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ISOLATION AND CHEMICAL CHARACTERIZATION OF DISSOLVED AND COLLOIDAL ORGANIC MATTER

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An overview of the methods used for the isolation and characterization of organic matter in natural waters is presented. Commonly used techniques for the concentration and isolation of organic matter from water, such as preparative chromatography, ultrafiltration and reverse osmosis, and the methods used to analyze the organic matter obtained by these methods are reviewed. The development of methods to obtain organic matter that is associated with fractions of the dissolved organic carbon other than humic substances, such as organic bases, hydrophilic organic acids and colloidal organic matter are discussed. Methods specifically used to study dissolved organic nitrogen and dissolved organic phosphorous are also discussed.

KEY WORDS: aquatic systems, organic matter, DOC, DON, DOP, sample preparation and analysis

INTRODUCTION

The importance of organic matter in aquatic systems has clearly been recognized during the past 30 years. Organic matter often controls geochemical processes by acting as a proton donor/acceptor and pH buffer, by affecting the transport and degradation of pollutants, and by participating in mineral dissolution/precipitation reactions. Dissolved and particulate organic materials may also serve as substrates for microbially mediated reactions. To understand more clearly the dynamics of carbon cycling and geochemical and biological processes involving organic matter, better definition of the processes controlling the distribution and fate of organic compounds and identifying the role these compounds play in biogeochemical systems is required.

The study of the nature and environmental significance of organic matter in natural waters, however, is hampered by the inherent chemical complexity of the organic matter. Once in the system, organic compounds can be truly dissolved, in association with solid substrates, or in association with suspended particles (including colloids). Organic matter in aquatic systems is, also, a complex heterogeneous continuum of high to low molecular weight species exhibiting different solubilities and reactivities. It is important to appreciate the analytical problems associated with this chemical complexity. Indeed, a major bottleneck at this time is the lack of sufficiently effective separation methods. To be effective, modern analytical techniques, such as mass spectrometry, require the separation of the analytes of interest from other components of the mixture. Otherwise data are obtained on numerous compounds at the same time, resulting in an overload of data that rapidly becomes indecipherable. It is for this reason that the development of sophisticated separation techniques, such as gas chromatography, has gone hand in hand with the development of detectors. Unfor-

tunately, the concentrations of identifiable compounds present in most natural waters is less than 10% of the total DOC (Thurman, 1985).

Even the basic determination of DOC concentration is not straightforward. Sugimura and Suzuki (1988) recently brought attention to possible problems with DOC data reported by marine chemists. The resulting controversy has led to renewed scrutiny of the methods employed for the determination of DOC (Toggweiler, 1988; Jackson, 1988; Williams and Druffel, 1988; Aiken, 1992; Hedges and Lee, 1992). Difficulties in the analysis of DOC arise for a variety of reasons. The quantities of organic matter may be low, as is the case for sea water and ground waters. In addition, the complex mixture of compounds that DOC comprises has a range of reactivities and oxidation efficiencies. Other problems include the presence in the sample of interfering substances, such as chloride.

Historically, organic matter in natural waters has been arbitrarily divided into dissolved and particulate organic carbon, based on filtration through a 0.45μ filter (Figure 1). No natural cut-off exists between these two fractions and the distinction is operational. Overlapping the dissolved and particulate fractions is the colloidal fraction, which consists of suspended solids that are operationally considered as solutes (Morel and Gschwend, 1987). Colloidal organic matter in natural waters is composed of living and senescent organisms, cellular exudates, and partially to extensively degraded detrital material, all of which may be associated with mineral phases (Ranville *et al.*, 1991).

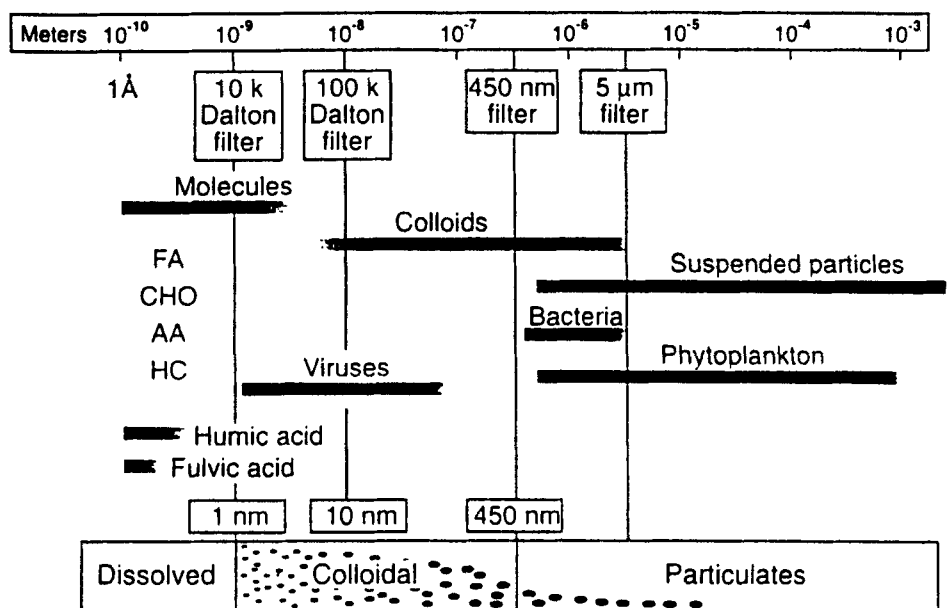


Figure 1 Continuum of particulate and dissolved organic carbon in natural waters. (FA = fatty acids, CHO = carbohydrates, AA = amino acids, HC = hydrocarbons)

