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STRUCTURAL AND BEHAVIORAL CHARACTERISTICS OF A COMMERCIAL HUMIC ACID AND NATURAL DISSOLVED AQUATIC ORGANIC MATTER

DOMENIC GRASSO*†, YU-PING CHIN††, WALTER J. WEBER, JR.†††

† Department of Civil Engineering and The Environmental Research Institute, The University of Connecticut, Storrs, CT 06269-3037

†† Ralph M. Parsons Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02135

††† Environmental and Water Resources Engineering, The University of Michigan, Ann Arbor, MI 48109

ABSTRACT

Dissolved organic matter (DOM) from two natural surface water sources and a commercial humic acid were analyzed using gel-permeation chromatography, high pressure reverse phase liquid chromatography, and ^{1}H-NMR spectroscopy. Results from the chromatographic studies show that the DOM of two natural waters consisted primarily of relatively low molecular weight, polar organic constituents, while large and relatively nonpolar macromolecules comprised a significant fraction of the commercial humic acid. The ^{1}H-NMR assays indicated that DOM from the two natural water samples was comprised of nonaromatic organic constituents, while the commercial humic acids tested contained both aromatic and aliphatic moieties. Based upon these composite results of the several different types of analysis employed, it is evident that the humic acid examined, and possibly others prepared in the same way, contain molecular structures which exhibit physical and chemical properties that do not reflect the true nature of DOM in real aquatic systems. Commercially available humic substances of this type may therefore not be suitable surrogates for naturally occurring DOM in laboratory investigations and analysis of geochemical and environmental transformation reactions.

INTRODUCTION

The dissolved organic matter (DOM) associated with natural waters is typically comprised of biogenic polyelectrolytic organic molecules and polymers. Humic and fulvic acids constitute approximately 25-50% of the total DOM found in both freshwater and marine environments (Thurman, 1985), while the remaining fractions are composed primarily of proteins, polysaccharides, and hydrophilic organic acids. These organic substances, derived from both allochthonous and autochthonous sources can substantially impact a variety of environmental and geochemical transformations. The presence of DOM has been observed to 1) play a major role in weathering processes and diagenesis (Drever,1988), 2) complex metals and alter their speciation in natural waters (Cabaniss and Shuman, 1988, Hering and Morel, 1988), 3) bind sparingly soluble nonelectrolytic organic solutes such as *n* -alkanes, polynuclear aromatic compounds, and pesticides (Boehm and Quinn, 1975, McCarthy and Jimenez, 1985, Morehead, *et al.*, 1986, Chiou *et al.*, 1987), 4) sensitize photochemical reactions in surface waters (Liefer, 1988), and 5) stabilize colloids (Grasso and Weber, 1988).

Although humic substances are often used to represent the labile fraction of DOM, isolation and preparation of significant amounts of humic substances from natural waters for use in laboratory investigations is time, labor, and cost intensive. Consequently, many researchers have resorted the use of commercially-available humic acids in their studies as surrogates for naturally-occurring organic materials. Recently, Malcolm and MacCarthy (1986) have reported that ^{13}C-NMR spectra, and van Krevelen plots of commercially-available humic acids suggest that these organic materials may not be representative of natural humic substances. They hypothesized that commercial humic acids from different suppliers may originate from the same source. These findings cast doubt on the validity of conducting experiments using commercially available humic acids as surrogates of aquatic DOM. Moreover, aquatic dissolved organic matter, in addition to its humic component, contains a myriad of other macromolecules derived from *in-situ* biological activity. It is likely that these constituents are relatively unaltered, and may figure significantly in a host of potential transformations in natural waters. Thus, the use of either commercial or extracted humic substances as surrogates for natural DOM makes it increasingly important to ensure that experiments, of geochemical and environmental interest, provide results that are truly reflective of processes they purport to emulate. For example, the nature and quality of dissolved organic matter may impact the fate and transport of hydrophobic organic compounds (HOC). A number of investigators (Gauthier *et al.*, 1987; Chiou *et al.*, 1987; Chin and Weber , 1989) have shown that DOM binding constants (K_b) of sparingly soluble organic pollutants are strongly dependent on target substrates. Furthermore, these studies have indicated that Aldrich humic acid - HOC K_bs are considerably higher than comparable natural aquatic DOM equilibrium binding constants.

This paper applies gel-permeation chromatography (GPC), reverse phase liquid chromatography (RPLC) and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy to the examination of the physicochemical properties and structural characteristics of a popular commercial humic acid and natural DOM isolated from two different water samples. Both GPC and RPLC discern behavioral characteristics while ^{1}H-NMR determines possible structural configurations. GPC is utilized for determination of apparent molecular weight distributions. While there are some inherent problems with gel-permeation chromatography in general, such as solute adsorption to the stationary phase, and electrostatic repulsion, it was found useful for elucidating major disparities between the apparent molecular weight distributions of the natural DOMs and the commercial humic acid as discerned by their relative behavior in the gel column.

