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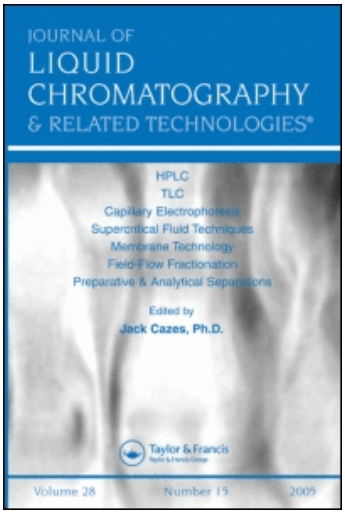

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MONITORING THE BIOLOGICAL AND PHYSICAL REACTIVITY OF DEXTRAN CARBOHYDRATES IN SEAWATER INCUBATIONS USING FLOW FIELD-FLOW FRACTIONATION

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ABSTRACT

In order to better understand the factors that modulate the size and reactivity of high molecular weight organic matter dissolved in seawater, fluorescently labeled dextrans were used as the model compounds whose molecular weight distributions were monitored by flow field-flow fractionation (flow FFF or FlFFF) during incubations in seawater matrices. Two fluorescein isothiocyanate (FITC) labeled dextrans (145k and 2M Da) were incubated in whole seawater (natural microbial population and natural dissolved organic matter (DOM) present), 0.02 μm filtered seawater (all microorganisms removed, but natural DOM

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largely unaltered), and UV-oxidized seawater (no microorganisms or natural DOM present). Flow FFF fractograms of the two FITC-dextrans incubated in UV-oxidized seawater showed no changes, signifying that the dextrans did not undergo any alteration or aggregation. The dextrans incubated in filtered seawater with natural DOM present resulted in fractograms that are shifted to higher retention times, consistent with aggregation of the dextran and natural DOM.

In the whole seawater incubations, the complex changes in the fractograms over time indicated that the dextrans underwent both aggregation with natural DOM and degradation by heterotrophic microorganisms. The latter was confirmed by microscopic examination of the collected fractions after selectively staining the microorganisms with the fluorescent dye 4,6-diamidino-2-phenylindole (DAPI). The FITC dextrans were observed to be "attached" to the bacteria's outer cell membrane where they are subsequently hydrolyzed into small low molecular weight fragments by extracellular enzymes. Some of these degraded dextrans are assimilated by the bacteria and the rest (~70%) are released into the seawater. Approximately half of the released degraded dextrans are of high enough molecular weight to be retained by flow FFF at the conditions employed in these studies. The data presented in this paper illustrate that, when used as both a qualitative and semipreparative tool, flow FFF can provide information on the relationships between natural and model DOM and microorganisms that would be difficult or impossible to obtain using other methods.

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INTRODUCTION

In seawater, dissolved organic matter is found at dilute concentrations that are typically less than one or two parts in a million, yet the large volume of the oceans hold a dissolved organic matter (DOM) pool that is of the same order of magnitude (0.60×10^{18} g carbon) as the atmospheric carbon dioxide pool (0.66×10^{18} g C) and the biomass contained within all terrestrial plants (0.95×10^{18} g C).[1] Due to the dilute nature of DOM in seawater and the fact that it is contained within an electrolyte-laden solution where the mass of salts outweigh organics by a factor of 35,000, exploring the dynamics of DOM has proven to be a challenging task. Adding further to the intrigue is the observation that most marine DOM is not readily available for biological consumption; the average "age" of DOM in the deep ocean (determined using ^{14}C) is ~6000

years,[2] long enough for DOM to cycle through the oceans several times prior to being degraded. Since the source of this dilute non-reactive DOM is ultimately the planktonic community at the ocean surface, links between the production, consumption, and preservation of DOM have been investigated for the past 70 years.[3]

The production and consumption of specific components of DOM (e.g. carbohydrates, proteins and lipids) by autotrophic and heterotrophic organisms are the primary forces that drive the oceanic DOM cycle. These processes can be monitored using labeled precursors and substrates.[4] For instance, the production and destruction rates of amino acids[5-7] and proteins[8,9] by organisms in seawater are now well established. However, physical and chemical (i.e., non-biological) processes may also affect the DOM cycle. Integrating studies of biological and physico-chemical influences on DOM have been difficult. Two central aspects of DOM cycling that have been especially difficult to study are the production of DOM intermediate in either size or bioavailability, and the abiotic (e.g. not biologically mediated) interactions of DOM with minerals or itself. These are important aspects of DOM cycling because they may provide insights about how DOM can become non-reactive, how it affects the light-scattering and radiative transferring properties of seawater (e.g. remote sensing),[10] and the role DOM may play in transferring carbon from the Earth's surface to the ocean's interior (where it would be out of contact with the atmosphere for hundreds to thousands of years). This last topic is currently receiving strong interest because the oceans are responding to the anthropogenic pumping of CO_2 into the atmosphere by increasing their carbon content (both inorganic and organic). One mechanism of transferring carbon from the ocean surface to depth is in the form of sinking particles formed by the aggregation of DOM to form particles of sufficient mass for sinking.[11,12]

