

Running Title: Marine Colloids and Trace Metals

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I. Introduction

A major fraction of organic carbon on the earth's surface exists as dissolved substances in seawater. These materials for the most part constitute the waste products of a dynamic cycling between carbon fixation and consumption in the oceans. They include reactive substances categorized broadly as proteins, polysaccharides and lipids, as well as biologically resistant heteropolycondensations (humic matter) and degradation products of these primary constituents. Overall, these dissolved substances harbour a myriad of charged sites that can bind trace elements. Because metal availability to microorganisms is in most cases proportional to the free ion concentrations of the metal, organic complexation decreases the immediate availability of nutrient metals. If retained in surface waters, these organic complexes serve to buffer metal ion activities at levels far below the total dissolved metal concentrations, and likely also slow the export of essential metals from the photic zone. The cycling and availability of metals to microorganisms therefore is intricately involved with the marine carbon cycle.

Metal availability strongly influences the abundance and types of phytoplankton that can flourish in a given location. Low metal availability favours picoplanktonic (0.2-2.0 μm) algal species over their larger counterparts because higher surface area:volume ratios and narrower diffusional layer thickness make small cells better at acquiring metals. In contrast, higher metal availability is required to sustain rapid growth of nanoplanktonic species (2.0-20 μm), particularly in the case of iron (e.g. Coale and associates, 1996; Hutchins and Bruland, 1998; Sunda and Huntsman, 1995a; Sunda *et al.*, 1991). The balance struck between pico- and nanoplanktonic algal production strongly affects the export of carbon and nutrients (including metals) from surface waters to the deep. A proportional increase in nitrate export over the long term can raise surface water alkalinity, resulting in lower $p\text{CO}_2$ in surface

waters, increased CO₂ exchange with the atmosphere and related consequences on global climate (Sigman and Boyle, 2000). Assessing the effect of dissolved carbon substances on the chemical speciation and transport of metals from surface waters is a key issue for understanding the global carbon cycle.

Dissolved organic carbon (DOC) concentrations in coastal and open ocean waters exceed that of bioactive trace metals by several orders of magnitude. But most of the metal-reactive functional sites on these organic constituents interact weakly with trace metals in seawater, and instead are occupied by major seawater ions (e.g., Mg²⁺, Ca²⁺). Organic ligands having high conditional stability constants for metal complexation comprise only a very small fraction of marine DOC. Nonetheless, these strong, effectively metal-specific organic ligands dominate the chemical speciation of most bioactive metals (e.g. Bruland, 1989; Coale and Bruland, 1988; Donat *et al.*, 1994; Gledhill and van den Berg, 1994; Moffett and Brand, 1995; Moffett *et al.*, 1990; Rue and Bruland, 1995; Wu and Luther, 1995). Some phytoplankton and heterotrophic bacteria release metal-complexing ligand molecules in response to metal stress, and grazing also is expected to release metabolites that contribute to metal complexation in seawater. It is anticipated that a tight bidirectional interaction between metal availability and ligand input likely shapes the magnitude and character of phytoplankton production in many coastal and offshore waters.

Up until the early 1990's, organic complexation was expected to help retain bioactive metals in surface waters by minimizing the adsorptive loss of dissolved metal ions to sinking particulates. However, there has been a broad recognition over the last decade that colloidal organic matter is extremely abundant in coastal and oceanic waters (e.g. Koike *et al.*, 1990; Leppard *et al.*, 1997; Santschi *et al.*, 1998; Wells and Goldberg, 1991; Wells and Goldberg, 1994). Earlier estuarine studies had demonstrated the importance of colloid aggregation for removing metals and carbon from estuarine waters (e.g. Sholkovitz *et al.*, 1978). But this

removal was driven by salt-induced destabilization of mainly lacustrine colloids, and it was not certain at the time whether colloidal cycling would be as important for marine-derived colloidal matter produced in shelf and offshore marine environments.

The immediate fate of colloidal organic carbon phases in ocean waters is a matter of continuing debate. There is direct and indirect evidence that aggregation of colloidal organic matter (and associated metals) can at times be rapid. But colloidal organic phases also are labile with respect to microbial degradation. Aggregations of these organic phases may not only provide a major fuel for sustaining microbial production but might also serve as a matrix locking this fuel and microbes together in tight, interactive neighbourhoods (Azam, 1998). While the relative importance of these opposing processes in coastal and offshore surface waters remains controversial, the outcome of the debate is significant because it bears upon how ocean systems might respond to perturbations in nutrient inputs associated with climate change.

The central goal of this chapter is to summarize how our understanding of colloidal carbon:metal interactions in seawater has evolved over the last decade. During this period the traditional estuarine studies of colloid behaviour have been expanded and extended to coastal and open ocean systems. To help set the stage, the definitions of marine colloidal matter and discussion of size separation methods are covered first. A summary is given next on which metals associate with marine colloids in nearshore and offshore waters and to what degree. Evidence is then presented on the chemical nature of the metal:colloid association along with the sources of these materials. The chapter finishes with a brief discussion of how the marine colloidal phase may influence the biological availability of metals. The key issue that eludes consensus at this time is whether the metal-reactive colloidal matrix is moving towards larger particles via aggregation, or if its immediate fate instead is microbial degradation, leading to the dissemination of metals into the soluble phase of seawaters.

II. Definition of Marine Colloids

One of the more notable issues in the study of marine colloids over the past decade has been the lack of agreement over what constitutes the marine colloidal phase. Thomas Graham (1861) coined the term “colloid” (meaning glue-like) to describe systems that displayed slow rates of diffusion through porous membranes (typical of glue solutions). Today, it is known that systems exhibiting “colloidal characteristics” contain finely divided matter generally sized between ~ 1-1000 nm in diameter (Vold and Vold, 1983), although these operational boundaries are not rigid. The lower size limit is the smallest dimension at which the internal environment of a substance theoretically can become markedly different from that of the surrounding milieu; hence an interface is established. The existence of an interface introduces the opportunity for other soluble or colloidal chemicals to interact both specifically and non-specifically at the surface (adsorption), or, depending on the chemical characteristics, partition across the interface into the colloid matrix (absorption). This surface must comprise enough point charges from ionized functional groups to create an electrostatic field sufficient to stabilize the colloidal substance with respect to spontaneous aggregation with other colloidal or (> 1 μm) particulate matter. The upper size boundary for colloidal matter lies at the juncture where gravity becomes the dominant force acting upon the particle. In essence then, a traditional view of colloids is that they are particles (not dissolved solutes) that do not sink unless they become entangled with other colloidal particles or sorb to sinking particulates.

A 1 nm spherical diameter roughly equates with macromolecules of ~1000 nominal molecular weight (or 1 kDa); a size equivalent to fulvic acids and marine porewater organic macromolecules (Chin and Gschwend, 1992). Organic biogeochemists traditionally categorize these and larger organic macromolecules as high molecular weight matter, and characterize it in terms of elemental and molecular composition rather than its bulk interface

characteristics. As a consequence, studies of the cycling of high molecular weight matter focus largely on specific molecular or biologically-mediated chemical transformations. Casting the veil of "colloid" over macromolecular constituents does not diminish the importance of these processes but simply adds to them a range of non-specific surface interactions that also might influence their behaviour. In fact, most of the current dispute over the immediate fate of colloidal/high molecular weight organic matter lies in whether its short-term behaviour is dominated by specific, biologically-mediated chemical transformations, or by rapid (and likely) non-specific aggregative processes.

By classical definition, the marine colloidal phase encompasses heterotrophic and phototrophic bacteria; termed "biocolloids". However, most oceanographers are dissatisfied with the concept of "biocolloids" so in most cases seawaters are filtered (0.2-0.8 μm) to arbitrarily separate matter into a "particulate" phase, containing cells and large detritus, and "dissolved" phase containing solutes and colloidal particles. Operationally then, marine colloids are a subset of the classical colloid fraction described above.

It has been argued recently that the definition of marine colloids instead should be based upon physicochemistry of the intra-colloidal matrix rather than a strict physical dimension (Gustafsson and Gschwend, 1997). In this "chemcentric" approach, the term colloid is applied only to those constituents that provide a molecular environment for the selective escape of chemicals from aqueous solution, by either partitioning into or onto the colloidal constituent. By this definition, the lower threshold separating solutes from colloids still corresponds to a physical dimension of ~ 1 nm (for the reasons outlined above) but the upper size threshold is constrained by environmental transport conditions rather than by arbitrary size delimitation (Fig. 1). In a refinement of the classical colloid definition, the size boundary between colloids and "gravitoids" is determined by the outcome of kinetic competition between aggregation and sedimentation. Gustafsson and Gschwend (1997) argue that this

size boundary shifts as a function of the total solids concentration, so the upper threshold delimiting colloidal matter will be several microns in coastal waters versus several tenths of microns in the deep ocean (Fig. 1). Another significant distinction is that not all substances larger than a nanometer are colloidal. High molecular weight polyelectrolyte molecules that assume an extended conformation in seawater would not meet the chemcentric criteria for colloids. This aspect is problematic for studying the marine colloidal phase because no methodologies currently exist for measuring the conformation of organic molecules in marine water samples. However, a functionally based definition is more adaptable to studying the effect of colloidal processes in natural systems (Gustafsson and Gschwend, 1997).

While the last decade has brought more dissention than consensus about what constitutes marine colloids, our understanding of the varied roles that colloids may play in coastal and offshore waters has nonetheless improved despite arbitrary and inconsistent size-based delimitations of the marine colloidal phase. The analytical methods underlying these studies are now considered.

III. Analytical Methods

The analytical approaches used to study marine colloids lie in two broad categories; determination of the abundance, elemental and molecular composition of marine colloids, and the assessment of colloid reaction rates, primarily with respect to their transfer into particulate phases. The methods used to quantify the abundance of colloidal matter will be considered now while the measurement and implications of colloid reaction rates are covered in Section VII.

A. Number Concentrations of Marine Colloidal Matter

Early studies established that a significant fraction of dissolved organic matter lay in the colloidal size range (e.g. Carlson *et al.*, 1985; Maurer, 1976; Moran and Moore, 1989; Ogura,

1977; Sharp, 1973). These findings catalyzed a burst of interest in marine colloids and their role in carbon and metal cycling. Koike *et al.* (1990) reported that the abundance of non-living organic particles sized between 0.38-1.0 μm were $\sim 10^7$ particles mL^{-1} in surface waters of the North Pacific. This finding was corroborated for coastal waters off Nova Scotia in a joint project using several different analytical approaches (Longhurst *et al.*, 1992). The concentration of these "Koike" particles decreased by 10x in deep waters, implying there was active production of colloidal matter in the photic zone. These flexible (difficult to filter) particles were 4-30 x more abundant than marine bacteria. Moreover, Koike *et al.* (1990) showed that bacteria were not a source of these colloids but that they likely originated with the activity of small flagellates. They estimated that these particles accounted for $\sim 10\%$ of the DOC, in agreement with estimates from earlier bulk separation studies (Sharp, 1973).

