

Analytical methods of size distribution for organic matter in water and wastewater

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Abstract—Organic matter (OM), such as natural organic matter (NOM) in surface waters, and effluent organic matter (EfOM) in wastewaters causes many problems. For example, color, taste and odor derogate potable water quality, while the presence of endocrine disrupting substances and the formation of disinfectant byproducts (DBPs) are public health concerns. Over the years various analytical methods have been developed to characterize organic matter in natural and wastewaters. However, it remains difficult to determine the properties and characteristics of various OM constituents. Since all OM components in water have their own specific sizes, size distribution is a useful analytical tool to characterize complex OM. The results also enable better interpretation of experimental results, determination of future research directions, and evaluation of the progress of investigations. This review presents the common analytical size distribution methods used to characterize OM present in waters and wastewaters.

Key words: NOM, Size Distribution, EfOM, DOC

INTRODUCTION

NOM originates from the contact of water with dead and living organic matter in the hydrologic cycle, and is a fundamental component of aquatic ecosystems. Organic matter has important roles in the treatment of waters and wastewaters. It is a major pollutant that produces harmful by-products with oxidants, increases chemical costs and deteriorates product water quality in the reticulation network.

While many previous researches have dealt with characteristics of NOM in surface waters, there are much fewer studies available on EfOM in biologically treated sewage effluent (BTSE). This may be due to the diverse, very complex composition of EfOM which varies from source to source and from season to season. However, with growing concerns related to wastewater discharges, new EfOM characterization methods have been envisaged. EfOM consists of NOM, soluble microbial products (SMPs), persistent organic matter (POP), emerging pollutants such as endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs). SMPs are a by-product of biological treatment, while POPs, EDCs and PPCPs originate from the use of synthetic organic matter (SOM) in daily life. The presence of these harmful chemicals in drinking water, even in trace amounts, is becoming a major concern; thus it is imperative to study the characteristics of EfOM in BTSE.

Aquatic NOM and EfOM are responsible for several problems in water, including color, taste, odor, increased chemical disinfectant demand and formation of DBPs. With the increasingly recognized importance of NOM and EfOM, new analytical methods have been developed to determine the organic content of natural and wastewaters. Nonetheless, it still remains difficult and challenging to measure the properties of many organic constituents present in various waters.

The analytical methods for different OM are divided into two broad groups: i) those which measure gross concentrations of OM greater than about 1 mg/L, and ii) those which measure trace concentrations in the range of nano- and micro sizes [Tchobanoglous and Burton, 1991]. The characterization of OM can also be classified into two categories: i) traditional analyses, and ii) advanced analytical methods [Her, 2002]. Most chemicals and also the physical characteristics of OM are normally analyzed with traditional methods (e.g., light absorptivity, DOC concentration, aromaticity, fluorescence, XAD fractionation) due to the difficulty and higher cost of detailed structure analysis. The advanced analytical approaches include nuclear magnetic resonance (NMR), gas chromatography and mass spectrometry (GC/MS), and attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR). These methods give more detailed information to characterize OM.

All OM constituents in water have their own specific sizes; therefore, size distribution is a useful analytical tool to characterize complex OM. Since 1950 a number of researchers have cited information of size distribution [Xu, 2000]. Size distribution has become an indispensable measurement in research, and many projects depend on information obtained from such examinations. The main objective of this paper is to present common analytical methods and their characteristics. Information on size distribution of OM gives a number of advantages: i) a more fundamental understanding of the complex interactions that occur in the unit operations and treatment process, ii) better process selection and evaluation to develop more effective treatment techniques, and iii) determination of MW cut-off (MWCO) for targeted pollutants in membrane separation processes. Therefore, it is essential to classify the analytical methods of size distribution to identify detailed OM.

CONSTITUENTS OF OM

Figs. 1 and 2 show typical organic compounds present in surface water and BTSE. Painter [1973] and Levine et al. [1985] reported

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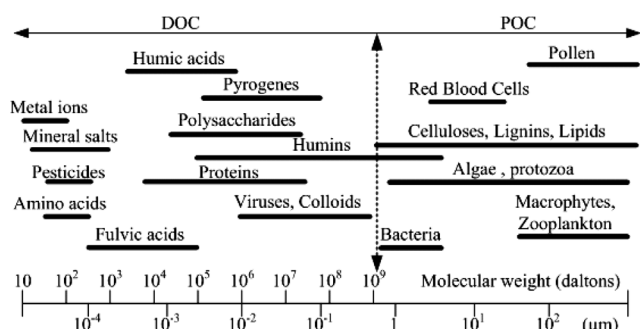


Fig. 1. Typical organic constituent in surface water and their size ranges [adapted from Thurman, 1985 and Schafer, 2001].

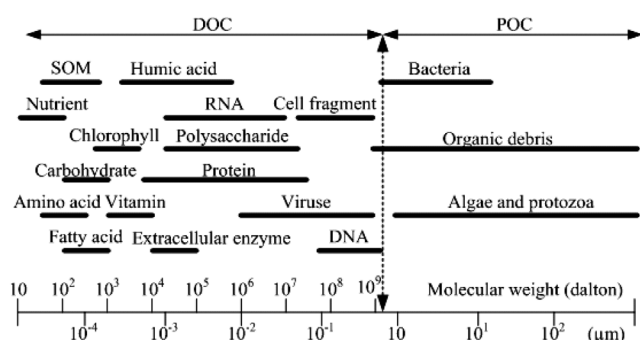


Fig. 2. Typical organic constituents in BTSE and their size ranges [adapted from Levine et al., 1985; Leenheer and Croue, 2003].

that organic contaminants range in size from less than 0.001 μm to well over 100 μm . While both surface water and BTSE include similar constituents, a significant difference is SMP and SOM levels [Shon et al., 2006]. In BTSE, SMP and SOM are preferentially present due to the origin of water and the biological treatment method involved. OM can be divided into dissolved organic carbon (DOC) and particulate organic carbon (POC) categories. Unfortunately, there is no commonly accepted understanding of the division size. For example, Levine et al. [1985] suggested 0.1 μm as the cut line, Metcalf and Eddy [1991] 1 μm , while Malpei et al. [1997] 0.45 μm . In this paper a 0.45 μm of cut line is considered because it is perhaps the most widely used in the literature.

