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Marine submicron particles

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ABSTRACT

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The qualitative and quantitative analyses of submicron particles from coastal surface seawaters off California involved their separation by ultracentrifugation, visual examination by transmission electron microscopy (TEM), and the determination of elemental composition by energy dispersive spectroscopy. Particles fell into three classes: organic, both living and detrital; inorganic, such as iron colloids; clay minerals. The greatest abundance of particles occurred in the smaller than 120 nm size fraction. The mass concentration for particles sized between 5 and 120 nm is estimated to range between 0.03 and 0.09 mg l^{-1}. Particle numbers in this size range were greater than 10^9 particles ml^{-1} with the total surface area being 8 m^2 per cubic meter of seawater, or more. Particle size distributions in the smaller than 120 nm fraction showed numbers to increase nearly logarithmically with decreasing size. TEM examination indicates that many of these particles are in fact aggregates of granules 2–5 nm in size.

INTRODUCTION

The involvement of submicron particles in the chemistries of the oceans is understudied, according to a recent evaluation by 100 chemists drawn from both the mother science and from a subgroup of marine chemists (Goldberg, 1988). It was argued that the components of the colloidal state (an operational definition for particles 1-1000 nm in size) are essentially unknown, both qualitatively and quantitatively, and that a systematic characterization of this material in seawater is needed.

The colloidal state may be substantially involved in all phases of marine science. Submicron particles, which include clay minerals and organic detritus, contribute to the scattering and absorption of light in seawater, well evidenced by the Tyndall effect. They may also serve as seeds for the aggregative formation of larger particles and, therefore, could strongly affect sedimentation processes (McCave, 1984). Additionally, the surface characteristics

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of colloidal particles may control the partitioning between free and adsorbed metals, and hence their behavior in seawater. For example, the aqueous chemistry of some radionuclides appears to be dominated by submicron particles (Santschi et al., 1987; Oriandini et al., 1990). Submicron particle surfaces may also be sites for photochemical reactions that alter the nature of organic and inorganic constituents (e.g. Kieber and Mopper, 1987; Wells and Mayer, 1991). Finally, living organisms also occur in the colloidal size range, including picoplankton, bacteria, and marine viruses, which are as small as 30 nm (Bergh et al., 1989).

This preliminary investigation involves a qualitative and quantitative survey of submicron particles in California coastal surface waters, with the associated goal of developing field and laboratory techniques for examining this size fraction.

METHODOLOGY

The analysis of submicron particles in ocean waters involved: (1) their separation from seawater by ultra-centrifugation; (2) visual examination by transmission electron microscopy (TEM); and (3) the determination of the elemental composition of some particles by energy dispersive spectroscopy (EDS).

Surface seawater was collected 2–3 km offshore of the Scripps Institution of Oceanography in 250 ml teflon bottles opened below the surface microlayer ahead of a slowly moving boat. Submicron particles were deposited directly on to TEM specimen grids (Ted Pella®, P.O. Box 2318, Redding, CA) using ultra-centrifugation in a procedure adapted from that of Nomizu et al. (1988). The 200 mesh Cu grids, coated with a 200 Å thick formvar substrate overlaid with 150 Å of elemental carbon, were treated with 60 s of glow discharge under vacuum to render them hydrophylic; charging improves particle recoveries and prevents particle agglomeration during drying (Fleisher et al., 1967). For each sample, two charged specimen grids were placed on to a specially designed support in the bottom of a 13 ml polyallomar centrifuge tube (Fig. 1) and 10 ml of seawater was added slowly by pipette. The seawater was centrifuged (Beckman LM-70) in a swing-bucket rotor (SW-41) at 41 000 rpm (288 000 *g*) for 4 h at 25°C.

After centrifugation, the supernatant was drawn slowly from the tubes with a peristaltic pump and the specimen grids were rinsed gently in place with 5 tube volumes of filtered (100 nm, Nuclepore®) glass-distilled water. Sample processing was completed within 8 h of collection. Particle recovery efficiencies were determined by centrifuging monodispersed suspensions of synthetic colloidal hematite (ca. 800 nm; Matijevic and Scheiner, 1978) and commercial latex microspheres (88 nm; SPI, P.O. Box 656, West Chester, PA); recoveries were $97 \pm 13\%$ and $93 \pm 0.2\%$, respectively.

