



The colloidal size spectrum of CDOM in the coastal region of the Mississippi Plume using flow field-flow fractionation

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Abstract

Chromophoric dissolved organic matter (CDOM) is a major component of the total dissolved organic matter in seawater, and it can interfere significantly with oceanographic remote sensing in nearshore waters. Coastal marine CDOM comprises an undefined, likely rapidly changing mixture of terrestrially and marine-derived substances. Flow Field-Flow Fractionation (FIFFF) offers a novel approach for separating the colloidal (high molecular weight) fraction of CDOM into broad size continuums that can offer insights to the characteristics and reactivity of CDOM constituents. FIFFF analyses of seawaters in and around the Mississippi Plume were performed during high and low flow conditions and the results compared to analyses of phytoplankton cultures and waters from a coastal estuary having low freshwater inputs. FIFFF fractograms displayed major differences in the size distribution of CDOM between offshore in the Gulf of Mexico (GOMex) and the Mississippi River plume. Colloidal size distributions in nearshore surface waters out of the immediate influence of the Mississippi Plume contained a mixture of features seen in offshore waters, plume waters, and in phytoplankton cultures. Plume waters had a high abundance of larger colloidal phases but continued dilution with coastal waters led to preferential losses of mid-sized colloidal matter. Colloidal size spectra in offshore waters of the GOMex during spring were similar to that observed in estuarine waters after the spring bloom. These preliminary findings suggest that the colloidal size spectrum of CDOM may provide information on its provenance and the relative mixtures of terrestrial and marine components.

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1. Introduction

Dissolved organic material (DOM) in seawater comprises a major portion of the global carbon budget, yet we have only rudimentary knowledge of its specific composition and reactivity. Sources of DOM in nearshore waters include terrestrially derived

substances carried mainly by rivers as well as production in situ by marine planktonic organisms. Colored, or chromophoric dissolved organic matter (CDOM) is the portion of DOM that absorbs visible and near-ultraviolet radiation, and its optical signal in coastal ocean waters can interfere with the remote sensing of oceanographic parameters. Significant gains in our understanding of CDOM characteristics have been achieved by studying its bulk optical (or chemical) features but these advances have been

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frustratingly slow due to limitations of the available analytical methods.

A large fraction of marine DOM is of high enough molecular weight that it lies within the colloidal size range (ca. 1–1000 nm, Vold and Vold, 1983), meaning that interfacial forces as well as biochemical and photochemical processes may affect the behavior of these substances (see Wells, 2002). Marine-derived colloidal organics on the whole tend to be more bioreactive than low molecular weight, or soluble, organic substances (Amon and Benner, 1996; Santschi et al., 1995). This observation is in sharp contrast to a major fraction of terrestrial colloidal humic substances that tend to be biologically recalcitrant. As a consequence, it will be important to tease apart these mixed pools of terrestrial and marine sourced CDOM if we are to understand their respective cycling and fates in nearshore environments.

Identifying the relative contributions of CDOM from different source regions using bulk optical characteristics has been difficult, due in large part to the presumed complex assemblage of molecules present. One approach that may offer new insights to the provenance and reactivity of CDOM is measurement of the spatial and temporal differences in size distributions of CDOM. A number of analytical methods exist for estimating the size distribution of colloidal organic matter, including light scattering, ultracentrifugation, gel permeation chromatography, size exclusion chromatography, mass spectrometry and cross-flow filtration, the latter being used frequently to study marine colloids (Benner et al., 1992; Buesseler et al., 1996; Buffle et al., 1992; Guo, 1995; Kepkay et al., 1993; Martin et al., 1995; Moran and Buesseler, 1992; Santschi et al., 1995; Sharp, 1973; Wells et al., 2000). Cross, or tangential flow filter methods typically employ 1–10 kDa molecular weight size cut-off membranes (Buesseler et al., 1996; Carlson et al., 1985; Guo and Santschi, 1996; Wells, 2002), although there are some uncertainties about the effective size cut-off of these techniques (e.g., Dai et al., 1998; Guo et al., 2000; Gustafsson et al., 2000). Even so, cross-flow filtration (CFF) remains the backbone for most marine colloid studies.

A key limitation of CFF methods for the study of the marine colloidal phase is that only a rudimentary size fractionation is obtained; that matter retained or not retained by a given membrane.

Multiple systems and samples must be processed to ascertain the broader size distribution of colloidal matter. In contrast, Flow Field-Flow Fractionation (FIFFF) is a chromatographic-like technique that can partition colloidal matter into its size continuum (Giddings et al., 1976). Early pioneering studies utilizing field-flow fractionation methods to study environmental samples (Beckett and Hart, 1993; Beckett et al., 1987, 1990) have sparked increasing interest in applying this novel approach to marine waters (Lyven et al., 1997; Ratanathanawongs-Williams and Keil, 1997; Vaillancourt and Balch, 2000; Zanardi-Lamardo et al., 2001, 2002).

In this study, FIFFF was applied to measure the size continuum of colloidal CDOM in coastal waters. Results are presented from two cruises to the Mississippi River delta region, and are compared to size profiles of colloidal CDOM produced in monoclonal phytoplankton cultures as well as from the Damariscotta River; an estuary on the coast of Maine having low freshwater inflows. FIFFF fractograms displayed major differences in the size distribution of CDOM between offshore in the Gulf of Mexico (GOMex) and the Mississippi River plume. Colloidal size distributions in nearshore surface waters out of the immediate influence of the Mississippi Plume contained a mixture of features seen in offshore waters, plume waters, and in phytoplankton cultures. These preliminary findings suggest that FIFFF may be a useful tool to ascribe the contributions of terrestrial and marine produced CDOM to bulk optical signatures in nearshore waters.

