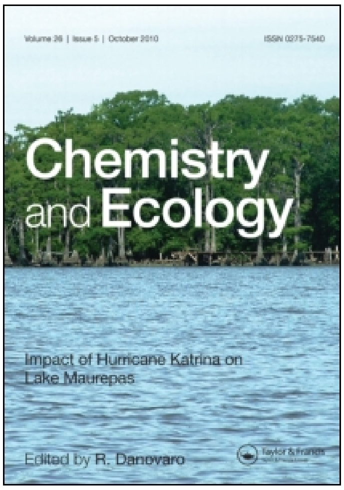

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THE ROLE OF ORGANIC MATTER IN NATURAL AQUATIC SYSTEMS: ANALYTICAL CONSTRAINTS

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The dissolved organic matter in aquatic systems consists of a heterogeneous mixture of compounds with wide ranging chemical properties and diverse origins. The presence of organic matter (OM) in surface and ground waters has important implications with regard to the mobility and therefore fate of many potential organic and inorganic pollutants. The majority of the analytical approaches in current use require exhaustive pre-treatment steps which may lead to the formation of artefacts within the sample. An analytical technique based upon gel permeation chromatography is proposed as a more reliable procedure for the characterisation of naturally occurring dissolved organic matter.

KEY WORDS: colligative properties, DOC, characterization, gel permeation chromatography

INTRODUCTION

Organic matter (OM) is present in most aquatic systems. Its origin is living organisms. Biological degradation and transformation leads to humic substances of complex structure (Christman, 1983; Thurman, 1988). The dissolved fraction plays a dominant role in the carbon cycle (Aiken, 1985) and participates in many physical, chemical and biological reactions (Perdue, 1990; Frimmel, 1988). Adsorption on natural particulates has to be seen in close relation to the transformation by micro-organisms (Meybeck, 1982; Sposito, 1986). Interactions with organic pollutants (Bollag, 1983; Chiou, 1986) and inorganic water constituents, especially heavy metals (Frimmel, 1983; Weber, 1988) are significant for the fate and transport of these substances in both surface water and ground water. In the photic zones of aquatic systems, light can be absorbed by OM and by this, oxidation reactions can be stimulated (Cooper, 1985; Zepp, 1988). Photochemically induced oxidation in waters has to be seen as a main path for the global carbon cycle. The numerous interactions in which OM are involved in aquatic systems are shown in Figure 1.

By its nature OM has to be multifunctional (Suffet, 1989). In addition, one has to keep in mind that OM is normally a mixture of different poorly-defined substances of a broad range of molecular size and weight (Hayes, 1989). Most of these properties are strongly dependent on the pH and on the ionic strength of the waters. This is most important for the comparison of results. The aim of this paper is to focus on the need for advanced characterization of organic matter in aquatic systems. In this context the chromatographic fractionation with multidimensional detection is discussed as a promising method.

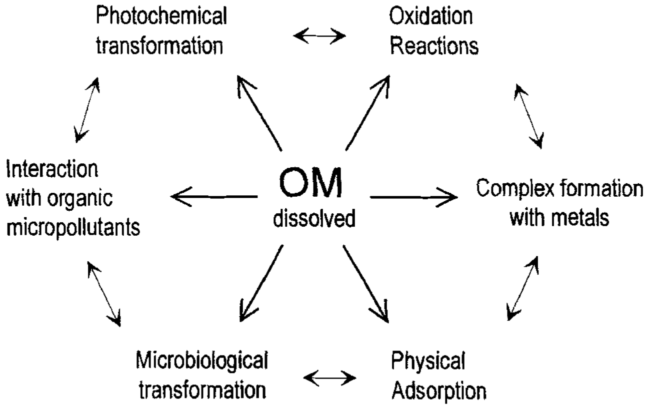

Figure 1 Important transformation pathways of organic matter (OM) in aquatic systems

CHARACTERIZATION OF ORGANIC MATTER

The polydisperse character and the complex structure of the material referred to as organic matter (OM) in literature makes it impossible to identify and determine OM in the sense of classical analytical terms. A useful approach uses quantification by

Table I Basic properties and analytical methods used for characterization of organic matter (OM)

Property of OM	*Analytical Method*	*Common Parameter*	*References*
Elemental composition	Elemental Analyzer	C, O, H, N, S, X metals, DOC	Steelink, 1985 Riise and Salbu, 1989
UV absorption	Spectroscopy	A (254 nm)	Ghosh and Schnitzer, 1979
Colour		A (436 nm)	Visser, 1983
Fluorescence		λ_{ex}, λ_{em}	MacCarthy and Rice, 1985
		Spectra	Bloom and Leenheer, 1989
Infrared absorption	FT-IR	Spectra	Bloom and Leenheer, 1989
Resonance	NMR liquid, solid ESR	^{1}H, ^{13}C, ^{15}N, ^{31}P	Wershaw, 1985 Wilson, 1989 Steelink *et al.*, 1989 Senesi and Steelink, 1989
Molecular size	Flow Field Fractionation Scattering techniques Gelchromatography	Nominal molecular weight	Beckett *et al.*, 1989 Swift, 1989 De Nobili *et al.*, 1989 Wershaw, 1989
Specific charge	Acid-Base titration	pK_a pH/pzc	Perdue, 1985
Complexation capacity	Polarographic titration	Cu-CC	Frimmel and Geywitz, 1983 Perdue, 1989
Polarity	HPLC (C-18) Phase distribution determination	Retention time	Saleh *et al.*, 1990
Biodegradability	Growth curve determination	AOC Yield factor	Van der Kooij, 1990 Hambsch, 1992

elemental analysis and characterization of typical properties on a mass specific basis. The choice of characterization methods will be determined mostly by the aim of the investigation. Some of the most important properties of OM and the methods by which they can be quantified are listed in Table I.

It is obvious that interpretation of the results is severely limited by the operational definitions and by the lack of well-defined authentic substances for calibration of the methods. As a consequence, characterization of OM has to be done by several independent methods. It is also attractive to analyse a whole sequence of samples from different sources or to use original samples and their products from well defined reactions to get meaningful data on the structure/property relations. Most important for the understanding of the genesis and fate of organic matter in aquatic systems is information on the kinetics of the transformations. However, few solid data have been reported.

ISOLATION AND CONCENTRATION OF OM

The analysis of OM in waters can be pursued in two ways: A 'preparative' way with sample concentration and isolation of defined fractions, and an 'analytical' way with only minor or no sample pretreatment (Abbt-Braun, 1991). The preparative way is suitable for most analytical methods since the compounds of interest are present in high concentrations, and unwanted matrix compounds can be separated. Moreover, the preparative way is time consuming and also may irreversibly alter the properties of the compounds of interest, or destroy their structure.

Several methods for the preparative enrichment of OM have been published and are reviewed by several authors (Aiken, 1985; Leenheer, 1985; Thurman, 1988; Peuravouri, 1991). Among the most common methods of enrichment, isolation fractionation includes the pH-controlled adsorption on macroporous XAD-resins (Mantoura and Riley, 1975; Frimmel, 1988; Daignault, 1988) and membrane separation methods like ultrafiltration, reverse osmosis and nanofiltration (Gjessing, 1970; Buffle, 1978; Abbt-Braun, 1991). The International Humic Substances Society (IHSS) has standardized the XAD-method for the isolation of humic and fulvic acid fractions (Figure 2).