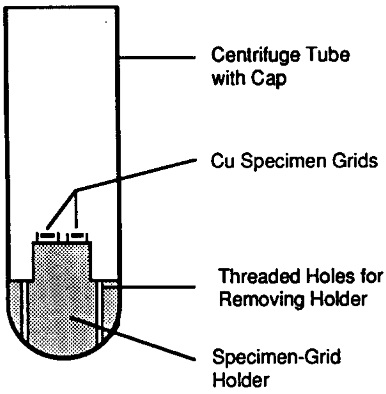

Fig. 1. The centrifugation apparatus.

The rinsed grids were dried in a vacuum dessicator overnight and examined at from 9000 × to 260 000 × magnification using a Phillips CM30 transmission electron microscope operated at 50–150 kV. Microscope fields were randomly selected from different grid openings and photographed. Particles in these photographs were separated into size classes on the basis of ruler measurements of the smallest diameter and counted; the rationale was that this dimension represents the smallest pore diameter through which a rigid particle could pass. These data were converted to particle numbers per cm^3 using the taper method for correcting for non-parallel particle trajectories during centrifugation (Mathews and Buthala, 1970). The elemental composition of individual particles was obtained qualitatively with an interfaced energy dispersive spectrometer (Link AN10) after reducing the spot size of the electron beam on to only the particle of interest. Owing to analytical limitations, particles smaller than ≈ 100 nm could not be characterized fully.

To minimize particle contamination, all pieces of equipment were washed with detergent and rinsed thoroughly with filtered (100 nm, Nuclepore®) glass-distilled water before use. Final rinses were carried out in a laminar flow air-bench (Class 100) and the samples were sealed during centrifugation. Analytical blanks were prepared by processing specimen grids in the same way as for the samples but without centrifugation. Very few, if any, particles were found in the blanks.

Particle sedimentation during centrifugation is a function of particle size and density and can be calculated by Stokes law (see Nomizu and Mizuike, 1986). The centrifugation times for quantitative sedimentation of 10, 15, and 20 nm diameter particles of differing density from seawater (6.9 cm fluid height) at 41 000 rpm are shown in Fig. 2. The 4 h of centrifugation used have been calculated to give quantitative recoveries of particles 15 nm and larger

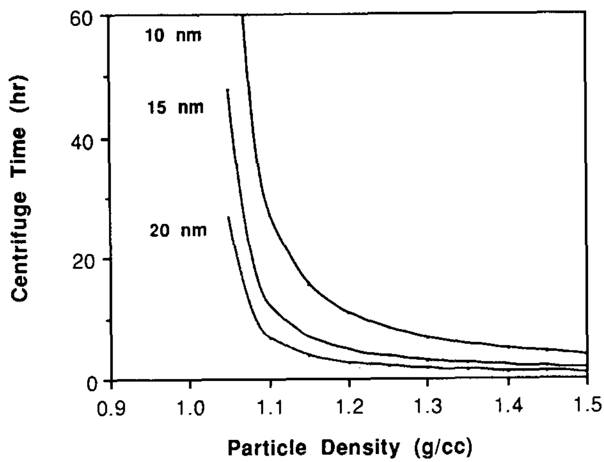

Fig. 2. The calculated centrifugation times for quantitative sedimentation of 10, 15 and 20 nm particles having different densities. Sedimentation times are calculated from Stokes settling rates in a 6.9 cm column of seawater centrifuged at 41 000 rpm at 25°C using the formulation in Nomizu and Mizuike (1988).

having densities of 1.22 g cm^{-3} or greater; smaller or less dense particles would be recovered non-quantitatively.

RESULTS AND DISCUSSION

The range of particle sizes observed on the TEM specimen grids extended from 5 nm to greater than 40 μm. Particle compositions were categorized into three groups; organic materials (both living and detrital), inorganic materials (e.g. iron colloids), and clay minerals (e.g. aluminosilicates).

The living organic matter in the submicron size range included relatively abundant, small bacteria and viruses, which were clearly identifiable in the EM photographs. These organisms were characterized by low electron densities (i.e. they were relatively transparent), by weak EDS signatures, and, in most cases, by the presence of phosphorous. An example of a marine virus and its associated EDS spectrum is shown in Fig. 3. The copper peaks (K_α and K_β) and, to a certain extent, the O peak are derived from the specimen grid and do not reflect the 'particle' composition. Those particles having little evidence of an organized structure (e.g. cell walls, regular shape, etc.) and an absence of element signatures, including phosphorus in the EDS spectra were considered to be non-living 'organic' matter; most of the particles examined fell into this category. Although organisms were not quantified separately from the non-living particles, their relative abundance in the samples illustrates that cell division and viral replication would be a significant source of submicron particles, a factor often ignored in discussions of the colloidal state in seawater.