Reverse phase liquid chromatography can be used to separate constituents of DOM based on relative polarity. Several investigators (Templeton and Chasteen, 1980, Hayase and Tsubota, 1984, Orem and Gaudette 1984, Saleh and Chang 1985) have successfully separated fulvic acids and dissolved organic matter from marine pore waters into a variety of polar and nonpolar moieties. Grasso and Weber (1988) have also applied RPLC for elucidating the polar/nonpolar properties of humic substances prior to and following chemical oxidation. This type of approach aids in the identification of DOM fractions that may participate in specific chemical reactions of environmental/geochemical interest, such as organic solute binding phenomenon and metal complexation (Landrum, *et al.*, 1984, Chiou, *et al.*, 1987, Mills and Quinn, 1981, Mills *et al.*, 1982).

Proton nuclear magnetic resonance spectroscopy was applied for purposes of qualitatively characterizing DOM components based on chemical structure. The composition of the organic skeleton and associated functional groups may be discerned via the absorption of electromagnetic radiation of protons in a magnetic field (Grant, 1977, Ruggiero, *et al.*, 1978, 1979, Wilson, *et al.*, 1981, Harvey, *et al.*, 1983). While ^{13}C-NMR is a more desirable for elucidating structural information because it examines the actual carbon skeleton rather than protons (Ruggiero, *et al.*, 1979, Hatcher, *et al.*, 1980, Lobatini and Tan, 1988), the availability of sufficient quantities of DOM samples prevented the application of the method.

MATERIALS AND METHODS

Humic acid purchased from Aldrich Chemicals, was selected to represent a typical commercially available DOM commonly used in environmental/geochemical reactivity studies. Stock solutions were made by adding specified amounts of Aldrich humic acid to distilled water, and raising the pH to 11 with 1.0 *N* NaOH to facilitate dissolution. After one hour of agitation, the pH was readjusted to 7 with 1.0 *N* HCl, and the solution filtered through two prewashed glass fiber filters (Gelman Science) to remove any fractions that may have precipitated. Final stock solution concentrations were assayed as total organic carbon using a Dohrmann UV/Persulfate organic carbon analyzer. Working concentrations of the humic acids were prepared by diluting the stock solutions with 10^{-3}*M* $NaHCO_3$ buffered water.

Huron River water (Ann Arbor, MI) and pore water samples from a site in Lake Michigan approximately 10 miles offshore South Haven, MI. were chosen to represent two different sources of natural DOM. The Huron River water (HRW) was pretreated by filtration through a 0.45μ membrane filter. Lake Michigan Pore Water (LMPW) was obtained by centrifuging sediments sampled using a box corer. The lacustrine sample appeared to be oxic and contained benthic organisms. The supernatant was carefully poured into a separate container, and passed through prewashed glass fiber and membrane filters to remove any remaining particulates. The organic carbon content of both natural water samples was determined using the method described previously.

Reverse phase liquid chromatography was used to separate the complex DOM samples into distinct fractions based on relative polarity. The instrument employed was comprised of a Waters M45 solvent delivery system, a Waters Lambda Max 480 variable wavelength UV detector set at 280nm, an Econosphere 15 cm, 5μ C_{18} reverse phase column, and a Hewlett Packard 3390A integrating recorder. Samples were introduced into the system with a 20μl Rheodyne rotary injector. The mobile phase was comprised of an isocratic binary methanol:water mixture (50:50 v/v) which provided good resolution, at a flow rate of 1cc/min.

Molecular weight distributions were determined using gel permeation chromatography (GPC). This technique was selected from among several other common methods; namely, small angle X-ray scattering (Thurman, 1985), ultrafiltration (UF) (Buffle *et al.*, 1978), and methods such as vapor pressure osmometry, isothermal distillation, and freezing point depression which are based on colligative properties (Reuter and Perdue, 1981).

GPC and ultrafiltration are by far the most popular techniques, but both have limitations. Although size exclusion is the primary mechanism operative in both techniques, steric hinderance, ion exclusion effects, and adsorption onto gel surfaces may impact the results (Amy *et al.*, 1987; Hine and Bursill, 1984). Ultrafiltration is a concentration-dependent process and filtrate concentrations have been found to increase linearly with feed concentrations (Macko, 1979). Concentration polarization is another problem commonly encounter in this analysis. Moreover, ultrafiltration membranes commonly contain a spectrum of pore sizes and consequently do not have precise molecular weight cutoffs (MWCO) (Amy *et al.*, 1987). Conversely, ion exclusion effects may result in artificially high measurements of molecular weight by GPC (Amy *et al.*, 1987). This problem may be circumvented by using weakly buffered mobile phases. One major advantage of GPC over ultrafiltration is that continuous molecular weight distributions can be obtained for a specific stationary phase from this type of analysis while only vague discretizations are obtained by the later technique (Grasso,1987). It was principally for this reason that GPC was selected for this study.

