CHAPTER 3. MAJOR BIOELEMENTS

WORKSHOP REPORT

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1. Introduction

The particulate matter pool in the ocean is comprised of both living and non-living components; the latter includes organic and inorganic fractions. However, most of the particulate matter in the ocean is of biotic origin. Thus biotic processes and transformations largely determine the types, size distributions, abundances and chemical compositions of particles in seawater. Carbon (C), nitrogen (N), oxygen (O), hydrogen (H), phosphorus (P) and sulfur (S) are the major components of living organisms and organic detritus. Accurate measurement of these "bioelements" is particularly important in assessing the chemistry and dynamics of particulate matter in the ocean.

During the discussion of the Bioelements Working Group, it became clear that a consensus exists within the community regarding the best analytical methods for measuring bioelements. Obtaining samples representative of nature and handling them so as not to alter their chemical composition was far more problematic. The following report begins by clarifying the nature of the diverse particle pools in the ocean. Sampling and handling problems unique to bioelements are then discussed. The report concludes with recommendations for the accurate chemical analyses of C, N, P and S. Details of these methods are discussed in the individual contributions. More detailed information can be obtained from articles provided in the reference list.

2. Origins and Pools of Marine Particles

Organic matter in the ocean exists along a continuum from small molecular weight compounds, e.g., hexoses, fatty acids, amino acids, nucleotides, to whale-sized particles [Sheldon and Parsons, 1967; Sharp, 1973]. Typically, an operational distinction is made between "dissolved" and "particulate" pools using either 0.2, 0.45, 0.7 or 1.2 μm pore size filters, depending upon the specific analytical measurements. It is important to note that by this operational definition, colloids and small particles including most bacteria and all viruses are sometimes included in the "dissolved" matter pool.

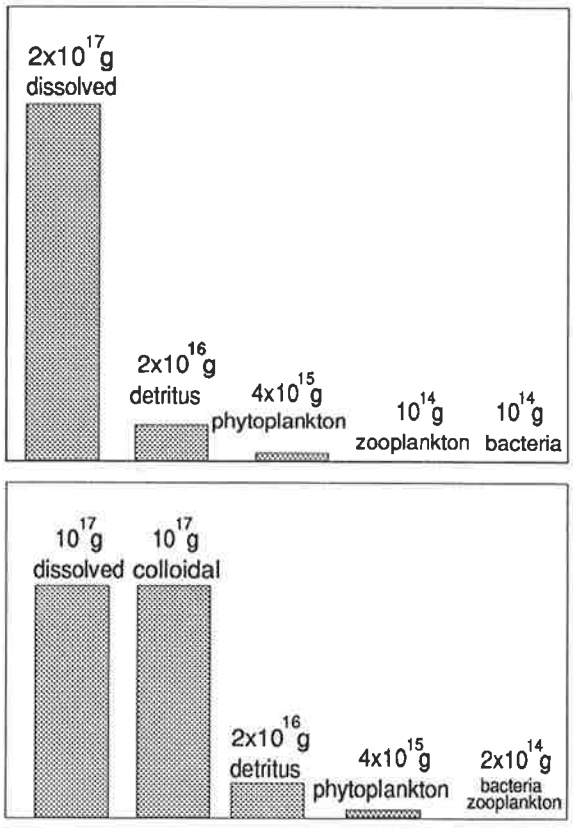


Fig. 3-1. Top: Distribution of organic carbon in the oceans [after Cauwet, 1978]. Bottom: Importance of organic colloids in seawater [after Cauwet, 1978].

