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Optimization of Method for Detecting and Characterizing NOM by HPLC−Size Exclusion Chromatography with UV and On-Line DOC Detection

NAMGUK HER,*,† GARY AMY,† DAVID FOSS,‡ JAEWEON CHO,§ YEOMIN YOON,† AND PAUL KOSENKA||

Environmental Engineering, University of Colorado, ECOT 441, Boulder, Colorado 80309, Wright Water Engineers, Inc., Denver, Colorado 80211, Kwangju Institute of Science and Technology, Kwangju, Korea, 500-712, and Ionics-Sievers Instruments, Boulder, Colorado 80301

High-performance liquid chromatography (HPLC)−size exclusion chromatography (SEC) with ultraviolet absorbance (UVA) and on-line dissolved organic carbon (DOC) detectors has been adapted and optimized under various conditions. An enhanced HPSEC−UVA system employing a modified commercially available DOC detector provides a better understanding of the qualitative and quantitative natural organic matter (NOM) properties in water samples by detecting aromatic and nonaromatic fractions of NOM as a function of molecular weight (MW). The most critical merit of this system is that the DOC detector is readily available and widely used. With only a few modifications, a commercially available TOC analyzer served as a DOC detector, integrated with the HPSEC to measure DOC along with UVA, and provided a specific UVA (SUVA) chromatograph that is useful information for drinking water plant design and operation. Without preconcentration, samples can be analyzed with a small amount of sample, with a DOC detection limit as low as 0.1 mg/L (as DOC).

Introduction

Organic matter in water originates from both natural and anthropogenic sources. A major fraction of the NOM present in surface waters or groundwaters is composed of humic substances, which are complex macromolecular products of the chemical and biological degradation of plant and animal residues, including lignin, carbohydrates, and proteins (*1*). HPSEC has been widely used to characterize NOM based on differential permeation of molecules due to its rapidity and reproducibility (*2*).

Molecular weight (MW) and aromaticity of dissolved organic matter (DOM) are important water quality parameters for predicting DBP formation potentials (*3*) and membrane fouling potential during water treatment. Several methods are used to determine MW; gel permeation chromatography, ultrafiltration, small-angle X-ray scattering, and electron microscopy (*4*). Chin et al. (*5*) used an HPSEC technique with UVA detection to analyze MW distribution and to calculate weight- and number-averaged MW values and polydispersivity using a modified silica column (Waters Protein-Pak 125) and sodium polystyrene sulfonates (PSS). However, HPSEC−UVA systems provide limited information because UV absorbance cannot detect carbon single bonds.

Recently, aliphatic and partial aromatic compounds in NOM have been shown to be important in water treatment. For example, polysaccharides and proteins were found as major foulants in nanofiltration (*6*, *7*). The use of HPSEC with sequential UVA and DOC detectors overcomes the limitations of UVA because the DOC analyzer detects virtually all of the organic carbon in the sample. The relative difference between two detectors also provides additional qualitative information (aromatic vs aliphatic) in addition to MW. In their pioneering work, Huber and Frimmel (*8*, *9*) developed an HPSEC−UVA system with a highly sensitive on-line organic carbon detector (OCD). Even though their organic carbon detector showed high performance, it may not be easily applied due to its availability, size, and complexity.

The objective of this research was to evaluate, optimize, and apply a newly modified DOC detector to the HPSEC−UVA system for characterization of NOM from a wide range of water samples, including treatment process train profiles. MW profiles can help optimize the performance of ozonation, granular activated carbon (GAC), and membrane processes by determining which NOM fractions can be preferentially removed by each process.

Methods and Materials

Analytical Setup. Experiments were performed with a high-performance liquid chromatograph (HPLC, LC600 Shimadzu) with UVA (SPD-6A Shimadzu) and DOC (Modified Sievers Turbo total organic carbon analyzer) detectors, as shown in Figure 1. Helium gas was sparged into a mobile-phase (eluent) reservoir to eliminate inorganic carbon and dissolved oxygen that can cause interferences or react with the mobile or the stationary phases. The DOC detector was connected directly to the UVA detector waste line. UVA at 254 nm and DOC data over time were collected every 4 s by digital signal processing using a modified Labview software.

Detectors. A modified Sievers Turbo total organic carbon analyzer is an instrument used to detect dissolved organic matter through UV/persulfate oxidation. A conductivity detector is utilized to measure CO_2 gas produced. The merit of this instrument is to the ability to detect any type of carbon-bonded chemicals. Samples are first acidified by adding phosphoric acid (6 M H_3PO_4) to convert all inorganic carbon to CO_2 (eq 1), which is then removed by vacuum during inorganic carbon removal (ICR). Ammonium persulfate (15% $(NH_4)_2S_2O_8$) is then added to the sample to oxidize organic constituents in the presence of UV light (eq 2). The conductivity detector quantifies CO_2 that diffuses through a semi-selective membrane.

$$2H^+ + CO_3^{2-} \rightarrow H_2O + CO_2 \quad (1)$$

$$DOC \xrightarrow{S_2O_8^{2-},\ UV} CO_2 + H_2O \quad (2)$$

The reagents are added to the sample stream using microprocessor-controlled syringe pumps. Typically, phosphoric acid is added at a flow rate of 0.2−1.5 μL/min to reduce the pH of the sample stream to pH <2. The persulfate can

* Corresponding author phone: (303)735-2433; fax: (303)492-7317; e-mail: her@ucsub.colorado.edu.
† University of Colorado.
‡ Wright Water Engineers, Inc.
§ Kwangju Institute of Science and Technology.
|| Ionics-Sievers Instruments.

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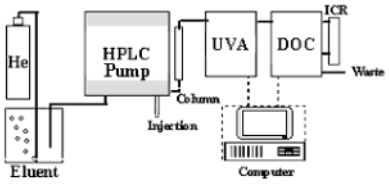

FIGURE 1. Schematic of HPLC−UVA/DOC.

TABLE 1. Characteristics of Tested Columns

type	column packing	particle size (μm)	separation range (Da)	column size (cm)
silica	Protein PAK 125	10	1000−30 000[a]	0.78 × 30
hydroxylated organic	TSK-50S	30	$<5 \times 10^6$	2 × 25
polyacrylamide	Bio-Gel P-6	90−180	1000−6000	0.5 × 90

[a] Random coil.

be added at a flow rate over 1.0 μL/min, depending on the DOC concentration of the sample.

The SPD-6A Shimadzu UVA is a sensitive photometer, which may be operated over a wide wavelength range. It exhibits high sensitivity and, in favorable cases, a few nanograms of a solute can be detected. Even though this UV detector is characterized by high sensitivity, good linearity, low detection limit, nondestruction of samples, and relatively small sample necessity, UVA requires aromatic double bonds or similar chromophores for sample detection. The work reported herein is based on a wavelength of 254 nm.