POC includes zooplankton, algae, bacteria, and debris organic matter from soil and plants, and usually represents less than 10% of total OM. In water treatment POC is of lesser concern than DOC because solid-liquid separation processes can remove it relatively easily. DOC is more difficult to deal with, as it can impart many adverse effects on water quality, and so it remains the focus of this paper.

The major macromolecules are polysaccharides, proteins, lipids and nucleic acids. DOC in the 1,000 to 1,000,000 Da molecular weight (MW) range usually includes humic and fulvic acids. DOC with MW smaller than 1,000 Da mostly includes carbohydrates, amino acids (AA), vitamins, and chlorophyll. POPs, EDCs, and PPCPs such as dichloro-diphenyl-trichloroethane (DDT), polychlorinated biphenyl (PCB) and other toxic substances of public health significance consist of low MW compounds [Stull et al., 1996; Pempkowiak and Obarska-Pempkowiak, 2002; Leenheer and Croue,

Table 1. Conversion between nm and Da units

| Size (Da) | Size (nm) |
|-----------|-----------|
| 500* | 0.39 |
| 1,000* | 0.50 |
| 5,000* | 0.85 |
| 7,000* | 0.95 |
| 10,000* | 1.1 |
| 20,000* | 1.3 |
| 100,000 | 10 |
| 500,000 | 50 |

* The equation used to compute the size is: $\text{Size (nm)} = \frac{0.1 * (\text{MW})^{0.3321}}{2}$

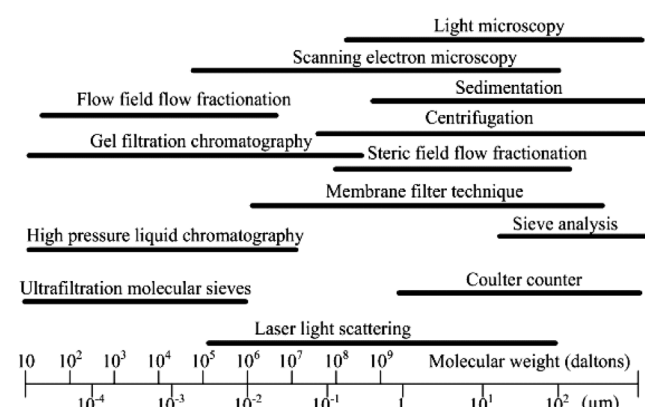


Fig. 3. Analytical techniques used for identification of the size of wastewater contaminants smaller than 100 μm .

2003].

OVERVIEW OF SIZE DISTRIBUTION

The two common units used to denote OM sizes are nm and Da. However, the conversion between these units is difficult due to the differences in organic geometric structures. Table 1 presents a practical conversion, based on Shon et al. [2006b], noting that other conversion methods are also available in the literature [Lentsch et al., 1993; Bowen and Mohammad, 1998; Singh et al., 1998; Combe et al., 1999].

Fig. 3 shows analytical methods and their range of application which are used to determine size distribution of OM. Evidently, alternative methods exist for both POC and DOC to determine size distributions in given ranges. For example, POC sizes can be determined by scanning electron microscopy (SEM), sedimentation, centrifugation, sieve, membrane filter technique and light scattering method. DOC can be analyzed by high-pressure size exclusion chromatograms (HPSEC), gel filtration, flow field flow fractionation (flow FFF), and ultrafiltration technique.

The conventional methods involved in POC size determination, such as sieve analysis, sedimentation analysis, centrifugation and membrane filter technique, are gradually being replaced by non-invasive methods which are based on light-matter interaction. In the case of DOC the conventional methods are ultrafiltration and gel filtration, which have been further developed to HPSEC and/or

substituted by flow FFF.

SIZE DISTRIBUTION OF POC

Size distribution of POC larger than 0.45 μm can be characterized by sieving, membrane filtration, steric field flow fractionation (FFF), particle counting, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Direct microscopic analysis can be used as an alternative way when POC sizes exceed 500 μm . POC of this range generally includes algae, protozoa, bacteria, organic debris from food and human waste and floc and/or aggregate of OM. Membrane filtration and FFF are also used in size distribution analysis of DOC.

SIEVING AND MEMBRANE FILTER ANALYSIS

Sieving and particulate membrane filter analysis are the oldest sizing methods. These both involve some kind of screens with uniform openings to separate different size fractions. Fractions of OM larger than screen openings are retained, and size distribution can be measured in terms of total organic carbon (TOC), biochemical oxygen demand (BOD), or chemical oxygen demand (COD).

Sieve analysis is usually used to measure the range of 5 μm to 10 cm. The standard methods of this technique can be found elsewhere [ISO, 1990; ASTM, 1995]. To analyze smaller size OM of POC, often series of polycarbonate membrane filters are used with pore sizes of 12, 8, 5, 3, 1 and 0.45 μm . The advantages of these methods are ease and affordability. The disadvantages of these techniques are that they are time consuming, require large sample volumes, and smaller pore sizes can interfere with correct separation due to sieve and membrane fouling, termed as self-rejection, [ASTM, 1995].

