

Lab 2: CDOM absorption**13 June 2023****1.1 Introduction and Lab Goals**

Our primary goal is for you to become familiar with the concept of absorption in its simplest case, i.e., a solution that lacks particle scattering. Our natural analogs are pure water and colored dissolved organic matter (CDOM; in older literature you may notice it referred to as gelbstoff – yellow matter – or gilvin). Absorbing particles will be the focus of lab 3; particle scattering will be the focus of lab 4. Much of what you will learn in this course is to identify and distinguish between what sensors measure versus the derived quantity of interest. In this lab, you will work with two different types of commercial sensors that provide estimate of *absorption*. Although they essentially measure the same optical configuration, the numbers you retrieve are at different stages of the absorption calculation (see section 1.3) and they require different data processing steps. We approach absorption by exploring the relationship described by Beer's Law (see appendix) and its application to a standard benchtop spectrophotometer and the WET Labs absorption and attenuation meters (ac-9 or ac-s).

Most instruments measure absorption relative to a reference material; pure water at a reference temperature is typically used to obtain absorption by the dissolved matter in the water. Measuring the absorption by pure water is very difficult but we have some great published results on which most people rely. For the latest on this subject see chapter 1 of a recent IOCCG report (https://ioccg.org/wp-content/uploads/2020/09/absorption_protocol_final-incl-cover_rev.pdf).

This lab explores factors that influence the absorption measurement, namely, sample concentration, optical pathlength, temperature, and salinity. You will explore how the absorption coefficient you derive depends upon those factors and the constraints that are necessary to achieve robust observations.

1.2 Activities*Rotations:*

The lab activity will take place in the teaching lab at multiple stations. The lab time is divided into two 90-minute intervals with a break in between. Students will be split into two groups, spending 90 minutes in each of the two lab sections. Section 1 is comprised of two activities: a simple active demonstration of Beer's Law and benchtop spectrophotometry. Students rotate through these two activities in 90 minutes. Section 2 is focused on using *in situ* absorption meters (ac-s). Students will be split into 3 subgroups; each group will have its own absorption meter to work on for the whole 90-minute interval. Your instructors will let you know when to rotate between sections.

Water samples: Water samples have been collected from two locations: (1) off the dock in the Harpswell Sound (HS) and (2) the Water Street Boat Launch (<https://www.brunswickme.org/Facilities/Facility/Details/Water-Street-Boat-Landings-45>) on the Androscoggin River (AR) in Brunswick. The Androscoggin River, while tidal at

this location, is what we call a freshwater ‘tea’ because of its brown color. The whole water samples were filtered through a 0.2 μm nominal pore sized filter. Water samples should be at room temperature because the absorption coefficient depends upon temperature and salinity. Always record the temperature and salinity of your sample and reference waters in case you need to apply a correction. In addition to these two natural samples, we will also use purified water at a range of temperatures and room temperature purified water with controlled additions of sea salt to demonstrate the effect of temperature and salinity on the absorption coefficient of water.

Each group will be responsible for a specific set of measurements during their rotation and for processing their measurements. Data synthesis requires the results from all of groups be combined. In this way, everyone has the opportunity to make one set of each type of measurement but does not have to collect all the observations nor process all the data. This is a *jigsaw approach* to collaborative learning. The analysis and data synthesis will be completed after dinner and the class will prepare a presentation of their results/findings for a debrief Wednesday morning.

Answer the following questions using the data you collected:

1. *How does the absorption spectrum of purified water vary with temperature?* Be specific about the wavelength ranges, compute the $\frac{\Delta a}{\Delta T}$ for specific wavelengths, or the $\frac{\Delta a}{\Delta T}$ spectrum. How does temperature-dependent absorption impact the absorption measurement for discrete samples in the lab? How will temperature-dependent absorption impact the absorption measurement in the ocean?
2. *How does the absorption spectrum of purified water vary with added salt (salinity)?* Be specific about the wavelength ranges, compute the $\frac{\Delta a}{\Delta S}$ for specific wavelengths, or the $\frac{\Delta a}{\Delta S}$ spectrum. How does the salinity-dependent absorption impact the absorption measurement for discrete samples in the lab? How will the salinity-dependent absorption impact the absorption measurement in the ocean?
3. *How does the dye concentration affect the absorbance of a solution?* Plot the absorbance versus added dye from the Beer’s Law demonstration. Is the relationship linear? If not, what is the dependence?
4. *How does geometric pathlength impact the measured absorbance and the derived absorption coefficient?* On separate graphs, plot both $A_{CDOM}(440)$ and $a_{CDOM}(440)$ versus the geometric pathlength of the cuvette (don’t forget to include the 25-cm acs readings in the $a_{CDOM}(440)$ graph). What conclusions can you draw from your results regarding the appropriate range of values for which you can accurately determine the absorption coefficient?

