Size Distribution and Fluorescent Characteristics of Colloidal CDOM: Indicators of the Provenance and Reactivity of CDOM in Coastal Waters?

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LONG-TERM GOAL

My long term research goal is to ascertain the nature and magnitude of optical effects (absorbance / fluorescence / scattering) in surface seawaters associated with the production and cycling of marine colloidal organic matter. I am particularly interested in determining how these effects are driven or modulated by the productivity dynamics of phytoplankton and marine heterotrophic bacteria.

OBJECTIVES

Marine chromophoric dissolved organic matter (CDOM) imparts highly variable optical signatures in surface waters over short spatial and temporal scales for reasons not yet understood. While considerable research efforts are currently underway on the specific absorption and fluorescence characteristics of the bulk CDOM, my primary objective is to follow the chromophoric signatures of different molecular weight fractions to determine if the production (or allochthonous input) and removal of colloidal organic matter contributes to the high variability in bulk CDOM. We know that a signficant fraction (10-40%) of non-living dissolved organic matter resides in the colloidal size fraction (1-1000 nm) (Benner et al., 1992; Buesseler et al., 1996; Chen and Schnitzer, 1989), and that this fraction is very reactive to both bio-degradation (to soluble substances) (Benner et al., 1992) and aggregation (to large sinking particles)(Baskaran et al., 1992; Chin et al., 1998; Moran and Buesseler, 1992) The challenge is to determine to what extent these dynamic, opposing processes influence the behavior of CDOM in surface seawaters, and how these biogeochemical effects interweave with photochemical degradation pathways.

APPROACH

My studies have focused on field measurements and laboratory incubation experiments aimed towards understanding how the optical characteristics of soluble and colloidal organic matter change under different phytoplankton growth conditions. Two research cruises have been conducted in and about the Mississippi River plume in the Gulf of Mexico during high (spring) and low (summer) flow conditions. Seasonal sampling (winter, spring, summer) has also been done in saline waters of the Damariscotta River estuary. These two sites (Mississippi and Damariscotta River) contrast regions where the marine dissolved organic matter is dominated by terrestrial inputs (Mississippi) and in-situ production by phytoplankton and benthic algae (Damariscotta River). We have investigated the differences and similarities in the molecular weight (colloid size) distributions between these regions and seasons.

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14. ABSTRACT
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In previous years the marine colloidal fraction was isolated from conventionally-filtered seawater (< 0.2 µm) using cross flow filtration (CFF) (or ultrafiltration). Seawater samples were recirculated under pressure (~ 10 psi) above a 1000 Dalton membrane, a small portion of the soluble phase passing through the membrane on each cycle. After ~2 hr, the large volume (20 l) sample was fully partitioned into soluble (membrane-permeable) and colloidal (membrane-retained) fractions that were retained for analysis. The optical signals of the bulk and size-fractionated CDOM then were characterized by acquiring 3 dimensional excitation/emmission matrices (EEMS) plotted as an intensity contour surface. 3D fluorescence spectroscopy has been shown to be an effective means for distinguishing between types of organic matter in seawater (Coble, 1996). In addition, absorbance scans were measured on each sample across the same excitation range to both characterize the absorbance characteristics and to enable the relative fluorescence efficiencies to be estimated.

Over the last year we have explored the use of Flow Field-Flow Fractionation (Flow FFF) for separting the colloidal CDOM fraction from seawater. Unlike CFF, which provides a single cutoff size, Flow FFF partitions organic phases into a size continuum, from soluble through to particulate (> 0.45 µm) sizes. Briefly, a flow field is applied at right angles to the channel flow within a shallow (~200 µm) ribbon-like chamber. Soluble fractions are driven through the membrane (1 KDa) on the accumulation wall, while colloidal components are driven to the accumulation wall. The resultant concentration gradient is opposed by diffusion (a function of colloidal size), resulting in colloids of different size being retained in different stream laminae. Unequal laminae velocities due to shear along the accumulation wall causes a well-defined separation of different colloidal size fractions according to the mean colloid proximity to the wall, which then is measured by UV absorption after the sample stream exits the flow chamber. A single wavelength detector (254 nm) has been primarily used for quantifying CDOM. In addition to yielding a soluble phase, the method provides a high resolution separation of organic matter into a continuous colloidal size spectrum.

Limitations of detection sensitivity has required that the entire colloid fraction be concentrated before analysis. We use on-line focusing for this purpose, whereby seawater (1-10 ml) is “focused” at the head of the channel before proceeding with the normal analysis mode. This approach has yielded sufficient sensitivity to measure the colloidal fraction without generating any detectable artifacts. We currently are interfacing a 50 cm waveguide/CARY 50 spectrophotometer system to the channel outflow. This will reduce the need for sample focusing and also will enable us to obtain multiple absorbance scans across the CDOM size continuum. The work here is among the first to apply this new tool to the study of marine systems.