For the GPC analyses a 1.6 cm diameter column, 34.5 cm in length, packed with Sephadex G-75 (MWCO ~10^5 to ~$10^{3.48}$ daltons) was connected to an Anspec AN203 UV spectrophotometer (254nm) in conjunction with a Hewlett-Packard 3390A integrating recorder. A flowrate of 0.44 cc/min was applied with a low-pressure positive displacement pump. The mobile phase was a filtered, degassed solution of $10^{-3}M$ $NaHCO_3$ adjusted to pH 8. This mobile phase was selected to minimize the impact of adsorption and ion exclusion (Swift and Posner, 1971). The column was calibrated with bovine serum albumin (67,000 daltons), ovalbumin (43,000 daltons), chymotrypsinogen A (25,000 daltons), and ribonuclease (13,700 daltons). Blue dextran 2000 (2 x 10^6 daltons) was used to determine the column void volume. Samples were injected directly into the column. Molecular weights for the DOM fractions were reported as apparent molecular weights (AMW) in that the structural characteristics and behavior of the samples are different from the proteins used to calibrate the instrument and may behave differently.

Proton nuclear magnetic resonance was used to obtain structural information. Aldrich humic acid was directly dissolved into the heavy water (deuterium oxide; D_2O) matrix. The organic matter concentrations in the natural water samples was separated from the aqueous phase using a one-liter vacuum flash evaporater (Buchner Instruments). The resultant residue was redissolved in D_2O, and all samples were analyzed using a Bruker WM-60 superconducting NMR with a spectral width of 5000 Hz and an acquisition time of 1.36 seconds. Each sample spectra represents an average minimum of 700 scans.

RESULTS AND DISCUSSION

Gel Permeation Chromatography

Figure 1 presents the correlation between the protein standard molecular weights and their chromatographic distribution coefficients, K_{av}:

$$K_{av} = \frac{V_e - V_o}{V_t - V_o} \qquad (1)$$

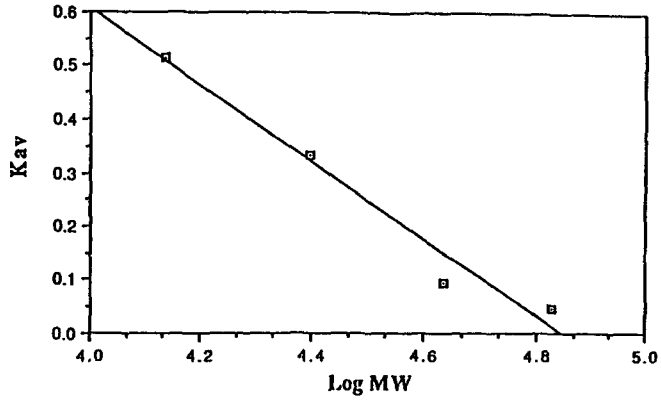

Figure 1: Relationship between K_{av} and log molecular weight.

Table 1: Summary of molecular weight modes for three different sources of dissolved organic matter.

	Molecular Weight Mode Assignment (Daltons)		
Sample	Mode 1	Mode 2	Mode 3
LMPW	>67,000	------	~ 3,000
HRW	>67,000	------	~ 7,000
AHA	>67,000	20,000-10,000	~ 5,000

where V_e, V_o, and V_t are the solute, void and total column volumes, respectively (Hayes and Swift, 1978). Regression of this data set yields:

$$K_{av} = 3.49 - 0.721(\log MW) \qquad r = 0.983 \qquad (2)$$

Equation 2 is linear only over a specific range as specified by the stationary phase molecular weight cutoff. Large molecules are excluded and elute at the same retention time (elution volume = 23cc) as blue dextran (the void volume tracer). Conversely, molecules smaller than the lower molecular weight cutoff will completely permeate the gel pores, and their elution volumes will be congruent with the maximum retention volume (V_p) for the column. The value V_p calculated from Equation 2, was found to be 70.4 cc. values for the three DOM samples were measured and molecular weights estimated using the protein calibration curve.

Figure 2 illustrates chromatograms representative of gel-permeation behavior for the samples studied. Corresponding molecular weight modes (as determined by protein standards) are reported in Table 1. Three molecular weight regions are clearly identified for the Aldrich humic acid, while the HRW and LMPW samples each yield two distinct peaks.

The validity of expressing these separated fractions on the basis of the size of the calibrating substances is questionable, however. Proteins tend to be globular while DOM macromolecules may be globular or radially coiled, depending on their composition. Humic molecules existing in a radially coiled state exhibit increases in their hydrodynamic radii thereby emulating behavior similar to higher molecular weight molecules. Therefore, actual molecular weights may be lower than reported in this paper because of structural conformations that may affect separation processes. The shapes and sizes of natural aquatic polymers are also highly dependent upon the pH and ionic strength of the aqueous phase because of their polyelectrolytic properties (Hine and Bursill, 1984, Cornel *et al.*, 1986, Hayes and Swift, 1978). The degree to which such effects impact the analysis can not accurately be determined *a priori,* and the mobile phase composition thus almost invariably affects the separation mechanism. Finally, specific solute-gel interactions (i.e., adsorptive processes) can also have pronounced effects, but these phenomena can be easily discerned by observing the existence of peaks and/or extensive tails that elute after the permeation volume.

