NOM Hydrophobicity

As performed at the University of Massachusetts, Environmental Engineering Research Laboratory

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Prepared by:
David A. Reckhow 413-545-5392
18 Marston Hall reckhow@ecs.umass.edu
University of Massachusetts
Amherst, MA 01003

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Standard Operating Procedures

NOM Hydrophobicity

This guidance document was prepared to assist research assistants, post-docs and lab technicians in conducting laboratory characterization of natural aquatic organic matter (NOM) with special focus on hydrophobic behavior. These tests are adapted to the particular resources and layout of the UMass Environmental Engineering research laboratories. It aspires to outline our standard operating procedures, as they exist at the present time. It also emphasizes elements of quality control that are necessary to assure high quality data. Please help me keep this document current by alerting me to any long-term changes in methodology or equipment.

Dave Reckhow

Faculty QC officer for NOM Hydrophobicity

Background

Natural organic matter (NOM) plays a very important role in the geochemical, ecological and engineered treatment systems. Dissolved Organic Matter (DOM) is that fraction of the NOM that passes through a standard glass fiber filter (nominal pore size is typically 0.45 um). NOM is especially important to water treatment engineers because it reacts with disinfectants such as chlorine, during the treatment process, and produces disinfection by-products such as trihalomethanes (THMs) and haloacetic acids (HAAs).

Dissolved organic carbon (DOC), the carbon contained in DOM, is often characterized by its hydrophobic properties using one of several resin adsorption protocols. The two-resin method of Aiken et al. (1992) is probably the most widely used of these protocols, and it results in three fractions: hydrophobic, mesophilic or transphilic, and hydrophilic. The hydrophobic fraction is that material retained by an XAD-8 resin column at low pH, the mesophilic (or transphilic) fraction is that which adsorbs to an XAD-4 column and the hydrophilic fraction is unretained. Data from the literature indicate that most raw waters have a preponderance of hydrophobic carbon, with smaller amounts of mesophilic and hydrophilic (see Table 1).

Table 1. Raw Water Fractions Based on DOC

Study	Hydrophobic	Mesophilic	Hydrophilic	Notes
Croue et al., 1993	41-56%	16-25%	21-34%	
Hwang et al., 2001	50-62%	18-24%	14-32%	k'=5
Goslan et al., 2002	61-79%	1-8%	13-34%	Used XAD-7
Labouyrie-Rouillier, 1997	45-56%	6-17%	27-42%	2 French Rivers
Croue et al., 1999	51-60%	18-27%	19-30%	5 samples
Hua & Reckhow, 2005	44-57%	23-29%	20-28%	3 samples

The hydrophobic fraction comprises fulvic and humic acids. These are strongly aromatic compounds with high specific UV absorbances (SUVA), and high specific disinfection byproduct (DBP) formation. Typically, the hydrophobic fraction is better removed during coagulation than the mesophilic or the hydrophilic fractions (Croue et al., 1993; Marahaba and Van, 2000). This fraction is also associated with soil organic matter, and residues from woody plant tissues. In contrast the hydrophilic fraction is more commonly associated with algal activity or aquogenic compound resulting from primary productivity.

Organic matter in water can also be characterized based on its nominal molecular weight. This can be accomplished by performing ultrafiltration using membranes that have a specific nominal molecular weight cutoffs. An alternative approach is to use size exclusion chromatography (SEC), or its modern adaptation to HPLC (HPSEC). In general, the higher the molecular weight of NOM, the more hydrophobic or humic-like it is (Mousset et al. 1997). However, the highest molecular weight material in NOM may be polysaccharidic (sugars and starches) and soluble only because of its high oxygen content and corresponding high level of hydrogen bonding. Natural organic matter with low molecular weight (few hundred to 1000 daltons) usually has a lower nitrogen concentration, by a factor of 2 to 3.5, relative to the higher molecular weight fraction (Rainer & Benner, 1996; Egeberg et al. 1998). Occasionally, high molecular weight NOM in water undergoes coagulation and sedimentation, naturally, at the source and settles leaving lower molecular weight NOM to make up most of the measured natural organic matter (Aouabed et al., 2001). THM modeling done by a group of researchers has shown that the THM yield coefficients increased when the NOM molecular weight decreased (Gang et al., 2002).

Affinity for Resin

The fundamental partition coefficient (K_P) is defined as the ration of the concentration on the solid surface (C_S; moles/cm^2 surface area) to the equilibrium water concentration (C_W; moles/liter of water). This is a fixed value for any particular solute (considering differences in protonation) and any particular resin.

$$K_P \equiv \frac{C_S}{C_W}$$

This is related to the capacity factor (k') with is defined as the ration of the mass of solute on the resin to the mass of solute dissolved in the water that resides in the column void volume. It can be expressed as follows:

$$k' = \frac{C_S A_R (1-\varphi)}{C_W \varphi}$$

Where A_R is the specific surface area of the resin (e.g., in m^2/m^3) and φ is the resin porosity (unitless). Substituting in for the partition coefficient, one gets:

$$k' = K_P \frac{A_R (1-\varphi)}{\varphi}$$

Thus, the k', like the K_P, is a fixed value for a given solute and a given resin, as long as the porosity and specific surface area are a constant. A small number of k' values have been determined experimentally assuming linear partitioning (Thurman and Malcolm, 1978). Comparison of these data show that they correlate well with published octanol-water partition coefficients (from Sangster, 1989; see Figure 1).