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Attempts to determine the molecular weight or size spectrum of DOM using size exclusion chromatography have generally been unsatisfactory[13] due to the high shear stresses that DOM is exposed to during separation and adsorption problems with the support matrix. Studies using ultrafiltration have generally limited separations to only a few broad size classes[14]. For example, Guo et al.[15] separated DOM into three fractions (<1000 Da, 1000-10,000 Da and >10,000 Da) and observed that 45% of the total organic carbon in the Gulf of Mexico was composed of DOM >1,000 Da in size. They and others[12,16] have observed that DOM exhibits spatial and temporal changes in quantity and molecular weight distribution. The causes of these changes (e.g., aggregation, degradation of specific components, sinking, etc.) could not be easily investigated because of the lack of a suitable technique capable of scanning the entire DOM pool and of isolating specific components. Amon and Benner[16] were able to determine that a carbohydrate-rich component of the >1000 Da

ultrafiltered DOM fraction was remineralized by bacteria more quickly than smaller material. However, isolation of, and discrimination between, the reactive and non-reactive DOM (>1000 Da) components remained elusive.

Field-flow fractionation (FFF) is a family of techniques that have not yet found routine application in marine systems. These separation techniques are similar in operation to chromatography. However, compounds are retained and separated in thin rectangular channels by interaction with an external field rather than an internal stationary phase. Separation occurs by differential retention of solute within a laminar flow stream bounded by thin parallel plates. With respect to traditional chromatographic techniques, advantages of applying an external force include elimination of adverse solute-stationary phase interactions and easily increased resolving power.[17] The theory behind flow FFF has been extensively presented elsewhere[17-23] and will not be discussed here.

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MATERIALS AND METHODS

Flow FFF

The flowrate-programmable FFF system used in this work has previously been described in detail by Ratanathanawongs and Giddings.[24] A strong field (high cross flowrate) is applied during the initial portions of the run and decreased over time. Programming the field strength is analogous to programming solvent strength in HPLC. The advantage of programming over isocratic conditions is most apparent when dealing with broad particle size distributions; particles that elute at retention time extremes can be adequately and quickly resolved.[25,26] In our dextran experiments, the cross flowrate was held constant for 20 seconds at 2.4 mL/min and then decreased to 0.2 mL/min according to the power decay function.[26] The channel flowrate was held constant at 2.5 mL/min.

The flow FFF channel had dimensions of 0.011 cm thickness, 2.0 cm breadth and a tip-to-tip length of 27.2 cm. The membrane had a nominal cutoff of 10,000 Da and was composed of regenerated cellulose (YM-10; Amicon Corp., Beverly, Massachussetts). After flow FFF separation, the channel effluent was sent through a UV detector (Applied Biosciences, Foster City, California) and a fluorometer (St. John and Associates, Inc., Beltsville, Maryland). After detection, fractions were collected using an FC-80K Microfractionator (Gilson Medical Electronics, Middleton, Wisconsin).

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Carrier Liquid

The carrier liquid for both the channel and crossflow was UV-oxidized seawater previously collected from 50 meters depth in central Puget Sound (see Experimental). The water was filtered through a nominal 0.7 μm glass fiber filter (GF/F, Gelman Scientific) to remove particulates and then UV oxidized for 6 hours. After oxidation, the water was placed in a sealed glass container for 1 week prior to experimental work in order to eliminate ozone and free radicals produced during UV oxidation. This procedure lowered the organic matter concentration of the water from 1.75 to ~0.15 mg/L organic carbon. Investigation by other researchers[27] indicated that much of this material is of very low molecular weight (<1,000 Da). Use of "organic-free" seawater allowed flow FFF studies of model compounds within a natural matrix. This is not a viable alternative for size exclusion chromatography, where mobile phases must be carefully controlled and high ionic strength solutions (>0.5M, such as seawater) cannot be used. For channel evaluation and standardization with polystyrene (PS) latex beads, surfactant- (FL-70; Fisher Scientific, Fair Lawn, New Jersey) containing seawater carrier fluid was used. For all the analyses involving dextran, no surfactant was added.