The molecular weight distributions reported in this paper do not represent absolute values because the samples may not be structurally identical to the proteins used to calibrate the GPC system. Hence the *apparent* molecular weight (AMW) values used for purposes of comparing behavioral differences between the three DOM samples probably do not indicate the absolute sizes of the separate fractions.

The AMW's for the Aldrich humic acid seemed primarily to be 20,000 daltons or less. A smaller, but significant portion of this sample (first peak) eluted between the blue dextran and bovine serum albumin, indicating the existence of fractions greater than 67,000 daltons in size. The HRW chromatogram exhibited a bimodal distribution in which an even smaller fraction of the organic matter had

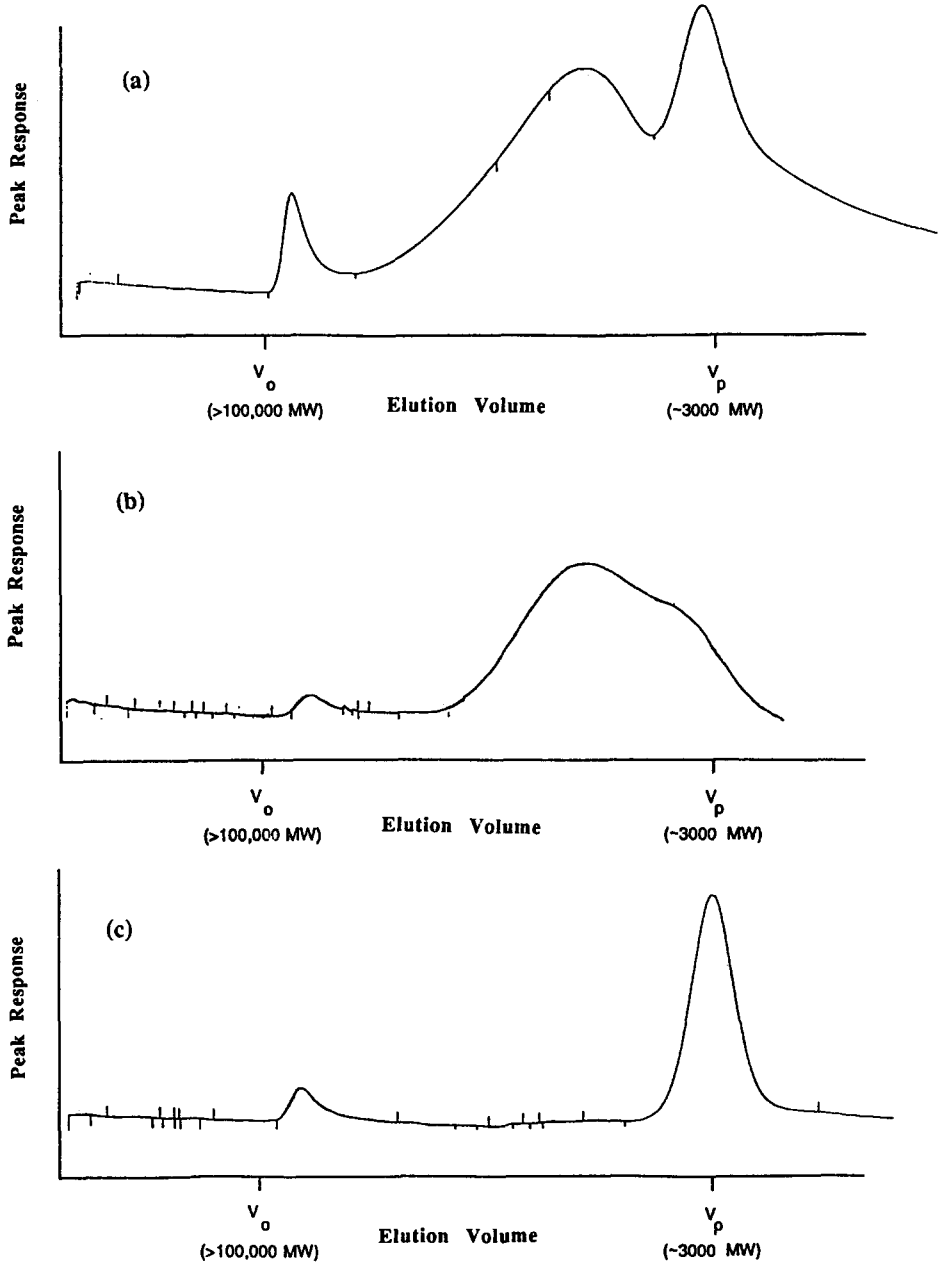

Figure 2: Gel-permeation chromatogram of an aqueous solution of (a) Aldrich humic acid; (b) organic matter isolated from Huron River water; and (c) organic matter isolated from Lake Michigan porewater.

AMW's greater than 67,000 daltons. Much of the HRW DOM is comprised of relatively low molecular weight polymers (3,000 to 10,000 range), as indicated by the broad unresolved second peak. The LMPW chromatogram yielded two distinct peaks indicating AMW distributions greater than 67,000 daltons and less than 3,000 daltons respectively. The large macromolecule fraction for this natural water sample is considerably greater than that for the HRW, but still comprises only a minor portion of the total organic matter present.

