

High Performance Size Exclusion Chromatography for NOM Characterization

Introduction

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

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

Background on HPSEC Methods

Conditions used for HPSEC-OCD

Group	Jekel	Huber	Kawasaki	Heitz			UMass
Column	Toyopearl HW50	TKS HW50S	TSKgel G3000SW _{XL}	Toyopearl HW50s			Biobasic SEC60
Dia (mm)		20		20			
Length (mm)		250		250			

Manufacturer	Tosoh Biosc.	Tosoh	Tosoh	Tosoh			Thermo electron
OCD	Huber	Huber	Shimadzu	(MS)			Sievers
Eluent A	pH 6.85 8.4 mM HPO ₄ 18.4 mM H ₂ PO ₄	pH 6.85 8.4 mM HPO ₄ 18.4 mM H ₂ PO ₄	pH 6.8 20 mM H _x PO ₄	pH 6.85 10 mM HPO ₄ 10 mM H ₂ PO ₄			pH 7.0 100 mM H _x PO ₄
Flow (mL/min)	1.0	1.1	0.5	1.0			1.0
Inj Vol (uL)	1000 or 100?	1000	100				
OC _{max} (min)	52	45					
End (min)	120	100					20

Ruhl and Jekel (2012) found that elution of low MW compounds (32-254 daltons) was spread out over the chromatogram from the location of a typical NOM peak to the full inclusion volume and beyond. Much of this suggests hydrophobic retention. The polycarboxylic aromatics eluted toward the end of the NOM profile. Many low MW compound showed dual peaks. Phenolics and aliphatics were often retained more than the polycarboxylic aromatics.

UMass Method for HPSEC/UV

High performance size exclusion chromatography (HPSEC) is used to obtain the apparent molecular weight distribution (AMWD) of natural organic matter (NOM). At UMass, we use an HPLC system (Waters 2690: Separations Module) with an auto sampler and a photodiode array detector (PDA, Waters 996) to separate and detect NOM of different molecular weight (or size) range in water samples. The autosampler, PDA data acquisition and absorbance chromatograms are controlled by Empower2 software (Waters Associates, Milford, MA). For HPSEC analysis, a ThermoElectron BioBasic SEC60 column, with pore size 60 Å, pore volume 0.7 ccg⁻¹ and particle size 5 µm, is used. The column consists of 5 µm silica particles, coated with a hydrophilic polymer to ensure separation solely based on solute size. A solution of 0.1M KH₂PO₄ in DI water adjusted to pH 7.0 with 50% NaOH is used as carrier solvent. A flow rate of 1.0 mL/min was used.

The relationship between AMW and elution time for HPSEC results must be determined by calibration using compounds of known MW. Because the separation process in HPSEC depends on factors such as the shape, charge, etc. of molecules, in

addition to MW, different calibrations are obtained when different materials are utilized. In work at UMass we have examined calibrations for two different sets of compounds. In one case the AMW for the HPSEC column effluent was calibrated with p-amino benzoic acid (PABA, MW 137.14 Da); bovine serum albumin (BSA, MW 66,432 Da); and 4300, 13000 and 32000 Da MW polystyrene sulphonate sodium salts (PSS) (Fluka Analytical, Buchs, Switzerland)) prepared at 0.1 g/L concentrations in the carrier solvent. The PSS standards were detected at 224 nm and all the other standards and NOM compounds were detected at 254 nm wavelength. The calibration curve obtained can be characterized by a second order equation of the form $\log(\text{MW}) = a + b(t) + c(t)^2$, where MW = the molecular weight; and t = the peak elution time. Chow et al. (2008) used PSS to calibrate AMW in the range of 50 to 50000 Da for HPSEC analyses of NOM using similar eluent conditions (but a different column). We have also determined a calibration curve based on uracil (MW = 112), a reference fulvic acid from IHSS (assumed MW of 15,000 at peak), a reference humic acid from IHSS (assumed MW of 30000 at peak), and BSA. Results for this set of compounds yield a linear calibration equation of the form $\log(\text{MW}) = a + b(t)$. It is important to realize that the AMW scale is very much “*apparent*” MW and is best used for comparative consideration within a set of results. Interlaboratory comparisons can only really be made for results obtained by the exact same experimental conditions (column, eluent, flow rate, detector, calibration compounds, etc.).