Column. Various columns and packing materials have been tested to determine optimum operating conditions for separation with high resolution. The columns employed were a Waters Protein-Pak 125 silica column, a Polyacrylamide Bio-Gel P-6 column, and a TSK-50S column (Table 1). Optimum performance of SEC packing material (stationary phase) involves high resolution and low column backpressure combined with good mechanical, chemical, and thermal stability.

In SEC, compounds are separated primarily on the basis of hydrodynamic molecular size. If potential hydrophobic and electrostatic interactions between the column resins and analyte molecules are ignored (*10*, *11*), the average retention time then depends on the effective size of the compounds. Molecules that are larger than the pore size of the packing material are excluded and elute first at the void volume. Smaller molecules can penetrate throughout the porous infrastructure and are attenuated, corresponding to a higher retention time. Short retention time corresponds to higher MW.

Samples. Different organic model compounds (Table 2) have been evaluated: albumin (with larger MW and relatively low aromaticity/UV absorptivity), Suwannee River humic acid (SRHA, with larger MW and high aromaticity), Suwannee River fulvic acid (SRFA, with lower MW and high aromaticity), and sucrose (with smaller MW and no aromaticity). These compounds were tested singularly and in mixtures. All mobile phases were produced using ultrapure water from a Millipore Milli-Q (MQ) system and phosphate buffer. Molecular weight calibration standards were comprised of sodium polystyrene sulfonates (PSSs: 1.8, 4.6, 8, and 3.5 kDa), and polyethylene glycols (PEGs: 200−10 000 Da).

Results and Discussion

The sensitivity and accuracy of both detectors were of major concern during the experiments. During optimization, different eluents (NaCl, NaF, $NaNO_3$, and Na_2SO_4), ionic strengths (MQ−0.15 M), injection loop sizes (150−500 μL), flow rates (0.4−0.8 mL/min), column-packing separation capabilities, and oxidizer concentrations for the DOC detector have been evaluated. Both synthetic and natural waters were analyzed by the system to test its robustness and range of application.

Effects of Injection Loop Size. Injection loop size is important because less or more than an optimum injected sample volume can cause weak intensity or significant band broadening, inducing a reduction of resolution and inaccurate MW measurements. Sample volume highly depends on column capacity and the mobile phase flow rate. For better detection, greater column capacity (cross-sectional area and length of column) can accommodate a larger sample. A maximum allowable sample volume should be less than one-third of the peak width volume.

Figure 2 shows a comparison of UVA and DOC peak heights for 150 and 500 μL sample volumes using a Waters Silica column (5 mg/L SRHA as DOC dissolved in MQ). A smaller sample volume is preferential in terms of reducing clogging or contamination of the column as well as to prolong column life. These results show that, at the lower sample volume, UVA is adequate in sensitivity; however, the DOC detector requires more sample volume to elucidate a good peak response. Peak height increased around 200% with little change in peak width when the sample volume increased from 150 to 500 μL (Table 3).

Standard curves at 150 and 500 μL sample volumes are compared in Figure 3. The slope at 500 μL is much steeper than that at 150 μL, implying that the DOC detector is more sensitive at a larger injection volume.

Effects of Eluent Type. Eluent composition significantly affects separation of components by HPSEC (*14*). Therefore, the choice of eluent is a critical factor in obtaining higher sensitivity and resolution (Table 4). The optimal eluent for use with each of the two on-line detectors should meet all conditions for both detectors at the same time. While detectors based on UV absorption are widely used, they exhibit some restricted application in gel permeation chromatography because some solvents have high UV absorptivity. In the case of the applied DOC instrument, it detects carbon dioxide formed by oxidation through conductivity. If the eluent reacts with the oxidizer and forms gases during oxidation, this adversely affects sensitivity and resolution.

A NaCl eluent is potentially problematical; Aiken (*15*) found that the presence of Cl^- in concentrations greater than 0.02 M interferes with the analysis of aqueous DOC concentrations by wet oxidation methods. Chloride competes with DOC for $S_2O_8^{2-}$, lowering the overall oxidation efficiency.

The DOC detector was designed to oxidize DOC to CO_2 by the persulfate oxidation reaction. But some persulfate reacts with chloride ions and produces hypochlorite (eq 3). Hypochlorite can also act as a strong oxidizer and reacts with DOC producing total organic halogen (TOX) (eq 4), resulting in an error in DOC concentration:

$$2Cl^- + S_2O_8^{2-} + 2H_2O \xrightarrow{UV} 2HOCl + 2HSO_4^- \quad (3)$$

$$DOC + HOCl \rightarrow TOX + H_2O \quad (4)$$

Figure 4 shows a comparison of UVA and DOC peaks with NaCl and Na_2SO_4 eluents. Separation between the main peak and the peak at the salt boundary is better with the NaCl eluent (6.7 min) than with the Na_2SO_4 eluent (3.9 min). Sensitivity is also better with the NaCl eluent (1.1 times) for the UVA detector, but the response of DOC is 2.5 times greater with Na_2SO_4 than with NaCl eluent. This implies that there are some interactions between chloride ions and the persulfate oxidizer with the NaCl eluent.

The Na_2SO_4 eluent also exhibits some minor interferences in that sulfate ion can serve as a nucleophilic attack bridge

TABLE 2. Molecular Weight, Aromaticity, and Major Chemical Bonds

name	MW	aromaticity	major chemical bonds	formula
SRHA		high	benzene ring, C=O, C−O, O−H[b]	
SRFA	M_w: 2310, M_n: 1360[a]	high	benzene ring, C=O, C−O, O−H[b]	
albumin	~70 000	low	C−N, N−H, C−C, C−H, C=O, C−O, O−H, C=C	
sucrose	342	none	C−C, C−O	$C_{12}H_{22}O_{11}$

[a] From ref *5*. [b] From refs *12* and *13*.

