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Studying Arsenite-Humic Acid Complexation Using Size Exclusion Chromatography-Inductively Coupled Plasma Mass Spectrometry

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Abstract

Arsenic (As) can form complexes with dissolved organic matter (DOM), which affects the fate of arsenic in waste sites and natural environments. It remains a challenge to analyze DOM-bound As, in particular by using a direct chromatographic separation method. Size exclusion chromatography (SEC) hyphenated with UV spectrophotometer and inductively coupled plasma mass spectrometry (ICP-MS) was developed to characterize the complexation of arsenite (As^{III}) with DOM. This SEC-UV-ICP-MS method is able to differentiate As^{III} -DOM complexes from free As species and has the advantage of direct determination of both free and DOM-bound As^{III} through mild separation. The suitability of this method for studying As^{III} -DOM complexation was demonstrated by its application, in combination with the Scatchard plot and nonlinear regression of ligand binding model, for characterizing As^{III} complexation with humic acid (HA) in the absence or presence of natural sand. The results suggest that, consistent with polyelectrolytic nature of HA, the As^{III} -HA complexation should be accounted for by multiple classes of binding sites. By loosely classifying the binding sites into strong (S1) and weak (S2) sites, the apparent stability constants (K_s) of the resulting As-DOM complexes were calculated as $\log K_{s1} = 6.5\text{--}7.1$ while $\log K_{s2} = 4.7\text{--}5.0$.

Keywords

Arsenic; SEC-UV-ICP-MS; Humic acid; Complexation; Apparent stability constant

1. Introduction

Arsenic (As), a toxic element of significant environmental concern due to leaching from hazardous wastes and geological materials [1-8], can interact with dissolved organic matter (DOM) to form As-DOM complexes. The formation of As-DOM complexes, which has been documented in a few studies [4-5, 9-13], can affect the fate and health risk of As [5, 8, 14-17]. For example, an appreciable fraction of arsenate (As^{V}) and arsenite (As^{III}) was observed to form aqueous complexes with DOM derived from different sources [5, 18]. Buschmann et al. [10] estimated that up to 26% of As^{III} and 62% of As^{V} could bind to DOM after spiked into Aldrich humic acid (HA) solution. The association between DOM and As^{V}

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was found to be involved by metal-bridged complexation between As^{V} , Fe^{III} , and DOM [9, 19]. Despite these studies, some fundamental parameters characterizing As-DOM complexation, e.g., stability constant (K_s) of As-DOM complexes, are scarce in the literature and only reported in very few studies [12, 20-21].

The determination of K_s for As-DOM complexes is a challenging problem since it is difficult to distinguish between free and DOM-associated As. The conventional separation method for As species, anion exchange high performance liquid chromatography (AE-HPLC), is able to determine only free As since DOM-complexed As is retained on the column [5]. The equilibrium dialysis and ion-exchange cartridge methods [22] were used to separate free and bound As in previous studies dealing with As-DOM interactions [4, 6, 9-10, 18]. These two methods, however, require additional time-consuming treatment to the solution containing As-DOM complexes. Moreover, these two methods determine DOM-bound As by calculating the difference between total and free As, which could introduce large errors, particularly in cases where DOM-bound As amounts to only a small fraction (e.g., less than 10%) of total As. A direct method that is able to simultaneously determine free and DOM-bound As is therefore needed for studying As-DOM interactions.

Size exclusion chromatography (SEC), in which analytes are separated based on their size, is a widely used technique for the purification and analysis of synthetic and biological polymers [23]. When coupled to element-specific detectors, e.g. inductively coupled plasma mass spectrometry (ICP-MS), SEC can be used to fractionate free and macromolecule-bound metals [24-26]. A few studies have employed this technique to study the interactions between metals and macromolecules, particularly biological macromolecules such as proteins [27-31]. SEC-ICP-MS has not been used for direct speciation of dissolved free and DOM-bound As, although it was used to determine As distribution in soil humic acids [24]. By analyzing the humic acid extracted from a Massachusetts apple orchard using SEC-ICP-MS, Newton et al. found that the majority of As was associated with the larger molecular mass fractions of the humic acid, yet no attempts were made to differentiate free and humic acid-bound As [24].