According to this procedure the original water sample is filtered through 0.45 μm filter to remove the particulate fraction and acidified (pH 2). A part of the OM is adsorbed on XAD-resin. The non-adsorbed fraction is operationally defined as non-humic substances. The adsorbed fraction is eluted with alkali solution (pH 13) and freeze-dried after cation exchange. With these methods about 40–70% of the total OM can be extracted. The fractions obtained have a very low salt content.

One of the main drawbacks of this method is the rigid chemical treatment with a large pH gradient. This can lead to irreversible denaturing (Malcolm, 1986; Daignault, 1988) and to the introduction of artefacts due to bleeding of the column resin. Also, minor fractions may be irreversibly adsorbed.

One way to circumvent these disadvantages is the use of membrane separation techniques; with these methods, about 60–90% of the OM can be isolated under more moderate conditions. One disadvantage is the parallel concentration of inorganic salts which may require further fractionation steps.

With both methods, only operationally defined OM-fractions with differing properties can be obtained. This applies more or less to all enrichment and isolation procedures. For characterization, the application of differing sample pretreatment methods can be useful. However, it should be stressed that a realistic evaluation of

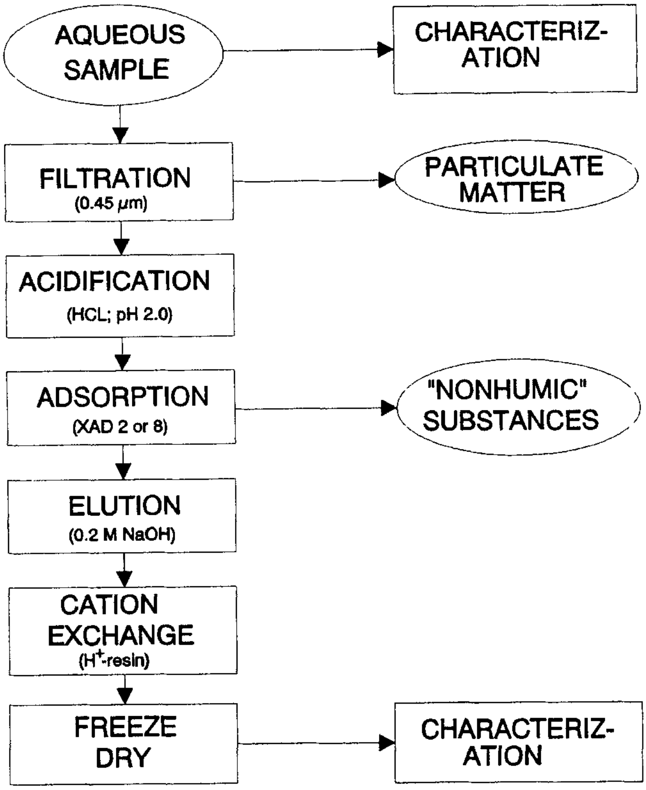

Figure 2 Scheme of the isolation procedure for aquatic humic substances (based on Mantoura & Riley 1975).

OM in aquatic systems can only be carried out in the original water sample. Unfortunately, this does not permit the application of many analytical tools.

COLLIGATIVE PROPERTIES FOR CHARACTERIZATION OF OM

One of the main fractions of OM is composed of dissolved substances. For this fraction, the term 'DOC' (dissolved organically bound carbon) is commonly used. DOC is one of the most fundamental sum parameters for the characterization of organic matter in aquatic systems because the DOC-value is a mass value which 'counts' the amount of organically bound carbon present in a sample. As such it has to be seen in comparison with other classical colligative properties, such as COD (Chemical Oxygen Demand), BOD (Biochemical Oxygen Demand) and spectral properties (e.g. light absorption, fluorescence). These parameters quantify only certain properties of DOC.

Due to the situation where little is known about the structure of DOC, elemental analysis can give insight into the chemical composition (Thurman, 1983; Steelink, 1985; Abbt-Braun *et al.*, 1990). The masses of C, O and H are in the range of 40–60%, 30–50% and 4–6% (weight-%), respectively. Further typical constituents are S (<2%) and N (1–6%). Generally, a high carbon content reflects a low degree of oxidation and a higher degree of hydrophobicity. The H/C-ratio gives information on the degree of saturation with respect to C, whereas the O/C-ratio describes the degree of oxidation and the acidity.

More details on the functionality and structural composition can be obtained by spectroscopic methods, such as UV/Vis-spectroscopy (Bloom, 1989), infrared spectroscopy (MacCarthy, 1985) and resonance spectroscopy (1H-NMR, 13C-NMR, ESR) (Wershaw, 1985; Steelink, 1989; Malcolm, 1989; Senesi, 1989; Peuravouri, 1990).

Absorption of light in the UV-range (200–400 nm) is caused by π-electrons and reflects aromatic, carbonylic and carboxylic electron systems and their conjugations. Absorption and emission (fluorescence) in the visible range (400 nm–800 nm) indicates higher conjugations of these electronic systems. Due to the heterogeneity, the UV/Vis spectra are very poorly structured. Infrared spectra can give more detailed information about functionality; the most important absorption bands are around 2900 cm^{-1} (C-H-groups), 1760 cm^{-1} and 1630 cm^{-1} (C=O and C=C-groups). Proton and carbon nuclear resonance spectroscopy allows the quantification of specifically bound elements. Determination of unpaired electrons via ESR cannot be used for structural analysis, but may serve to identify free radicals.

VALUE OF COLLIGATIVE PROPERTIES IN ECOLOGICAL STUDIES

It is widely accepted that in many natural aquatic environments a close relationship between most common sum parameters prevails. (Smart *et al.*, 1976). This makes it possible to assess many chemical properties of OM by the determination of one single parameter only.

Figure 3 shows an example of the correlation between UV-absorption (254 nm) and DOC for humic matter isolated from some different aquatic systems. As can

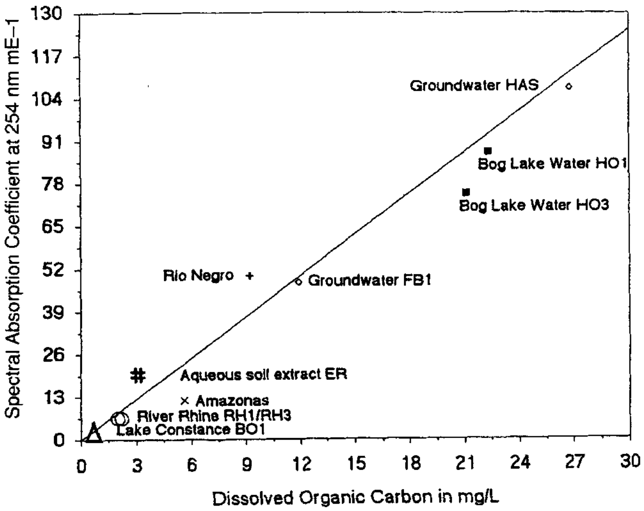

Figure 3 Correlation between UV-absorption (254 nm) and DOC for some natural waters of different origin.

be seen, there is an excellent correlation for waters of very different origin. In cases like these, UV-absorption may well be used as a surrogate parameter for the more difficult and often less accurate determination of the DOC (Armstrong, 1961). However, it seems that at least two prerequisites limit the possibility of replacing the DOC by UV-absorption. The waters should be homogenized waters of different origin: in this case, the measured UV-values are 'sum'-values of a wide distribution of individual compounds with different UV/DOC-ratios. Secondly, the waters should contain free oxygen in order to have stable conditions for sample collection and analysis.