In most oceanic environments, the inventory of organic matter, usually expressed as organic carbon, is dominated by dissolved materials (Figure 3-1). Consequently, one cannot ignore the presence of dissolved organic matter (DOM) when analyzing and interpreting particulate matter distributions in the sea. Recently, the accuracy of historical measurements of DOM in seawater has been called into question [Sugimura and Suzuki, 1988; Williams and Druffel, 1988]. Apparently, there exists a large pool of unidentified carbon compounds that are difficult to oxidize by conventional analytical procedures. It is conceivable that an analogous

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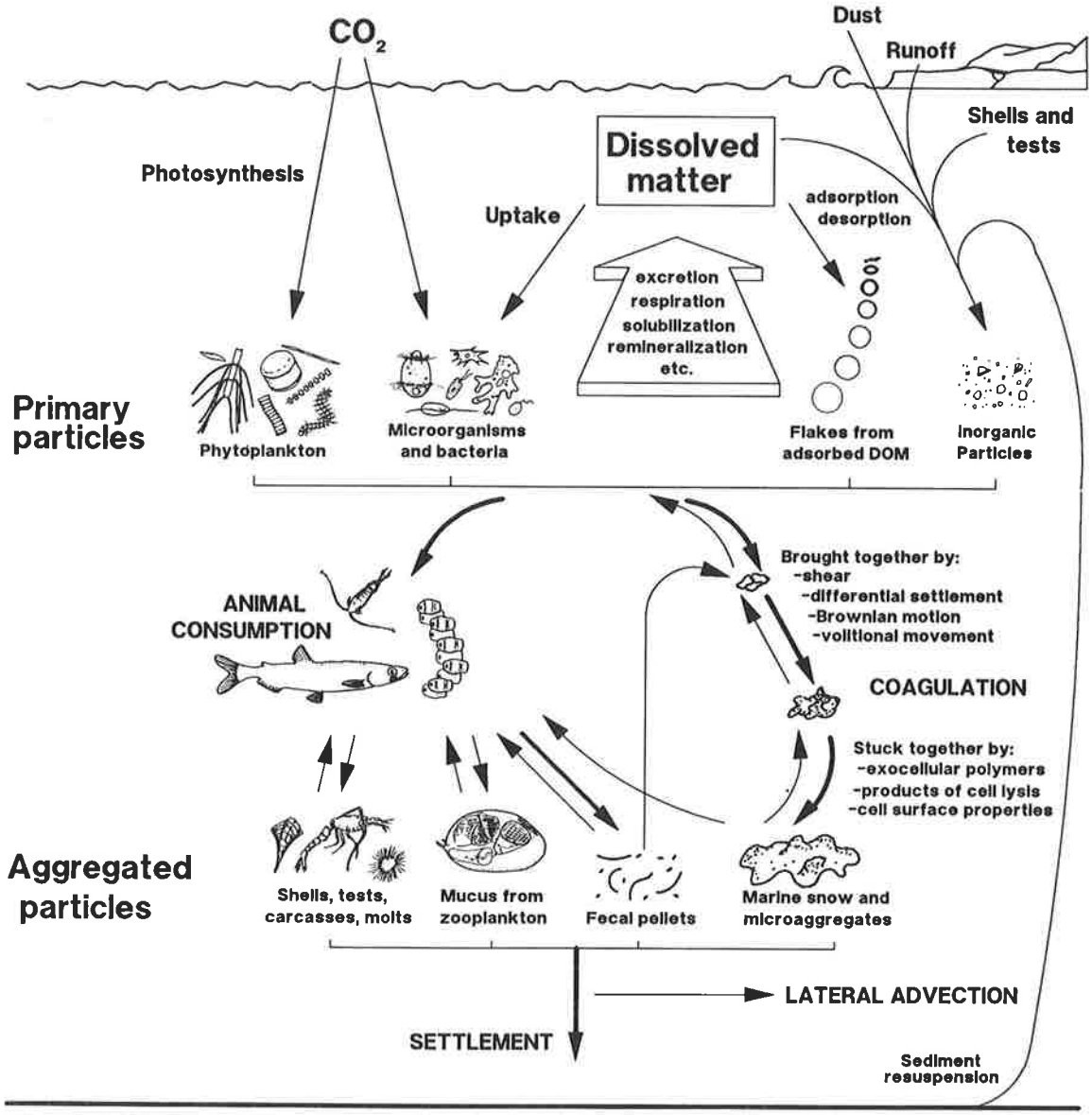


Fig. 3-2. Major sources of particles in seawater. Primary particles are inorganic particles or organic particles formed directly from inorganic or dissolved organic sources via carbon fixation, microbial uptake or adsorption. Aggregated particles are formed by the coagulation of primary particles or by animal consumption.

component of chemically-resistant particulate organic matter (POM) also exists in seawater.