WORK COMPLETED

Two recent cruises have been completed and these data have been processed as part of this project: 1) June, 2000, Mississippi plume region (ONR Optics cruise), and 4) April 2001, Mississippi plume (ONR Optics cruise). The new Flow Field-Flow Fractionation instrumental approach was applied during these cruises. Water samples collected from an underway pumping system were concentrated in the channel by reverse flow before proceeding with the size separation. UV absorbance (254 nm) of the channel outflow was monitored as a measure of the amounts of organic matter, the time trace giving a spectrum of colloidal sizes present in the sample. In addition to studying spatial variations, vertical profiles also were analyzed, providing an opportunity to examine the differences between allochthonous and attochthonous derived matter. In some cases, aliquots of the outflow stream were retained for 3D excitation/emission spectra determinations. In addition, EEMS were measured on whole dissolved (< 0.4 uM) seawater samples, both for comparison with size fractionated aliquots and also to provide spatial mapping of CDOM fluorescence characteristics in conjunction with various other survey parameters monitored by other members of the ONR funded science party.

Seasonally distinct surface water samples also have been, and continue to be, collected in the Damariscotta River estuary for analysis by Flow Field-flow fractionation (as above). In addition, monoclonal cultures of several different phytoplankton species are being reared in synthetic seawater and the colloidal CDOM size spectrum measured (by FFFF) at different growth stages (early exponential, late exponential, senescence) to determine the size characteristics of phytoplantkon-derived CDOM.

The key individuals assisting me with this work are Kathy Hardy (research specialist), Peggy Hughes at the Institute of Marine Sciences, UCSC, Sheri Flogi (M.Sc. student, UM) and Jennifer Boehme (Postdoctoral Scientist), who just recently joined our laboratory.

RESULTS

1. Flow-FFF provides a distinct and reproducible measure of the size continuum of molecular weight standards added to seawater. Even so, retention times depend strongly on the hydrodynamic diameter of the molecules, so molecules of similar molecular weight but different architectures will have different retention times. Molecular weight standardization therefore is best used to assess the integrity of the FFFF system, and only provides a rough estimate of the molecular weights of natural compounds separated by FFFF.

2. On-line focusing of increasing volumes (100 µl to 10 ml) greatly increases the signal strength of molecular weight standards without significantly altering their colloidal size distribution. Similarly, on-line focusing does not alter the size spectrum of naturla colloidal matter in seawater. These findings indicate that Flow-FFF with on-line focusing provides an effective means for obtaining the size fractionation of colloidal CDOM in seawater.

3. The abundance and size distributions of colloidal CDOM changes significantly between winter and summer in saline waters of the Damariscotta River estuary (Figure 1). Colloidal CDOM is dominated by very small colloids (< ~10 kDa) during winter (low phytoplankton production) and by large colloidal matter (> ~150 kDa) during summer (high phytoplankton production).

4. Large differences in colloidal CDOM abundance were measured in an around the Mississippi River plume during high and low flow conditions. Paradoxically, larger amounts of colloidal CDOM were observed during low rather than high flow conditions.

5. While there was a consistent decrease in the abundance of colloidal CDOM over inshore to offshore transects, this loss was not uniform across the size spectrum. Smaller-sized colloidal matter (~7-150 kDa) disappeared preferentially over larger-sized colloidal CDOM (Figure 2).

6. Marine CDOM off the Mississippi plume was largely soluble (< 1 kDa) in subsurface waters during spring (high flow conditions) while colloidal CDOM was much more abundant later in the season (low flow conditions). The low flow conditions conincided with higher phytoplankton abundance (as indicated by chlorophyll concentrations).

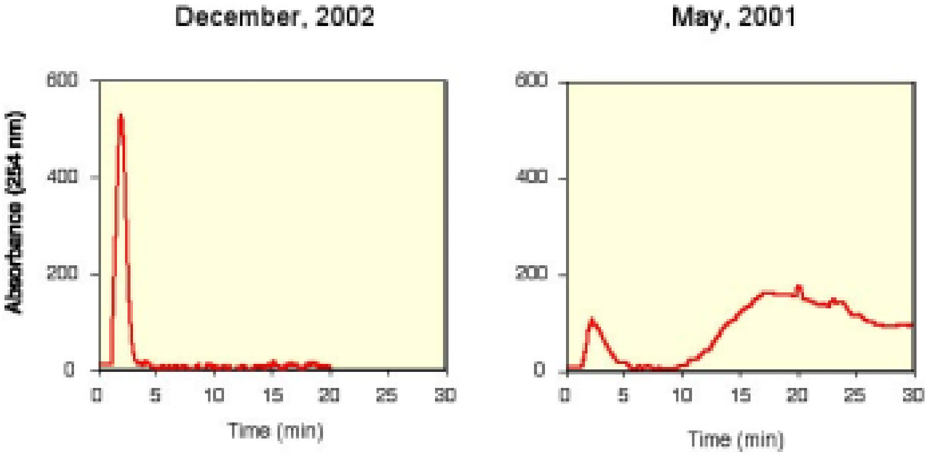

Figure 1. Flow Field-Flow Fractionation size spectra of colloidal matter in surface seawaters from the Damariscotta River during winter and early summer. Plots show absorbance (@254 nm) of the channel outflow with time. Smaller colloids exit earlier than larger colloidal phases (i.e., increasing time depicts increasing size of the CDOM). Clear differences are observed between seasons, with low molecular weight colloidal matter dominating during winter while higher molecular weight colloidal matters dominates during summer.