Some studies, however, showed that the determination of a single colligative property may not suffice to determine the organic content of a natural water. A clear-cut picture of the real situation is not available at present.

Upland streams or brooks can have very different UV/DOC-ratios even when they belong to similar catchment areas (Edwards, 1987). Also, short-term climatic changes can influence the ratio. Smart *et al.* (1976) studied the relationship between fluorescence (λ_{ex} = 365 nm, λ_{em} = 400–600 nm) and DOC in waters from different natural or human-influenced sources. The correlations were found to be satisfactory. Therefore, the determination of fluorescence as a surrogate parameter for DOC was suggested.

Quite different were the results of studies in which the usefulness of optical properties for replacing the DOC were investigated. By means of gel permeation chromatography, Steward and Wetzel (1981) found that fluorescence values can be influenced by fluorescence quenching effects. The reliability of UV-values was also questioned, since it was found that the alkalinity of the water, in particular the concentration of calcium in the sample, may influence UV-absorption. In another paper, distinct differences between the UV/DOC values and the fluorescence/DOC values were used to investigate the genesis of OM in a Swiss alpine lake (Zumstein and Buffle, 1989). The authors were able to attribute most of the OM to a pedogenic origin whereas aquagenic organic matter was not stable and underwent rapid sedimentation.

Between the COD (chemical oxygen demand) and the DOC, good correlation but high variation due to oxidisable inorganic constituents was found (Sontheimer, 1978). In a study of eutrophic lakes, strong differences could be seen between the oxic and the anoxic zones due to sulphide-containing material (Prochatzkova, 1984). The replacement of COD by DOC as a surrogate parameter was discussed and was favoured for sewage water (Hein, 1991). Finally, Mrkva (1983) found good correlation between the UV-absorption and the COD.

No good correlation was found between the BOD and DOC (Davis in Maier, 1978; Servais, 1987). This was to be expected since the BOD is strongly dependent on the residence time of OM in a biologically active environment.

CHROMATOGRAPHY OF ORGANIC MATTER

The characterization of OM becomes quite complex when the material is broken down into fractions by chromatographic means. In routine analysis this method has not found much support because of tedious pretreatment procedures and not easily interpretable results. The chromatograms obtained in this way have generally the character of 'fingerprints'. In many cases, acceptable results could be obtained only by comparison of waters which underwent exactly the same pretreatment steps.

In a pioneer paper, Gjessing and Lee (1967) characterized surface waters of different origin by this method. They detected in a quasi-continuous fashion the UV, the COD and the DON (organically bound nitrogen). As expected, they found clear differences between the waters studied. DeHaan and DeBoer (1978) discussed an association formation of fulvic acids with hydrocarbons and amino acids and found a strong binding affinity of fulvic acids. Davis and Gloor (1981) compared the DOC of surface water (River Rhine) before and after adsorption on aluminium oxide. Steinberg and Stabel (1978) studied metal complexation in the fractions. Fuchs (1985/86) compared distribution patterns of DOC-chromatograms along the river Rhine. Kalouskova (1978) used gel permeation chromatography to study the association of a pesticide to OM.

All authors could show that OM is comprised of a variety of substances with very different optical and chemical properties. The studies made clear that the determination of colligative properties alone was not satisfactory for correct characterization of OM, and as a consequence, for their description in ecosystems. In all the examples mentioned above, preconcentration of OM was necessary. Artefact formation resulting from this was not under the control of the investigators. In order to minimise the risk of artefact formation and in order to reduce the analysis time drastically, direct chromatographic methods should be appealing for this kind of research.

APPLICATIONS

Chromatography of Natural Waters

In the past few years we have investigated the possible use of chromatographic techniques for the analysis of DOC in natural water samples. The aim was to develop chromatographic tools which would allow the characterization of DOC by simply injecting small amounts of sample on to a column. This seemed to be the most promising method to get meaningful data for many samples within a relatively short time, with minimal alterations in the DOC. In the second part of this paper, some of these results will be discussed.

We have used two chromatographic systems, a conventional high pressure liquid chromatography (HPLC) system with 'Diode-Array' (DAD) and fluorescence detection, and a laboratory built (LC) system. Our liquid chromatography examples should be regarded as case studies and not all chromatographic data are given. More experimental details can be found in the literature cited.

CONVENTIONAL CHROMATOGRAPHY WITH SPECTROMETRIC DETECTION

With conventional chromatographic systems, a direct characterization of DOC-rich-surface waters is possible in some cases. This will be illustrated with a sample of natural brown water from Lake Hohloh (Black Forest, Germany, 1000 m above sea level, DOC about 17 mgl^{-1}). The original water and preconcentrated fractions obtained with the XAD-method were chromatographed (Gremm, 1992).

The gel chromatogram (245 nm) of the fulvic acid isolated from Lake Hohloh (see Figure 5) is fairly featureless compared with the chromatogram of the original water (Figure 4). At short retention times the latter shows a significant fraction (fraction 1) which does not fluoresce. The DAD spectra are practically the same for all fractions and the spectral absorbance decreases continuously from $\lambda = 210$ nm

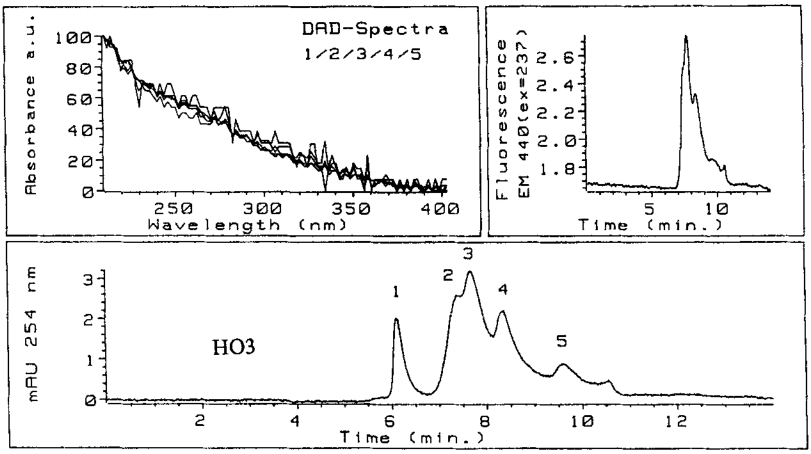

Figure 4 Gel permeation chromatograms (TSK G 2000 SW) of original water from a brown water lake (HO3) detected at $\lambda = 254$ nm milli absorbance units (mAU) and by fluorescence $\lambda_{ex} = 237$ nm, $\lambda_{em} = 440$ nm. The DAD absorbance spectra (a.u. = normalized absorbance units) are also given for the numbered fractions.