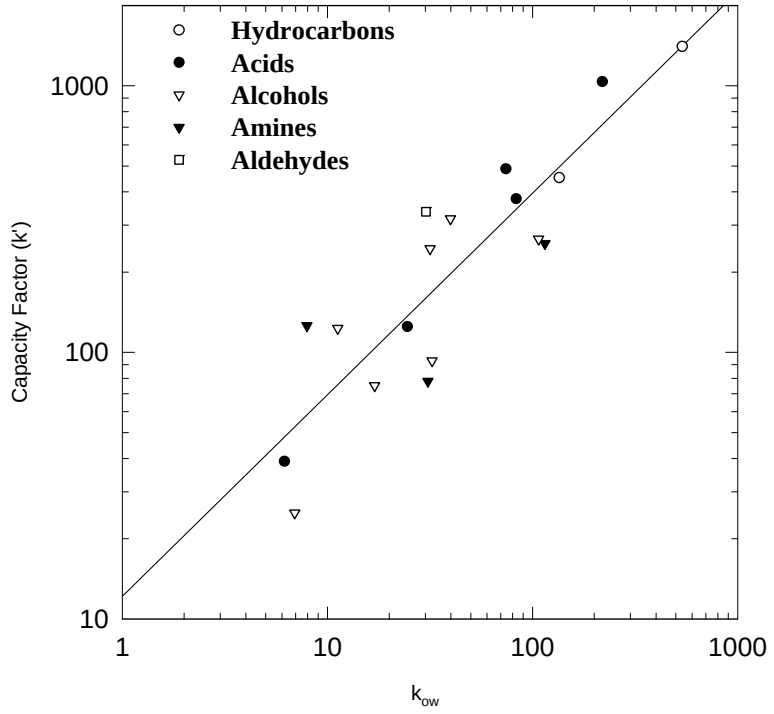


Fig. 1 Relationship between XAD Resin and Octanol Partitioning

Column Capacity

Once the resin is packed into a liquid chromatographic (LC) column, resin volume, surface area and porosity are fixed. At this point the only operational parameters left are sample volume (V) and flow rate (Q). Presuming good plug flow through the

column, solutes will have exhibit near ideal frontal chromatrogaphy as in Figure 1. For each solute there is a "breakthrough" sample volume (or breakthrough effluent volume, V_E), sometimes called the elution volume where the frontal wave of saturated pore water just reaches the column exit. Chromatographic theory holds that the elution volume is related to the void volume (V_0) and the capacity factor as follows:

$$V_E = V_0(1 + k')$$

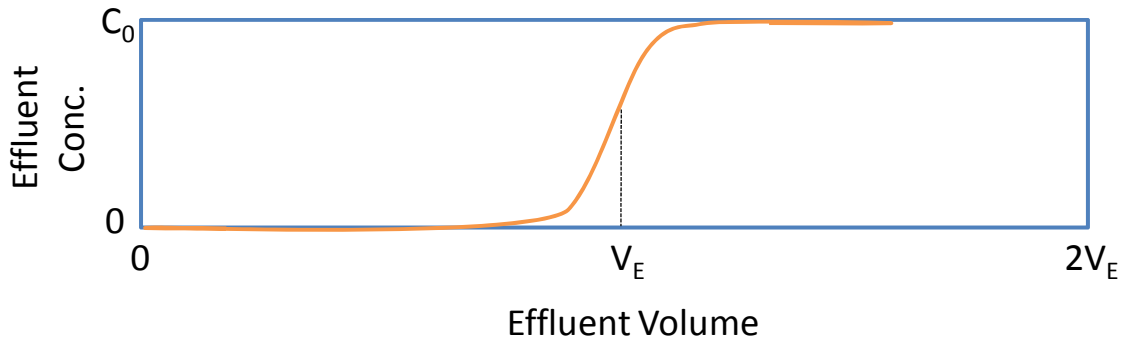


Figure 1. Frontal Chromatography Breakthrough Curve (redrawn from Leenheer, 1981)

In an ideal column, the solute will not appear in the effluent until this point, and reaches the influent concentration (C_0) very quickly. This at two times the breakthrough volume, half of the solute is in the effluent water and half still resides on the resin surface. So the volume to 50% retention ($V_{0.5r}$) is just twice the elution volume and:

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

Since the void volume is a simple function of the total column volume (V_C, only up to the top of the resin) and the porosity

$$V_0 = V_C\varphi$$

$$V_{0.5r} = 2V_C(\varphi + K_P A_R(1 - \varphi))$$

Which approaches the following for well retained solutes:

$$V_{0.5r} \cong 2V_C K_P A_R(1 - \varphi)$$

Leenheer (1981) reports that XAD-8 columns have a void volume (porosity) of about 65%.

Scope

This method has been used in the UMass Environmental Engineering Laboratory for laboratory analysis of NOM aimed at assessing its hydrophobic behavior. It has been found to meet data quality criteria with all raw and treated drinking for which it has been tested. This method should not be used for other media without further validation.

Method Overview

Non-ionic resin fractionation by XAD resin adsorption chromatography is used to determine the DOC distribution of operationally defined hydrophobic, mesophilic (or transphilic) and hydrophilic DOC fractions. The methodology is scaled down from the design employed by Aiken et al. (1992). Two sequential columns containing XAD-8 and XAD-4 resins are used to adsorb (the column distribution coefficient, $k'_{0.5r}$, is set equal to 50 for both XAD-8 and XAD-4 resins, $V_{0.5r} = 2V_0(1+k'_{0.5r})$ with V_0 : Void volume) hydrophobic and transphilic (or mesophilic) DOC, respectively. The XAD-8 resin is an acrylic ester polymer and the XAD-4 resin is a styrene divinylbenzene copolymer. Hydrochloric acid is used to acidify samples to pH ~ 2 prior to application to the columns. Acidified samples are first passed through a column containing XAD-8 resin at an approximate flow rate of 3 mL/min, and then subsequently passed through an additional column containing XAD-4 resin at the same flow rate. DOC measurements of influents and effluents of columns are used to perform a carbon mass balance, which yields hydrophobic, mesophilic and hydrophilic DOC fractions. Hydrophobic DOC are compounds that adsorb onto XAD-8 resin, mesophilic DOC are compounds that adsorb onto XAD-4 resin but not onto XAD-8, and hydrophilic DOC are compounds that pass through both columns. Columns are desorbed with 0.1 N NaOH following each test. The eluate is typically analyzed as an estimate of recoverable phobic and meso NOM.

Reproduced below is a simple, step-by-step outline of our NOM Hydrophobicity method for quick reference.