The extensive tailing beyond the total permeation volume (V_p) observed for the last peak of the Aldrich humic acid sample is indicative of the occurrence of specific solute-stationary phase interactions. This suggests the existence of nonpolar and possibly large moieties that sorbed onto the gel and separated by hydrophobic interactions rather than by size exclusion mechanisms. The two natural water samples did not exhibit this phenomenon.

All three samples contained relatively small amounts of polymers having AMW values greater than 67,000 daltons. With respect to the Aldrich humic acid, the lowering of the pH during the preparation of the stock solutions may have precipitated some of the large base soluble polymers. It is also possible that the amount of high molecular weight polymers may be artificially low due to adsorption of hydrophobic components to the stationary phase, as discussed previously.

The natural samples were taken from oxic environments where their organic constituents are constantly undergoing mineralization. It is likely that much of the DOM present is comprised of relatively small, polar and partially oxidized moieties. The large molecular weight fractions present in both samples may be comprised of more recalcitrant components that appear to have undergone a greater degree of polymerization.

Reverse Phase High Performance Liquid Chromatography

RPLC chromatograms for the systems studied are illustrated in Figure 3. Relative polarity is defined in this study in terms of the affinity of the compound for the nonpolar stationary phase. The more *polar* the compound, the less the attraction for the column packing and hence, the shorter the retention time. Early peaks are, therefore, representative of more polar compounds.

The peaks identified for the LMPW sample represent approximately comparable amounts of DOM based on the integrated output. The HRW sample can also be characterized by two fractions with retention times similar to LMPW organic matter. The more polar of the two peaks in this sample contains a majority of the DOM based on integrated areas. It is apparent, nonetheless, that both natural water samples contain DOM constituents that are hydrophilic.

The commercially available humic acid (AHA) was separated into four fractions. It appeared to be composed primarily of relatively less polar fractions than the natural water samples with the appearance of a large relatively nonpolar peak that eluted at 2.28 minutes. This supports the hypothesis developed from the gel permeation chromatography study where the presence of large macromolecules and extensive tailing of the sample beyond the total permeation volume (V_p) may be evidence for the existence of nonpolar moieties in the humic acid sample. The presence of a relatively nonpolar component may also