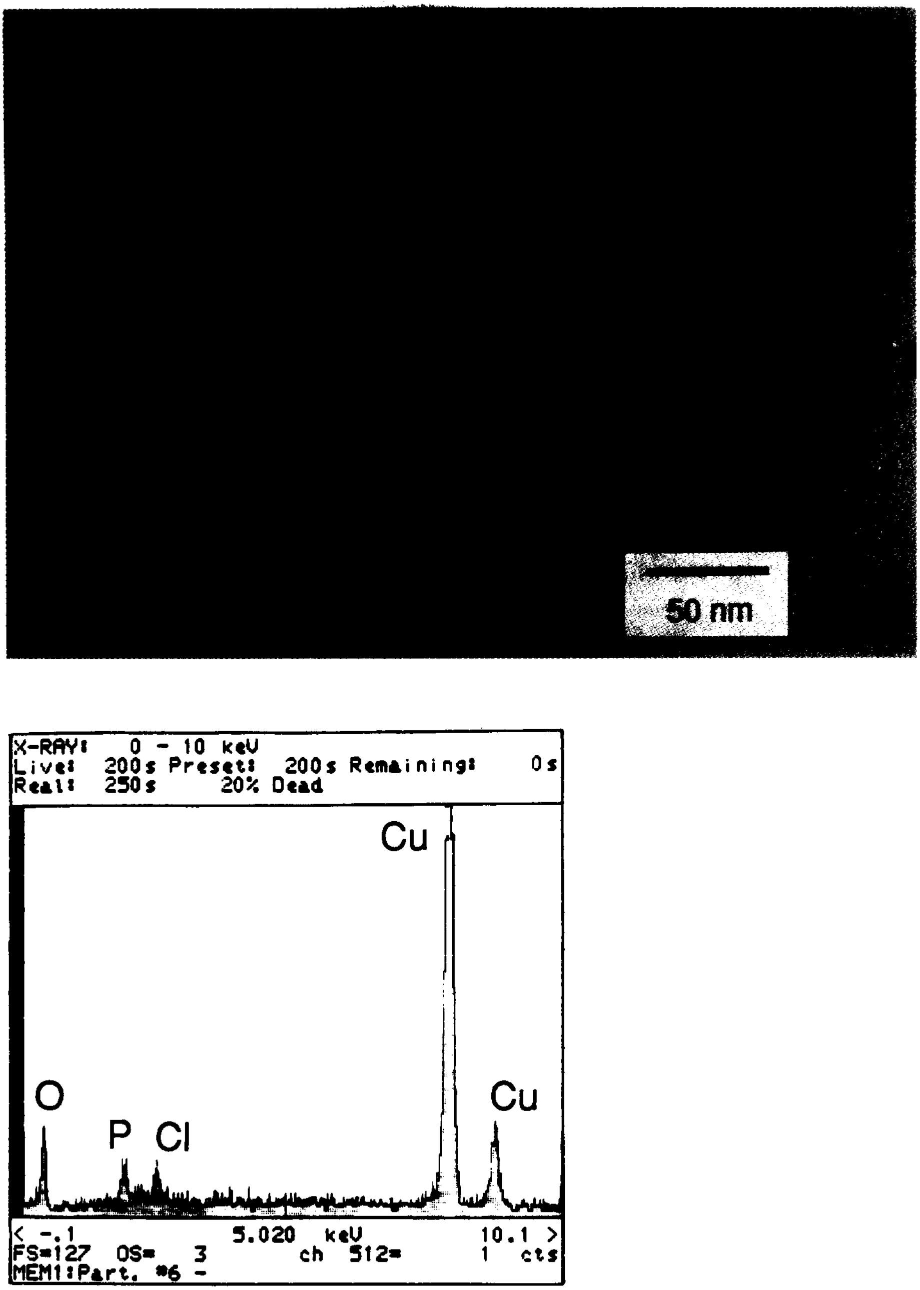

Fig. 3. A TEM micrograph of a marine virus and its associated EDS spectrum showing the presence of phosphorus. The large copper peak, and probably much of the oxygen peak, are from the specimen grid and formvar coating, respectively: residual chlorine (but not sodium or magnesium) is sometimes found, perhaps reflecting anion adsorption on the charged grids.