Number concentrations of marine colloids in seawater were measured for sizes down to ~ 5 nm using a combination of ultracentrifugation and transmission electron microscopy (Wells and Goldberg, 1991; Wells and Goldberg, 1992; Wells and Goldberg, 1994). Colloid concentrations increased logarithmically with decreasing size in coastal California seawaters, with numbers being on the order of 10^9 colloids mL^{-1} . Similar abundances were observed in surface and deep waters of the North Atlantic, equatorial Pacific, and the Southern Ocean (Wells and Goldberg 1994 and unpublished data), demonstrating the widespread distribution of marine colloidal matter. At each station, the mean particle size tended to be larger near the base of the thermocline, suggesting a different source or intensity of colloid production in this region. In all cases, the globular-shaped colloidal particles exhibited heterogeneous electron densities, suggesting they perhaps are aggregates of smaller molecules (Fig 2). In subsequent studies, resin embedding methods were used to ensure that molecules maintained their configurations upon drying, and stains were applied to improve the visibility of the colloidal phase (e.g. Heissenberger and Herndl, 1994; Leppard *et al.*, 1997). These studies

confirmed the presence of the granular colloids noted above and showed that additional, more amorphous colloidal matter also was present. TEM studies also showed the presence of large aggregates of colloidal matter (Leppard *et al.*, 1997; Wells and Goldberg, 1993) that can become incorporated into marine snow aggregates (Leppard *et al.*, 1996).

More recently, Santschi *et al.* (1998) used a combination of TEM and atomic force microscopy to show that fibrillar colloids, 1-3 nm in width and 100-2000 nm in length, comprise a significant fraction of colloidal organic matter in coastal and offshore seawaters. These fibrils, rich in polysaccharides (Santschi *et al.*, 1998), are clearly colloidal by the standard size definition but may be non-colloidal based upon a chemcentric view (Gustafsson and Gschwend, 1997). Regardless, these fibrils form aggregates up to several micron in size, often incorporating globular-shaped colloids (Santschi *et al.*, 1998). Fibrillar "particles" therefore are likely to be important in colloid cycling.

Dynamic light scattering (DLS), also known as photon correlation spectroscopy, has been successfully applied recently to the study of colloidal abundance and formation in seawater (Chin *et al.*, 1998). With dynamic light scattering, the time dependence of light scattered from a laser-illuminated volume of solution is measured over tenths of a microsecond to milliseconds. These fluctuations are a function of the diffusion rate of molecules and particles within this volume (that is, Brownian motion). The time dependence of scatter therefore can be used to calculate the diffusion coefficient of particles if a number of conditions can be met. In favourable cases there are methods available for treating the time-dependent fluctuations in the scattered light intensity to extract the "hydrodynamic" (or "Stokes radius") colloid size distribution. Chin *et al.* (1998) used this capability to measure short-term changes in the abundance of colloids a few nm to a few microns in size (see below). This methodology is certain to be applied more frequently in future studies on marine colloid dynamics.

B. Isolation of Colloidal Matter for Bulk Analysis

Analysis of the constituents that comprise marine colloidal matter generally requires the separation and preconcentration of colloids from conventionally filtered (e.g., 0.2 μm) seawater. Although it is possible to analyze elemental compositions of individual particles using TEM/energy dispersive spectroscopy (e.g. Chin *et al.*, 1998), the low sensitivity of the method and non-homogeneity within and amongst individual particles strictly limits the quantitative value of this approach (Wells and Goldberg, 1991). The primary method at present for preconcentrating colloidal matter for bulk chemical analysis is cross-flow (or tangential flow) filtration (CFF). This approach is attractive for its operational simplicity and the high concentrations factors (> 100x) that can be achieved. However, the separation of molecules by pore size exclusion is strongly influenced by molecular conformation, interaction with the membrane and interaction with other soluble and colloidal substances near the membrane surface (Buffle *et al.*, 1992).

Aside from conformational and molecular flexibility issues that cloud the accuracy of size exclusion methods, solvent flow through the membrane leads to the accumulation of macromolecular substances near the membrane surface; a process that is countered by back diffusion of molecules from the membrane. This concentration "polarization" can enhance colloid-colloid and colloid-solute interactions. Small solutes that should otherwise pass through the membrane might then become associated with larger colloids that do not, altering the apparent size fractionation. Directing sample flow tangentially across the membrane surface reduces the thickness of the concentration polarization layer, decreasing but likely not entirely eliminating the possibility for self-aggregation (Buffle *et al.*, 1992). The lowering of the osmotic barrier also increases permeate (filtrate) flow rates. In practice, the retentate solution containing the macromolecules is swept from the membrane and recycled through the retentate reservoir. This reservoir usually encompasses the entire

starting sample volume, but in a few cases a small retentate reservoir instead is continuously replenished with fresh sample water as CFF proceeds (e.g. Gustafsson *et al.*, 1996). The latter approach, termed sampling mode (Dai *et al.*, 1998), minimizes the exposure of the colloidal constituents to the CFF system and may yield better estimates of the retention coefficient of a molecule.

Determination of the percent colloidal fraction of carbon or metals in conventionally filtered (0.2 - 0.7 μ m) "dissolved" samples typically is done in one of two ways. The more straightforward method is to subtract analyte concentrations in the membrane permeate from those in the starting filtrate solution. But this approach potentially can bias the determinations because any sorption of truly soluble metals or organic molecules to the CFF system is then quantified as being colloidal, thus overestimating the colloid fraction. Conversely, the colloidal fraction could be underestimated if there is low-level carbon or metal contamination of the permeate from the CFF system. Nonetheless, once system leaching and sorption problems are verified to be minimal for a given water type, ultrafiltration cartridges can offer a viable straightforward approach for determining colloidal metal concentrations (Nishioka *et al.*, 2001)

The preferred analytical approach is to determine mass balance for each sample separation to ascertain if there are contamination or sorption problems. In this case, analyte concentrations are measured in the starting filtrate, the membrane permeate and the membrane retentate fractions. Colloidal metal concentrations are then calculated by subtracting the permeated concentration from the retentate and dividing the result by the concentration factor. Mass balance can then be assessed by comparing the sum of the analyte soluble and colloidal concentrations with that in the conventionally filtered starting solution. While preferable over the simple difference approach, mass balance determinations

still are relatively insensitive and could mask significant sorption problems (Gustafsson *et al.*, 1996).

A measured loss of analyte to the CFF system may not be due to sorption. Incomplete flushing while extracting the CFF retentate will leave a large portion of colloidal material in the concentration polarization layer, leading to a low estimate of the colloidal metal concentration (see in Buffle *et al.*, 1992). For example, it is recommended that once CFF processing is complete, the retentate solution should be recirculated for some time with the permeate flow turned off to enhance recovery of the colloidal material (Buesseler *et al.*, 1996). In practice, any “missing” analyte usually is attributed to incomplete colloid recovery, the identical result as taking the simple difference between permeate and starting solution concentrations. Nonetheless, determining mass balance provides an indication of which results should be interpreted with added caution.

The increasing use of CFF in colloid studies during the early 1990's led to an intercomparison study to assess whether different CFF systems provided well-defined and operationally reproducible results (Buesseler *et al.*, 1996). This “colloid cookout” study was conducted using 14 different CFF systems representing 5 different manufacturers, with the central criterion being the size fractionation of organic carbon with 1 kDa membranes. Large volumes of homogenized surface waters off Woods Hole, MA and mid-depth (600 m) waters off the National Energy Laboratory of Hawaii were processed on-site (Buesseler *et al.*, 1996). Although the primary focus was testing the separation of colloidal organic matter, the outcome is summarized here because it has direct significance to the study of colloidal trace metals.

There were two primary findings of the intercomparison study. First, extremely long cleaning and flushing times are required to reduce the DOC blanks of new cartridges. Second, the degree of colloid retention by the 1 kDa membranes varied dramatically among

manufacturers, but was similar among different groups using the same brand of membrane. Even so, retention efficiencies can vary among CFF membrane batches from a single manufacturer (Dai *et al.*, 1998, P. Santschi, pers. comm.), reflecting the shortcomings of using industrial-based separation technologies outside the limits of their design application. For systems displaying high retention efficiencies of marine colloids (e.g., Amicon), permeate DOC concentrations increased significantly with the concentration factor. In contrast, this “breakthrough” of organic carbon was not apparent in systems equipped with membranes having lower colloidal retention efficiencies. A similar increase in the permeate concentration of trace metals has been observed with Amicon 1 kDa membranes (Guo *et al.*, 2000b; Wen *et al.*, 1996) but not with Filtron 1 kDa membranes (Powell *et al.*, 1996; Wells *et al.*, 2000).

Although there is uncertainty about the cause of changing analyte concentrations in the permeate during processing (Buesseler *et al.*, 1996), it may be due to increased membrane transport of soluble (< 1 kDa) molecules held in the concentration polarization layer (Guo and Santschi, 1996; Gustafsson *et al.*, 1996). Guo *et al.* (2000b) experimented with standard molecules and showed that > 40% of 0.5 kDa rhodamine 6G and 0.6 kDa glutathione are retained by the 1 kDa Amicon S10N1 membrane, even at concentration factors of ~50. They suggested that the reverse problem, breakthrough of high molecular weight standards, was not significant (but see below). Retention of soluble (< 1 kDa) substances would imply that colloidal fractions may be overestimated when large changes occur in permeate analyte concentrations over time. As a result, Guo *et al.* (2000b) argue that high concentration factors (> 40) help to minimize the retention of soluble organic phases, contrary to earlier recommended protocols (e.g., Buesseler *et al.*, 1996). They also showed that diafiltration with deionized water further reduced the retention of their < 1 kDa molecular probes.

But the mechanistic interpretations by Guo *et al.* (2000b) for the increasing permeate concentrations with higher concentration factors rely on a permeation model for single

discrete molecules that assumes the sorption of molecules to the membrane is negligible (see in Kilduff and Weber, 1992; Logan and Juany, 1990). However, sorption of certain substances can be significant (e.g. Dai *et al.*, 1998; Gustafsson *et al.*, 1996). This simplified model also may not apply equally well to complex mixtures of individual compounds or to colloidal assemblages of discrete molecules, both of which likely comprise the marine colloidal phase. The decreasing ionic strength during diafiltration of the retentate also may alter the conformation of natural colloidal organic molecules enough to affect their retention. For example, decreasing Mg^{2+} and Ca^{2+} activities causes disaggregation of natural colloidal polymers in coastal waters (Chin *et al.*, 1998).

The question of the breakthrough of organic molecules during CFF is an issue of continuing debate. Dai *et al.* (1998) compared the performance of the Amicon 1 kDa membrane used by Guo *et al.* (2000b) with the Millipore Prep/Scale 1 Kda membrane with standard molecules as well as nearshore and offshore seawaters. This comparison is particularly useful because the Amicon 1 kDa membrane, a mainstay for marine colloid studies, became no longer commercially available after Amicon merged with Millipore. From these data, Dai *et al.* (1998) concluded that breakthrough of both high and low molecular weight matter occurs during processing as a consequence of the CFF membrane itself as well as physical/chemical interactions of specific organic constituents with the membrane. Breakthrough varied among the oceanographic sites likely due to differences in molecular composition and concentrations of COC. They attributed the bulk of this breakthrough to high molecular weight colloids, in contrast to the findings of Guo *et al.* (2000b), and recommended keeping CFF concentration factors <5 to minimize breakthrough artifacts. It is likely that the same recommendation would apply to the retention of colloidal trace metals, although it is not known whether the minute subset of COC responsible for binding trace metals behaves similarly to the bulk COC.

