Lab 3: Particle absorption   21 July 2021

1.1 Introduction and Lab Goals

Particulate absorption measurements provide the second part of the determination of absorption by aquatic systems, dissolved measurements in Lab 2 being the first. Suspended particulate absorption is obtained in two ways: first, by difference between paired spectrophotometric measurements of total and filtered sample in cuvettes (Slade et al. 2010), and second by filtration onto glass fiber filters and direct spectrophotometric analysis. This lab builds upon Lab 2 to demonstrate three ways of measuring particulate absorption spectra, with ac-meters, in cuvettes placed inside an integrating sphere, and on filters placed inside an integrating sphere.

The major absorbers in seawater are water itself, CDOM, and absorbing particles. These two components are separated easily by filtration. The total particulate absorption (designated a_{part} or a_p), consists of phytoplankton, other living organic particles such as viruses, bacteria, zooplankton, dead or detrital organic particles, and inorganic minerals or sediments. The particulate absorption component is separated operationally via extraction with a strong polar solvent such as methanol (Kishino et al. 1985) into phytoplankton (designated as $a_{phyt}, a_{phi}, a_{ph}$, or a_{ϕ}), and non-algal particles (a_{nap}, a term introduced by Babin et al. 2003 that replaced the designation a_d, detrital absorption, because *detrital* has different meanings in geology and biology, neither of which captures the true nature of particles in this fraction). The solvent extraction step effectively removes the phytoplankton pigments while leaving all other particles (organic and inorganic, living and non-living) and the non-pigmented portion of phytoplankton cellular matter intact; this material is identified as NAP. While a_{nap} consists of this large range of materials, the grouping is about more than extractive techniques. The living and dead organic and inorganic constituents within NAP all have a generally exponentially-decaying absorption coefficient which is very different from the absorption spectra of the in vivo phytoplankton pigments, which exhibit strong features associated with distinct pigments.

Absorption coefficients are additive, hence:

$$a_{part}(\lambda) = a_{phyt}(\lambda) + a_{NAP}(\lambda) \qquad (1)$$

The phytoplankton absorption is determined by difference between the measured $a_{part}(\lambda)$ and $a_{NAP}(\lambda)$, after computation of absorption from absorbance. This method provides the best estimate of absorption by phytoplankton pigments, as they were *in vivo* (specifically, as they were packaged in the chloroplasts of living cells). Phycobilipigments associated with cyanobacteria are not extractable with solvents and must be extracted with hot water or phosphate buffer, otherwise a_{NAP} will contain pigment features and be overestimated, leaving a_{phyt} underestimated. These extractions, when gently performed, do not disrupt the particulate matter, including cells.

1.2 Activities

Rotations: As with Lab 2, the class will be divided into two groups of 8 and 9 students, Group 1 and Group 2. Each group will spend half the lab working with the spectrophotometer (and preparing the filtered samples) and half the lab working with the ac-meters.

Samples: We will perform particulate absorption analysis on Harpswell Sound (HS) water (whole water and filtrate for the ac-meters, filter pad samples for the spectrophotometer) and phytoplankton cultures. We will measure chlorophyll via extractive fluorescence analysis on cultures and HS samples.

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

Answer the following questions using the data you collected:

1. How do the absorption spectra of the phytoplankton culture measured in suspension in the spectrophotometer, on the filter pad, and with the acs compare? Describe similarities and differences in magnitude and spectral shape.
2. How does the particulate absorption measured on filter pads vary as a function of volume filtered? What is the uncertainty spectrum for each sample based upon volume? What conclusions can you draw concerning the optimum volume filtered?
3. How do the NAP and phytoplankton absorption spectra pads vary as a function of volume filtered? Are their differences in magnitude and spectral shape?
4. How does the particulate absorption measured in the spectrophotometer compare to that measured in the acs? Describe similarities and differences in spectral shape.

1.3 The instruments

The **benchtop spectrophotometer** from Lab 2 now has an integrating sphere accessory which provides estimates of absorbance, A. Samples are placed inside the sphere so that radiant power scattered by particles or the filter are detected in the transmitted beam, leaving the only loss of radiant power due to absorption.