DOC can be further fractionated according to chemical characteristics using chromatographic techniques into six fractions: hydrophobic acids, bases, and neutrals; and hydrophilic acids, bases and neutrals (Leenheer, 1981). DOC fractionation data for a variety of freshwater systems are given in Table I.

Table I Dissolved organic carbon fractionation data (mg C/l) for selected freshwater systems of the United States

| Sample | Date | DOC | Hydrophobics | | | Hydrophilics | | |
|------------------------------------|-------|------|----------------------|---------|----------|--------------|---------|----------|
| | | | Acids | Bases | Neutrals | Acids | Bases | Neutrals |
| Black Lake, North Carolina | 11/81 | 8.3 | 3.4(41) ^a | 0.1(1) | 1.5(19) | 0.7(8) | 2.6(31) | 0.0 |
| Ohio river at Cincinnati, Ohio | 6/81 | 3.7 | 1.2(32) | 0.0 | 0.9(25) | 1.3(36) | 0.3(7) | 0.0 |
| Missouri River at Sioux City, Iowa | 8/81 | 3.4 | 0.7(19) | 0.8(22) | 0.0 | 1.7(51) | 0.3(8) | 0.0 |
| Suwannee River at Fargo, Georgia | 12/82 | 38.2 | 16.0(42) | 0.2(1) | 1.0(2) | 19.2(50) | 1.4(3) | 0.6(1) |

^a Numbers in parentheses are the percent of the total DOC of that fraction

This approach is useful for evaluating the reactivity and fundamental chemical properties of each fraction. The major fractions of the DOC pool of most natural waters are refractory organic compounds that are operationally defined as aquatic fulvic acids and hydrophilic acids (Aiken *et al.*, 1992). Aquatic fulvic acids are yellow in colour, heterogeneous, biologically recalcitrant organic acids, and usually make up 20–80% of the DOC.

In this paper a discussion of the methods used for the isolation and characterization of organic matter in natural waters will be presented. By necessity, the discussion is limited to techniques currently employed and methods that are in the developmental stages. Fairly recent reviews of the methods used for the study of organic matter in water (Aiken *et al.*, 1985; Thurman, 1985; Aiken, 1988) provide a more in depth discussion of the details and drawbacks of the methods used for this purpose. In the future, the study of organic matter in aquatic systems will require more attention to be paid to colloidal material and fractions of the DOC other than humic substances.

REVIEW

Isolation Methods

A common approach to the study of DOC is to isolate functionally distinct DOC fractions to determine fundamental chemical properties of each fraction, ultimately relating structural and chemical information to the biogenesis and environmental roles of these materials. This approach has been successfully employed in the study of DOC in natural waters and has led to many advances in the field, especially in the study of aquatic humic substances. A number of concentration methods have been used ranging from sorption on chromatographic media to separation based on other factors such as molecular size. Unfortunately, no method is universally applicable for all fractions of organic matter. Each method has drawbacks, such as potential sample alteration and contamination (Aiken, 1988), and the question of how representative these materials are of the DOC as a whole (Shuman, 1990). Often the method of choice is dictated by the questions to be addressed and by the chemical matrix of the water sample of interest.

Resin isolation methods

Since the purpose of isolating and fractionating DOC is to elucidate chemical properties, it is desirable to have an isolation approach based on the chemical properties of the materials of interest. Adsorption chromatography on both ion exchange resins (Leenheer, 1981; Miles *et al.*, 1983) and nonionic macroporous resins (Mantoura and Riley, 1975; Thurman and Malcolm, 1981; Aiken *et al.*, 1992) has been successfully employed for this purpose, with most of the emphasis placed on hydrophobic organic acids (e.g. aquatic humic substances). As for DOC in general, organic acids in water are a complex mixture of both high and low molecular weight species that exhibit different chromatographic behavior on resin sorbents. The efficiency of sorption of these organic acids is a function of the aqueous solubility of the solute, and the nature of the sorbent.

There are a number of advantages associated with the chromatographic methods of isolation. First, it is possible to fractionate and isolate a total of, approximately, 55–90% of the DOC from a variety of aquatic environments. The method has been used successfully to isolate the hydrophobic and hydrophilic acid fractions from waters having low DOC concentrations such as ground waters, from saline waters such as sea water and saline lakes, from waters contaminated with organic acids from the microbial degradation of organic contaminants, and from “black” waters with high DOC concentrations, such as the Suwannee River.