SEM AND TEM

Microscopic methods cover the size range of 0.001-200 μm . Based on the energy of involved electrons, and the way of electron collection, the methods are divided into TEM (0.001-5 μm) and SEM (0.02-200 μm) [Xu, 2000]. SEM probes the surface of particles which are first covered with a very thin layer of gold and then bombarded by electrons. TEM can show the internal structure of particles, and therefore provides a more detailed characterization. Particle shapes and sizes can be deliberately measured by using these techniques; however, they are costly and time consuming [Levine et al., 1985].

STERIC FFF

Separation in steric FFF is related to the physical properties of particle size, shape, density, and water viscosity in the 1-100 μm range. The theory of steric FFF is based on different elution mechanisms of OM in terms of sizes. The procedure utilizes the laminar flow which exists in the measuring channel, and POC is separated by size in the flow streamlines, like in chromatography [Tong and Caldwell, 1995]. Here, the diffusion of POC is negligible in retention. TOC and/or SEM can further analyze the separated POC. This technique is relatively new but well suited for POC size determinations.

PARTICLE COUNTER WITH ELECTRONIC PULSE

Particle counters using electronic pulses are widely used in laboratories. There are three instrumental counters, classified according to type of sensor involved: i) electrical sensing zone, ii) light blockage, and iii) light scattering [Eaton et al., 1995]. Size distribution of POC is measured by changes of the voltage, current, or resistance of electronic pulses.

When a particle moves through an orifice, it occupies some volume to cause a detectable change in an electrical field. The change of a current or resistance is proportional to the size of the particle. The measuring range starts from about 0.7 μm , and the maximum particle size is about 20% of an orifice diameter. The light blocking method consists of a measurement zone and a coupled photovoltaic cell. When POC passes through the sensor, the blockage of light produces voltage changes in the photovoltaic cell. With this method it is possible to measure the range from 0.1 μm to the employed orifice size. The light scattering method provides unique distribution patterns in terms of POC size. The measuring principle involves the shining of a laser (He-Ne) beam on POC. The diffraction light pattern is proportional to POC size, and correlated by the Fraunhofer or Mie theory. This theory concerns the refractive indices of the particles, of the dispersion media and the imaginary part of the refractive index of the particles. The range of the size measurement is from 0.02 to 2000 μm .

The disadvantages of these instruments are that i) gas bubbles and electronic noises cause interference, ii) the sample should be diluted, and iii) color interference in methods which involve light.

SIZE DISTRIBUTION OF DOC

In most waters DOC represent the dominant, larger part of OM. It is also recognized that a large amount of DOC is associated with vital or toxic chemicals. Aquatic humic substances contribute to over 50 percent of DOC in most natural waters, [Amy et al., 1987]. Also, DOC is less easily removed than POC by liquid separation processes.

DOC smaller than 0.45 μm can be separated by dialysis [Buffle et al., 1992], laser desorption Fourier transform mass spectrometry (LDFTMS) [Novotny et al., 1995], vapor pressure osmometry (VPO) [Aiken and Malcolm, 1987], ultracentrifugation [Reid et al., 1990], X-ray scattering [Thurman et al., 1982], ultrafiltration (UF) technique [Cai, 1999], flow FFF [Beckett et al., 1987], gel permeation chromatography (GPC) [Levine, 1985] and HPSEC [Zhou et al., 2000].

Table 2 summarizes the main characteristics of these analytical methods. Separation of DOC by dialysis is simple, with the main force being diffusion. However, it is also a time-consuming process, and the need for large sample volumes limits the application of this technique. LDFTMS, VPO and ultracentrifugation are rarely applied to size distribution determinations in water. In contrast, UF, HPSEC and flow FFF are widely employed; thus we focus on these methods.

ULTRAFILTRATION

The UF technique is affordable and thus widely used to separate species by molecular size, shape and charge. This method covers the range of MW distribution from less than 1,000 to more than

Table 2. Characteristics of methods used for the determination of DOC size distribution [adapted from Thurman et al., 1982; Levine, 1985; Aiken and Malcolm, 1987; Beckett et al., 1987; Reid et al., 1990; Buffle et al., 1992; Novotny et al., 1995; Cai, 1999; Zhou et al., 2000; Kim and Koo, 2002]

| Technical method | Advantage | Disadvantage |
|---------------------|--|---|
| Dialysis | <ul style="list-style-type: none"> - Simple application - Natural force (diffusion) | <ul style="list-style-type: none"> - Time consuming - Large sample volume required - Limited range of size distribution (2-5 nm) - Careful handling of membrane |
| LDFTMS | <ul style="list-style-type: none"> - Independent on the material being characterized - Relatively accurate size | <ul style="list-style-type: none"> - High power required - Concentrated samples required - Small MW measured |
| VPO | | <ul style="list-style-type: none"> - Limited range of size distribution - Yield only a number-average MW - Corrections for ionizable compounds |
| Ultracentrifugation | <ul style="list-style-type: none"> - Various molar mass (M_w, M_n, and M_z) | <ul style="list-style-type: none"> - Diffusion coefficient required - Swamping of charge effects; absorptivity varies with MW |
| UF | <ul style="list-style-type: none"> - Relatively inexpensive - nondestructive and reagent-free - Simple application - High reliability | <ul style="list-style-type: none"> - Effect of self rejection - Broad range of size distribution - Large sample volume required - Influence of ionic strength, pH and concentration polarization - Difficult separation with high concentration |
| Flow FFF | <ul style="list-style-type: none"> - Identification of interaction between membrane and OM | <ul style="list-style-type: none"> - Sorption on the membrane |
| HPSEC | <ul style="list-style-type: none"> - Small sample volume required - Specific range of size distribution - Automatic analysis - A number of compatibilities - Relatively inexpensive | <ul style="list-style-type: none"> - Errors due to chemical interactions among OM, column packing and eluent - Electrostatic interaction - Sorption - Calibration required - Specific analysis depending on detectors used - Effect of pH |

100,000 Da. UF involves the selective rejection of solutes by convective flow through a membrane. The targeted MW can be selected in terms of MWCO of membranes. Solute of larger than the specified MWCO are quantitatively retained, while solutes of smaller MW pass the membrane in the permeate.