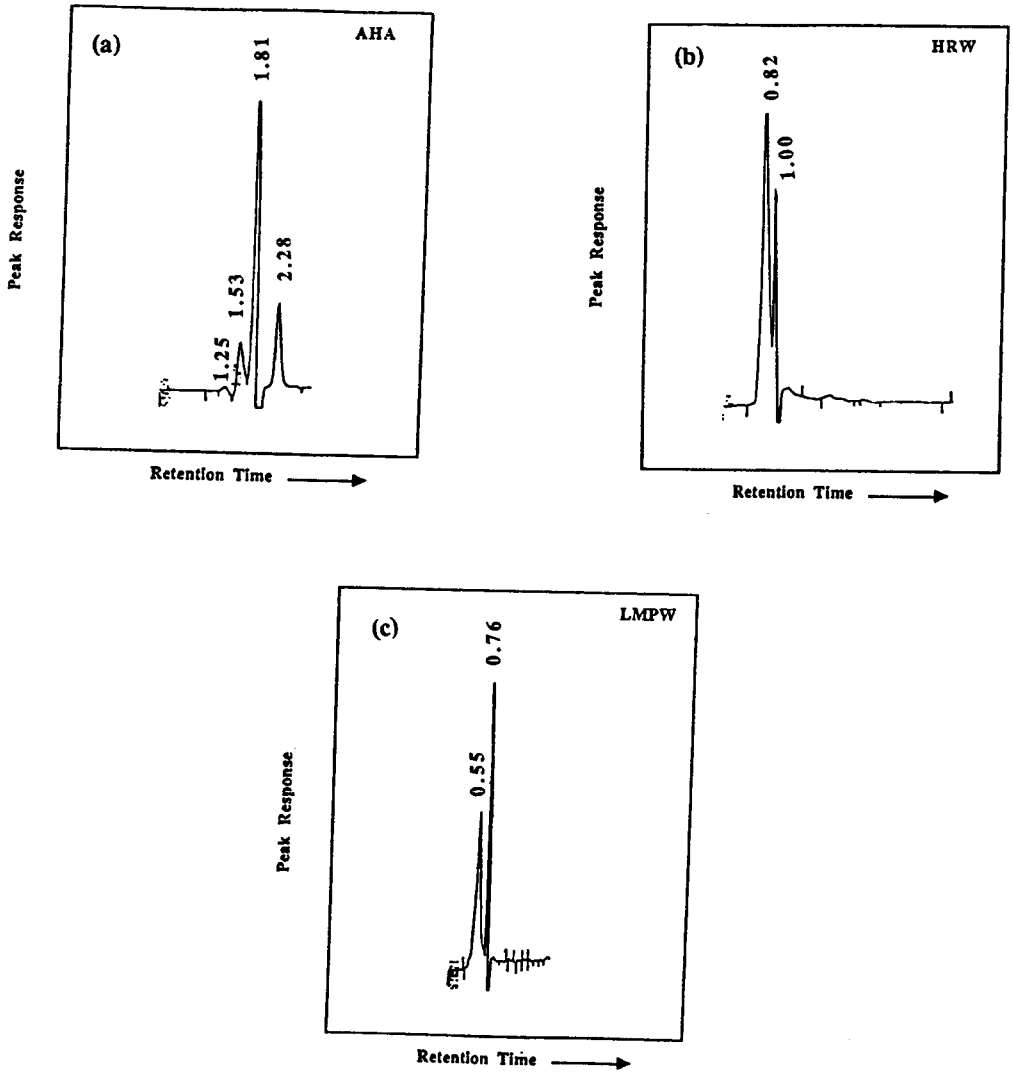

Figure 3: Reverse phase liquid chromatogram for (a) Aldrich humic acid solution; (b) Huron River water dissolved organic matter; and (c) Lake Michigan pore water dissolved organic matter.

explain why sparingly soluble organic substances partition more favorably into commercial humic acids than to DOM derived from natural sources (Carter and Suffet, 1983, Chiou *et al.*, 1987).

The relative polarity determinations appear to support at least some of the findings regarding apparent molecular weight distributions. Results from both studies identified large differences in polarity and size between Aldrich humic acid and the two natural DOM samples. Differences between the two natural samples were more difficult to discern, but revealed the presence of more polar and partially oxidized organic components. These findings agree with those reported by Templeton and Chasteen (1980) who observed increases in polarity and decreases in molecular weight for marine porewater DOM after oxidation.

^{1}H-NMR Spectroscopy

Proton NMR spectra for the three aqueous samples are shown in Figure 4. Table 2 summarizes the major peaks and possible corresponding structures. The natural water samples were prepared by evaporation and subsequent redissolution of the residue in D_2O. The Aldrich humic acid ^{1}H-NMR spectra revealed strong resonances in the upfield (0.8 to 3.0 ppm) chemical shift region, which is characteristic of protons associated with aliphatics and carbons adjacent to either aromatics, double bonds, carbonyls or other electron withdrawing groups (Grant, 1977, Stuermer and Payne, 1976, Hatcher, *et al.*, 1980, Wilson, 1981, Gillam, *et al.*, 1987). Another strong signal occurs in the downfield region (6.5 to 8.6 ppm chemical shift) which is indicative of hindered and unhindered protons directly attached to aromatic substances. The signal at 8.4 ppm has been identified as formate, and can be formed by the hydrolysis of lignin (Wilson, *et al.*, 1988) There is a lack of specific peaks in the 3.0 to 4.0 ppm range, which would be indicative of carbohydrates, lactones, amines, and methoxy type structures (Wilson, 1981). The absence of such a signal may be the result of the preferential removal of associated organic compounds (cellulose, proteins etc.) by extraction techniques used by the manufacturer. It may also be indicative of the origin of such commercial substances. Raw materials such as peat and lignite may have undergone a mild degree of diagenesis in which biotic and abiotic processes have broken down amino acids and carbohydrates leaving only resistant organic matter such as lignins and waxy substances. Thus, it is plausible that the lack of a well-defined signal in this chemical shift range is possibly due to both the manner in which commercial humic substances are prepared, and their source materials.

The proton NMR spectra of the Huron River water exhibits the same characteristics as the Aldrich samples in the downfield region of the scan, where strong signals at 1.15 and 1.14 ppm chemical shift indicate the presence of protons associated with aliphatic chains connected to aromatics or polar functional groups at the β or γ carbon (Wilson, 1981, Ruggiero, *et al.*, 1978). Signal responses at 1.73 are indicative of protons associated with carbon α to aromatic compounds (Wilson, 1981). A new, but relatively weak resonance seemingly absent in the commercial humic acid appears between 3.0 and 3.9 ppm, indicating the possible existence of organic structures summarized in Table 2, and discussed in the preceding paragraph. While a significant portion of the organic matter found in Huron River water is derived from land runoff (which also provides a large influx of inorganic nutrients), much of it is autochthonous because of high primary productivity occurring during the spring and summer months

