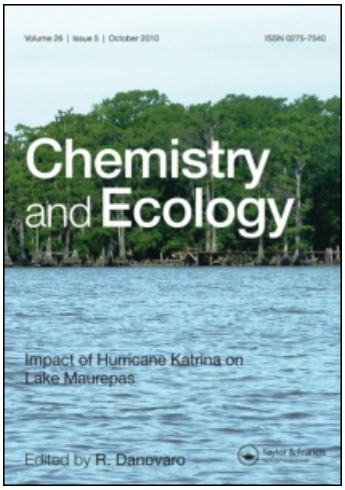

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ORGANIC AND INORGANIC CHARACTERIZATION OF MARINE COLLOIDS

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Isolates of the organic matter in the particulate, colloidal and dissolved states were obtained by tangential flow ultrafiltration through 0.40 μm polycarbonate and ~1 nm (1000 NMWL*) regenerated cellulose membranes and by solid-liquid reverse phase extraction techniques. The material was analyzed qualitatively by mass spectroscopy, proton nuclear magnetic resonance, Fourier transform infrared spectroscopy, high performance liquid chromatography with fluorescence detection of labelled primary amines and amino acids, and inductively coupled plasma/mass spectroscopy. All three states are characterized by similar organic chemistries. The marine colloidal state in coastal waters off the Californian coast contains primarily carbohydrates, fatty acids, minor amounts of proteinaceous compounds and electropositive elements including aluminium and iron. Aromatic molecules and olefinic functional groups are in low concentration. The colloidal state differs qualitatively from the particulate and dissolved states. Yet all three could be derived, with degradation, from algal or macroalgal surface components.

KEY WORDS: Colloids, coastal waters, qualitative analysis.

INTRODUCTION

Comparative studies of the nature of the organic matter in the particulate, colloidal and dissolved states isolated from the same sea water sample, have not been sought until now, yet they are clearly important in understanding the chemistry of the carbon cycle. The characterization of soluble and suspended organic matter in the marine environment is not a new endeavour (Kalle, 1937). The marine organic material has been described as phenolic (Meyers-Schulte and Hedges, 1986), aliphatic (Steurmer and Harvey, 1978; Harvey *et al.*, 1983; Harvey *et al.*, 1984; Ishiwatari, 1992), aromatic (Steurmer and Harvey, 1978) and polysaccharidic or polyhydroxy alkyl (Benner *et al.*, 1992; McCarthy *et al.*, 1993; Burney and Sieburth, 1976; Johnson and Sieburth, 1976; Steurmer and Payne, 1976).

Local marine flora has been long suspected as the source of this organic material (Sieburth and Jensen, 1968; Sieburth and Jensen, 1969; Sieburth, 1969). Brown algae are known to contain carbohydrate polymers (Mabeau and Kloareg, 1987; Percival and Mian, 1972; Vreeland, 1974), phenolic polymers (Koch *et al.*, 1981; Craigie and McLachlan, 1964; Faulkner, 1992), and both cyclic and linear aliphatic moieties

* Nominal Molecular Weight Limit

(Faulkner, 1992). The most abundant class of compounds found in brown algae are the carbohydrates (Faulkner, 1986; Zeller and Gray, 1992; Zeller, 1993).

The organic components of the colloidal fraction in sea water are, by far, the most important quantitatively (Wells and Goldberg, 1993). The erratic concentration profiles of the colloids with depth, which can differ by factors of a thousand for differences of as little as 20 metres, strongly suggest that the colloids are highly reactive, with quite short residence times as a consequence. Inhomogeneities were observed both above and below the thermocline in the Northern Atlantic and Southern Oceans. An understanding of the reactivity of the colloids may be gained by studies of their chemical nature. Here we describe our approach to this problem using a variety of analytical techniques. The physical characteristics of the marine colloidal state provide the starting point for this work with the development of an isolation procedure along with an initial comparison of the organic matter in the particulate, colloidal, and dissolved fractions. The colloidal fraction was also assayed for more than 60 elements.

SAMPLING PROTOCOLS

Surface water was obtained with the use of a submersible, polyvinylchloride bilge pump with a flow of 2000 l h^{-1} about three km off the La Jolla, California coast (32° 52'N, 117° 17.6'W) on March 16, 1993, April 6, 1993, June 17, 1993, and September 13, 1993. Sampling occurred only when the water was calm, clear blue and the Secchi disk depth was greater than 10 m. The water was piped through precleaned, nitric acid washed, polypropylene tubing (Cat. No. 14-176-155)[1] or silicone tubing (Cat. No. G-06411-83)[2] into polycarbonate carboys (Cat. No. 02-961-55C; Nalgene No. 2213-0050, Fisher Scientific). The carboys were rinsed thoroughly with sample water prior to filling. The pump was hand-held about 0.5 m below the surface of the water on the up-current side of the boat. The Monterey Bay sample was obtained on March 27, 1993 from 10 m depth at 36° 50.13' N; 122° 20.86' W. About 20 l of sample water was drained from 10 l modified Niskin bottles mounted on a CTD rosette into a polycarbonate carboy. The modified Niskin bottles had silicone rubber "springs" installed. This sample was sent overnight via surface transport to La Jolla packaged in a polyethylene bag. It was processed on the day following its arrival.