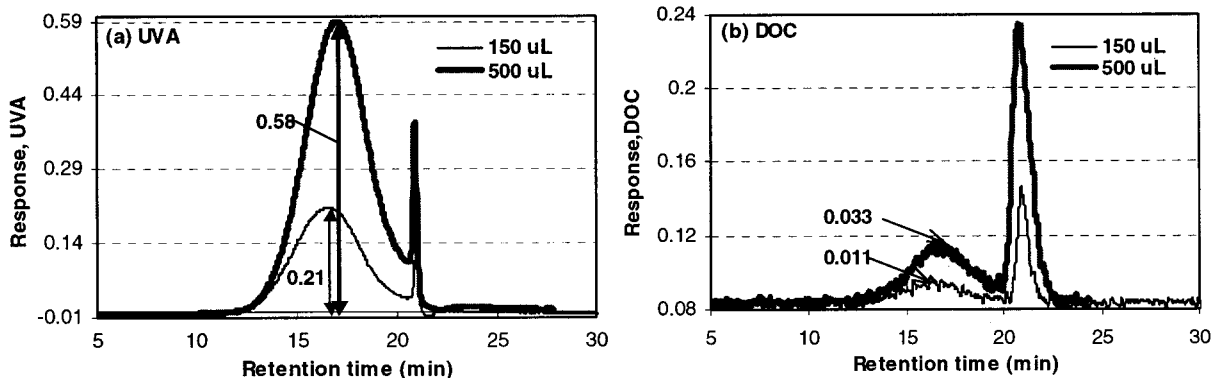

FIGURE 2. Effects of sample volume on (a) UVA and (b) DOC detector. Waters column (0.78 × 30 cm), 5 mg/L (as DOC) SRHA, 0.6 mL/min of NaCl eluent (0.1 M), acid 0.75 μL/min, and oxidizer 2.5 μL/min for DOC.

TABLE 3. Variation of Peak Height and Width at 150 and 500 μL Sample Volumes

	peak height (intensity)		width (min)	
sample vol	UVA	DOC	UVA	DOC
150 μL	0.21	0.011	6.67	8.5
500 μL	0.58	0.033	6.67	9.3
difference	0.37	0.022	0	0.8
increase (%)	176	200	0	9.4

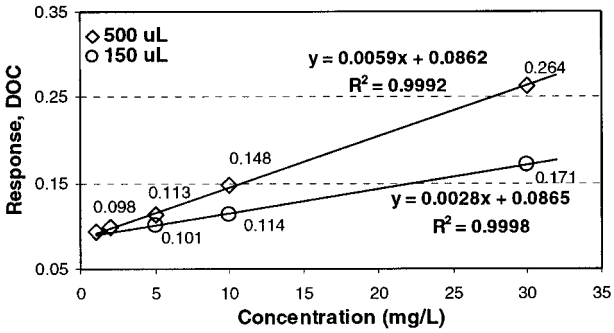

FIGURE 3. Standard curve comparison at different sample volumes. Waters column (0.78 × 30 cm), 0.6 mL/min of NaCl eluent (0.1 M), acid 0.75 μL/min, and oxidizer 2.5 μL/min for DOC.

between two compounds that contain partial positive charge depicted by resonance structure. Such a bridge results in a larger molecule than the original compound. Figure 5 shows the bridging model for acetone (*16*). When acetone was injected into the DOC analyzer, two peaks were detected. One is the original acetone (MW 58 Da) peak, and the other one is the peak of the bridged molecule (MW 214 Da) that is produced due to the binding of two acetone molecules by sulfate ion (Figure 6).

NaF was also tested as an eluent for UVA and DOC. To eliminate inorganic carbon, the Sievers TOC adds 6 M phosphoric acid to reduce the pH to below 2. This pH converts NaF (i.e., F^-) to HF ($pK_a = 3.2$) gas within the eluent. HF gas permeates the semipermeable membrane and then increases the conductivity like CO_2 gas, causing an unstable high baseline for the DOC detector (Figure 7). The $NaNO_3$ eluent strongly absorbs UV at 254 nm, and it was excluded for this system.

Effects of Ionic Strength. To reduce the interactions between the column resin and NOM (charge interaction), a strong ionic strength is necessary. The effects of ionic strength were tested with water ranging from pure water to 0.15 M using NaCl, with 5 mg/L (as DOC) SRHA injected into the Waters column (1 mL/min flow rate and 0.5 mL sample volume). Table 5 shows increasing elution time with increasing ionic strength, corresponding to a smaller molecular size. However, the elution time was almost the same at ionic strengths greater than 0.1 M, indicating a plateau in the steric configuration effects induced by mobile phase conductivity.

Effects of Eluent Flow Rate. Eluent flow rate exhibits a significant influence on the resolution of a column and efficiency of the DOC detector. Optimum flow rate for the column can be obtained depending on the cross-sectional area of column, pore size of resin, and mass transfer characteristics of the sample. Within the efficient flow rate range of the column, the sensitivity of the DOC detector was evaluated at different flow rates.

Figure 8 shows the effects of flow rate on the DOC detector. The Waters Protein-Pak 125 silica column was used with 10 mg/L (as DOC) of SRHA. The applied amount of acid and oxidizer in the DOC detector was 2.5 μL/min. The peak areas are similar at 0.7 and 0.8 mL/min flow rates, yet the area and peak response obtained with the 0.6 mL/min flow rate is increased substantially. This difference may be caused by the reaction time between NOM in the sample and the oxidizer; therefore, the amount of oxidizer must be considered in optimizing flow rate.

Effects of Column Packing Material. A polyacrylamide gel column is commonly used in SEC. The charge of polyacrylamide is neutral, although the amide functional groups display acidic properties that impart hydrophilicity to the gel (*17*). This produces hydrogen bonding between the amide functional group and aromatic compounds. The peak of SRFA is extremely broad due to the interactions between aromatic components of SRFA and the polyacrylamide column gel despite high ionic strength (Figure 9a). Swift and Posner (*18*) suggested that the use of a borate

TABLE 4. Evaluation of Eluents for UVA and DOC Detectors

eluent	UVA detector (254 nm)	DOC detector
NaCl (0.075 M)	low absorption	interference by Cl^-
NaF (0.075 M)	low absorption	interference by HF gas formation
$NaNO_3$ (0.075 M)	high absorption	interference by NO_2 gas formation
Na_2SO_4 (0.025 M)	moderate absorption	good