1.3 The instruments

The **Beer's Law demonstration** provides an estimate of absorptance, which is the ratio of the absorbed radiant power, Φ_a , to the incident radiant power, Φ_o [Absorptance is often denoted by capital letter A , however, this is also the symbol for Absorbance. Because we will be discussing Absorbance much more than Absorptance, we will use the bolded A for Absorptance]. The absorbed radiant power cannot be measured directly, but because, by conservation of energy, $\Phi_o = \Phi_t + \Phi_a + \Phi_b$, and because we are assuming there is no scattered radiant energy, it is calculated by the difference between incident and transmitted, Φ_t , radiant power through a sample. Absorptance is:

$$A = \frac{\Phi_o - \Phi_t}{\Phi_o}$$

The **benchtop spectrophotometer** provides estimates of absorbance, A , which is also dimensionless. Sometimes called optical density, it is the \log_{10} of the ratio of incident to transmitted radiant power:

$$A = \log_{10} \left(\frac{\Phi_o}{\Phi_t} \right)$$

It is related to absorptance by:

$$A = -\log_{10} \left(\frac{\Phi_t}{\Phi_o} \right) = -\log_{10} \left(1 - \frac{\Phi_o - \Phi_t}{\Phi_o} \right) = -\log_{10}(1 - A) = \log_{10} \left(\frac{1}{1 - A} \right)$$

Absorbance is measured spectrally typically over the UV to visible range (e.g., 200nm to 800 nm), resolved to narrow wavelength bands (e.g., 1-2 nm). Most often, as we do here, we measure absorbance *relative* to another substance (reference material, think about what reference material makes the most sense):

$$A_{sample} - A_{ref} = -\log_{10} \left(\frac{\Phi_t}{\Phi_o} \right)_{sample} + \log_{10} \left(\frac{\Phi_t}{\Phi_o} \right)_{ref} = -\log_{10} \left(\frac{\Phi_{t,sample}}{\Phi_{t,ref}} \right)$$

The **in situ absorption meters** provide estimates of the absorption coefficient, a , with units of m^{-1} and based on the natural log (\log_e). Typically, a is reported as the *spectral* absorption coefficient with the designation (λ). Note the conversion between A ($\log 10$) and a (natural log) is:

$$a(m^{-1}) = \frac{A}{\log_{10}(e) \times L} = 2.303 \frac{A}{L}$$

where L is the geometric pathlength of the instrument cuvette measured in m .

1.4 Sample preparation and processing

CDOM analyses should be carried out as soon as possible after water collection and filtration. Samples are collected in acid washed amber glass bottle. Whole water samples are collected from the filtrate of 0.2 μm plastic filters (e.g., Sartoris filters) or 0.7 μm glass fiber filters (e.g., Whatman G/FF filters). *Think about each of these steps and consider why they are recommended? What factors should you consider in sample collection, processing, and analysis to retrieve the most robust estimates of a_{CDOM} ?*

1.5 What to do at each station.

1.5.1 Beer's Law Demonstration Effect of concentration.

Position the microscope light on one side of the tank and a light detector on the other. Maintain the positions throughout the experiment. You will be measuring the transmitted light through the tank for sequential additions of a dye. The initial measurement of the pure water in the tank is the reference measurement, which represents the incident radiance power (Φ_o). Subsequent measurements with dye represent the transmitted radiant power (Φ_t).

Add 1 mL drop of green dye to the tank, mix, measure and record transmitted light (Φ_t).

Continue adding dye until $\Phi_t = 0.5 \Phi_o$. Plot A vs. *Cumulative* $V_{dye\ added}(ml)$.