Despite intensive study then, we remain at odds over whether large or small concentration factors improve the accurate retention and isolation of marine colloidal organic matter. The answer, if it is truly one or the other for all marine colloidal constituents, likely will depend in large part upon the molecular architecture of the colloidal organic matter and the degree to which it is structurally rigid or amorphous. Those colloids less susceptible to structural deformation would have a low probability of permeating through the membrane pores while the reverse would be true for more amorphous or even linear structures, such as reported by Santchi *et al.* (1998). Strict calibration of CFF membranes is useful for assessing membrane characteristics and performance over time, but this “calibration” shifts when standards of equivalent molecular weight but different molecular architectures are used (see in Gustafsson *et al.*, 1996). Combined with the recognition that compositional fractionation of the colloidal retentate will occur as concentration factors increase, it is likely that highly accurate colloidal size separations may never be achieved by CFF. Nonetheless, CFF remains an extremely useful tool for assessing the broad characteristics and abundance of the marine colloidal phase.

An alternate method for the size separation of molecules is Flow Field-Flow Fractionation (Flow FFF), and this technique is only just beginning to be applied in the study of marine colloidal matter (Beckett and Hart, 1993; Beckett *et al.*, 1990; Gustafsson *et al.*, 2001; Hassellöv *et al.*, 1996; Hassellöv *et al.*, 1999). In Flow FFF, a flow field is applied at right angles to a channel flow in a shallow (~200 μm) ribbon-like chamber. Soluble fractions are driven through a membrane (1 kDa) at the accumulation wall, while colloidal components are driven towards the membrane surface. The resultant concentration gradient is opposed by diffusion (a function of colloidal size), resulting in colloids of different sizes being retained in different stream laminae (Fig. 3). The smaller the colloids the higher they diffuse into the parabolic velocity profile and thus the faster they elute. The timing at which colloidal matter

exits the flow chamber then is proportion to its size (hydrodynamic diameter). In addition to yielding a soluble phase, the method provides a high resolution, continuous size spectrum of colloidal matter, contrary to CFF which yields only bulk separation of those materials retained by the membrane. A central disadvantage of Flow-FFF is that the sample is greatly diluted within the channel, decreasing the ability to measure the resultant size fractionation.

Sample “focusing” in which a larger volume of sample (e.g., 10 ml) can be preconcentrated within the channel before initiating flow separation (Hassellöv *et al.*, 1996) may partially offset this shortcoming but it remains unclear whether this focusing introduces artifacts surrounding the size distribution of the colloidal phase. For example, preconcentration of riverine colloids can enhance colloid-colloid and colloid-solute interactions that potentially could alter colloidal size separations by Flow-FFF (Lead *et al.*, 1997), but these aggregation problems have not yet been shown to be significant at the high ionic strength of seawater.

Thus while Flow-FFF techniques are not be suitable for the bulk isolation of large quantities of marine colloidal matter, they may be ideally suited for studying constituents that can be detected with high sensitivity. Flow FFF also affords an opportunity to perhaps investigate degradation or aggregation process with high definition. For example, the system components can be constructed of “metal-free” polymers suitable for trace metal studies. Hassellöv *et al.* (1999) coupled the channel outflow of Flow FFF on-line with a low resolution ICP-MS to determine the colloidal size continuum of >20 elements in stream waters. Unfortunately, the high salt content of seawater precludes this simple approach for all but perhaps estuarine waters, but interfacing Flow-FFF with flow injection analytical methods might still provide a similar capability for studying colloidal trace metals in marine waters. While the very limited use of Flow FFF in marine waters has prevented close examination of potential methodological artifacts influencing the size separations, these techniques are certain to garner attention in the future.

The differing and imprecise size retention of marine colloids by CFF membranes challenges our ability to quantitatively compare findings among studies from disparate locations and times. While this situation is unfortunate, it is important to remember that these shortcomings are not restricted to CFF systems. Conventional filtration of seawater also suffers major artifacts in size selectivity, even when using etched membrane filters (e.g., Koike *et al.*, 1990; Stockner *et al.*, 1989). Nonetheless, CFF presently remains the only effective method for accumulating enough high molecular weight substances from seawater to examine the broad molecular and metal composition of the marine colloidal phase. For that reason, CFF will continue to serve a key role in colloid studies in the foreseeable future.

IV. Metal Content of Marine Colloidal Matter

The tremendous increase in study of colloid-associated trace metals over the last decade has considerably expanded the database for estuarine and nearshore waters (Table 1). Even so, this database comprises < 25 articles with generally only a few samples each. Typically, CFF processing has been conducted with either 1 or 10 kDa molecular weight cutoff membranes. Many early studies either did not determine mass balances or suffered from low metal recoveries. However, the qualitative picture that emerged from this work has been refined with recent improved methods.

The collective findings establish that a significant component of bioactive, or nutrient metals (Mn, Fe, Co, Ni, Cu, Zn, Cd) occur in the colloidal phase along with numerous other trace metals. Sigelo and Helz (1981) showed that estuarine colloidal material contained more than 30 elements, while Bertine and Vernon-Clark (1996) found a similarly large range of colloidal elements off the California coast. Post-collection desalting procedures were used in both of these studies, so colloid fractions may have been underestimated if there were significant ionic strength mediated changes in molecular conformations. But as noted above,

Guo *et al.* (2000b) argue that any “losses” during desalting instead result in more accurate colloidal size separations.

Despite the different cutoff size and filter types used for conventional filtration and subsequent CFF's, several consistent patterns appear in these estuarine and coastal water data. The most straightforward observation is that the proportion of colloidal to soluble metals tends to decrease from the upper estuary to coastal waters, as has been described previously (e.g., Sholkovitz *et al.*, 1978). Even so, colloid-associated metals constitute a significant fraction of “dissolved” metals in coastal, high salinity waters (Table 1), also consistent with the results of earlier studies (e.g., Mayer, 1982).

With respect to nutrient trace metals, a high proportion of dissolved Fe is colloidal in many estuarine waters (e.g., Dai and Martin, 1995; Guieu *et al.*, 1998; Powell *et al.*, 1996; Wen *et al.*, 1996) and coastal embayments (e.g., Benoit *et al.*, 1994; Martin *et al.*, 1995; Wells *et al.*, 1998; Wells *et al.*, 2000; Wen *et al.*, 1999; Whitehouse *et al.*, 1990). Dissolved Cu is only slightly less dependent on the colloidal phase, with 1-90% of dissolved Cu appearing in the various colloidal size fractions in these waters (Table 1). Ni and Cd follow, with each being up to ~ 78 % colloidal (Dai and Martin, 1995; Dai *et al.*, 1995; Martin *et al.*, 1995; Muller, 1998; Powell *et al.*, 1996; Sanudo-Wilhelmy *et al.*, 1996; Wells *et al.*, 1998; Wells *et al.*, 2000; Wen *et al.*, 1996; Wen *et al.*, 1999). Wen *et al.* (1999) found that 5-32 % of dissolved Co was colloidal in Galveston Bay. Santschi *et al.* (1987) showed that 30-60% of Se, a metal required by some phytoplankton, was colloidal in Narragansett Bay, RI.

In contrast to these metals, dissolved Zn normally is predominantly soluble (Benoit *et al.*, 1994; Sanudo-Wilhelmy *et al.*, 1996; Wells *et al.*, 1998; Wells *et al.*, 2000; Wen *et al.*, 1996). A notable exception is in Galveston Bay where > 85 % of dissolved Zn was retained by a 1 kDa (Amicon) membrane (Wen *et al.*, 1999). Dissolved Mn also occurs predominantly as soluble species in seawater (Benoit *et al.*, 1994; Guentzel *et al.*, 1996; Powell *et al.*, 1996; Sanudo-

Wilhelmy *et al.*, 1996; Wells *et al.*, 2000; Wen *et al.*, 1996; Whitehouse *et al.*, 1990), the exception being in Venice Lagoon where ~ 50% of dissolved Mn was colloidal (Martin *et al.*, 1995). This unusual result may be related to the very high DOC concentrations in these lagoon waters.

The marine colloidal phase also contains non-nutrient trace metals. From 2-100 % of conventionally filtered Al is colloidal in coastal embayments and nearshore waters (Benoit *et al.*, 1994; Sanudo-Wilhelmy *et al.*, 1996). Dissolved Pb shows a similar preference for the colloidal phase (1-100%) in these environments (Benoit *et al.*, 1994; Dai and Martin, 1995; Kozelka *et al.*, 1997; Martin *et al.*, 1995; Muller, 1998; Wells *et al.*, 1998; Wen *et al.*, 1996; Wen *et al.*, 1999). In addition, a major fraction of dissolved Hg is colloidal in various Texas estuaries and the Ochlockonee estuary in Florida (Powell *et al.*, 1996; Stordal *et al.*, 1996).

There are far fewer studies of colloid-associated metals in offshore waters (Table 2). Moran and Moore (1989) used some of the first trace metal clean CFF techniques to measure a 1-15 % colloidal fraction of Al in North Atlantic surface waters. Similar values were observed in deep (600 m) waters off Hawaii during the colloid intercomparison study (Reitmeyer *et al.*, 1996; Wen *et al.*, 1996). The colloidal fraction of Fe typically is higher, ranging from 10-50 % in the North Atlantic, North Pacific and equatorial Pacific (Nishioka *et al.*, 2001; Wells, in press; Wu and Luther, 1994). The colloidal phase comprises a significant portion of dissolved Ni, Cu, Cd, and Pb in surface waters of the Gulf of Maine and in deep waters off Hawaii (Greenamoyer and Moran, 1996; Reitmeyer *et al.*, 1996; Wen *et al.*, 1996). Although sparse in comparison to nearshore data, these limited findings suggest that the percent colloidal component of dissolved metals may not be substantially different between open ocean and coastal environments. Yet these two environments differ greatly in total dissolved metal concentrations. These findings may further suggest that colloids contain chemical species that interact selectively with different metals.

The underlying cause for the large differences in percent colloidal metal measured among similar water types is difficult to assess. This variability probably attributes partly to the use of different molecular weight cutoff membranes and variable concentration factors (Gustafsson *et al.*, 1996). For example Wen *et al.* (1999) found the highest colloidal fraction of Zn to date in a coastal embayment using a membrane brand known to have higher colloid retention efficiencies than other brands (Buesseler *et al.*, 1996). But a large range in colloidal metal content also is found among environments when uniform sampling methodologies are employed (e.g. Guo *et al.*, 2000a). Moreover, seasonal and depth-related changes in the fraction of colloidal metal have been measured at single sampling sites (Kuma *et al.*, 2000; Nishioka *et al.*, 2001). For example, Kuma *et al.* (2000) showed that the change in colloidal fraction of Fe at 2 m over the spring bloom was as large as that from surface to deep (ranging from 6-70%). Thus while analytical issues can be problematic, they likely overlay fundamental trends in the composition and abundance of the colloidal metal fraction in seawater.