A number of particles in the 100–500 nm size range were composed almost exclusively of iron (Fig. 4). These particles often were visually distinguishable as aggregates of elliptically shaped particles. In some cases, weak electron diffraction patterns were observed demonstrating that these iron particles were crystalline in nature, although their mineralogies were not determined. Single particles containing only titanium or silicon were also observed. Aluminosilicates occurred in the 200–1000 nm size fraction and significant amounts of aluminum and silicon were also detected in some particles in the smaller than 100 nm fraction; the latter were too small to be characterized fully by EDS analyses.

While a full range of submicron particle sizes was observed, particles smaller than 120 nm were by far the most abundant. The following discussion deals mainly with this small size fraction. Here, particle numbers increased nearly exponentially with decreasing size classes in each of the three samples, with total particle numbers being greater than 10^9 particles cm^{-3} (Figs 5(A)–(C). A similar particle size distribution occurred in seawater pumped from the end of the Scripps Institution of Oceanography pier, just offshore of the surf zone, although the number of smaller than 120 nm sized colloids was an order of magnitude greater (data not shown). These particle numbers likely represent a lower estimate of actual values because the smaller colloids may not have been sedimented quantitatively during centrifugation (see below). It should be noted that the extent to which particle sizes were altered as a result of settling on to the specimen grid and subsequent dehydration is unknown. Verification of these size measurements awaits the development of methods capable of measuring in situ the dimensions of very small colloids.

The high abundance of small submicron particles does not appear to be related to the break-up of larger aggregates during sample processing. Gentle (< 3 p.s.i.) prefiltering (400 nm Nuclepore®) of the seawater did not alter significantly the number or size distribution of colloids. Moreover, because shear forces across submicron distances are negligible (McCave, 1984), physical disruption of smaller aggregates (120–400 nm) also is unlikely. Indirect evidence that small particles were not disrupted during centrifugation is that the latex microspheres used to determine recovery efficiencies remained clumped unless ultrasonicated immediately before centrifuging.

The size distributions of marine particles and atmospheric dusts in the 1–100 μm size range generally follow the power-law distribution:

$$N = kd^{-\beta} \text{ (particles cm}^{-3}\text{)} \qquad (1)$$

where N is the cumulative number of particles greater than d, the particle diameter, k is a constant with units of particles cm^{-3} and β is a dimensionless constant (Junge, 1969; McCave, 1984). Plotting N vs. d on log–log graphs yields β as the slope of the straight line portion of the size spectrum. A value