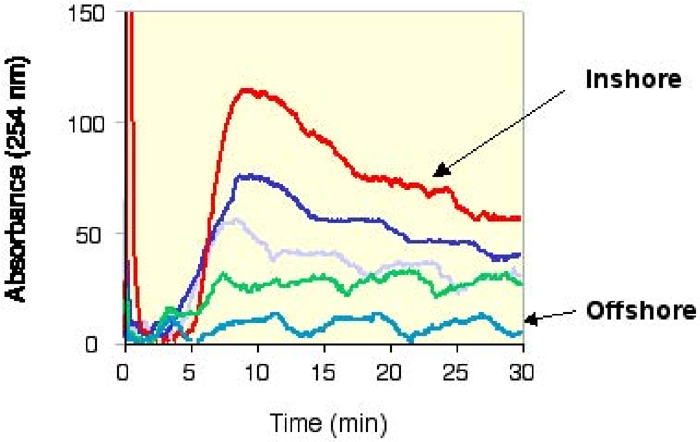

Figure 2. Relative changes in the abundance and size distribution of colloidal CDOM (absorbance @ 250 nm) moving from inshore (upper trace) to offshore (lower trace). Results show a preferential loss of smaller-sized colloidal constituents with distance from shore, with the offshore trace being from neashore "blue" waters.

7. Laboratory culture experiments demonstrate that marine phytoplankton can be a direct source of small and large colloidal CDOM (Figure 3), with production rates being highest during the latter stages of culture growth. That is, nutrient-stressed phytoplankton produced larger quantities of colloidal CDOM than rapidly growing cells. This finding is in agreement with earlier experiments with natural population cultures in upwelling waters (see previous annual reports).

8. Previous measurements the fluorescence characteristics of the colloidal phase (colloidal FDOM) used CFF to obtain the needed sample volumes. The sample stream from FFFF requires the use of very low volume (< 100 µL) samples. Integration of sample outputs over longer times (that is, collection of multiple CDOM peaks) has not illustrated any large differences in FDOM characteristics with size. However, we are now attempting to improve this capability by working with low volume cells.

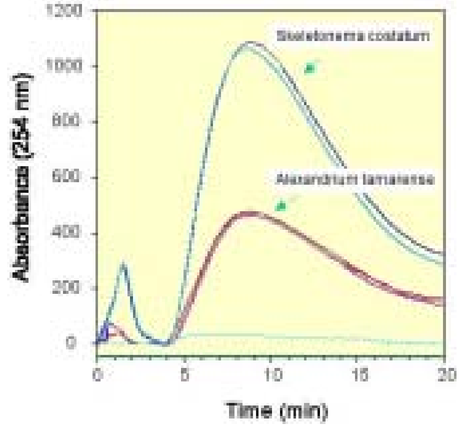

Figure 3. Size fraction of colloidal CDOM produced by monoclonal cultures of the diatom <u>Skeletonema costatum</u> (top trace) and the dinoflagellate <u>Alexandrium tamarense</u> (bottom trace). Results show triplicate runs of culture filtrates (<0.2 µm) and illustrate that phytoplankton can be a direct source of the sizes of colloidal matter observed in natural waters.

IMPACT/APPLICATION

These findings support the view that a significant fraction of the CDOM fluorescence component is associated with the marine colloidal phase. This colloidal fraction of CDOM is a dynamic pool that can change over time scales ranging from days to months. The laboratory findings demonstrate that phytoplankton abundance, their growth rates, and the ambient nutrient conditions all will be important factors controlling the in-situ production of colloidal CDOM. Marine colloidal matter as a whole is recognized to both aggregate to form large sinking particles, and to serve as a carbon resource for heterotrophic bacteria, both processes of which could influence the bulk CDOM signature of seawater over short time scales. Previous work on this project has demonstrated that the colloidal organic phase harbors hydrophobic microenvironments (based on dye absorption experiments). Partitioning of fluorophores into these reservoirs conceivably could cause fluorescence characteristics of these molecules to shift; a change that might mistakenly be attributed to diagenesis. These findings provide insights to the underlying biogeochemical mechanisms affecting the magnitude and character of CDOM in seawater and complement those studies examining direct photochemical effects.

TRANSITIONS

The application of flow field-flow fractionation methods to the study of the marine organic phase is in its infancy. We anticipate that publication of our findings in the planned special issue of Marine Chemistry dealing with marine CDOM will stimulate further use of this novel tool.

RELATED PROJECTS

None

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Wells, M. L. 2002. Interactions among marine colloids and metals in seawater: A review. Chapter 11 in “Biogeochemistry of Marine Dissolved Organic Matter”, Ed. D.A. Hansell and C. A. Carlson