Examining marine particulate organic matter at sub-micron scales using scanning transmission X-ray microscopy and carbon X-ray absorption near edge structure spectroscopy

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Abstract

Marine sinking particulate organic matter (POM) represents the link between surface primary production and burial of organic matter in marine sediments. As such, the nature of this material has been the subject of numerous studies attempting to characterize its composition. The results of these studies have shown that a significant proportion of POM is not recognizable as known compounds, and that the proportion of uncharacterized material increases with age/depth/diagenesis. However, few studies have examined the spatial heterogeneity of this material. This study uses a new tool, scanning transmission X-ray microscopy (STXM), together with carbon X-ray absorption near edge structure (C-XANES), to examine POM collected from sediment traps deployed at one location in the Arabian Sea as part of the JGOFS program. The results indicate that POM is composed primarily of four distinct phases: protein, an aliphatic rich phase, a carboxylic-acid-rich phase, and a phase with complex unsaturated and quinone character. This last phase may be a condensation product between carbohydrates and proteins, or from degraded plant pigments. Many particles consisted of a single chemical phase; however, in particles with mixed compositions, individual domains retained distinctive chemical signatures at the instrument’s resolution limit (50 nm). All major chemical phases were observed in sediment trap particles from 531 to 3369 m depth, supporting the hypothesis that non-selective degradation dominates particle remineralization, and that overall particle compositions are determined by near surface processes. Only one particle, out of more than 60 examined, exhibited soot-like
composition. The lack of a significant black carbon/soot component may be attributable to sampling during the winter monsoon period.

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1. Introduction

The nature of organic matter leaving the euphotic zone and its fate have been the subject of much debate (Lee et al., in press). Wakeham et al. (1997) were able to identify many compounds directly by HPLC, GC, and GC/MS, yet, their work pointed to an inability to identify the majority (>50%) of carbon in sinking POM. The presence of “analytically uncharacterized” material in sinking POM (Hedges et al., 2000) has led to a number of hypotheses about the nature of this material. Sources may include: algaenan or waxy insoluble material (Hwang and Druffel, 2003), soot or ash-derived black carbon (Deuser et al., 1983), transparent exopolymers formed from polysaccharides (Chin et al., 1998; Passow et al., 2001; Lee et al., in press), or organic matter that has been rendered unidentifiable by internal polymerization/crosslinking (Hedges, 1978; Qian et al., 1992). Attempting to reconcile between these competing hypotheses is difficult as different methods examine only selective fractions of POM. There is also the unstated assumption that compounds are homogenously distributed within samples, but there is very little information to support or refute this.

One reason that spatial information content of samples has been largely ignored in biogeochemistry is that the analytical techniques most commonly used in this field do not lend themselves to acquiring this information. Studies of cells using various microscopy techniques (Mitchell, 2001; Sommer and Franke, 2002; Jamin et al., 2003) serve as reminders that organic matter is produced with chemical heterogeneity on nanoscales. Although the composition of individual structures may be tremendously altered by diagenesis, it is not a given that these structures will be homogenized. In special cases, plant cell structures have been shown to survive burial for hundreds of millions of years, with some features still identifiable by their distinct chemical signatures (Boyce et al., 2002).

Electromagnetic-radiation-based micro-spectroscopy methods such as infrared and X-ray microscopy are increasingly used as tools to investigate problems in environmental chemistry and biochemistry (Myneni, 2002; Dumas and Miller, 2003; Miller et al., 2003). These spectromicroscopy methods can generate information on functional group distributions within samples with enough spatial and chemical resolution to minimize “signal averaging” problems, and with minor sample handling (e.g., no derivatization or extraction). Infrared microscopy is diffraction limited to spatial resolutions of roughly 3–10 μm, while X-ray microscopy has a theoretical resolution limit of less than 10 nm, with current instruments limited to about 30 nm (Jacobsen and Kirz, 1998).