In contrast to most studies that analyze volume-based concentrations of colloidal trace metals (e.g., nM), Guo *et al.* (2000a) characterized metal and carbon contents of diafiltered, freeze-dried CFF colloidal isolates from three marine regions; the Mid Atlantic Bight, the Gulf of Mexico, and Galveston Bay. Their study is the most comprehensive to date on metal colloid associations in different coastal waters. They show that the bioactive metals Cu, Zn, Ni, and Fe have average concentrations $\geq 10 \mu\text{g/g}$ colloidal mass while Pb, Al, Mn, V, Ba, and Ti have average concentrations between 1-10 $\mu\text{g/g}$. Concentrations of Cd, Co and Be were $< 1 \mu\text{g/g}$ of colloidal mass. These data have been influenced by a significant loss of colloidal metals during the diafiltration process. In Galveston Bay, metal recoveries from the retentate after diafiltration were ~50% for Cu, ~36% for Zn, ~32% for Ni and only ~9% for Pb (Wen *et al.*, 1999). It remains to be determined whether these losses reflect more efficient

flushing of low molecular weight colloids from the concentration polarization layer (Guo *et al.*, 2000b) or de-salting effects on the conformation of colloidal molecules.

Despite these uncertainties, the work of Guo *et al.* (2000b) is important because the identical methodologies employed permit closer examination of the differences in colloidal metal fractions in surface waters of Galveston Bay, the Gulf of Mexico and the Mid Atlantic Bight. In general, the inshore to offshore concentrations of colloidal metals ($\mu\text{g Me/gC}$) either decreased (Cu, Co, Ni, Cd, Fe, Al, Mn, Ba, Ti), or showed no obvious trend (V, Pb, Zn, Cr, Be) in the Gulf of Mexico (Guo *et al.*, 2000b). In contrast, most colloidal metal concentrations ($\mu\text{g/g}$) increased from nearshore to offshore in the Mid Atlantic Bight for Fe, Al, Mn, V, Ti, Ba, Co, Ni, Pb, Zn, Cd, Cr and Be. In almost all cases the average colloid metal concentrations were higher in the Mid Atlantic Bight than in the Gulf of Mexico. This pattern is consistent with the higher terrestrial and aerosol input to North Atlantic waters relative to other open ocean regions (Duce and associates, 1991). These comprehensive data provide clear evidence that colloidal metals are a significant component of the dissolved metal fraction in coastal and open ocean waters. The findings also provide the first quantitative evidence that spatial distribution of colloidal metals in offshore waters is likely coupled to the prevalence of terrestrial dust inputs (e.g., higher in the Western N. Pacific and equatorial Atlantic surface waters). In contrast, there are no clear indications of the temporal variations in colloidal metals.

V. The Chemical Form of Colloidal Metals

One of the more obvious changes in the bulk composition of colloidal matter moving from estuarine to shelf and offshore waters is a progressive decrease in mineralogical content. While clays and metal oxyhydroxides can be abundant colloidal constituents in riverine and estuarine waters (Sigleo and Helz, 1981; Sigleo and Means, 1990), they are only occasionally

identified in coastal waters (Wells and Goldberg, 1992, Wells, unpublished data) and are extremely rare in offshore surface and deep environments (Benner *et al.*, 1997; Leppard *et al.*, 1997; Wells and Goldberg, 1994). Instead, the colloidal phase is almost exclusively organic matter, the composition of which is discussed in detail by R. Benner (this volume). It is sufficient here to recognize that 10-78% of "dissolved" organic carbon in seawater is colloidal, based upon CFF size fractionation. In most cases then, metals are trace constituents that accompany this bulk carrier phase.

Metal associations with organic matter can be characterized by ligand exchange reactions, whereby cations associate with electronegative or negatively-charged functional groups of the molecule (or "surface"). These likely include -COOH, -OH, and R-N-R or R-S-R groups distributed within the organic matrix. For example, colloidal organic matter in Galveston Bay and the Gulf of Mexico contains 1.4 meq/g of proton reactive sites, or ~1-10 μM (Santschi *et al.*, 1995). Taken singly, these sites add hydrophilicity to the molecules but bind trace metals only weakly if at all due to competition with the major seawater cations (e.g. Mg^{2+} , Ca^{2+}). But when two or more of these functional groups occur in close proximity to one another, or along molecules flexible enough to bring them into close proximity, then strong "chelates" might form with trace metal ions depending upon the architecture of the ligand site. Unfortunately, measurement of bulk proton reactivity gives no indication of how strongly trace metals will associate with organic matter.

Nonetheless, various studies have found positive correlations between colloidal organic carbon (COC) and colloidal metal concentrations. Stordal *et al.* (1996) showed colloidal Hg in Galveston Bay was strongly related to COC concentrations, perhaps bound to thiol functional sites (Guentzel *et al.*, 1996). Colloidal Cu, Ni, Zn, Co, Cd and Pb also correlated with COC in Galveston Bay (Guo *et al.*, 2000a; Wen *et al.*, 1999). Colloidal Fe, Ni, and Cu were linearly correlated with COC in the Ochlockonee estuary (Powell *et al.*, 1996). However, in each case

the correlations differ distinctly among the metals. On average, colloidal metal:COC correlations in offshore waters in the Gulf of Mexico and the Middle Atlantic Bight are weaker but still suggested for Cu, Zn, Ni, and Co (Guo *et al.*, 2000a). But these relationships also differed substantially among the three environments (Guo *et al.*, 2000a) making it clear that the colloidal fraction of any given trace metal is not coupled tightly to the abundance of colloidal carbon.

It is now widely accepted that the chemical speciation of most bioactive metals in seawater is regulated by strong complexation with natural organic chelators (see in Bruland *et al.*, 1991; Donat and Bruland, 1995). These ligands are present in very low concentrations (nM) but effectively form metal:specific complexes having very high conditional stability constants in seawater; orders of magnitude above that of simple cation sorption to charged surface sites. The cycling of bioactive metals therefore is intrinsic to the behaviour of this subset of organic constituents.

While it was anticipated that these strong ligands would be low molecular weight, recent work demonstrates that Cu, Zn, Cd, and Pb complexing ligands are partly colloidal in both Narragansett Bay and estuarine and coastal waters off England and Scotland (Muller, 1996; Muller, 1998; Muller, 1999; Wells *et al.*, 1998). In Narragansett Bay, there is a distinct separation in the size classes of Cu and Pb ligands, with colloidal Cu ligands being 1-8 kDa and colloidal Pb ligands being mainly 8 kDa-0.2 μm in size (Wells *et al.*, 1998). These findings indicate that metal specific reactions control bioactive metal:colloid associations, rather than simple sorption processes. Muller (1998) arrived at the same conclusion for Cu and Pb based upon ligand spatial distributions and comparative ligand/colloid characteristics in the Firth of Clyde, Scotland. Muller (1999) suggested that colloidal Cu and Pb complexing ligands might form *in-situ* as weakly bound assemblages of soluble metal-specific ligand molecules. A

possible mechanism for generating such assemblages is discussed by Chin *et al.* (1998) (see below).

Further evidence that metal:colloid interactions are controlled by metal-specific reactions are observations that the conditional stability constants for metal complexation vary with the size of organic ligands. The stronger class of Cu-complexing organic ligands (L_1) are more prominent in the soluble phase of Chesapeake Bay (Gordon *et al.*, 1996) and in the coastal waters of Southern England (Muller, 1996). But both the strong and weaker classes of Cu-complexing ligands appear to be split between the soluble and colloidal phases in Narragansett Bay (Wells *et al.*, 1998). It is not clear if this difference is real or if it simply reflects the different size-separation methodologies used in these studies. In contrast, strong Pb-complexing organic ligands often are predominantly colloidal in these environments (Muller, 1996; Muller, 1998; Wells *et al.*, 1998). These findings contribute to the view that the marine colloidal phase is not a uniform substrate for metal associations (Wells *et al.*, 2000).

There is some question though whether the soluble ligand model is appropriate for colloidal ligand sites. Muller (1998) found that Pb bound to colloidal matter was not in a state of dynamic equilibrium with the solution phase, and that "occupied" and "unoccupied" colloidal ligand sites were not equivalent. In other words, Pb association and dissociation kinetics were different when reacting with colloidal matter compared to soluble ligand complexes. Muller suggested this situation developed with aging of marine colloids in the water column (Muller, 1998). Sunda and Huntsman (1991) showed that a major fraction of "dissolved" Cu in Narragansett Bay surface waters was not released after acidification to pH 2.0 for 19 days but was released 2-8 months later. UV oxidation eliminated this delay, indicating that the organically complexed Cu was not in dynamic equilibrium with the solution phase. The distribution of these kinetically inert complexes closely followed the distribution

of colloidal Cu (Wells *et al.*, 2000), indicating that the kinetically inert Cu was bound in colloidal complexes. Slow kinetics of equilibration could arise if the soluble metal-complexing ligand molecules incorporate into, or onto colloidal organic matrices and then become “internalized” by either hydrophobic partitioning or by the continued sorption of new organic matter. In this case the true conditional stability constant (and specificity) of the metal-ligand complex is unchanged but the ability to readily exchange with solution species becomes restricted.

In a dissenting voice, Mackey and Zirino (1994) suggested that observations of organic ligands capable of forming highly specific, strong complexes with trace metals were probably artifacts of the voltammetric methods used. They hypothesized instead that strong ligand signatures arise when weak, metal-binding compounds of low and high molecular weights coalesce, entrapping the weakly bound metals within a colloidal organic matrix (the “onion” hypothesis). They argued that the resultant physically restricted exchange between colloidal metals and the electrode surface would appear as a strong complex. They proposed this model based in large part to puzzlement of how strong chelators could be so specific for a given metal, or why metals and their ligand concentrations should be so closely matched in seawater. But biological systems have evolved highly tuned molecular architectures for the specific complexation of individual bioactive metals (e.g. Crumbliss 1991; Silva and Williams 1991), and there now is clear evidence that microbes are a direct source of some metal specific complexing ligands in seawater (see below). In addition, the “onion” hypothesis requires that the “strength” of metal complexation increase with increasing ligand size, which is the opposite of what is measured for Cu, Zn, and Cd in Narragansett Bay (Wells *et al.*, 1998). Even so, colloidal Pb was bound more strongly than by soluble ligands in Narragansett Bay (Wells *et al.*, 1998). It may be then that metal-colloid associations in seawater include both strong, highly specific ligands as well as perhaps less specific and

weaker ligands that persist due to their internalization within the colloidal matrix. A progressive internalization of either ligand could help explain why the lability of colloidal metals might change with time in the water column (Muller, 1998; Sunda and Huntsman, 1991).