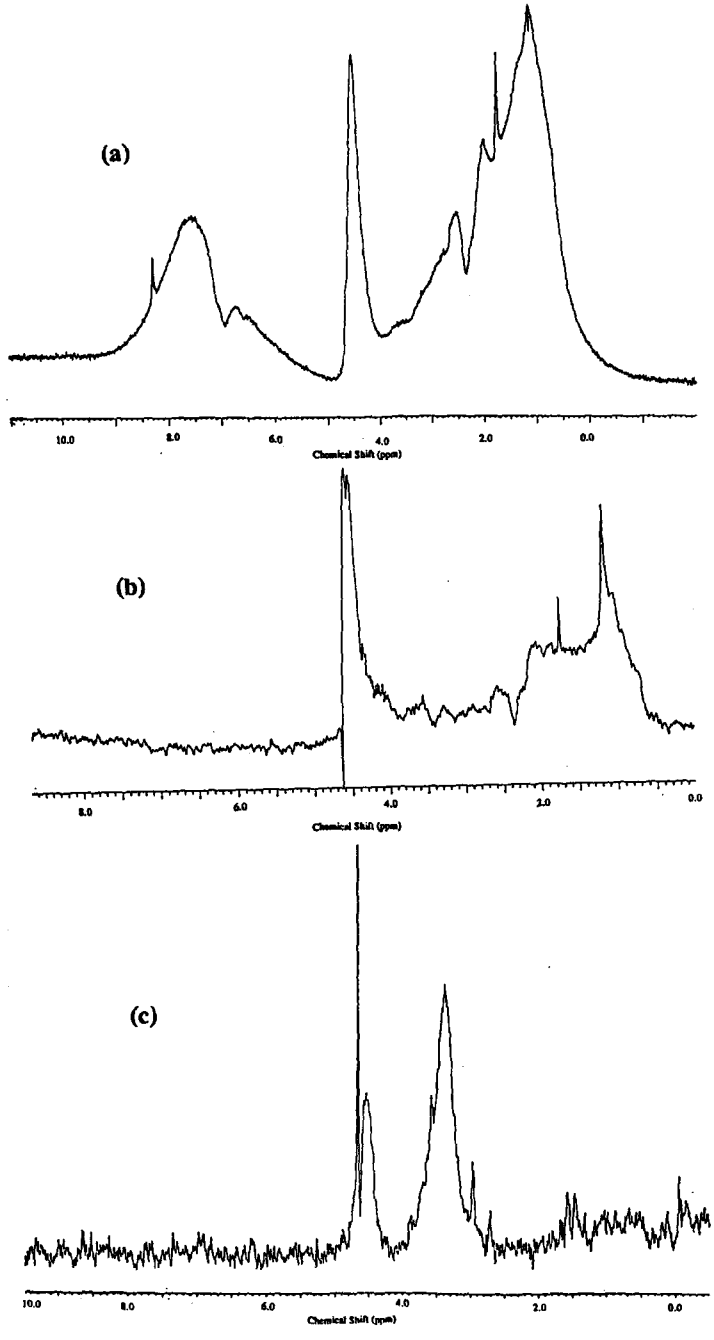

Figure 4: ^{1}H-NMR spectra for (a) Aldrich humic acid in D_2O; (b) Huron River dissolved organic matter in D_2O; and (c) Lake Michigan porewater dissolved organic matter in D_2O.

Table 2: Observed proton resonances and possible molecular configurations for dissolved organic matter from different sources.

		Observed Resonances[2]		
Chemical Shift (δ)	Assignment[1]	AHA	HRW	LMPW
0.8 -1.0	terminal methyl groups of methylene chains	0.8-1.11	0.7	none
1.0 - 1.4	methyl protons of a chain β or γ to aromatic ring	1.11	1.15 + 1.14	none
1.4-1.7	methylene of alicyclic compounds	1.7	none	1.5 + 1.6
1.7 - 2.0	protons on carbons α to aromatic rings	2.0	1.73	none
2.0 - 3.3	protons of aliphatics α to aromatics; or α to carboxylic functional groups or carbonyls	2.4-2.7	2.5-2.3	2.7 +
3.3 - 5.0	protons α to carbons to oxygen groups such as methoxy bonds, and aldehydes; aromatic amines,carbohydrates, aromatic hydroxyl polymers, and protons associated with lactones (cyclic esters) exchangeable protons associated with phenols, and carboxylic functional groups, and traces of water	none	3.1-3.4 3.5-3.9 4.0-4.4	3.0-3.9 4.3-4.6
6.5 - 8.1	unhindered aromatic protons including those associated with phenols and quinones	7.0 - 8.6	none	none
8.1 - 9.0	sterically hindered aromatic protons; nitrogen heteroaromatics, formate	8.3-8.6	none	none

[1] Chemical shift assignments of this table was compiled from the following references:
Wilson (1981), Aiken, et al. (1985), Steurmer and Payne (1976)

2 AHA = Aldrich Humic Acid
HRW = Huron River Water
LMPW = Lake Michigan Pore Water

(Grasso, 1987). Thus a major contributor to the organic budget of this body of water would be comprised of extra-cellular products, and decomposing microbial biomass materials, which would release carbohydrates, porphyrins, sterols, amino acids, fatty acids, and other such compounds to the water column. A portion of this organic matter will be immediately utilized by other microorganisms, while the remainder may be incorporated into macromolecules. The two peaks in the 4.3 to 4.6 chemical shift region may be due to lactones, matrix impurities such as H_2O, or organic molecules capable of participating in acid/base reactions.

