

## Isolation of hydrophilic organic acids from water using nonionic macroporous resins

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**Abstract**—A method has been developed for the isolation of hydrophilic organic acids from aquatic environments using Amberlite\* XAD-4 resin. The method uses a two column array of XAD-8 and XAD-4 resins in series. The hydrophobic organic acids, composed primarily of aquatic fulvic acid, are removed from the sample on XAD-8, followed by the isolation of the more hydrophilic organic acids on XAD-4. For samples from a number of diverse environments, more of the dissolved organic carbon was isolated on the XAD-8 resin (23–58%) than on the XAD-4 resin (7–25%). For these samples, the hydrophilic acids have lower carbon and hydrogen contents, higher oxygen and nitrogen contents, and are lower in molecular weight than the corresponding fulvic acids. <sup>13</sup>C NMR analyses indicate that the hydrophilic acids have a lower concentration of aromatic carbon and greater heteroaliphatic, ketone and carboxyl content than the fulvic acid.

**Key words**—hydrophobic acids, aquatic fulvic acid, XAD-resins, natural waters, DOC, isolation, chromatography

### INTRODUCTION

The study of the nature and environmental significance of dissolved organic carbon (DOC) in natural waters is often hampered by the inherent chemical complexity of the organic carbon to be studied. A complementary approach to studying whole samples is to isolate functionally distinct DOC fractions from natural waters to determine fundamental chemical properties of each fraction, ultimately relating structural and chemical information to the biogenesis and environmental roles of these materials. While this approach has some 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), many advances have been made in the field, especially with respect to the study of aquatic humic substances.

Organic acids in water are a complex heterogeneous continuum of high to low molecular weight species, exhibiting varying chromatographic behavior on resin sorbents. Sorption efficiency of these organic acids is a function of the aqueous solubility of the solute, and the nature of the sorbent. No single absorbent can isolate the entire suite of organic acids present in any given natural water sample. According to the DOC fractionation scheme of Leenheer (1981), organic acids in natural waters are fractionated into the hydrophobic acid fraction and the hydrophilic

acid fraction (Table 1). Aquatic fulvic acid is the major component of the hydrophobic fraction, ranging in concentration from 20 µg C/l in groundwater to over 30 mg C/l in surface water. Methods utilizing various Amberlite XAD resins to isolate humic substances from other classes of organic carbon have been extensively used (Mantoura and Riley, 1975; Aiken, 1985). Techniques for the isolation and fractionation of the hydrophilic acid fraction, which can account for 30–50% of the DOC (Aiken, 1985; Leenheer, 1981), are inherently more challenging because of problems encountered in separating these solutes from inorganic salts dissolved in the water sample. These difficulties have recently been demonstrated by the isolation of hydrophilic organic solutes from saline waters by a method utilizing zeotropic distillation developed by Leenheer *et al.* (1987). As a result, a significant portion of the DOC in natural waters has not been well studied.

This paper describes the use of a two column array of Amberlite XAD-8 resin and Amberlite XAD-4 resin to isolate a large portion of the hydrophilic acid fraction from natural waters in preparative quantities. It also discusses the factors controlling sorption of natural organic acids on XAD resins. The two column setup is an extension of the method of Thurman and Malcolm (1981) that allows isolation and separation of both aquatic fulvic acid (hydrophobic acid fraction) and a portion of the hydrophilic acid fraction from a given water sample, while maintaining the operational definition for aquatic humic substances established by Thurman and Malcolm (1981).

\*Use of trade names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

Table 1. Operational definitions of different fractions of organic acids comprising dissolved organic carbon that are obtained using XAD-8 and XAD-4 resins

Hydrophobic acid fraction	That portion of the DOC that sorbs on a column of XAD-8 resin at pH 2 under conditions where $k'_{\text{cutoff}} = 50$ for the column, and are eluted at pH 13. This fraction can contain aliphatic carboxylic acids of 5-9 carbons, one- and two-ring aromatic carboxylic acids, one- and two-ring phenols, and aquatic humic substances.
Hydrophilic acid fraction	That portion of the DOC contained in the XAD-8 resin effluent at pH 2 that sorbs on a column of XAD-4 resin under conditions where $k'_{\text{cutoff}} = 50$ for the column, and are eluted at pH 13. This fraction can contain polyfunctional organic acids and aliphatic acids with five or fewer carbon atoms.