The ionic strength and pH of all the water samples are pre-adjusted to make them similar to the HPSEC carrier solvent; 136 mg of KH_2PO_4 is added to 100 mL of each of the samples, and the pH is adjusted to 7 by addition of 50% NaOH solution. Each sample is filtered with 0.45 μm Whatman GF/F filters prior to HPSEC analysis.

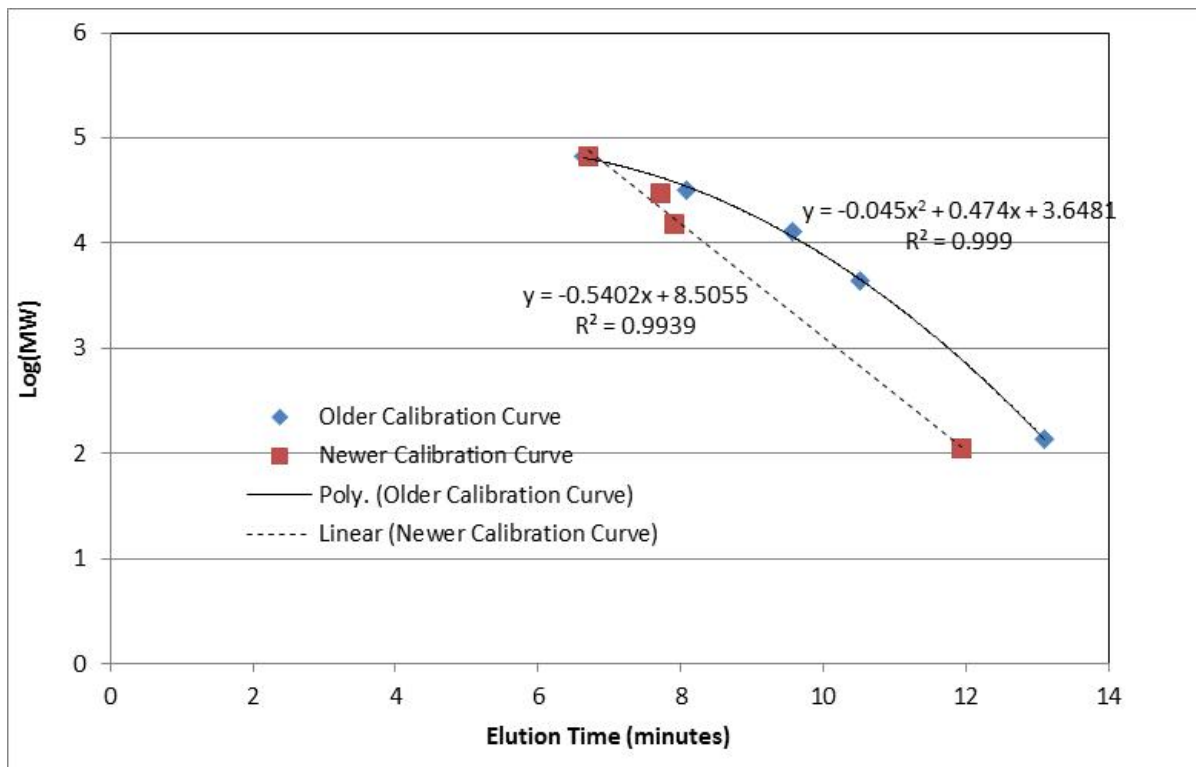