Second, the fractionation is carried out on the original water sample without using a preconcentration step, such as ultrafiltration, reverse osmosis, or vacuum evaporation, maintaining fractionation consistency and comparability between samples. This is an important factor for two reasons:

1. Increasing the concentration of the original sample can change the nature and extent of interactions between chemical constituents present in the original sample, possibly affecting the behaviour of the sample on the resin. An example of this type of effect would be increased aggregation of the sample.
2. The fractionation of organic solutes on resin sorbents is dependent on the concentration of material in the sample. At this time, the effect of increasing the concentration of a mixture of compounds on the affinity of these compounds for the resin has not been clearly defined, and it is possible that the interactions of the sample with the resin will be altered, even without undergoing aggregation.

Disadvantages of this approach include:

1. Potential sample alteration associated with the use of various solvents and pH conditions necessary to bring about the sorption/desorption of the compounds of interest. Examples of these types of reactions are (1) ester hydrolysis reactions of labile functional groups under basic conditions, (2) re-esterification of hydroxyacids to lactones under acid conditions, (3) incorporation of nitrogen into organic compounds resulting from interactions with ammonia, and (4) alcoholysis reactions of organic acid and ester functional groups that occur in the presence of alcohols (Aiken, 1988). Almost all resin-based extraction schemes involve contact of the sample with either base (NaOH or NH_4OH) or methanol, and it is likely that some degree of alteration occurs.
2. The fractions of organic matter obtained using preparative isolation methods are not sharply defined. There is, therefore, some overlap between fractions. For instance, some of the hydrophilic acids in a sample will be concentrated along with

the hydrophobic acid fraction. These compounds are eluted in the same manner as humic substances and will be included in the final extract.

Separations based on molecular size

Ultrafiltration and reverse osmosis are membrane separation techniques by which solutes are separated from the water as a function of molecular size. The two methods differ in molecular size (often referred to as molecular weight) cut-off. Ultrafiltration membranes of various molecular weight cut-offs (500–100,000 daltons) are available. Reverse osmosis has a lower cut-off, and, theoretically, all the solutes present in the sample are concentrated. Both methods have been used to isolate humic substances and to concentrate organic matter from water (Milanovich *et al.*, 1975; Kopfler *et al.*, 1975; Odegaard and Koottatep, 1982; Serkiz and Perdue, 1990; Nanny and Minear, 1993). These methods have the advantage of utilizing ambient conditions to minimize the possibility of destructive chemical reactions, a high percentage of the DOC can be obtained, and the method is relatively rapid. Problems associated with these methods are:

1. There is evidence that the exclusion efficiency is dependent on membrane type, membrane pore size, volume concentration factor, and ionic strength (Odergaard and Koottatep, 1982; Nanny and Minear, 1993). These factors can result in both increased breakthrough and sorption effects on the membranes.
2. Subsequent analyses of isolated organic matter may require further separation from inorganic salts that are concentrated along with the samples of interest. This problem is more significant for the more hydrophilic fractions of organic matter because of the difficulties involved in desalting hydrophilic organic isolates, and is a drawback for using this method unless the original water sample has relatively low salt content (Serkiz and Perdue, 1990).
3. Subsequent analyses may require fractionation of the isolated organic matter into chemically distinct fractions. As noted previously, chemical information obtained from complicated mixtures is difficult to interpret. The average properties of the entire organic matter isolate are likely to be less meaningful than for chemically defined fractions.

Methods of Analysis

In the following section a brief review of the methods commonly used to analyze organic compounds in water is presented.

Specific compound analysis

Advances in the field of mass spectrometry coupled with the development of sophisticated chromatographic techniques have provided valuable tools for the analysis of individual compounds in water. In general, the amounts of identifiable compounds present in aquatic samples are low, less than 10% of the DOC. Thurman (1985) reviewed the classes and amounts of material reported for a number of distinct compound classes found in natural waters including carboxylic acids and phenols, amino acids, carbohydrates and hydrocarbons. These compound classes are generally analyzed using techniques that result in the isolation of the compounds of interest, followed by treatment of the sample to enhance chromatographic separation, such

as the derivitization of organic acids to the corresponding methyl esters. The compounds of interest are then separated by either gas or liquid chromatography and quantified using a detector such as a mass spectrometer.

Complicated mixture analysis

Typically, the complicated mixtures obtained from the fractionation of DOC into compound classes are characterized by elemental, molecular weight, acid-base titration and amino acid analyses, and by ^{13}C -NMR, ^1H -NMR and IR spectroscopy. The fact that the fractions are complex, heterogeneous mixtures limits the amount of information that can be obtained about the composition of the mixture using each of the above methods, but these techniques do provide valuable structural and functional group information, that, taken as a whole, can be used to establish the nature and source of organic isolates. In addition, these data have been used to generate average structural models for aquatic fulvic acid (Leenheer *et al.*, 1989a). A critical overview of these methods can be found in Aiken *et al.*, 1985. In recent years, there has been a dramatic increase in the use of ^{13}C -NMR, and a number of review articles describing its application to environmental problems have been published (Wershaw and Mikita, 1987; Preston, 1987; Steelink *et al.*, 1990).