## EXPERIMENTAL PROCEDURES

### Resin preparation

The Amberlite XAD resins were obtained from Rohm and Haas. The resins were cleaned by first washing the beads (20-50 mesh) in 0.1 N NaOH and then rinsing the resin with distilled water. The resin was then placed in a Soxhlet extractor and sequentially extracted for 48 h each with methanol and acetonitrile. This sequence was repeated twice. Clean resin was stored in methanol. Glass columns were packed with a H<sub>2</sub>O-resin slurry and rinsed with distilled water to remove methanol. The resin was further cleaned with 3 successive 0.1 N NaOH-0.1 N HCl rinses immediately before using.

### Determination of capacity factors

Capacity factors for the model compounds on each of the XAD resins were determined by frontal chromatography. Solutes were dissolved at concentrations of  $10^{-4}$  molar and passed through a 20-ml column of resin at 4 ml/min until effluent and influent concentrations were equal. At this point, the column was eluted with the appropriate solvent, and the amount of solute in the eluate was quantified by DOC analysis or gas chromatography; this quantification represented the amount of material adsorbed by the column. The void volume was measured with non-sorbed solutes, and capacity factors ( $k'$ ) determined using the following equation:

$$k' = \frac{\text{grams of solute on resin}}{\text{grams of solute in column void volume.}}$$

### Methodology and sample characterization

Procedures used for isolating fulvic and hydrophilic acids from water were similar to those employed by Thurman and Malcolm (1981). In brief, 2-l columns of XAD-8 and XAD-4 (Fig. 1) were connected in series by Teflon tubing. Samples were filtered through a Balston glass fiber filter type AAH (0.3  $\mu$ ) and acidified to pH 2. One hundred and twenty liters of sample were then passed through the XAD-8/XAD-4 column pair. Each column was separately back eluted with 4 l of 0.1 N NaOH. NaOH

eluates were immediately acidified with concentrated HCl to pH 2 to minimize alteration of the sample at high pH. Eluates were reconcentrated on the appropriate resin, hydrogen-saturated using AG-MP 50 cation exchange resin obtained from Biorad, and lyophilized.

Samples were characterized by elemental, molecular weight, titration and <sup>13</sup>C-NMR analyses. A review of the methods used for the determination of each element has been published by Huffman and Stuber (1985). Number-average molecular weights were determined by vapor pressure osmometry with water as solvent. Details of the method and correction of the data for dissociation have been published by Aiken and Malcolm (1987).

Samples were prepared for NMR analysis by dissolving 70 mg of the hydrophilic acid in 1.5 ml D<sub>2</sub>O, and 180 mg of the fulvic acid in 2.0 ml D<sub>2</sub>O, in 10 mm NMR tubes; the pH was adjusted to 7.0. The <sup>13</sup>C NMR spectra were recorded on a Varian XL300 NMR spectrometer at 75.4 MHz. The acquisition parameters included a 50,000 Hz spectral window, 45° pulse angle, 0.2 s acquisition time, 10.0 s pulse delay, and inverse gated decoupling; a line broadening of 100.0 Hz was applied to the free induction decays. Dioxane, assumed to be 67.4 ppm, was used as an internal reference standard. Peak areas of the <sup>13</sup>C NMR spectra were measured by electronic integration.