Using marine particulate organic matter (POM) from the Arabian Sea, we have applied scanning transmission X-ray microscopy (STXM) together with concurrent measurements of carbon X-ray absorption near edge structure (C-XANES) spectroscopy to examine the potential for identifying particle compositions and mapping particle heterogeneity. This approach allows us to examine the composition of POM on a particle-by-particle basis. Although there are limitations in examining large particles (>200 μm diameter particles were not analyzed), and particles need to be dried and briefly heated to embed them in S8 prior to sectioning, our approach examines all particles without extraction or derivatization and thus provides an overall view of particle compositions.

2. Background on X-ray microscopy

X-ray absorption edges arise when an incident X-ray photon exceeds the threshold energy needed to
completely remove (ionize) an electron from an inner-shell orbital. The result is a step-like rise (the \(Q\) edge in X-ray absorption near edge structure spectroscopy) in the absorption cross section. Photons with energies just below the ionization edge (280–300 eV in the case of carbon 1s electrons) can promote core electrons into a variety of bound states that correspond to unoccupied or partially occupied molecular orbitals (Fig. 1). These near edge absorption bands are sensitive indicators of the local chemical bonding environment surrounding the atom in question (Stöhr, 1992). In the case of C (1s) XANES, the absorption spectrum can be used to estimate the abundances of different functional groups, subject to the caveat that carbon in different electronic environments may have vastly different absorption coefficients, and in many cases absorption bands from different organic functional groups overlap.

Extensive C-XANES studies of polymers and other well-characterized materials provide generally robust peak assignment criteria. In the case of carbon K-shell electrons, the near edge absorption bands span from 280 to 295 eV and correspond to transitions to both unoccupied \(\pi^*\) (antibonding) and low lying \(\sigma^*\) orbitals. As a general rule, the peak width is given by the lifetime of the photoexcited transition. Transitions at lower energies relative to ionization edge have longer lifetimes, and since the 1s–\(\pi^*\) transitions occur at lower energy than do 1s–\(\sigma^*\) transitions, the 1s–\(\pi^*\) transitions tend to have a narrow band width. Conjugated \(\pi\) orbitals present multiple unoccupied \(\pi^*\) orbitals to which the photoexcited electron can be promoted. In general, however, these higher energy 1s–\(\pi^*\) transitions are considerably weaker than the lowest energy 1s–\(\pi^*\) transition. Table 1 lists the peak energy assignments for common carbon functional groups. The lowest absorption bands, occurring at around 284–285 eV, correspond to molecules with unusually low energy \(\pi^*\) states, e.g., quinones. Aromatic carbon that is bonded to hydrogen or reduced carbon exhibits a strong 1s–\(\pi^*\) transition at \(\sim\)285 eV. With the addition of electron withdrawing elements in the ring (e.g., pyridinic nitrogen) or substituted on the ring (e.g., carbonyl or oxygen) the binding energy of the 1s electron is increased shifting the 1s–\(\pi^*\) transitions of aromatic carbon to higher energies, e.g., \(\sim\)286 eV for carbonyl and nitrogen, and \(\sim\)287 eV for oxygen (e.g., phenols). In the case of carboxyl functional groups, the effect of two oxygens results in a shift of the 1s–\(\pi^*\) transition up to 288.5 eV; carboxyamide’s 1s–\(\pi^*\) transition lies slightly lower at 288.2 eV (see discussions by Urquhart and Ade, 2002). Saturated carbon exhibits a relatively strong absorption band near 287.5–288 eV, when substituted with oxygen (e.g., ethers and alcohols), this transition shifts up to \(\sim\)289.5 eV. Although an overlap exists between different functional groups (most notably in the 286–289 region), in marine