The macrophyte *Macrocystis pyrifera* was analyzed as a representative of the plant community. This particular plant was chosen due to its high abundance near the site of sea water collection. The *Macrocystis pyrifera* sample was obtained by capturing a free floating, living frond from the lowered stairs at Scripps Institution of Oceanography pier on July 7, 1993. The mucus was removed by washing and gently squeezing the kelp frond with clean nitrile gloves (Cat. No. 11-388-33, Fisher Scientific) and eluting with ASTM Type I water (This ~ 18 megohm/cm water was produced in a SuperQ Plus system which uses one Super-C Carbon, two Ion Ex, and one Organex-Q Cartridge with a Millipak filter.[3])

[1] Nalgene No. 8020-0937, Fisher Scientific, P. O. Box 9800, Tustin, CA 92681
[2] Cole-Parmer Instrument Company, 7425 North Park Ave., Niles, IL 60714
[3] Millipore Corp., 448 Grandview Dr., South San Francisco, CA. 94080

METHODS

The scheme of analysis and the isolation techniques are given in Figure 1. The particulate and colloidal phases were isolated by a 0.4 μm polycarbonate membrane[4] and two *ca.* 1 nm regenerated cellulose membrane filters (1000 NMWL).[5] The dissolved organic components of sea water were removed with reverse phase chromatography by a C-18 Empore Disk[6] at pH 2 with 0.5% methanol and then eluted with methanol. We recognize that this isolation is not quantitative as some highly polar compounds will be missed, for example at pH 2 carboxylic acids would be retained preferentially over amines in the salt water medium. There is extensive literature regarding the isolation of known compounds on ultrafiltration membranes (Buffle *et al.*, 1992, and references therein).

The operations were carried out in a laminar flow hood. During the separation steps the complete removal of sample water and elution of sea salts was carried out with the use of Type I water. All sample collection and transfer containers were washed thoroughly, kept at least 24 h with 5% HNO_3, rinsed and filled with Type I water and left for an additional 24 h or more. The Millipore membranes were cleaned with 0.1 N NaOH, 50 ppm NaOCl at pH 8, 0.2% Terg-a-zyme (Alconox, Inc., New York, NY), 1% citric acid at pH 3, and 0.1 N HNO_3 in accordance with the recommendations of the manufacturer. The Poretics polycarbonate membranes were cleaned similarly but without the strong base. Subsequently, the membranes were rinsed with at least 20 l of

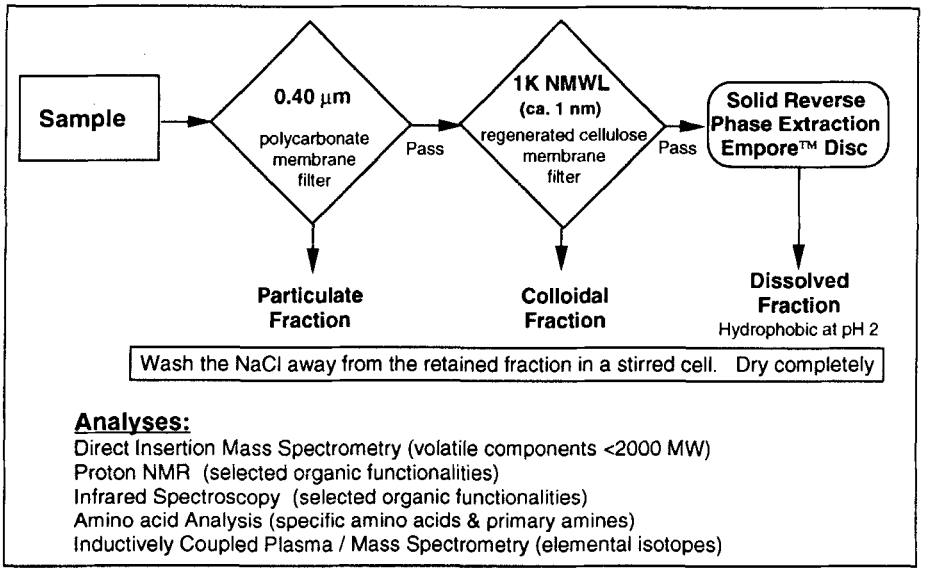

Figure 1 Flow chart of separation and analytical methods

[4] (Cat. No. 19401, Poretics Corporation, 111 Lindberg Ave., Livermore, CA 94550)
[5] Cat. No. CDUF006LA. Millipore Corp & 1000 MWCO Cat. No. 2060814. Amicon Inc. 172 Cherry Hill Drive. Beverly MA 01915
[6] Varian, 24201 Frampton Ave. Harbor City, CA 90710