## RESULTS AND DISCUSSION

Differences in the sorption characteristics of XAD-8 and XAD-4 can be used to isolate a large fraction of the natural organic acids present in an environmental water sample by using a two column array consisting of a column of XAD-8 followed by a column of XAD-4 (Fig. 1). After filtration the sample

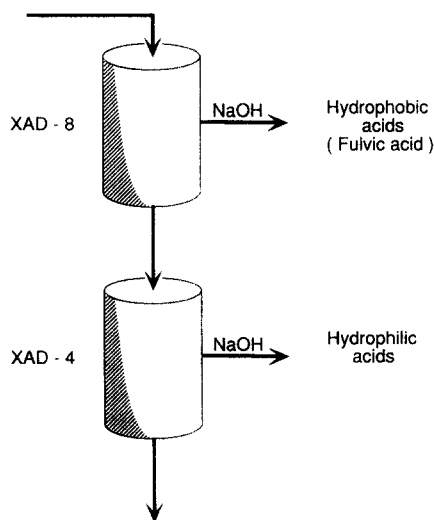


Fig. 1. Schematic diagram of the XAD-8/XAD-4 isolation scheme.

Table 2. Fractionation of dissolved organic carbon (DOC) from a variety of aquatic environments

Location	DOC (mg C/l)	Percent fulvic acid	Percent hydrophilic acid
Lake Fryxell, Antarctica (depth—7.5 m)	5.2	23	7
Lake Hoare, Antarctica (depth—12 m)	2.0	23	9
Yakima River, Wash.			
Cle Elum	1.6	26	8
Kiona	2.4	23	12
Bemidji, Minn. (contaminated groundwater)*	16	42	22
Suwannee River, Ga	58	58	25

\*Sample contains high concentrations of organic compounds resulting from the microbiological degradation of crude oil.

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 XAD-4 column, wherein a fraction of the hydrophilic acids is sorbed. Each column is separately back-eluted with 0.1 N NaOH to obtain the hydrophobic acid fraction and the retained portion of hydrophilic acids. DOC fractionation data from a variety of aquatic environments where this method has been employed are presented in Table 2. In each case, a total of 30–83% of the DOC has been isolated on the XAD-8/XAD-4 array, with the retainable hydrophilic acids accounting for 7–25% of the DOC, a significant fraction of the total hydrophilic acids.

#### Chromatographic aspects

Given that each of these fractions is operationally defined, further discussion of the rationale for the method is warranted. Sorption characteristics of XAD-8 and XAD-4 resins are dependent primarily on chemical composition, resin surface area and resin pore size (Table 3). Resin surface area strongly affects adsorption efficiency. Data presented in Table 4 demonstrate that for five XAD resins, XAD-4, which possesses the greatest surface area, has the greatest capacity for low molecular weight solutes. A resin's capacity for a low molecular weight solute is, in part, a function of the solute's aqueous solubility and the resin's usable surface area. The importance of solubility in the sorption process has been documented for various chromatographic systems (Locke, 1974; Karger *et al.*, 1976; Thurman *et al.*, 1978a). It was previously found that the logarithm of the capacity

factor ( $k'$ ) of an organic solute with molecular weight less than 500 daltons varies linearly with the log of its aqueous solubility ( $S$ ) on XAD-8 (Thurman *et al.*, 1978a) and on XAD-2 (Thurman *et al.*, 1978b). This relation also holds for XAD-4 resin. The log  $k'$ -log solubility plots for both XAD-4 and XAD-8 appear in Fig. 2. From the data plotted, we found that for XAD-4,  $\log k' = 2.6 - 0.46 \log S$  ( $r^2 = 0.84$ ) while for XAD-8,  $\log k' = 1.8 - 0.50 \log S$  ( $r^2 = 0.80$ ). Given the aqueous solubility of a compound, these relations can be used to estimate the  $k'$  of that compound on these resins to a first approximation. While the slopes are similar between resins, the large difference in intercepts reflects differences in surface area and capacity. It is clear that XAD-4 is significantly more effective than XAD-8 for the low molecular weight solutes examined.

