Evaluating the size dependence of metal-organic matter complexation: quality control considerations

Authors: Erica R. McKenzie, Thomas M. Young*

Department of Civil and Environmental Engineering
University of California, Davis
One Shields Avenue
Davis, CA, 95616

*Corresponding author:
email: tyoung@ucdavis.edu
Phone: (530) 754-9399
Fax: (530) 752-7872

email addresses of other authors:
ermckenzie@ucdavis.edu
Abstract

Size exclusion chromatography (SEC), which separates molecules based on molecular volume, can be coupled with online inductively coupled plasma mass spectrometry (ICP-MS) in order to explore metal-natural organic matter (NOM) complexations. In order to effectively use this dual detector system, the operator should be mindful of quality control measures. C, Al, Cr, Fe, Se, and Sn all exhibited columnless attenuation, which indicates unintended interactions with system components. ICP-MS measured elements were evaluated based on signal-to-noise ratio and peak reproducibility between duplicate analyses. Among the environmental samples used in this study, consistent peak time and height were for Mg, Cl, Mn, Cu, Br, and Pb. Al, V, Fe, Co, Ni, Zn, Se, Cd, Sn, and Sb were less consistent over all, however did produce consistent measurements in select samples. Sample separation and storage were also evaluated. Ultrafiltering and centrifuging produced similar peak distributions, but glass fiber filtration produced more high molecular weight peaks. Storage in glass also produced more high molecular weight peaks than did plastic bottles.

Introduction

Size exclusion chromatography is a liquid chromatography technique that separates molecules based on molecular volume, and it has been applied to analysis of natural organic matter (NOM; e.g., Cabaniss et al. 2000; Her et al. 2002). SEC results have been found to be highly dependent on the chromatography system, the standards employed, and the data processing approach (Zhou et al. 2000); owing to these discrepancies, SEC is widely described as semi-quantitative. Though typically coupled with UV-visible absorbance detection (λ=254 nm; Zhou et al. 2000), analytical power has been increased via online-coupling with other analytical techniques including fluorescence (Huber and Frimmel 1992; Her et al. 2003), total organic carbon (Her et al. 2002), and inductively coupled plasma mass spectrometry (ICP-MS) (Schmitt et al. 2001; Casartelli and Miekeley 2003).

Aromatic organic carbon and co-eluting metals can be detected with dual online detectors – UV-visible absorbance (λ=254 nm) and ICP-MS, respectively. Conventional sampling collection and processing wisdom prescribes different practices for the analysis of organics compared with metals. Chief among these differences are that metal or glass instruments/containers are typically employed when trace organics/NOM are the constituents of interest, while plastic is typically used when metals are the constituents of interest. In each case, the material is selected to minimize sources and sinks for the constituents of interest. Additional challenges arise when both organics and metals are to be analyzed in a single sample.

The objective of this work is to investigate quality control factors in an SEC system with dual UV-visible absorbance and online ICP-MS detectors (SEC – ICP-MS), and to consider how sample processing will influence SEC – ICP-MS results.