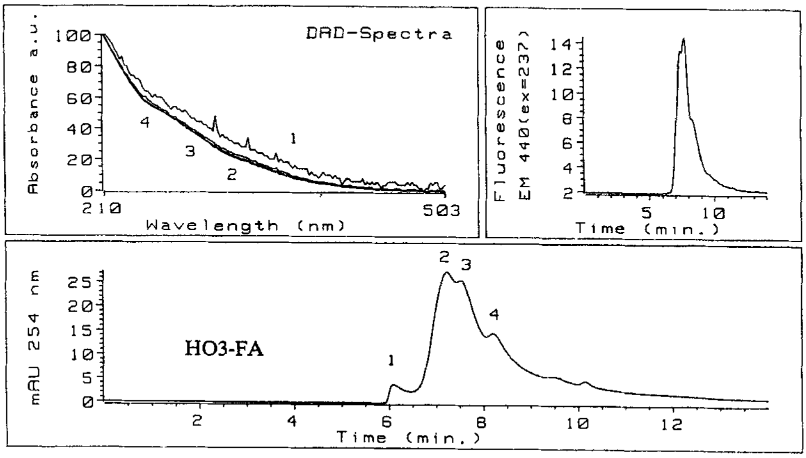

Figure 5 Gel permeation chromatograms (TSK G 2000 SW) of isolated fulvic acids (HO3-FA) detected at $\lambda = 254$ nm milli absorbance units (mAU) and by fluorescence $\lambda_{ex} = 237$ nm, $\lambda_{em} = 440$ nm. The DAD absorbance spectra (a.u. = normalized absorbance units) are also given for the numbered fractions.

to longer wavelengths. Even though the chromatograms are not well resolved due to the polydisperse character of 'mixed' humic substances, comparison of the chromatograms of the isolated fulvic acid and the original brown water shows clearly that there are differences.

The non-humic substances (NHS) which were not adsorbed on XAD are shown in Figure 6. The main fractions appear at slightly longer retention times compared with the chromatograms of the original brown water. This is an indication of the presence of low-molecular-weight organic compounds or more readily adsorbed substances. All the main fractions also fluoresce.

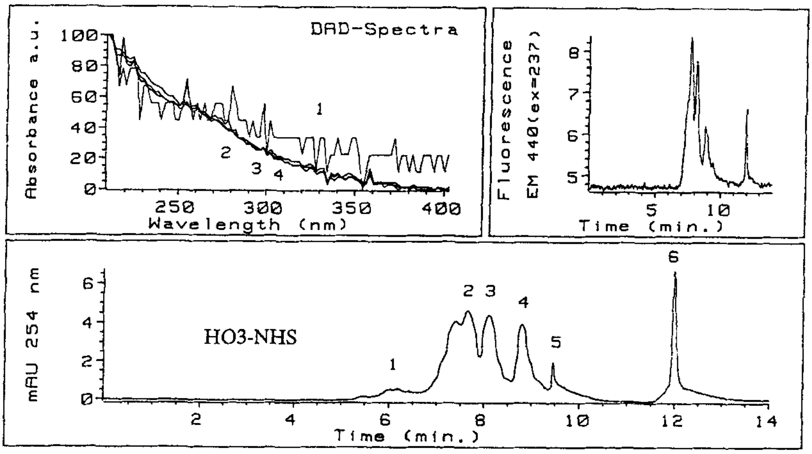

Figure 6 Gel permeation chromatograms (TSK G 2000 SW) of the XAD non-adsorbed substances (HO3-NHS) detected at λ = 254 nm milli absorbance units (mAU) and by fluorescence λ_{ex} = 237 nm, λ_{em} = 440 nm. The DAD absorbance spectra (a.u. = normalized absorbance units) are also given for the numbered fractions. The fraction eluting at about 12 min also appears in the system control of the TSK column and is therefore assigned to impurities.

The UV-Vis spectra for most fractions are similar to those of the fractions of the chromatogram in Figure 4. Only the fastest eluting fraction (T_R ca. 6 min) shows a somewhat higher absorbance at higher wavelengths, and the slowest eluting fraction (T_R ca. 12 min) shows a weaker absorbance at shorter wavelengths. The presence of the latter points out the necessity of applying background corrections which can only be made using a very sensitive detection system. Whether the correction can be neglected or not has to be examined carefully from case to case.

DIRECT CHROMATOGRAPHIC ANALYSIS WITH DOC-DETECTION

The direct chromatographic separation of organic material in natural waters with a low DOC content is not a technical problem but detection systems with sufficiently high detection sensitivity were probably difficult to find in the past. So we have no information of reports in which organic constituents of natural waters were chromatographed without the sample preconcentration step.

Detectors for UV-Vis and fluorescence with the required sensitivity have become commercially available in recent years. Also, a high-sensitivity detector for the continuous determination of the DOC and inorganic carbon (IC) in the low ppb to ppm concentration range with the help of a UV-thin-film reactor has been developed (Graentzel, 1984). The DOC-detector can play an integral role in combined chromatographic systems. One such system has been developed and used in various studies (Huber *et al.*, 1990; Huber and Frimmel, 1992). A detailed description of the DOC-detector and its performance was given by Huber and Frimmel (1991).

Figure 7 shows the general outline of the fully automated system. It consists of two UV-Vis detectors, a fluorescence detector, two infrared detectors, two HPLC pumps, a UV-thin-film reactor and two UV-batch reactors for the supply of low-organic-carbon water. The thin-film reactor is used for the oxidation and separation of organic and inorganic carbon. At the reactor inflow side, UV-Vis and fluorescence

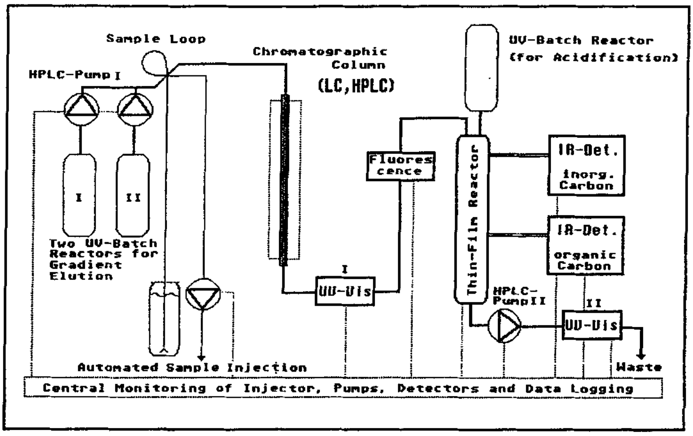

Figure 7 General outline of a fully automated LC/HPLC system with the simultaneous detection of UV-Vis, fluorescence, organic carbon, inorganic carbon and organically bound nitrogen in the ppb to ppm concentration range.

properties are measured. In the reactor outflow, oxidized reaction products can be further analyzed. In addition, a second UV-Vis detector (210 nm) was set in the reactor outflow. It is planned to determine organically bound nitrogen (DON) as secondary nitrate. Primary nitrate present in the sample is retarded by a chromatographic size exclusion process, whereas DON may be oxidized to strongly UV-absorbing nitrate. Other heterocyclic elements, like phosphorus or sulphur, would be oxidized to (non-UV-absorbing) phosphate and sulphate salts. Initial results compared to elemental analysis suggest that all DON is oxidized to nitrate.

