



## TEP and coagulation during a mesocosm experiment

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**Abstract**—Transparent exo-polymeric particles (TEP) have been associated with the aggregation of diatoms. Comparisons of TEP concentrations to those of conventionally-determined particles during a mesocosm experiment show that particles larger than about 30  $\mu\text{m}$  had concentrations very similar to, if not the same as, those of conventionally-determined particles. Smaller TEP were present mostly at concentrations less than those of non-TEP. Furthermore, their relative concentrations decreased over the course of the experiment. These comparisons suggest that the dominant interaction of TEP with algae in marine snow formation is to affect general particle stickiness rather than to aggregate by themselves.

### INTRODUCTION

Coagulation theory, developed to understand the transformation and removal of particles, is proving a useful tool in understanding the dynamics of particles in the ocean (Hunt, 1980; McCave, 1984). The aggregation of diatoms during a bloom to form large aggregates known as marine snow is a fairly simple example of this (e.g. Jackson, 1990; Jackson and Lochmann, 1992; Hill, 1992). However, the application of coagulation theory to even these simple oceanic systems has required its modification in ways that are not always simple or obvious. Examples include the need to account for the heterogeneous sources of particles, the porous nature of aggregates, and the non-spherical aspects of algal spines and chains (e.g. Jackson and Lochmann, 1993). Perhaps the most intriguing problem has been discerning the precise role of transparent exo-polymeric particles (TEP).

TEP material itself is not detectable by traditional particle sizing techniques, such as Coulter Counters and photographic analysis (Alldredge *et al.*, 1993), although particulate matter associated with it may be. However, it has been found associated with active formation of aggregates in algal systems and appears to play an important role in algal coagulation (Alldredge *et al.*, 1993; Passow *et al.*, 1994; Kiørboe and Hansen, 1993).

TEP offer several challenges for using coagulation theory to describe algal aggregation. Key to coagulation theory is knowing particle size distributions over the size ranges of importance. Undetected particles interacting with known particles can result in underestimates of particle concentrations and confound calculations of aggregation rates. Furthermore, if TEP have different physical and chemical properties than algae, they can affect important physical properties of aggregates, such as their densities and settling speeds, as well as chemical properties affecting the probability that two particles stick together when they collide (stickiness).

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There have been two methods proposed to account for TEP in algal aggregation. The first assumes that TEP are much stickier than diatoms. By colliding and sticking to algal particles that are relatively non-sticky when by themselves, TEP could enhance effective algal stickiness and facilitate algal aggregation (Kjørboe and Hansen, 1993). Support for this possibility has come from observations of the relationship between the TEP to chlorophyll ratio and the empirically measured stickiness rate (Dam and Drapeau, 1995). The second possible mechanism is that TEP coagulation itself is the process dominating the formation of large marine snow aggregates, with algal coagulation only a by-product of the process (Logan *et al.*, 1995). This has been supported by estimates of aggregation rates involving TEP particles. In either case, the effect would be to enhance aggregation rates.

These different interpretations of the role of TEP in aggregation have important implications for how marine aggregation should be studied. If the major role of TEP is to modify algal stickiness, the role of TEP might be assessed by measuring particle stickiness in conjunction with measurements of TEP and non-TEP. If TEP-TEP aggregation dominates, TEP dynamics should be the dominant concern.

The SIGMA (Significant Interactions Generating Marine Aggregates) program has provided a detailed description of the events occurring while an algal population grows and aggregates in a controlled system (this volume). Results have included the measurement of particle size distributions using conventional aperture impedance techniques as well as a photographic/image analysis method (Alldredge *et al.*, 1995; Dam and Drapeau, 1995; Li and Logan, 1995). Particle size distributions from these techniques have been combined (Jackson *et al.*, 1995) and used to test aggregation models (Jackson, 1995). TEP size distributions also have been measured (Passow and Alldredge, 1995). Logan *et al.* (1995) and Dam and Drapeau (1995) have offered different interpretations of the role of TEP in the process using the mesocosm data set. This paper uses the particle size spectra as a basis for considering their ideas.