Model DOM

There are currently no acceptable analytical methods available to measure the low quantities of DOM found in natural systems after fractionation. The predominant technique, high temperature combustion to CO_2 followed by infrared detection,[28] is sensitive down to approximately 0.05 mg/L. A flow FFF separation of DOM would produce fractions that are at concentrations approximately equal to this or lower, making detection extremely difficult. For this reason, a model compound that mimics many of the characteristics of naturally occurring DOM was used. Dextran, a globular carbohydrate, was chosen because it is thought to be somewhat representative of the dissolved organic matter present in seawater,[27,29,30] of which >50% is complex carbohydrate. Additionally, these globular dextrans do not appreciably adsorb to regenerated cellulose membranes in seawater.[30,31] Benner et al.[27] have observed only low levels (<10%) of adsorption of natural DOM to regenerated cellulose membrane filters. The model DOMs used in the study were 145,000 and 2,000,000 Dalton globular dextrans (145k and 2M Da; Sigma Chemical Co., St. Louis, Missouri) that had been labeled along the chain with fluorescein isothiocyanate (FITC). A nonlabeled 9.4M Da dextran (Sigma Chemical Co., St. Louis, Missouri) and 20 nm, 54 nm, and 107 nm polystyrene latex standards (Duke Scientific, Palo Alto, California) were also used for evaluating and standardizing the channel.

EXPERIMENTAL

Seawater was collected from the south-west expansion of Useless Bay in central Puget Sound, Washington, USA. Seawater used as the carrier liquid was collected from a depth of 50 m using a pre-cleaned 30 L Go-Flo™ bottle approximately two weeks prior to sample collection. Seawater samples for the reactivity experiments were similarly collected from a depth of 10 m, but were then filtered through a 63 μm mesh screen to remove large organisms and particles. The primary population of organisms in the water were heterotrophic bacteria (~0.5 μm average size) and mixotrophic flagellates (~3-10 μm average size). The water was transfered to a clean 20 L polystyrene carboy, stored in the dark at *in situ* temperature (15°C) and immediately transported to the laboratory in Utah. All experimental work was started within 36 hours of sample collection.

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Two 1 L cleaned polycarbonate bottles were filled with 63 μm filtered seawater (hereafter referred to as "whole" seawater because neither the heterotrophic microoganisms nor the DOM pools were disturbed), two bottles received 0.02 μm filtered water (all microorganisms removed, but DOM largely unaltered), and two bottles received UV-oxidized water (no microorganisms or DOM present). Three of the bottles (one each of the whole, filtered and oxidized water) received additions of the 2M Da fluorescently labeled dextran, and three bottles received additions of 145k Da fluorescently labeled dextran (Table 1). Additions were at a concentration of 0.01 mg/L, or approximately 1/175th the dissolved organic carbon of the water (as measured by a Shimadzu TOC5000 high-temperature catalytic oxidation analyzer). The bottles were then incubated in the dark at *in situ* temperature and subsamples were drawn after 0, 2, 4, 8, 12 and 23 h. Surfactants were not added to the carrier fluid of the flow FFF system during analysis of these samples so that artificially induced aggregation or disaggregation would not be an issue.

The experimental design allowed several possible fates for the added dextran. In the UV-oxidized seawater, all the organisms and >90% of the naturally occurring DOM was removed. Model DOM added to the UV-oxidized seawater could conceivably aggregate with itself, interact with dissolved metals and minerals in the water, or undergo no interactions. In the 0.02 μm filtered seawater, natural DOM was present but no microorganisms. This adds the potential that the model DOM could interact with the naturally occurring DOM to form abiotically produced aggregates. In the whole seawater, the two dominant organisms, mixotrophic flagellates (microorganisms that can act as either autotrophs or heterotrophs depending on conditions) and heterotrophic bacteria, were present at approximate densities of 10^4 cells/mL and 10^6 cells/mL, respectively. Flagellates are known predators

of heterotrophic bacteria, and have also been shown to consume DOM of very high molecular weight.[29] Heterotrophic bacteria are the primary consumers of DOM. Model DOM added to whole seawater could undergo any of the previous interactions, plus be consumed by either the bacteria or the flagellates. Because bacteria cannot assimilate large molecules across their cell membranes, they degrade macromolecules extracellularly and import the resulting smaller components into the cell. Often, extracellular enzymatic activity is membrane-associated, that is, the bacteria attach the substrate to their cell wall for degradation rather than releasing enzymes into the seawater.[33] It is an open question as to whether bacteria release a large portion of the hydrolyzed substrate into the water during hydrolysis and before uptake.[9] Degradation of the dextran by flagellates should be easily observed as fluroescence in the phagocysts of the flagellates.