Type I water. The polycarbonate membranes were placed in the Pelicon acrylic filter holder with linear flow channel (Cat. No. PSSPL2004, Millipore Corp.) and filtrate separator screens (Cat. No. PSSP00F11, Millipore Corp.). The 1000 NMWL regenerated cellulose membranes are contained in a spiral-wound configuration. Both arrangements provide large cross-membrane flow with low trans-membrane pressures in a tangential flow filtration process (Pelicon Tangential Flow Filtration Apparatus Cat. No. XX 42 PEL 60, Millipore Corp.). The trans-membrane pressures were 7 kPa for the 0.4 μm and 27 to 35 kPa for the 1000 NMWL membranes. The goal with such low trans-membrane pressures is to obtain a complete isolation of the colloidal material. With large tangential flow forces and minute trans-membrane pressures, artefacts due to concentration polarization, destruction of aggregates, the bursting of cells and the forcing of large pieces through small holes are kept to a minimum. All such artefacts can be present with much larger trans-membrane pressures (Buffle *et al.*, 1992).

Final separation of water from the samples was accomplished in a stirred cell apparatus (400 ml), where slightly higher pressures (up to 400 kPa) were used.[7] The colloidal and particulate fractions were washed off the membrane with Type I water into teflon containers and subsequently dried under 0.40 μm filtered air and infrared light. The removal of sea salts was essentially complete as indicated by the low concentration of sodium in the colloidal isolates (less than 7 nM or 10^{-6} percent of that in sea water). The 20–70 l samples intended for ICP/MS assay were kept separate from the 60–80 l samples analyzed for organic material. The ultrafiltration apparatus used for the isolation of the material studied by ^{13}C NMR is similar.

In the development of this method, we expected initially to isolate *ca.* 1 mg l^{-1} of colloids from the sea water. This was over-optimistic by a factor of four to eight. So larger samples were collected but as the sample size increased, the processing time lengthened greatly. To minimize the processing time, the 0.40 μm membrane surface area was increased from 661 to 1,980 cm^2 and the $\sim$ 1 nm (1000 NMWL) membrane surface area increased from 0.0926 to 1.11 m^2. The processing time, from sampling to final isolation of the solid, was reduced from two weeks to one day. Of the samples reported here, calcium and magnesium concentrations in the colloidal isolates were less than 40 μM and 19 nM respectively. We recognize that microbial activity could have altered the nature of the isolates during processing, especially when this was lengthy. There are, however, no observable differences in the spectra obtained from the samples processed with longer or shorter isolation times. This implies that any changes which might have occurred did not vary these bulk characterizations significantly. There is some support for this observation from the work of Wheeler (1976) who showed that processing time up to 8 h yielded no detectable alterations.

Inherent in this approach is the possibility of selective removal of different organic molecules to the surfaces of the sampling bottles, tubing, and other components of the apparatus. Any surface, such as glass and Teflon® (Goldberg *et al.*, 1988; Weiss *et al.*, 1991), can sorb both organic and inorganic material. This may be reduced by rapid processing and rinsing. The materials used were chosen to minimize metallic contamination and to withstand vigorous cleaning.

[7] (Costar, Nucleopore, 17035 Commerce Circle, Pleasanton, CA 94588)

RESULTS

Direct Insertion Mass Spectrometry: This technique involves the high temperature (up to 350 °C), high vacuum (10^{-4} Pa) volatilization of the organic phases with subsequent ionization by electrons. The mass/charge ratios of the resulting fragments are detected by a quadrapole mass spectrometer. The instrument, Hewlett-Packard 5988A, could assay masses up to 2000 daltons although the maximum observed fragment weight was 1841 in one particulate fraction. The instrument was run in the electron impact mode at 70 eV.

The spectra from the dissolved, particulate and colloidal phases of La Jolla coastal water taken in June were similar but each possessed unique characteristics (Figure 2). There were marked periodicities with separations of 14 atomic mass units (daltons) up to about 250 daltons, most likely due to the loss of CH_2 groups upon fragmentation. This offers permissive support, though not conclusive evidence, for the presence of aliphatic compounds. Other compounds, such as steroids, which also have methylene carbons (CH_2 groups) could produce similar patterns. There are common molecular weight fragments for all three fractions (e.g. masses 149, possibly $C_8H_4O_3$, 213 and 353). Although odd-valued intact molecular ions can indicate the presence of nitrogen, the absolute amount of nitrogen is estimated to be low from the amino acid analysis (see below). Thus, the odd mass ions are most likely indicative of fragments of larger, nitrogen-free molecules. Higher mass fragments were found in the particulate isolates than in the colloidal phases or dissolved phases: particulate-1841; colloidal–446; dissolved–336.