Table 2: Summary of Procedure for NOM Hydrophobicity; double resin test

1. **Prepare apparatus and labware (clean resin, bottles, blanks)**
2. **Acidify Sample & collect representative subsample**
3. **Apply sample to XAD-8 Column**
4. **Collect representative sample of XAD-8 effluent**
5. **Apply XAD-8 effluent to XAD-4 column**
6. **Collect representative sample of XAD-4 effluent**
7. **Back elute both columns separately with NaOH**
8. **Collect representative samples of the eluents & acidify to pH <3 with H_3PO_4**
9. **Analyze all 5 samples for TOC, and UV absorbance**
10. **Perform calculations and mass balance checks**

Detailed Procedures

Basis for Method

There is no single, generally-accepted method for determining hydrophobicity in aquatic organic matter. Nevertheless there is significant precedent in the literature.

Elements of the well-established hydrophobic resin methods of Aiken and co-workers (1992), and Malcolm and MacCarthy (1992) can be found in this SOP. The Aiken publication is included in the appendix of this document. It should be consulted by the analyst prior to running a hydrophobic test for the first time.

There have been a number of modifications of Aiken's method in the recent literature. Some have used alternative resins (e.g., XAD-7HP in place of XAD-8) when availability has become a problem (Goslan et al., 2002).

UMass Detailed Procedures

Sample Volumes and Preparation of Resin Columns

Elements of the hydrophobicity test procedure and equipment used will depend on the sample size to be analyzed. This decision itself depends on the experimental objectives (types of data desired). In most cases, a sample size of 200 mL will be used.

1. **Decide on analyses to be performed on the fractionated sample**
   - Volumes needed per sample can be determined as follows:

Test	Volume needed per replicate (mL)		Typical # replicates
	Typical	Minimum	
TOC	25 mL	10 mL	2
UV abs.	10 mL	5 mL	2
Chlorine residual	100 for fixed resid. test 20-50 for fixed dose test	25 for fixed resid. test 5-10 for fixed dose test	1
THMs	40[1]	10	2
HAAs	30	15	2
TOX	50-100	25	2

   - Typical volume of 200 mL permits analysis of TOC and UV absorbance only
   - Larger volumes may be used, but they require use of higher volume resin columns
   - Smaller volumes may be used, and these may require special considerations regarding resin columns, flow rate, etc.

2. **Decide on resin volumes needed**
   a) Most tests will use "micro" setup which involves 300 mL sample volumes, and as a result , 5 mL resin volumes. These can be used with the 10 mm glass columns. These columns, and associated pump and tubing are currently located in the last bay in Room 301.
   b) Other sample volumes may require larger or smaller resin volumes and LC columns.
      - We have a "mini" set of columns and resins (15 mm columns with about 10 mL of resin)

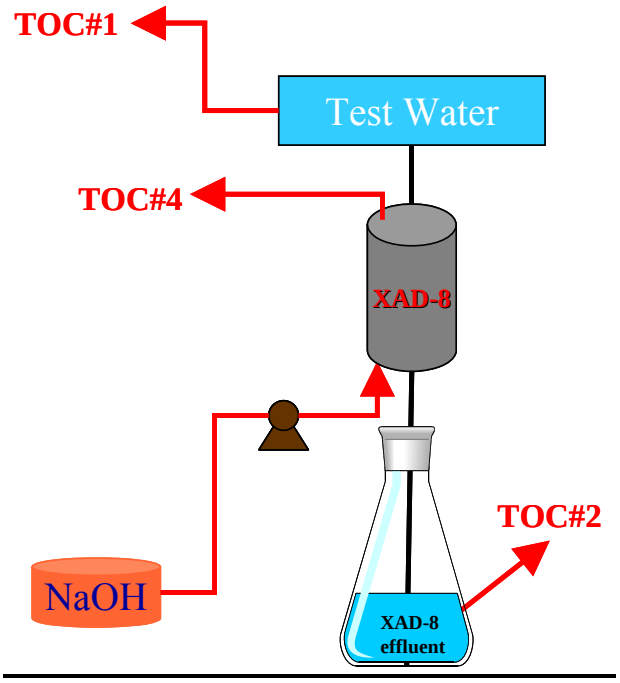
[1] this is needed to fill a 40-mL vial headspace-free, although only 20 mL of this are used for THM analysis.

- We also have the "macro" columns (2000 mL) in room 308

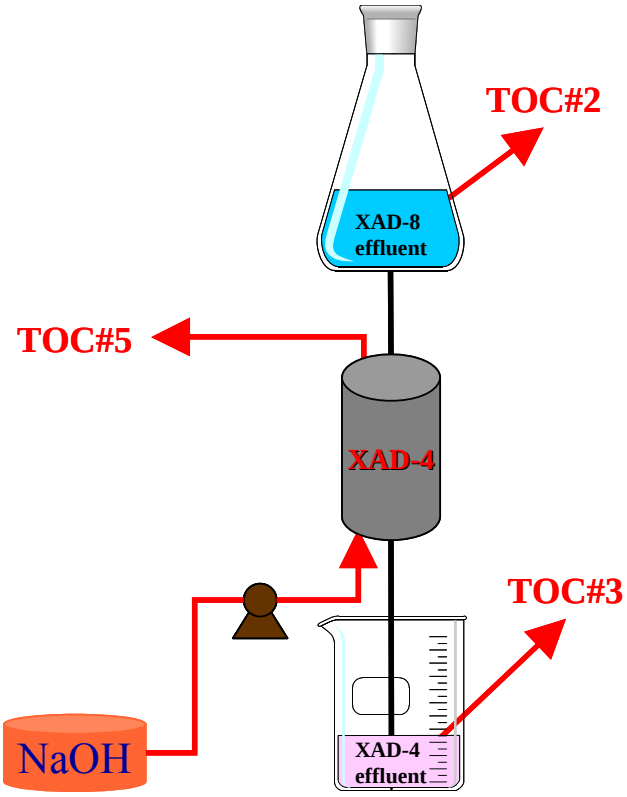
3. **Prepare resin and LC column**
   - Back elute resin columns separately with about 10 mL 0.1 N NaOH
   - Collect eluent until UV absorbance drops below 0.010 cm^{-1}.
   - Re-acidify columns by applying 3 bed volumes of pH 2 super-Q water (adjusted with H_3PO_4) in the downward direction. Check UV absorbance of eluent versus feed water (i.e., the pH 2 Super-Q water). The difference should be <0.010 cm^{-1}.