Methods

Metal complexation dependence on NOM molecular weight was investigated using size exclusion chromatography (SEC) with dual online detectors: diode array detector (DAD) and inductively coupled plasma mass spectrometer (ICP-MS). SEC measurements were made using
a Phenomenex s2000 column (300 x 4.6 mm) with a mobile phase containing phosphate buffer (4 mM NaH$_2$PO$_4$) with NH$_4$NO$_3$ for additional ionic strength (0.1 total ionic strength); pH adjusted to 7.0 and pumped at 0.8 mL min$^{-1}$. The sample injection volume was 100 µL. An Agilent 1200 HPLC system was used with a DAD which was simultaneously monitoring $\lambda$ = 210, 230, 254, and 280 nm; unless otherwise noted, data evolution and presentation is for response at 254 nm, as recommended by Zhou et al. (Zhou et al. 2000). ICP-MS analysis was conducted on an Agilent 7500i, and the following elements were analyzed (m/z monitored): C (13), Mg (24), Al (27), Cl (35), V (51), Cr (52, 53), Mn (55), Fe (57), Co (59), Ni (60), Cu (63, 65), Zn (64, 66), As (75), Se (77, 82), Br (79), Cd (111), Sn (118), Sb (121), and Pb (208). Pd (105) and Pt (195) were previously investigated and no peaks were observed. The column eluent was divided after the DAD, prior to the ICP-MS, so that 0.4 mL min$^{-1}$ was retained for ICP-MS analysis, and the other 0.4 mL min$^{-1}$ was wasted or collected for future work. Prior to sample analysis, SPEX 2A standards, containing 27 elements were ICP-MS analyzed, and 18, 8, 4.6, and 1 kDa sodium polystyrene sulfonate (PSS), as well as salicylic acid (138 Da), were included as molecular size standards with charge and coiling properties similar to those of NOM (Zhou et al. 2000).

To avoid clogging the column, large particulate matter is removed either via flow through centrifuge (calculated particle size cutoff 0.3 µm), ultrafiltration (Pall, Microza Polysulfone, 10 kDa) or glass fiber filter (0.7 µm). Sample ionic strength and solution chemistry were adjusted to closely match that of the mobile phase by adding the appropriate volume of 10x mobile phase stock; sample pH was adjusted to 7.0. Samples were analyzed in duplicate; for each sample 1800 absorbance data points (per wavelength) and 330 elemental concentration data points (per element) were collected. Small solvent peak/dip was observed in the DAD data for MW ~ 1500-2400 Da, making this MW range less sensitive for the detection of OM, however detected positive peaks were typically much larger than the solvent dip; this region was linearly interpolated to overcome the solvent peak. ICP-MS data was observed to be fairly noisy, especially for elements that were present in low concentrations; to more clearly visualize peaks, a five-point moving average was used to smooth elemental chromatograms. The results were baseline corrected (least squares linear fit) to improve consistency (Zhou et al. 2000) and the results of the duplicate runs were averaged. ICP-MS counts per second data were converted to ppb using slopes derived from the analysis of bulk multi-element standards. Per Zhou et al. (2000), peak lower molecular weight cutoffs were set to 2% peak height or 100 Da, and high molecular weight cutoffs were set to 1% peak height or 300 kDa.

**Results and Discussion**

**Signal attenuation in a columnless system**

In an ideal chromatography system, constituents only interact with the stationary phase, and not with any other system components. To determine whether unwanted interactions occurred in the system, all samples were injected when the column was absent from the system, and their “elution” was monitored. The distribution of the elemental columnless peak elution times is shown in figure 1. Interactions with the other system components results in a retarded columnless elution. In the case of SEC, this might bias the measured molecular weights downward. C, Al, Cr, Fe, Se, and Sn were all periodically retarded in the columnless system – with the SEC column in place, these elements may co-elute with smaller molecules. The current
study was not able to determine what caused these elements to be inconsistently retarded, however the implications of this retardation should be kept in mind as the elemental-OM size distribution is measured.

Figure 1: Columnless elution distribution (n=32)

Control and duplicate analysis

Buffer controls and “injectionless” samples did not display any obvious elemental peaks. Duplicate analysis results varied by the element. All elements were evaluated based on three criteria: signal-to-noise which was operationally defined as the 95th percentile (signal) divided by the 20th percentile (noise); reproducibility of peak chromatogram location; reproducibility of peak response (height). The latter two metric were evaluated qualitatively compared to all elements included in the study, and results are shown in table 1. Peak placement was more reproducible than peak height.
Elements such as Mg, which had isolated clearly defined peaks with minimal noise, had very consistent peak elution times (typically identical). Even among the most consistent element, peak height varied noticeably (~20%). A sample duplicate chromatogram is shown in Figure 2. Mg, Cl, and Br were the most consistent, followed by Mn, Cu, Pb, Al, V, Ni, and Cd. Some elements such as Al, Cd, and Ni were consistent in their elution timing, but less consistent in the peak height.