Figure 1: Typical AMW calibration for HPSEC analysis (UMass, ca. 2009)¹

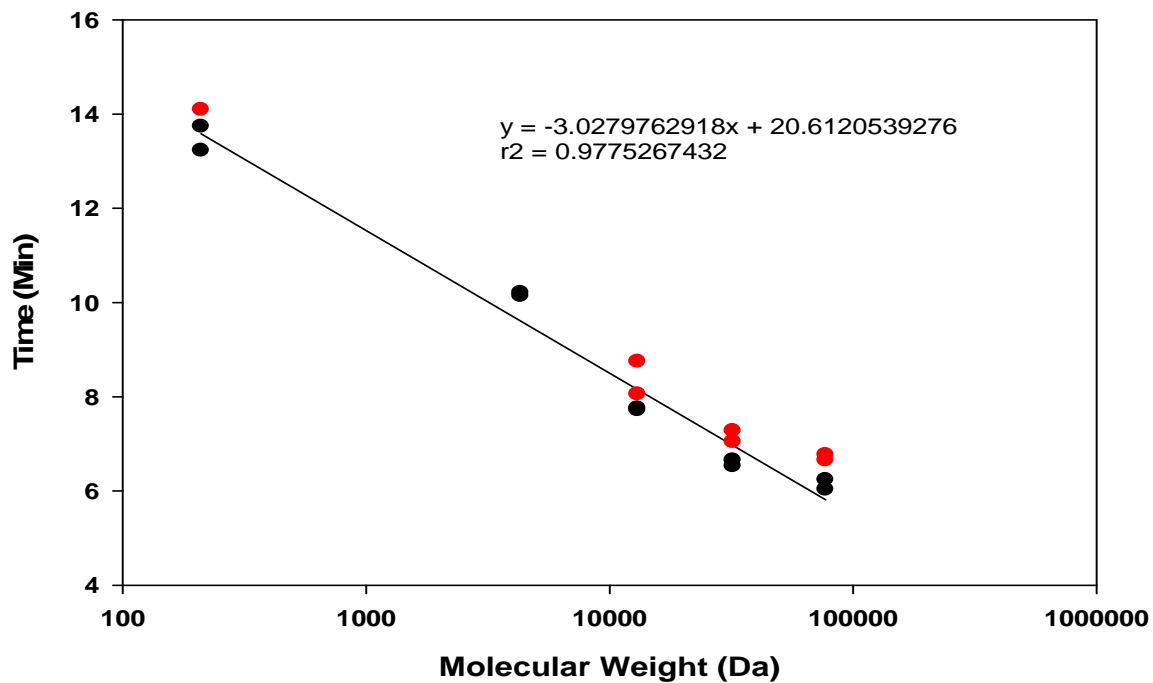


Figure 2. Calibration Curve from December 2011 (prepared by Larry Kramer)²

¹ This may have been prepared using a different column/program than the one described above.

² Prepared using the procedure described above (SEC60 column)