## METHODS

The particle size spectrum  $n$  is the standard description of particle size distributions. If  $N(d)$  is the number of particles larger than particle diameter  $d$ , then

$$n = -\frac{dN}{dd} \quad (1)$$

TEP size measurement techniques for the SIGMA mesocosm have been discussed by Passow *et al.* (1995). Particles, captured on filters and stained, were counted using a microscope. Particles were categorized by size and shape. Concentrations for all shapes in a given size range were summed for this analysis. Values of  $N$  at requisite diameters were calculated using the original data and linear interpolations of log-log transformed data. These values were used to calculate values of  $n$  at the same diameters as the spectra in Jackson *et al.* (1995). The results are shown as particle size spectra for Days 7 to 14 of the mesocosm experiment. Concentrations of particles in the larger size ranges are less reliable because there were fewer particles in them and the relative variability in replicate measurements was greater.

The conventional particle spectra in the mesocosm were determined by combining measurements using aperture impedance instruments, such as those by Coulter (Li and

Logan, 1995) and Elzone (Dam and Drapeau, 1995) particle counters, with measurements using photographs and an image analysis system (Alldredge *et al.*, 1995). Uniting the two types of measurements involved making assumptions about the fractal nature of aggregates (Jackson *et al.*, 1995). The spectra shown here have been transformed to the size expected to be visible, twice the radius of gyration.

## RESULTS

TEP size spectra show different patterns than those of particles determined by conventional means (Fig. 1). TEP concentrations increased through time for particles larger than about  $30\ \mu\text{m}$  diameter, as did particle concentrations measured by conventional methods, but smaller particle concentrations decreased, unlike the other particles.

The ratios of particle spectra accentuate the difference between small and large TEP (Fig. 2). With the exception of Day 7, concentrations of TEP less than  $20\ \mu\text{m}$  were a relatively small fraction of those of conventional particles. Concentrations of TEP in this size range systematically decreased relative to those of the conventional particles from Day 7 to Day 11, staying low until Day 14. The relative concentrations of particles larger than about  $30\ \mu\text{m}$  remained within a factor of 2–3 of those of the conventional particles. TEP concentrations in the larger size ranges are more uncertain because of the small number of particles involved. For example, the number of particles counted for the two replicate samples within the  $284\text{--}768\ \mu\text{m}$  size range were 7 and 0 on Day 9 and 27 and 36 on Day 10 and 28 and 14 on Day 11, and 23 and 23 on Day 12. Given the resulting uncertainties in TEP concentrations, TEP and non-TEP concentrations for the large size range are not distinctly different.

TEP volume (calculated as encased volume) was predominantly in the larger size fraction [Fig. 3(A)]. This was even more true than for the conventional particles, which had a small peak at small radii (Jackson *et al.*, 1995). By number, however, most TEP were in the small size range [Fig. 3(B)].

## DISCUSSION

The similarity of large ( $>30\ \mu\text{m}$ ) TEP and conventional particle concentrations suggests that they are determined by the same processes and are probably the same particles. Because TEP can form aggregates with algal particles (e.g. Passow and Alldredge, 1995; Kiørboe and Hansen, 1993), such aggregates should be observed by the conventional particle counting methods, particularly imaging techniques. There may, however, be discrepancies between TEP and conventional particle size determinations because of TEP transparency.

The different behaviour of small ( $<20\ \mu\text{m}$ ) TEP is intriguing. It suggests that, while TEP may not be detectable by instruments such as Coulter Counters, their concentrations are too low to affect measurements of particles size distributions significantly. Furthermore, the higher concentrations of small, non-TEP imply that TEP are more likely to collide with them than with other TEP. Passow *et al.* (1994) made a similar observation for the mesocosm system. If TEP are more likely to collide and stick with other particles, then TEP aggregation rates will be controlled by the concentrations of the other particles. If the other particles are relatively non-sticky, then concentrations of pure TEP could be