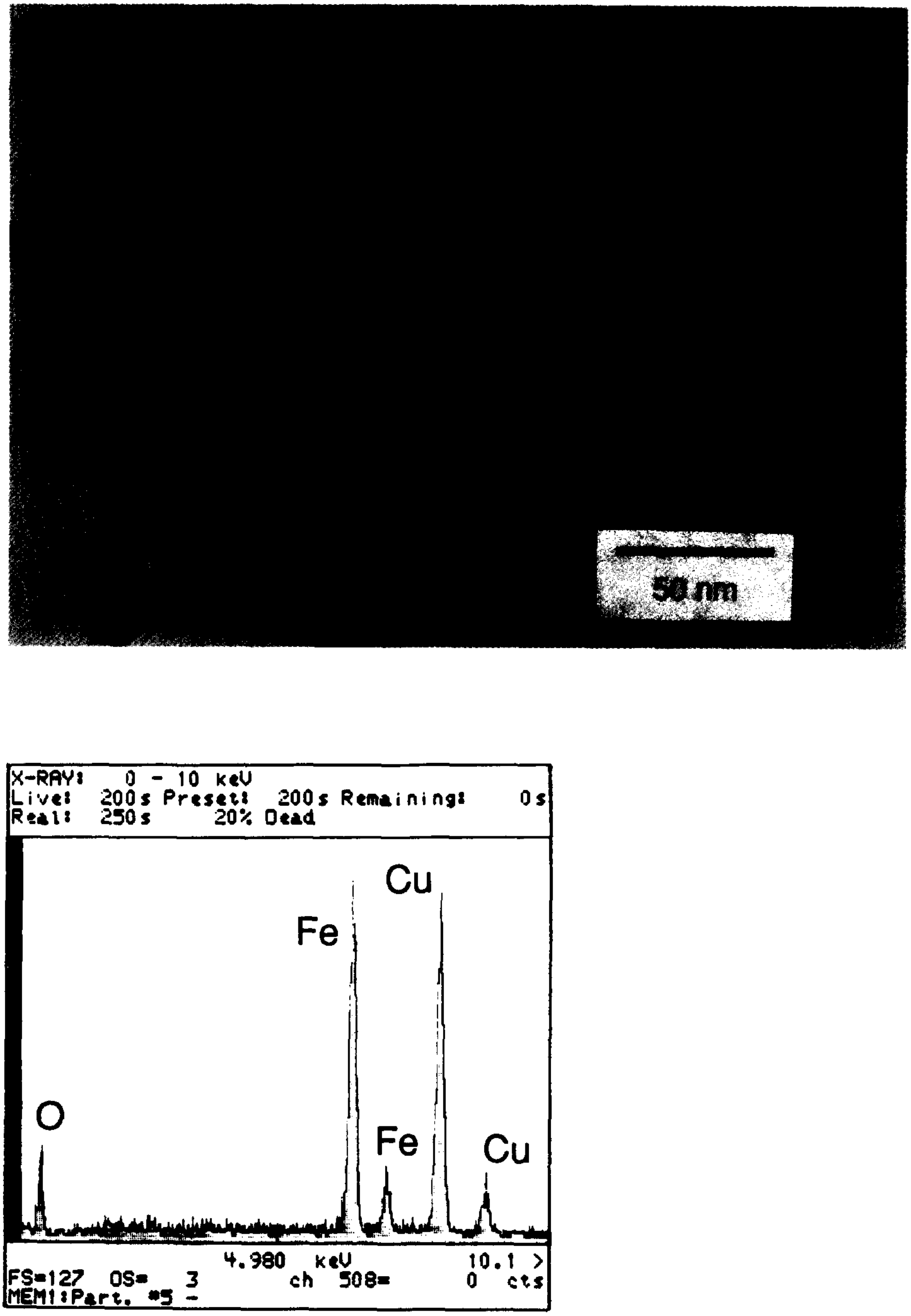

Fig. 4. A TEM micrograph of a submicron marine aggregate and its EDS spectrum indicating that it is composed largely of iron. These characteristic eliptical particles were commonly observed and they exhibited electron diffraction patterns indicative of a crystalline matrix.

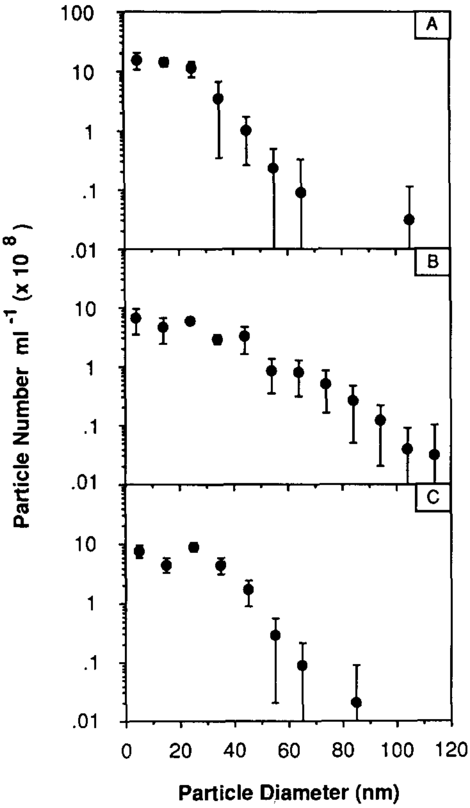

Fig. 5. The size distribution of submicron particles in the smaller than 120 nm fraction in surface coastal seawater. Dates of collection are: (A) 17 May 1988, (B) 25 June 1990, and (C) 29 June 1990. Total particle numbers in this size range were 4.5×10^9, 2.6×10^9 particles ml^{-1}, and 2.8×10^9 particles ml^{-1}, respectively. Error bars represent one standard deviation ($n = 8$).

of $\beta = 3$ indicates that total particle volumes are equal in logarithmically decreasing size fractions.