These spectral similarities suggest there are some common components of all three phases. Fractionation from a common source could allow the formation of their unique

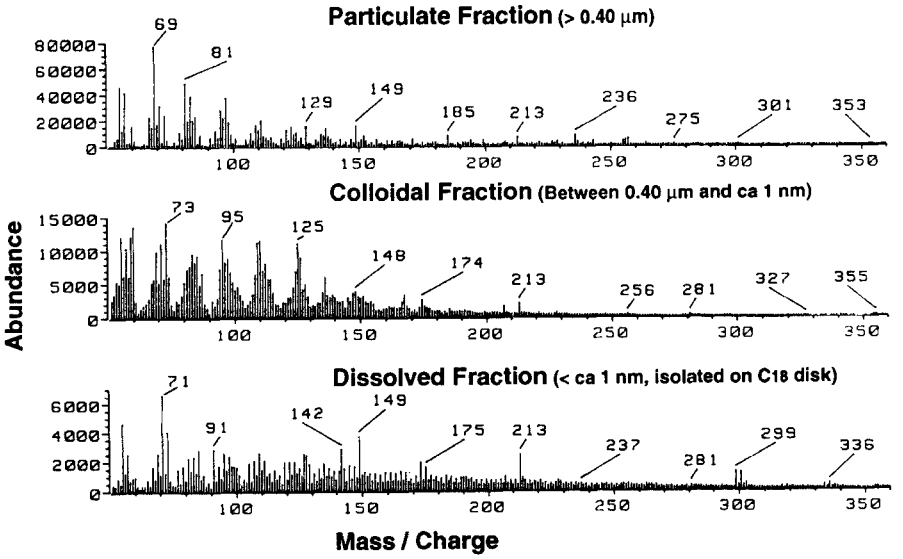

Figure 2 Mass spectra of the three fractions isolated from a 68.3 l sample of La Jolla coastal surface water in June, 1993. Identical 50–360 amu windows were chosen for the detail. ("amu" = atomic mass units).

characteristics. The aggregation of the smaller colloids into particulates (Wells and Goldberg, 1993) also supports this finding.

Proton NMR: Among other things, this technique can give the nature of the organic functionality or group with which the hydrogens are associated. The dried isolates were dissolved in D_2O and analyzed using a simple one-pulse sequence with a large number of transients added on a 500 MHz Varian Unity Instrument to provide an additional characterization of the dissolved, particulate and colloidal states from the June La Jolla coastal water (Figure 3) and of the colloidal states from both the La Jolla coastal water and the Monterey Bay water (Figure 4).

Using peak integration as a guide to relative abundance, the ^1H NMR spectra were divided into seven regions which may characterize classes of compounds on the basis of the site of the hydrogen: 1) on the carbon next to an electropositive element, 2) on an alkyl carbon, 3) on the carbon next to a carbonyl, 4) on the carbon next to an ether or alcohol, 5) in the solvent, 6) on an olefinic carbon, and 7) on an aromatic carbon. While not universal, these divisions allow a reasonable approach to the data. Some of the hazards which are inherent in this analysis were discussed in Steurmer and Payne, (1976).

The relative percentages of compounds in each region, except the solvent, for all three fractions of the June La Jolla coastal water and the colloidal fractions for the *Macrocystis pyrifera* surface slime, the April La Jolla water, and the March Monterey Bay water are given in Table I.

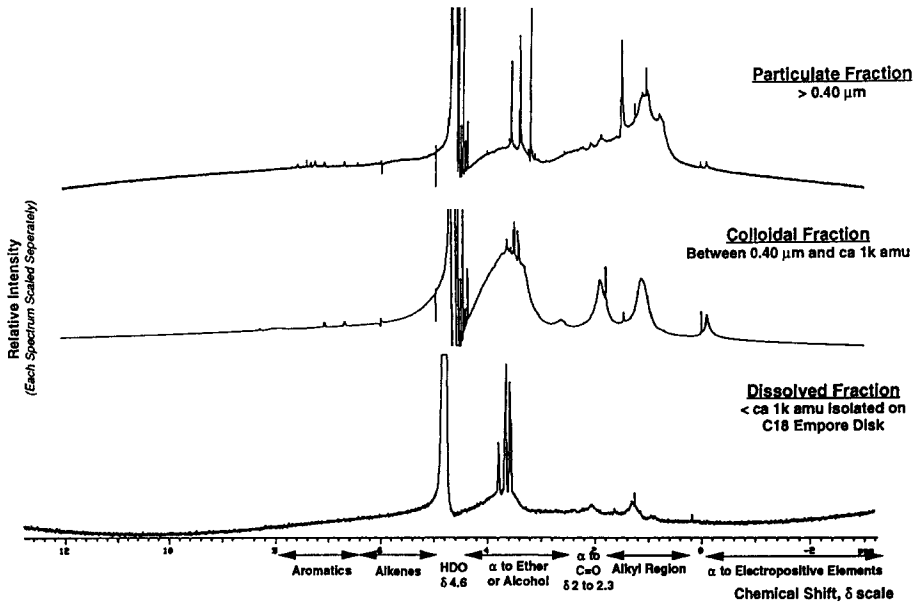

Figure 3 All three fractions isolated from the June, 1993 La Jolla surface water sample. Proton NMR at 500 MHz in deuterium oxide with a one-pulse sequence.