The main phenomena involved in UF separation are advective flow and molecular diffusion. The solute flux is related to the area of membrane, concentration gradient and diffusion. Stevenson [1982] observed that the extent of interactions between OM and UF depended on concentration, ionic composition, presence of polyvalent cations and pH. Cai [1999] also reported that retention or rejection of solute was a function of MW size and shape, also influenced by a number of factors, such as solute concentration, ionic strength, concentration polarization and pH. Macko et al. [1979] suggested that pH and ionic strength should be held constant for uniform results. By contrast, Brock [1983] reported that varying pH, electrolyte concentration and pressure did not change the results, but OM concentration affected the solute retention. Increasing the OM concentration decreased the solute retention [Ogura, 1974]. Buffle et al. [1978] reported that an increase in the initial OM concentration resulted in an increase in the retention of large MW, but a decrease in the retention of small MW. Macko et al. [1979] investigated ways to reduce concentration polarization, and found that vigorous mixing

and dilution of the feed solution were advantageous. Brock [1983] suggested that the phenomena of the Donnan effect might lead to an unequal distribution of ions so that proteins may be unable to pass the membrane.

In general, this method is reproducible and accurate. Reproducibility can be maintained with proper cleaning, and accuracy can be improved by using larger sample volumes.

HPSEC

Since 1958 HPSEC, also known as gel permeation chromatography (GPC), and gel filtration chromatography (GFC), has been one of the most commonly used methods to determine size distribution of DOC. It is a versatile method having relative ease of application, modest equipment requirements, and ability to generate both average and distributed size information [Poole, 2003]. The origin of modern HPSEC can be traced to the introduction of cross-linked poly(dextran) and poly(saccharide) gels used for the size separation of water-soluble biopolymers and of semi-rigid, porous cross-linked poly(styrene) gels for the separation of organic polymers. These developments occurred in parallel with the separation of water-soluble biopolymers (GFC), mainly a concern of biochemists, and the separation of organic polymers (GPC) by polymer chemists. The

Table 3. Molecular weight statistics

| Reference | Calculation method |
|-------------------|---|
| Poole, 2003 | <p>Suitable terms are the number average MW,</p> $M_n = \sum_{i=1}^n (N_i M_i) / \sum_{i=1}^n (N_i)$ <p>the weight average MW</p> $M_w = \sum_{i=1}^n (N_i M_i^2) / \sum_{i=1}^n (N_i M_i)$ <p>or the z-average MW</p> $M_z = \sum_{i=1}^n (N_i M_i^3) / \sum_{i=1}^n (N_i M_i^2)$ <p>where N_i is the number of molecules having a molecular weight M_i and i is an incrementing index over all molecular weight present.</p> |
| Beri et al., 2001 | <p>The viscosity average molecular weight:</p> $M_v = \left[\sum_{i=1}^n (h_i M_i^{1+a}) / \sum_{i=1}^n (h_i M_i) \right]^{1/a}$ <p>where a is the Mark-Houwink exponent as defined as either prior knowledge of the Mark-Houwink constants or determination of a universal calibration curve with molecular weight standards and Peak maximum molecular weight (M_p), n is the number of chromatographic slices, h_i is the SEC curve height at the ith volume increment, M_i is the molecular weight of the species eluted in the ith retention volume increment.</p> |

division is redundant today, and size-exclusion chromatography is the preferred term for all separations resulting from the size-dependent distribution of sample molecules between a mobile phase and a porous stationary phase [Poole, 2003].

Solute separation in terms of MW distribution is based on the different abilities of the various solutes to enter the pores of the stationary phase via molecular diffusion. Solute of large MW cannot enter the stationary phase and moves quickly through the void volume of the bed, whereas solute of small MW can enter the gel pores; thus its movement through the column is retarded [Amy et al., 1987b].

Column materials for use in HPSEC are rigid and include carbohydrates, methacrylates, silicas and polystyrene. Silica, zirconium-stabilized silica, and controlled pore glass are extremely rigid materials that can withstand the relatively high pressures used in high performance liquid chromatography (HPLC). Carbohydrates and some polystyrenes are considered to be microporous, while silicas and some polystyrenes are regarded macroporous. Many researchers [Kainulainen et al., 1994; Shaw et al., 1994; Cho, 1998; Pelekani et al., 1999; Her, 2002; Shon et al., 2005] have used various silica-based gels in order to characterize water and wastewater samples. These include Sephadex, Waters Protein-Pak 125 and TSK gel. Chin et al. [1994] and Shon et al. [2004] also used a modified silica column (Protein-Pak 125, Waters Co.) to analyze MW distribution of DOC and weight-averaged MW values.

Standard solutions of different polystyrene sulfonates with known MW (PSS: 210, 1,800, 4,600, 8,000, and 18,000 Da) are typically used to calibrate the HPSEC equipment [Her et al., 2002]. The MW distribution is represented by a UV response (mV intensity) with time. Results can also be presented as normalized fraction percentages, obtained by dividing each incremental height of the chromatogram with a sum of the heights when the chromatogram was divided into incremental mass intervals [Cho et al., 2000; Lee et al., 2002].