As molecular weight increases, however, the effect of resin pore size on capacity becomes more significant. Aiken *et al.* (1979) demonstrated that for high molecular weight solutes size exclusion occurs on XAD resins. For polyacrylic acids of increasing molecular weight, distribution coefficients were shown to decrease markedly on XAD-4 and XAD-2 (pore sizes of 50 and 91 Å, respectively), whereas the effect on XAD-8, which has a pore diameter of 250 Å, is less dramatic. In addition, pore size also effects the rate of sorption of large molecules (Aiken *et al.*, 1979). In batch experiments designed to measure rates of adsorption of soil fulvic acid onto XAD resins, XAD-8 attained equilibrium at a significantly faster rate than XAD-4. Intraparticle diffusion was concluded to be the rate limiting step. Using a soil fulvic acid to compare the effectiveness of XAD resins for isolation of fulvic acid from water, Aiken *et al.* (1979) concluded that XAD-8 adsorbed fulvic acid more efficiently than XAD-4 due to its large pore size.

One important purpose that the XAD-8 column serves in this isolation scheme is to remove aquatic fulvic acid from the sample before it contacts the XAD-4 resin. It was noted in past work (Aiken *et al.*, 1979) that the acrylic-ester (XAD-7, XAD-8) resins elute humic substances more efficiently than the styrene divinylbenzene resins (XAD-1, XAD-2, XAD-8). While recoveries of 98% were obtained on XAD-8 and XAD-7, only 70% was recovered from the styrene divinylbenzene resins. The styrene-divinylbenzene copolymers are aromatic in character, hydrophobic, and possess no ion exchange capacity. The acrylic-ester resins are nonaromatic, more hydrophilic than the styrene divinylbenzene resins, and

Table 3. Properties of the XAD resins studied

Resin	Composition	Average pore diameter (Å)	Specific surface area (m <sup>2</sup> /g)	Specific pore volume (cm <sup>3</sup> /g)
XAD-1	styrene divinylbenzene	200	100	0.69
XAD-2	styrene divinylbenzene	90	330	0.69
XAD-4	styrene divinylbenzene	50	750	0.99
XAD-7	acrylic ester	80	450	1.08
XAD-8	acrylic ester	250	140	0.82

Table 4. Capacity factors of various organic solutes on XAD resins

Compounds	Solute capacity factors				
	XAD-1	XAD-2	XAD-4	XAD-7	XAD-8
<i>Low molecular weight</i>					
<i>p</i> -Toluic acid	625	1800	—	—	1037
Aniline	131	157	684	—	126
Benzoic acid	177	450	1700	345	488
Caproic acid	320	775	1963	249	377
Phenol	63	109	720	88	245
Benzaldehyde	501	770	—	218	337
Valeric acid	73	215	756	42	125
Cyclohexanoic acid	—	690	—	—	390
Heptanoic acid	—	1950	—	—	960
<i>p</i> -Nitrophenol	—	—	1350	—	—
Butyric acid	—	—	196	—	39
<i>High molecular weight</i>					
Polyacrylic acid-2000	175	580	735	—	945
Polyacrylic acid-5000	138	475	175	—	1500
Polyacrylic acid-90,000	35	30	0	—	350
Soil fulvic acid†	475	515	332	1480	604

\*Determined by batch technique.

†Extracted from spodic horizon of Lakewood soil series near Wilmington, N.C.

have a measurable cation exchange capacity (on the order of 10–65 microequivalents per gram of resin, respectively). The excellent elution efficiencies of the acrylic ester resins for humic substances result, in part, from charge repulsion, as both the resin and the fulvic acid are anionic at pH 13. The styrene divinylbenzene resins, on the other hand are neutral at all pH values, and have been shown to interact strongly with fulvic acid, even at pH 13. These interactions were attributed to charge transfer interactions between the resin and the fulvic acid (Aiken *et al.*, 1979).