Detailed Test Procedures

1. **Fill feed reservoir with sample and acidify**
   a. **Usually 300 mL for "micro" setup**
   b. **Add 1 mL of 1M HCl per 100 mL of sample**
      i. **For 'micro" it is 3 mL for a 300 mL sample**
   c. **Remove about 40 mL of this for UV & TOC analysis**
2. **Pump though first column (XAD-8)**
   a. **First pump about 20-25 mL to waste (about 7 min @ 3.3 mL/min)**
   b. **Switch valve and pump through XAD-8 column**
   c. **Stop as reservoir volume drops to just a few mLs**
3. **Collect effluent sample from first column for analysis**
   a. **Usually about 40 mL for TOC and UV abs**
   b. **Need to have about 195 mL left for XAD-4 separation**
4. **Elute NOM from first column (XAD-8) with NaOH solution**
   a. **Back elute to waste at first**
      i. **Micro: About 12 mL or 4 min @ 3.1 mL/min**
   b. **Switch to collection**
      i. **Eluate #1: first 10 mL or ~3min at 3.1 ml/min – this starts just before the initial wave of organics comes throught and contains most of the NOM**
      ii. **Eluate #2: second 10 mL or ~3min at 3.1 ml/min**



5. **Pump effluent through second column (XAD-4)**
    a. **First pump about 20-25 mL to waste (about 7 min @ 3.3 mL/min)**
    b. **Switch valve and pump through XAD-4 column**
    c. **Stop as reservoir volume drops to just a few mLs**
6. **Collect effluent sample from second column for analysis**
    a. **This time collect and save entire volume**
7. **Elute NOM from second column (XAD-4) with NaOH solution**
    a. **Back elute to waste at first**
        i. **Micro: About 12 mL or 4 min @ 3.1 mL/min**
    b. **Switch to collection**
        i. **Eluate #1: first 10 mL or ~3min at 3.1 ml/min – this starts just before the initial wave of organics comes throught and contains most of the NOM**
        ii. **Eluate #2: second 10 mL or ~3min at 3.1 ml/min**



XAD-8 Adsorption

starting volume =	300	mL		
sample volume =	40	mL	for TOC & Uvabs	
remaining =	260	mL		
		time @	3.1	mL/min
Target applied volume =	255	mL =	82	min
initial to waste =	22	mL =	7	min
volume collected =	233	mL =	75	min

XAD-8 Elution	@	3.1	mL/min
back elution to waste =	12 mL =	4	min
collection of eluate =	20 mL =	6	min
regeneration volume =	40 mL =	13	min

XAD-4 Adsorption

starting volume =	233	mL	
sample volume =	40	mL	for TOC & Uvabs
remaining =	193	mL	
		time @	3.1 mL/min
Target applied volume =	167	mL =	54 min
initial to waste =	22	mL =	7 min
volume collected =	145	mL =	47 min

XAD-4 Elution	@	3.1	mL/min
back elution to waste =	12	mL =	4 min
collection of eluate =	20	mL =	6 min
regeneration volume =	40	mL =	13 min

Sample Preservation

1. **Refrigeration.**
   - Place samples for hydrophobicity testing in a clean refrigerator designated for storage of drinking water samples
   -

2. **Acidification**
   - Add 1 mL of 1M HCl to each 100 mL of water sample using a borosilicate pipette (301 Elab II; bay F)

3. **Addition of biocide**
   - May be required of some protocols

Data Analysis & QC Reporting

1. **Data Analysis begins with assessment of raw sample.**
   - Measure raw sample TOC and UV absorbance
   -

2. **Lab Water Blanks**
   At least one in every 10 samples must be a laboratory blank. Results of lab water blanks and any proposed corrective action must be reported by email to the graduate QC officer or his/her designee if he/she is not available.

   - The message must also include the address of the Faculty QC officer in the "cc:" line (reckhow@ecs.umass.edu).
   - The subject line of this email message must simply read "QC report for hydrophobicity"

3. **Final Documentation of Hydrophobicity QC**
   - The graduate QC officer or his/her designee then must send an email message to the faculty QC officer stating whether the QC data are within control limits, and if they are not, what actions will be taken.
     - Again, the subject line of this email message must simply read "QC report for hydrophobicity".
     - This must be done as soon as possible, but no later than 24 hours from the time of receipt of the analyst's QC report (per instructions on Lab Water Blanks above).

4. **Evaluate all other QC data**
   - This must be done as soon as possible, but no later than 24 hours from the end of the analytical run. Send an email report in accordance with the analytical SOP.

Data Interpretation

Calculation of NOM fractionation is quite simple mathematically. In principle it is just the difference between the various initial, intermediate and final measurements.

Standard Solutions, Solvents and Supplies

Solutions

Sample Bottles and other labware

All glassware must be rendered free from contamination that might interfrer with this test. In many cases, they are also used for subsequent analysis of DBPs, and must therefore be free from trace halogenated contaminants.

Cleaning of bottles and other glassware

See general laboratory SOP for glassware cleaning. Final cleaning is as:

a) Acid wash by soaking in a covered acid bath[2]
b) rinse thoroughly with Super-Q water
c) place overnight in a covered chlorine bath (when FP tests are to follow)
d) rinse thoroughly with Super-Q water
e) dry in a high-temperature oven.[3]

Cleaning of septa

- Septa must be washed with detergent, rinsed with reagent water, wrapped in aluminum foil and dried in 100ºC oven.