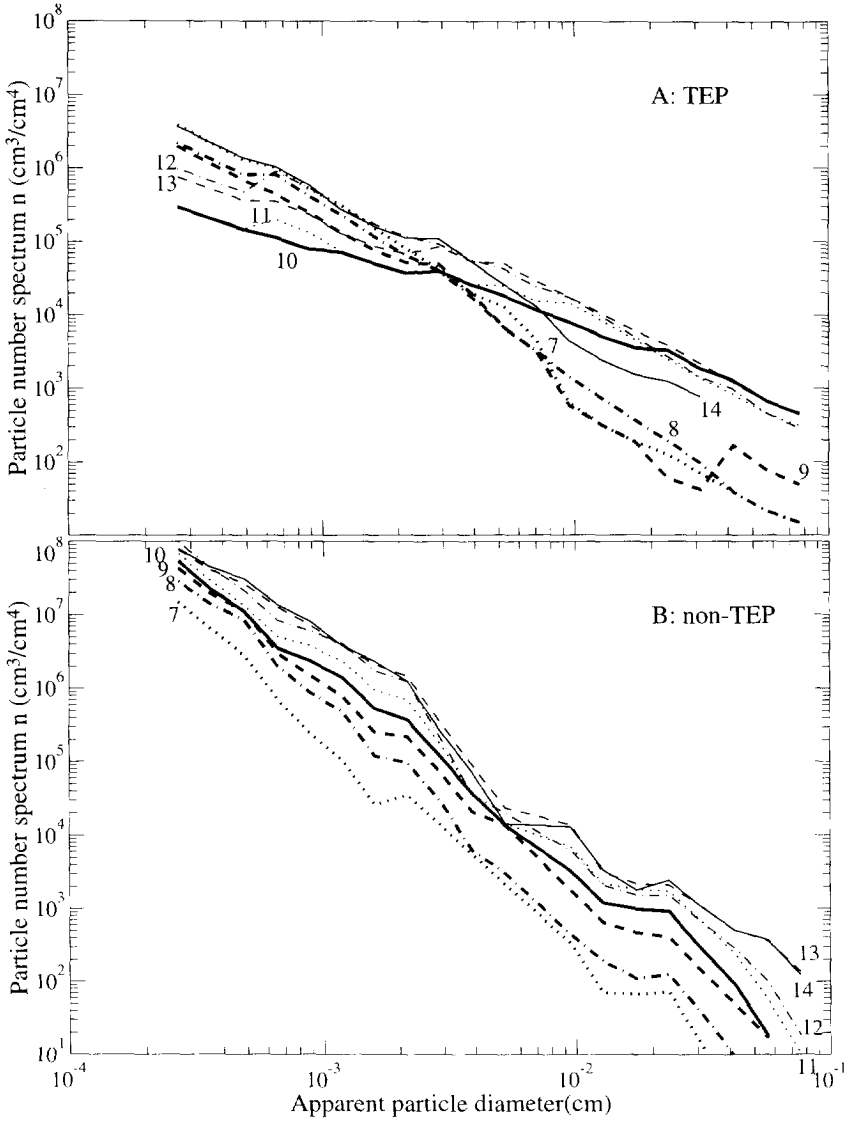


Fig. 1. Particle size spectra for the mesocosm experiment. (A) TEP (data courtesy of U. Passow); (B) particles determined using conventional techniques (data from Jackson *et al.*, 1995). Numbers on lines indicate the sample day. The apparent diameter is the measured particle diameter for TEP and twice the radius of gyration for the conventional techniques. The conventional measurements were made using an aperture impedance particle counter (Elzone) and a photographic/image analysis system. There was a gap in the ranges covered by the two instruments between about 40 and 70  $\mu\text{m}$  that was filled by interpolation between the two sets of measurements.