As noted by Leenheer (1981),  $k'$  for a solute that is 50% retained at the hydrophobic–hydrophilic break, known as the  $k'$ -cutoff, is given by the following expression:

$$V_{0.5r} = 2V_0(1 + k')$$

where,  $V_0$  is the void volume of the column and  $V_{0.5r}$  is the effluent volume at which 50% of the total mass of solute has been retained. For the isolation of humic substances from water, sample volume and XAD-8 column size are chosen such that a solute with  $k' = 50$  is 50% retained by the column. In the present method, these conditions have been established for both the XAD-8 and XAD-4 resins. The fraction of organic acids that sorb to the XAD-4 resin is dependent both on the  $k'$ -cutoff of the column and on the solubility characteristics of the organic acids in the sample. For example, butyric acid (Table 3) has a  $k'$  of 39 on XAD-8, and will pass through the first column as a hydrophilic acid when the  $k'$ -cutoff = 100. On XAD-4, butyric acid has a  $k' = 196$ , and, under the same chromatographic conditions, will be retained on the XAD-4 column. Solutes that exhibit a high aqueous solubility such as acetic acid and gluconic acid have low  $k'$  values of XAD-4 and will not be retained. Note, however, that fractions separated on XAD resin sorbents by the type of preparative chromatographic method used here to isolate DOC components from water are not

sharp. This is a complicating factor that results in some overlap between fractions. Undoubtedly, the XAD-8 and XAD-4 each remove some of both the hydrophobic and hydrophilic acid fractions. However, each fraction is dominated by specific components. The critical issue is that the method reproducibly isolate compositionally distinct fractions from the molecular continuum observed in natural DOC.

Advantages associated with this method of isolation are as follows. First, the method is applicable across a wide range of natural matrices and potential interferences. For instance, XAD-8 and XAD-4 have no affinity for inorganic anions. Salt contents upto 0.5 M NaCl were found to have no effect on the distribution coefficients of organic acids in the neutral form on XAD resins (Pietrzyk and Chu, 1977). Consequently, XAD resins are often used to isolate organic acids from saline environments (Aiken, 1985). Anion exchange resins, on the other hand, have strong affinities for inorganic anions. Inorganic anions compete for binding sites with organic acids on the anion exchange resin, and are concentrated and eluted with the organic acids. Additional desalting steps are required to obtain the organic acids of interest (Leenheer, 1981). In the presence of appreciable salt content, the effectiveness of the anion

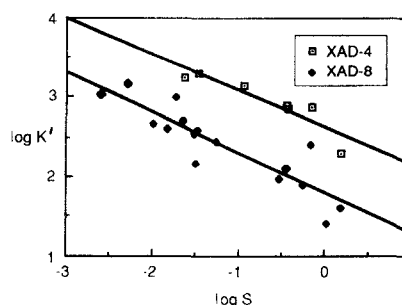


Fig. 2. Log  $k'$ –log solubility plots for low molecular weight solutes on XAD-4 and XAD-8.

Table 5. Elemental and molecular weight data for fulvic acid and hydrophilic acids isolated from the Yakima River near Kiona, Wash. (elemental data presented as percent)

		C	H	O	N	S		Carboxyl content (meq/gm)	Molecular weight* (daltons)
Sample	Fraction	(ash free)					Ash		
Yakima River	FA	56.1	4.95	35.5	2.2	0.97	1.1	5.4	650
at Kiona	HPIA	50.5	4.4	40.6	3.0	1.2	3.9	5.9	411
Yakima River	FA	57.2	4.9	35.9	1.0	0.6	8.53	5.2	—
at Cle Elum	HPIA	52.2	4.6	40.8	1.5	0.6	3.1	5.9	—
Lake Fryxell	FA	55	5.5	34.9	3.1	1.3	1.0	—	—
(7.5 m)	HPIA	49.3	4.8	39.2	4.8	1.8	8.9	—	—
Bemidji groundwater	FA	61.9	5.7	30.7	0.3	—	0.9	—	335
(contaminated)	HPIA	53.0	5.6	38.8	1.1	—	3.12	—	—

\*Determined by vapor pressure osmometry in water.

exchange resins for isolating organic acids is greatly decreased (Aiken, 1987). Using the XAD-8/XAD-4 method, hydrophobic and hydrophilic acid fractions have been isolated from waters having low DOC concentrations such as groundwaters, from saline waters such as seawater 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 or reverse osmosis, maintaining fraction consistency and comparability between samples. In the authors' opinion, this is an important factor for two reasons:

(1) Concentrating the original sample can change the nature and degree of interactions that can take place between chemical constituents present in the original sample, possibly affecting sample behavior on the resin. An example of this type of effect would be increased sample aggregation.