2. Materials and methods

2.1. Sampling

The study region lay in and around the Birdfoot region of the Mississippi River delta and the adjacent Atachafalya River (Fig. 1). Two cruises were conducted, one during low river flow conditions (June 2000) and the second during high flow conditions (April 2001). Underway surface water samples (2–4 m) were collected either with a metal-free surface pumping system (June 2000) or directly from the EcoShuttle on-line pumping system (April 2001) described by Chen et al. (this issue). Vertical profiles

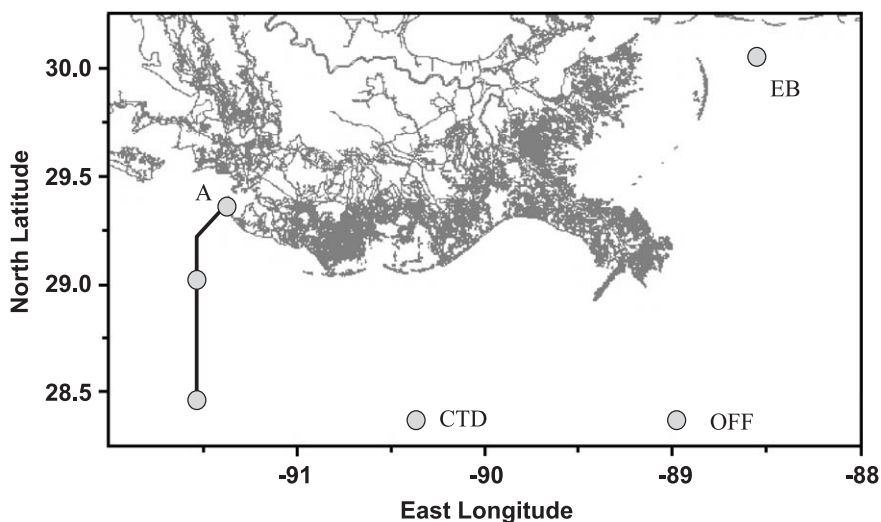


Fig. 1. The sampling region showing the sampling sites for the study reported here. See Chen et al. (this issue) for the broader range of transects conducted during the two cruises. Data in the figures were obtained from the sampling sites labeled: (A) Atachafalya transect (2000 cruise), (CTD) depth profile station, (OFF) offshore “Blue” water site (2001 cruise), (EB) east of the Mississippi River Birdfoot delta (2000 cruise).

were obtained from a CTD rosette sampler at a site influenced by plume waters (Fig. 1). Water samples were gently filtered ($0.2\ \mu\text{m}$, Poretics) by vacuum and the filtrates retained for FIFFF analyses.

2.2. Size fractionation

FIFFF is one of a family of field-flow techniques pioneered by the late Giddings (1993) and his colleagues where a field is applied to separate particles and macromolecules during passage along a thin open channel. In FIFFF, these colloidal particles are separated on the basis of their respective diffusivities. Ceramic frits comprise the top and bottom panels of the ribbon-like channel with a semipermeable molecular weight cutoff membrane lining the bottom accumulation wall. The carrier solution is pumped through the channel by two precision pumps; one along the channel and a second across the channel and out through the cutoff membrane. The flow streams down the channel are laminar and parabolic, with the streams being slower closer to the channel boundaries.

An HPLC injection port is used to introduce the sample to the lead end of the channel. As it enters the channel, the along-channel flow is stopped briefly to allow colloids to migrate to their equilibrium height

above the membrane against the cross-channel flow field. Dissolved substances smaller than the membrane cutoff (i.e., soluble matter) are swept through the membrane to waste. At equilibrium, the cross-flow velocity balances colloid diffusion, so that colloids having greater diffusivities migrate higher across the thin axis of the channel than do larger colloids having lower diffusivities. As the channel flow resumes after this equilibration, colloidal matter is carried down the channel in the lamellar flow. Due to the parabolic flow streams, the smaller colloids move faster down the channel and exit the system before larger colloidal constituents contained in the lower velocity flow streams. The retention time of an idealized particle is related precisely to its diffusion coefficient that, in turn, can be related to particle diameter through the Stokes–Einstein equation (Giddings et al., 1976).

One major difficulty in applying FIFFF to the study of marine waters is the inherent dilution of the sample during processing. Typical injection volumes in normal operation are on the order of $10\text{--}50\ \mu\text{l}$ while carrier volumes are several tens of milliliters. Most FIFFF studies of organic colloids in natural waters have examined material preconcentrated from surface waters, or waters already high in DOC concentrations (e.g., Beckett and Hart, 1993; Vaillancourt and Balch,

2000) but more recently on-channel preconcentration methods have been developed, whereby sample volumes up to 1 l can be preconcentrated by reverse flow into the channel before proceeding with normal FIFFF analyses (Lee et al., 1998; Lyven et al., 1997). Although as with any preconcentration method there is the potential that enhanced colloid–colloid interactions could alter natural size distributions (Buffle et al., 1992; Wells, 2002), the likelihood of these artifacts can be evaluated with FIFFF techniques (Lyven et al., 1997).

Although FIFFF theory is well described (Giddings, 1993), the linkage between colloid size and retention time depends critically upon whether the Stokes–Einstein equation adequately describes the behavior of unknown natural constituents in the flow channel. For example, the discovery that colloidal fibrils are highly abundant in nearshore waters (Santschi et al., 1998) raises significant questions about interpretation of FIFFF colloid retention times. Coupling of the channel detector outflow to secondary more sophisticated detectors would increase the information on the accuracy of molecular weight inferences from FIFFF (Jiang et al., 2000). Until then though, molecular weight standards will be best utilized in FIFFF environmental studies to signal changes in channel characteristics over time and to facilitate the comparison of findings among different studies. For this reason, fractogram profiles are presented here in terms of only retention times, rather than in terms of molecular weight estimates.

