



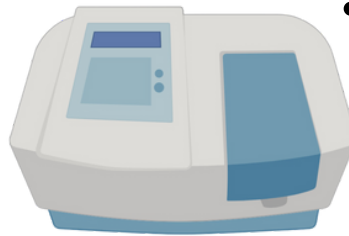
Reading the Water Sample with the Spectrophotometer: Getting Started

Contact: Shelby Brown brownsh@si.edu or 443-482-2270
Pat Neale nealep@si.edu

STEP 01

Prepare your materials.

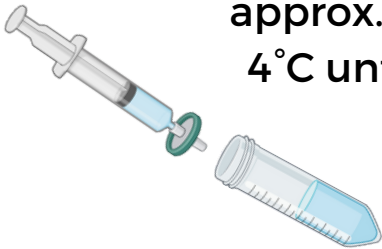
- Water sample
- Empty sample tube (ex. Falcon 50mL centrifuge tube)
- Luer lock syringe
- GDX syringe filter 0.45 μm
- Dual-beam spectrophotometer
- Quartz or silica cuvette
- Deionized water



STEP 02

Filter the sample.

Fill the syringe with sample water, attach GDX 0.45 μm filter, and express a few drops to rinse the filter. Express a few more drops into your empty, clean, filtrate container to rinse it. Filter approx. 40mL into the tube. You can store filtered samples at 4°C until analysis which should be < 2 weeks from sampling time to avoid loss due to degradation.



STEP 03

Set up the spectrophotometer.

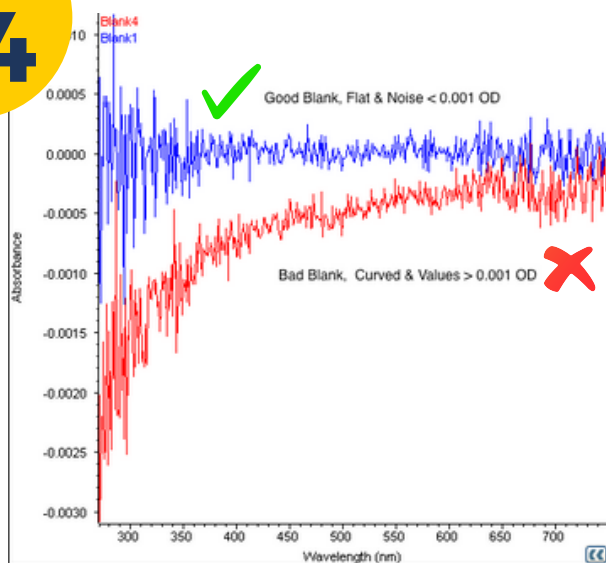
Let the samples warm up to room temperature in the dark (can cover with aluminum foil). Let spectrophotometer warm up for at least 30 mins. While it warms up, set up your blank to baseline the optical density (OD) of the instrument. Use deionized water in your quartz or silica cuvette, set the machine to 270-750 nm, 1 nm interval, and bandpass (2 nm bandpass is OK if 1 nm is not available).



Reading the Water Sample with the Spectrophotometer: Analysis

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STEP 04



Establish baseline.

After the baseline, run a DI blank to verify a "flat" baseline. Typically noise should be less than 10^{-3} OD. For each sample, we will run a blank and 3 replicate scans. After the last sample scan, run a final blank so that each set of scans has a flanking set of blanks. Watch the blanks for drift, if it exceeds 10^{-3} , re-baseline the instrument before proceeding.

STEP 05

Correct and analyze readings.

CWW uses a Matlab script that we are happy to share that can be used to correct, screen, and convert the data. Otherwise, correct scans by subtracting the average of pre- and post-blank scans, and subtract any residual offset at 750 nm. Review each of the scans for replication, reject scans showing deviation, and then average the remaining replicate scans. Convert to absorption coefficient in units of m^{-1} using the following formula: **Abs = 230.3 * OD (in 1cm cuvettes).**