### Table 1

<table>
<thead>
<tr>
<th>Approximate energy ranges for primary absorption peaks at the carbon 1s edge</th>
<th>Functionality</th>
</tr>
</thead>
<tbody>
<tr>
<td>284</td>
<td>quinone</td>
</tr>
<tr>
<td>284.9–285.5</td>
<td>unsaturated/aromatic</td>
</tr>
<tr>
<td>285.8–286.4</td>
<td>aromatic C–OH</td>
</tr>
<tr>
<td>286.6</td>
<td>ketone C=O</td>
</tr>
<tr>
<td>287.1–287.4</td>
<td>aliphatic</td>
</tr>
<tr>
<td>287.7–288.6</td>
<td>carbonyl</td>
</tr>
<tr>
<td>289.3–289.5</td>
<td>alcohol</td>
</tr>
<tr>
<td>290.3–290.6</td>
<td>carbonate</td>
</tr>
</tbody>
</table>

Sources: Francis and Hitchcock (1992), Hitchcock et al. (1986, 1992), Hitchcock and Ishii (1987), Hitchcock and Stöhr (1987), and Urquhart and Ade (2002).
organic matter one can use other information, e.g., previous NMR studies, to rule out the presence of significant aldehyde or ketone functionalities (Hedges et al., 2001).

Spectra at the full 30–50 nm resolution limit of present-day X-ray microscopes can be obtained either by using cross-correlation methods for post-facto alignment (Jacobsen et al., 2000), or laser-interferometer scanning stage control (Kilcoyne et al., 2003), to acquire a large series of images over a range of energies spanning the XANES spectral region. This stack of images yields a data set with spatial dimensions $X$ and $Y$, and energy dimension $E$. All of the particle data presented in this paper were collected from such three-dimensional data sets. By taking the ratio of data from images collected below and at a particular absorption edge, one can map the concentration of specific functional groups across a sample. For example, calculation of the ratios of image maps from 285 and 280 eV yields a map of the distribution of unsaturated carbon within a sample, while a ratio of 298 eV (above the XANES regions) to 280 eV yields a carbon density or optical density map.

Because we obtain data with 100–200 spectral points, and several tens of thousands of spatial locations, multivariate statistical analysis methods are used to conduct detailed sample analysis. Principal component analysis (PCA) provides a means of highlighting successive themes of correlation of spectra among the $(X, Y, E)$ data (King et al., 1989; Bonnet et al., 1999; Osanna and Jacobsen, 2000), and to reject poor correlations which are dominated by photon statistics noise only. We have used PCA as a method of generating a noise-filtered, orthogonal space for cluster analysis of these data (Jacobsen et al., 2003; Lerotic et al., 2004). By grouping pixels together based on their spectroscopic signature and with PCA noise-filtering, one can obtain spectra from representative regions in the sample with a vastly improved signal to noise ratio. At the stage of development employed here, cluster analysis has not always succeeded in finding few-pixel regions with different spectroscopic signatures, although efforts to improve this in future work are underway. This paper therefore makes use of both cluster analysis and user-selected spectromicroscopy analysis.

3. Methods

Archived sediment trap samples from the Arabian Sea JGOFS program were analyzed from station M4 (531, 814, 2222, and 3369 m). The collection methodologies are described in Lee et al. (1998) and Wakeham et al. (2002). Small subsamples (~1 mg) were embedded in reagent grade elemental sulfur by briefly heating (~120 °C) a mixture of fine S$_8$ grains with the samples on a clean glass slide until the mixture melted into a drop. The drop was then subsampled into 70–100 nm thick sections using a Leica Ultra-Cut™ ultramicrotome and a diamond knife. Sections were transferred to silica monoxide supported TEM grids for storage and analysis. Images and stacks were collected at the scanning transmission X-ray microscope located on beamline X1-A (outboard branch) at the National Synchrotron Light Source in Brookhaven National Laboratory (Winn et al., 2000). Full details on the microscope design, operation and capabilities are given in Feser et al. (2000) and Feser et al. (2001).