The **in situ absorption meters** provide estimates of the particulate absorption coefficient by difference between measurements of whole water absorption minus measurements of the filtrate of that whole water (the $a_{CDOM}(\lambda)$ samples measured in Lab 2):

$$a_{part}(\lambda) = a_{whole}(\lambda) - a_{filt}(\lambda)$$

1.4 Sample preparation and processing

Particulate absorption analysis should be performed as soon as possible after water collection. Filtered samples for spectrophotometry can be stored in liquid nitrogen if it is not possible to measure them immediately. However, once thawed, analysis should be immediate. Filter pads for NAP absorption can then be extracted without immediacy but should not be allowed to dry out. All samples should be kept in the dark and at cool temperatures. *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_{part}, a_{phyt} *and* a_{NAP}*?*

1.5 What to do at each station

We have three absorption meters and one spectrophotometer. Students will be broken up into two groups, each group spending approximately 90 minutes in spectrophotometry and 90 minutes with absorption meters, with a break in between.

1.5.1 **Benchtop Spectrophotometer**

For each 90-minute interval, students will be divided into two groups, one will focus on filtering samples, the other on measuring absorption in the integrating sphere. Note that you will be provided with samples filtered by another group. Each group will measure the absorption of their culture suspension in a cuvette and filtered onto a glass fiber filter as well as a Harpswell Sound water sample filtered onto a glass fiber filter (each group will have a different volume filtered). Details are found in Table 1.

Table 1. Particulate absorption samples to be run by each of the four groups.

Group	Sample	V_{filt} (ml)	Sample	V_{filt} (ml)	cuvette
1	HS	250	Culture1	5	1-cm
2	HS	500	Culture2	5	1-cm
3	HS	800	Culture3	5	1-cm
4	HS	1000	Culture4	5	1-cm

Spectrophotometer Protocols

- Spectrophotometer Setup: Menu: **wavelength scan**; Mode: **Abs**; scan from **750 nm** to **350 nm** at **1 nm intervals** with **slow** scan speed. Automatically subtract Baseline.
- The photomultiplier tube (PMT) is very sensitive to light, if exposed to room light levels while scanning it will fry it and we are done for the duration of the course. **Never** click *scan* without the sphere cover in place and the box cover closed. The PMT also sits directly beneath the sample. Thus, it is imperative that no liquid is spilled from the cuvette nor any water drips from the filter. If you have any questions, please ask for help.
- Place the 1-cm cuvette with filtered culture water in the cuvette. Collect **Baseline scan**.
- Collect **Zero scan**, press Start to run the Milli-Q water as a baseline-corrected scan (this is the minimum resolution achievable).
- Collect **Sample scan** with the phytoplankton culture.
- Remove the cuvette and replace with the Plexiglas filter holder.

- Place a blank filter in the holder, top towards the beam. Collect **Baseline scan**.
- Collect **Zero scan**, press *Start* to run the baseline filter as a baseline-corrected scan (this is the minimum resolution achievable).
- Collect **3 blank scans**, press *Start* to run the blank filters as a baseline-corrected scan (this provides uncertainty associated with filter pads).
- Collect **Sample scan**, press *Start* to run the sample as a baseline-corrected scan
- Place the filter back into the filter cup, extract with 10 ml hot methanol as described by your instructor. Rinse with MQ. Collect as **Sample scan** as above.

Compute spectral absorption coefficients from spectral absorbance for each scan. For the blank filter scans, assume the volume filtered is the same as for your samples. The equation to compute particulate absorption from filter pad samples is derived in section 1.6.

1.5.2 **In situ absorption meters**

As in Lab 2, each of the two groups that rotate though this lab will break into 2 subgroups (A, B) of 4-5 students and will collect absorbance spectra of the samples in Table 2. Make sure you record the temperature and salinity of the whole water and filtrate readings.

Table 2. Samples to be run by each subgroup and the instrument to use. Fill in the associated temperature and salinity.

Group	Water sample	Instrument	Temperature (C)		Salinity (ppt)	
			Whole	Filtrate	Whole	Filtrate
1A	HS	acs				
1B	Culture	acs				

If time permits, run a second culture (out of the 3 we have).

Ac meter Protocols

- Clean the sensor windows and tubes prior to measurements with lens paper and ethanol or isopropyl alcohol. 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 calibration (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 sample in both the a-tube AND c-tube of the ac-meter. Measure and record the temperature and salinity.
- Repeat for the whole water sample.

- Collectively you will be able to compare a_{part} of HS water between an ac9 and an acs, a_{part} of HS and culture with an acs, and compare $a_{part}(\lambda)$ for HS and culture with the spectrophotometric observations (filter pad and culture suspension). Remember to save the files in your group's folder

Instructions and code for processing data will be provided at the lab.