(2) Langmuir isotherms for organic acids on XAD resins over large concentration ranges have been shown to be L-shaped (Gustafson and Paleos, 1971) indicating a decreasing affinity of the resin for organic acids as the degree of sorption increases with increasing concentration. However, at low concentration, less than  $10^{-3}$  moles per liter, the isotherms are linear, which is likely the case for the low concentrations of organic acids normally found in natural waters. The  $k'$  data and  $\log k' - \log S$  relations presented in this paper have been established for samples with DOC concentrations in the range of 0–25 mg C/l, well within the linear regions of the isotherms. At this time, the effect of concentrating mixtures of compounds on sample fractionation has not been clearly defined. However, it is clear that, even without undergoing aggregation, sample interactions with the resin would be altered.

#### Practical application

The fulvic acid and hydrophilic acid fractions isolated from a number of locations using the XAD-8/XAD-4 isolation scheme have been characterized and the results are presented here to illustrate the nature of the hydrophilic acid fraction. Factors

such as molecular weight, heteroatom content and carboxyl content are important in determining the overall aqueous solubility of organic molecules. Given the nature of the isolation scheme employed to isolate these samples, it is expected that the hydrophilic acid fraction would be of lower molecular weight and have greater heteroatom and carboxyl content than the corresponding fulvic acid.

Results of elemental and molecular weight analyses (Table 5) show that the hydrophilic acids have consistently greater amounts of oxygen, nitrogen, and sulfur, with lesser amounts of carbon and hydrogen than the hydrophobic acids, and for the case of the Yakima River sample collected near Kiona, Wash., a lower number average molecular weight than the corresponding fulvic acid. With the exception of the sample collected from the Yakima River near Cle Elum, Wash., the hydrophilic acids also have higher ash contents than the fulvic acids. In general, semi-quantitative analyses of the ash in fulvic acid and hydrophilic acid samples indicate that the major constituents in the ash are sodium and silicon.

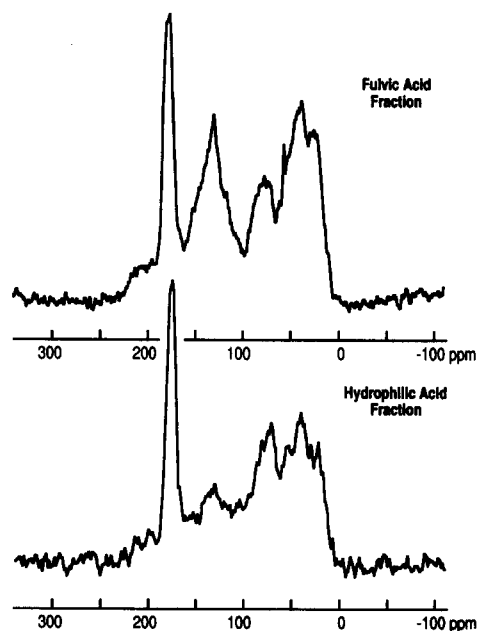


Fig. 3.  $^{13}\text{C}$ -NMR spectra for fulvic acid and the hydrophilic acid fraction isolated from the Yakima River.

Table 6. Peak areas as percentage of total spectrum area for quantitative  $^{13}\text{C}$ -NMR spectra of fulvic acid and hydrophilic acids

Sample	Fraction	Aliphatic I 0–60 ppm	Aliphatic II 60–90 ppm	Aromatic 90–160 ppm	Carboxyl 160–190 ppm	Ketone 190–220 ppm
Yakima River (at Kiona)	FA	34.2	12.8	30.1	19.3	3.6
	HPIA	32.0	18.0	23.4	22.8	3.8
Yakima River (at Cle Elum)	FA	33.7	13.8	33.1	17.2	4.1
	HPIA	30.52	20.5	22.1	21.0	5.8
Bemidji groundwater	FA	55	7	19	15	3
(contaminated)	HPIA	43	19	13	19	6

Sodium is incorporated into the sample due to incomplete ion exchange during the hydrogen saturation step. Dissolved silicic acid present in the water sample can interact directly with both organic acid fractions by hydrogen bonding, and can also interact with the resins. The aqueous solubility of silicic acid is strongly pH dependent, and it is expected that at pH 2 silicic acid can interact directly with resins. The solubility of silicic acid is greatly enhanced at high pH resulting in coelution of silicic acid with the organic acid fractions with an alkaline eluent. It has been noted that silicic acid behaves in a similar fashion to the hydrophilic organic acids, in particular, and is difficult to separate from them (Leenheer *et al.*, 1987).