The statistics of MW distribution by HPSEC have been well defined (Table 3). There are four average values of MW: i) M_n (num-

ber average MW), ii) M_w (weight average MW), iii) M_z (z-average molecular weight), and iv) M_v (viscosity average MW). Among these statistics of MW distribution, the weight average MW (M_w) is commonly used. The number average MW (M_n) is obtained by multiplying the number of chains of a certain length with their MW and adding this to the number of a second class of chain multiplied by their MW, and so on, then dividing by the total number of chains [Mulder, 1996]. The use of weight fraction ($N_i M_i$) instead of the number of MW (N_i) results in the weight average MW (M_w). A distribution can be expressed in terms of the polydispersity ($P=M_w/M_n$).

HPSEC separation is dependent on i) the degree and method of concentration of OM, ii) the type and grade of the gel, iii) the standard biochemicals or synthetic chemicals used to calibrate the column, and iv) the composition of the eluent. Cai [1999] suggested that significant errors might occur due to chemical interactions among the column packing, the eluent and the organic components. Further, the extent of interaction may be influenced by the degree of DOC concentration, the presence of polyvalent cations and the ionic strength [Amy et al., 1987b]. Amy et al. [1987b] also reported that this method was more profoundly affected by pH, suggesting that the UF method rather than HPSEC technique may be more appropriate when pH conditions are important. While comparing the MW distribution of DOC in raw waters and drinking waters, Nissinen et al. [2001] found that the HPSEC method was sensitive to different water sources.

Gjessing and Lee [1967] reported that the shapes and sizes of the small MW components could determine the extent of their penetration into the gel beads, leading to an underestimation of low MW components. Thurman and Malcolm [1979] observed that negatively charged MW traveled faster than non-charged DOC of similar size through the gel column. Increased pH increased the solubility of humic molecules as a result of ionization of functional groups, and reduced hydrogen bonding with the resin. Swift and Posner [1971] observed that adsorption between gel and DOC strongly correlated with the degree of gel cross-linking. Cameron et al. [1972]

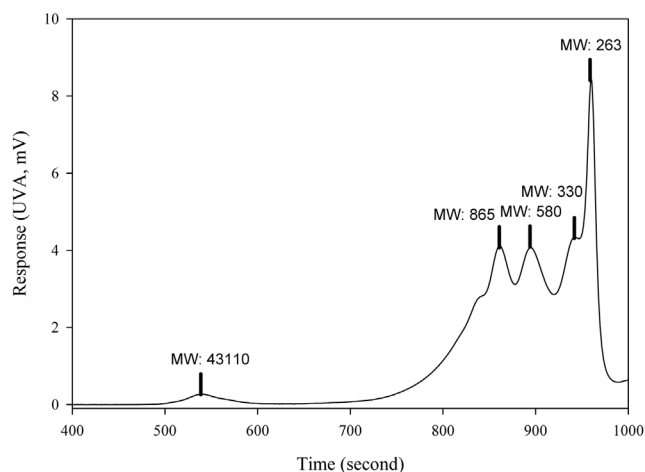


Fig. 4. MW distribution of EfOM in BTSE by HPSEC technique.

found that the behavior of humic acids on gels could not be predicted from the calibration curves obtained for proteins or dextrans.

MW distribution was rigorously related to hydrodynamic sizes of the OM rather than to MW [Stevenson, 1982]. Amy et al. [1987b] suggested that it is important to maintain the same experimental conditions in analyzing different water sources. Zhou et al. [2000] observed that this method did not always provide reproducible results for humic substances, showing 10–20% variability. While the method of baseline correction of large MW cutoff of the HPSEC chromatogram was not important, the choice of small MW cutoff could greatly affect M_n and P . Although the PSS standards are commonly used in combination with acetone, they suggested the inclusion of salicylic acid as a charged small MW standard. Also, they recommended UV detection wavelengths between 230 and 280 nm for reasonable results, noting that higher wavelengths could bias larger MW matter. In general, a 254 nm wavelength is suitable, except for samples of low carbon concentration where 230 nm provides better sensitivity. As such, they resulted in excellent reproducibility (2–3%) of M_n and M_w .

Fig. 4 shows a typical chromatogram for MW distribution of DOC. The MW of EfOM in BTSE ranged from 260 to about 43,110 Da, with the highest fraction being 263 to 870 Da. The compound of 43110 Da may be polysaccharide; 580 Da and 865 - humic substances; 330 Da - building blocks; 250 Da - acids, and less than 200 Da - amphiphilics [Huber, 1998].

COUPLING OF UV AND FLUORESCENCE DETECTORS

Since UV at 254 nm is preferentially absorbed by π -bonded molecules of organic matter, HPSEC with the UV detector is chiefly applied to MW estimations of humic and fulvic acids, and the aromatic hydrophobic portion of DOC. This has the limitation of detecting low UV-absorbing components, such as proteins and polysaccharides. Thus, fluorescence detectors are often employed to interpret protein-like substances. Fluorescence detectors are used at two wavelengths, excitation (279 nm) and emission (353 nm) [Her, 2002]. Leenheer and Croue [2003] classified more detailed major fluorescent components in excitation and emission in terms of different organic types. The analysis set-up of the assembly is pre-

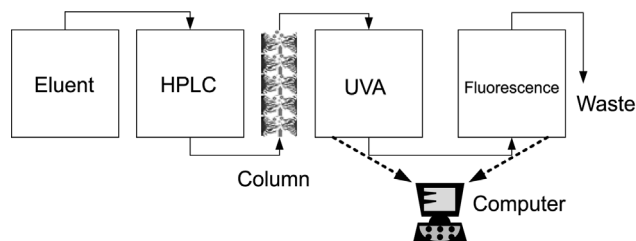


Fig. 5. Schematic diagram of HPSEC coupled with UV-fluorescence detector.