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Fluorescence Microscopy

In addition to measuring the molecular weight distribution via flow FFF, fractions of the column effluent were collected for microscopic analysis. Magnification used on the fluorescence microscope (X1001; Zeiss Inc., Hamburg) varied between 100-5000×. Fractions were analyzed for heterotrophic microorganism abundances after staining with 4,6-diamidino-2-phenyl indole (DAPI). DAPI and FITC fluoresce in different regions,[29] thereby facilitating discrimination between the dextran and the microorganisms. The percentage of fluorescence associated with the bacterial and flagellate size classes was measured by filtering material collected from the void peak through 1.0 and 0.2 μm filters and measuring changes in fluorescence (Table 2). Non-associated fluorescence was defined as the fluorescence in the 0.2 μm filtrate. Flagellate-associated fluorescence was defined as the total fluorescence minus the fluorescence in the 1 μm filtrate, and bacteria-associated fluorescence was defined as 1 μm filtrate fluorescence minus non-associated fluorescence. No attempt was made to correlate fluorescence of the fractions to the total fluorescence added to the incubations.

RESULTS AND DISCUSSION

An important aspect of this study was to identify suitable particle standards that can be used to evaluate flow FFF channel performance while using seawater as a carrier liquid. Initial experiments with polystyrene (PS) latex standards showed complete adsorption to the membrane wall (no elution). This problem was resolved by adding 0.1% (v/v) of FL-70 surfactant to the seawater carrier liquid. The resulting separation is shown in Figure 1a.

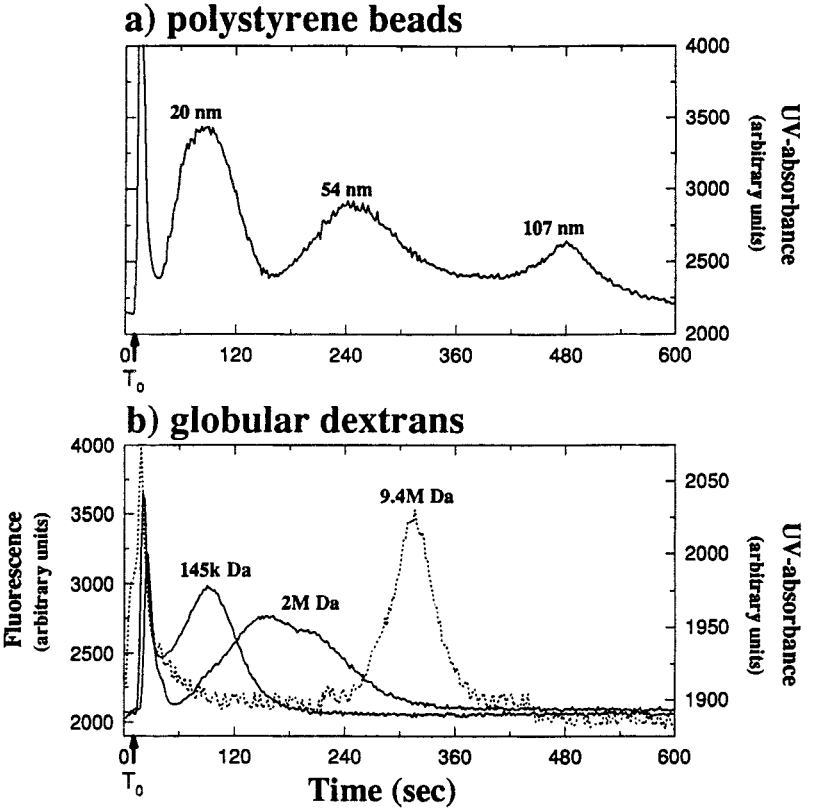

Figure 1. A) Separation of polystyrene latex standards in a flow FFF system using seawater as a carrier fluid with surfactant (FL-70) added at 0.1% (v/v). The channel and cross flowrates were 2.6 and 3.0 mL/min, respectively. B) Separation of dextran standards in a seawater carrier without added surfactant. The channel flowrate was held constant at 2.5 mL/min and the cross flowrate was programmed from 2.4 mL/min to 0.2 mL/min with a 20 s delay time using a power decay function with $p=2^{26}$.

