



Alternate Calibration Methods for the YSI 6025 Chlorophyll Sensor

For calibrating the YSI 6025 chlorophyll sensor, the sonde software offers the option of either zeroing the generic fluorescence parameter (% FS) or of performing 1-point, 2-point, or 3-point calibration procedures in $\mu\text{g/L}$ of chlorophyll. Two formulations of standards can be used for calibration of the YSI chlorophyll system: algal suspensions and dye solutions. In Section 5, Principles of Operation of the YSI 6-Series Operations Manual, the dye that is emphasized for this purpose is **acridine orange**. However, **rhodamine b**, a more common dye for environmental studies, can also be used to calibrate the sensor and some users of the YSI 6025 chlorophyll sensor have requested that YSI provide instructions on the use of this alternative calibrant. This information is provided below.

Note that use of the rhodamine b standard is likely to be less accurate than using samples of algae whose chlorophyll has been determined by extractive analysis. The rhodamine B standard should only be used as an approximation that can be confirmed or later adjusted using the extractive analysis results from grab samples acquired during the study. The rhodamine B standard is very useful in assuring the overall optical stability of the chlorophyll sensors in post-study quality control procedures.

Rhodamine B: Preparation & Use

CAUTION: Rhodamine B is listed as a possible carcinogen/mutagen and should be handled with gloves. Be certain to read the safety instructions provided by the supplier with this chemical before preparing solution standards from the solid material.

Preparation

Use the following procedure to prepare a rhodamine B solution that will roughly correlate with *in situ* chlorophyll values:

1. Rhodamine B dye is a commonly used marker reagent and can be purchased from chemical supply houses such as Sigma/Aldrich. YSI used Rhodamine B from Aldrich Chemical Company (Item # R95-3) and, since this dye can vary with regard to its purity, we recommend that the user purchase this exact item if possible. Purchase the minimum quantity possible since only very dilute solutions of the dye will be required.

2. Accurately weigh 0.0500 g of the Rhodamine B solid and quantitatively transfer to a 500 mL volumetric flask. Dissolve the solid in purified (distilled or deionized) water and then fill the flask to the top graduation. This solution contains 100 mg of Rhodamine B per 1000 mL of water.

3. Accurately transfer 5.0 mL of the solution prepared in the above step to a 1000 mL volumetric flask and then fill the flask to the top graduation with purified water. Mix well to obtain a solution, which is 0.5 mg/L in water (a 200:1 dilution of the concentrated solution).

4. Store the concentrated standard solution in a darkened glass bottle in a refrigerator to retard decomposition. The dilute standard prepared in the previous step should be used within 24 hours of its preparation.

When Rhodamine standards are required in the future, perform another dilution of the concentrated Rhodamine solution after warming it to ambient temperature. Our experience has indicated that the concentrated solution that has been kept at cold temperatures is much more stable than the dilute solution stored at room temperature.

Temp, °C	$\mu\text{g/L}$ Chl to Enter
30	72.6
28	74.1
26	75.6
24	77.0
22	79.4
20	82.0
18	86.4
16	90.8
14	93.2
12	95.1
10	98.0
8	100.0

Approximate algal chlorophyll equivalent of 0.5 mg/L rhodamine B as a function of temperature.

Use

It is well known that the intensity of the fluorescence of rhodamine B shows an inverse relationship with temperature. The effect must also be accounted for when calibrating the YSI chlorophyll sensor with rhodamine B. To properly set the sensitivity of the sensor toward algae at 20°C using the 0.5 mg/L rhodamine B standard, enter the calibration value from the table below corresponding to the temperature of the standard.

WARNING: The “Chl Tempco” factor in the Advanced|Sensor menu, MUST BE SET TO ZERO, when calibrating with Rhodamine B.

Rhodamine WT: Preparation and Use

CAUTION: Be certain to read and follow all the safety instructions and MSDS documentation which is supplied with the dye before proceeding.

(continued)

Preparation

Use the following procedure to prepare a Rhodamine WT solution that will roughly correlate with in situ chlorophyll values.

1. Rhodamine WT dye is usually purchased in solution form and can vary somewhat in nominal concentration. YSI uses Rhodamine WT from the supplier noted below and recommends that the user purchase this exact item if possible. The solution is approximately 2 % in Rhodamine WT.

Fluorescent FWT Red Dye (Lot# 257201; 16 Fl. Oz.)
 Kingscote Chemicals
 9676 N. Looney Road
 Piqua, OH 45356
 1-800-394-0678
 Fax: 937-773-7994

2. Accurately transfer 5.0 mL of the Rhodamine WT solution into a 1000 mL volumetric flask. Fill the flask to the volumetric mark with deionized or distilled water and mix well to produce a solution that is approximately 100 mg/L of Rhodamine WT. Transfer this standard to a glass bottle and retain it for future use.

3. Accurately transfer 5.0 mL of the solution prepared in the above step to a 1000 mL volumetric flask and then fill the flask to the volumetric mark with deionized or distilled water. Mix well to obtain a solution, which is 0.5 mg/L in water (a 200:1 dilution of the concentrated solution).

4. Store the concentrated standard solution produced in (6) above in a glass bottle in a refrigerator to retard decomposition. The dilute standard prepared in the previous step should be used within 24 hours of its preparation.

When Rhodamine standards are required in the future, perform another dilution of the concentrated Rhodamine solution after warming it to ambient temperature. Our experience has indicated

that the concentrated solution that has been kept at cold temperatures is much more stable than the dilute solution stored at room temperature.

Use

It is well known that the intensity of the fluorescence of Rhodamine WT shows an inverse relationship with temperature. The effect must also be accounted for when calibrating the YSI chlorophyll sensor with Rhodamine WT. To properly set the sensitivity of the sensor toward algae at 20 C using the 0.5 mg/L Rhodamine WT standard, enter the calibration value from the table below corresponding to the temperature of the standard.

Temp, °C	µg/L Chl to Enter
30	100
28	103
26	106
24	110
22	113
20	118
18	122
16	126
14	131
12	136
10	140
8	144

Approximate algal chlorophyll equivalent of 0.5 mg/L Rhodamine WT as a function of temperature

WARNING: The “Chl Tempco” factor in the Advanced|Sensor menu, MUST BE SET TO ZERO, when calibrating with Rhodamine WT.

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