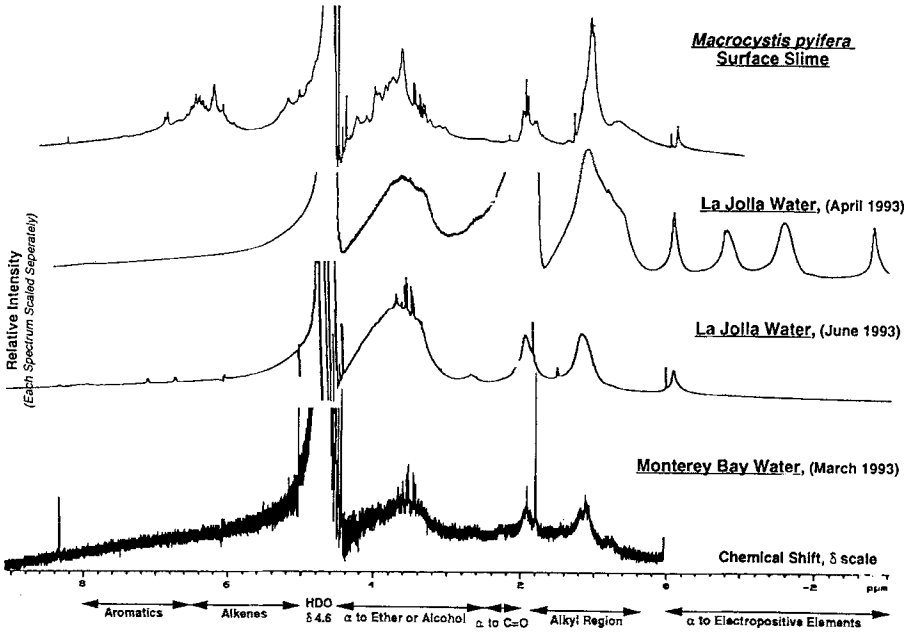

Figure 4 Colloidal fractions of surface sea water and kelp surfaces. Proton NMR at 500 MHz in deuterium oxide with a one-pulse sequence.

Table I Relative percentages of the integrated ^{1}H NMR signals.

Chemical Shift δ (ppm) Regions →	*-2 to 0.5 α to an Electropositive Element*[a]	*0.5 to 1.8 Alkyl*	*1.8 to 2.5 α to Carbonyl*	*2.5 to 4.5 α to Ether or Alcohol*	*5 to 6.5 Alkene*	*6.5 to 8 Aromatic*
June La Jolla coastal water sample						
Particulate Fraction	5	47	10	28	< 1	10
Colloidal Fraction	8	23	12	53	< 1	4
Dissolved Fraction	< 1	20	13	67	< 1	< 1
Colloidal fractions of the other samples						
Macrocystis pyrifera surface slime	3	22	10	32	14	19
April, La Jolla coastal water	6	12	70	11	< 1	2
March, Monterey Bay water	7	35	2	55	< 1	1

[a] α "means the hydrogen on a carbon next to"

The spectra of the colloidal, particulate and dissolved phases from La Jolla (April and June 1993) and Monterey are quite similar (Figure 3 and Table I). The three distinct peaks, upfield of 0 ppm in the April La Jolla sample, are unique and as yet unexplained (Figure 4). All of the samples have a large aliphatic character (δ 0.5 to 1.8 ppm) with

a large component of hetero-atom substitution, giving rise to ethers, alcohols and carbonyls, for example δ 1.8 to 4.5 ppm. Except for the April La Jolla sample, about half of the proton signal (peak area) from each colloidal isolate appears to be carbohydrate (δ 2.5 to 4.5 ppm) in nature. Each of the colloidal and dissolved isolates showed only small quantities of aromatic compounds (δ 6.5 to 8 ppm) with the colloidal amounts greater than those of the dissolved phase. This is the region in which signals from polynuclear aromatic compounds would appear. The largest contribution of aromatics from the sea water samples was contained in the particulate fractions.

The particulate fraction contains significantly more aromatic and alkyl compounds than either the colloidal or dissolved fractions. The colloidal fraction contains a larger contribution of material with hydrogens on a carbon next to an electropositive element (8%)than does the dissolved ($< 1\%$).The dissolved fraction has the greatest amount of material with hydrogens on a carbon next to an ether or alcohol. The dissolved fraction also has the same relative proportions of carbonyl ($\sim 12\%$)and alkyl (20%)functionalities as the colloidal fraction.

The colloidal fraction of the *Macrocystis pyrifera* surface slime possesses alkene (δ 5.0 to 6.5 ppm) and aromatic (δ 6.5 to 8 ppm) functionalities which distinguish them from the La Jolla coastal and the Monterey Bay sea water extracts (Figure 4 and Table I). This spectrum is consistent with a mixture of known compounds extracted from brown algae (Faulkner, 1992). It is evident from this figure that components of the organic extracts from all three states could have originated from algal or macroalgal components. Future investigations will also explore extracts from single cultures of phytoplankton for comparison. The olefinic groups are more liable to destruction through hydration or oxidation processes (Harvey *et al.*, 1983, 1984). Clearly, sterically unencumbered olefinic molecules may not long survive chemical and biological processes in the ocean environment.