In the following examples, the signals obtained for the UV-absorption, fluorescence and DOC are shown. All chromatograms were obtained by using chromatographic columns with lengths between 20 cm and 90 cm and a diameter of 1.6 cm. The columns were packed with polyacrylate gel TSK HW 40 (S) (Merck, Germany) with an average particle size of 40 μm and pores in the 40 nm to 80 nm size range. The separation principle is a filtration process with the high molecular material eluting first and the low molecular material eluting later. The chromatographic separation conditions are restricted since organic modifiers cannot be used. Best separation results were obtained when the aqueous phase contained moderate amounts of buffer salts (28 mM phosphate buffer) at almost neutral pH values (pH 6.58). Calibration of the columns was performed with polyethylene glycols. However, because authentic materials for calibration are not available, only relative sizes can be discussed.

Biodegradation of DOC

Gel chromatographic studies can also be used to distinguish fractions of DOC in biodegradation experiments which characterize the biodegradable and the non-biodegradable fractions of DOC. In Figure 8, the DOC-chromatograms of the acid soluble fraction of an aqueous soil extract before and after biological treatment in an open batch reactor are shown. For inoculation, a small amount of the original soil was added to the solution. The experiment was stopped after 5 days' incubation time.

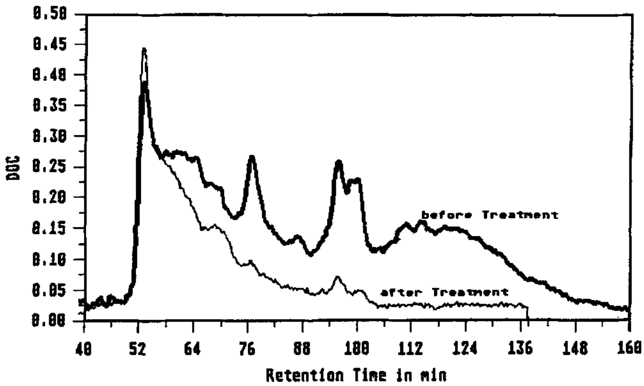

Figure 8 DOC-Chromatograms of the acid soluble fraction of an aqueous soil extract before and after biological treatment.

The original soil extract was composed of a mixture of high-molecular-weight material (T_R: 50–70 min) and low-molecular-weight material (T_R: 70–150 min). Four sharp peaks can be distinguished which may originate from well structured compounds. The peaks are superimposed on a very broad shoulder of unresolved DOC material. After biological treatment, three of the four peaks have almost disappeared. In contrast, the first peak has increased showing that some high-molecular-weight material was newly formed during the biological process. The broad shoulder has diminished by about 50%, showing that this material was only in part biodegradable within the timeframe of the experiment.

More specific information can be obtained when the DOC-chromatograms are compared with the chromatograms obtained for UV-absorption. In Figure 9, the results for the untreated sample are shown. It is striking to see that almost no correlation exists between UV and DOC. The highest UV-absorption values are found in a fraction where no specific responses can be found for DOC. Only two of the four DOC-peaks are UV-active. The broad DOC-shoulder contains only in part UV-active structures.

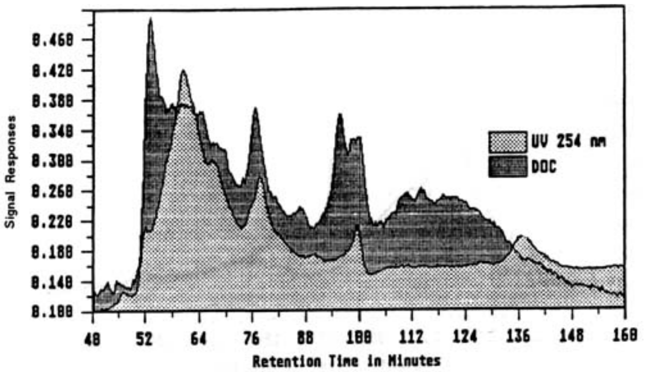

Figure 9 Chromatogram of the untreated sample given in Figure 8 with UV- and DOC-detection

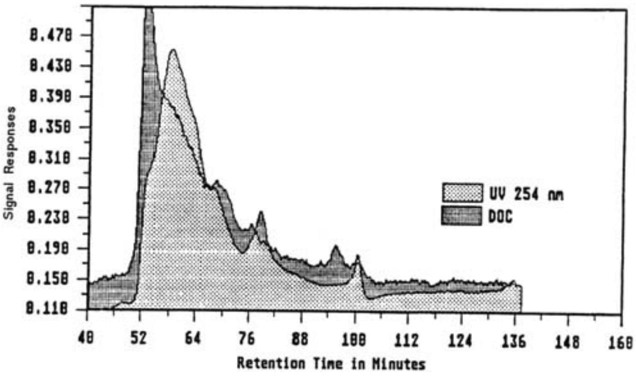

Figure 10 Chromatogram of the biologically treated sample given in Figure 8 with UV- and DOC-detection

In Figure 10, the results after biological treatment are shown with generally good correlation between DOC and UV. The UV-chromatogram resembles closely the previous chromatogram (Figure 9) showing that the UV-active structures were completely resistant to the biological attack. Moreover, some new UV-active material was formed in the high-molecular-weight range suggesting the biological formation of humic substances by condensation processes.

Comparison of a Surface Water with a Bank Filtrate

The system can also be used for the comparison of the DOC of surface water and adjacent bank filtration water. The example in Figure 11 shows a sample of River Rhine water near Karlsruhe (thick line) and water which was collected from a well about 200 m off the river bank (thin line). For the River Rhine water, a DOC of about 3 mgl^{-1} was determined by integration over the entire chromatogram. For the filtrate water, a DOC of about 1 mgl^{-1} was found. For better comparison both chromatograms are superimposed and adjusted to similar intensities.

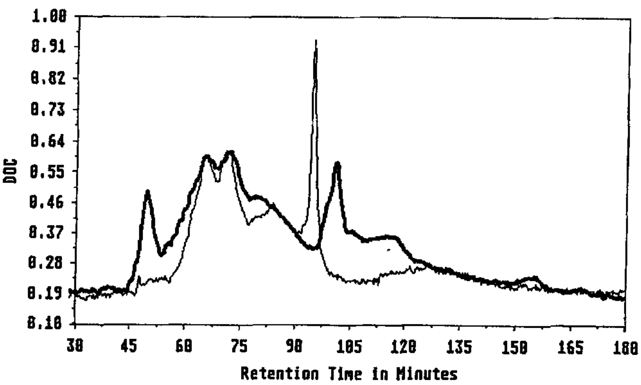

Figure 11 Overlain DOC-Chromatograms of River Rhine water (near Karlsruhe, thick line) and adjacent bank filtration water (thin line). The samples show the heterogeneity of the DOC in natural aquatic systems.

The heterogeneity of the DOC in natural surface waters is well illustrated in the chromatograms. Several peaks can be distinguished. Due to the limited resolution of the column (number of theoretical plates about 500) many more peaks could be expected if the resolution was better. In general there is good correlation between the surface water and the filtrate water. After passage through the river bank, some compounds or classes of compounds have been removed in the low- and in the high-molecular-weight range. The reason for this may have been biological degradation and/or adsorption. The signals seen for UV were very similar. This again shows that the 'dynamic' portions of DOC are more likely found in the non UV-absorbing, aliphatic fraction of the DOC.