VI. Particulate-based Estimates of Colloidal Metal Concentrations

The inherent difficulties in determining colloidal metal concentrations in seawater has spawned early attempts to model colloidal metal content based upon the composition of the particulate phase (e.g. Honeyman and Santschi, 1989; Moran and Buesseler, 1993; Moran et al., 1996). In this approach, a first order approximation of colloid mass is obtained using projected relationships with the measured particulate mass present in seawater (e.g., $C_c/C_p = 2 - \log C_p$ – Moran and Moore, 1989, or $C_c = 0.7 \cdot \log C_p - 2.6$ – Honeyman and Santschi, 1989, where C_c and C_p are colloid and particulate masses (mg liter^{-1}) respectively). Without going into detail here, these equations have been derived to explain the non-linear inverse log relationship between the dissolved:particulate partition coefficient (K_d) and particulate mass (C_p) at high particle mass concentrations (that is, where colloids and particulate matter compete in the sorption of dissolved metals, e.g. Honeyman and Santschi, 1989). Unfortunately, these relationships remain unverified because colloid mass cannot yet be unequivocally determined in seawater. The resultant colloid mass estimates are combined with assumed colloid:metal partition coefficients (K_c) to arrive at first order predictions for colloidal metal concentrations without *a priori* knowledge of the processes controlling the colloidal organic phase (e.g., Moran et al., 1996). Preliminary comparisons of these estimates with measured colloidal metal concentrations have not yielded close agreement (Wells et al., 2000), although very few data so far are available for this assessment.

There are reasons to expect that bulk methods, while attractive from a methodological approach, will not provide accurate predictions of colloidal metal concentrations anytime in the near future. In addition to the need for stricter characterization of the mechanistic bases underlying any relationship between the particulate and colloidal phases, the selection of values for K_c remains problematic. While it has been generally assumed that $K_c \approx K_d$ for bioactive metals (as appears to be the case for Th – Guo and Santschi, 1997), measurement of highly specific metal complexation by colloidal matter [e.g., Muller, 1996 #294; Wells, 1998 #441] would suggest that $K_c \gg K_d$, assuming that K_d is controlled largely by non-specific sorption reactions. While this may be true for nearshore waters (Wen et al., 1999), where the particulate phase can be heavily influenced by mineralogical fragments, it may not be as true for offshore seawaters, where living biomass and non-living cellular fragments comprise much of the particulate phase. In that case, organic particulate matter likely contains remnant (or still functioning) highly specific metal transport sites having high conditional stability constants for bioactive metals. If so, K_d might be expected to vary significantly among water masses, complicating the straightforward application of bulk methods for estimating colloidal metal concentrations. It remains to be seen how effective a sentinel the particulate phase will be for marine colloidal matter.

VII. Sources of Metal-Complexing Colloidal Ligands

The direct source of strong complexing ligands that control metal speciation in seawater is not well understood. There is clear evidence that Cu-complexing organic ligands can be released by living prokaryotic and eukaryotic phytoplankton in response to Cu stress (Croot *et al.*, 2000; Gonzalez-Davila *et al.*, 1995; Leal *et al.*, 1999; Lombardi and Vieira, 1999; Moffett and Brand, 1995). Heterotrophic marine bacteria and fungi also have this capability (Schreiber *et al.*, 1990; Sunda and Gessner, 1989). A handful of marine prokaryotes now are

known to release siderophore (Fe-complexing) molecules having conditional stability constants that match the stronger of the two Fe ligand classes measured in seawater (Lewis *et al.*, 1995, Rue and Bruland, unpublished, Rue and Trick, unpublished; Reid and Butler, 1991; Reid *et al.*, 1993). The direct source of organic chelators specific for other bioactive metals (e.g., Ni, Zn, Co, Cd) remains unknown. Some metal ligand complexes could be byproducts of intracellular metal detoxification mechanisms (Huges and Poole, 1989; Olafson *et al.*, 1988), although this source likely could be significant only in estuarine and coastal regions impacted by high metal availabilities.

It is very unlikely that colloidal ligands are released directly by microbes. The upper size for transport of molecules by bacteria is ~700 daltons, although hydrodynamic volume of the molecule is more relevant (Payne, 1980). For example, most siderophore molecules are well below this threshold (Crumbliss, 1991). There is no reason to suspect that the capabilities of marine phytoplankton would be any different. While these actively-released compounds presumably are hydrophilic, and thus water soluble, is conceivable that they could become incorporated into the marine colloidal phase without loss of their metal-complexing functionality.

If colloidal metal-specific complexing ligands indeed are sequestered from the soluble phase, then this incorporation should be strongly influenced by the character of the colloidal matrix. Muller (1998) showed that the fraction of strong Cu-binding ligands associated with the colloidal phase increased approximately linearly with increasing colloid charge density; that is, colloids having low charge densities contained less of the strong Cu chelators.

In addition to *in-situ* formation, colloidal metal-complexing ligands may also represent recycled components of cellular metabolism (e.g. Koike *et al.*, 1990; Stramski *et al.*, 1992). Inputs related to cell lysis, protozoan excretion, sloppy feeding by grazers, and viral infection all are suspected at this time. For example, molecules of the weaker Fe ligand class in

seawater have conditional stability constants similar to the porphyrin complexes (tetrapyrroles) that are the most abundant Fe chelators in cellular metabolism (Hutchins *et al.*, 1999). A number of metal-requiring metabolites within cells are membrane-bound and so might be expected to appear at least initially associated with higher molecular weight matter upon recycling. Jaïry *et al.* (1999) found the colloidal proportion of Cu increased during a phytoplankton bloom in the river Marne, and similar increased colloidal fractions of bioactive metals have been measured for blooms in upwelling regions (Wells, unpublished data). The breakdown of biogenic detrital particulates might also release metal complexing colloidal phases (Smith *et al.*, 1992). Alternatively, inputs of colloidal ligands from terrestrial and lacustrine sources may be significant in localized regions, as indicated for Pb-binding ligands in the Firth of Clyde (Muller, 1998).

VIII. Measurement of Colloid Reaction Rates

The kinetics of colloid-colloid and colloid-particulate interactions are likely to strongly influence the residence time of colloidal organic matter in seawater. Based on the findings from estuarine environments, it had been expected that aggregation would be the major fate of marine colloidal matter. Certainly, there is evidence of colloid aggregation in surface and deep ocean waters from transmission electron microscopy (Leppard *et al.*, 1997; Wells and Goldberg, 1993). Two broad morphologies of (globular) colloid aggregates observed at different sites indicate that both very rapid (diffusion-limited) and slower (reaction-limited) aggregation regimes can occur (Wells and Goldberg, 1993). Rapid aggregation, characterized by open, tenuous colloid aggregate architectures (Lin *et al.*, 1989), was only observed in surface waters at primarily nearshore sites (Wells and Goldberg, 1993). Induction of very rapid colloid aggregation is believed to be a critical precursor for formation of marine snow (e.g. Kepkay, 1994; Mopper *et al.*, 1995; Passow and Aldredge, 1995; Passow *et al.*, 1994).

What effect do these colloid processes have on the transport and fate of trace metals?

^{234}Th has been used widely as a tracer for particle cycling and removal from surface waters (e.g. Bacon and Anderson, 1982; Coale and Bruland, 1985; Honeyman *et al.*, 1988). Its high affinity for interfaces, short half-life (24 days) and natural *in-situ* radiogenic source (^{238}U) make it an excellent tracer for quantifying rates of particle cycling over time scales up to 100 days, which spans that of planktonic processes operating in oceanic systems. These attributes made ^{234}Th an ideal starting point for the study of metal scavenging by colloid aggregation in seawater systems (Honeyman and Santschi 1989).

The proportion of dissolved ^{234}Th that is colloidal ranges from 2-16% in shelf waters off New England (10 kDa - Moran and Buesseler, 1993), 1-47 % in surface waters of the Gulf of Maine (1 kDa - Greenamoyer and Moran, 1997), 12% in the northeast Pacific Ocean (10 kDa - Huh and Prahl, 1995), ~10% in the Sargasso Sea (10 kDa - Moran and Buesseler, 1992) and 10-78% in the Gulf of Mexico (10 kDa - Baskaran *et al.*, 1992). In most cases, these studies used a three-box scavenging model to estimate the residence times of ^{234}Th in the soluble, colloidal and particulate size fractions, and then extrapolated to calculate colloid turnover (Fig. 4). By these estimates colloid residence times are on the order of a few hours in nearshore waters and only slightly longer in offshore environments (Baskaran *et al.*, 1992; Moran and Buesseler, 1992; Moran and Buesseler, 1993). In comparison, residence times for soluble ^{234}Th species ranged from several days to weeks in coastal and offshore waters, respectively (Moran and Buesseler, 1993). Such rapid turnover of colloidal matter implies that colloid cycling could substantially influence the transport and fate of metals even if only a few percent of dissolved metals became associated with the colloidal phase.

However, there are several major assumptions in the simplified models used to estimate colloid turnover times. First, the models require a steady state distribution of tracer among the soluble, colloidal and particulate size classes, so the effects of changing phytoplankton

production on colloid cycling rates cannot be examined (e.g. Niven *et al.*, 1995). Second, Th sorption to the colloidal phase is assumed to be irreversible; a condition that is oversimplified for particulate matter (Bacon and Anderson, 1982). Third, these models do not consider direct sorption of soluble ^{234}Th to the particulate phase, making colloid aggregation the only means for transferring Th from the soluble to particulate phase. Improved modelling efforts now account for the direct sorption to particulates and desorption from both particulates and colloidal matter but other necessary parameterizations are still missing (see Burd *et al.*, 2000).

A major shortcoming of the ^{234}Th -based colloid cycling models to date is that microbial degradation is not considered. Microbial activity on particulates could release colloidal matter that, in turn, might be degraded to soluble molecules (e.g. Karl *et al.*, 1988; Smith *et al.*, 1992); each step transferring sorbed ^{234}Th to smaller size classes. For example, Amon and Benner (1996) showed that while bacterial growth efficiencies on soluble organic matter were consistently higher than sustained by colloidal matter, the utilization rates of colloidal carbon were up to 4 x greater. They suggested that the colloidal organic phase is more bioreactive and less diagenetically altered than the bulk of soluble organic matter. This view is supported by carbon isotopic evidence that the colloidal carbon pool as a whole is more recent than the soluble organic phase (Santschi *et al.*, 1995). Benner *et al.* (1997) used extensive data of size-fractionated carbon and nitrogen from the Pacific and Atlantic oceans to argue the “upward” movement of colloidal material into large particulates is less significant than the “downward” size shift from degradation (see Benner, this volume). Metals bound strongly to colloidal ligands, or colloid-associated ligands, presumably also could follow this pathway.

A significant degradation pathway is not entirely at odds with ^{234}Th -based modelling of colloid transport. For example, it is feasible that colloidal ^{234}Th could be tracing the

decomposition of large biogenic matter; that is, the particle cycling models may be operating in reverse (Moran and Buesseler, 1993). As a further complication, ^{234}Th also may not trace bulk colloidal carbon but rather only specific carbon components. Niven *et al.* (1995) used results from a coastal phytoplankton bloom to argue that ^{234}Th associates primarily with the polymer exudates of living phytoplankton; substrates that are thought to be essential precursors for the formation of marine snow (Mopper *et al.*, 1995; Passow and Aldredge, 1995). Because it is unlikely that these carbohydrate polyelectrolytes will form highly stable, metal-specific complexes, ^{234}Th cycling (in either direction) may not be very useful for assessing colloid effects on bioactive metals (Wells *et al.*, 2000).