Carbon-13 NMR: This technique is useful for the determination of the functionality and structure of organic compounds. Dried isolate from ~ 2301 of La Jolla coastal surface water was placed in an NMR tube with D_2O and analyzed with broad band decoupling on a 500 MHz Varian Unity Instrument to provide characterization of the colloidal state. The spectra acquired were consistent with a mixture of carbohydrates. There were no other significant resonances observed.

Infrared Spectroscopy: This analytical technique provides for identification of many functional groups in a semiquantitative way. Fractions from the La Jolla isolates, pelletized in KBr, were examined using a Perkin-Elmer 1600 FTIR. The infrared spectra of each of the three size fractions are very similar (Figure 5). All of the fractions demonstrate overlapping absorbances consistent with alkanes, amino acids, carboxylic acids, amines, alcohols, esters, amides, ethers and sulphonates. From these data alone one could not rule out the presence of alkenes or aromatic compounds in the colloidal material. Even so, all three fractions, from their spectra, display remarkable similarity which supports the view that they are from common sources.

Amino Acid Analysis: This technique provides an analysis of specific compounds and a measure of the relative contribution of proteinaceous matter to the colloidal fraction.

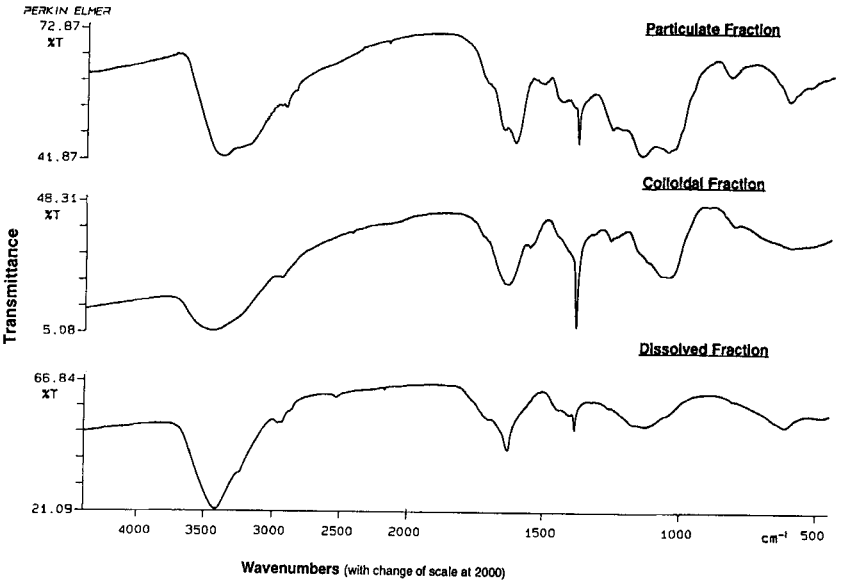

Figure 5 Infrared spectra of all the fractions from June, 1993, La Jolla surface water. The samples were dried in a vacuum oven and pelletized in KBr.

Evaluation of the April La Jolla colloidal fraction only using the reaction of *o*-phthalaldehyde as a fluorescent label, with primary amines in the presence of a chiral alkyl thiol, was undertaken (Nimura and Kinoshita, 1986; Zhao, 1991). This analysis was carried out after a standard 24 h reflux hydrolysis in concentrated HCl (Figure 6). Identification of the hydrolyzed amino acids was accomplished by comparison of the peak retention times from the hydrolyzed material with those of amino acid standards as determined by a fluorescence detector after separation on a reverse phase (C_{18}) HPLC column. The amino acids alanine, glycine, leucine, valine, aspartic acid and glutamic acid were found (Table II). Quantification of the total amount of hydrolyzed amino acids used alanine as a standard. The sum of the peak areas of the total hydrolyzed amino acids was compared with the peak area of a known amount of alanine. Although this is not exact, it can provide some measure of the protein content. Each identified amino acid was quantified by comparison of its peak area with that of the corresponding standard. Beside the compounds found, the standards used for comparison included serine, isoleucine, hydroxyethyl amine, methyl amine, isopropyl amine, and *n*-propyl amine. The methyl amine peak, identified in Figure 6, is an artefact, and also present in the blank. Two analyses of this sample were completed, with identical results. The part of the hydrolyzed colloidal material that contains amino acids is less than one percent. This may represent a lower limit as incomplete hydrolysis of the sample would result in fewer amines or amino acids being found. About 60% of the total peak area on the chromatogram was identified.

With the exception of the smallest amino acids, alanine and glycine, the compounds found are in very similar ratios to those in proteins (Table II). It is possible that fractionation or degradative processes may enrich these compounds relative to the others.