Supplies

Item	Catalog #	Approx. Price	Approx # used/run[4]
Pasteur Pipettes	Fisher: 13-678-20A	720/ $46.10	10
DIUF Water	Fisher: W2-20	$32.29	Not normally used
H_2SO_4	UMass Stockroom		

[2] may substitute overnight detergent (e.g, Fisher FL-70, 4%) soak
[3] preferably at 140 C or higher
[4] Assuming about 10 samples analyzed

Quality Assurance/Quality Control

General Approach

Quality assurance is an essential and integral part of a research study. The purpose of any QA plan is to insure that valid and reliable procedures are used in collecting and processing research data. The procedures outlined are designed to eliminate or reduce errors in experiments, sample preparation and handling, and analytical methods. Attention must be paid throughout one's lab work to incorporating the QA plan into all ongoing research projects.

Any equipment and experimental procedures that are used to provide numerical data must be calibrated to the accuracy requirements for its use. Records are to be kept of all calibrations. Calibration schedules are generally established for all aspects of physical and chemical measurements and these must be strictly followed. Physical standards and measuring devices must have currently valid calibrations, traceable to national standards. Most chemical standards are acquired from commercial suppliers, and they should be of the highest purity available. When necessary, standards unavailable from commercial suppliers should be synthesized using the best methodology available.

As a general rule, experiments should be replicated to assure reproducibility. All data reported should include a statement of its uncertainty, and the means for the determination and assignment of such limits. Standard reference materials are used for this purpose where possible. Statistically established confidence limits and an analysis of sources of systematic error are to be used in the absence of experimental demonstration of limits of inaccuracy.

All data will be subject to review by the faculty QC officer before being formally accepted. The analysts involved will certify reports as well as all who review them. All analysts and QC officers must attest that the data and associated information contained in the report are believed to be correct and that all quality assurance requirements have been fulfilled, unless exceptions are approved and noted. Careful and detailed laboratory records will be maintained by each analyst, including source of reagents, meticulously detailed procedures (referring to a traceable SOP, and any departures or clarifications), instrumentation and conditions of analysis, failed experiments, etc.

Regular meetings will be held to review the results and project progress, and to plan further experiments. The results will be analyzed promptly and summarized by means of internal reports or formal reports for external distribution. The experimental and analytical procedures will be reviewed for their performances and changes will be made as necessary. The quality assurance program as described in this document must be strictly observed.

Quality Assurance Objectives

Precision, accuracy and repeatability are evaluated to the extent possible, and where there are existing protocols, held within the control limits set forth in the accepted references (e.g.,APHA et al., 1999; USEPA-EMSL, 1990; ASTM, 1994). In addition to the analysis of sample replicates, a minimum of 10 percent of the time is typically involved in experimentation that is devoted to quality control. The precision or reproducibility of each process test is determined through analysis of sample replicates. These are commonly presented in the form of control charts (e.g. Section 1020B of APHA et al., 1999).

Process tests generally involve a measurable outcome (e.g., chemical demand, dose vs. response relationship). The accuracy of some process tests can be determined by testing samples that have been fortified with a standard having a known and measurable influence on the test. Recovery is then calculated as the incremental effect of the presence of this standard as compared to the sample when it is absent. The recovery will be calculated and will be considered acceptable if it falls within the control limits determined for the particular test. For new methods developed at UMass or for modifications of existing methods, we will have to establish criteria on acceptable control limits. Where possible, external performance standards will also be run. This serves as a measure of accuracy both for the analysis and for standard preparation. When this is not possible or practical, independently prepared standards will be used instead (e.g., standards prepared by different analysts at different times using different reagents & equipment. These are sometimes referred to as "calibration check" standards).

Data generated by the QA program will be incorporated into a Quality Control (QC) archive that is used to monitor the fluctuations in precision and accuracy so that chance or assignable causes of error can be determined. Control charts such as X-charts for simple successive samples or cumulative sum techniques may be employed to record both precision and accuracy data (Taylor, 1987).

General Procedures

General sample collection and handling will be in accordance with the guidelines of Section 1060 of Standard Methods (APHA et al., 1999). All previously established analytical methods used in laboratory research will follow approved methods in the standard compilations (e.g., ,APHA et al., 1999; USEPA-EMSL, 1990, or ASTM, 1994).

Reagent grade chemicals or higher quality when needed will be used throughout the research. Laboratory-grade water (purified by reverse osmosis, deionization, and carbon adsorption) will be used for preparation of reagents, sample blanks, and dilution water. Where necessary, this water will be further purified using batch UV irradiation. All glassware used in the experiments and in analytical analyses will be thoroughly cleaned with a sequence of detergent wash, acid soak, and extensive rinsing to prevent interferences from trace contaminants. Where necessary, degreasing and oxidative treatments (persulfate) will precede the standard washing protocol

Procedures specific to NOM Characterization Tests

General QC

Data quality objectives for NOM characterization is assured by: (1) use of blanks; (2) analysis of duplicates; and (3) analysis of a matrix standard. Two types of blanks should be run with each set of samples: (1) laboratory water blanks; and (2) field blanks. The first blank is of critical importance to resin-based analytical methods. This is because all resins will bleed monomer to some extent. It is therefore, extremely important that laboratory grade water be run through the entire fractionation procedure with each set of samples to establish the extent of monomer bleed. The DI column effluents are analyzed for TOC in the same way that the samples are, and these data provide the best estimate of background TOC that must then be subtracted from the sample values. This second type of blank is prepared by transporting laboratory reagent-grade water to the study site, and transferring it to a labeled sample vial at the time of general sample collection. In most laboratory experimentation, the laboratory water blank can also serve as a "field blank". Matrix standards (e.g., mix of known organic compounds) are prepared and analyzed by each new research assistant, post-doc or technician. We prefer to use a mixture of cinnamic acid and synapic acid as a highly reliable matrix standard solution.

This outlines our general QA philosophy for characterization and process tests. Many specific details relating to the individual procedures may be found in the cited references, and other particulars will have to be adopted as new methods are developed.

Many types of QC procedures are required as indicated in the preceding text. The guidelines below are prepared assuming that samples are run in groups, whereby a "daily" frequency refers to once every day that the analytical method is being used.

