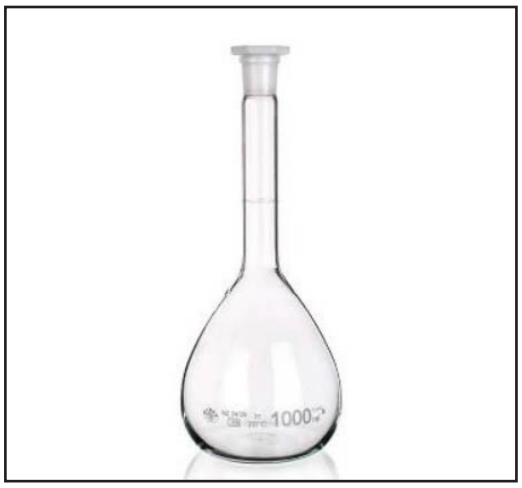
	Target Calibration Reading (Fixed)	Measured Temp (From Proteus or Thermometer)	Reference Temp (Fixed)	Temp Correction Coefficient (Fixed)
CDOM	100	9	20	-0.016
Tryp	100	17	20	-0.039
Chlorophyll-A	200	16	20	-0.014
		RAW Value (CDOM)	RAW Value (Tryp)	RAW Value (Chl)
		117.6	111.7	211.2

(3) Insert value from Row 6 as calibration standard value during the second calibration

12. Enter the Temperature Corrected value created by the calculator into the software box for the Second Calibration Point.
13. When stable select 'OK'.
14. A message should be displayed confirming the calibration was 'successful'. Select 'okay' to return to the main scrolling page (this may happen automatically after a few seconds).
15. With the Proteus still submerged in the 100ppb fluid, review the values in the scrolling data. The Temperature Corrected value (TempCorr) should read 100ppb. The value obtained in stage 9 for the second calibration point should appear in the column 'CDOM ppb'. This parameter should be removed from the parameter list so it does not appear in the main scrolling page.

Appendix

Equipment Examples



1 Litre volumetric

1 ml pipette

250 ml Squeezy Bottle

Weigh Dish/Boat

Laboratory Scales

Magnetic Stirrer