2.3. Apparatus

An F-1000 FO Universal Fractionator (PostNova Analytics, Salt Lake City, UT) was employed for this study after modification for on-channel preconcentration (Lee et al., 1998; Lyven et al., 1997). Three Alltech HPLC pumps were used; one controlling the channel flow, one the channel cross-flow, and one for sample focusing. The channel dimensions in this system are 29.4 (tip to tip) \times 2.0 cm with a thickness of 254 μm . A 1 kDa regenerated cellulose acetate membrane lined the accumulation wall of the channel. Molecular weight standards were injected through a Rheodyne 9725 injector using a 20 μl loop. The channel void volume,

calculated from the retention time of bovine serum albumin, was 1.41 ml.

The channel was equipped with a frit outlet to further increase the concentration of the colloidal sample components eluting from the channel during analysis. During normal operation, the perpendicular (field) flow keeps the colloidal constituents of the sample within a few microns of the accumulation wall as they move down the channel. The frit outlet split off the upper portion of the channel flow (carrier solution only) at the end of the channel while the lower portion of the channel flow was directed to a Hyper Quan UV detector. A split flow ratio of 6:1 was used for this work, yielding a six times enhancement of the detector signal. Keeping this split ratio <10:1 ensures complete transfer of sample components from the channel to the detector for even poorly retained samples (Jiang et al., 2000). Absorbance in the channel outflow was measured at 254 nm and is used to define CDOM for the purpose of this study.

2.4. Channel cleaning

The FIFFF channel required occasional cleaning, as indicated by changes in the shape or retention time of standards, or by increased system pressure (indicative of membrane fouling). In this case, the channel was opened and a small amount of dilute detergent (MICRO™) was applied directly to the membrane and frit surfaces, followed by gentle rubbing with a gloved finger. The system was reassembled after rinsing with copious amounts of deionized water (Millipore Element™), and then flushed for several hours with carrier solution before recalibration with size standards.

2.5. Carrier solution

The carrier solution is used for both focusing and transport of the colloidal concentrates along the channel to the detector. It is important then that this solution does not alter the stability of the natural colloidal sol, or itself contain significant amounts of colloidal contaminants. Offshore ultrafiltered (<1 kDa) seawater (Millipore Prep Scale cartridges) was freshly prepared and used as the carrier during each cruise while similarly prepared Damariscotta seawater was used for the Maine samples and analyses of

phytoplankton cultures. Experiments with synthetic ocean water as a carrier solution yielded results indistinguishable from that with natural seawater carrier solutions but was less convenient to prepare on-board ship. Surfactants often are added to low ionic strength carrier solutions to minimize sorption but were not used in these experiments. Early work indicated that sorption was not a significant problem, and surfactants conceivably could generate artifacts by disaggregating any colloidal scale molecular assemblages present in the samples.

2.6. Procedure

All analyses followed that same three-step procedure; sample loading and focusing, relaxation within the channel, and then elution. Samples (5 ml) were loaded into the FIFFF channel from the backward end using an injection loop and the colloidal constituents focused into a narrow band by the opposing flows from both ends of the channel. During this stage, no flow is allowed to enter the channel through the topside frit, and all of the focus flow passes through the 1 kDa membrane on the accumulation wall. A flow ratio of 12.5:1 for the backward focus flow (5.0 ml min^{-1}) to forward channel flow (0.4 ml min^{-1}) was applied for 4 min: a sufficient time for focusing the colloidal matter into a band at the very lead end of the channel (Lyven et al., 1997). This positioning was confirmed by comparing the retention times of molecular weight standards by direct injection (normal) and by sample focusing; that is, retention times of focused and directly injected standards were the same. After focusing, the software program control was manually triggered to begin the relaxation step.

During the relaxation, or equilibration step, the channel and focus flows are stopped and the field flow (4.0 ml min^{-1}) initiated. Colloidal constituents are forced towards the accumulation wall by this field flow. Brownian motion compensates this migration so that the population of a given colloidal size forms an exponential distribution with distance from the accumulation wall. The mean distance of the colloids from the wall is directly proportional to the particle diffusivity. After equilibration, the channel flow (2.0 ml min^{-1}) was initiated and the resulting fractogram UV absorbance profiles acquired. Cross-flow rates were maintained at 4.0 ml min^{-1} for the entire separation

(30–40 min). Baseline fractograms, obtained by focusing carrier solution in the same way as for the samples, were normally flat, although broad curved baselines sometimes were noted. Sample fractograms were corrected when necessary for these curvatures and normalized according to the absorbance immediately following the injection peak ($\sim 45 \text{ s}$). As noted above, these FIFFF data are presented as a function of retention times, rather than calculated hydrodynamic diameters or molecular weights.

3. Results

Fractogram profiles of polystyrene sulphonate (PSS) molecular weight standards measured with a seawater carrier solution are shown in Fig. 2a. The profiles for standards routinely exhibited sharp Gaussian-shaped peaks and the log of retention time increased with the log of molecular weight (e.g., Lyven et al., 1997; Fig. 2b). These findings confirmed that the FIFFF system behaved according to theory using the seawater carrier solution.

Changing the sample volume from 1 to 10 ml had no appreciable effect on the size distribution of natural colloidal CDOM in Damariscotta seawater (Fig. 3a). Fractogram profiles show a single large peak of small-sized colloidal matter, and this peak increased in magnitude with increasing sample volumes. There were no indications that focusing initiated or enhanced any aggregation of this material; fractogram profiles remained near baseline levels across the larger size range. The increase in sample peak area was linear with respect to sample volume (Fig. 3b); however, colloid recoveries were $\sim 35\%$. System pressures remained constant during these runs and subsequent injections with standard molecules were normal, indicating that the membrane and system were functioning well.