Wen *et al.* (1997) measured the uptake of ^{59}Fe , ^{54}Mn , ^{65}Zn , ^{60}Co , ^{109}Cd , ^{113}Sn , ^{110}Ag , and ^{133}Ba associated with natural colloidal matter by particulates in estuarine waters of Galveston Bay (S=22). They observed two distinct reaction scales; a fast transfer during the first few hours followed by a slower reaction over subsequent days. They suggested the fast reaction was consistent with a colloid (Brownian) "pumping" mechanism (Farley and Morel, 1986; Honeyman and Santschi, 1989) while the slower transfer involved trace (colloidal) organic constituents with ligand groups having different trace metal affinities. This view requires the colloidal phase to be non-homogeneous with respect to metal content, which is supported by later findings (Wells *et al.*, 1998; Wells *et al.*, 2000).

Recent evidence adds strong support to the hypothesis that colloid aggregation is prevalent and rapid in ocean waters. Chin *et al.* (1998) used dynamic light scattering to show that aggregation of soluble and colloidal organic matter quickly forms particulates several microns in size once the ambient particulates are removed by conventional filtration (Fig. 5). They went on to demonstrate that these molecule associations were stabilized by salt bridges, as evidenced by aggregate disintegration upon the addition of EDTA to bind Ca^{2+} and Mg^{2+} ions. The pH-induced phase transitions of these particulates were consistent with the

tangled network theory of polymer gel formation (Edwards, 1986), indicating that these aggregates were in true equilibrium with soluble and colloidal-sized polymers. If so, these findings turn previous concepts of marine colloid aggregation upside down (Wells, 1998). Rather than colloid aggregation being driven by instability of the colloidal phase, it instead would be controlled by an equilibrium-based relationship with particulate gels. Aggregation kinetics then would be a direct function of the rate that particulate gels are lost from surface waters via sinking or perhaps microbial degradation, as indicated by the reformation of microgels after repeated filtrations (Chin *et al.*, 1998). The particulate and colloidal gel phase would remain in pseudo-steady state as long as gel precursors were present in (or added to) the soluble phase. This process will complicate efforts to establish whether the release of organic matter by actively growing phytoplankton assemblages directly alters the dynamics of colloidal trace metal cycling in surface waters (e.g. Kuma *et al.*, 2000; Wells *et al.*, 2000).

If microgels formed by this process are more readily intercepted and utilized by heterotrophic bacteria (e.g., Johnson and Kepkay, 1992; Kepkay, 1994; Kepkay and Johnson, 1988), then tangled network theory of polymer gels may provide a novel mechanistic insight to how marine microbes degrade organic matter. By degrading particulate endmembers efficiently, bacteria might facilitate the consumption of reactive soluble and colloidal organic counterparts by drawing these constituents into the particulate phase (Fig. 6). If metal-complexing organic molecules also are incorporated during gel formation, and a significant fraction of these particulate gels are removed by sinking and grazing, then microbial degradation of particulates ironically could indirectly enhance the removal of dissolved bioactive metals from surface waters, which is counter to present expectations.

IX. The Biological Availability of Colloidal Bioactive Metals

A key but largely unanswered question is how metal-colloid associations affect the availability of bioactive metals to prokaryotic and eukaryotic microbes. In most cases, metal uptake by phytoplankton is a function of the free metal ion activity in solution (e.g. Sunda and Guillard, 1976). Complexation by soluble (or colloidal) metal chelators therefore decreases metal availability, in some cases lowering it to levels optimal for organism growth (Sunda and Huntsman, 1995b). (The chemical speciation constraints for Fe (and Co) uptake are considerably more involved – see Nolan *et al.*, 1992.; Wells *et al.*, 1995) This complexation also serves to buffer metal ion activity, so that metal is released to the ionic pool in response to biological uptake (Huntsman and Sunda, 1980). It also is expected that complexation slows the rate of metal loss from surface waters by limiting the direct sorption of metals to sinking particulates (Honeyman and Santschi, 1989; Jannasch *et al.*, 1996) (but see above).

We now know that some portion of these highly specific, strong chelators of bioactive metals occur in marine colloidal matter (Muller, 1996; Muller, 1998; Wells *et al.*, 1998). Presumably these colloidal ligands also modulate free metal ion activities and thus influence the availability of bioactive metals (but see Mackey and Zirino, 1994). Wang and Guo (2000) found that addition of marine colloidal matter to cultures of a diatom and dinoflagellate decreased Zn uptake but increased apparent Cr uptake. However, cell surfaces were not washed to remove extracellular metals (e.g. Hudson and Morel, 1989) so it is uncertain whether the enhanced Cr uptake was due to metal assimilation or metal sorption. Nonetheless, decreased Zn uptake is consistent with colloidal complexation of free Zn^{2+} and the subsequent buffering of its subsequent availability. Nishioka and Takeda (2000) found that the growth of an oceanic *Chaetoceros* sp. appeared to be fueled by colloidal Fe rather than soluble Fe species in cultures using nutrient amended offshore seawater. But it is

uncertain whether this colloidal Fe existed as colloidal-sized organic complexes or as reactive Fe(III) oxyhydroxides, which can readily dissolve to re-supply soluble chemical species taken up by phytoplankton (Rich and Morel, 1990; Wells *et al.*, 1983).

While it is unlikely that phytoplankton and heterotrophic bacteria can take up colloidal matter directly (but see Maranger *et al.*, 1998), the colloidal phase still might be an important direct source of metals to heterotrophic flagellates (Barbeau and Moffet, 2000; Tranvik *et al.*, 1993) and filter-feeding tunicates (Flood *et al.*, 1992) and bivalves (e.g. Roditi *et al.*, 2000, Goldberg and Wells, unpublished data). Much more work is needed before the significance of the colloidal pathway for metal assimilation by animals can be assessed.

If the immediate fate of colloidal matrices is microbial degradation, then the associated metals would be recycled to the soluble phase, either as free ions or in liberated ligand complexes. The net result would be that colloidal processes act to retain bioactive metals in solution by slowing their sorption to sinking particulates. However, if the metal ligand complexes instead are associated with colloidal constituents that rapidly aggregate, the marine colloidal phase may act to strip bioactive metals from surface waters. The effect of marine colloidal matter on metal availability thus depends upon the balance struck between these opposing fates. The nature of this balance will depend upon the type and quantity of organic matter released by phytoplankton that, in return, may be controlled by nutrient availability and the character of the algal assemblage. It is likely then that the fate of colloidal metals will shift in response to seasonal and short-term episodic perturbations in the biogeochemical cycle.

X. Summary

The marine colloidal phase is a major component of DOC in the oceans and contains with it a wide range of metals that affect the growth of marine phytoplankton. Given that this

association will alter the biological availability of dissolved metals, colloid-metal interactions almost certainly influence biological production and thus the generation of COC. Advances over the last decade have established this bi-directional coupling but details of the cycling remain obscure. For example, while the ultimate source of COC is marine phytoplankton, it is unclear whether discrete colloidal particles arise from aggregation of soluble organic constituents or by direct release from degradation of living and detrital matter. While the last ten years has seen a sharp increase in the study of colloidal metals in nearshore and estuarine waters, there have been far fewer studies in the open ocean where the bulk of carbon fixation occurs.

Our ability to study the marine colloidal phase has been challenged over the last decade by the limited number of methods available to isolate marine colloids from soluble constituents, and by a lack of consensus over how the colloidal phase should be defined. Recently, novel techniques have been applied to study colloid composition and the kinetics of colloid cycling, and these will undoubtedly attract considerable interest over the next decade.

Despite these difficulties, a picture is emerging of the distribution and significance of marine colloids with respect to bioactive (nutrient) metals as well as other geochemically important elements. These colloidal metal associations can be attributed in large part to specific and strong metal complexation rather than non-specific metal sorption to the bulk colloid interface. This specificity greatly complicates efforts to generate meaningful generalizations and predictions of the colloidal metal contents of different seawaters. Early indications are that global scale processes such as aerosol transport can influence the concentrations and metal composition of the marine colloidal phase, not by direct deposition but indirectly by influencing the biological processes that produce and maintain the pool of marine colloidal organic matter.

Perhaps the single greatest unresolved issue is deciphering the predominant fate of marine colloidal matter and its associated metals; aggregation to larger particle sizes, microbial degradation to soluble constituents, or both depending on the variable influence of other factors. Given the anticipated short residence time of marine colloids in surface waters with respect to either degradation or aggregation, the processes responsible for the outcome almost certainly are highly dynamic. With respect to trace metals, the central challenge facing scientists over the next decade will be to ascertain whether the marine colloidal phase serves primarily to buffer bioactive metals, helping to retain them in surface waters, or instead hastens metal removal from surface waters, thus impacting the marine phytoplankton community.

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Table 1. Compilation of the % colloidal fraction of metals in dissolved (generally < 0.45 µm) estuarine and coastal waters.

Location	Salinity	Cutoff	Al	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb	Se
Nearshore													
Bothnic Bay, Baltic Sea (Gustafsson <i>et al.</i> , 2000)	1.4 – 3	3 kDa			84-96								
Buzzard's Bay (Bertine and VernonClark, 1996)		1 kDa	2		6		1	5					
Danube River Estuary (Guieu <i>et al.</i> , 1998)	0 - 17	10 kDa		3 - 70	0 - 88			10 - 45			0 - 43		
Firth of Clyde (Muller, 1998)	18-33	3 kDa						39 - 73			0 - 31	0 - 85	
Funka Bay, Japan (Kuma <i>et al.</i> , 2000)	32 - 34	> 0.025 µm			3-70								
Galveston Bay (Benoit <i>et al.</i> , 1994) ^a		10 kDa	100	2	100			4	2			100	
Galveston Bay (Wen <i>et al.</i> , 1999)	0 - 34	1 kDa			44 -96	5 - 32	12 -47	48 -89	84 -97		12 - 58	51 -88	
Southern England estuarine and coastal waters (Muller, 1996)	22-36	3 kDa						22-51			10-56	28-64	
Halifax Harbour (Whitehouse <i>et al.</i> , 1990)	30	10 kDa		22	78			35					
Narragansett Bay (Santschi <i>et al.</i> , 1987)	30	1 kDa			10-70				2-20	2-20			30-60
Narragansett Bay (Wells <i>et al.</i> , 1998; Wells <i>et al.</i> , 2000)	25 -30	1 kDa		1 – 5	93 - 99		19 - 40	35 - 53	2 - 14		3 - 37	35 - 41	
Ob and Yenisey Estuarine Systems (Dai and Martin, 1995)	0 – 35	10 kDa			30 - 97		50 - 60	17 - 65			1 - 76	22 – 52	
Ochlockonee Estuary (Guentzel <i>et al.</i> , 1996; Powell <i>et al.</i> , 1996)	0 - 29	1 kDa		0 - 30	80 - 98		0 - 78	20 – 90			20 -67		
Rhone Delta (Dai <i>et al.</i> , 1995)	0-38	10 kDa					0 - 18	21 - 39			0 -38		
San Francisco Bay (Kozelka <i>et al.</i> , 1997; Sanudo-Wilhelmy <i>et al.</i> , 1996)	0 - 32	10 kDa	3 - 99	2 – 20	4 – 88		0 – 2	1 – 18	1 – 3		0 – 9	1	
Texas Estuaries (Stordal <i>et al.</i> , 1996)	0 - 30	1 kDa											
Trinity River, Texas (Wen <i>et al.</i> , 1996)	28	10 kDa		7	75		38	66	9		15	15	
Venice Lagoon (Martin <i>et al.</i> , 1995)	0 - 35	10 kDa		54	87		18	46			34	58	

^a Colloidal metal concentrations not corrected for dissolved component left in the retentate

Table 2. Compilation of the % colloidal fraction of metals in dissolved (generally < 0.45 µm) offshore waters.