### Table 1: evaluation of element quality control measures

<table>
<thead>
<tr>
<th>Element</th>
<th>Signal/noise</th>
<th>Duplicate analysis</th>
<th>Peak timing</th>
<th>Peak height</th>
</tr>
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<tbody>
<tr>
<td>C</td>
<td>13</td>
<td>Poor</td>
<td>Poor</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>2394</td>
<td>Good</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>22</td>
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<td>Mediocre</td>
<td></td>
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<tr>
<td>Cl</td>
<td>224</td>
<td>Good</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>25</td>
<td>Good</td>
<td>Mediocre</td>
<td></td>
</tr>
<tr>
<td>Cr (52)</td>
<td>27</td>
<td>Poor</td>
<td>Poor</td>
<td></td>
</tr>
<tr>
<td>Cr (53)</td>
<td>15</td>
<td>Poor</td>
<td>Poor</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>49</td>
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<td>Good</td>
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<tr>
<td>Fe</td>
<td>16</td>
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<td></td>
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<tr>
<td>Co</td>
<td>28</td>
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<tr>
<td>Ni</td>
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<td></td>
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<tr>
<td>Cu (63)</td>
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<td>Poor</td>
<td></td>
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<td>Zn (64)</td>
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<td>Zn (66)</td>
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<td></td>
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<tr>
<td>As</td>
<td>17</td>
<td>Poor</td>
<td>Poor</td>
<td></td>
</tr>
<tr>
<td>Se (77)</td>
<td>7</td>
<td>Poor</td>
<td>Mediocre</td>
<td></td>
</tr>
<tr>
<td>Br</td>
<td>38</td>
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<td>Good</td>
<td></td>
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<tr>
<td>Se (82)</td>
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<td>Poor</td>
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<td></td>
</tr>
<tr>
<td>Cd</td>
<td>18</td>
<td>Good</td>
<td>Mediocre</td>
<td></td>
</tr>
<tr>
<td>Sn</td>
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<td></td>
</tr>
<tr>
<td>Sb</td>
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<tr>
<td>Pb</td>
<td>185</td>
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</tbody>
</table>

* Average P(95)/P(20)

Bold values indicate comparatively strong performance
Figure 2: Sample chromatogram for duplicates (Al from an urban creek; centrifuged and stored in plastic)

**Total detected signal**

To assess the ability of the system to produce quantitative results, identified peak area was compared to several other measures. Working backwards, the background subtraction and peak identification software was validated by comparing total peak area (limited to areas of the chromatogram identified as peaks) with total area (all chromatogram areas). For most elements, the results were strongly linearly related. Elements with low signal to noise ratio, most notably Cr, were less strongly related, which is to be expected. When this relationship was restricted to UF samples and elements with associated standards, the slope and $R^2$ were both near unity ($m=0.9618$ and $R^2=0.9999$). Identified peak area was also compared to the columnless injection detected area. Similar to the previous comparison, the relationship was improved when it was restricted to UF samples, however the overall $R^2$~0.75. Individual element peak area-columnless area typically were poorly related ($R^2$<0.5) except Br which demonstrated moderate agreement ($R^2=0.79$). Centrifuged and glass fiber filtered samples are likely to contain larger colloids that may be removed by the pre-column (or column) and that would not be observed at the detector. As shown below, even the UF samples contained macro-molecules/colloids at least up to 300 kDa, so this situation may also apply, but to a lesser extent, with the UF samples.