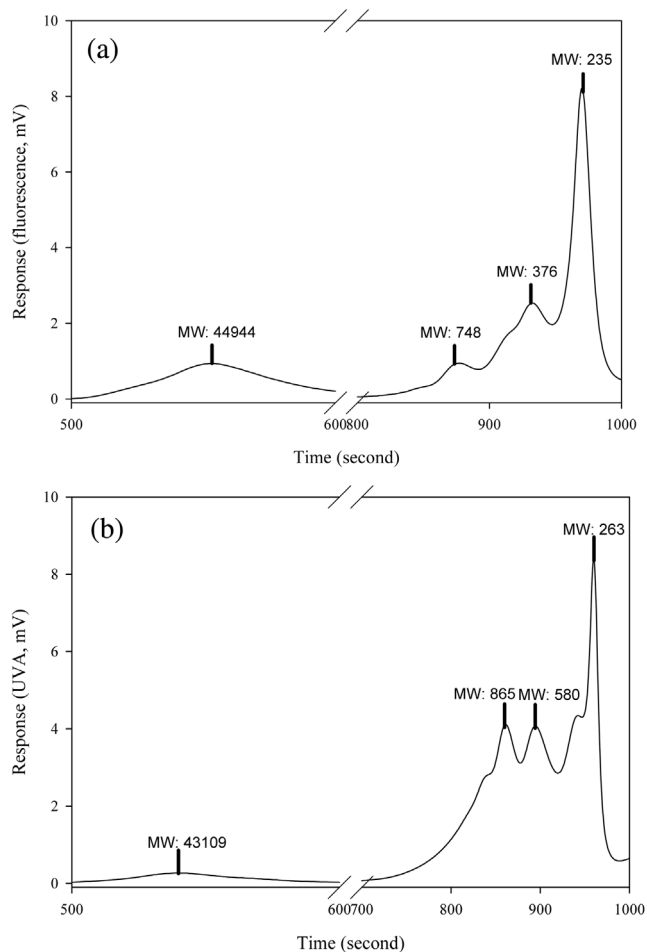


Fig. 6. Comparison of (a) fluorescence chromatogram and (b) UV chromatogram for MW distribution of EfOM with BTSE (initial DOC concentration=6.5 mg/L).

sented in Fig. 5. The calibration of the equipment is generally conducted with the same standards (PSS).

Fig. 6(a) shows a sample by the fluorescence chromatogram at 279 nm excitation and 353 nm emission. For comparison, the MW distribution by the 254 nm UV detector is also shown in Fig. 6(b). Fig. 6(a) detects the high responses at 44,944 and 235 Da, which could be protein-like substances. However, the MWs of 376 and 748 Da have low intensity, suggesting that these peaks may be due to humic substances (humic and fulvic acids).

COUPLING OF UV AND DOC DETECTORS

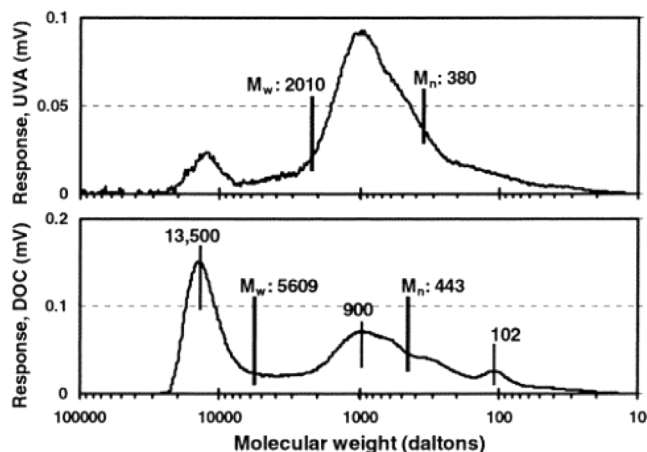


Fig. 7. HPSEC-UV-DOC chromatograms for Barr Lake in USA [adapted from Her et al., 2002].

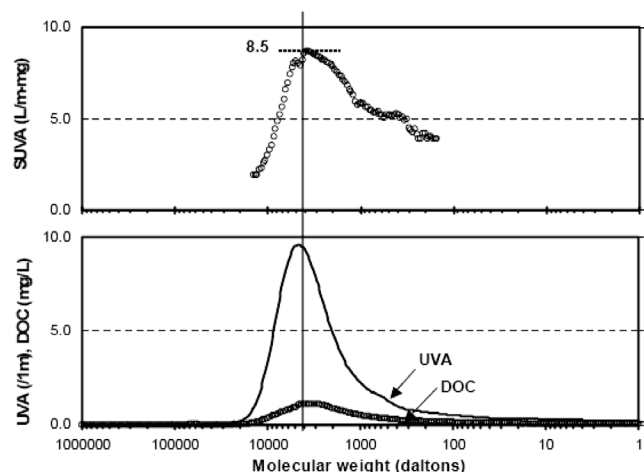


Fig. 8. SUVA values by HPSEC-UVA-DOC [adapted from Her, 2002].