ONGOING RESEARCH

Hydrophilic Species

In natural waters, from 10–50% of the DOC may consist of hydrophilic substances that are difficult to isolate by reverse-phase chromatography on low polarity sorbents. Hydrophilic DOC is much more difficult to isolate and purify than is hydrophobic DOC because inorganic salts have similar hydrophilic properties. There is no single technique that can concentrate, desalt, and purify hydrophilic substances in water. Not only the organic matrix, but also the inorganic matrix of each specific sample must be considered in designing a customized approach that will result in the isolation of hydrophilic substances of sufficient purity to meet specific research objectives. There are several techniques that can be used in a complementary manner to isolate hydrophilic substances quantitatively from water. Following are several methods and approaches that have been used to isolate hydrophilic dissolved organic matter. With most of these techniques, an implicit assumption is that the hydrophobic fractions have been removed from the water sample before the hydrophilic organic matter is recovered.

Use of Amberlite XAD-4

Differences in the sorption characteristics of XAD-8 and XAD-4 can be used to isolate a large fraction of the organic acids present in a water sample by using a two column array consisting of a column of XAD-8 followed by a column of XAD-4 (Figure 2; Aiken *et al.*, 1992). After filtration the sample is acidified to pH 2 and passed first through the XAD-8 resin. The hydrophobic acid fraction containing the humic substances is retained before the sample contacts the XAD-4 resin. The effluent from the XAD-8 resin contains hydrophilic acids, bases and neutrals. It is subsequently passed through the column of XAD-4, wherein a fraction of the hydrophilic acids sorbs to the XAD-4 resin. This fraction of the hydrophilic acid fraction contains a large amount of humic-like compounds and might be classified

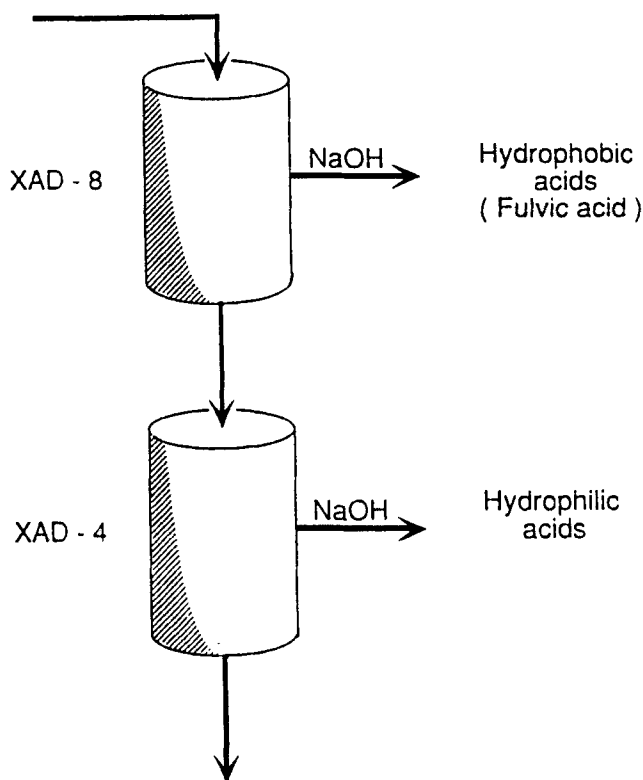


Figure 2 Schematic diagram of the XAD-8/XAD-4 isolation scheme.

as “hydrophilic fulvic acid”. Each column is separately back-eluted with 0.1 N NaOH to obtain the hydrophobic acid fraction and a portion of the hydrophilic acid fraction of the DOC. DOC fractionation data from a variety of aquatic environments where this method has been employed are presented in Table II. In each case, a total of 55–83% of the DOC has been isolated on the XAD-8/XAD-4 array, with the

Table II Fractionation of Dissolved Organic Carbon (DOC) from a variety of aquatic environments.

| <i>Location</i> | <i>DOC (mg C/l)</i> | <i>Percent Fulvic Acid</i> | <i>Percent Hydrophilic Acid^a</i> |
|--|-------------------------|------------------------------------|---|
| Lake Fryxell, Antarctica (depth – 7.5 meters) | 5.2 | 23 | 7 |
| Lake Hoare, Antarctica (depth – 12 meters) | 2.0 | 23 | 9 |
| Yakima River, Washington | | | |
| Cle Elum | 1.6 | 26 | 8 |
| Kiona | 2.4 | 23 | 12 |
| Bemidji, Minnesota (contaminated groundwater) ^b | 16 | 42 | 22 |
| Suwannee River, Georgia | 58 | 58 | 25 |

^a As defined by isolation on XAD-4 resin.

^b Sample contains high concentrations of organic compounds resulting from the microbiological degradation of crude oil.

retainable hydrophilic acids accounting for 14–25% of the DOC, a large portion of the hydrophilic acid fraction in these samples.

Lowering the column capacity factor (k') to 1 on Amberlite XAD-8

The ratio of the volume of water processed to the amount of resin sorbent determines k' (Leenheer, 1981). Typical applications of reverse-phase chromatography to isolate hydrophobic organic fractions isolate solutes that have k' values greater than 10 to 100. It is possible to recover and desalt solutes that have k' values as low as 1 on Amberlite XAD-8.