SEC identified peak area and columnless areas were also compared to bulk element concentration, and the results are shown in figure 3; a 1:1 line is included for comparison. Columnless peak-bulk concentration resulted in a strong relationship ($R^2$~0.92), however with a slope ~0.5 (unadjusted for dilution and sampling frequency however fit was not improved by accounting for them). A likely explanation for the consistent yet not equal concentration
measurements using these two methods is that the standard calibration for the bulk samples includes an intercept, while only the standard calibration slope is used in the case of SEC concentrations. This employment of a standard calibration intercept resulted in 0-value bulk measurements, which are graphically displayed as 0.001 ppb. The use of the standard calibration intercept with the SEC data was explored, however this resulted in too many 0-value time points, even when obvious peaks were present in the raw data.

Figure 3: Relationship between bulk concentration and detected areas with/without SEC column

Sample processing and storage

In this study, eight storm event samples were collected, and were subsequently processed with a flow-through centrifuge (13,000 Gs; expected particle retention for \(d_p>0.3\ \mu m\)) with subsequent ultrafiltration (Pall, Microza Polysulfone, 10 kDa) or glass fiber filtration. Storm event subsamples (various time points) were stored in either polyethylene or glass containers. For SEC analysis purposes, all samples were transferred to glass autosampler vials.

In order to holistically evaluate the effect of sample processing and storage, all identified sample peaks (regardless of peak size) were plotted as a function of cumulative probability density, as shown in figure 4. The expected distribution for UF samples would not include peaks above 10 kDa, however approximately 40% of detected peaks in UF samples were detected at MW>10 kDa. Samples processed by centrifugation produced a peak distribution that was statistically similar to those observed in UF processed samples. Among the samples collected, only 10-30% of the OM in the centrifuge effluent was removed by the UF, so from that perspective, it is not surprising that the distributions are similar. Additionally, though both UF and centrifuge samples contained peaks distributed across the MW range of interest, most of the larger peaks (as % of total detected peak area for a given element) were observed for MW<10 kDa. Sample centrifugation and UF processing were pursued promptly following sample collection, however samples were not analyzed with SEC for several months following collection, and likely OM aggregation lead to similar distributions. NOM has been observed to aggregate over time (Schmitt et al. 2000), and thus efforts should be made to conduct SEC
analysis promptly. By contrast, GF filtration had comparatively more HMW peaks. Interestingly, even though the expected removal cutoffs for centrifugation and GFF are 0.3 and 0.7 µm respectively, which are both well above the column upper separation limit of 300 kDa (~3 nm), centrifugation resulted in fewer HMW peaks.

Since GFF displayed a different peak distribution, storage effects on size distribution analysis was restricted to only centrifugation and UF samples. In spite of the fact that the glass and plastic cumulative distribution function appear similar, storage in glass containers resulted in a statistically significant increase in high molecular weight peaks, which may indicate that that the HMW macro-molecules/colloids are less polar and therefore are more susceptible to adsorption to the plastic.

![Graphs](image)

Figure 4: Cumulative probability density functions: A) separation method; B) storage method

Conclusion

SEC – ICP-MS is a powerful tool for evaluating macro-molecular metal-OM complexations. However care needs to be taken to ensure reliable results. Consistent simple processing and storage are important components for any study, but particularly in the face of trace analysis of multiple constituents. Duplicate analysis is also an important component to aid to which elements are producing strong and reproducible chromatograms. In this study, which included OM collected from highway stormwater runoff and in-stream OM from three associated land uses (urban, agricultural, and less impacted), Mg, Cl, Mn, Cu, Br, and Pb were the most consistent, while Al, V, Fe, Co, Ni, Zn, Se, Cd, Sn, and Sb are all worth considering but should be used with caution.
Acknowledgments

The authors wish to acknowledge Dr. Peter Green for his assistance with configuring and troubleshooting SEC – ICP-MS interface. The project described was supported by the Research and Education in Green Materials fellowship (REGM) for E.R. McKenzie and by Award Number P42ES004699 from the National Institute of Environmental Health Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of REGM or the National Institute of Environmental Health Sciences or the National Institutes of Health.

References