HPSEC technique coupled with UV-DOC detector to provide qualitative information (e.g., a specific UVA (SUVA) chromatogram as a function of MW by the relative ratio between UVA at 254 nm and DOC) in addition to quantitative information on MW distribution. With an on-line DOC detector, all organic compounds can be recognized and the SUVA values can be monitored in real-time (Figs. 7 and 8). As can be seen in Fig. 7, the UV and the DOC chromatograms gave completely different results. The UV detector showed higher response at 900 Da than at 13,500 Da, while the DOC detector showed an opposite pattern. This indicates that the compound of 900 Da could be more aromatic or hydrophobic than the compound of 13,500 Da. Nonetheless, this system is not sufficient to identify chemical and physical properties of a particular DOC compound. The combination (HPSEC-UVA-Fluorescence-DOC) helps to discriminate DOC components such as specific biopolymer, aromaticity and DOC fraction. Huber [1998b] reported that this approach was successfully applied to other fields of water analysis, like highly mineralized table waters, marine waters, wastewaters and soil extracts.

UV ABSORBANCE RATIO INDEX (URI)

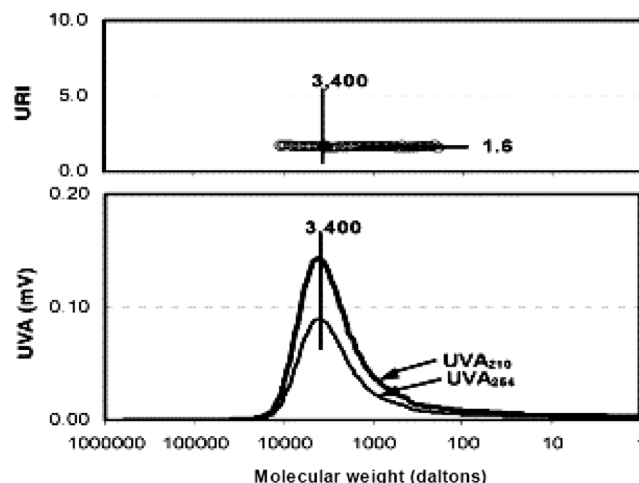


Fig. 9. URI and HPSEC-UV chromatograms for humic acid [adapted from Her, 2002].

URI is the ratio of UV absorbances at 210 nm and 254 nm (UVA_{210}/UVA_{254}), which provides qualitative information on the relative proportion between UV-absorbing functional groups and unsaturated compounds. On the one hand, unsaturated compounds effectively absorb UV light at both 254 and 210 nm wavelengths (Fig. 9). On the other hand, functional (amino) groups absorb UV light more effectively at 210 than at 254 nm. Therefore, a higher density of functional (e.g., amino) groups result in a higher absorption at 210 nm, and subsequently produces a higher URI. Her [2002] found that proteins and amino acids have higher URI than humic substances. The URI values are the lowest for humic acids with the highest aromaticity (1.59), medium for fulvic acids with intermediate aromaticity (1.88), and the highest for proteins of bovine serum albumin with lowest aromaticity (13.50). Therefore, the URI analysis effectively distinguishes protein-like substances from other DOC components. Fig. 9 presents HPSEC-UV chromatograms (at 210 and 254 nm) and URI values (UVA_{210}/UVA_{254}) for humic acids. A URI value at 3,400 Da indicates the presence of high density of UV absorbing functional groups. The URI value of 1.6 implies the lowest proportions of functional groups with the highest proportions of aromatic rings, which could be humic acids.

FLOW FFF

Flow FFF is a fractionation method based on a channel with walls consisting of a ceramic frit material. It has been traditionally developed to determine MW distribution, diffusivity and hydrodynamic sizes of DOC. The method does not require chromatography packing material. Steric FFF is used to measure POC sizes by collecting samples with retention time, while flow FFF measures DOC with combined UV absorbance and fluorescence detectors, in real-time.

There are two liquid flows in the flow FFF method: i) channel flow and ii) second cross-flow at 90° to the channel (Fig. 10). A semi-permeable membrane is placed on one side of the channel, allowing the cross-flow to pass but not the compounds of interest. Flow FFF separates DOC according to molecular sizes. Diffusion coefficients are calculated from the retention time and channel oper-

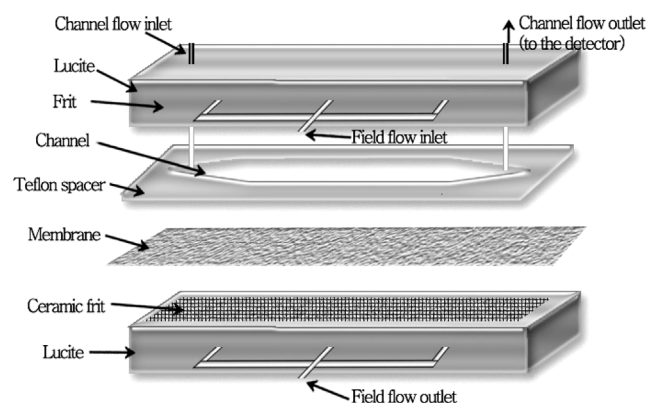


Fig. 10. Schematic diagram of a flow FFF channel [adapted from Assemi et al., 2004].

ating conditions. With appropriate standards such as PSS, MW distributions are determined [Zanardi-Lamardo et al., 2002]. A major advantage of this technique is the identification of the interactions between a semi-permeable membrane and DOC [Hartmann and Williams, 2002].