Location	Salinity	Cutoff	Al	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb	Hg
Offshore													
North Atlantic (Moran and Moore, 1989)	31-34	1 kDa	1 - 15										
North Atlantic (Wu and Luther, 1994) ^a	35 – 36.7	0.2 µm			20 - 45								
Hawaii – Deep (Reitmeyer <i>et al.</i> , 1996; Wen <i>et al.</i> , 1996)		1 kDa	0 - 11				9	63			2	9	
Gulf of Maine (Greenamoyer and Moran, 1996)		1 kDa					0 - 45	34			6 - 67		
North Pacific (Nishioka <i>et al.</i> , 2001)					13 - 50								
Equatorial Pacific (Wells, in press)	35	1 kDa			10								

^aThe colloidal size range of 0.2-0.4 µm is much larger than normally accepted but the results are included here for reference.

List of Figures

Figure 1. A graphical depiction of a chemcentric definition for colloidal matter. Here, the effect of particle dynamics processes on the mass distribution over the various size classes is illustrated. Steady state particle size distributions (mass-based) are shown for three aquatic regimes having large differences in solids concentrations. The inflection point of the each line where the slope changes is the functional distinction between colloids and "gravitoids" (sinking particles). The shaded areas depict the traditional, operational boundaries between soluble, colloidal and "particulate" fractions. Based on a chemcentric approach, the upper size boundary of colloidal matter shifts in conjunction with the total solids concentrations in the water. (from Gustafsson and Gschwend, 1997)

Figure 2. Transmission electron microscope images of marine colloidal matter from a depth profile in the Sargasso Sea. Colloids were settled directly onto charged TEM grids by ultracentrifugation (see Wells and Goldberg, 1994). Differences in colloid size, morphology and abundance are readily apparent, with the larger colloids being most prevalent near the base of the photic zone (100 m) and immediately below (300 m). These globular colloidal particles as well as more electron-transparent colloidal particles are seen in nearshore waters when embedding and staining methods are employed (Leppard *et al.*, 1997). Scale bars denote 100 nm.

Figure 3. The components and theory of FFF separation. A) The ribbon-like channel has a breadth of a few centimeters and a thickness of ~100 μm . The outflow is directed to a detector for various optical or metal analyses. B) The lengthwise cross section of the channel shows the different distributions of arbitrary components X and

Y across a parabolic flow profile and the unequal flow velocities that result. The characteristic mean elevations of the X and Y “clouds” are indicated. C) Illustration of the flow field across the channel in Flow FFF. (Adapted from Giddings, 1993)

Figure 4. The 3 box scavenging model used by Moran and Buesseler (1992; 1993) to calculate the residence times of ^{234}Th in the dissolved, colloidal and particulate size classes. The variables J_{th} , C_{th} , and P_{th} , are the net removal rates of ^{234}Th from the various size classes and λ is the ^{234}Th decay constant. In this simplified approach, the processes of ^{234}Th desorption from particulates and colloids and microbial degradation are not considered. (From Moran and Buesseler, 1993)

Figure 5. Polymer gel size in seawater as a function of time after conventional filtration ($<0.2 \mu\text{m}$). The hydrodynamic diameter of assembling gels was measured by Dynamic Light Scattering (filled circles, solid line) and by flow cytometry (empty squares, dashed line). Poisoning the seawater with 0.02% sodium azide did not alter the outcome of the assembly process (empty circles). A control experiment, where 10 mM EDTA was added to chelate Ca^{2+} and Mg^{2+} showed inhibition of polymer assembly (empty triangles), giving an average size of 1-2.5 nm. This inhibition of polymer assembly suggests that aggregate formed by salt-bridging among the constituent polymers. (From Chin *et al.*, 1998)

Figure 6. Schematic of the dynamic interplay between marine gel formation via colloid aggregation and the microbial degradation of particulate gels. Thickness of the arrows depicts the expected relative reaction rates. According to polymer gel theory, the particulate gel phase is in equilibrium with the low molecular weight gel

constituents, so that removal of particulate gels by sinking or microbial degradation results in further aggregation. (from Wells, 1998)