Incident, Φ_o , light reading _____

$V_{dye\ added} (ml)$	<i>Cumulative</i> $V_{dye\ added} (ml)$	Transmitted Light reading, Φ_t	$-\log_{10} \left(\frac{\Phi_{t,sample}}{\Phi_{t,ref}} \right)$
0	0		
1	1		
1	2		
2	4		

Notes:

1.5.2 Benchtop Spectrophotometer

The four groups that rotate through this lab will collect absorbance spectra of the following samples:

Group	Water sample	cuvette length (cm)	Temperature (C)	Salinity (ppt)
1	Pure water	10	T _{fridge} =	0
2	Saline water	10	T _{fridge} =	30
3	Pure water	10	T _{room} =	0
4	Saline water	10	T _{room} =	30
1	Androscoggin	10	T _{room} =	0
2	Androscoggin	5	T _{room} =	0
3	Androscoggin	2	T _{room} =	0
4	Androscoggin	1	T _{room} =	0

Spectrophotometer Protocols

- Cuvettes are pre-cleaned and ready for use. Clean them when you have completed your scans. Wear gloves and never touch the optical faces of the cuvettes. Use only lens paper, not Kim Wipes. Rinse cuvette with sample 3 times before filling 75% full for scanning. Always start with your cleanest, lowest CDOM water samples, transitioning to your highest CDOM samples. Visually inspect the cuvette to ensure there are not particles or bubbles in the sample and that the windows are clean and dry. Maintain cuvette orientation in the cuvette holder (don't flip around).
- Spectrophotometer Setup: Open up the method for our course (ag_TR_2023_OpticsCourse). If you select Setup you can see the following configurations:
 - Cary Tab: Xmode: Nanometers 850nm-300 nm. Ymode: Abs, Factor 1.0, Ymin-Ymax -0.05 to 0.5. Cycle not selected. Scan Controls: Ave time 0.2 s, Data Interval 1.0 nm, Scan rate 300 nm/min.
 - Options Tab: SBW **2.0 nm**. Beam mode **Double**. Energy 1.0. Signal to noise not selected. Source UV-Vis change over 350.00 nm. Display Options **Overlay data**.
 - Baseline Tab: Correction **Baseline correction**.
 - Reports Tab: Autoconvert **Select for ASCII (csv)**.
 - Auto Store Tab: Storage **Storage on (prompt at end)**

- Protocol for sample collection
 - Collect **Baseline scan**. For pure and saline water samples, use air without a cuvette as your reference. For Androscoggin River samples, use pure water (MilliQ) at room temperature as your reference. Run 100% Abs scan as a baseline.
 - Collect **Zero scan**, press Start to run the reference again against the baseline (essentially running reference against itself). This provides an estimate of the best achievable resolution.
 - **Sample scan** for each assigned sample relative to the reference baseline.
- Compute spectral absorption coefficients from spectral absorbance.

Notes:

1.5.3 In situ absorption meters effect of temperature and salinity

The two groups (1 and 2) that rotate through this lab section will each break into 3 subgroups (A, B, C) of 3-4 students to will collect absorption spectra of the following samples:

Group	Water sample	Instrument (serial no)	Temperature (C)	Salinity (ppt)
1A	Pure water	acs ____	$T_{\text{fridge}} =$	$S = 0$
1B	Saline water	acs ____	$T_{\text{fridge}} =$	$S = 30$
1C	Pure water	acs ____	$T_{\text{room}} =$	$S = 0$
1A	AR	acs ____	$T =$	$S = 0$
1B	AR	acs ____	$T =$	$S = 0$
1C	AR	acs ____	$T =$	$S = 0$
2A	Pure water	acs ____	$T_{\text{fridge}} =$	$S = 0$
2B	Saline water	acs ____	$T_{\text{fridge}} =$	$S = 30$
2C	Saline water	acs ____	$T_{\text{room}} =$	$S = 30$
2A	HS	acs ____	$T =$	$S =$
2B	HS	acs ____	$T =$	$S =$
2C	HS	acs ____	$T =$	$S =$

Ac meter Protocols

- Clean the sensor windows and tubes prior to measurements with lens paper and ethanol or isopropyl alcohol followed by rinsing with DIW. Measure the temperature and salinity of every sample (even those entered as target values).
- Focus on getting good pure water calibrations: each student should run her/his own Milli-Q water cal (either a-tube or c-tube of the ac-meter, or both). Save files in your group's folder. Note the magnitude and shape of the pure water spectra; they should be repeatable to within the instrumented resolution between calibrations (i.e. 0.005 m^{-1} for absorption; 0.01 m^{-1} for attenuation).
- Run the filtered water samples in both the a-tube AND c-tube of the ac-meter. Collectively you will be able to compare a_{CDOM} of AR water between with an acs, and $a_{CDOM}(440)$ as a function of pathlength for the acs and benchtop spectrophotometer. Remember to save the files in your group's folder.

- Instructions and code for processing data will be provided in lab.