Comparison of a Tap Water (Ground Water) with a Thermal Water

An example which was chosen to show an extreme case of heterogeneity of natural DOC is given in Figure 12. The chromatograms of a tap water (based on groundwater) sample (Karlsruhe) and of a thermal water ('Friedrich-Quelle', spa Baden-Baden) are overlain. The signals for fluorescence (above, λ_{ex} = 258 nm, λ_{em} = 430 nm) and DOC-detection (below) are shown.

The tap water sample consists mainly of high molecular weight material which is almost completely absent in the thermal water sample. The first peak at 15 min corresponds to the exclusion limit (>4000 Daltons, as determined by polyethylene glycol). Material of the humic type (humic acid, fulvic acid) is present mainly in this peak. Characterization of the other two peaks is not possible. The nominal size range is between 1500 and 500 Daltons. It is assumed that small fulvic acids and other hydrophilic acids are eluted in this range. A broad band caused by unresolved material shows up in the time span between 23 min and 40 min. This material eluted in part after the permeation limit of the column (around 23 min), an indication that some interaction with the column material must have taken place. Again, classification and further interpretation is not reasonable at this time.

In the thermal water sample, several peaks in the lower molecular range were found. It is striking that no material eluted close to the exclusion limit suggesting that humic-like substances were totally absent in this water sample. This is the first example of a DOC-containing natural water completely devoid of humic-like material found by us.

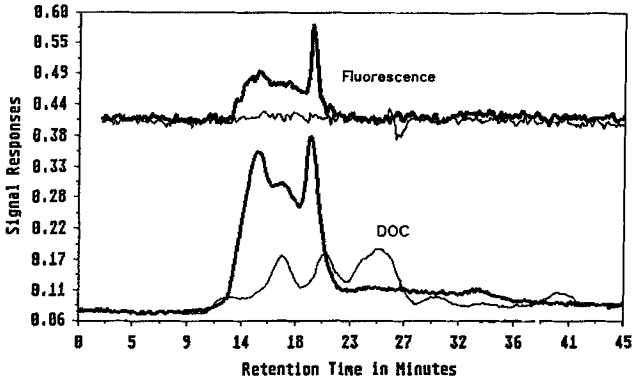

Figure 12 Chromatograms of tap water from Karlsruhe (thick line) and thermal water from a spa, Baden-Baden ('Friedrich-Quelle', thin line), with fluorescence detection (above) and DOC-detection (below).

The corresponding fluorescence intensities for the tap water are in relatively good agreement with the DOC-signals. Only the broad low-molecular weight DOC-fraction was not fluorescent. In the thermal water sample no fluorescing properties are present. This leads to the assumption that, in the thermal water, extreme reaction conditions under elevated temperatures have altered the composition of the DOC. This may have resulted in a breakdown of high-molecular-weight material and in the destruction (oxidation) of fluorescing centres.

CONCLUSIONS

Our knowledge of the composition, structure and ecological behaviour of dissolved organic matter in natural aquatic systems is still quite limited. The main reason for this is the fact that our analytical techniques are simply not good enough to be able to 'see' what is in the water. Most analytical approaches for fractionation require tedious pretreatment steps which may lead to the formation of severe artefacts. Conversely, direct analytical approaches are limited to the determination of colligative properties. A literature review has shown that these are insufficient for proper characterization of natural aquatic systems.

We believe that gel permeation chromatography is a good method for characterization of natural dissolved organic matter. The preconcentration step can be omitted when the sensitivity of the detectors is sufficient. The chromatograms can be run at pH-values and ionic strengths relatively close to natural conditions. The fractionation process in the column is very gentle and the risk of the formation of artefacts is minimal. The continuous detection of the DOC allows a relatively good quantification of individual fractions of organic matter. In conjunction with the advanced DOC determination, other parameters like UV-absorption and fluorescence can now be much better evaluated. Additional characterization methods may be used for quantitative interpretation of organic material and for understanding its role in aquatic systems. Methods for continuous monitoring at low concentration levels are especially needed.

References

Abbt-Braun, G., Schmiedel, U. and Frimmel, F.H. (1990) Elementaranalytische Untersuchung isolierter Fulvinsäuren unterschiedlichen Ursprungs. *Vom Wasser* **75**, 59–73.

Abbt-Braun, G. and Frimmel, F.H. (1991) Spektroskopische Strukturaufklärung aquatischer Huminstoffe. *Vom Wasser* 77, 291–303.

Abbt-Braun, G., Frimmel, F.H. and Lipp, P. (1991) Isolation of organic substances from aquatic and terrestrial system comparison of some methods. *Zeitschrift Wasser Abwasser Forschung* **24**, 285–292.

Aiken, G.R. McKnight, D.M., Wershaw, R.L. and MacCarthy, P. (1985) An introduction to humic substances in soil, sediment and water. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L. and MacCarthy, P. (Eds): *Humic Substances in Soil, Sediment and Water*, John Wiley and Sons, Chichester, pp. 1–9.

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

Armstrong, F. and Boalch, G. (1961) The ultra-violet absorption of sea-water. *Journal Marine Biological Association U.K.*, **41**, 591–57.

Beckett, R., Bigelow, C.J., Jue, Z. and Giddings, J.C. (1989) Analysis of humic substances using flow field-flow fractionation. In: Suffet, I.H. and MacCarthy, P. (Eds): *Aquatic Humic Substances. Influence on Fate and Treatment of Pollutants*, Advances in Chemistry, Series 219, American Chemical Society, Washington DC, pp. 65–80.

Bloom, P.R. and Leenheer, J.A. (1989) Vibrational, electronic and high-energy spectroscopic methods for characterizing humic substances. In: Hayes, B.H.M., MacCarthy, P., Malcolm, R.L. and Swift, R.S. (Eds): *Humic Substances II. In Search of Structure*, John Wiley and Sons, Chichester, pp. 409–446.

Bollag, J.-M. (1983) Cross-coupling of humus constituents and xenobiotic substances. In: Christman, R.F. and Gjessing, E.T. (Eds): *Aquatic and Terrestrial Humic Substances*, Ann Arbor, Michigan, pp. 127–141.

Buffle, J. and Deladoey, P. and Haerri, W. (1978) The use of ultrafiltration for the separation and fractionation for organic ligands in fresh waters. *Analytica Chimica Acta* **101**, 339–357.

Chiou, C.T., Malcolm, R.L., Brinton, T.I. and Kile, D.E. (1986) Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. *Environmental Science and Technology* **20**, 502–508.

Christman, R.F. and Gjessing, E.T. (Eds) (1983) *Aquatic and Terrestrial Humic Materials*, Ann Arbor, Michigan, USA.

Cooper, W.J. and Herr, F.L. (1985) Photochemistry of environmental aquatic systems – introduction and overview. In: Zika, R.G. and Cooper, W.J. (Eds): *Photochemistry of Environmental Aquatic Systems*, Advances in Chemistry, Series 327, American Chemical Society, Washington DC, pp. 1–10.