The cumulative number data are not linear through the entire 5–120 nm size range, though particle sizes greater than 40 nm do appear to be consistent with the power-law relationship in eq. (1) (Figs. 6(A)–(C). In all three samples, particles between 40 and 120 nm in size yielded $\beta \geqslant 5$, signifying that the intergrated particle volume increased with decreasing particle size. By comparison, marine macroparticles generally have $\beta \approx 3$ (Sheldon et al., 1972; McCave, 1984). Harris (1977) obtained a slope of 1.65 for the smaller than 100 nm size fraction in Gulf of Mexico waters retained on interpore surfaces of 450 nm pore-sized filters. The considerably lower value of β he found may be due largely to incomplete particle recoveries, particularly in the smallest

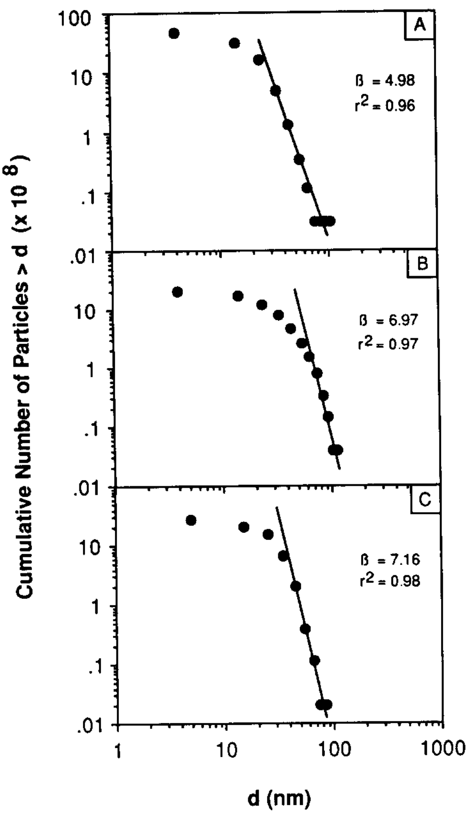

Fig. 6. The cumulative number distribution of submicron particles of diameter greater than *d*. Data were taken from Fig. 3. The power-law distribution (eqn. 1) is fitted to these data for the linear portion of the size spectrum (> 40 nm). The negative slope of the line is given as β (see text).

size fractions. However, large intersite differences in the submicron size spectra cannot be ruled out.

Size spectra such as in Figs. 6(A)–(C), where the number of small particles (< 40 nm) falls below that predicted by eqn. (1), are also found in the macroparticle (1–100 μm) size range and usually are attributed to the aggregation of smaller particles into larger size classes (Lerman, 1979). Here, it is not clear whether the pronounced curvatures are due to aggregation, to poor recoveries of the smaller particles, or to both. An incomplete sedimentation of smaller than 40 nm particles after 4 h of centrifugation would infer that they had densities of $\approx 1.07\,g\,cm^{-3}$ or less, thereby implying a largely organic nature. However, these calculations of particle sedimentation rates rely upon Stokesian assumptions, which have been shown to underestimate micron-sized particle sinking rates in natural seawater by up to an order of magnitude (Chase, 1979). It is, therefore, not clear whether there was incomplete recovery of the smaller than 40 nm size fraction even if particle densities were indeed less than 1.07 $g\,cm^{-3}$.

Given that particle densities likely differ among submicron size classes, and that the abundance of colloids greater than 120 nm in size was not quantified, a complete distribution of submicron particle mass cannot be determined. However, the data in Fig. 5 permit calculation of a lower limit for the colloid mass concentration in the smaller than 120 nm size class. For spherical particles with an assumed density of 1.07 $g\,cm^{-3}$, the integrated particle mass in the smaller than 120 nm size fraction is on the order of 0.03–0.09 $mg\,l^{-1}$. This mass concentration, combined with that of larger, more dense inorganic colloids (i.e. iron oxides, aluminosilicates, etc.) suggests that suspended solid concentrations in seawater may in some cases be significantly underestimated according to current operational definitions (typically greater than 400 nm).