Without surfactant, the high ionic strength of seawater collapses the electric double layer and thus promotes sorption of the PS beads to the regenerated cellulose membrane. The surfactant prevents adsorption through a steric stabilization mechanism.[34]

Retention times for the 20, 54, and 107 nm particles are 0, 9 and 17% longer than predicted by theory, reflecting the increasing effect of particle-membrane interactions with increasing proximity to the membrane.

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Though the peaks are broader than usually observed in flow FFF runs using lower ionic strength carriers, the particles do not appear to be aggregating with each other.

In theory, since normal mode FFF retention is calculated from first principles,[17,18] it is possible to obtain size or molecular weight information directly from the retention times provided the flowrate, field strength, and channel void volume are known. However, in this case where particle-membrane interactions cause deviations from theoretical retentions, the PS standard run is only used to provide an approximate relationship between retention time and particle diameter and to provide a baseline run for qualitative comparisons with fractograms obtained at a later date.

It is important to note that, while the PS standards required addition of surfactant to the seawater carrier liquid, the presence of surfactant in these runs does not mitigate the use of measured retention times as size or molecular weight markers.

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Fractograms of 145k, 2M and 9.4M Da dextrans are superimposed in Figure 1b. By programming the field strength, the separation times are kept below ten minutes. The order of elution of the dextran is as expected for normal mode operation of flow FFF, with the smaller (higher diffusion coefficient) components eluting first. Also, as expected for polydisperse globular dextrans, the three peaks are very broad (Figure 1b). Repeat injections of the standards throughout the experimental period illustrated that the elution profile was stable (data not shown). The amount of dextran recovered after a separation was calculated by ratioing the peak area of a normal run to that of a channel bypass run. The latter involves rerouting the flow path to directly connect the injector and the detector.

Assuming that the peak area resulting from the channel bypass run corresponded to 100% recovery of dextran, the recovery based on the peak area of the normal run was 85 ± 15%. The small interaction between the dextran and the regenerated cellulose membrane in the flow channel is similar to that reported by other groups.[27,30]

The generalized fate of the 145k and 2M Da dextrans under the three incubation schemes is outlined in Table 1. Dextran added to UV-oxidized seawater (no microorganisms or DOM present) showed no changes in fluorescene intensity or distribution (Figures 2a and 3a). This indicates that the dextran was not aggregating with itself during the experimental period and that the flow FFF system was stable and reproducible throughout this study. Slight differences in the spectra were attributed to the lack of an autoinjector or

Table 1

Experimental Matrices Used to Evaluate the Effects of Aggregation and Microbial Degradation of Fluorescent Dextrans in Seawater Incubations[a]

	Medium		
Dextran	**UV Oxidized sH_2O[b]**	**0.02 μm Filtered sH_2O[c]**	**Whole sH_2O[d]**
145k Da	aggregation: **no** degradation: **no**	aggregation: **yes** degradation: **no**	aggregation: **yes** degradation: **yes**
2M Da	aggregation: **no** degradation: **no**	aggregation: **yes** degradation: **no**	aggregation: **yes** degradation: **yes**

[a] sH_2O = seawater; [b] no DOM or microorganisms present; [c] DOM present but no microorganisms; [d] DOM and microorganisms present.

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sample loop, and likely reflect our ability to repeatedly inject the same amount of sample. Although it is theoretically possible for the dextran to interact with metal ions and very fine mineral components of the seawater, these interactions are not measurable with the instrumentation used in this work.

Dextran added to the 0.02 μm filtered seawater (no microorganisms, but natural DOM largely intact) did not undergo significant change during the first twelve hours of incubation (Figures 2b and 3b). After 12 hours the distribution shifts and broadens toward higher molecular weights. Since the seawater was filtered, the shift to higher molecular weights may reflect interaction (aggregation) of the dextran with other organic matter or with other small colloidal materials present in the seawater. Such interaction has been hypothesized as a mechanism that might alter the lability of DOM in seawater,[35] and could ultimately lead to the formation of large organic aggregates (marine snow).[11]

Additions of dextran to whole seawater showed the most dynamic patterns (Figures 2c and 3c). For both the 145k and 2M Da dextrans, decreases in total fluorescence were observed over the 6-23 h period. The 145k Da dextran showed the greatest changes in molecular weight (Figure 2c). In the first 4 h, a shift to higher molecular weight (as indicated by longer retention times) was observed, but by 6 h the trend had reversed and the molecular weight of the dextran (or any dextran-DOM complex) appeared to be decreasing. By 23 h, the fluorescence intensity of the 145k Da dextran had decreased by half