The diverse origins of particles in seawater are summarized in Figure 3-2. Primary particles are those materials that arise directly from either inorganic or dissolved organic sources. While some primary particles in the ocean are inorganic, originating from windblown dust, terrigenous run-off and resuspension of bottom sediments, the majority are organic particles. The contribution of inorganic sources to the primary particle pool depends upon location and is particularly sensitive to proximity to the shore or bottom.

Organic primary particles arise from a variety of sources but most are produced through photosynthesis by eucaryotic and procaryotic algae and cyanobacteria. Transformation of dissolved organic matter into particles can also occur through processes that may be quite significant but poorly quantified. Monomeric organic compounds are known to bind abiotically to dissolved macromolecules [Carlson et al., 1985], thereby affecting the molecular weight distribution of organic matter in seawater. High molecular weight DOM adsorbed to bubble surfaces becomes flakes of particulate matter ranging from a few to tens of microns as the bubbles

burst or dissolve [Johnson and Cooke, 1980; Kepkay and Johnson, 1988, 1989]. Humic acids and other dissolved organic and colloidal materials form particles via salt flocculation as river water mixes with seawater [Sholkovitz, 1976; Fox, 1983]. It is also well known that bacteria can transform DOM into organic particles [Parsons and Strickland, 1962; Azam et al., 1983] and there is some evidence that DOM can also chemically precipitate in standing seawater [Riley, 1970]. Finally, the role of photochemical processes in the formation and transformations of organic matter in the marine environment also needs to be considered [Mopper and Stahovec, 1986; Kieber et al., 1989].

Once formed, primary particles in seawater undergo numerous transformations, most of which involve alteration and aggregation into new particles with substantially different properties. Some living primary particles die and thereby directly contribute to the formation of detritus. Most, however, become aggregated through two major pathways: consumption by organisms and physical coagulation [Alldredge and Silver, 1988]. Primary particles and other aggregated particles are consumed by organisms, especially protozoans, zooplankton and nekton, and converted to new particulate phases including animal biomass such as living animal bodies, calcareous or siliceous tests, molts, eggs, carcasses, fecal pellets and feeding webs and to dissolved and inorganic phases through respiration, excretion and bacterial decomposition. Primary particles may also aggregate into larger particles via physical coagulation processes. Physical transport mechanisms such as Brownian motion, turbulent shear and differential settlement join smaller particles together. These components stick together at rates dependent upon their surface properties and the nature of the "sticky" substances produced by microbes living on their surfaces. Coagulation processes may be particularly important in aggregating phytoplankton blooms and in producing larger sinking particles.

The multiple origins of particles in seawater serve to graphically illustrate the diverse nature of the types of particle pools which exist simultaneously in any parcel of seawater. Moreover, transformations between these pools are bidirectional and dynamic. The diverse and changing nature of these pools, both in terms of chemical composition and size, must be kept in mind when designing any program for the chemical analysis of marine particulate matter. It is particularly important when considering analyses of bioelements. Interpretation of the results of chemical analyses may ultimately depend upon the investigator having a quantitative understanding of the types of particles making up a sample of filtered seawater or a sediment trap sample.

3. Problems of Sample Collection and Handling Unique to Bioelements

Particulate material in the water column is segregated into suspended particles and sinking particles, which can be characterized by their respective residence times and sinking speeds in the water column [Riley, 1970]. Traditionally, suspended particles are collected by either water bottles or by in situ filtration. Although most of the problems associated with the collection and sample handling of bioelements are common to marine particles in general, the following aspects deserve particular attention:

1.) The cleaning of equipment appropriate to the particular analysis by removing the surface films, adsorbed trace elements etc.;
2.) Homogeneity of the sample must be maintained. This can be achieved by either filtration of the total contents of the water bottle or by gentle agitation of the water sample prior to taking a sufficient subsample. Care must be taken to avoid de novo formation of particles via shaking.
3.) The presence of high concentrations of macrozooplankton may bias the suspended sample. Prescreening of the sample may reduce this problem. However, care must be taken that mucilaginous algae (e.g., *Phaeocystis*) or diatom chains are not excluded by this process.
4.) The choice of filter must be compatible with the size spectrum of particles to be sampled.
5.) The application of vacuum/pressure filtration should be conducted so as to avoid sample damage such as cell lysis.
6.) If samples cannot be analyzed in reasonable time, they can be stored either dry or deep frozen.