There are a number of possible sources for the abundant 'organic' submicron particles found in these waters. They may be derived from larger particulate matter as a result of microbial degradation, the 'sloppy' feeding practices or metabolic wastes of protozoa and larger zooplankton, or the sluffing off of materials from cell surfaces. Marine viruses may be another important mechanism for generating these 'organic' particles; viral-induced lysis (rupture) of the host bacteria and phytoplankton would release cytoplasmic materials to seawater. Alternatively, submicron particles could be expected to form by aggregation from a milieu of smaller materials, including dissolved substances. Indeed, many of the particles appeared in the TEM micrographs to be aggregations of smaller granules 2–5 nm in size, though low contrast in the unstained particles made it difficult to resolve internal structure unambiguously. Some of these inclusions were darker than others (i.e. more electron dense), indicating that these aggregates may have formed from a non-homogeneous mixture of substances (Fig. 7). An aggregation mechanism of formation also would be consistent with the cumulative number data in Figs. 6(A)–(C).

The possible aggregative formation of these small submicron particles raises questions about the stability, or reactivity of these suspensions in seawater. Particle aggregation in marine waters is a function of Brownian motion, turbulent shear, differential settling, and zooplankton feeding rates, among other factors. For particles smaller than 1000 nm, aggregation rates are dominated by Brownian motion, which determines the collision frequency, and by the coalescence efficiency, which is the fraction of collisions which result in particle adhesion (McCave, 1984). McCave suggested that particles in the Brownian range (< 1000 nm) are pumped rapidly into larger size classes by aggregation. This rapid removal is predicated on the coalescence efficiency of the colloids being near 0.1, a value determined for natural clay sediments (ca. 1–2 μm) in artificial seawater (Edzwald et al., 1974). According to McCave (1984), the coagulation half-time (t_c) can be calculated

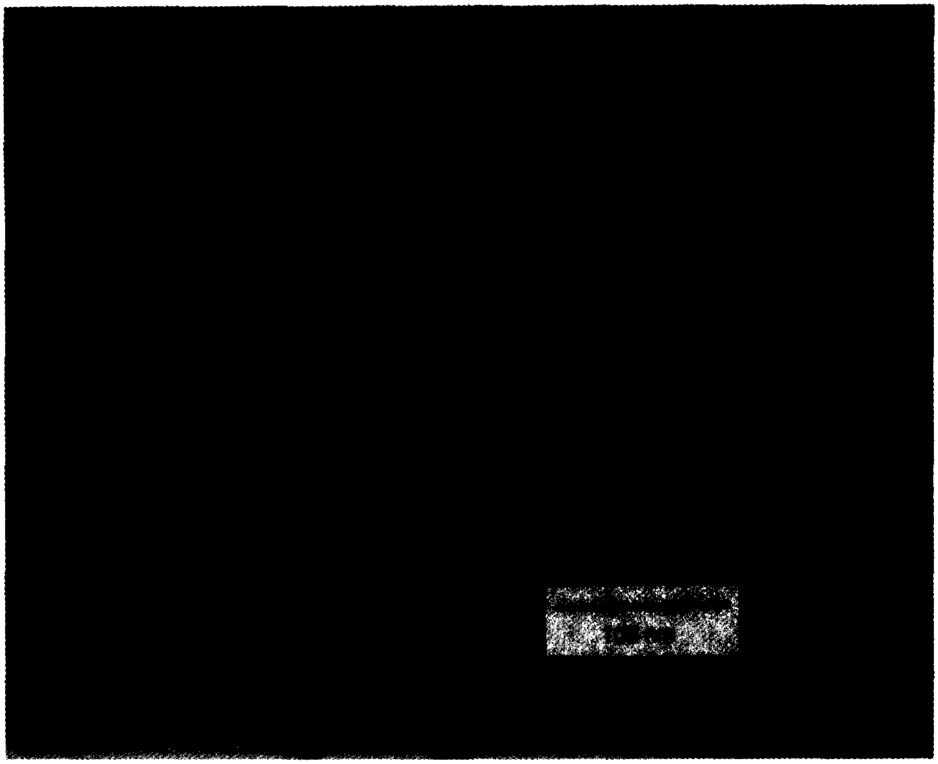

Fig. 7. A TEM micrograph of submicron particles categorized as 'organic'. The dark inclusions within these particles, along with the evidence of other internal structuring, suggest that these particles may be conglomerates of materials 2–5 nm in size.