To obtain recoveries of solutes with k' as low as 1, the sample is first acidified with HCl to pH 4 (to eliminate carbonates), vacuum evaporated to the point of salt saturation, and the pH adjusted to 2. After humic acids are separated by centrifugation, the concentrate is applied to an XAD-8 column and is followed by a rinse of 0.01 N HCl. To retain solutes with k' as low as 1, the volume of the sample plus the 0.01 N HCl rinse should not exceed the bed volume of the column. The point at which inorganic salts are eluted from the column is determined by monitoring the specific conductance of the column eluent during the rinse phase (Leenheer, 1981). Hydrophilic substances are then eluted from the column with 75% acetonitrile, 25% 0.01 N HCl. The acetonitrile/water eluent is removed by vacuum evaporation. Acetonitrile forms a low boiling azeotrope with water, and HCl can be quantitatively removed by repeated evaporations of acetonitrile from the hydrophilic solutes.

A comparative recovery study on DOC in the Mississippi River was performed using Amberlite XAD-8 with k' cut-offs for hydrophobic solutes at 10 and 1 (Leenheer *et al.*, 1993). For $k' = 10$, the average DOC recovery was 60%; for $k' = 1$, the average recovery was 66%. Thus, only 6% of the dissolved hydrophilic compounds were shifted into the hydrophobic fraction by lowering the column capacity factor.

Anion exchange resins

Anion exchange resins adsorb the hydrophilic acid fraction that constitutes the majority of the hydrophilic DOC. There are two significant problems associated with the use of anion exchange resins for the isolation of hydrophilic acids: first, most anion exchange resins are difficult to elute, and, second, inorganic anions are concentrated and eluted along with the organic acids of interest. A review of the application of anion exchange resins to the DOC isolation problem is given by Leenheer (1984).

An anion exchange technique worthy of specific mention is the use of formic acid as an eluent (Aue *et al.*, 1972). Formate has a low exchange constant for basic exchanger groups, and does not displace strong-acid inorganic anions; only weak-acid anions such as carboxyl groups are displaced. This technique has been used by numerous researchers to isolate EDTA, NTA, and other organic acids from sewage effluents and river waters (Schaffner and Giger, 1984). Unfortunately, hydrophilic acids containing phenolic groups do not elute with formic acid. Experience in the author's (Leenheer) laboratory has found that about one third of the hydrophilic acids in natural waters can be isolated and desalted on weak-base anion exchangers with formic acid elution. As formic acid is volatile, it is easily removed by vacuum evaporation or freeze-drying.

Coprecipitation

Several methods exist for coprecipitating DOC with metal hydroxides (Al^{+3} , Cu^{+2} , Fe^{+3} , Mn^{+2} , and Pb^{+2}) (Leenheer, 1984). These methods are selective for the acid fraction of the DOC, and work especially well on the acids that form complexes with metals, such as citric acid. Relatively low concentrations of metal ions (30–50 mg/l) have been found to remove about half of the DOC from fresh and sea water samples. Despite the relative promise of coprecipitation as an isolation technique, most of the early work (1960's and 70's) has not been improved and optimized. None of the published studies using coprecipitation has attempted to recover and purify the DOC from the precipitate.

Recent work (Leenheer) with cupric hydroxide found that about two thirds of the hydrophilic acid fraction of the DOC from the Mississippi River could be isolated. In this procedure, the hydrophilic eluent from the XAD-8 resin is vacuum evaporated to salt saturation. The pH of the concentrate is adjusted to pH 1, and sulphate is removed by precipitation with barium chloride. Cupric chloride (10 millimoles) is added and the cupric hydroxide precipitate is formed by adjusting the pH to 7.0 with NaOH. The precipitate is washed three times with deionized water to remove chloride and nitrate, and the precipitate is redissolved by adding formic acid to pH 3. This solution is then passed through a H-saturated cation exchange resin to remove cupric ion, and the aqueous formic acid is removed by vacuum evaporation. In addition to isolating hydrophilic acids, silicic acid, boric acid and phosphoric acids are isolated. These inorganic acids can be removed by specific evaporation, precipitation, and selective dissolution techniques discussed later in this report. Sulphate has to be removed prior to the coprecipitation because it is partially coprecipitated with cupric hydroxide, and the resulting sulphuric acid after the cation exchange step damages the isolate after it is dried.

Zeotropic distillation

Zeotropic distillation is the separation of two miscible solvents that do not form azeotropes by distillation. In the case of water, it can be distilled from higher boiling NN-dimethylformamide (DMF) or acetic acid. Solutes originally dissolved in water are transferred into an organic solvent; inorganic acids are generally less soluble than hydrophilic organic acids. This procedure has been used to isolate hydrophilic DOC from water and partially desalt this isolated DOC using DMF (Leenheer *et al.*, 1987) or acetic acid (Leenheer *et al.*, 1993). The procedure works well to remove inorganic chlorides and sulphates that constitute the bulk of the inorganic salts, but weak inorganic acids (boric, silicic and phosphoric acid), nitrates, and hydrated magnesium cations are not removed.