The sinking particulate pool can be sampled by pumps (see sampling section), by sediment traps or directly using SCUBA or submersibles. The sampling of macroaggregates (e.g., marine snow) is specifically discussed by Alldredge (this chapter, page 46).

Since sediment traps were first introduced in the 1970's, a number of problems have arisen with respect to their use. Examples of such concerns include trap design, various hydrodynamic considerations, appropriate sample preservation, mode of deployment (e.g., moored vs. free floating) and potential contamination from the

inclusion of organisms not considered as part of the passively sinking flux [i.e., swimmers; Knauer et al., 1979, 1984]. All of these issues have been discussed in detail in the U.S. GOFS report #10 [1989] and some are reiterated in this chapter (see reports by von Bodungen et al., Karl et al., Knauer, and Wassmann, pages 47, 73, 81 and 99). The latter reports include the use of traps in both eutrophic and oligotrophic ocean waters, appropriate blank preparation and the use of various types of density solutions.

4. Chemical Analyses of Specific Bioelements

The major bioelements are routinely analyzed in many oceanographic laboratories. In most cases similar standard methods of analysis are employed. The working group recommends the following procedures for the chemical analysis of each bioelement. Details of the methods used in individual laboratories may be found in the individual contributions.

4.1. Analysis of Particulate Carbon and Nitrogen

Particulate carbon (PC) and nitrogen (PN) are the most commonly measured bioelements in the ocean, partially because of the convenient simultaneous determination of these two elements with commercial elemental analyzers. Consequently, of the three elements commonly reported as components of the "Redfield ratio" (i.e. C, N, P), particulate phosphorus (PP) is the least frequently determined.

Both organic and inorganic forms of PC are found in seawater. Relatively easy separation protocols (e.g., acidification) have been established so that particulate organic carbon (POC) can be directly distinguished from particulate inorganic carbon (PIC). There are no common marine nitrogen minerals, but ammonium can adsorb to or form in particles, creating an inorganic pool of PN. This pool is not usually considered to be significant. Phosphate can adsorb to particles, and phosphate minerals do exist in seawater. Sequential extractions have been used to separate particulate phosphorus (PP) phases (see individual papers in this chapter). Since much of the phosphorus bound in plankton can be lost with inorganic extraction protocols, such separations are not satisfactory for determining particulate organic phosphorus (POP).

4.1.1. Method.

Particulate matter on a filter or in powdered form, from sediment traps or sedimentary material, is analyzed by high temperature oxidation usually followed by detection of carbon as CO_2 and nitrogen as N_2 by thermal conductivity detection (TCD) gas chromatography using a commercial elemental analyzer. The advantage of the TCD is that both elements can be determined in a single sample.

4.1.2. Sample acquisition.

With bottle sampling, care should be taken to assure that quantitative recovery of a representative aliquot of the full sample or the full sample itself is used since suspended material may be unevenly distributed [Wangersky, 1974]. Although metal-free cleaning procedures [Fitzwater et al., 1982] are not necessary, care should be taken to assure that C and N contamination does not occur during sample collection. Sample volume should be dictated by the sensitivity of the analyzer used and the concentration and distribution of the suspended material in the environment being sampled. If infrequent large particles are encountered on filters, prescreening of water samples or removal of such particles may be desired and, if done, should be reported.

There are unique problems with sediment trap sample preparation (e.g., contamination, "swimmers", sample splitting) that are discussed below. If samples are collected by in situ pumps, considerations about particle destruction and/or production should be made.

4.1.3. Collection on filters.

Commonly, glass fiber filters (e.g., Whatman GF/F or GF/C) are used to collect particulate matter, although silver membrane and quartz fiber filters are sometimes used. Recent applications of aluminum oxide filters, i.e., Anopore, have been made with apparent good retention of submicron particles, although the filters are expensive and difficult to handle [Altabet, 1990].