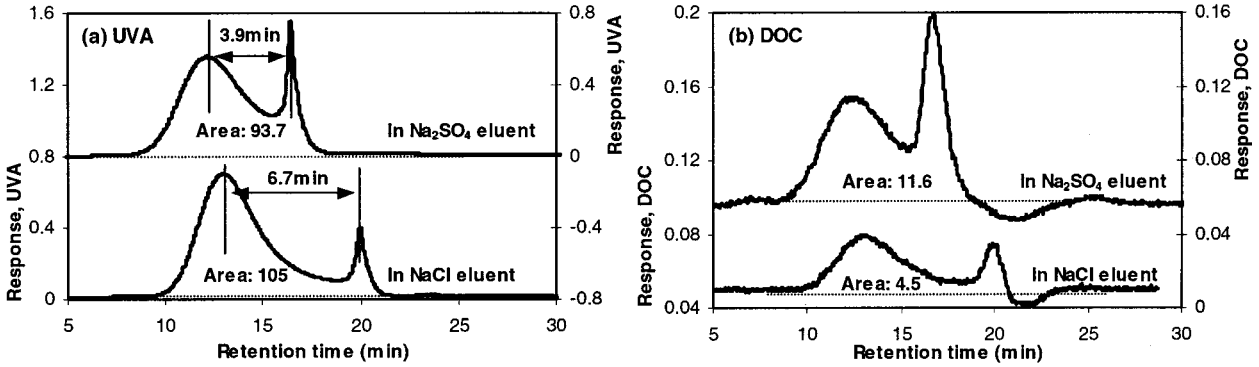

FIGURE 4. Comparison of (a) UVA and (b) DOC peaks with NaCl and Na_2SO_4 eluents. Waters column (0.78 × 30 cm), 7.5 mg/L (as DOC) SRHA dissolved in MQ, 0.6 mL/min of eluent, acid 0.75 μL/min, and oxidizer 2.5 μL/min for DOC, phosphate buffer, ionic strength 0.1 M.

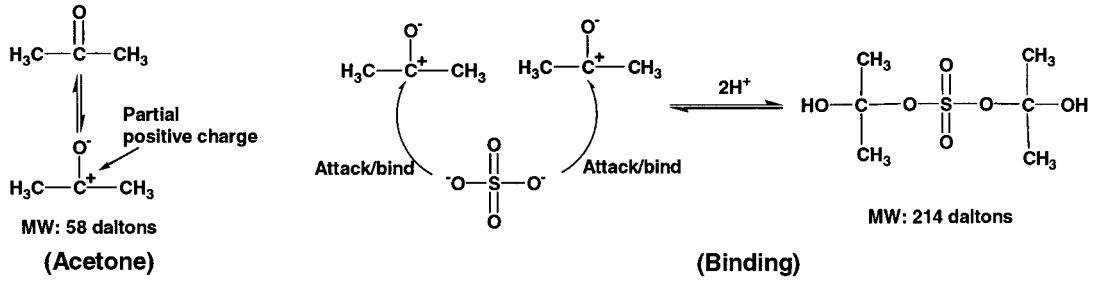

FIGURE 5. Bridging model between acetones by sulfate ions.

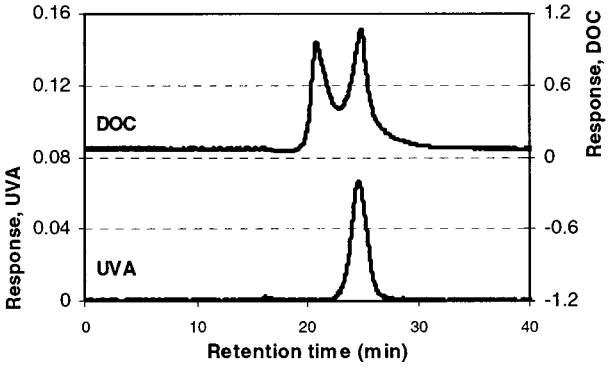

FIGURE 6. UVA/DOC peaks of acetone. Waters column (0.78 × 30 cm), acid 0.75 μL/min, and oxidizer 1.5 μL/min for DOC, 1 mL/min of eluent, phosphate buffer, ionic strength 0.1 M.

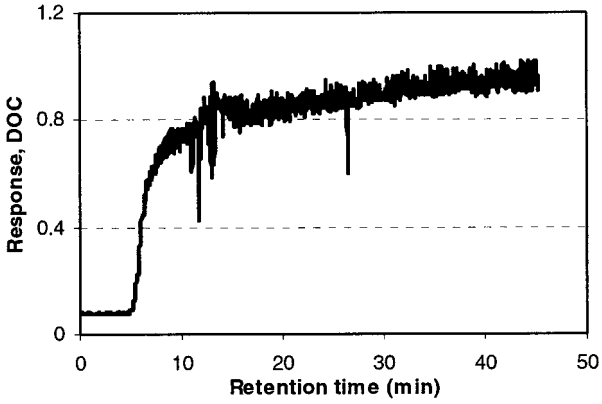

FIGURE 7. DOC detector baseline with NaF eluent. Waters column (0.78 × 30 cm), acid 1.5 μL/min, and oxidizer 1 μL/min for DOC, phosphate buffer, ionic strength 0.1 M, 0.6 mL/min.

buffer resulted in elimination of aromatic interactions of SRFA with polyacrylamide gel; however, the peak of SRFA still showed adsorption and was broad even with a borate buffer (Figure 9b).

The Waters Protein-Pak 125 silica column has high separation capability, but it has a limitation of sample volume due to the column capacity (0.78 × 30 cm); 0.5 mL of SRHA (1 mg/L of DOC) was injected into the Waters column, but the resolution of DOC was poor (Figure 10a). The TSK 50S column (BIAX-Crom) has a high cross-sectional area and volume (2 cm radius, 25 cm length); 2 mL of sample was injected, and the DOC response was significantly increased with this column, resulting in a lowered detection limit (Figure 10b).

Comparisons of Four Different Types of Compounds. Albumin (bovine), SRHA, SRFA, and sucrose have been evaluated by the HPSEC−UVA/DOC system. With all present either separately (7.5 mg/L as DOC for each sample) or in a mixture (30 mg/L as DOC), Figure 11 shows that the order according to MW, as expected, is albumin, SRHA, SRFA, and sucrose. Aromaticity effects can be discerned from this figure. The aromaticities of SRHA and SRFA are larger because they exhibit relatively higher peaks of UVA as compared to the other compounds. The UVA peak of sucrose is virtually nonexistent within the error range (no double bond), but the DOC response represents a relatively high concentration (Figure 11). In the case of sample mixtures, the peaks of

TABLE 5. Elution Time of SRHA at Different Ionic Strengths

ionic strength	elution time (min)	ionic strength	elution time (min)
MQ water	6.02	0.1 M	10.1
0.01 M	6.67	0.15 M	10.3
0.05 M	9.53		

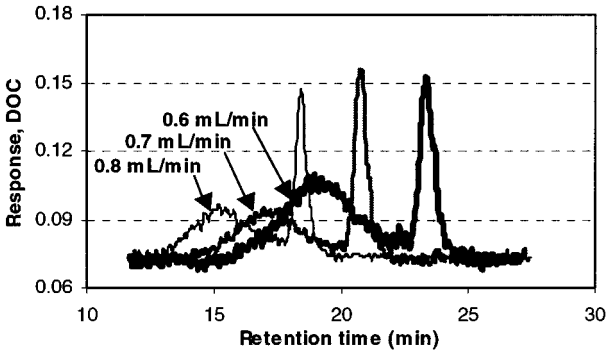

FIGURE 8. Effects of flow rate on DOC detector. Waters column (0.78 × 30 cm), 10 mg/L (as DOC) SRHA, phosphate buffer, ionic strength 0.1 M (NaCl), sample loop 0.5 mL, acid 2.5 μL/min, and oxidizer 2.5 μL/min for DOC.