4. Results

Rather than present spectral libraries of possible model organic compounds present in sinking POM, we present below representative spectra found in an investigation of more than 60 POM particles analyzed. Although material from four traps was analyzed, the number of particles observed ranged from 7 (531 m trap) to 20 (2222 m trap). The limited number of observations, necessitated by an average analysis time of 2 h per observation (one to five particles per observation), makes combining all observations the best choice for the overview analysis presented here. Particle chemical identifications are based upon peak positions (Table 1) and relative intensities. The spectra shown in this paper are in units of optical density, which is the negative of the logarithm of the ratio of transmitted to incident flux, $-\ln(I/I_o)$. As a reference, a 110-nm-thick, 2 g/cm$^3$ carbon film would have an optical density of 1.0 at 300 eV. The $x$-axis is plotted as X-ray energy in electronvolts. The following organic matter types were observed.
4.1. Protein

Proteins exhibit both a 285 eV peak, due to the presence of aromatic amino acids phenylalanine, tyrosine and tryptophan, and a strong 288.2 eV peak (Fig. 2A). The 288.2 eV peak is due to the presence of amide-linked carbonyl carbon, and is shifted down in energy by 0.2–0.3 eV from the 1s–π* transition of carboxylic acids. As noted below, protein may also be present in several of the other particle types identified. However, for the purposes of identifying the majority chemical composition, the presence of a 288–288.2 eV peak was considered diagnostic for the dominance of protein within a particle.

4.2. Material with a strongly acidic nature

In many particles with a dominant absorption peak in the 288–289 eV region, the peak center was observed at 288.5 eV, characteristic of carboxylic acids (Table 1; Fig. 2B). This material exhibited consistent spectra, namely a small peak in the 285–286 eV region followed by a primary peak centered at 288.5 eV. The small 285 eV peak indicates the presence of either aromatic or olefinic carbon. The relatively broad nature of the 288.5 eV peak, with significant energy in the 288.0–288.3 eV range likely records some protein content. A protein spectrum is overlaid in Fig. 2b to illustrate the spectral differences between these particle chemistries. The molar absorption coefficient of aliphatic carbon is much weaker than that of carbonyl carbon; nonetheless, there is a significant absorption component in the 287–288 eV region, as well as some spread of the 288.5 eV energy into the 289.5 eV region. Material containing a very prominent and narrow 288.5 eV peak and consisting of long, thin “strings” was found at 531 and 814 m depths. Spectrally and structurally, this material strongly resembled lipid-rich cell walls, although an absolute confirmation of its source could not be made. All acid-rich particles typically had a 288.5 eV peak that was 20–30% greater in intensity than the 290–300 eV range.

4.3. Aliphatic particles

Some particles were notable for lack of a strong peak in the 287–288 eV region, instead exhibiting a rise/plateau spectrum in this energy range. This suggests a chemical composition dominated by aliphatic carbon (Table 1). This material also contained significant energy absorption in the 286 and 287 eV ranges, but little or no absorption in the aromatic region. The high C/O ratio and presence of peaks in the ketone/alddehyde region suggest a waxy or paraffinic nature of this material.

4.4. Carbohydrates

Sinking particulate organic matter contains a significant proportion of carbohydrates (Wakeham et al., 1997). Carbohydrates are dominated by a 289.5 eV absorption peak (Cody et al., 1996; Cody and Sághi-Szabó, 1999) (Fig. 2D). There is also a minor 286.5 eV peak that may be an artifact of radiation damage or diagenesis. Although we used techniques designed to minimize this problem (scanning from low to high energy range, using minimal dwell times and imaging the particle as few times as possible prior to spectral analysis) carbohydrates (as well as other compounds) are susceptible to radiation damage (Foster et al., 1992; Cazaux, 1997; Cherezov et al., 2002; Ito et al., 2002; Ravelli et al., 2002), and this damage appears as both a decrease in the 289.5 eV absorption (mass loss) and an increase in the 286.5 eV absorption. The increase at 286.5 eV is presumably due to the formation of aromatic heterocycles like furans (Newbury et al., 1986). The formation of phenolic carbon is unlikely as there is an insignificant component present at 285 eV.