Data Analysis

CDOM Spectral Slope:

The CDOM absorption spectrum decays exponentially from blue to red given by:

$$a_{CDOM}(\lambda) = a_{CDOM}(\lambda_{ref}) \times e^{(-S_{CDOM} \times (\lambda - \lambda_{ref}))}$$

Where S_{CDOM} is the exponential slope with units of (nm^{-1}) . The slope has been used to qualitatively describe CDOM composition, with steep slopes associated with older and more refractory compounds and flatter slopes with fresher, more labile compounds (Carder et al., 1989; Blough and Del Vecchio, 2002; Simeon et al. 2003).

Calculate the a_{CDOM} spectral slope, S_{CDOM} , for DRE and Biscay Pond water. For the ac-meters, the slope will be for the visible range only; the benchtop spectrophotometer extends into the UV range.

Be prepared to discuss the variations in slope as a function of water type, instrument, and wavelength range used.

Table of computed S_{CDOM} values

$\lambda_{min} - \lambda_{max}$	Sample	Androscoggin River	Harpswell Sound
300 nm – 700 nm			
300 nm – 650 nm			
350 nm – 700 nm			
350 nm – 650 nm			
412 nm – 700 nm			
412 nm – 650 nm			

Note on slope calculation

The best method to calculate S_{CDOM} is to minimize the difference between the exponential model and the data (possibly weighed by a different error in each wavelength if the uncertainty varies as function of wavelength, e.g. due to variability in source intensity as function of wavelength). The relative (percent) error is not constant spectrally; in the red the absorption is low and the signal-to-noise high. Slope measurements often exclude red wavelengths due to its sensitivity to temperature.

You may write your own code to determine the slope by non-linear exponential regression (we will also supply code: <http://misclab.umeoce.maine.edu/software.php>).

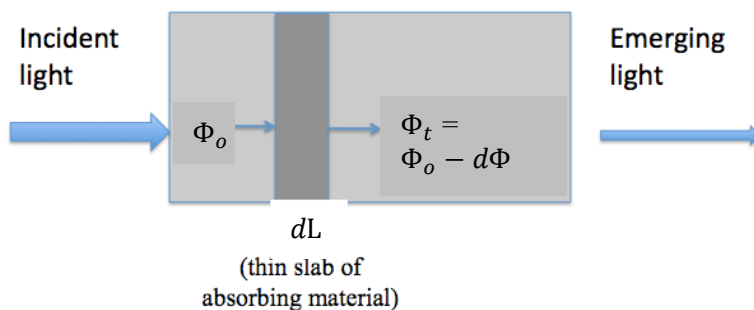
Less rigorously, you may determine S_{CDOM} by plotting the natural-log-transformed values of a_{CDOM} vs. wavelength using Excel and adding a trend line (this is the same as if you fit an exponential curve in Excel, try it). If you use the latter method, *are the slope the same with both methods?*

REFERENCES

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APPENDIX 1 REVIEW OF BEER'S LAW

<http://teaching.shu.ac.uk/hwb/chemistry/tutorials/molspec/beers1.htm>



$$-d\Phi = \Phi_o \times (\varepsilon \times C) \times dL$$

$$-\left(\frac{d\Phi}{\Phi_o}\right) = \varepsilon \times C \times dL$$

Integrate over the pathlength, from 0 to L

$$-\ln\left(\frac{\Phi_t}{\Phi_o}\right) = \varepsilon \times C \times L = a \times L$$

$$\left(\frac{\Phi_t}{\Phi_o}\right) = e^{-\varepsilon \times C \times L}$$

$$\Phi_t = \Phi_o \times e^{-\varepsilon \times C \times L}$$

$$a = -\ln\left(\frac{\Phi_t}{\Phi_o}\right)/L = \varepsilon \times C$$

where:

Φ radiant power

$d\Phi$ infinitesimal loss of radiant power over infinitesimal path dL

Φ_o radiant power incident on the sample,

Φ_t radiant power transmitted through sample,

ε molar absorption coefficient ($m^2 mol^{-1}$), a measure of how much radiant power is absorbed by a 1Molar solution

C molar concentration of the absorbing solution ($mol m^{-3}$)

L pathlength through sample (m)

a sample absorption coefficient (m^{-1})

NB: we combine terms " $\varepsilon \times C$ " into a single term a , the absorption coefficient (m^{-1}).

Notice that the Beer's Law equation is written in \log_e (natural logarithm, \ln). However, spectroscopists historically used \log_{10} , rather than \log_e . The principle is the same but A , the absorbance output from the spectrophotometer, is \log_{10} . Also, chemists include pathlength in A , because measurements are typically made using same 1-cm pathlength.