The size distribution of CDOM produced in cultures of the coastal pennate diatom *Pseudo-nitzschia australis* entering stationary phase contained both small and larger sized colloidal materials (Fig. 4). The smaller material at retention time of $\sim 2 \text{ min}$ was very similar to that measured in the Damariscotta seawater sample (Fig. 3a). This material was followed by a broader profile of larger sized colloidal matter having a peak retention time of $\sim 7 \text{ min}$ but tapered

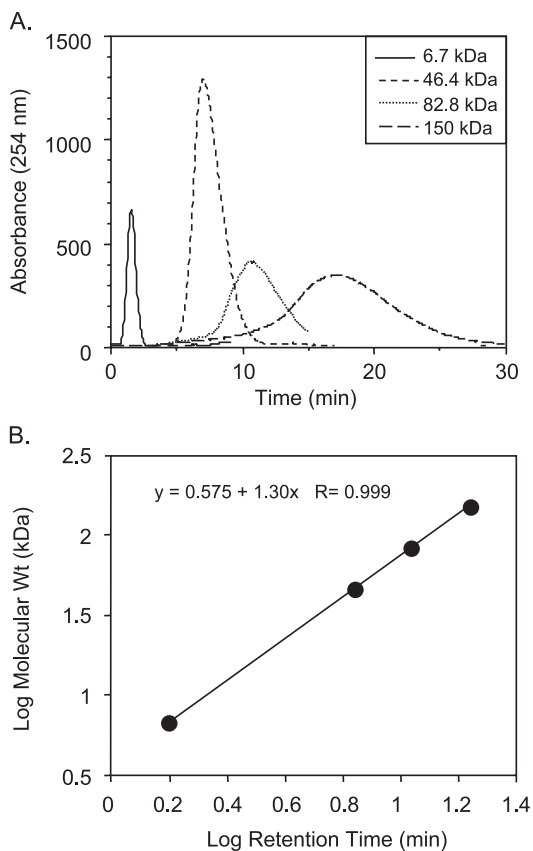


Fig. 2. (A) A composite of fractogram profiles for different sized polystyrene sulphonate (PSS) molecular weight standards analyzed with an ultrafiltered (<1 kDa) seawater carrier solution. (B) The molecular weight of PSS standards as a function of peak retention times (peak maximum). Samples were analyzed using stop-flow injection.

slowly across retention times up to 30 min. Aging of the senescent culture under continued growth conditions (light, temperature) yielded a substantial increase in the secondary colloidal CDOM peak but had little effect on the apparent amount of smaller sized material. Similarly, there was little increase in the largest size fraction with aging. These profile features and their changes with aging also were observed in replicate cultures (data not shown).

Fractogram profiles obtained from low salinity (<5) outflow waters of the Atachafalya River during low flow conditions (June) differ markedly from those of the pure phytoplankton cultures (Fig. 5a). The Atachafalya sample profiles have no detectable

matter with ~2 min retention times, but instead showed a very sharp and large increase in UV absorbing material after 7 min. The profiles peak at a retention time of ~15 min and then slowly taper off for >80 min before eventually returning to baseline (not shown). The shape of colloidal profiles obtained for low salinity (<5) waters of the Mississippi River were indistinguishable from that shown in Fig. 5a. The same characteristic profiles were measured during high flow conditions (April) but the magnitude of the peaks was less (data not shown), perhaps indicating a decrease in the concentrations of colloidal CDOM associated with the higher volume of water transported. These profiles

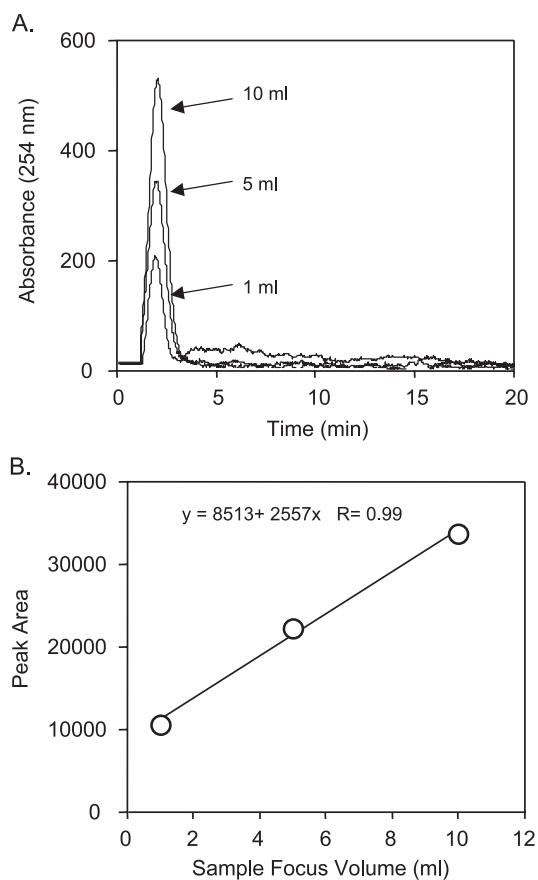


Fig. 3. (A) A composite of individual fractograms showing the effect of increasing focus volumes of Damariscotta river seawater on sample size profiles. (B) The linear relationship between sample focus volumes and peak heights of the 2 min retention time peaks shown in (A).

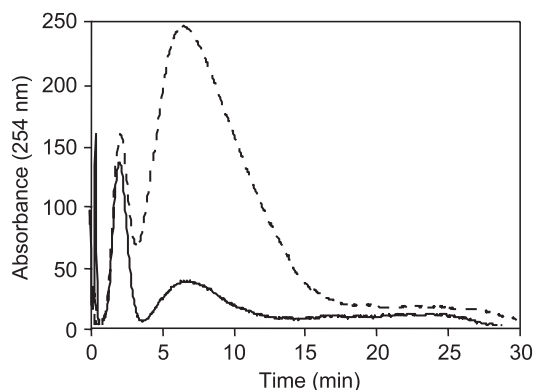


Fig. 4. Fractogram profiles for filtered ($<0.2 \mu\text{m}$) growth media from a culture of the pennate diatom *P. australis* (solid line) as it approached senescence (stationary phase). The fractogram of this senescent culture medium ($<0.2 \mu\text{m}$) after 1.5 weeks of aging is shown by the dashed line.

indicate that terrestrial CDOM has a very wide continuum of colloidal size.