A relatively low pressure differential (0.3 to 0.5 atmospheres) should be used in vacuum or pressure filtration to minimize cell disruption. Care should be taken to prevent the filters from running dry until the end of the filtration. A common problem for glass fiber filters is a change in the nominal pore size with loading of particulate matter on the filter because of accumulation of particles on the surface and in the interstices of the filter. Therefore, attempts should be made to maintain consistent filter loading.

Sea salt residues in the filters are not ordinarily a problem. Rinsing, for example with deionized water, might compromise the integrity of the sample (e.g., cell lysis) and is not required for CNP analysis and therefore is not ordinarily recommended, however see Knauer (this chapter, page 81) for exceptions with trap collected POC

and PON. In certain environments (e.g., coccolithophorid blooms, coral reef environments), carbonate minerals can contribute a significant portion of PC. Under such circumstances, removal of PIC by direct acidification can be done to separate between total PC and POC. Fuming is not recommended. The acid used must be strong enough to dissolve carbonate particles embedded in organic matrices. Total PC can be analyzed on an untreated filter while POC can be measured after calcium carbonate removal, with PIC determined by difference. Differential temperature techniques for separation between PIC and POC [Telek and Marshall, 1974; Hirota and Szyper, 1975] are not recommended because there can be significant portions of the PIC (particularly high-Mg calcite) that decompose below the recommended 500° C and significant portions of the POC that survive temperatures above that.

Several of us have seen evidence of losses (up to 40%) of PN and POC with acid treatment. If acid rinsing is used, the rinse should be collected and analyzed for dissolved organic carbon and nitrogen (DOC, DON; Walsh et al., this volume, page 93). The recommended method for acidification in other than high carbonate environments is addition of acid in a modified sample analyzer cup (Cutter, this chapter, page 57).

4.1.4. Sample storage.

It is desirable to minimize sample handling in order to avoid contamination. Therefore, it is best to store samples in a vacuum desiccator directly in sample cups for the instrumental analysis. As an alternative, samples can be frozen with care to minimize contamination in the freezer and subsequently desiccated before analysis. There is no indication of time limits for storage.

4.1.5. Instrumentation.

Although much of the PC and PN analyses reported in the literature were performed on manual instruments, primarily Hewlett-Packard [Sharp, 1974] and Perkin-Elmer [Gordon and Sutcliffe, 1974], instruments available today are automated full gas chromatographic instruments (Carlo Erba, Europa, Perkin-Elmer, Leeman - see individual papers in this chapter). Operation should follow manufacturer recommendations with modifications determined by empirical evidence and from revisions from manufacturer and other users.

4.1.6. Standards and blanks.

The primary standard should be stable and not hygroscopic; it should have a C/N ratio appropriate to the material being analyzed (commonly used compounds are EDTA, acetanilide, and sulfanilamide). Because these pure compounds are easily analyzed and may not adequately represent instrument performance with more recalcitrant compounds under all conditions, it has been recommended that an appropriate natural material reference standard be used. For example, National Institute of Standards and Testing (NIST) "Orchard Leaves" and "Estuarine Sediment" have been used. Broader use of such reference standards is strongly recommended and it is suggested that other appropriate NIST standards be established for use by the oceanographic community.

Appropriate blank correction is necessary because of contamination of samples from handling and storage, and contamination from dissolved organic matter retained on filter pads. The instrument blank can usually be accounted for during calibration. The best method to determine the handling and storage contamination is to treat the filter blank exactly as a sample. A filter can retain seawater which includes a small but significant amount of dissolved carbon; there is no comparable nitrogen blank problem because of much lower concentrations of dissolved nitrogen. If a filter blank is wetted with filtered seawater before treatment like a sample, this will account for the retained carbon. If there is concern about dissolved organic matter (DOM) contributing a blank problem (e.g., organic compounds adhering to the matrix of the filter and aggregation of colloidal particles from turbulence during filtration), appropriate blank corrections should be made. One method to approach this problem is to use an additional filter (or filters) below the sample collection filter in the filtration step [Menzel, 1967; Holm-Hansen et al., 1966]. It has been suggested that filtering large volumes of water in low particle environments could decrease the influence of the DOM adhesion by increasing the amount of particulate matter on the filter. However, this could also indicate that the low, relatively invariant particulate content in the deep ocean might be only dissolved contamination.