SRHA and SRFA are not distinguished due to the limitation of separation capability of the column.

Effects of Oxidizer Concentration. Oxidation of various types of organic carbon material is not the same. The oxidation reaction rate of complex aromatic compounds, long-chain hydrocarbons, and simple single-bond hydrocarbons differ. Goulden and Anthony (*19*) found that different organic molecules exhibited different oxidation efficiencies.

Reaction rate depends on temperature, catalyst, concentration of reactants, reaction time, and properties of the reactants. Two different properties of reactants (potassium hydrogen phthalate (PHP), an aromatic compound with MW 204 Da, and sucrose, a single-bond compound with MW 346 Da) were compared under different flow rates of oxidizer (at room temperature). Figure 12 shows the structures of PHP and sucrose.

Because of the bridging of PHP by SO_4^{2-}, the elution time of PHP is earlier than that of sucrose. Sucrose was completely oxidized by UV irradiation even without the oxidizer (persulfate). However the height of PHP continuously changed with increasing amount of oxidizer. The maximum peak height is shown at 3 μL/min of oxidizer addition, and it was slightly diminished at 4 μL/min (Figure 13). If a large amount of excess persulfate is present, UV-promoted decomposition of persulfate to produce oxygen will occur. The oxygen will collect as gas bubbles that can cause problems in the measurement of CO_2 using the membrane-based detection system, causing poor reproducibility and low DOC readings. Optimizing the oxidizer addition as a function of the DOC concentration and mobile phase flow rate remains the subject of continuing research.

Effects of Sample Pretreatment. A strong mobile phase ionic strength is desirable for the HPLC−SEC system for reducing interactions between NOM and the column packing material, as discussed earlier. Therefore, samples should also be prepared as similar to the mobile phase as possible because they are mixed during the separation within the column. Particularly, pH and ionic strength should be considered.

Figure 14 demonstrates the effects of sample conditions. The SEC chromatogram of the SRHA dissolved in MQ displays two peaks. However, the salt boundary peak was significantly lowered or disappeared for the SEC chromatogram of the SRHA dissolved in eluent. Therefore, the ionic strength of samples should be adjusted before SEC analysis. Highly concentrated salts of the mobile phase can be utilized to adjust the ionic strength of the sample using a conductivity meter.

Overall, our results suggest that the TSK 50S column, which allows a larger sample volume, is better suited for DOC detector sensitivity. Na_2SO_4 with phosphate buffer as an eluent provided good ionic strength adjustment and reduced interactions between column resin and NOM as well as minimized interference for both the UVA and the DOC detectors. To reduce the salt boundary peak, ionic strength adjustment of the sample is required as a pretreatment step for optimal SEC chromatograms. On the basis of the above optimization results, an HPSEC−UVA system with a modified DOC detector has been applied for NOM analysis to demonstrate the range of applications.

Applications

Molecular Weight Determination. Relative molecular size distributions of NOM in aqueous solution were determined by HPLC−UVA/DOC. The calibration standards were PSSs and PEGs. PSS standards are more similar to NOM than PEG standards in SEC characteristics (hydrodynamic radii, viscosity, etc.) (*20*). Unfortunately, PSSs display interaction with the TSK 50S column in a Na_2SO_4 eluent. Therefore, PEGs were applied to obtain a standard curve. The calibration curve (Figure 15a) was semilog linear over the range obtained by PEG standards and was used to estimate MW of natural water samples. The HPSEC mobile phase was prepared with 0.004

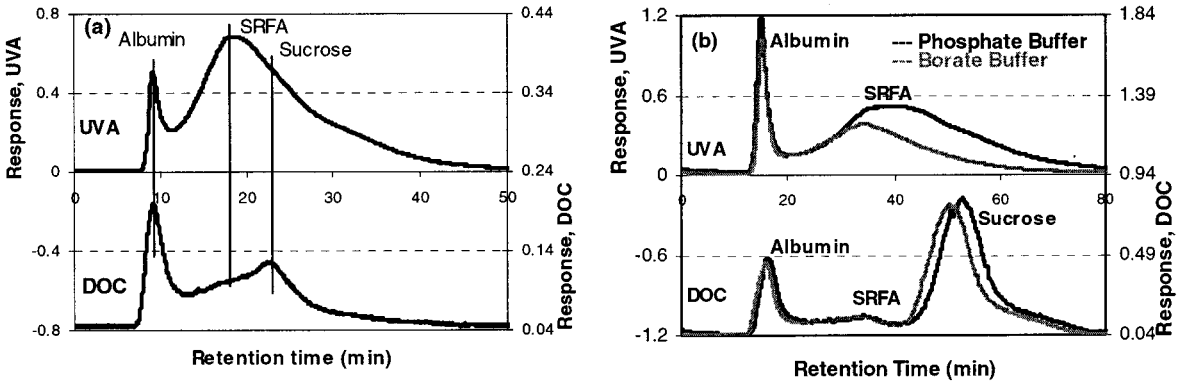

FIGURE 9. (a) Separation of albumin, SRFA, and sucrose by polyacrylamide Bio-Gel P-6 (0.78 × 30 cm). NaCl eluent (0.075 M), phosphate buffer (0.004 M), flow rate (0.6 mL/min), acid 0.75 μL/min, and oxidizer 2.5 μL/min for DOC. (b) Comparison of peaks with different buffers by polyacrylamide Bio-Gel P-6 (0.5 × 90 cm). NaCl eluent (0.075 M), phosphate buffer (0.004 M), borate buffer (0.004 M), flow rate (0.4 mL/min), acid 0.75 μL/min, and oxidizer 2.5 μL/min for DOC. Samples were dissolved in MQ.