The magnitude and shape of these size profiles changed rapidly with distance from the Atachafalya region (Fig. 5a). Surface waters in this region were outside the immediate proximity of the Mississippi Plume. Total peak areas decreased substantially with distance from shore but this disappearance of colloidal material was not uniform across the fractogram profile. The retention times of the initial fractogram peaks shifted to lower values, suggesting that the mid-sized colloidal CDOM was lost preferentially from surface waters. This loss is illustrated by the change in absorbance at 15 min relative to 40 min (Fig. 5a). Samples from the outermost shelf station had a largely bimodal size distribution of peaks with ~ 10 and ~ 30 min retention times. This size profile is similar to those obtained in surface waters near the outer periphery of the plume, although peak heights were notably higher (e.g., Fig. 8a).

Fractogram profiles in offshore “blue” waters of the GOMex during April display remarkable similarities to those measured in surface waters of the Damariscotta River estuary in May (Fig. 6a, b), although the overall magnitude of signal was larger in Damariscotta samples. Both profiles show a low molecular weight colloidal constituent having a retention time of ~ 2 min. Peaks with the same retention times were observed in diatom cultures but were

absent from the riverine and shelf waters in the Atachafalya transect. Both profiles show a second broad and asymmetric peak having a retention time of ~ 20 min. This larger sized colloidal matter was not observed in the fractogram profiles of Damariscotta seawater collected during winter (Fig. 3a).

The vertical profiles at the CTD station (Fig. 1) obtained during low flow conditions showed a strong stratification, with a lower salinity ($S=21$) surface lens (5 m) overlying a higher salinity ($S=28$), well mixed surface layer (Fig. 7). Chlorophyll fluorescence was low in surface waters but increased to a maximum near the base of the photic zone. A nephloid layer, identified by a sharp decrease in water transmissivity (not shown), lay immediately below this chlorophyll feature. Four depths were sampled for FIFFF analyses (Fig. 7). The colloidal CDOM profile measured in water collected at 20 m, near the base of the well-mixed surface layer, showed an initial small peak with a retention time of ~ 2 – 3 min that was followed by a

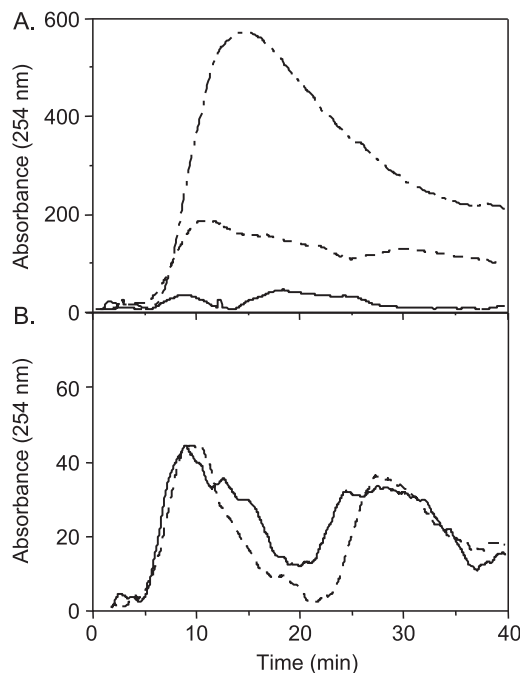


Fig. 5. (A) The change in colloidal size profiles in surface waters along the Atachafalya River transect shown in Fig. 1. The inshore station shown the largest amount of colloidal CDOM which decreased progressively with distance from shore. (B) Fractogram of the outermost station (different from A) showing a replicate analysis.

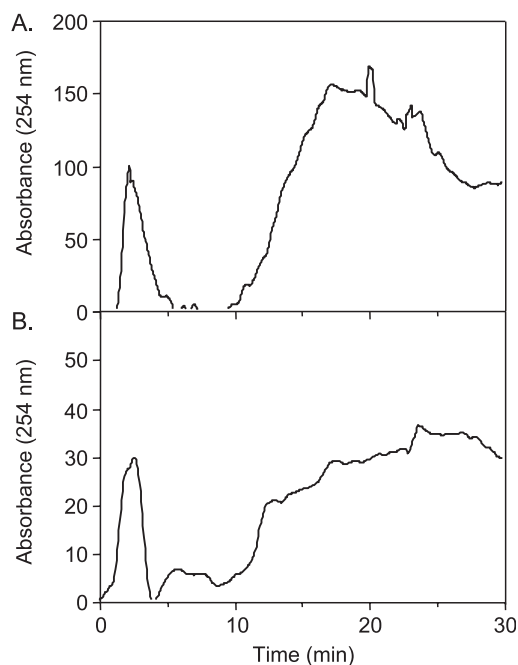


Fig. 6. (A) Colloidal CDOM size profile obtained for saline Damariscotta water during May 2001. (B) A fractogram from offshore “blue” waters of the GOMex measured during the April 2001 cruise showing similar features. Note the different absorbance scales used here.

groupings of larger sized colloidal matter at ~ 9 and ~ 26 min. This profile was strikingly similar to those from shallow (4 m) surface samples taken from the lateral peripheries of the Mississippi Plume (data not shown). There is some indication that even larger sized CDOM material was exiting the channel when the software program ended data collection. The colloidal size profile from within the nephloid layer (56 m) displayed similar features (without the small peak) but with lower abundance (Fig. 8a). The low transmissivity in this bottom layer presumably reflects a resuspension of recently deposited and remineralized organic matter from the sediments.

The surface and bottom water fractogram profiles differed substantially from those obtained from mid-depth waters (Fig. 8b). Waters from within the deep chlorophyll maximum (47 m) and immediately above this feature (37 m) showed a large abundance of mid-sized colloidal matter having a peak retention time of ~ 22 min. In addition, the 37 m sample contained a symmetrical peak having ~ 3 min retention time.