Table 7. Summary of QC Elements as Applied to NOM Characterization Tests

Types of Samples or Standards	Purpose	Frequency	Timing	QC data
Laboratory Water Blank	Assess cleanliness of water, reagents, and glassware	1 for every 10 samples	Beginning of a new study and randomly thereafter	
Initial Demonstration of Capability (IDC)	To show that an analyst's technique and equipment are adequate for NOM characterization tests	One set of model compounds analyzed when first learning method, otherwise not		Match to expected fractionation

		done		
Field Reagent Blank (FRB)	Test all field conditions for interferents or contaminants	1 per set, if sampling occurred outside of the lab	mid day	
Spiked sample, or Laboratory Fortified Sample Matrix (LFM)	To test analyte recovery in the sample matrix	1 for every 10 samples	Depends on study objective	% recovery, mean and standard deviation
Unknowns or "samples"	This is what you really want to measure	As many as desired	Mixed throughout day	Reproducibility

Initial Demonstration of Capability (IDC)

This should be done whenever a new student or technician is first learning the procedure for NOM characterization. The analyst should record all details on solution preparation, fractionation and analysis in a permanent lab notebook. This should be done in such as way that it is understandable to other students and faculty.

1. **Conduct IDC lab experiment**
   a) Prepare 2 liters of a solution containing the following reagent-grade organic compounds in reagent-grade water and buffered at pH 7.0.
      - 0.1 mM Cinnamic acid.
      - 0.1 mM Synapic acid
      - 0.1 mM (maybe PEG?)
      - 0.1 mM ??

   b) Run this mixture through the standard resin fractionation scheme
   c) Collect the usual samples for analysis
      - Dissolved Organic Carbon
      - UV absorbance
      - Analysis of specific compounds by LC/MS

2. **Evaluate IDC data**
   - Report all data to the faculty QC officer (David Reckhow) in an MS excel spreadsheet
   - Include determination of DOC and UV absorbance
   - Calculate the "NOM fraction" abundances, based on DOC and UVabs

3. **Compare with data quality criteria**
   - The faculty QC officer will check the data and compare with the quality objectives for this tests
   - He will also consult the LC/MS data

- Depending on the results, you may be asked to re-do the test

QC Protocols after IDC

Table 8 shows a recommended sequence for a typical run of about 12 samples. The first two samples require immediate attention, as they are simple indicators of unacceptable QC. When these show abnormally elevated demands, the operator must intervene before proceeding. The problem must be diagnosed, solved and the sequence restarted at sample #1.

Table 8: Typical NOM Testing Sequence

Sample #	Sample type	QC objectives
1	Lab Water Blank	To check for gross contamination of water or lab environment, and establish background
2-5	**Analytical Samples**	
6	Lab Water Blank (or field blank)	Check DOC bleed from resins
7-10	**Analytical Samples**	
11	Lab Water Blank (or field blank)	Check DOC bleed from resins
12-15	**Analytical Samples**	
16	Lab Water Blank (or field blank)	Check DOC bleed from resins

Quality control data must be analyzed as soon as possible. The best practice is to have the QC data tabulated and evaluated as the run is underway. However, it is recognized that there will be times when this is impossible (e.g., for some complex experiments). QC and calibration data must always be analyzed and reported within 24 hours of completion of a run (see section on Data Analysis & QC Reporting, page 14). Quantitative criteria (Table 9) must be applied, and violations must be immediately reported to the faculty QC officer. The graduate and faculty QC officer along with the analyst will then work out a plan for returning the analysis to acceptable levels of QC.

In several cases, quantitative criteria are based on long term trends, and these must be monitored by means of appropriate control charts. Laboratory water blanks and field blanks are documented over time in this way. All summarized QC data (tabular and graphical) must be kept in a notebook in the Elab II NOM lab (room 308). A duplicate set must be deposited with the faculty QC officer (D. Reckhow).

Table 9: Quantitative Criteria for Judging Data Acceptability

Types of Samples or Standards	Frequency	Timing	QC data Acceptance Criteria
			❖
Laboratory Water Blanks	1 for every 10 samples		❖ Average DOC value of demand ≤0.5 mg/L ❖ Max value ≤1 mg/L
Unknowns or "samples"	As many as desired	Mixed throughout day	❖ Replicates = ±10%

Sampling Custody

In most cases analyses will be performed immediately upon return from the field or after preparation of samples in the laboratory. Problems with sample custody are minimized, because the same people who receive (or sometimes, collect) the samples also analyze them. In general sample collection, handling, and preservation will be in accordance with the guidelines of Section 1060 of Standard Methods (APHA et al., 1999). All samples must be fully labeled with the sample identity, date, and name of researcher.

Sample Collection and Storage

Samples are collected and stored in clean borosilicate (e.g., Pyrex, Kimax) glass containers. Containers must be capped with either Teflon-lined septa or ground glass stoppers. Exceptions are made for large volume samples which may be stored and shipped in clean polyethylene carboys. Glass containers are cleaned with detergent, followed by 5% sulfuric acid soak, and final rinsing with reagent-grade water. These containers are dried in a 150 C oven.

Samples for NOM characterization and subsequent analysis must be kept in the dark, and in a refrigerator from the time of fractionation until the start of analysis. Some NOM consituents are biodegradable, so a biocide must be added if the samples are to be kept for more than 2 days prior to analysis.

Handling and Storage of Standards and Reagents

Data Reduction, Validation and Reporting

To ensure the accuracy and permanency of collected data, all research data are recorded with permanent ink in bound notebooks and all QC data (precision, accuracy) are recorded in instrument log notebooks. Summary QC graphs and tables are reviewed at least quarterly by the Faculty QC officer to observe noteworthy trends or inconsistencies. These are maintained in loose leaf notebooks for subsequent use in preparing progress reports, final reports, and theses. Major concerns and conclusions are reported to the external Project Officer via the progress reports.