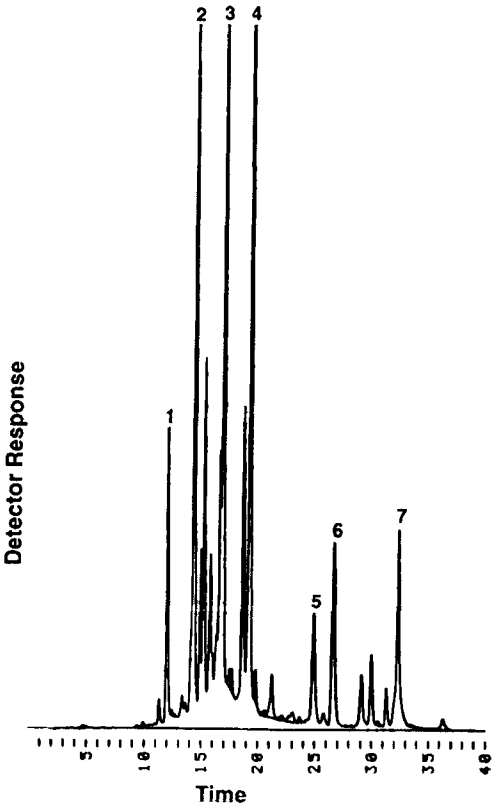

Figure 6 Amino acid analysis of the colloidal fraction after hydrolysis from April, 1993, La Jolla coastal water.

Table II Amino acids identified from the hydrolyzed colloidal fraction taken from the April La Jolla coastal water.

Identified Amino Acids	*Molar Percentages of the Identified Amino Acids*[a]	*Molar Percentages of Amino Acids in Proteins*[b]
Alanine	33	9.0
Aspartic Acid	8.8	5.5
Glutamic Acid	12.5	6.2
Glycine	31	7.5
Leucine	7.5	7.5
Valine	6.7	6.9

[a] Percentages of the total identified amino acids
[b] Values from Chothia (1975).

Inductively Coupled Plasma/Mass Spectrometry: ICP-MS measures the amounts of isotopes of the elements by mass spectroscopy. For our initial survey of the inorganic nature of the marine colloidal state, the guiding criterion was to determine as many elements as possible with lower precision, rather than a few elements with greater

precision. ICP-MS enabled us to analyze for 69 elements between the masses of lithium and uranium with the exception of a few elements such as silicon which has both a N_2 and a CO interference. Analyses were made with a VG plasmaquad PQII$^+$ ICP-MS instrument. A 100 ppb solution of Be, Mg, Co, In, Pr, Ho, Bi and U (multi-element standard prepared from 1000 ppm atomic absorption standards) was used to generate an instrumental response curve. One hundred ppb of In was added to all samples and blanks as an internal standard. Blanks were prepared by running 20–70 l of Type I water through the same separation procedure as carried out for the colloidal fractions. Two procedural blanks were run for the colloidal isolation step, one prior to isolation of each sample and one after. Each sample and blank were run twice on the instrument and the results averaged. The multi-element standard was run after every two samples (or blanks) and used to correct for drift. Where possible, more than one isotope of an element was assayed. If the results were similar, all isotopes were averaged to compute a concentration. If not, the lower value was chosen because of the possibility of spectral overlaps. A comparison of concentrations determined for isotopes of the same element may be used to give a measure of the precision of the analysis. The two isotopes of nickel agreed within 17%;3 isotopes of zinc within 11%;3 isotopes of barium within 20%and 2 isotopes of molybdenum within 25%. Of the elements analyzed iron has one of the greatest spectral overlaps; ArO and ArOH produce peaks which interfere with iron masses 56 and 57 respectively. These interferences result in large correction factors (large apparent blanks) in the iron determinations. However, despite this, the two isotopes agreed generally within 30%. The precision for some elements may exceed this due to a combination of spectral interferences, very low concentrations, blank corrections and single isotope elements. Only the minor elements whose concentrations in the colloid fractions exceeded their blank concentrations by 50% or more are reported in Table III.

Table III Inorganic analyses of colloids from California coastal surface seawaters.

Element	*Literature Values for "dissolved" < 0.45 μm per litre*[a]	*4/6/93 pM (23 l)*	*6/17/93 pM (46 l)*	*9/13/93 pM (72 l)*
Al	5 nM	3,500	nd[b]	740
P	< 1–3.5 μM	1,100	4,800	12,000
Cr	2–5 nM	25	31	71
Mn	0.2–3 nM	33	7.3	36
Fe	0.1–2.5 nM	1,100	320	640
Ni	2–12 nM	nd	140	140
Co	0.01–0.1 nM	nd	85	22
Cu	0.5–6 nM	79	63	140
Zn	0.05–9 nM	nd	550	nd
As	15–25 nM	1.3	1.3	2.7
Mo	0.11 μM	3.1	nd	1.0
Cd	0.001–1.1 nM	nd	2.7	nd
I	0.2–0.5 μM	3.2	10	130
Ba	32–150 nM	nd	8.7	4.4
Pb	5–175 pM	nd	2.9	2.9
U	14 nM	4.6	1.3	3.4

[a] Data from Bruland (1983).
[b] nd = not detected

We compare the measured concentrations of the elements in the colloidal phase with their concentrations in the $< 0.45\,\mu m$ "dissolved" phase, which contains both the colloidal and dissolved contributions to the elemental concentrations (Bruland, 1983) (Table III). The values in the colloidal fraction are less than those in the $< 0.45\,\mu m$ "dissolved" fraction.