The advantage of zeotropic distillation is that the majority of the salts can be removed from hydrophilic concentrates, brines and sea water without losing hydrophilic dissolved organic material. After this salt removal, other techniques such as ion exchange or coprecipitation can be used for final isolation and purification. Zeotropic distillation with acetic acid has been found to be superior to the use of DMF because it has a lower boiling point and can be easily removed by vacuum evaporation. Acetic acid is also available in high purity and does not contain hydrolysis products as does DMF.

Specific desalting procedures

Sulphate

Sulphate ions can be removed from hydrophilic concentrates by addition of barium chloride or barium acetate at pH 1 to precipitate sulphate. The pH must be as low as 1 to assure that all the organic acids are protonated and will not coprecipitate with barium sulphate (Fischer, 1961). If inorganic acids precipitate at this low pH, addition of acetonitrile (50% by volume) will prevent organic acid precipitation and still allow barium sulphate to precipitate. Excess barium ions can then be removed by cation exchange resin.

Borate

Boric acid often co-isolates with hydrophilic organic substance. It can be easily removed from the concentrate by adding methanol and evaporating to dryness. Volatile trimethyl borate is formed by reaction with methanol. If the hydrophilic acids are in the hydrogen form, the treatment must be rapid and at low temperature to prevent methanol from forming methyl esters with organic acids.

Silicates

Silicic acid occurs in equivalent concentrations to DOC in natural waters, and often co-isolates with hydrophilic DOC. It can be easily removed from the dried concentrate by suspending the concentrate in 75% acetonitrile, 25% water. The organic hydrophilic substances will dissolve and the silicic acid can be filtered out as an insoluble hydrophilic gel.

Phosphates

Phosphate can be precipitated from neutral aqueous solution as magnesium ammonium phosphate by addition of magnesium ammonium acetate solution buffered at pH 8 (Fischer, 1961). All other cations except the alkali metals and ammonium ions must be absent during precipitation, and the pH must not be allowed to rise above 8 or so to prevent undesirable side reactions of hydrophilic DOC with free ammonia. Excess ammonium and magnesium ions can be removed after phosphate precipitation by a cation exchange resin. This procedure has to be used only occasionally because phosphate concentrations in natural waters are usually too low to interfere.

Chloride

Hydrochloric acid forms a low boiling azeotrope with acetonitrile and can be easily removed from the sample by repeated evaporation of the isolate with anhydrous acetonitrile.

Nitrate

Nitrate anion is the biggest problem in obtaining an inorganic-free isolate from the zeotropic distillation procedure. No specific precipitation procedures exist to remove it, and nitric acid is not sufficiently volatile to be removed by vacuum evaporation. The current approach is to remove it by coprecipitating hydrophilic acids with cupric hydroxide. Hydrophilic neutral and hydrophilic acids that do not coprecipitate can

then be passed through a weak-base anion exchange resin, and eluted with formic acid. By controlling the pH and elution volume, nitrate will be retained and the hydrophilic organic acids and neutrals will be eluted.

Organic Bases

Cation exchange resins

The hydrophilic base fraction usually constitutes 5–10% of the DOC in water. This fraction is composed of amino acids, amino sugars, peptides, and proteins, and it is operationally defined by its adsorption on cation exchange resins. Both strong acid (Degens and Reuter, 1964; Gardner and Lee, 1973; Leenheer and Noyes, 1984) and weak acid exchangers (Sirotkina *et al.*, 1974) have been used. The hydrophilic base fraction from strong acid exchangers is typically desorbed with base, typically 3N NH_4OH or 1 N NaOH. The ammonia can be removed by vacuum evaporation, but undesirable side reactions of ammonia with carbonyl groups in sugars and humic substances are possible. Elution with NaOH is accompanied by difficulties in removing the NaOH. Lithium hydroxide may have promise as an eluent because it can be removed by neutralizing it with phosphoric acid to pH 10 and precipitating lithium phosphate.

Weak-acid carboxy methyl cellulose exchangers have been used to adsorb and recover the hydrophilic base fraction by eluting with 0.1 N HCl (Sirotkina *et al.*, 1974). However, other metal cations are also eluted with acid and additional desalting procedures are necessary.

Lastly, ligand exchange chromatography has been used to specifically isolate α -amino acids that complex with cupric ions immobilized on an ion exchange resin (Siegel and Degens, 1966). Amino acids in sea water were recovered by this method using NH_4OH to elute the amino acids from the resin.

Dissolved Organic Nitrogen and Dissolved Organic Phosphorus

Dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) are fractions of organic matter that can be important in the overall nitrogen and phosphorus budgets of aquatic systems. To fully understand the aquatic cycles of these elements, the identity and the chemical and physical behaviour of DON and DOP must be known. This task is difficult due to the low concentrations of these materials in aquatic systems and to the many chemical compounds that N and P can be incorporated into. As a result, little is known regarding the identity and characteristics of DON and DOP in aquatic environments.