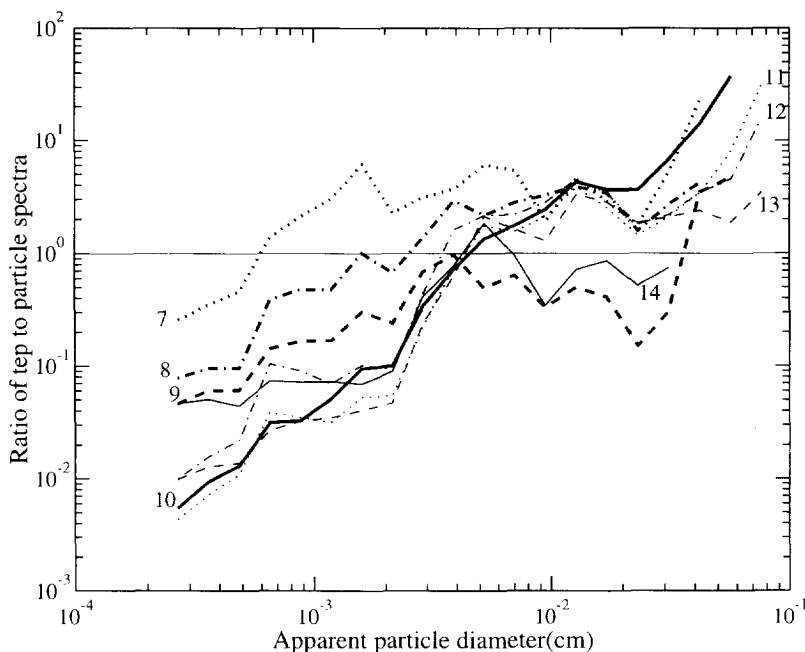


Fig. 2. Ratio of TEP to conventional particle spectra. The original spectra are in Fig. 1.

selectively removed and their concentrations decreased relative to non-TEP. Such a scenario is consistent with the observed TEP concentrations (Fig. 2).

The relationship between the amount of TEP per unit plant material and the stickiness that Dam and Drapeau (1995) developed suggests that one of the main observable effects of TEP is to change particle stickiness. If TEP did not stick to non-TEP, this relationship would be difficult to explain. The relatively low concentrations of the small TEP that would interact with non-TEP suggests that the collision rate between TEP and non-TEP is a less important aspect of their relationship. For this reason, measurements of particle stickiness could allow the incorporation of TEP effects while using particle size spectra measured with conventional instruments. An analysis of coagulation in the mesocosm experiment using the full particle spectrum did not explicitly consider TEP concentrations but did incorporate experimental measurements of particle stickiness (Jackson, 1995).

The focus on diatom coagulation in models tends to obscure the role that coagulation can have in other circumstances. McCave (1984) decided that coagulation in the interior part of the ocean is too slow to be important, but he specifically excluded surface waters from his analysis because he expected organism-feeding interactions to dominate there. Hill (1992) analyzed coagulation rates and resulting particle removal rates under non-bloom conditions in surface waters, finding that particle removal was slower than for bloom conditions but that it could still be significant because other particle removal rates were also slower. Hill's results highlight the fact that coagulation and subsequent formation of marine snow are not about presence or absence of coagulation but are about coagulation rates.

Algal coagulation in the mesocosm turns out to have been a remarkably complicated