For most elements, the colloidal fraction contains only a fraction of the reported "dissolved" ($< 0.45\,\mu m$) concentrations of sea water. Iron and aluminium are usually evident, in agreement with the SEM/EDAX results of Wells and Goldberg (1993). Iron ranged in our samples from 320 to 1100 pM, which is similar to the range of concentrations in the "dissolved" fraction of sea water (100 to 2500 pM, from Table III). However, given the range of values in the colloids and the fact that iron was not determined in both the colloidal and $< 0.45\,\mu m$ "dissolved" fraction of the same sample, it is impossible to say what percent of the total iron in sea water is present in the colloidal phase.

Aluminium in the colloidal fraction varies from undetectable to 3500 pM (Table III). This range encompasses the concentration of aluminium in colloids isolated from near-shore waters of the North West Arm off Nova Scotia (1400 pM) (Moran and Moore, 1989).

The high concentration of phosphorus determined in the colloids is consistent with their organic nature (Wells and Goldberg, 1993).

DISCUSSION

The marine colloidal state, from work in this laboratory, can be characterized primarily as organic, reactive and inhomogeneously distributed in the water column. Its organic components differ but slightly from its dissolved and particulate counterparts and in principle all could be derived primarily from the same general sources, probably algae. Recent work by Benner (1994) suggests that the major source of carbohydrates in the Gulf of Mexico are phytoplankton; our La Jolla investigation suggests that macroalgae could be the source material.

The organic colloids in sea water can serve as a food source for organisms, can reduce components in oxidized states, can provide complexing groups for metals and can aggregate to non-colloidal particulates (Wells and Goldberg, 1993). It is possible that there is a continuous flow of material between the colloidal, particulate and dissolved states.

In the colloidal phase we find evidence for carbohydrates (IR: C—O, C=O, and O—H stretches, ^{1}H NMR: δ 2.5 to 4.5 ppm), aliphatics (MS: 14 dalton periodicities, IR: C—H stretches; ^{1}H NMR: δ 0.4—1.8 ppm), sulphonates (IR: 1384 cm^{-1}) and amines and proteins (via amino acid analysis). We have found only traces of aromatic components, a major characteristic of terrestrial humic and fulvic materials. Aluminium and iron are usually present in marine colloids.

These results for the colloidal fraction are in accord with the previous investigations of Benner *et al.* (1992) and McCarthy *et al.* (1993) where a major class of compounds in the colloidal fraction was identified as carbohydrate, or polyhydroxy compounds. They also agree with the published spectra of the early NMR work by Steurmer and

Payne (1976) who isolated humic material by adsorption at pH 2 on Amberlite XAD-2 resin.

The results of ultrafiltration and affinity chromatography by previous investigators for the isolation of aquatic substances suggest that a major portion of the DOC has a molecular weight of less than 1000 daltons. Wheeler (1976) isolated material using 1 k, 30 k and 100 k nominal molecular weight cutoff membranes for samples from the marshes and coastal waters of Georgia. Ogura (1970) used 0.45 μm and 0.1 μm membranes initially, and then 100 k and 500 MW membranes (Ogura, 1974) to study Tokyo Bay water. Although Ogura was able to remove about 40% of the DOC between the 100 k and 500 MW membranes, Sharp (1973), using samples from the western North Atlantic, found that about 80% of the DOC passed through a 50 k MW membrane. Both Amon and Benner (1994) and Bauer *et al.* (1994) find, by DOC measurements, that most marine organic material classed as "dissolved" (< 0.45 μM) is small enough to pass through a 1000 dalton membrane, 55% and 70% respectively. With the Amberlite XAD-2 isolated humic material from the north-western Sargasso Sea and the coastal water near Woods Hole, Steurmer and Harvey (1974) noted that the greatest portion of the fulvic acid was less than 700 MW when measured by size exclusion gels. Our analyses (e.g. Figure 3) suggest that this low molecular weight fraction may encompass a narrow range of compounds.

The concept of a continuum of organic molecules whose molecular weights link them to the dissolved, colloidal or particulate states offers an explanation for some recent observations relating to the marine carbon cycle. For example, the apparent discrepancy between the old ages (thousands of years) of organic matter in the colloidal and dissolved states (Druffel *et al.*, 1992) and the short residence time of colloidal particles (Wells and Goldberg, 1993) may relate to the relative non-reactivity of the dissolved state and the ready availability of colloids for chemical and biological reactions. Higher solubilities in part relate to larger numbers of functional groups that can hydrogen-bond. If this explanation is correct, then there would be little exchange between the molecules in the dissolved and those in the particulate or colloidal states.

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