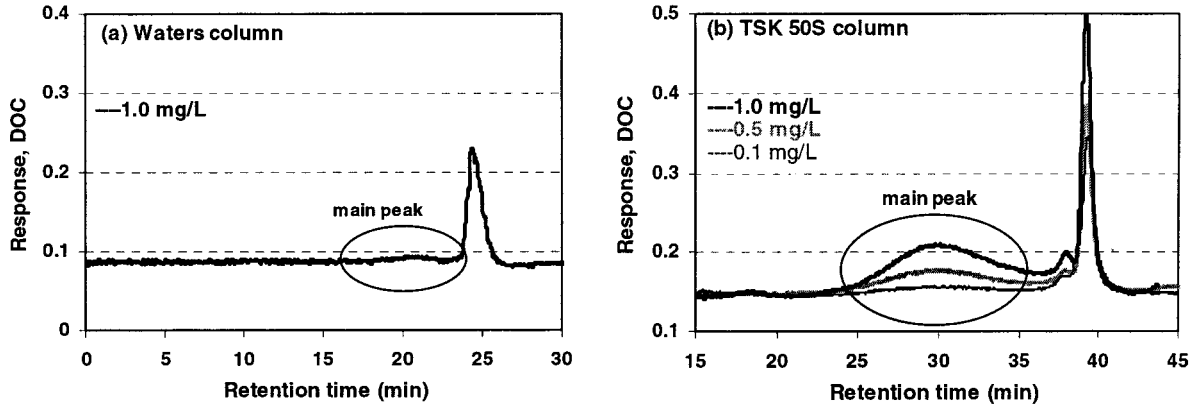

FIGURE 10. Comparison of resolution: (a) Waters and (b) TSK 50S columns. SRHA dissolved in MQ, NaCl eluent (a), Na_2SO_4 eluent (b), acid 0.75 μL/min, and oxidizer 1 μL/min for DOC, phosphate buffer, ionic strength 0.1 M, sample loop 0.5 mL (a), sample loop 2 mL (b).

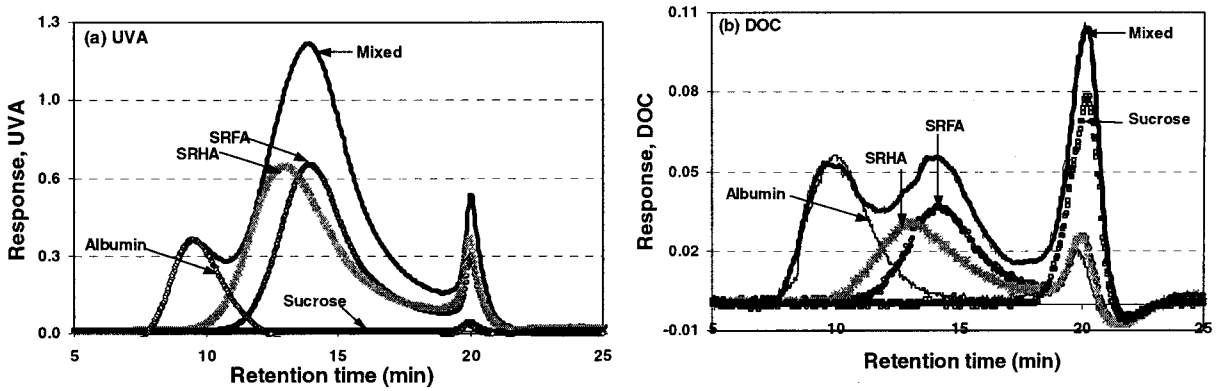

FIGURE 11. (a) UVA and (b) DOC peaks of albumin, SRHA, SRFA, and sucrose (7.5 ppm). TSK 50S column (2 × 25 cm), Na_2SO_4 eluent, acid 0.75 μL/min, and oxidizer 1.5 μL/min for DOC, phosphate buffer, ionic strength 0.1 M.

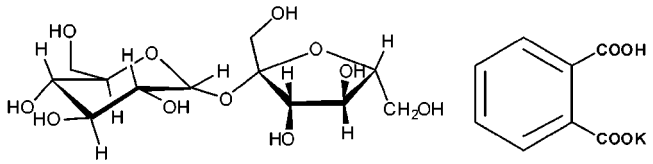

FIGURE 12. Structures of sucrose and PHP.

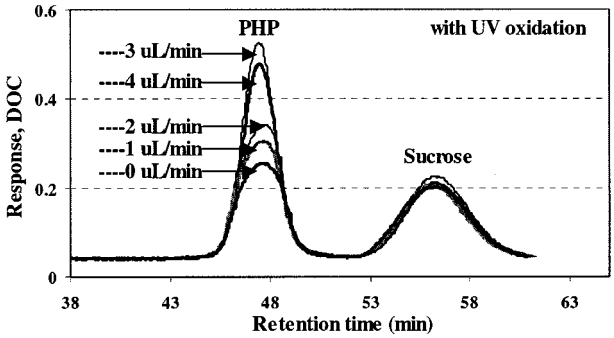

FIGURE 13. DOC peaks of PHP and sucrose under different amounts of oxidizer. TSK 50S column (2 × 25 cm), 5 mg/L (as DOC) of PHP and sucrose, Na_2SO_4 eluent, 1 mL/min of eluent, acid 0.75 μL/min for DOC, phosphate buffer, ionic strength 0.1 M.

M phosphate buffer (pH 6.8) and 0.025 M sodium sulfate, producing an ionic strength of 0.1 M.

MW is a fundamental property for understanding physical and chemical characteristics of natural organic matter. MW distributions can be represented by number-averaged MW (M_n), weight-averaged MW (M_w), and polydispersity (M_w/M_n). M_n and M_w values for Oise River water (Figure 15b) were determined using the following equations (*21*):

$$\text{number-averaged MW:} \quad M_n = \sum_{i=1}^{N} h_i / \sum_{i=1}^{N} (h_i/M_i)$$

$$\text{weight-averaged MW:} \quad M_w = \sum_{i=1}^{N} (h_i M_i) / \sum_{i=1}^{N} h_i$$

where h_i is the detector response at the MW corresponding to the elution time (i). Table 6 shows the calculated MW obtained by UVA and DOC detectors. Polydispersity measured by the DOC detector is larger than that by the UVA detector due to differences of the detector capability (i.e., DOC detects all organic compound while UVA only detects compounds with aromatic or double bonds). The NOM of the Oise River water exhibits lower aromaticity over higher and lower MW ranges.