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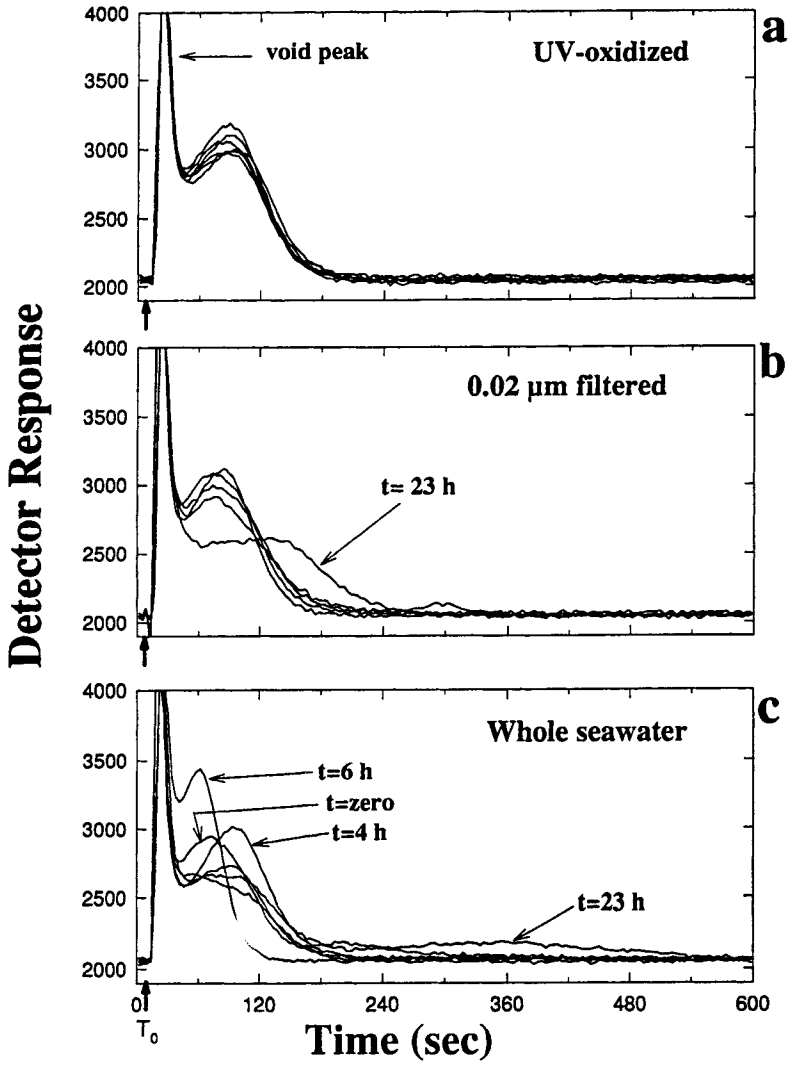

Figure 2. A) Incubation of 145k Da dextran in UV-oxidized seawater. B) Incubation in 0.02 µm filtered seawater. C) Incubation in whole seawater. FFF experimental conditions as described in Figure 1B.