In a recently published study, Qualls and Haines (1991) reported on the geochemistry of DOP and DON in a forest ecosystem. Samples of throughfall, soil interstitial water, and stream water were fractionated using a modified version of the preparative isolation scheme of Leenheer and Noyes (1984). Most of the DON, in order of decreasing importance, was found in the hydrophobic acid, hydrophilic acid and hydrophilic neutral fractions, whereas, most of the DOP was found in the hydrophilic acid, hydrophobic acid and hydrophilic neutral fractions. Organic bases, which were expected to contain cationic amino groups were found to be less than 2.5% of the DOC in this study. No attempt was made to identify the nature of the DON in these fractions.

Most of the research on the distribution and nature of DON has focussed on amino acid content. Amino acids have been fractionated into the dissolved -free amino acids and dissolved-combined amino acids (Thurman, 1985). In one of the few studies on the distribution of amino acids in aquatic systems, Lytle and Perdue (1981) found that greater than 96 percent of the dissolved amino acids were dissolved-combined amino acids associated with dissolved humic material. Unfortunately, hydrolysable amino acids account for only a small percent of the total N content of dissolved humic substances. For instance, the hydrolysable amino acids account for 8.7 percent of the fulvic acid N and 26 percent of the humic N in the Suwannee River (Thurman and Malcolm, 1989). Little is known about the remaining N associated with humic substances, whether in aquatic systems or in soils (Schnitzer, 1985).

DOP has been studied using gel permeation chromatography, isotopic studies, bioassays and HPLC (Nanny and Minear, 1993). These studies have been largely ineffective in elucidating the chemical nature of DOP. As with the study of other organic components associated with DOC, the concentration of DOP is often low, typically 5–100 $\mu\text{g P/l}$ (Nanny and Minear, 1993). Samples therefore require pre-concentration before the application of many methods that might prove useful in studying this fraction of the organic matter. Among the methods employed to isolate DOP are anion exchange and lanthanum hydroxide precipitation, freeze concentration and lyophilization. Each of these methods involves chemical and physical transformations of the sample and/or long processing times. Of particular significance is the possibility of hydrolysis reactions in the presence of alkaline solutions.

In an effort to learn more about the nature of DOP, Nanny and Minear (1993) have reported recently the use of ^{31}P -NMR to analyse DOP obtained from the epilimnion of a small lake. Ultrafiltration and reverse osmosis were used to concentrate the sample for NMR analysis. While individual compounds were not conclusively identified, mono- and diester phosphates were detected in the DOP, and the presence of DNA was suggested. In addition, temporal changes in the spectra were noted over the sampling period. It was concluded that the use of ^{31}P -NMR, in combination with other analytical techniques, has the potential to provide useful information about DOP.

Colloidal Organic Matter

The colloidal state of matter has properties intermediate between the dissolved and particulate states. It is not the intent of this report to provide a comprehensive review of colloids in water, which has been covered in detail recently by Hiemenz (1986). Rather, recent research on colloid isolation and characterization will be presented.

Colloids have been obtained from water samples using cross-flow (also known as tangential flow) ultrafiltration. This method has been used to concentrate phytoplankton (Barthel *et al.*, 1989) and suspended sediments from rivers (Hernandez and Stallard, 1988; Leenheer *et al.*, 1989b), lakes (Ranville *et al.*, 1991) and marine waters (Whitehouse *et al.*, 1986). Ranville *et al.*, (1991) used cross-flow ultrafiltration to study the importance of sources of organic material in determining the characteristics of the colloidal and particulate organic matter in Pueblo Reservoir (Colorado). The sample was size fractionated using settling to obtain the silt fraction, and ultrafiltration using polysulfone membranes to obtain the colloid fraction (Figure 3). The colloids were concentrated in the ultrafiltration retentate and on the membrane surface. Colloids were removed from the membranes by gently massaging the membranes with some retentate in a Teflon bag. Final isolation of the colloids was by laboratory centrifugation to remove the mineral colloids, followed by lyophiliz-

ation of each of the fractions. The samples were analysed to determine physical and chemical characteristics, including organic carbon functionality and lipid content.