4.1.7. Accuracy, precision and detection limits.

Due to the unknown nature of organic matter in seawater, we consider accuracy difficult to assess. If the instrument for CN analysis is properly calibrated, the accuracy must be 100%.

Analytical precision (replicability of a homogeneous standard material) should be within ± 5-10%. The coefficient of variation of replicate sample filters of suspended matter has been shown to be approximately ± 10%.

The analytical detection limits of the various instruments vary somewhat, and are about 0.1 to 0.4 μM C or N. Samples containing greater than 0.5 μM N and 1 μM C can be run with confidence on most of the instruments.

4.2. Analysis of Phosphorus

4.2.1. Method.

Particulate matter on a filter or in powdered form such as from sedimentary material of sediment traps is usually analyzed by high temperature combustion and acid hydrolysis followed by detection by standard colorimetric analysis of phosphate. Various other oxidation methods (e.g., UV irradiation, persulfate oxidation) have been used previously; these have been shown to suffer from variable and incomplete conversion of bound phosphorus to orthophosphate (see individual contributions in this chapter). Moreover, the PP methods discussed here undoubtedly miss some of the mineral phosphate pools (e.g., some of the apatite minerals).

4.2.2. Sample acquisition.

Sample acquisition procedures used for PC and PN are also appropriate for PP. No unique precautions are noted.

4.2.3. Collection on filters.

If a desired objective is direct comparison of P data to C and N data, it is important to follow the same sample filtration procedures (e.g., filter type, filter loading) used for carbon and nitrogen. There is not a sea salt problem with phosphorus comparable to that with carbon due to the comparatively low dissolved phosphate concentration in seawater.

4.2.4. Sample storage.

It is desirable to minimize sample handling to avoid contamination. It is convenient to place wet filters directly in analysis vials or tubes and dry in an oven immediately after collection, before storing the samples in a desiccator at room temperature. As an alternative, samples can be frozen with care to minimize contamination in the freezer. There is no indication of time limits for storage.

4.2.5. Analysis.

The high temperature method for destruction of organic phosphorus is commonly used and convenient [see individual papers in this chapter]. The UV oxidation method [Armstrong et al., 1966] was suspect for incomplete recovery of phosphorus from organic phosphorus compounds (Solorzano, personal communication, 1980). However, a UV method with higher output lamp appears to compare favorably with high temperature combustion (Harrison, this chapter, page 71). Persulfate digestion for PP analysis has been found by several laboratories (e.g., Smith and Walsh, unpublished, 1981) to yield non-quantitative recovery.

4.2.6. Standards and blanks.

Because complete recovery of phosphate from organic compounds has been well established [Solorzano and Sharp, 1980], a number of laboratories do routine standardization with orthophosphate only. Other laboratories do daily standardization with organic phosphorus compounds such as NIST Orchard Leaves or glucose-6-phosphate.

Blanks can be run by treating an unused filter in the same fashion as a sample. When this is done carefully, the final colorimetric absorption is similar to an orthophosphate analysis deionized water blank. For routine analysis, it is advisable to run a filter blank with a batch of samples to verify that there is no contamination problem.

4.2.7. Accuracy, precision and limits of detection.

With complete recovery of organic standards, the accuracy of the method is 100%. The analytical precision of the method is nominally the same as that of the orthophosphate method [Strickland and Parsons, 1972]. The precision for particulate phosphorus on filters has been determined in several laboratories at about ± 10%. Strickland and Parsons [1972] report the limits of detection for the orthophosphate method as 0.03 μM (μM L^{-1}). The detection limits for the particulate phosphorus method will depend upon the concentration of phosphorus in the final product water after all sample handling (influenced by volume of water filtered, dilution, etc.).