for uniform-sized particles as:

$$t_c = 3\mu/4kTN_0E \quad (2)$$

where μ is viscosity of seawater in poise, k is the Boltzmann constant, T is the absolute temperature, N_0 is the initial particle number, and E is the coalescence efficiency. Assuming a coalescence efficiency of 0.1 and uniform-sized particles at concentrations of 10^9 particles ml^{-1}, then $t_c \approx 40$ min. However, the abundance of the submicron particles in samples preserved with 1 μM of $HgCl_2$ does not diminish markedly after even 4 days (data not shown). Clearly, the coalescence efficiency of these submicron particles must be considerably below 0.1. There is, however, visual evidence that these particles do adhere to one another (Fig. 7) and that large agglomerations having fractal-like structures can be produced (Fig. 8). Nevertheless, given the apparent stability of these suspensions, these submicron particles may be distributed widely by large-scale oceanic transport processes before removal to the bottom sediments.

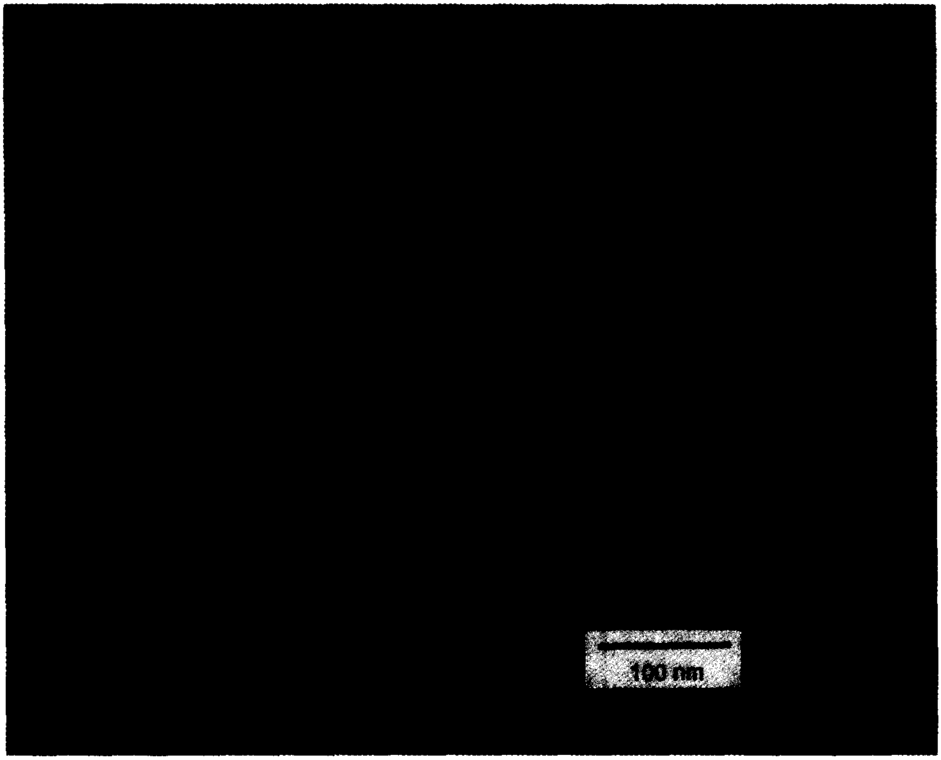

Fig. 8. A TEM micrograph of a submicron, marine floccule. The floccule is composed partly of materials similar to the smaller particles in the background.

Particles smaller than 120 nm are extremely abundant in coastal surface waters, 3–6 orders of magnitude more abundant than either bacteria or phytoplankton, respectively (F. Azam, personal communication, 1990). The surface area associated with these particles is on the order of 8 m^2 per cubic meter of water, assuming spherical geometry. This value does not take into account the internal surface area if these particles are highly porous. Hence, the distribution and behavior of submicron particles could heavily influence the chemistry and cycling of dissolved species. For instance, Honeyman and Santschi (1989) suggest that the apparent 'sorption' of trace metals to particulates ($>$400 nm) is due in large part to the aggregation of submicron, metal-containing particles into filterable sizes. Also, if these submicron particles have nutritional value they may be grazed by bacteria or protozoans, and serve, therefore, as a vehicle for returning carbon into the food chain. Alternatively, these particles may be a repository of refractory, or difficult to oxidize, carbon; Sugimura and Suzuki (1988) attribute the discrepancy between traditional measurements of dissolved organic carbon in seawater and their high-temperature method to high molecular weight (i.e. nanometer-sized) compounds. We are currently working to characterize the distribution of submicron particles in marine waters.

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