Performance Measurement of HPSEC−UVA/DOC with Silver Lake Surface Water. Silver Lake surface water (SL-

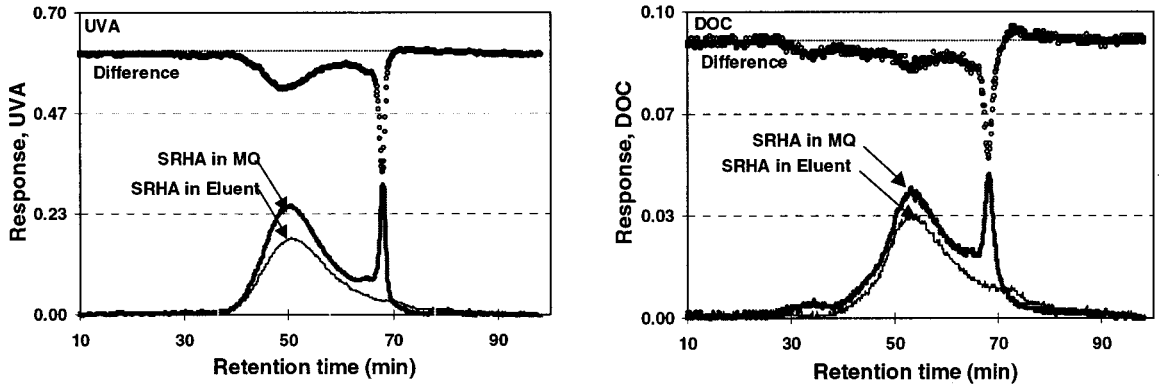

FIGURE 14. UVA and DOC peaks of SRHA isolate diluted in MQ and eluent. TSK 50S column (2 × 25 cm), SRHA, Na_2SO_4 eluent, 1 mL/min of eluent, acid 2 μL/min, and oxidizer 2 μL/min for DOC, phosphate buffer, ionic strength 0.1 M.

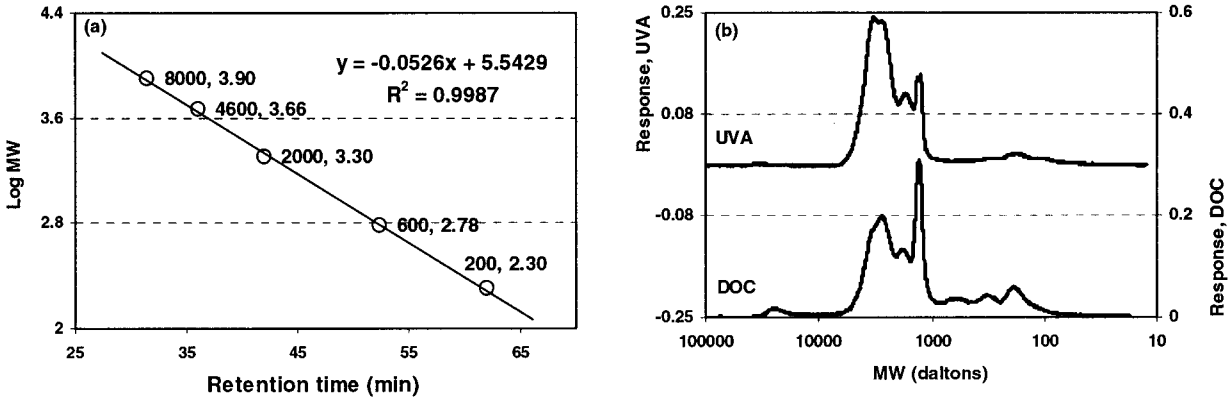

FIGURE 15. (a) Standard curve using PEG and (b) MW distribution of Oise River water. TSK 50S column (2 × 25 cm), Na_2SO_4 eluent, acid 0.75 μL/min, and oxidizer 1 μL/min for DOC, phosphate buffer, ionic strength 0.1 M.

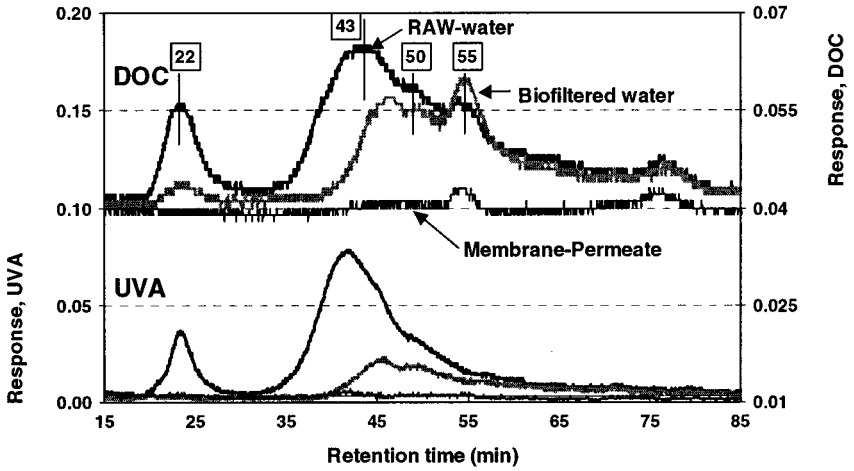

FIGURE 16. UVA and DOC peaks of raw and treated waters (SL-SW). TSK 50S column (2 × 25 cm), Na_2SO_4 eluent, acid 0.75 μL/min, and oxidizer 1 μL/min for DOC, phosphate buffer, ionic strength 0.1 M, flow rate 1 mL/min.

TABLE 6. MW Measured by UVA and DOC

detector	number-averaged MW (M_n)	weight-averaged MW (M_w)	polydispersivity (M_w/M_n)
UVA	1109	1762	1.59
TOC	694	2018	2.91

SW), used as source water at the Boulder, CO, water treatment plant, was employed to check the performance of the HPSEC−UVA/DOC. The DOC concentration of the raw water was 3.6 mg/L as measured using a standard curve that was produced with PHP standards (Figure 17), and the UVA of raw water (Table 7) was 0.058 cm^{-1} (SUVA: 1.6 L/m-mg). The peak area of UVA represents the intensity of UV absorbance of the sample and that of DOC directly shows the concentration of organic carbon.

Figure 16 clearly shows the performance of two treatment processes: biofiltration and membrane filtration. The biofiltration sample was collected from a pilot-scale biologically active rapid sand filter (BARSF) consisting of a series of three 2.54 cm × 60 cm glass columns operated at a 4.2 m/h hydraulic loading and a 17-min empty-bed contact time after ozonation (1.3 mg of O_3/mg of DOC); and membrane permeate was obtained from dead-end stirred-cell filtration with a nanofiltration (NF 200) membrane. SL-SW raw water can be described according to four general fractions.