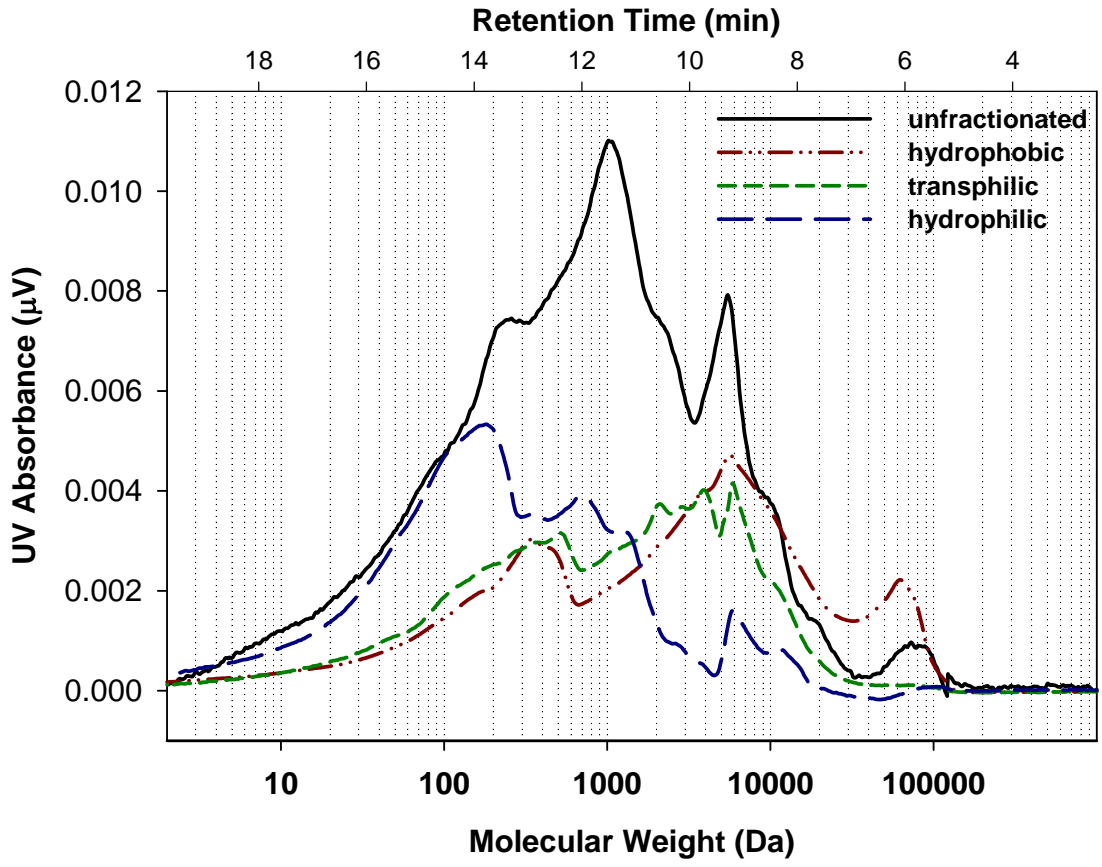


Figure 3: Example HPSEC/UV Data from Oak Bluffs Project (Kramer, December 2011). Note reverse elution presentation.

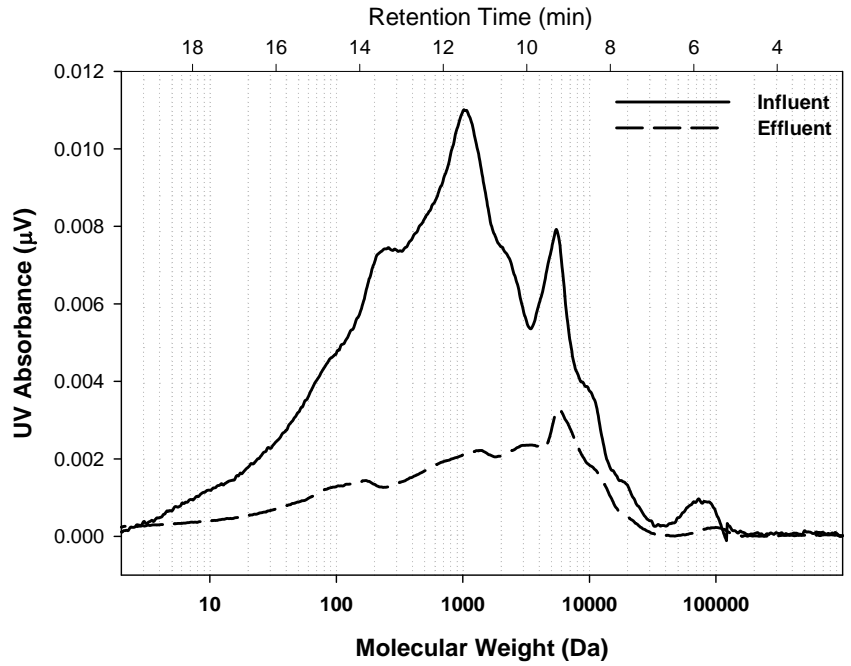


Figure 4: Example HPSEC/UV Data from Oak Bluffs Project (Kramer, December 2011)