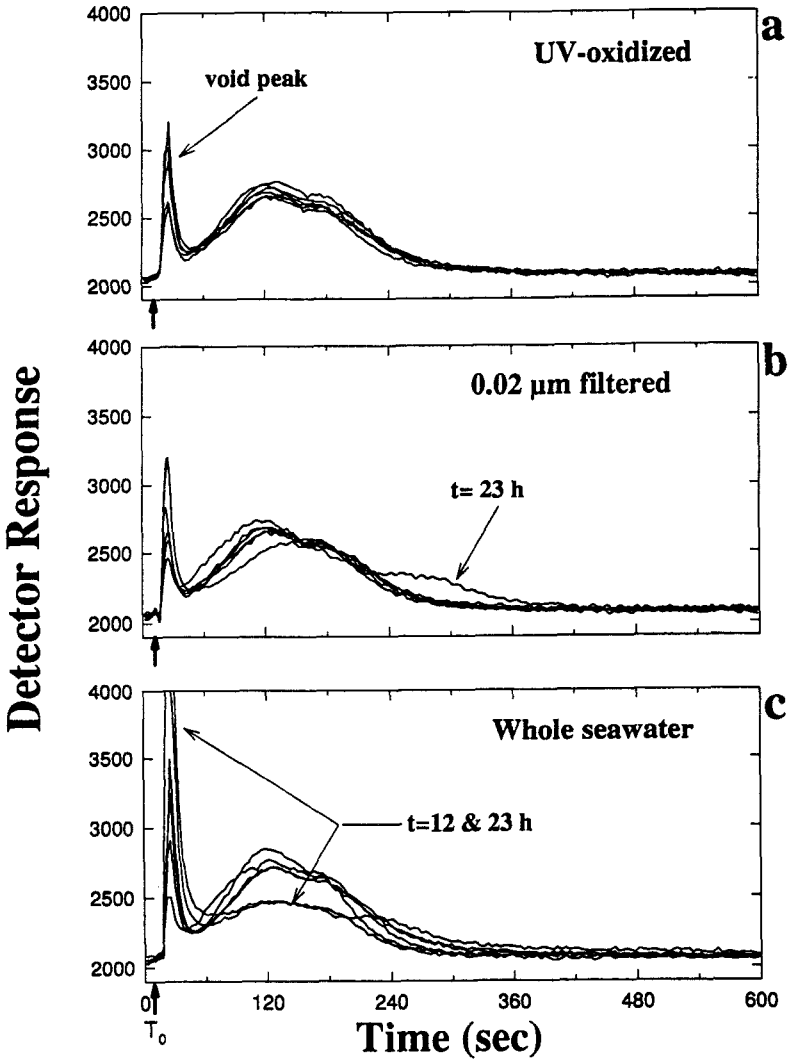

Figure 3. A) Incubation of 2M Da dextran in UV-oxidized seawater. B) Incubation in 0.02 µm filtered seawater. C) Incubation in whole seawater. FFF experimental conditions as described in Figure 1B.

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compared to that at time-zero, and a broad peak was observed in the region of higher molecular weights (Figure 3c). This broad peak could be due to aggregation with DOM, such as the incorporation of the labeled dextran into high molecular weight carbohydrate mucus,[11] or to the release of high molecular weight digestive fragments during flagellate grazing.[36] The 2M Da dextran showed a consistent decrease in fluorescence intensity as a function of incubation time (Figure 3c). This decrease in the 2M Da dextran was accompanied by an increase in the fluorescence of the void peak (Figure 3c).

Thus far, the normal mode of flow FFF has been discussed and demonstrated. A second mode of operation, called steric FFF, becomes important when the particles being separated are large relative to their diffusion distance from the accumulation wall, or when the experimental conditions employed cause particles to reside directly on the membrane surface.[20,22] In these cases, larger particles will protrude further into the center of the channel (higher in the flow stream) than the smaller particles. Consequently, the larger particles will elute earlier than smaller particles, opposite to that of normal mode. The transition from normal to steric mode, or vice versa, occurs in the size range of ~0.2-3 μm,[20,37,38] and is determined by the field strength, channel dimensions, flowrate, carrier liquid viscosity and particle density.[20,37-39] FFF separation of samples that span this range is complicated because both normal and steric modes are in operation. For a polydisperse sample possessing a continuous range of particle sizes, coelution of particles of two different sizes will occur. A fractionation technique would be used to separate the particles into two size classes prior to FFF analysis. In the case of a polydisperse sample with two distinct populations it is possible to select experimental conditions that allow the two different populations to separate in the two different modes over two different time periods. In the situation studied here, the two populations are the smaller dextrans and the larger microorganisms. The operating conditions we employed caused bacteria and microflagellates to be rapidly swept through the column and eluted with the void peak (i.e., no retention) and the dextrans to be separated in the normal mode.

The sharp void peak at the beginning of each run contains both very large (bacteria and flagellates, etc.) and very small species. Consequently, changes in the fluorescent intensity of the void peak may reflect association of the dextran with bacteria, ingestion of dextran by flagellates, or degradation of the dextran to small FITC-labeled compounds of a mass between ~10,000 Da and ~5 nm (the channel membrane molecular weight cutoff and the approximate lower limit of separation, respectively). Two approaches were used to distinguish between these three possibilities. First, a portion of the void peak was examined microscopically after filtration and staining with DAPI (and using both blue and UV excitation).[30] At time-zero, we observed no association

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of FITC fluorescence with either the bacteria or flagellates. After longer incubation times, FITC fluorescence was observed to be associated with both the bacterial and flagellate populations. The amount of FITC-fluorescence associated with both microorganisms was quantified as the proportion of cells with FITC fluorescence over the total number of cells in the sample. This is a rough measure of the percentage of the populations that assimilated the dextrans (Table 2; %cells). It should be noted that FITC fluorescence associated with flagellates could result either directly via ingestion of labeled dextran, or indirectly via ingestion of bacteria that had previously assimilated labeled dextran. Significant association of the FITC with microorganisms was only observed after 12 hours (Table 2). A higher proportion of the flagellate population (~16%) accumulated fluorescence when incubated with the 2M Da dextran than with the 145k Da dextran (<10%). The proportion of the bacterial population that assimilated fluorescence was also slightly higher when incubated with the larger dextran (Table 2).