Pages from the laboratory data books are regularly duplicated so that a file copy of raw data can be placed in safe storage in the event that the book is lost or destroyed. At the end of the project, all bound data books and any loose leaf data will be stored by the project team for at least ten years. Summary data files will be put on magnetic media so that statistical analysis of the data can be done. Our laboratory has several personal computers that can be used for this purpose.

References

Aiken, G.R., Mcknight, D.M., Thorn, K.A., and Thurman, E.M. (1992) Isolation of Hydrophilic Organic-Acids From Water Using Nonionic Macroporous Resins. *Organic Geochemistry* **18** (4), 567-573.

Aiken, George R., Thurman, E. Michael, Malcolm, Ronald L., and Walton, Harold F. Comparison of XAD Macroporous Resins for the Concentration of Fulvic Acid from Aqueous Solution. Analytical Chemistry 51[11], 1799-1803. 1979.

Belin, C, Quellec, C., Labotte, M, Ewald, Marc, and Simon, P. Characterization by Fluorescence of the Dissolved Organic Matter in Naturual Water. Application to Fractions Obtained by Tangential Ultrafiltration and XAD Resin Isolation. Environmental Technology 14, 1131-1144. 1993.

Croué, J.P, Lefebvre, E, Martin, B and Legube B. (1993) Removal of Dissolved Hydrophobic and Hydrophilic Organic Substances during Coagulation/ Flocculation of Surface Waters. *Water Science and Technology* 27(11): 143-152.

Croue et al., 1999 [in Formation and Control of DBPs in Drinking Water, PC Singer, ed.]

Goslan, E.H., Fearing, D.A., Banks, J., Wilson, D., Hills, P., Campbell, A.T., and Parsons, S.A. (2002) Seasonal Variations in the Disinfection by-Product Precursor Profile of a Reservoir Water. *Journal of Water Supply Research and Technology-Aqua* **51** (8), 475-482.

Hautala, K., Peuravuori, J., and Pihlaja, K. (1997) Estimation of Origin of Lignin in Humic Dom by Cuo-Oxidation. *Chemosphere* **35** (4), 809-817.

Hua, G. and Reckhow, D.A Proceedings, AWWA June 2005, San Francisco

Hwang, C.J. et al. (2001) Polar NOM: Characterization, DBPs, Treatment. Final project report, AWWA Research Foundation, Denver, CO.

Kaiser, K., Guggenberger, G., Haumaier, L., and Zech, W. (2001) Seasonal Variations in the Chemical Composition of Dissolved Organic Matter in Organic Forest Floor Layer Leachates of Old- Growth Scots Pine (Pinus Sylvestris L.) And European Beech (Fagus Sylvatica L.) Stands in Northeastern Bavaria, Germany. *Biogeochemistry* **55** (2), 103-143.

Labouyrie-Rouillier, 1997 [Poitiers dissertation]

Leenheer, Jerry A., Rostad, Colleen E., Barber, Larry B., Schroeder, Roy A., Anders, Robert, and Davisson, M. Lee. Nature and Chlorine Reactivity of Organic Constitutents from Relcaimed Water in Groundwater, Los Angeles County, California. Environmental Science and Technology 35[19], 3869-3876. 2001.

Malcolm, Ronald L. and MacCarthy, Patrick. Quantitative Evaluation of XAD-8 and XAD-4 Resins Used in Tandem for Removing Organic Solutes from Water. Environment International 18, 597-607. 1992.

Marhaba, T.F and Van, D. (2000) The Variations in Mass and Disinfection By-Product Formation Potential of Dissolved Organic Matter Fractions along a Conventional Surface Water Treatment Plant. *Journal of Hazardous Materials* 74(3): 133-147.

Maurice, P.A., Pullin, M.J., Cabaniss, S.E., Zhou, Q.H., Namjesnik-Dejanovic, K., and Aiken, G.R. (2002) A Comparison of Surface Water Natural Organic Matter in Raw Filtered Water Samples, Xad, and Reverse Osmosis Isolates. *Water Research* **36** (9), 2357-2371.

Sangster, J., Octanol-Water Partition Coefficients of Simple Organic Compounds, Journal of Physical and Chemical Reference Data, 1989, 18(3)1111-1229.

Thurman, E.M., R. L. Malcolm, G. R. Aiken, Prediction of Capacity Factors for Aqueous Organic Solutes Adsorbed on a Porous Acrylic Resin. Analytical Chemistry, 1978, 50 (6), 775-779

Appendix

Aiken et al., 1992

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Isolation of hydrophilic organic acids from water using nonionic macroporous resins

G. R. AIKEN,[1] D. M. MCKNIGHT,[1] K. A. THORN[1] and E. M. THURMAN[2]
[1]U.S. Geological Survey, MS 458, Box 25046, Denver Federal Center, Denver, CO 80225 and
[2]U.S. Geological Survey, 4821 Quail Crest Place, Lawrence, KS 66049, U.S.A.

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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. ^{13}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 zeotrophic 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 $k'_{cutoff} = 50$ 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 $k'_{cutoff} = 50$ 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_2O-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 nonsorbed solutes, and capacity factors (k') determined using the following equation:

k' = grams of solute on resin/
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 μ) 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 acidifed 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 ^{13}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_2O, and 180 mg of the fulvic acid in 2.0 ml D_2O, in 10 mm NMR tubes; the pH was adjusted to 7.0. The ^{13}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 ^{13}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

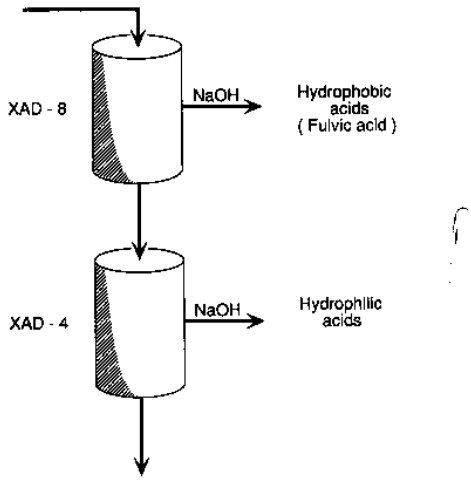

Fig. 1. Schematic diagram of the XAD-8/XAD-4 isolation scheme.