Only traces of this smaller sized material were measured at the chlorophyll maximum (Fig. 8b). Matter with this retention time also was seen in phytoplankton cultures, offshore waters of the GOMex and in the Damariscotta River estuary.

No distinct subsurface chlorophyll maxima were observed in CTD profiles during high flow conditions in April. Instead, the surface mixed layer was dominated by low salinity plume waters, resulting in restricted light penetration (Chen et al., this volume). The characteristic river profile was observed throughout most surface waters, with the amplitude of the profiles decreasing rapidly with distance from the

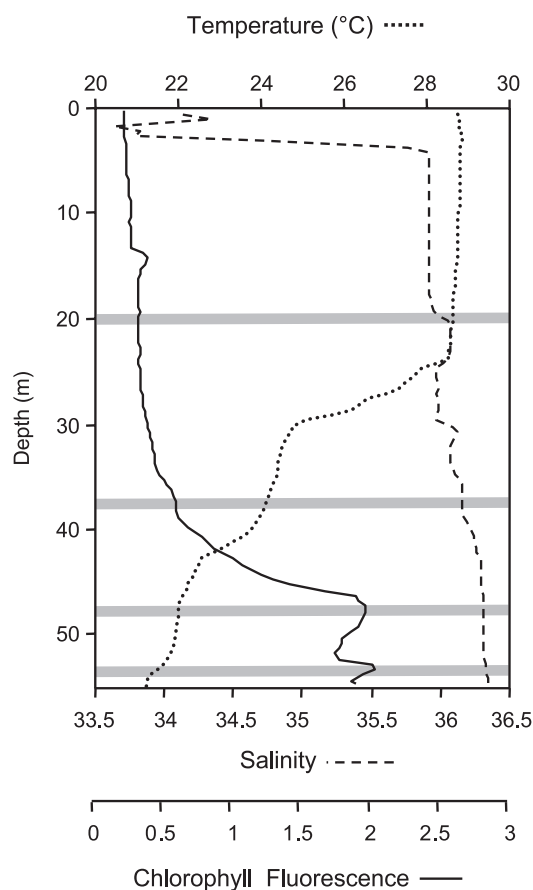


Fig. 7. Profiles of temperature, salinity and chlorophyll fluorescence at CTD Station #3. Samples were collected for FIFFF analyses at the based of the mixed layer (20 m), immediately above the subsurface chlorophyll maximum (37 m), within the chlorophyll maximum (47 m), and from a bottom nephloid layer (56 m), as indicated by the grey bars.

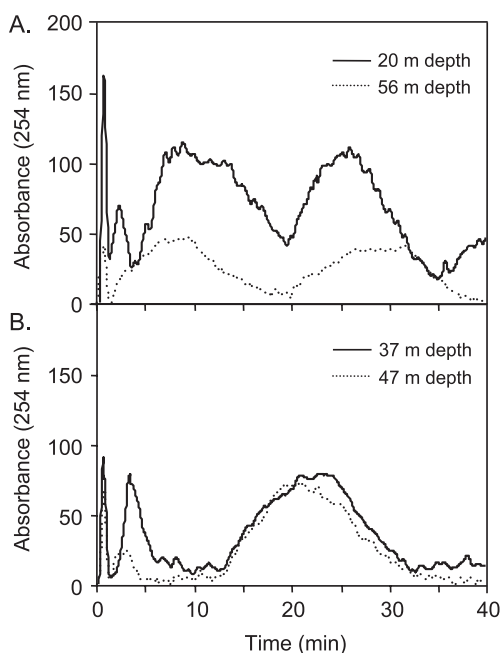


Fig. 8. (A) Fractogram profiles of colloidal size distributions in samples from the base of the mixed layer and the bottom nepheloid layer shown in Fig. 7. (B) Size distributions of colloidal CDOM in samples immediately above and within the subsurface chlorophyll maximum from the same depth profile.

plume and with depth (not shown). No peaks were identified in the high salinity waters underlying the plume, even when the detector sensitivity was adjusted to maximum levels.

The path of the Mississippi Plume was westward of the Birdfoot delta during both cruises, although surface waters were optically dense throughout the region. CDOM characteristics in shallow waters east of the Birdfoot region might then be expected to have been influenced more by interactions with the benthic sediments and in situ biological processes than by direct river inflow. Fractogram profiles in surface waters east of the Birdfoot during June displayed trimodal colloidal size distributions; features that differed substantially from that measured on the western side of the delta (Fig. 9a). No samples were collected east of the Birdfoot during the April cruise. As was observed in low salinity waters of the Mississippi and Atachafalya, the greatest amount of colloidal material appeared in the smaller size classes. However, the mean retention time for initial peaks

were ~ 7 min, which was considerably lower than the ~ 15 min retention time for the peak size class observed in low salinity waters of the Mississippi and Atachafalya rivers (Fig. 9a). The initial colloidal size class had nearly identical retention times as the larger sized colloidal matter produced in diatom cultures (Figs. 4 and 9b). This latter peak was found to increase dramatically upon aging of senescent, nutrient-starved cultures.