1.6 Data analysis for spectrophotometric filter pad absorption

Recall from lab 2 that absorption, $a(m^{-1})$, is computed from the absorbance, A, obtained from the spectrophotometer, where L is the geometric pathlength of the cuvette (m):

$$a(m^{-1}) = 2.303\,\frac{A}{L} \tag{1}$$

The geometric pathlength is equivalent to quotient of the volume of sample filtered (*ml*) to the effective area of the filter (*cm*^{2}), essentially a cylinder of water the volume of which is the filter volume, V, and the geometry of which is the effective filter area, $\pi\,r^2$, times the height, H; the height is the geometric pathlength:

$$H = \frac{V}{\pi\,r^2} \tag{2}$$

Thus, the absorption is computed as:

$$a(\lambda)(m^{-1}) = 2.303\ \times 100\ \frac{A(\lambda)}{\frac{V}{\pi\,r^2}} \tag{3}$$

Where 100 is the conversion between *cm* and *m*. Recall that the sample absorbance measured on the filter pad has been corrected for its blank (baseline scan with a blank filter):

$$A_{pad}(\lambda) = A_{sample}(\lambda) - A_{blank}(\lambda) \tag{4}$$

The advantage of the center-mounted integrating sphere technique is that there is no scattering error in the measurement. Thus, any signal observed in the near IR is due to absorption. Note how this might change your interpretation of the ac-meter scattering correction. See Pegau et al. (2002) for an extensive discussion of "issues" with selecting a null wavelength where theoretically absorption is zero or negligible. Also see Babin and Stramski (2002) for a discussion on null wavelength correction for filter pad absorption.

We typically use a high-quality caliper to measure the effective filter diameter. Today we will use a ruler. Make sure you note your uncertainty in the measurement, we will work on including uncertainty in all our measurements as we go through the course.

Effective filter diameter _______________ cm; uncertainty in diameter _________________cm
In addition to concentrating the particles, the glass fiber filter also provides a highly diffusing light field for the spectrophotometric measurement and increases the optical pathlength that photons travel between the source and detector. The consequence is higher probability of photon absorption and an overestimated absorption coefficient due to "pathlength

amplification”. The absorption calculation must correct for pathlength amplification (Stramski et al. 2015):

$$A_{pad_{corr}}(\lambda) = 0.323 \times A_{pad}(\lambda)^{1.0867} \quad (5)$$

Where $A_{pad_{corr}}(\lambda)$ is substituted for $A(\lambda)$ in the absorption equation (3) above.

1.7 References:

Babin, M., and D. Stramski,. 2002. Light absorption by aquatic particles in the near-infrared spectral region. *Limnol. Oceanogr.,* 47(3), 2002, 911–915.

Babin, M., D. Stramski, G.M. Ferrari, H. Claustre, A. Bricaud, G. Obolensky, and N. Hoepffner. 2003. Variations in the light absorption coefficients of phytoplankton, nonalgal particles, and dissolved organic matter in coastal waters around Europe. Journal of Geophysical Research Ocean 108 (C7): article number 3211 , doi: 10.1029/2001JC000882.

Kishino, M., N. Takahashi, N. Okami, and S. Ichimura, 1985: Estimation of the spectral absorption coefficients of phytoplankton in the sea. *Bulletin of Marine Science.* 37, 634-642.

Pegau, S., J.Ronald V. Zaneveld, B. Gregg Mitchell, James L. Mueller, M. Kahru, J. Wieland and M. Stramska. 2002. Ocean Optics Protocols For Satellite Ocean Color Sensor Validation, Revision 4, Volume IV: Inherent Optical Properties: Instruments, Characterizations, Field Measurements and Data Analysis Protocols. Edited by Mueller, J. L., G.S. Fargion and C R. McClain. NASA/TM-2003-211621/Rev4-Vol.IV. Greenbelt, Maryland.

Roesler, C., D. Stramski, E. D’Sa, R. Röttgers, and R.A. Reynolds. 2018. Chapter 5: Spectrophotometric measurements of particulate absorption using filter pads. In Neeley, A. R. and Mannino, A. [eds.], IOCCG Ocean Optics and Biogeochemistry Protocols for Satellite Ocean Colour Sensor Validation, Volume 1.0. IOCCG, Dartmouth, NS, Canada. doi: 10.25607/OBP-119

Slade, W.H, E. Boss, G. Dall'Olmo, M.R. Langner, J. Loftin, M.J. Behrenfeld, C. Roesler, and T.K. Westberry, 2010. Underway and moored methods for improving accuray in measurement of spectral particulate absorption and attenuation. Journal of Atmospheric and Oceanic Technology, 27:10, 1733-1746.

Stramski, D.**,** R. A. Reynolds, S. Kaczmarek, J. Uitz and G. Zheng. 2015. Correction of pathlength amplification in the filter-pad technique for measurements of particulate absorption coefficient in the visible spectral region. Applied Optics, 54(22): 6763-6782.