4.5. Calcium carbonate

Carbonates exhibit a very characteristic strong absorption peak at 290.5 eV (Fig. 2E) (Urquhart and Ade, 2002). In pure CaCO₃, only a few other minor absorption peaks are observed (Fig. 2E), but in our sediment trap samples, the presence of organic matter was often detected intermixed with the mineral.

4.6. Particles with quinone/unsaturated character

There were a significant number of particles exhibiting strong absorbance in the 284–286 eV range associated with unsaturated or aromatic carbon (Fig. 2F). This organic matter is distinguished by several
Fig. 2. Carbon XANES spectra of different classes of marine particulate matter. (A) Protein, (B) acid-rich (with protein spectrum as a comparison), (C) aliphatic-rich, (D) carbohydrate, (E) CaCO₃, (F) quinone/unsaturated, (G) soot/black carbon, and (H) non-carbon mineral.
spectral features: (1) presence of a significant 285 eV peak with components at 284 and 286 eV associated with unsaturated carbon, phenols, quinone, and polynuclear aromatic carbon (Horsley et al., 1985; Francis and Hitchcock, 1992; Hitchcock et al., 1992; Hitchcock and Mancini, 1994); (2) significant absorption in the 287–288 eV range associated with aliphatic carbon; (3) a lack of a strong absorption peak in the 288 or 289 eV range. Some particles do exhibit small peaks in the 288.5 and 289.5 region, suggesting that they also may contain organic acids and alcohol moieties.

4.7. Black carbon/soot

The presence of soot or combustion products in sinking POM has been observed (Schmidt and Noack, 2000). Of the ~60 particles that we have examined from station M4 in the Arabian Sea, only one has unambiguously fit the profile of a combustion source particle (Fig. 2G). This particle contained a large and well-defined 285 eV peak, followed by peaks in the 288.5 and 287.5 eV range. This spectrum is characteristic of combustion-derived soot (see spectra of NIST standard soot at http://xray1.physics.sunysb.edu/data/spectra.php), with the exception of a minor 288.5 peak that may be the result of adsorbed marine carbon or UV induced oxidation.

4.8. Non-carbon minerals and elements

Mineral grains and SiO2 frustules have “flat” spectra with no absorption peaks present (Fig. 2H). The presence of mineral in organic matter is expressed by a uniform increase in optical density, which is especially noticeable in the region below the C edge (<283 eV). Thus, if a particle contains a significant amount of mineral, the optical density will be shifted upwards by >0.5 (compare the baseline in Fig. 2D with that in Fig. 2A for an example). The only common element with an absorption peak in the 280–300 eV range is potassium, this exhibits 2s→π* shell doublet peaks at 295 and 297 eV. This energy range lies above the region of interest for organic carbon; however, it can complicate efforts to determine particle densities based upon techniques that integrate the region immediately above the absorption edge at ~290 eV.

5. Discussion

5.1. Overall distributions

The roughly 60 particles from Arabian Sea sediment traps examined by C-XANES were dominated by acid-rich (~25%), aliphatic (15%), protein-rich (15%), and quinone/aromatic (10%) compositions (Fig. 3). In many cases, particles consisted of adjoining regions with different chemical compositions, such as carbohydrate/acid, protein/acid, and algaenan/acid; in several cases, particles consisted of three or more dominant chemical types distributed non-homogenously (denoted as Mix, Fig. 3). Our estimate of particle chemical distributions is preliminary; without detailed spectral deconvolution within each region, we cannot quantify exact chemical proportions. The presence of a noticeable (>0.1 optical density) peak at 290.5 eV on organic particles was considered diagnostic for the presence of CaCO3. Most of the CaCO3-associated material fell into the acidic particle category (Fig. 3). This is consistent with the known preferential association of acidic amino acids and other compounds with CaCO3. The presence of protein in some of the acid-rich particles

![Image](https://example.com/image.png)

Fig. 3. Particle chemistry histogram. Dark bars represent particles observed without any sign of a CaCO3 peak at 290.5 eV, while speckled bars represent particles with a CaCO3 peak.
is also likely, as many of these particles contained non-trivial (>0.05 optical density) 285 eV peaks associated with aromatic amino acids (although unsaturated fatty acids and other olefins are also a possibility). Thus, our estimate of the number of protein-containing particles is an underestimate. The same is true for the aliphatic class; without spectral deconvolution, it is impossible to assign a proportion of this composition to particles containing a significant amount of protein or acidic materials.