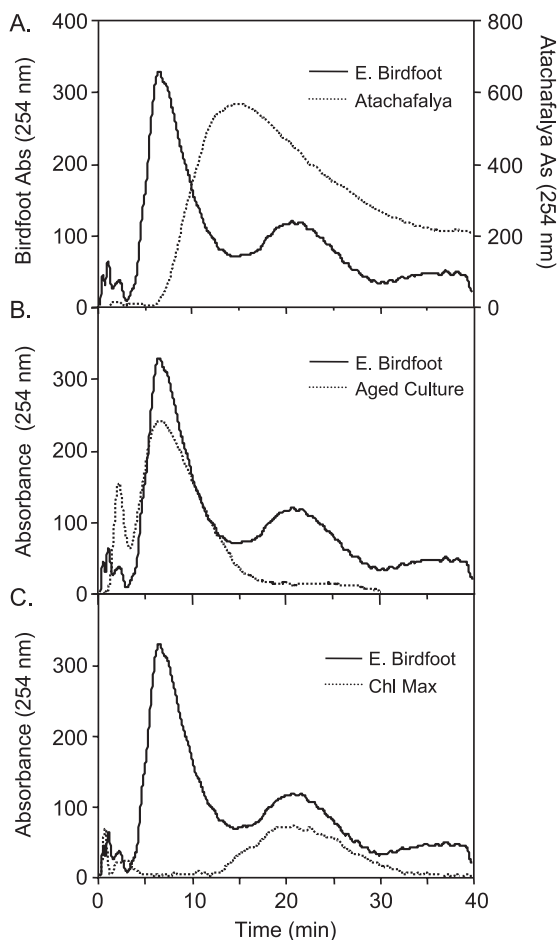


Fig. 9. Three comparison panels of a sample fractogram from east of the Birdfoot region relative to other sites. (A) Representative example of colloidal size distribution of CDOM east of the Birdfoot delta (solid line) in comparison to the characteristic fractogram profile from low salinity riverine waters (dashed line). (B) Comparison of this CDOM (solid line) with colloidal size profiles measured in pennate diatom cultures (dashed line), and (C) comparison of this CDOM (solid line) with fractogram profiles obtained in the deep chlorophyll maximum (dashed line).

The second peak in colloid profiles from east of the Birdfoot showed retention times on the order of ~ 21 min, although the abundance of this material varied at different sites (not shown). While this distinct size class was not prominent in samples west of the Birdfoot, traces of it did in some cases appear to be superimposed on the broad shoulder of the river-type profiles in waters inshore of the plume during the April cruise (data not shown). The retention time of this material matches that of colloidal CDOM measured near and within the subsurface chlorophyll maximum (Figs. 8b and 9c).

4. Discussion

The marine colloidal phase is one of the larger pools of reactive carbon on the earth's surface and its involvement in global biogeochemical processes has attracted increasing interest over the last decade. Complex organic molecules and molecular assemblages comprise the major portion of this colloidal matter yet there is little understanding or consensus about its direct sources, residence times, or even what the immediate fates are for marine colloidal matter. The challenge of explaining the abundance and distribution of CDOM lies upon this imperfect foundation. Assessing the size distribution of the colloidal portion of CDOM, and how this distribution changes across spatial and temporal scales, offers potential for new insights to its behavior and cycling in marine systems. Cross-flow filtration methods provided much of the initial evidence for the importance of colloidal processes in carbon cycling, but the use of single size cutoff filters has practical limitations for determining the size continuum of colloidal CDOM. FIFFF has the benefit of being able to measure a wide spectrum of colloidal sizes (1–1000 nm), but there have been few studies using this method in marine systems. The work here adds to this small database evidence that FIFFF provides a unique look at the temporal and spatial differences of colloidal CDOM constituents in nearshore waters.

CDOM is defined here by absorbance at 254 nm, which is at a higher energy than the near UV range (300–400 nm) where CDOM imparts its influence in the marine environment. This lower wavelength was chosen to optimize analytical sensitivity; CDOM

absorbance increases exponentially with decreasing UV wavelengths (see Blough and Del Vecchio, 2002). This increased sensitivity was necessary for the analysis of offshore and some subsurface waters. A small subset of nearshore (high CDOM) samples were measured at both 254 and 290 nm and yielded very similar fractogram patterns but it is important to remember that changes in absorbance may not exactly reflect fluctuations in absorbance at higher (300–400 nm) wavelengths.

FIFFF theory is well described and predictable size separations can be obtained with model substances (Giddings, 1993) but applying a theoretical diffusivity/size linkage for unknown colloidal particles requires the assumption that they are roughly spherical shaped. That is not to say that the relationship will not hold for natural colloidal particles, but it requires independent verification using coupled FIFFF analytical techniques (e.g., Jiang et al., 2000) before being used in data interpretations. Although many marine colloids have a generally globular architecture in EM preparations (Heissenberger et al., 1994; Leppard et al., 1997; Wells and Goldberg, 1991, 1994), many others have diffuse shapes (Leppard et al., 1997) or needlelike forms (Santschi et al., 1998). There is reason therefore to question whether the behavior of marine colloids in the FIFFF channel will hold to theoretical predictions. As a consequence, "calibration" of FIFFF systems using molecular weight standards seems unlikely at this stage to provide accurate colloid sizes for the presumably diverse assemblage of natural colloidal particles. For this reason, the results here are presented only in terms of colloid retention times, which itself is reproducible with both idealized standards and natural samples. Even so, the work here shows that FIFFF can be useful for identifying differences in the colloidal phase among environments and over time.

A uniform carrier solution of synthetic seawater or ultrafiltered offshore surface waters was used for all of the samples here. Ideally, ultrafiltered sample water would serve as the carrier, so that potential changes in colloid configurations due to ionic strength, pH, etc., would be minimized. However, this approach would require long and inconsistent delays in sample processing, and because sample storage can significantly affect colloid size distributions (S. Floggi, unpublished data), fast sample processing with a

uniform carrier solution was the only practical approach.

An additional likely complication in interpreting FIFFF fractograms was raised by Vaillancourt and Balch (2000). They showed that UV detector responses can have a strong change in sensitivity with particle size, so that particles smaller than 10 nm were under detected with respect to larger ones. Some level of caution therefore is needed when assessing relative abundances of larger and smaller colloidal CDOM based solely upon peak sizes in FIFFF fractograms.