The flow FFF can be applied to environmental and biological matrices and to the detection of polymers and inorganic colloids [Gimbert et al., 2003]. Various detectors can be coupled, depending on the targeted DOC. In general, UV absorbance by DOC is monitored at 254 nm, and fluorescence is measured at excitation and emission wavelengths of 228 and 360 nm, respectively. Both the retention time and the peak area are obtained. This method covers the size range from about 500 Da to 1 μ m. The precisions for both detectors, after a flow FFF run, are from 1.4–2.5%, based on

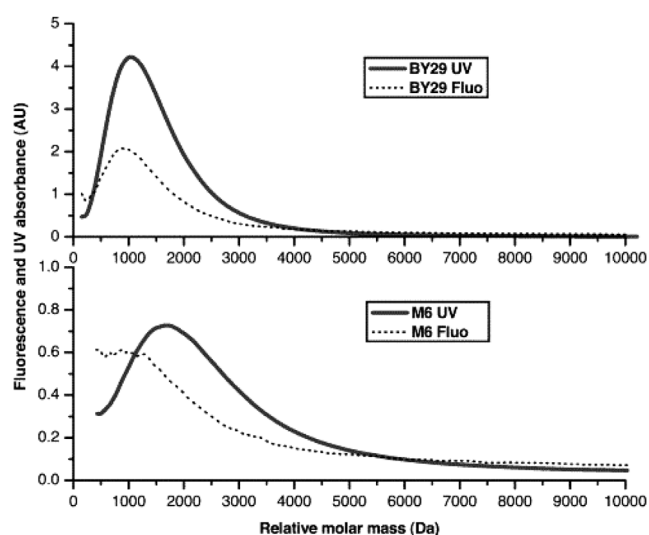


Fig. 11. Relative molar mass distributions of fluorescence and UV absorbance from two contrasting surface water samples from station marine (M6) and brackish (BY29) [adapted from Hassellöv, 2005].

the variation of the retention time, and 3.3–3.9%, based on the peak areas [Zanardi-Lamardo et al., 2001]. A method of characterizing DOC by asymmetrical flow field-flow fractionation with on-line UV and DOC detection is described and applied to standards and natural water samples [Reszat and Hendry, 2005]. MW determinations in the samples and standards were 6–30% lower with DOC analysis than with UV analysis. This difference was due to the insensitivity of the latter technique to non-aromatic carbon, suggesting

Table 4. Environmental applications [adapted from Gimbert et al., 2003]

| Analyte | Crossflow | Membrane | Carrier liquid | Detector |
|---|-------------------|--|---|------------------------------|
| Colloids (in coastal seawater) | Recirculating | Regenerated cellulose, 10,000 Da nominal MWCO | Seawater with addition of biological non-ionic surfactant (Pluronic F68) to final concentration of 0.1% (v/v) | UV (254 nm) |
| Dissolved organic material (colored, in river and coastal waters) | Recirculating | Regenerated cellulose, 3000 Da nominal MWCO for globular compounds (FFFractionation) | 0.005% FL-70, 0.05 M Trisma and 0.029 M HCl prepared in organic-free distilled water, to give pH 8 and ionic strength of 0.08 M | UV (330 nm) and fluorescence |
| Dissolved organic carbon (in fresh and marine waters) | Non-recirculating | Modified polyether sulfone Membrane (Omega), 1000 MWCO optimum | (i) 25 mM Tris, 20 mM sodium chloride (ii) 10 mM borate, 20 mM sodium chloride - optimal carriers | UV (270 nm) |
| Dissolved organic matter (pulp and paper mill effluents) | Non-recirculating | Cellulose acetate, (manufactured in laboratory) | Distilled deionised water with 0.05 M tris buffer adjusted to pH 8.0 by addition of HCl. Ionic strength ~0.03 M | UV (254 nm) |
| Dissolved organic matter (in seawater) | Recirculating | Regenerated cellulose (YM-10, Amicon), 10,000 Da nominal MWCO | UV-oxidised seawater | UV and fluorescence |

that MW determined with the DOC detector is a more accurate representation of the actual MW of DOC. A normalized intensity comparison method was applied to yield an average aromatic content of the bulk DOC and to detail the aromatic content over a range of MW.

Table 4 summarizes the application of flow FFF to environment matrices. In this method regenerated cellulose membrane are used most often, with UV detection being in the range of 254 nm to 330 nm. Fig. 11 shows relative molecular weight (RMM) distributions of surface water samples by fluorescence and UV detectors. The representative chromatogram of flow FFF is similar with that of HPSEC.

The coupling of flow FFF with other detectors, such as flow injection-spectrophotometry incorporating selective derivatisation reactions and inductively coupled plasma mass spectrometry (ICP-MASS), will produce novel multi-dimensional information [Gimbert et al., 2003].

CONCLUDING REMARKS

Size distribution is an important analytical tool to characterize OM. For size distribution of POC (above 0.45 μm), sieving, membrane filtration technique, steric FFF, particle counting with electronic pulse, SEM and TEM are available. Light scattering techniques, such as particle counting, are replacing the conventional methods of sieving and membrane filter technique for size distribution of POC.

Size distribution of DOC has drawn more attention than that of POC. This is partly because DOC imparts many adverse effects to water, and partly because solid-liquid separation processes more easily remove POC. For size distribution determinations of DOC the available methods include dialysis, LDFTMS, VPO, ultracentrifugation, X-ray scattering, ultrafiltration, flow FFF, GPC, and HPSEC. The conventional methods of ultrafiltration and GPC have gradually been replaced by flow FFF and HPSEC with coupled detectors. The use of HPSEC is widespread for several reasons, including small sample volumes, specific range of size distribution, automatized analysis, and relatively small costs. Still, HPSEC has limitations, but it has been improved by coupling it with other analytical methods, such as fluorescence and UV absorbance ratio index (URI), resulting in better reproducibility. A more deliberate classification of OM with real time is also possible. However, chemical interactions among OM, column packing, and eluent should be carefully considered. Although HPSEC characterizes size distribution of most OMs, it cannot detect emerging compounds such as POPs, EDCs, and PPCPs.

Flow FFF is becoming increasingly important with the wider use of membrane technologies. With this method, it is possible to investigate the fouling interactions between DOC and targeted membranes.

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