Titration data have also been obtained for the Yakima River samples (Table 5). The titration curves (not shown) for the FA and hydrophilic acid fractions are similar in shape. However, the hydrophilic acid fraction, with a greater oxygen content, also has a greater amount of carboxyl functional groups compared to the fulvic acid as determined by titration. Further analysis of the titration data for the Kiona sample indicates that the apparent  $\text{pK}$  ( $\text{pK}_{\text{app}}$ ) for the hydrophilic acid fraction is less than for the fulvic acid.

More detailed structural differences between the two fractions are apparent in the quantitative solution state  $^{13}\text{C}$  NMR spectra for the Yakima River (Kiona) samples presented in Fig. 3. Each spectrum is comprised of the five major bands characteristic of humic substances. General assignments for these major bands are as follows.

- (1) Aliphatic I (0–60 ppm)—primarily  $sp^3$  hybridized carbons bonded to other carbons.
- (2) Aliphatic II (60–90 ppm)—hetero-aliphatic carbons, primarily  $sp^3$  hybridized carbons bonded to oxygens, including ether, alcohol, and carbohydrate carbons.
- (3) Aromatic (90–160 ppm)—primarily aromatic and olefinic carbons.
- (4) Carboxyl (160–190 ppm)—primarily carboxylic acid carbons.
- (5) Ketone (190–220 ppm)—ketone carbons.

The broad-banded nature of the spectra indicate that both fractions are complex mixtures. In all samples (Table 6), the hydrophilic acids have greater carboxyl, aliphatic II and ketone carbon contents than

the corresponding fulvic acids. For the Yakima River (Kiona) sample, approx. 23% of the carbon in the hydrophilic acid fraction is carboxyl carbon, compared to 19% for the fulvic acid. This data supports the titration data presented in Table 5. With respect to aliphatic I carbon, the two fractions are similar, with the hydrophilic acid fraction having slightly less (32%) than the fulvic acid (34%). Major differences between the two fractions are apparent in the aromatic and aliphatic-II regions. The fulvic acid has a greater amount of aromatic carbon (30%) and a lesser amount of aliphatic II carbon (about 13%) than the hydrophilic acid fraction (23 and 18%, respectively). From the  $^{13}\text{C}$  NMR data, it is apparent that the major structural differences between the two fractions are that the hydrophilic acid fraction is less aromatic than the fulvic acid, and has a greater amount of carboxyl and heteroaliphatic carbon.

#### SUMMARY

A goal of isolating different fractions of DOC from aqueous environments using preparative chromatography is to separate distinct components from the more complicated chemical matrix and to obtain sufficient amounts of these fractions for subsequent analysis. As demonstrated by the comparison of characterization data for the hydrophilic acids and fulvic acids isolated from a variety of environments, this goal has been met by using the XAD-8/XAD-4 array. Compared with the hydrophobic acid fraction, the hydrophilic acids are lower molecular weight, with greater heteroatom and carboxyl content. However, there appear to be a number of similarities between the two fractions, with the hydrophilic acids appearing to be rather humic-like in nature. To date, this large fraction (as much as 25% of the DOC in some samples) of humic-like compounds has been largely ignored in the study of aquatic humic substances, due, in large part, to problems associated with the efficient isolation of this material. Given the greater heteroatom and carboxyl content relative to the hydrophobic acid fraction, these compounds may be of considerable geochemical significance, playing an important role in such processes as metal binding, mineral weathering, and water acidification.

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