While there is some uncertainty about the fine scale interpretations of fractogram profiles, the system provides clear size separation of idealized standards in a seawater matrix (Fig. 2). Focusing of seawater samples gave a linear increase in peak areas but recoveries of the small-sized matter were ~ 35% (Fig. 3b). These recoveries were higher than reported for seawater by Zanardi-Lamardo et al. (2001) but lower than the optimized method of Lyven et al. (1997) for freshwaters. The likely cause for the lower recoveries here is that the smallest colloidal matter was forced through the 1 kDa membrane under the flow conditions used (Lyven et al., 1997); a factor likely not to be as significant for larger size colloidal substances. Subsequent testing and optimization increased recoveries significantly by decreasing the focus flow rates (J. Boehme and S. Flogii, unpublished data). Even so, recent experience indicates that the recovery of very small-sized colloidal matter may decrease with membrane use, even though the retention times of calibration standards is stable. Combined with detector bias then, the contribution of small-size matter to the colloidal size continuum may well be underrepresented in the profiles shown here. Perhaps more importantly though, there were no indications of a change in size distributions with increasing focus volumes, which suggests that sample focusing created no aggregation artifacts. More extensive testing with a wide range of standards supports this finding (Boehme, unpublished data; Flogii, unpublished data).

Pennate diatoms of the genus *Pseudo-nitzschia* produce two size classes of colloidal CDOM, a narrow size range of low molecular weight matter having ~ 2 min retention times, and a broadly sized milieu of larger molecules having retention times

spanning 5–15 min. The abundance of this larger material increased dramatically upon aging of the senescent cultures but aging had little apparent effect on the amount of smaller colloidal matter (Fig. 4). The release of organic matter by senescent and stressed phytoplankton is well described and is the likely reason for the increased amount of mid-sized colloidal matter. Fractogram peaks with ~ 2 min retention times also were prominent in the Damariscotta river estuary, near the subsurface chlorophyll maximum off the Mississippi River plume, and offshore in the GOMex. In contrast, this small-sized material was absent in profiles from surface waters of the plume and immediately adjacent regions. It is tempting therefore to speculate that this size of material may be a marker for recent in situ production of CDOM.

There were other marked differences in fractogram profiles that separated plume-influenced seawaters from more marine-type end members. The size spectra of colloidal CDOM in offshore waters of the GOMex were remarkably similar to those in saline waters of the Damariscotta river estuary (Fig. 6a, b). The Damariscotta estuary has relatively small freshwater inputs so CDOM signatures here should be influenced more by in situ production, as would be the case in offshore waters. In sharp contrast, the terrestrial-sourced CDOM in surface waters near the Atachafalya and Mississippi rivers had major peaks with retention times of ~ 15 min (Fig. 5a); material that apparently is of a smaller size than the bulk of colloidal CDOM measured in offshore waters. This riverine-type profile was an effective tracer of plume waters during the cruises.

During the June cruise, surface waters east and west of the Birdfoot delta had consistently different fractogram profiles. On the western margin of the delta, where the bulk of freshwater inflows were directed, the size profiles changed with proximity to the plume. The abundance of the mid-sized colloidal matter decreased with proximity to the freshwater inflow, resulting in fractograms containing two roughly equal sized peaks with ~ 10 and ~ 30 min retention times (Figs. 5 and 8a). In other words, the mixing of CDOM-rich riverine waters with coastal waters led to changes in colloid size distributions rather than a simple conservative decrease in the overall abundance of colloidal matter. As noted

above, these profiles still differed from that in offshore waters, indicating that the changes in size distributions with aging of the plume could not be explained by simple mixing. Moreover, subsurface waters near the deep chlorophyll maximum had strikingly different CDOM fractogram profiles from either plume-influenced surface waters or “blue” waters that lay offshore. The colloidal size distribution of this presumably in situ produced CDOM also was substantially different from that in diatom cultures, possibly due to microbial processes or perhaps differences in the phytoplankton assemblage. In contrast, the fractogram profile from the nephroid layer bore a strong resemblance to size profiles in diluted plume waters. This agreement would be consistent with expectations that much of this resuspended organic matter was from a riverine source. Alternatively, these colloid profiles may reflect the degree of bacterial processing of freshly produced colloidal matter. Experiments are underway in our laboratory to examine this possibility.

Surface waters east of the Birdfoot delta, less diluted with riverine waters and possibly more representative of shallow coastal conditions, had trimodal profiles. Peak retention times in several samples from this region were constant but the total and relative peak heights varied somewhat with location (not shown). It seems reasonable to expect that in situ biological processes, both phytoplankton growth and bacterial remineralization, could have had more time to influence CDOM characteristics in these waters compared to west of the Birdfoot, where freshwater inflows were greater. The FIFFF data here would appear to support this contention. Retention times of initial peaks in these profiles are the same as colloidal CDOM produced in diatom cultures (Fig. 9b). While this agreement provides no insight to whether this material comprises similar organic constituents, it might indicate the fresh release of phytoplankton-derived colloidal CDOM. Certainly, it suggests a different source for this material than Mississippi River water. The second peak observed in profiles from east of the Birdfoot had retention times nearly identical to the major peaks measured near the subsurface chlorophyll maximum, which itself presumably resulted from a combination of in situ production and processing. Traces of this mid-sized material also were found in

very shallow waters west of the Birdfoot but inshore of the plume.

These findings show the potential for FIFFF to ascertain the differences in colloidal CDOM size distributions among discrete and mixed nearshore water masses. Fractogram profiles differed substantially across spatial and temporal scales, indicating that the size continuum of colloidal CDOM is fluid. The preliminary indications are that these size profiles may contain signals that help identify the provenance or reactivity of colloidal CDOM; however, these relationships have yet to be fully investigated. Interfacing the FIFFF channel outflow to more sophisticated detectors offers a unique opportunity to begin assessing the specific optical and compositional attributes across the colloidal size spectrum. For example, Hassellöv et al. (1999) linked a similar FIFFF system to an inductively coupled plasma mass spectrometer (ICP-MS) to obtain multi-element measurements across the size spectrum of freshwater colloids. Zanardi-Lamardo et al. (2002) have measured both absorption and fluorescence across the size spectrum of marine CDOM using FIFFF and also have found differences between freshwater and coastal marine waters. The findings here add further evidence that FIFFF is a useful new tool for helping to assess the provenance and composition of CDOM in nearshore waters.

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