Daignault, S.A., Noot, D.K., Williams, D.T. and Huck, P.M. (1988) A review of the use of XAD resins to concentrate organic compounds in water. *Water Research* **22**, 803–813.

Davis, J.A. and Gloor, R. (1981) Adsorption of dissolved organics in lake water by aluminium oxide. Effect of molecular weight. *Environmental Science and Technology* **15**, 1223–1229.

DeHaan, H. and DeBoer, T. (1978) A study of the possible interactions between fulvic acids and carbohydrates from Tjeukemeer, based on gel filtration. *Water Research* **12**, 1035–1040.

DeNobili, M., Gjessing, E.T. and Sequi, P. (1989) Sizes and shapes of humic substances by gel chromatography. In: Hayes, B.H.M., MacCarthy, P., Malcolm, R.L. and Swift, R.S. (Eds): *Humic Substances II. In Search of Structure*, John Wiley and Sons, Chichester, pp. 561–591.

Edwards, A.C. and Cresser, M.S. (1987) Relationships between ultraviolet absorbance and total organic carbon in two upland catchments. *Water Research* **21**, 49–56.

Fischer, A.M., Winterle, J.S. and Mill, T. (1985) Primary photochemical processes in photolysis mediated by humic substances. In: Zika, R.G. and Cooper, W.J. (Eds): *Photochemistry of Environmental Aquatic Systems*, Advances in Chemistry, Series 327, American Chemical Society, Washington DC, pp. 141–157.

Frimmel, F.H. and Niedermann, H. (1980) Komplexierung von Metallionen durch Gewässerhuminstoffe 1. Ein Braunwassersee als Huminstofflieferant. *Zeitschrift Wasser Abwasser Forschung* **13**, 119–124.

Frimmel, F.H. und Geywitz, J. (1983) Zur koordinativen Bindung von Metallionen an Gewässerhuminstoffe. Fresenius *Zeitschrift Analytische Chemie* **316**, 582–588.

Frimmel, F.H. and Christman, R.F. (Eds) (1988) *Humic Substances and Their Role in the Environmnent*, John Wiley and Sons, Chichester.

Frimmel, F.H. (1990) Characterization of organic acids in freshwater. A: Current status and limitations. In: Perdue, E.M. and Gjessing, E.T. (Eds): *Organic Acids in Aquatic Ecosystems*, John Wiley and Sons, Chichester, pp. 5–23.

Fuchs, F. (1985) Gelchromatographische Trennung von organischen Wasserinhaltsstoffen. Teil I. *Vom Wasser* **64**, 129–144.

Fuchs, F. (1986) Gelchromatographische Trennung von organischen Wasserinhaltsstoffen. Teil II. *Vom Wasser* **65**, 93–105.

Fuchs, F. (1986) Gelchromatographische Trennung von organischen Wasserinhaltsstoffen. Teil III. *Vom Wasser* **66**, 127–136.

Ghosh, K. and Schnitzer, M. (1979) UV and visible absorption spectroscopic investigations in relation to macromolecular characteristics in humic substances. *Journal of Soil Science* **30**, 373–379.

Gjessing, E.T. and Lee, G.F. (1967) Fractionation of organic matter in natural waters on Sephadex columns. *Environmental Science and Technology* **1**, 631–638.

Gjessing, E.T. (1970) Ultrafiltration of aquatic humus. *Environmental Science and Technology* **4**, 437–438.

Gräntzel, A. (1980, 1982, 1986) Apparatur zur Bestrahlung strömungsfähiger Medien zum Erzeugen photochemischer Reaktionsprodukte. German Patent P3422553C1 (1980), U. S. Patent 4201917 (1982), Japanese Patent J 1121663 (1986).

Grasso, D., Chin, Y.-P. and Weber, W. (1990) Structural and behavioral characteristics of a commercial humic acid and natural dissolved aquatic organic matter. *Chemosphere* **21**, 1181–1197.

Gremm, T. (1992) Zur Verteilung polycyclischer aromatischer Kohlenwasserstoffe (PAK) und zinnogranischer Verbindungen in gewässerelevanten Mehrphasensystemen. Dissertation University of Karlsruhe, Germany.

Hambsch, B. (1992) Untersuchungen zu mikrobiellen Abbauvorgängen bei der Uferfiltration. Dissertation University of Karlsruhe, Germany.

Hayes, E.H.M., MacCarthy, P., Malcolm, R.L. and Swift, R.S. (Eds) (1989) *Humic Substances II: In Search of Structure*, John Wiley and Sons, Chichester.

Hein, D. (1991) Kann der TOC den CSB in der Abwasserüberwachung ersetzen? *Korrespondenz Abwasser* **38**, 1358–1361.

Huber, S., Gremm, T. and Frimmel, F.H. (1990) Chromatographische Trennung natürlicher organischer Wasserinhaltsstoffe mit UV-, Fluoreszenz- und DOC/TOC-Detektion ohne Probenvoranreicherung. *Vom Wasser* **75**, 331–342.

Huber, S.A. and Frimmel, F.H. (1992) A liquid chromatographic system with multi-detection for the direct analysis of hydrophilic organic compounds in natural waters. *Fresenius Journal of Analytical Chemistry* **342**, 198–200.

Huber, S.A. and Frimmel, F.H. (1991) Flow injection analysis of organic and inorganic carbon in the low ppb concentration range. *Analytical Chemistry* **63**, 2122–2130.

Kalouskova, N. (1978) Interactions of humic acids with atrazine. *Journal Environmental Science and Health* **22**, 113–123.

Leenheer, J.A. (1985) Fractionation techniques for aquatic humic substances. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L. and MacCarthy, P. (Eds): *Humic Substances in Soil, Sediment and Water*, John Wiley and Sons, Chichester, pp. 409–429.

MacCarthy, P. und Rice, J.A. (1985) Spectroscopic methods for determining functionality in humic substances. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L. and MacCarthy, P. (Eds): *Humic Substances in Soil, Sediment, and Water*, John Wiley and Sons, Chichester, pp. 527–559.

Maier, W.J. and Swain, W.R. (1978) Organic carbon—a nonspecific water quality indicator for Lake Superior. *Water Research* **12**, 523–529.

Malcolm, R. and MacCarthy, P. (1986) Limitations in the use of commercial humic acids in water and soil research. *Environmental Science and Technology* **20**, 904–911.

Malcolm, R.L. (1989) Application of solid state 13C-NMR spectroscopy to geochemical studies of humic substances. In: Hayes, B.H.M., MacCarthy, P., Malcolm, R.L. and Swift, R.S. (Eds): *Humic Substances II. In Search of Structure*, John Wiley and Sons, Chichester, pp. 339–372.

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

Meybeck, M. (1982) Carbon, nitrogen, phosphorus transport by world rivers. *Journal of the American Chemical Society* **282**, 401–450.

Mrkva, M. (1983) Evaluation of correlations between absorbance at 254 nm and COD of river waters. *Water Research* **17**, 231–235.

Perdue, M.E. (1985) Acidic functional groups of humic substances. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L. and MacCarthy, P. (Eds): *Humic Substances in Soil, Sediment and Water*, John Wiley and Sons, Chichester, pp. 493–526.