Pore water extracted from Lake Michigan sediments seems to lack aliphatic character as indicated by the absence of any strong resonances in the 0.8 to 1.0 ppm chemical shift region. It does however exhibit two peaks at 1.5 and 1.6 ppm which may be methyl groups associated with alicyclic compounds (Wilson, 1981). It is plausible that many of the larger hydrophobic aliphatic hydrocabons (i.e.waxy substances from leaves, porphyrins, long chained fatty acids etc.) have partitioned onto the sediments, and could not be removed by centrifugation. Due to the limited amount of pore water samples available to us, we were unable to process large quantities, and did not specifically extract constituents (i.e. humic and fulvic acids) in order to preserve, as best possible, the chemical structure of the remaining DOM. Thus any of these substances still present in the aqueous phase may be below the instrument's limit of detection.

Other definable signals do occur at δ values of 2.7 and 2.9 ppm suggesting the existence of aliphatics attached to aromatic structures or polar functional groups at the α carbon (Wilson, *et al.*, 1988) The best defined resonance occurs between chemical shifts of 3.0 and 3.9 ppm and is attributable to methyls and methylenes associated with electronegative atoms such as oxygen and nitrogen, and may be comprised of carbohydrates, methoxy compounds, carboxylic acids, and organic amines. This could be evidence for the existence of polysaccharides and proteinaceous substances. The large peak further downfield at 4.5 ppm may also contain carbohydrates, but can also be caused by proton donor/acceptor substances such as carboxylic acids and phenols. All three samples also contained an HDO or water peak in the 4.6 to 4.7 chemical shift region. Ruggiero and co-workers (1978, 1979) observed that acidic (particularly carboxylic and hydroxyl functional groups) as well as amphoteric substances such as trace amounts of HDO or water in the D_2O matrix may yield an exchangeable proton absorption band in this region where:

$$\delta_{ep} = \chi_{H_2O}\delta_{H_2O} + \Sigma(\chi_{COOH}\delta_{COOH})_i + \Sigma(\chi_{ROH}\delta_{ROH})_j + \Sigma(\chi_{AROH}\delta_{AROH})_k + \ldots \quad (3)$$

where δ_{ep} is the exchangeable proton chemical shift, and χ and δ are the fraction and chemical shifts of the proton donor/acceptor components, respectively. Removal of all traces of water would shift the OH, COOH protons downfield which may result in a potential interference in the aromatic absorption region. (Ruggiero, *et al.*, 1979). It is unlikely, however, that the downfield region is affected by this artifact because the presence of trace amounts of HDO and water would cause all the exchangeable proton signals to coalesce in the 4.5-4.7 ppm peaks.

Signals in the aromatic chemical shift region (6.5 to 8.1 ppm) for Huron River water and Lake Michigan porewater were either absent or very weak, while the Aldrich humic acid sample exhibited a strong and broad peak. This is indicative of direct proton association with aromatic compounds. The lack of such a signal in the natural water samples may be due to a *masking* effect where all the sites on an aromatic structure can be substituted by elements other than hydrogen (halogens for example) or by functional groups. The presence of absorption bands in the 1.7 to 3.0 chemical shift region for these samples does provide some evidence of the presence of methyl aromatic structures, as discussed previously.

The proton NMR spectra of aquatic organic and humic substances are difficult to interpret precisely given the relatively broad and poorly resolved resonances. This is largely due to the molecular size, complexity, and nonrepetitive subunit structure of the samples. Nonetheless some generalized structural information can be determined. While the NMR spectra for all three organic polymers studied indicated that they are all remarkably different in composition, there exist some structural common denominator that is common to all of the samples analyzed.

SUMMARY

Aquatic systems commonly involve complex mixtures of dissolved organic matter that impact the transformations of environmentally important substances. Difficulties associated with extracting DOM from natural waters for use in experiments designed to identify and quantify such processes have led to the increased use of commercially available DOM surrogates. Several types of analytical techniques may be required to properly characterize the DOM of either synthetic or natural systems. Relatively small differences in system composition may impact DOM behavior in geochemical and environmental transformation reactions. Recognizing the temporal and spatial dependency of a given system, several observations can be made regarding the aquatic systems investigated in this work.

° The behavior [apparent molecular weight distributions], of DOM indigenous to pore waters extracted from an offshore site in Lake Michigan, in gel permeation columns appears to yield a bimodal distribution with fairly narrow variances while the behavior of river water DOM (Huron River, Ann Arbor, MI) also yields a bimodal distribution but with much greater variances. In contrast, the apparent molecular weight distribution of a commercial humic acid (Aldrich) is trimodal with relatively high variances. This may be due to the origin and nature of the Aldrich HA or the processing of the product.

° High pressure reverse phase liquid chromatograms indicate that both natural water aliquots are relatively more polar [as defined in this paper] than the Aldrich humic acid sample.

° ^{1}H-NMR spectra for both natural system samples provided no evidence of direct proton association with aromatic groups. However, the commercial humic acid sample spectra revealed a strong signal in the downfield region (δ = 6.5 - 8.6 ppm), indicative of hindered and unhindered protons associated directly with aromatic groups. All three samples indicated the presence of high concentrations of aliphatic groups either free or attached to aromatic groups.

° Based on the composite results of the several types of analyses performed in this investigation, it appears that this and likely other commercial humic acids may not be a suitable surrogate for natural aquatic DOM in the study and analysis of environmental/geochemical transformation processes.

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