4.3. H,O and Total Caloric Content

The measurement of carbon-specific H, O and caloric content of suspended and sediment trap-collected organic matter is of intrinsic interest in the assessment of the available energy content. These measurements are fundamental to studies of trophodynamics and energy flux [Paine, 1971], but are rarely conducted in oceanographic studies.

In theory, quantitative biochemical composition (i.e., total protein, lipid and carbohydrate) of bulk organic matter should be stoichiometrically related to H elemental analyses and to the bomb caloric value. Of these determinations, CHN elemental composition appears to be the most straightforward analysis. It requires only

small amounts of particulate organic matter (≤ 250 μg dry weight) and can be measured simultaneously with C and N using commercially-available instruments. Gnaiger and Bitterlich [1984] found excellent agreement between proximate biochemical composition and CHN determinations for a variety of organic materials, including detritus and algae.

Because quantitative measurements of H and O are not routinely conducted, we have decided against making any recommendation regarding detailed analytical procedures. The fact that several investigators have published data on CHN content of particulate matter [Hallegraeff, 1978; Gnaiger and Bitterlich, 1984; Martin et al., 1987] indicates that these measurements are possible. A major source of error in H measurements is the presence of residual water in the sample materials. Consequently, it is imperative that all samples are thoroughly dried and retained in an absolutely dehydrated condition until final analysis. Gnaiger and Bitterlich [1984] provide a comprehensive discussion of the "residual water" problem and provide analytical procedures designed for the Carlo Erba Model #1160 Elemental Analyzer to satisfy these objectives.

4.4. Sulfur

4.4.1. Method description.

The basis of determining sulfur in marine particles is the high temperature combustion of filters or powdered material (e.g., from sediment traps) to produce sulfur dioxide. This SO_2 is then quantified using sulfur-specific detectors (Matrai, this chapter, page 83) or gas chromatography with thermal conductivity detection (GC-TCD; Cutter, this chapter, page 57). Although the GC-TCD method has higher detection limits, it is also capable of simultaneously determining carbon and nitrogen. It is important to recognize that particulate sulfur can exist in many chemical forms, e.g., organic compounds such as methionine and inorganic compounds such as pyrite. Therefore, the combustion efficiency must be evaluated for a wide class of compounds and materials in order to assure accurate determinations of total sulfur. In this respect, standard reference materials must always be analyzed.

4.4.2. Instrumentation.

Two commercially available systems are capable of determining sulfur in marine particulate matter: the Antek 771 pyroreactor with 701C sulfur detector [Matrai, 1989], and the Carlo Erba NA-1500 elemental analyzer (Cutter, this volume, page 57). Generally, these instruments can be used with minimal modifications.

4.4.3. Sample acquisition and storage.

Although there are many techniques for acquiring suspended particles in the water column, three important considerations are:

1) the method should collect samples that are representative of marine suspended matter;
2) the method must not compromise the chemical composition or speciation of a sample;
3) the method should be logistically acceptable, e.g., relatively low cost, simple to use at sea.

The procedures recommended here and in the Carbon and Nitrogen section generally meet these criteria.

The choice of filter type and size is a difficult one and requires certain compromises. The simultaneous determination of carbon, nitrogen and sulfur excludes the use of most filters with sub-micron pore-sizes (e.g., Nuclepore polycarbonate filters). Aluminum oxide and silver filters are too costly for most laboratories and too fragile to be folded into the tin sample cups used with the Carlo Erba or quartz spoon used by the Antek instrument. Thus, it is necessary to use quartz or glass fiber filters (nominal pore size ≥ 0.7 μm). In the case of the Carlo Erba analyzer, the size of the sample cups and the capability of the instrument also limit the filter diameter to 13 millimeters.

It is recommended that samples be filtered at least in duplicate, and preferably in triplicate, in order to assess overall precision. After filtration, the only sample pretreatment for sulfur that is performed on board ship is to remove inorganic sulfate which might remain on the filter after the seawater evaporates; we wish to determine the sulfur in the particles and not that from evaporated sea salts. Any rinse must be of a similar ionic strength in order to avoid cell rupture and the potential loss of sulfur. For the specific determination of particulate sulfur, Matrai [1989] used ammonium formate, but for simultaneous CNS determinations (i.e., Carlo Erba) this reagent may contaminate the sample with excess nitrogen and carbon. Thus, the Carlo Erba procedure utilizes a simple sodium chloride solution. With either desalting method the filter is immediately frozen in order to minimize artifacts due to bacterial growth and degradation of the organic matter.