Perdue, M.E. (1989) Effects of humic substances on metal speciation. In: Suffet, I.H. and MacCarthy, P. (Eds): *Aquatic Humic Substances. Influence on Fate and Treatment of Pollutants*, Advances in Chemistry, Series 219, American Chemical Society, Washington, 281–297.

Perdue, E.M. and Gjessing, E.T. (Eds) (1990) *Organic Aids in Aquatic Ecosystems*, John Wiley and Sons, Chichester.

Peuravuori, J. and Pihlaja, K. (1990) Humus and its structure. 1. A systematic characterization of lake humus. *Humus Uutiset (Finnish Humus News)* **2**, 4–15.

Peuravuroi, J. Pihlaja, K. (1991) Isolation and fractionation of humic substances in lake waters. In: Allard, B. *et al.* (Eds) *Humic Substances in the Aquatic Environment*, Lecture Notes in Earth Sciences, 33, Springer Verlag, Berlin, pp. 123–134.

Rise, G. and Salbu, B. (1989) Major and trace elements in standard and reference samples of aquatic humic substances determined by instrumental neutron activation analysis (INAA). *Science of the Total Environment* **81**, 137–142.

Prochaztkova, L. and Blazka, P. (1984) Relationship between organic carbon and chemical oxygen demand. *Acta Hydrochimica et Hydrobiologica* **12**, 383–392.

Saleh, F.Y. and Ong, W.A. (1989) Structural features of aquatic fulvic acids. Analytical and preparative reversed-phase high performance liquid chromatography separation with diode-array-detection. *Analytical Chemistry* **61**, 2792–2800.

Senesi, N. and Steelink, C. (1989) Application of ESR spectroscopy to the study of humic substances. In: Hayes, B.H.M., MacCarthy, P., Malcolm, R.L. and Swift, R.S. (Eds): *Humic Substances II. In Search of Structure*, John Wiley and Sons, Chichester, pp. 457–476.

Servais, P., Billen, G. and Hascoet, M.C. (1987) Determination of the biodegradable fraction of dissolved organic matter in waters. *Water Research* **21**, 445–450.

Smart, P.L., Finlayson, B.L., Rylands, W.D. and Ball, C.M. (1976) The relation of fluorescence to dissolved organic carbon in surface waters. *Water Research* **10**, 805–811.

Sontheimer, H., Frick, B., Fettig, J., Hörner, G., Hubele, C. and Zimmer, G. (1985) *Adsorptionsverfahren zur Wasserreinigung*, DVGW Forschungsstelle at the Engler-Bunte-Institute, University of Karlsruhe, Germany.

Sontheimer, H., Fuchs, F. and Kühn, W. (1978) Beurteilung der organischen Belastung von Fließgewässern. *Vom Wasser* **51**, 1–10.

Sposito, G. (1986) Sorption of trace metals by humic materials in soils and natural waters. *Critical Reviews Environmental Control*, **16**, 193–229.

Steelink, C. (1985) Implications of elemental characteristics of humic substances. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L. and MacCarthy, P. (Eds): *Humic Substances in Soil, Sediment, and Water*, John Wiley and Sons, Chichester, pp. 281–308.

Steelink, C., Wershaw, R.L., Thorn, K.A. and Wilson, M.A. (1989) Application of liquid-state NMR spectroscopy to humic substances. In: Hayes, B.H.M., MacCarthy, P., Malcolm, R.L. and Swift, R.S. (Eds): *Humic Substances II. In Search of Structure*, John Wiley and Sons, Chichester, pp. 281–308.

Steinberg, C. and Stabel, H.H. (1978) Untersuchungen über gelöste organische Substanzen und ihre Beziehungen zu Spurenmetallen. *Vom Wasser* **51**, 11–32.

Steward, A.J. and Wetzel, R.G. (1981) Asymmetrical relationships between absorbance, fluorescence, and dissolved organic matter. *Limnol. Oceanogr.* **26**, 590–597.

Suffet, E.H. and MacCarthy, P. (Eds) (1989) Aquatic Humic Substances. *Influence and Fate and Treatment Pollutants*, Advances in Chemistry, Series 219, American Chemical Society, Washington DC.

Swift, R.S. (1989) Molecular weight, size and shape, and charge characteristics of humic substances. Some basic considerations. In: Hayes, B.H.M., MacCarthy, P., Malcolm, R.L. and Swift, R.S. (Eds): *Humic Substances II. In Search of Structure*, John Wiley and Sons, Chichester, pp. 449–465.

Thurman, E.M. and Malcolm, R.L. (1983) Structural study of humic substances. New approaches and methods. In: Christman, R.F. and Gjessing, E.T. (Eds) *Aquatic and Terrestrial Humic Materials*, Ann Arbor, Michigan, pp. 1–23.

Thurman, E.M. (1988) Isolation of soil and aquatic humic substances (group report). In: Frimmel, F.H. and Christman, H.F. (Eds): *Humic Substances and Their Role in the Environment*, Dahlem Workshop Reports. John Wiley and Sons, Chichester, pp. 31–43.

Van der Kooij, D. (1990) Assimilable organic carbon (AOC) in drinking water. In: McFeters G.A. (Ed). *Drinking Water. Microbiological Progress and Recent Developments*, Springer Verlag, New York, pp. 126–134.

Visser, S.A. (1983) Fluorescence phenomena of humic matter of aquatic origin and microbial cultures. In: Christman, R.F. and Gjessing, E.T. (Eds): *Aquatic and Terrestrial Humic Materials*, Ann Arbor, Michigan, pp. 183–202.

Weber, J.H. (1988) Binding and transport of metals by humic materials. In: Frimmel, F.H. and Christman, H.F. (Eds): *Humic Substances and Their Role in the Environment*, Dahlem Workshop Reports, John Wiley and Sons, Chichester, pp. 165–178.

Wershaw, R.L. (1985) Application of nuclear magnetic resonance spectroscopy for determining functionality in humic substances. In: Aiken, G.R., McKnight, D.M., Wershaw, R.L. and MacCarthy, P. (Eds): *Humic Substances in Soil, Sediment, and Water*, John Wiley and Sons, Chichester, pp. 561–591.

Wershaw, R.L. (1989) Sizes and shape of humic substances by scattering techniques. In: Hayes, B.H. M., MacCarthy, P., Malcolm, R.L. and Swift, R.S. (Eds): *Humic Substances II. In Search of Structure*, John Wiley and Sons, Chichester, pp. 545–559.

Wilson, M.A. (1989) Solid-state nuclear magnetic resonance spectroscopy of humic substances. Basic concepts and techniques. In: Hayes, B.H.M., MacCarthy, P., Malcolm, R.L. and Swift, R.S. (Eds): *Humic Substances II. In Search of Structure*, John Wiley and Sons, Chichester, pp. 309–338.

Zepp, R.G. (1988) Environmental photoprocesses involving natural organic matter. In: Frimmel, F.H. and Christman, R.F. (Eds): *Humic Substances and Their Role in the Environment*, Dahlem Workshop Reports, John Wiley and Sons, Chichester, pp. 193–214.

Zumstein, J. and Buffle, J. (1989) Circulation of pedogenetic and aquagenetic organic matter in an eutrophic lake. *Water Research* **23**, 229–239.