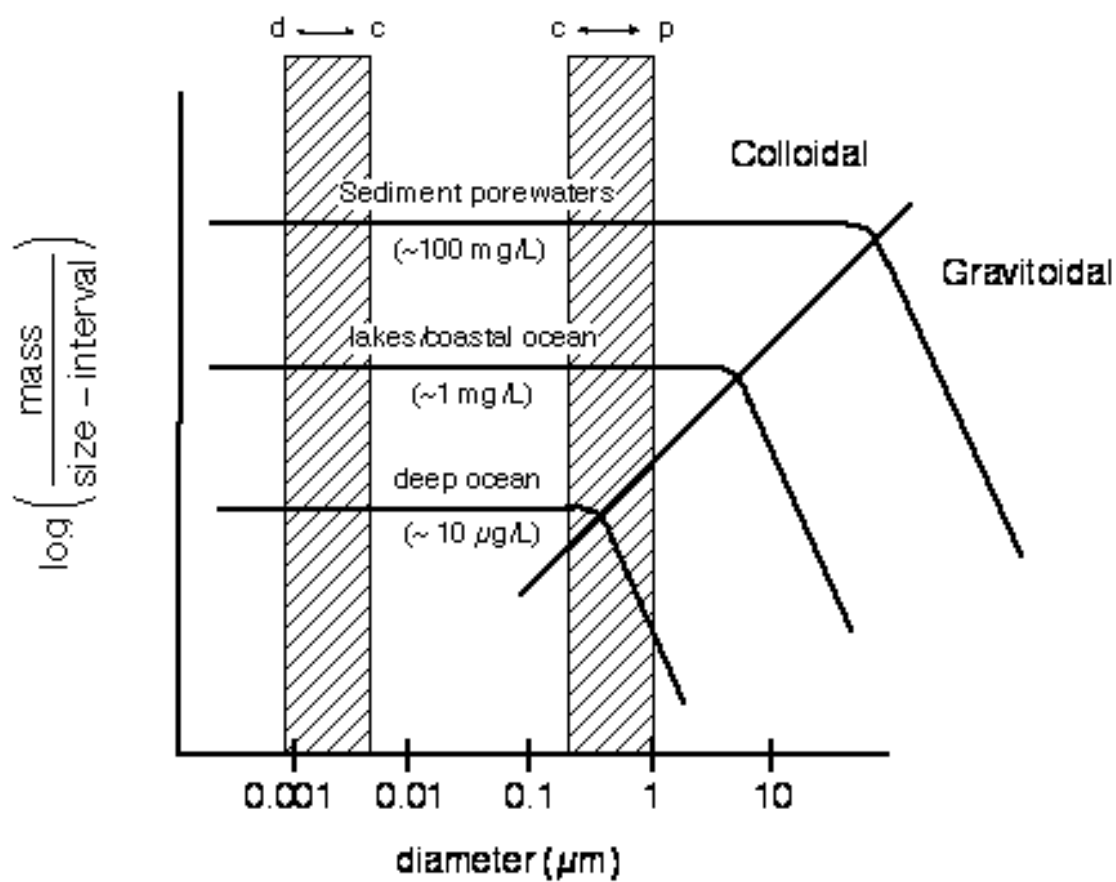


Figure 1

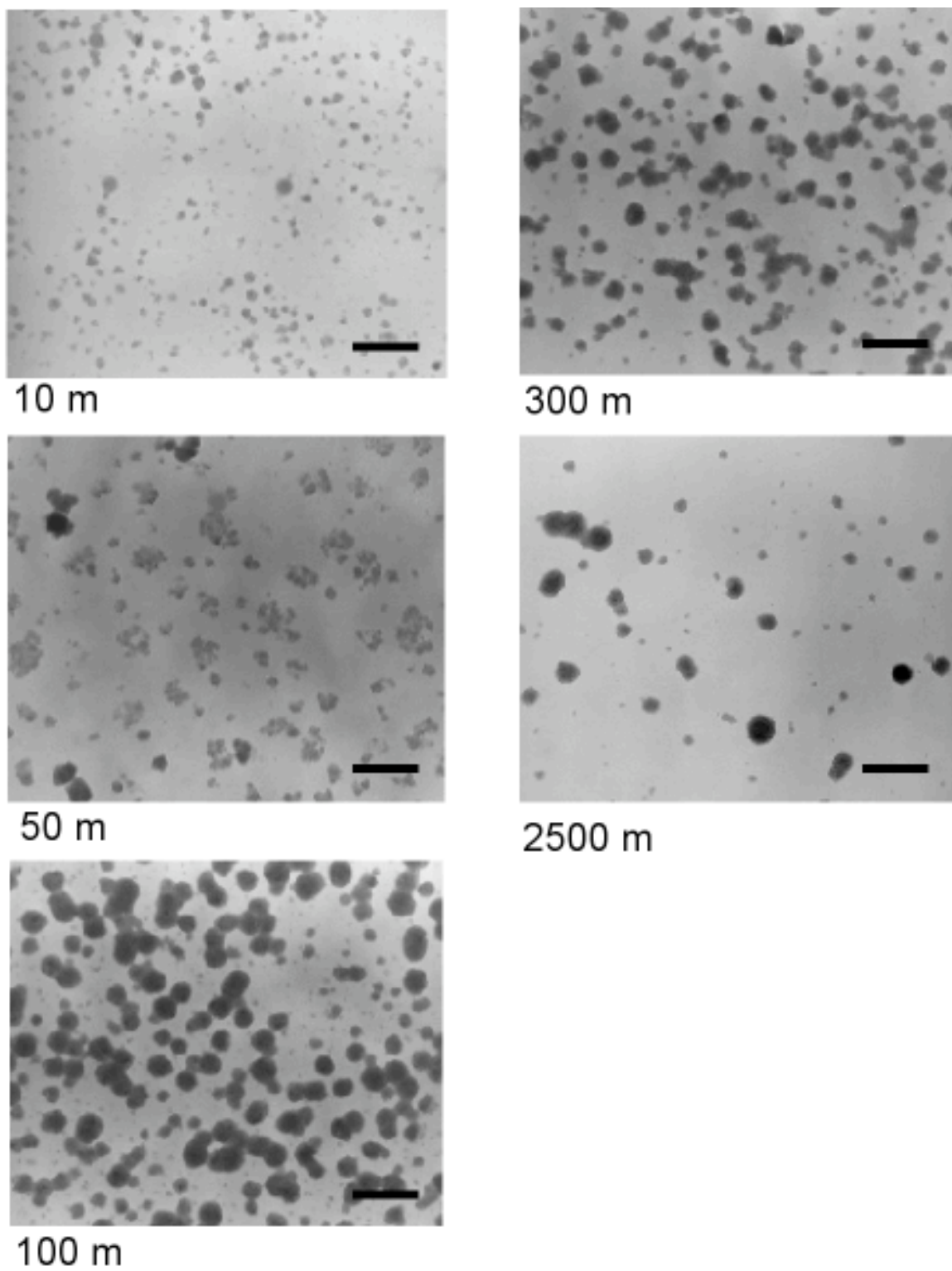


Figure 2

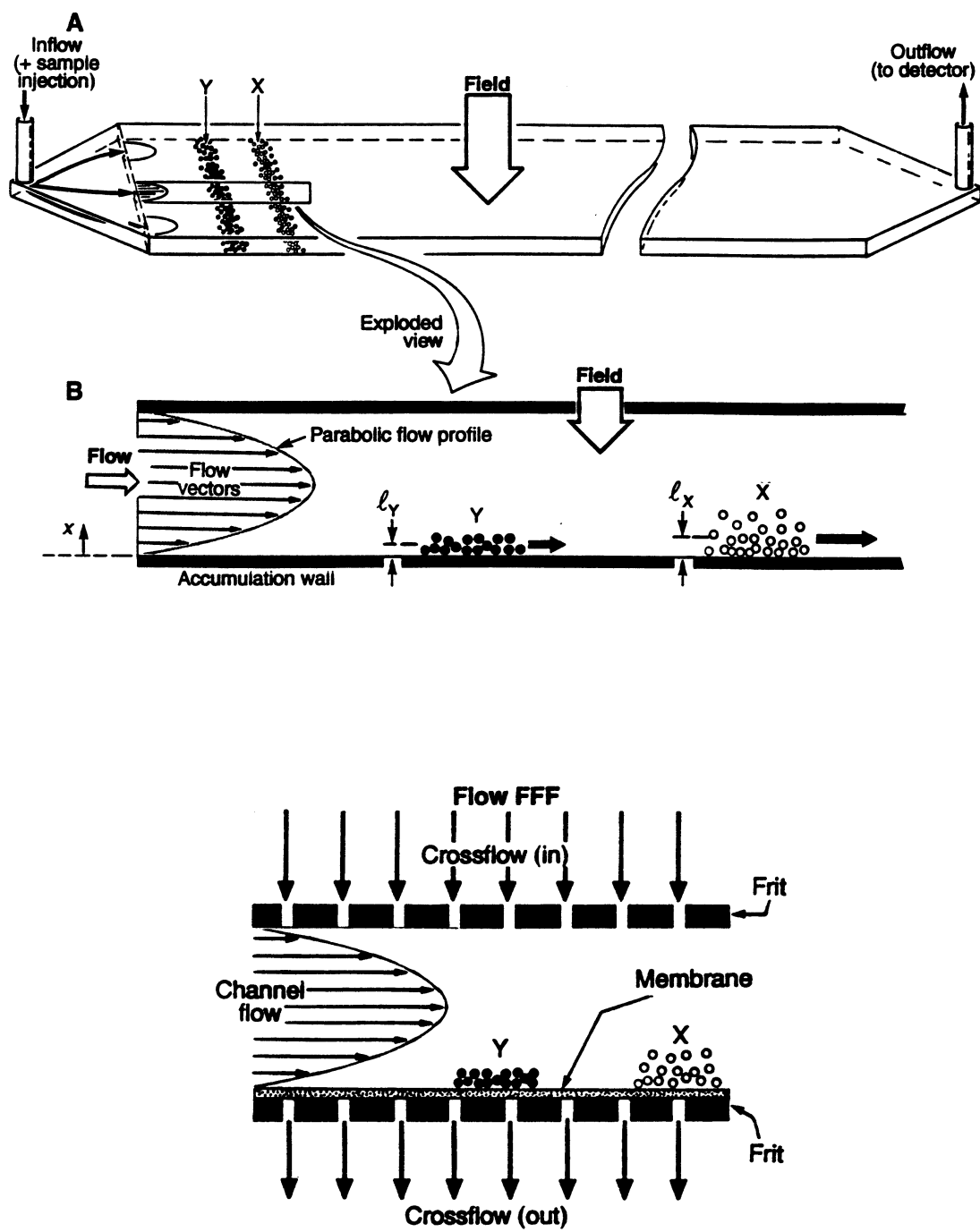


Figure 3

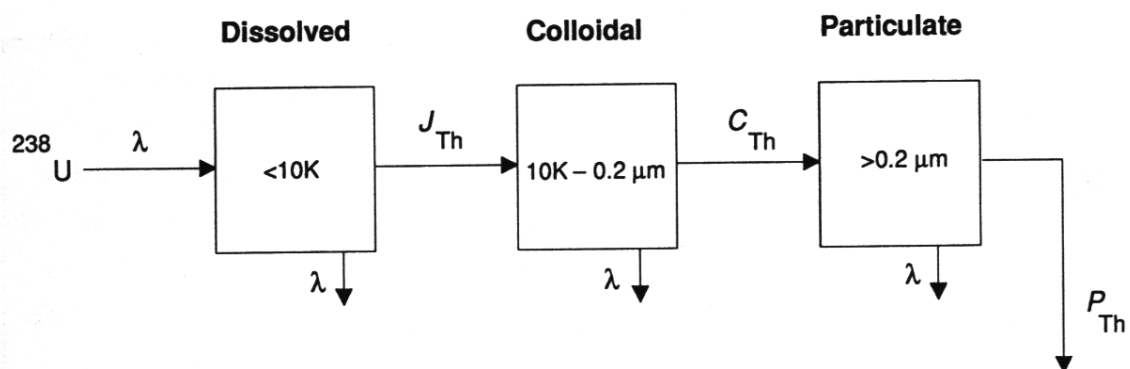


Figure 4

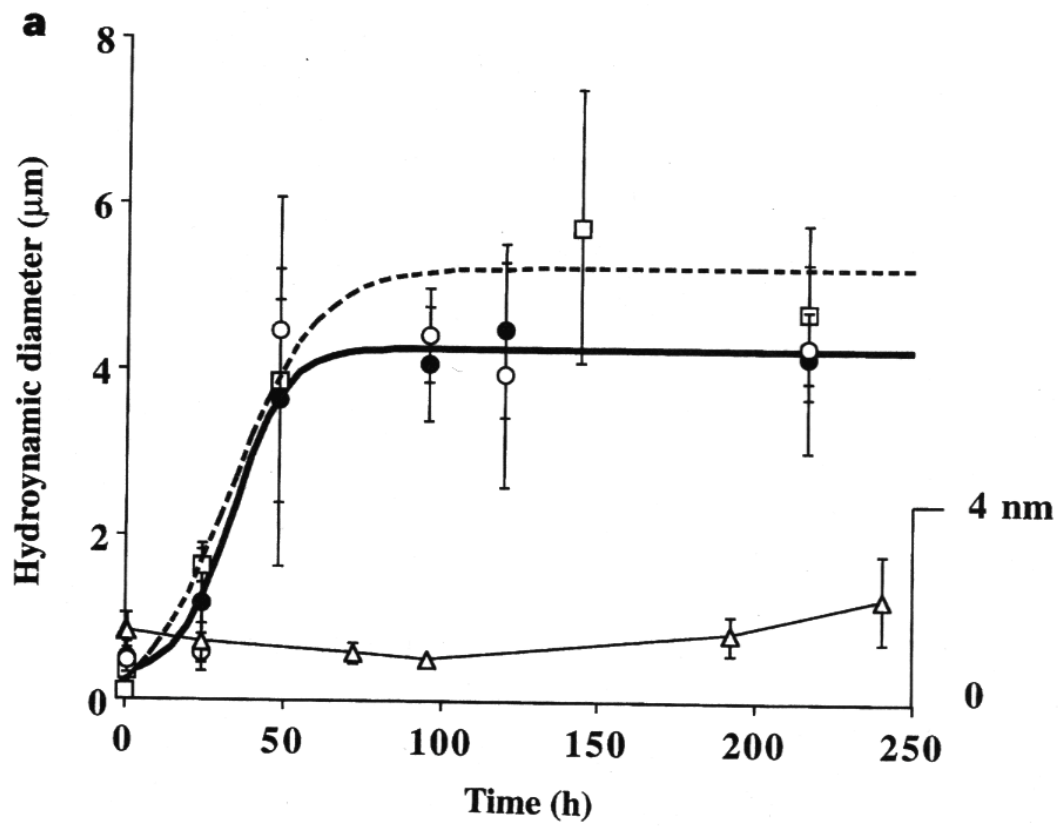


Figure 5

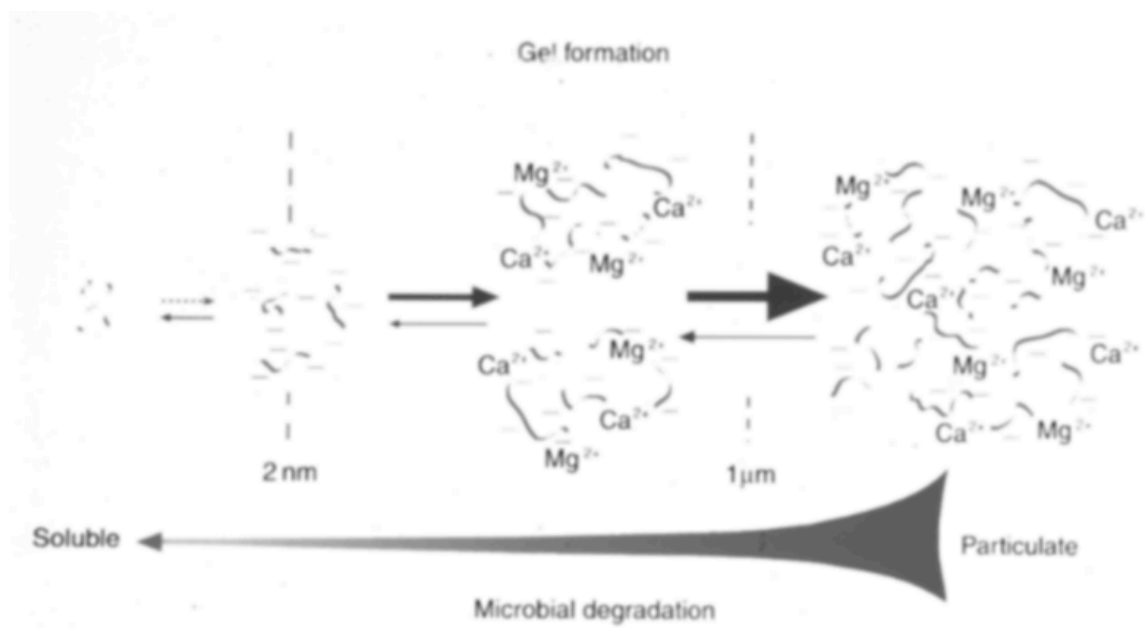


Figure 6