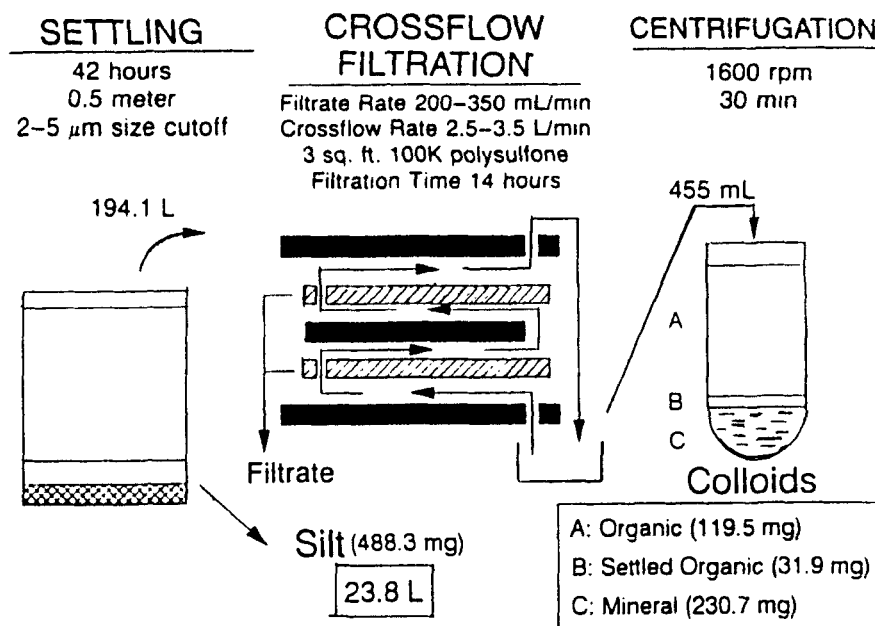


Figure 3 Diagram showing the operation of crossflow ultrafiltration for the collection of colloid samples.

A similar approach was employed in a study of the Mississippi River conducted by the U.S. Geological Survey (1987–1992) to assess contamination in the river, and to determine the transport of these contaminants in the dissolved, colloidal, suspended sediment and bed sediment phases. Water samples up to 1000 l were sampled representatively and processed sequentially on board ship to separate sand by sieving through a 63 μm sieve, silt by continuous-flow centrifugation, colloids by cross-flow ultrafiltration, and dissolved organic matter by adsorption on XAD-8 resin (Leenheer *et al.*, 1989b). Centrifugation rather than filtration or settling was chosen to separate suspended sediment from the colloids because more operationally consistent particle separations based on size and density were obtained. Particles as large as one micron elutriated from the centrifuge, but most of the elutriated particles were in the 0.1–0.5 μm range (Rees *et al.*, 1991).

As part of this study, several types of ultrafiltration membranes were tested. Deacetylated cellulose with a 0.005 μm pore size was found to have the best combination of high water flux rates, good contaminant blank characteristics, and it did not appreciably adsorb the dissolved organic and inorganic contaminants being measured (Leenheer *et al.*, 1989b). After combining the retentate with colloids obtained by massaging the membranes, the mineral colloids were separated from the sample by laboratory supercentrifugation. The organic colloids that remained suspended in the supernatant were dialyzed against distilled water in a 1000 dalton cellulose dialysis bag to remove salt, and then freeze dried.

The colloids were characterized by infrared spectrometry, solid state ^{13}C -NMR spectrometry, elemental analysis, scanning electron microscopy, and x-ray fluorescence spectrometry. Colloidal organic carbon concentrations ranged from 5–20% of the DOC concentrations. Generally speaking, the organic constituents on the mineral colloids (5–15% organic carbon) were predominantly carbohydrates and proteins. The organic colloids (15–40% organic carbon) freeze dried as a spongy white material, shown to be predominantly bacterial cells by scanning electron microscopy. The spectrometric characterization of organic colloids revealed lipid, protein, and carbohydrate structures. Both colloid fractions were compositionally very different from the dissolved organic matter fractions.

FUTURE DIRECTIONS

A significant need at this time is for the development of methods to isolate dissolved organic matter without pH adjustment. Certain covalent linkages in dissolved organic matter in water are exceptionally labile with changes in pH. Sulphate ester and phosphate ester conjugates with dissolved organic matter, for instance, are easily hydrolyzed by acid or base. Ultrafiltration can isolate these conjugates without pH adjustment, but solutes smaller than 1000 daltons are not efficiently separated from hydrated salt ions by membrane separations. Other methods that might be explored for isolating DOC without pH adjustment are ion-pair chromatography and affinity chromatography based on bonded borate supports.

Another need is the development of methods to isolate dissolved hydrophilic substances from sea water. Sea water differs from fresh water, not only in its salt concentration and ionic composition, but the DOC in sea water is predominantly hydrophilic in nature. Whereas XAD resins can isolate a large per cent of the DOC in fresh water in the hydrophobic acid fraction, only about 15–20% of marine organic material can be isolated in this way. Given the present importance of global carbon cycle research, priority should be given to the isolation and geochemical characterization of DOC in sea water, one of the largest reservoirs of organic carbon in the world.

A significant need also exists to understand in more detail the nature of organically bound N and P both in soil and water. In soil, organic nitrogen plays an important role in soil biogeochemistry and soil fertility (Schnitzer, 1985), and dissolved organic matter has been noted as a major vehicle for the translocation and loss of N and P (Qualls and Haines, 1991). Similarly, in aquatic systems, DON and DOP may play important roles in metal binding and the availability of N and P to the ecosystem. Presently, little is known about the chemical nature and behaviour of these fractions of dissolved organic matter.

Finally, a significant, albeit poorly understood, mechanism for transport of hydrophobic organic species in aquatic systems is via colloidal interactions. For instance, the organic matter of the colloids present in the Mississippi River was found to be more active in partitioning PCB contaminants than organic matter in the silt fractions (Rostad, personal communication). Approximately half of the PCB load of the Mississippi River is transported in association with the colloid fraction. While the potential significance of colloids in facilitated transport has been recognized (McCarthy and Zachara, 1989; Capel and Eisenreich, 1989), a number of problems associated with sampling and analysis of colloids have hampered research in this area.

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