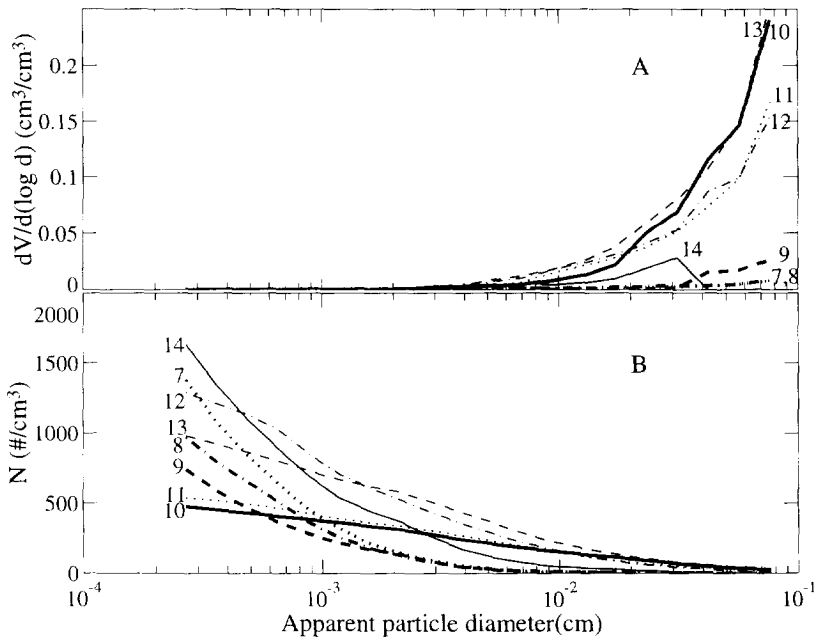


Fig. 3. Distribution of TEP volume (A) and cumulative number  $N$  (B) as functions of particle diameter. The area under a curve in (A) is proportional to the total volume of TEP in the given size range.  $V$  is the total TEP volume greater than diameter  $d$ . It is calculated as  $\pi/6 d^3 n$ . The TEP volume has not been corrected for changes in particle density resulting from particle fractal nature.

Note that  $N$  is the total number of particles larger than a given particle size.

situation. Besides the TEP role, disaggregation was as important as coagulation in controlling the concentrations of large aggregates (Jackson, 1995). Coagulation occurred continually over the entire size. Coagulation in the ocean is even more complicated, with organisms removing food particles and adding faeces and with larger particles settling out of any layer.

Given the complexity of the interaction potentially involved in a coagulating system, it is not surprising that simpler diagnostic parameters have been suggested to predict conditions of rapid coagulation. Kiørboe *et al.* (1994) found that an estimate for the maximum phytoplankton concentration derived for a simple bloom model in Jackson (1990) worked well to predict the maximum phytoplankton concentrations in a fjord and that these periods of maximum phytoplankton concentration coincided with periods of maximum vertical particle flux. They did have to modify the critical concentration index to include interactions between different diatom species. Dam and Drapeau (1995) used it to predict phytoplankton concentrations in the mesocosm, finding that predictions agreed well with observed maximum phytoplankton concentrations.

Logan *et al.* (1995) found that the predicted maximum concentration did not work well to predict maximum particle fluxes for a lake system. They focussed on the role of TEP in promoting aggregation, proposing an index that used only TEP concentrations and that assumed that TEP could be characterized as if monodisperse (all particles of the same size). Because this index gave greater collision rates than those of an algal-based index, they decided it was a superior diagnostic.

Both the algal and the TEP indices offer incomplete measures of the myriad of interactions among particles. The maximum phytoplankton concentration does not explicitly account for the presence of other particles, such as TEP, although the effect of TEP could be incorporated in experimentally derived values of particles stickiness. Furthermore, the concept of algal size is poorly determined in a world where algal cells have spines and form chains. The TEP index ignores interactions with other particles, such as algae, and assumes that the TEP are monodisperse. That two such different indices of coagulation potential, one based on algal concentrations, the other based on TEP concentrations, should prove useful in predicting the coagulation potential of marine systems is amazing and may indicate the robust nature of aggregation.

Both types of information, TEP and algal, need to be incorporated in a more complete description of system dynamics. Kiørboe and Hansen (1993) showed that algal interactions with TEP in laboratory cultures varied with the species. Some species did not need TEP to aggregate, other species did not aggregate even in the presence of TEP. Just as not all algae act the same, there is no reason to believe that all TEP will act the same.

Further evidence for multiple sources of TEP come from descriptions of its morphology. Wells and Goldberg (1994) have observed colloids that have the characteristic cluster structure that is described using fractals. In contrast, Alldredge *et al.* (1993) describe TEP as frequently being films. Kiørboe and Hansen (1993) describe mucus secreted in sheets by diatoms. These differences in TEP shape suggest that many TEP are created not from colloids but as relatively large particles. As such, they would have different dynamics, both in terms of their ability to coat algae and change their stickiness, as well as how they interact with other particles. Furthermore, the creation of aggregates composed of films rather than something constructed from aggregated colloidal fractals should affect physical properties of aggregates such as the rate of water flow through them.

Other important questions also remain. If TEP originate from mucus excreted by diatoms, does the mucus also stick to them and how does it affect their stickiness? If TEP and algae do not stick to each other when they collide, how can TEP collect algae as they coagulate?

It will be difficult to understand how TEP interact with algal particles until we know more about the details of their generation and interaction with other particles. Such information will be crucial for the development of a comprehensive theory of algal coagulation.

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