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A differential filtration method was used to determine the cause of increased fluorescence in the void peak over time. The percentage of fluorescence associated with bacteria and flagellates was measured by filtering material void peak material through 1.0 and 0.2 μm filters and measuring changes in fluorescence (Table 2). Initially, nearly all the fluorescence was in the <0.2 μm size class (i.e. not associated with bacteria or flagellates), but after 12 hours, nearly half the fluorescence signal was associated with either the bacterial or flagellate size classes. Heterotrophic flagellates also had measurable fluorescence associated with them as ingested particles, although at levels below that observed by Tranvik et al.[30] Similar to the result of direct observation, the association of 145k Da dextran with flagellates was much weaker than that of the 2M Da dextran (Table 2). In the 2M Da incubation, the percentage of non-associated fluorescence (fluorescence in the <0.2 μm filtrate) increased between 12 and 23 hours. This may be due to an increase in the degradation and release of small fluorescent materials relative to the rate of association or ingestion of the larger dextran. Taken as a whole, there was a good correlation between the change in the molecular weight and quantity of fluorescently labeled dextran in solution and the increase of bacterial- and flagellate-associated dextran in the void peak.

The incubations in the 0.2 μm filtered water illustrated significant interaction of the dextran with other organic matter in the same nominal molecular weight range (100K - 10M Da). This is one of the first pieces of direct evidence that dissolved organic matter may aggregate and form large conglomerates in seawater without the assistance of bubbling or shaking of the water, and on the same time scale as microbial degradation occurs. Whether these organic-organic interactions are also typical of natural organic matter

Table 2

FITC Fluorescence Associated With Bacteria or Flagellates During Whole Seawater Incubations of 145k Da and 2M Da Labeled Dextrans[a]

Time (h)	Flagellates		Bacteria		Non-Associated
	% Fluor	% Cells	% Fluor	%Cells	% Fluor
			145k Da Dextran		
0	1	0	1	0	97
2	2	0	8	<10	91
4	3	0	14	<10	81
8	3	0	28	<10	74
12	5	<10	44	24	49
23	5	<10	74	31	20
			2M Da Dextran		
0	1	0	2	0	101
2	2	0	6	<10	90
4	2	0	14	17	85
8	5	0	23	21	71
12	8	13	65	43	26
23	13	16	50	39	38

[a] Data are expressed as the percent of total fluorescence intensity (%Fluor) in the void peak (standard error of ±3%). Non-associated fluorescence is defined as the % of total fluorescence in the 0.2 μm filtrate. Heterotrophic flagellate-associated fluorescence is the total fluorescence minus the fluorescence in the 1 μm filtrate, and bacteria-associated fluorescence is the 1 μm filtrate fluorescence minus non-associated fluorescence. Microscopy-based data are also tabulated for the percent of total cells with associated FITC fluorescence (%cells).

remains to be investigated. The incubations with whole seawater illustrate that the dominant force acting upon the dextrans was degradation by heterotrophic microorganisms. The observation that the bacteria efficiently assimilate the extracellular breakdown products of the dextran (they cannot transport large molecules directly across their cell membranes) and that only small quantities of dextran of intermediate molecular weight accumulate in the medium is

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consistent with observations made using radiolabeled proteins[9] and illustrates the efficiency of the bacterial degradation scheme. Finally, the assimilation of the dextran by heterotrophic flagellates is consistent with and extends the findings of Sherr[29] and Tranvik et al.[30]

This feasibility study illustrates that the processes acting upon dissolved organic substances in seawater can be fruitfully examined using flow FFF analysis coupled with other experimental and analytical techniques. Our results confirm previous studies of model DOM behavior in marine systems and for the first time include small relative changes in molecular weight that were not observable in earlier studies. Although this study leaves many unanswered questions about the factors that promote or limit changes in natural and model DOM, it is the first to show that these changes can be observed semi-quantitatively over a broad molecular weight range using natural seawater as a carrier fluid. Flow FFF analysis can potentially provide a great deal of information on the relationships between dissolved organic matter and microorganisms in seawater that would be difficult or impossible to obtain with other methods because seawater can be used as a carrier and because flow FFF can potentially fractionate both DOM and heterotrophic microorganisms in a single analysis.

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