Method of **Francisco J. Rodríguez, Luis A. Marcos and Luis A. Núñez**

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Molecular weights of humic substances were determined using high-performance size exclusion chromatography (HPSEC) (Zhou et al., 2000). HPSEC instrumentation (LDC Analytical) comprised of a solvent pump, a variable wavelength detector and a rotary injector valve equipped with a 20 μ L sample loop. A column (Waters Ultrahydrogel 250) packed with hydroxylated polymethacrylate-based gel was selected for HPSEC because of its minimal nonsize exclusion effects. Mobile phases used in this study were comprised of Milli-Q water buffered with phosphate to a pH of 7.0, and sodium chloride was added to yield an ionic strength equivalent to 0.1M NaCl. Humic substances were detected at a wavelength of 224 nm. A range of sodium polystyrene sulfonate (PSS) macromolecules (1K, 5K, 13K, 20K) were used as standards in this study. Some investigators have shown that globular proteins standards tend to overpredict the molecular weight of humic substances and have advocated the use of sodium polystyrene sulfonate macromolecules as standards (Chin et al., 1994). PSS macromolecules are more flexible than aquatic humic substances but several authors have demonstrated that a mobile-phase composition with an ionic strength equivalent to 0.1M NaCl can be used with PSS standards because their coiled configuration (Fuoss effect) appeared to be nearly identical to that of aquatic humic substances.

1. Chin, Y.P. et al. "Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances". *Environ. Sci. Technol.* **28**:1853 (1994).
2. Zhou, Q. et al. "Considerations in the use of high performance size exclusion chromatography (HPSEC) for determining molecular weights of aquatic humic substances". *Wat. Res.* **34**:3505 (2000).