In three cases, we observed carboxylic acid-rich linear or branched structures (two each in the 814 m samples, one in the 531 m samples). Examples of two of these are given in Fig. 4. The strong peak at 288.5 eV in this material is consistent with the presence of fatty acids. This material may include the remnants of cell walls, and indeed in Fig. 4A, a series of linked 4–5 μm length cells is presented. This material appears devoid of debris or other cellular remains.

Most particles examined appear to be composed of only a single chemical phase. About a quarter of particles consisted of two or more closely intermingled phases. This suggests that the processes of digestion and aggregation that lead to POM formation do not normally homogenize or mix this material on tens of nanometer scales, although smaller scale mixing is possible. Principal component analysis on one mixed particle indicated that, although some of the components appear primarily to be variations in thickness of the same material (e.g., Fig. 5D and E), there are at least four chemically distinct phases present. The material mapped in Fig. 5C, D, and E are dominated by aliphatic components, although the outer edges appear to have some added acidic or proteinaceous character. There is also evidence of carbohydrate (Fig. 5G,H), CaCO₃ intermixed at a sub-micron scale with acidic or proteinaceous organic material (Fig. 5I), and inorganic, presumably siliceous, particles (Fig. 5J). The carbohydrate-rich material appears more closely associated with the non-CaCO₃ containing particles, while the other fractions appear to be randomly distributed.

In the case of the three-component system shown in Fig. 6, an inorganic core is surrounded by an aliphatic-rich layer, followed by a transition to a proteinaceous/acrid outer layer. The presence of defined peaks in the background spectrum shown in Fig. 6C indicates that the PCA software has integrated some regions with organic matter in them. However, the intensity of such peaks is very small (compare the maximum peak intensity in Fig. 6C at ~0.08 optical density, with that in Fig. 6D at ~1.2 O.D.). Thus, although some organic matter has been integrated into the average spectrum, it is not a major component and represents very thin regions around the periphery of the particles in Fig. 6. There is also a small CaCO₃ dominated particle in the upper central portion of the image (Fig. 6G). Even in cases where compounds are
mixed, we observed distinct chemical “zones” within particles (Figs. 5 and 6). It is interesting to speculate whether the presence of heterogeneous chemical “domains” within particles might lead to entanglement or encapsulation of reactive compounds that could make it difficult for enzymes to recognize organic matter. For example, in Fig. 6, there exists a gradient between acid-rich and aliphatic-rich compositions within a single particle.

5.2. Particle character

Our results suggest that marine sinking POM from the Arabian Sea is a mixture of phases,
including several that could be difficult to analyze by liquid or gas chromatography. One such phase could be algaenans. The suggestion that algaenans and other waxy or aliphatic cross-linked phases are abundant in sinking POM (Hwang and Druffel, 2003) is supported by the presence of a significant component of aliphatic-rich particles (15%, Fig. 3). If one includes all particles containing some fraction of this material, the proportion rises to >20%. While recognizing that particle abundances do not necessarily correlate to particle mass, this still suggests that this material is a major component of the sinking POM. The lack of a strong 288 or 289 eV peak in this material indicates that it is extremely oxygen poor, and thus hydrophobic. As the oscillator strength of carboxyl groups is quite high, even a small amount of COOH functionality would result in a noticeable 288.5 eV peak, which was not observed in most cases (Fig. 2C).