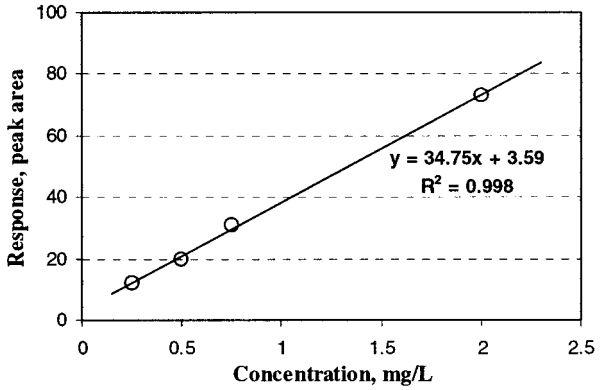

FIGURE 17. Standard curve using PHP for measurement of DOC concentration (by DOC detector). TSK 50S column (2 × 25 cm), Na_2SO_4 eluent, acid 0.75 μL/min, and oxidizer 2.5 μL/min for DOC, phosphate buffer, ionic strength 0.1 M, flow rate 1 mL/min.

TABLE 7. Quality of Silver Lake Surface Water and Treated Water

	raw water	biofiltered water	membrane permeate
UVA (cm^{-1})	0.058	0.018	0.004
DOC (mg/L)	3.6	1.25	0.22

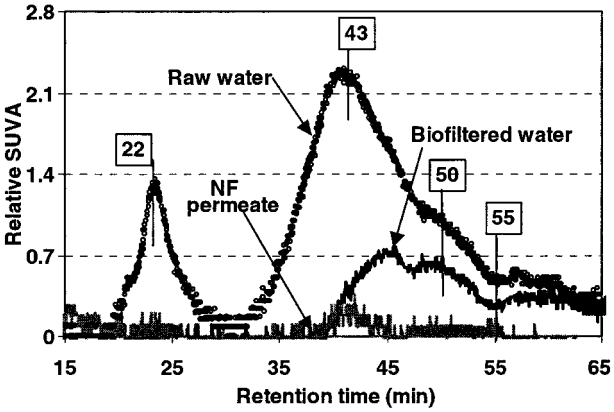

FIGURE 18. Relative SUVA values as a function of MW. TSK 50S column (2 × 25 cm), Na_2SO_4 eluent, acid 0.75 μL/min, and oxidizer 2.5 μL/min for DOC, phosphate buffer, ionic strength 0.1 M, flow rate 1 mL/min.

The first peak at 22 min, indicative of high molar mass, shows relatively high UVA (intermediate values of relative SUVA in Figure 18). Both membrane and O_3/BARSF processes effectively remove this fraction, possibly proteinaceous material.

The second broad peak around 43 min, representative of humic substances, is characterized by highly aromatic components with high relative SUVA values (Figure 18). These components, which are predominant in all natural waters, were moderately reduced by O_3/BARSF and almost completely removed by the membrane process (Figure 16). The peak around 50 min represents components that may be fulvic acids or aliphatic diprotic organic acids. The peak at 55 min is likely monoprotic organic acids and other low MW organic acids including small size fulvic acids (*22*). Even though small organic acids tend to be more biodegradable than large organic acids, there are likely two mechanisms affecting this fraction in the O_3/BARSF processes. First, ozonation creates a greater portion of smaller molecules from the larger mass compounds. Second, BARSF biodegrades these smaller entities.

Environmental Significance and Future Work. The modified DOC detector, derived from a commercially available TOC analyzer, has shown high sensitivity for the HPSEC–UVA/DOC system detecting NOM fractions based on MW. The detector achieves good reproducibility and allows relatively quick analysis. The technique is currently being demonstrated with natural waters subjected to various treatments. Direct qualification and quantification of NOM will be helpful in characterizing drinking water samples. This research will provide drinking water utilities with a better understanding of NOM characteristics and elucidate easily removable or refractory NOM fractions by different treatment processes.

Current research is focused on the application of this method to analyzing samples obtained throughout various water treatment process trains. The amount of oxidizer as a function of DOC concentration and flow rate for DOC detector is the subject of ongoing research.

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Literature Cited

(1) Nystrom, M.; Ruohomaki, K.; Kaipia, L. *Desalination* **1996**, *106*, 71.

(2) Vuorio, E.; Vahala, R.; Rintala, J.; Laukkanen, R. *Environ. Int.* **1998**, *24*, 617.

(3) Amy, G. L.; Collins, M. R.; Kuo, C. J.; King, P. H. *J. Am. Water Works Assoc.* **1987**, *79*, 43.

(4) Thurman, E. M.; Wershaw, R. L.; Malcolm, R. L.; Pinckney, D. J. *Org. Geochem.* **1982**, *4*, 27.

(5) Chin, Y.; Aiken, G.; O'Loughlin, E. *Environ. Sci. Technol.* **1994**, *28*, 1853.

(6) Cho, J.; Amy, G. L.; Pelligrino, J.; Yoon, Y. *Desalination* **1998**, *118*, 101.

(7) Speth, T. F.; Summers, R. S.; Guesses, A. M. *Environ. Sci. Technol.* **1998**, *32*, 3612.

(8) Huber, S. A.; Frimmel, F. H. *Int. J. Environ. Anal. Chem.* **1992**, *49*, 49.

(9) Huber, S. A.; Frimmel, F. H. *Environ. Sci. Technol.* **1994**, *28*, 1194.

(10) Specht, C. H.; Frimmel, F. H. *Environ. Sci. Technol.* **2000**, *34*, 2361.

(11) Fuchs, F.; Heidt, A. *Acta Hydrochim. Hydrobiol.* **1994**, *22*, 121.

(12) Leenheer, J. A.; Wershaw, R. L.; Reddy, M. M. *Environ. Sci. Technol.* **1995**, *29*, 399.

(13) Thorn, K. A. *Sci. Total Environ.* **1987**, *62*, 175.

(14) Linder, H.; Helliger, W. *Chromatographia* **1990**, *30*, 518.

(15) Aiken, G. *Environ. Sci. Technol.* **1992**, *26*, 2435.

(16) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry*, 3rd ed.; Plenum: New York, 1990; p 447.

(17) Jacobs, J. A.; Christman, R. F.; Johnson, J. D. *J. Chromatogr.* **1988**, *450*, 433.

(18) Swift, R. S.; Posner, A. M. *J. Soil Sci.* **1971**, *22*, 237.

(19) Goulden, P.; Anthony, D. *Anal. Chem.* **1978**, *50*, 953.

(20) Perminova, I. V.; Frimmel, F. H.; Kovalevskii, D. V.; Abbt-Braun, G.; Kudryavtsev, A. V.; Hesse, S. *Water Res.* **1998**, *32*, 872.

(21) Yau, W. W.; Kirkland, J. J.; Bly, D. D. *Modern Size Exclusion Chromatography*; Wiley-Interscience: New York, 1979; pp 318–326.

(22) Huber, S. A.; Frimmel, F. H. *Vom Wasser* **1996**, *86*, 277.

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