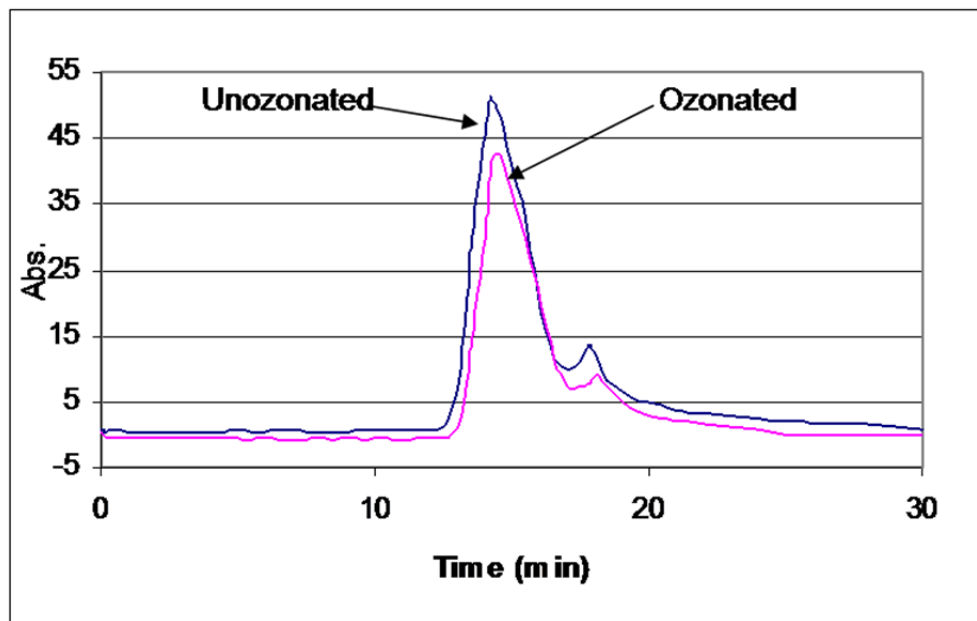


Figure 2. HPSEC chromatogram of natural fulvic acids

References

- Mousset, B, Croué, J.P, Lefebvre, E. and Legube B. (1997) Distribution and Characterization of Dissolved Organic Matter of Surface Waters. *Water Research* 31(3): 541-553.
- Rainer, M.W.A, Benner, R. (1996) Bacterial Utilization of Different Size Classes of Dissolved Organic Matter. *Limnol.Oceanogr* 41:41-51.
- Egeberg, P.K, Eikenes, M and Gjessing, E.T (1998) Organic Nitrogen Distribution in NOM Size Classes. *Environmental International* 25 (2/3): 225-236.
- Aouabed, A, Ben Aim, R and Hadj-Boussaad, D.E. (2001) Extraction and Characterization of Organic Matter from Surface Waters (reservoir at keddara in Algeria). *Environmental Technology* 22(5): 597-606.
- Gang, D, Clevenger, T.E, and Banerji, S.K. (2002) Relationship of Chlorine Decay and THMs Formation to NOM Size. *Journal of Hazardous Materials* 96(1): 1-12.

- Allpike, B.P., A. Heitz, C.A. Joll, and R.I. Kagi. 2007. A new organic carbon detector for size exclusion chromatography. *Journal of Chromatography A* 1157:472-476.
- Baghoth, S.A., S.K. Sharma, M. Guitard, V. Heim, J.P. Croue, and G.L. Amy. 2011. Removal of NOM-constituents as characterized by LC-OCD and F-EEM during drinking water treatment. *Journal of Water Supply Research and Technology-Aqua* 60:412-424.
- Huber, S.A., A. Balz, and M. Abert. New method for urea analysis in surface and tap waters with LC-OCD-OND (liquid chromatography-organic carbon detection-organic nitrogen detection). *Journal of Water Supply Research and Technology-Aqua* 60:159-166.
- Huber, S.A., A. Balz, M. Abert, and W. Pronk. 2011. Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography - organic carbon detection - organic nitrogen detection (LC-OCD-OND). *Water Research* 45:879-885.
- Hutta, M., R. Gora, R. Halko, and M. Chalanyova. 2011. Some theoretical and practical aspects in the separation of humic substances by combined liquid chromatography methods. *Journal of Chromatography A* 1218:8946-8957.
- Kawasaki, N., K. Matsushige, K. Komatsu, A. Kohzu, F.W. Nara, F. Ogishi, M. Yahata, H. Mikami, T. Goto, and A. Imai. 2011. Fast and precise method for HPLC-size exclusion chromatography with UV and TOC (NDIR) detection: Importance of multiple detectors to evaluate the characteristics of dissolved organic matter. *Water Research* 45:6240-6248.
- Lankes, U., M.B. Mueller, M. Weber, and F.H. Frimmel. 2009. Reconsidering the quantitative analysis of organic carbon concentrations in size exclusion chromatography. *Water Research* 43:915-924.
- Ruhl, A.S., and M. Jekel. 2012. Elution behaviour of low molecular weight compounds in size exclusion chromatography. *Journal of Water Supply Research and Technology-Aqua* 61:32-40.
- Warton, B., A. Heitz, B. Allpike, and R. Kagi. 2008. Size-exclusion chromatography with organic carbon detection using a mass spectrometer. *Journal of Chromatography A* 1207:186-189.
- Yan, M.Q., G. Korshin, D.S. Wang, and Z.X. Cai. 2012. Characterization of dissolved organic matter using high-performance liquid chromatography (HPLC)-size exclusion chromatography (SEC) with a multiple wavelength absorbance detector. *Chemosphere* 87:879-885.