The most intriguing particle composition category is the quinone/aromatic type (Fig. 2F). This is a significant particle category (10%, 15% if mix particles are included). The major cellular biochemicals: protein, carbohydrate, lipids, and nucleic acids, do not exhibit an absorption peak in the 284–285 eV (quinone) region. However, certain photosynthetic reaction centers use quinonic moieties for electron transport, and certain condensation reactions can also produce quinones. The dissimilarity in spectra between chlorophyll $a$ (Fig. 7) and the samples...
indicates that this POM category is not simply undegraded chlorophyll or even a simple degradation product of this pigment (e.g., phaeophorbide). Only minor amounts of pigments and their intact degradation products are found in sinking POM (Welschmeyer and Lorenzen, 1985; Lee et al., 2000). Another possible reaction pathway that could produce quinone moieties is the reaction between proteins and carbohydrates, the Maillard reaction (Hedges, 1978). Several studies have shown that this reaction is capable of providing a wide range of polynuclear aromatic, quinone and unsaturated compounds (e.g., Olsson et al., 1977, 1978; Miller et al., 1984). Indeed, C-XANES of the reaction product of lysine and glucose (e.g., melanoidin) contains significant spectral content in the 284–285 eV range (Fig. 7A), but is again, not spectrally identical to this POM class. There is also a spectral feature at around 286.3–287.0 in Fig. 2F that may correspond to a peak at 286.4 eV in the melanoidin spectrum. Without further work, it is not possible to distinguish between these two sources, or to rule out another pathway that can produce this material.

Surprisingly, a particle class that does not appear to form a major component of sinking POM in Arabian Sea samples is soot/black carbon (BC). In this study, we observed only one such particle (out of >60); if combustion products made up a significant percentage of sinking POM, then one might expect to observe many more. The quinone/aromatic fraction described above may be ruled out as soot or combustion derived BC based upon two observations: (1) Most of that particle class had a morphology inconsistent with soot, being composed of strings and membrane-like structures; and (2) soot and graphitic material does not, in our experience, exhibit a significant sub-285 eV component. It must be noted that the Arabian Sea is a marine productivity dominated regime, and that samples from sites closer to urban population centers may contain larger amounts of soot and ash derived black carbon. These samples were also taken during the winter monsoon period, when prevailing winds come from Somalia. The presence of significant amounts of soot and combustion products in aerosols originating from the Indian subcontinent (Dickerson et al., 2002; Menon et al., 2002) would presumably generate a greater abundance of black carbon in particles collected when winds from the NE are prevalent. It also may be possible that very small aerosol particles of BC would be overlooked by our method (although stack analysis targeting aromatic rich particles was done for each data set). However, very small particles would not sink unless adsorbed or otherwise attached to larger particles.

Given the high proportion of protein identified in sinking POM by Wakeham et al. (1997) and Lee et al. (2000), it is not surprising to see protein as a major particle composition class (15%, or 27% including mixtures, Fig. 3). Some particles within the acid-rich particle class (25%, Fig. 3) also undoubtedly contain significant amounts of protein, based upon broadening of the primary peak in the

![Carbon XANES spectra of (A) chlorophyll a and (B) melanoidin produced by the reaction of lysine with glucose.](https://example.com/figure7.png)
288.0–288.3 region. It must be noted that other, non-protein, compounds can give spectra quite similar to the acid-rich type particles shown in Fig. 2B. For example, peptidoglycan and chitin both have primary absorption peaks centered at 288.5 eV. The chief spectral differences between pure peptidoglycan and the particle spectra in Fig. 2B are that peptidoglycan exhibits no absorption in the aromatic region (285 eV) and has a significant 289.5 eV peak associated with alcohol (peptidoglycan, chitin and other model compound spectra can be found at the X1A online spectral library http://xray1.physics.sunysb.edu/data/spectra.php). The lack of strong 289.5 eV peaks in the acidic particles indicates that either alcohol functionalities are lost early in diagenesis or that the material began with a lower alcohol/carbon ratio than typical for carbohydrates.