Sample processing at the shore-based laboratory includes drying the filters or sediment trap materials. This must be done at low temperatures (40 to 60° C) to avoid the loss of volatile sulfur compounds such as dimethylsulfide. Furthermore, lyophilization should be

avoided since very high losses of total sulfur have been observed in lacustrine sediments [Amaral et al., 1989].

4.4.4. Standards and blanks.

An important consideration in the determination of particulate sulfur is the choice of standards. Because pure sulfanilamide can be obtained commercially, is stable during storage, and is not hygroscopic, it makes an excellent primary standard. Similarly, methionine is a stable sulfur standard. However, neither is very representative of marine particles. In this respect, standard reference materials such as NIST "Estuarine Sediment" may be useful for evaluating accuracy. Estuarine Sediment has an uncertified sulfur value of 0.96%, and using data over the last 5 years from the Cutter lab, has a sulfur concentration of 0.96 ± 0.04% (n=114).

Filter blanks should be processed in a manner similar to that of samples. This can include soaking the filter in filtered seawater, and then processing as a sample (i.e., wash, freeze, and dry).

4.4.5. Accuracy, precision and detection limits.

The absolute detection limit (3 times the standard deviation of blanks) for the Carlo Erba instrument is 0.50 μg S. Assuming that 2 L of seawater are filtered, this translates to a relative detection limit of 7.8 nM S L^{-1}. For solid samples (i.e., sediment trap material), an average of 10 mg is analyzed, giving a relative detection limit of 1.6 μM S g^{-1}. The precision of the method for suspended (filtered) particles, expressed as the relative standard deviation, is 12% for sulfur at the 0.06 μM S L^{-1} level. For solid phase samples (i.e., sediments), precision (as RSD) is 2% at 0.3 mM S g^{-1}. Accuracy using the Carlo Erba is assured by the analysis of Estuarine Sediment for every 12 samples or one standard for each tray on the autosampler.

The absolute limit of detection for the Antek instrument is 0.01 μg (11% CV) at the highest sensitivity setting available, corresponding to a relative detection limit of 0.3 nM S L^{-1} for a 1 L sample. The precision of the method is approximately 4% (CV) for sulfur signals greater than 2 μg and 11% for the smallest amount routinely tested (0.5 μg S). The accuracy of the Antek has been tested by analyzing known amounts of sulfur in several compounds chosen to represent various sulfur-containing metabolite pools present in phytoplankton, as well as directly analyzing a SO_2 gas standard. No significant differences have been observed among the standard curves of S-methionine and other organic sulfur-containing compounds tested [Matrai, 1989]. Inorganic sulfur, such as that present in sodium sulfate and the ester sulfate carrageenan, has shown a significantly lower signal. This reduces the possibility of sea salt-sulfur contamination.

5. Analysis of Total Mass

Methods and problems associated with total mass are discussed in detail in the inorganic section and in the contributions of Bishop, Karl et al. and Sherrell (this volume, pages 229, 73 and 289). Certain aspects of the measurement of total mass by the methods described there are particularly important for bioelements. We often wish to know the mass of a particular bioelement as a function of total mass. Two problems arise here. First, where possible the sample for mass should be taken from the same collecting bottle as the sample for C, N, P and S. This will reduce variability due to real variability in nature expressed by two different bottle samples. Second, mass is most accurately determined on Nuclepore filters while C, N, P and S are usually measured using material collected on glass fiber filters. These filters have different retention characteristics and care must be exercised in selecting a membrane filter of appropriate pore size to match the retention characteristics of the glass fiber filters. Sheldon [1972] reports a nominal pore size of 0.7 μm for GF/F glass fiber filters. Recommendations for comparable Nuclepore pore sizes range from 0.2 to 0.8 μm depending upon the volume of sample to be filtered and the nature of its contained particles.

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