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^2/g)	Specific pore volume (cm^3/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
Low molecular weight					
p-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
p-Nitrophenol	—	—	1350	—	—
Butyric acid	—	—	196	—	39
High molecular weight					
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

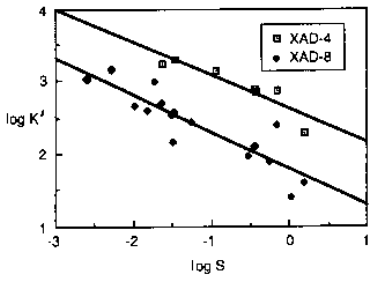

Fig. 2. Log k'–log solubility plots for low molecular weight solutes on XAD-4 and XAD-8.

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)

Sample	Fraction	C	H	O	N	S	Ash	Carboxyl content (meq/gm)	Molecular weight* (daltons)
		(ash free)							
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.

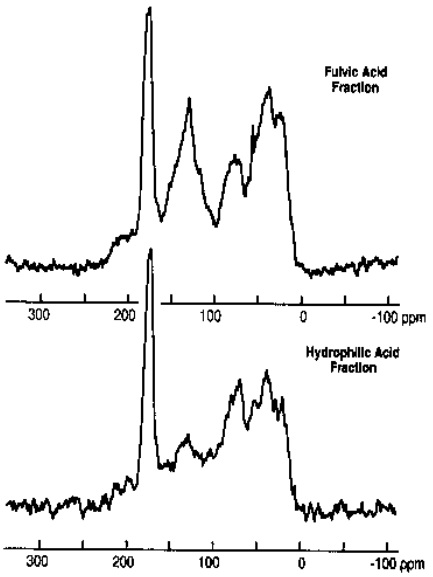

Fig. 3. ^{13}C-NMR spectra for fulvic acid and the hydrophilic acid fraction isolated from the Yakima River.

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Table 6. Peak areas as percentage of total spectrum area for quantitative ^{13}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 (contaminated)	FA	55	7	19	15	3
	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 pK (pK_{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}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}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.

REFERENCES

Aiken G. R. (1985) Isolation and concentration techniques for aquatic humic substances. In *Humic Substances in Soil, Sediment, and Water* (Edited by Aiken G. R., McKnight D. M., Wershaw R. L. and MacCarthy P.), pp. 363–385. Wiley, New York.

Aiken G. R. (1987) Isolation of organic acids from large volumes of water by adsorption chromatography. In *Proc. of the 1984 ACS symp. Concentration of Organic Solutes from Water for Biological Testing* (Edited by Suffet I. M. and Malaiyandi M.), pp. 295–307.

Aiken G. R. (1988) A critical evaluation of the use of macroporous resins for the isolation of aquatic humic substances. In *Humic Substances and Their Role in the Environment* (Edited by Frimmel F. H. and Christman R. F.), pp. 15–30.

Aiken G. R. and Malcolm R. L. (1987) Molecular weight of aquatic fulvic acids by vapor pressure osmometry. *Geochim. Cosmochim. Acta* **51,** 2177–2184.

Aiken G. R., Thurman E. M., Malcolm R. L. and Walton H. F. (1979) Comparison of XAD macroporous resins for the concentration of fulvic acid from aqueous solution. *Anal. Chem.* **51,** 1799–1803.

Gustafson R. L. and Paleos J. (1971) Interactions responsible for the selective adsorption of organics on organic surfaces. In *Organic Compounds in Aquatic Environments* (Edited by Faust S. F. and Hunter J. V.), pp. 213–238. Marcel Dekker, New York.

Huffman E. W. D. Jr. and Stuber H. A. (1985) Analytical methodology for elemental analysis of humic substances. In *Humic Substances in Soil, Sediment, and Water* (Edited by Aiken G. R., McKnight D. M. and MacCarthy P.), pp. 433–455. Wiley, New York.

Karger B. L., Grant J. R. Hartkopf A. and Weiner P. H. (1976) Hydrophobic effects in reversed-phase liquid chromatography. *J. Chromatogr.* **128,** 65–78.

Leenheer J. A. (1981) Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. *Environ. Sci. Technol.* **15,** 578–587.

Leenheer J. A., Brown P. A. and Stiles E. A. (1987) Isolation of nonvolatile, organic solutes from natural waters by zeotrophic distillation of water from *n,n*-dimethylformamide. *Anal. Chem.* **59,** 1313–1319.

Locke D. C. (1974) Selectivity in reversed-phase liquid chromatography using chemically bonded stationary phases. *J. Chromatogr. Sci.* **12,** 433–437.

Mantoura R. F. C. and Riley J. P. (1975) The analytical concentration of humic substances from natural waters. *Anal. Chim. Acta* **76,** 97–106.

Pietrzyk D. J. and Chu C. (1977) Amberlite XAD copolymers in reversed phase gravity flow and high pressure liquid chromatography. *Anal. Chem.* **49,** 757–763.

Shuman M. S. (1990) Carboxyl acidity of aquatic organic matter: possible systemic errors induced by XAD extraction. In *Organic Acids in Aquatic Ecosystems, Report of the Dahlem Workshop on Organic Acid in Aquatic Ecosystems*, Berlin 1989, 7–12 May (Edited by Perdue E. M. and Gjessing E. T.), pp. 97–109.

Thurman E. M. and Malcolm R. L. (1981) Preparative isolation of aquatic humic substances. *Environ. Sci. Technol.* **15,** 463–466.

Thurman E. M., Malcolm R. L. and Aiken G. R. (1978a) Prediction of capacity factors for aqueous organic solutes on a porous acrylic resin. *Anal. Chem.* **50,** 775–779.

Thurman E. M., Aiken G. R. and Malcolm R. L. (1978b) The use of macroreticular resins to preconcentrate trace organic acids from water. *Am. Chem. Soc., Proc. of the 4th Joint Conf. on the Sensing of Environmental Pollutants*, New Orleans, November 1977, 630–634.