5.3. Implications for the alteration of sinking POM

Given the results presented above, one can begin to put different results in the context of the large body of work examining POM decomposition. Hedges et al. (2001) suggested that the overall chemical composition of sinking POM was consistent with passage through the water column. Although we did not measure enough particles to make definitive statements about the effect of early diagenesis upon particle composition (less than 15 particles were measured at each depth), we did observe all of the major particle chemical categories at each depth. This would suggest that each particle type is labile to some extent, as there will be a significant loss of material between the 531 and 3369 m traps (Lee et al., 1998). In addition, even material that would presumably be extremely labile, such as proteins, is present at depths where over 2/3 of the total POM flux has been remineralized (Wakeham et al., 1997; Lee et al., 1998). Thus, the overall pattern of particle chemistry, including the presence of presumably highly altered material, appears to be set early on in the lifespan of POM. The large proportion of analytically uncharacterizable material in deep-water POM is consistent with the presence of waxy, “algaenan”-like material (Hwang and Druffel, 2003) as well as heavily degraded, presumably crosslinked quinone/aromatic containing organic matter possibly derived from reactions between proteins and carbohydrates. The apparent absence of soot or black carbon in this marine organic matter, as opposed to previous studies (Masiello and Druffel, 1998; Middelburg et al., 1999; Tsapakis et al., 2003), may be due to a lack of input of these materials during the sample period, or it may be an artifact of the chemical and physical isolation procedures used.

Many questions still remain and new ones are raised by this work. Chief among these is why different compound classes appear to be remineralized with equal alacrity by heterotrophs. When one considers the spectrum of particle character, from cell walls and protein-rich particles to waxy/algaenan-like and highly condensed quinone/aromatic containing molecules, the expectation would be that some material would be removed/remineralized preferentially, consistent with the results obtained by molecular-level studies (Wakeham et al., 1997; Lee et al., in press). Again, we cannot make definitive statements as to particle class abundance with depth, but clearly, given the limited subset of data presented here, we can state that no one class is completely removed by diagenesis at depth. It is not possible, given the overall mass losses experienced by sinking POM, to state that any of the chemical types presented in Fig. 2 is immune from degradation. A plausible explanation is provided by Armstrong et al. (2002), where material that reaches the deep sea is dominated by mineral-ballasted organic matter. It may be hypothesized that all of the organic matter particle types presented here are susceptible to degradation, but at a rate slow enough so that particles with mineral ballast can reach the deeper ocean with their initial and overall chemistries intact.

6. Conclusions

The use of synchrotron-radiation-based soft X-ray spectromicroscopy has enabled the examination of individual particle compositions from sediment trap samples with minimal sample alteration. Particles collected in traps from 531, 814, 2222, and 3369 m depths from station M4 in the Arabian Sea JGOFS program (Lee et al., 1998) showed a variety of individual particle phases, but were dominated by four types. Particles with strong 288.5 eV peaks
characteristic of carboxylic acids formed the highest percentage of material, but protein, waxy, and quinone/aromatic particle chemistries were also observed. Intact cell walls were observed in trap samples down to a depth of 814 m, but not seen below that depth. All other particle classes were observed down to the lowermost trap samples at 3369 m depth. The results suggest that much of the “molecularly uncharacterized” fraction described by Wakeham et al. (1997) and Hedges et al. (2000) is composed of aliphatic-rich and quinone/unsaturated material. We hypothesize that this latter particle composition is the result of Maillard-type reactions between proteins and carbohydrates and or pigments that take place early in the particle’s history. Only 1 out of more than 60 particles fit the spectral profile of soot or other “black carbon” combustion derived materials, suggesting that this material is not a major component of sinking organic matter in the Arabian Sea during the winter monsoon period. The presence of all four major particle classes in even the deepest trap samples also suggests that no one particle composition is particularly susceptible to degradation, rather, the survival of individual particles may be a balance between slow degradation of all particle classes and sinking rates influenced by the presence or absence of inorganic minerals. Future work involving detailed spectral deconvolution or “line shaping”, as well as examination of more particles collected from different regions and seasons, will provide a more complete assessment of particle chemical compositions.

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