Our objective in this study was to develop a SEC-UV-ICP-MS method capable of directly determining DOM-bound As and further to demonstrate the suitability of this method for studying As^{III} complexation with HA. We coupled SEC for separation of As^{III} -DOM complexes to UV for DOM monitoring and ICP-MS for As detection to develop the As^{III} -DOM complexes speciation method and then conducted experiments to demonstrate the application of this method in studying As-DOM complexation. We determined simultaneously free and DOM-bound As^{III} in As^{III} -HA systems and employed the Scatchard plot and nonlinear regression of ligand binding model to calculate K_s .

2. Materials and Methods

2.1. Instrumentation

A SEC-UV-ICP-MS method was developed, where SEC column was used to separate free As^{III} and As^{III} -DOM complex, followed by monitoring DOM peak using UV and As detection using ICP-MS. Detailed information on the instrumental setup and ancillary analytical methods can be found in Supplementary data.

2.2. Materials

As^{III} standard solutions were prepared by dissolving appropriate amounts of sodium meta-arsenite (98%, Aldrich) in deionized water (DIW) to achieve a concentration of 1000 mg/L (as As), followed by diluting this solution to desired concentrations. Aldrich HA (sodium salt) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of HA were

prepared by dissolving 0.5 g of HA in 100 ml of potassium hydroxide (0.1 M). A natural sand (NS) was collected from Lake County, Florida and used as a solid phase in As^{III}-DOM complexation experiments. Major quartz and clay fractions in this sand include gibbsite (Al(OH)₃), hydroxy-interlayered vermiculate ((Mg,Fe⁺⁺,Al)₃(Al,Si)₄O₁₀(OH)₂·4(H₂O)), kaolinite (Al₂Si₂O₅(OH)₄), and goethite (FeO(OH)) [18]. All other chemicals used were of analytical or trace metal grade unless specified otherwise. Water (18 M Ω -cm) prepared with a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout. All glassware was acid cleaned using 10% HNO₃ prior to the experiments.

2.3. Detection of DOM-bound As^{III}

When used for preparation of As^{III}-DOM complex, 5 ml of HA stock solution was diluted in 495 ml DIW and filtered through 0.45 μ m PTFE membrane filters (Corning Inc., Corning, NY, USA). After dilution, the HA solution contains 14.4 mg/L of DOC, 19.3 μ g/L of Al, 20.0 μ g/L of Mg, 32.5 μ g/L of Mn, and 72.1 μ g/L of Fe. To a HDPE centrifuge tube 50 ml of this HA solution was added, followed by adding 25 μ l of As^{III} standard solution to produce an As concentration of 50 μ g/L. Sodium azide (0.02%) was then added to the centrifuge tubes to minimize microbial oxidation of As^{III}. The centrifuge tubes were shaken continuously at 300 rpm for 24 h, after which time the concentrations of DOM-bound As^{III} will not increase as predetermined in preliminary experiments, to let As^{III} form complexes with DOM. The mixtures were then centrifuged at 1700 g for 10 min and the supernatants were decanted, filtrated (0.45 μ m), and analyzed by SEC-UV-ICP-MS for free and DOM-bound As^{III}. Since DOM and free As were expected to elute from the SEC column at different times due to their different molecular sizes, we should be able to differentiate free As^{III} from DOM-bound As when UV spectrometer was used to record the DOM peak and ICP-MS to detect the As simultaneously.

After initially observing the coelution of an As peak with DOM (in addition to free As^{III} peak at a different retention time), which was assumed to be As^{III}-DOM complex, further experiments were conducted to verify this As species is DOM-bound As^{III}. In order to exclude the possibility of misidentification of other As species (e.g., As^V, which might be formed during the course of the experiment) as As^{III}-DOM complex, the solutions were analyzed using AE-HPLC-HG-AFS for As^{III}, As^V, MMA, and DMA. In addition, concentrated acid (HCl or HNO₃) was added to the solutions (final acid concentration was 10%, v:v) to release As from DOM. After acidification, the solutions were analyzed again using SEC-UV-ICP-MS and AE-HPLC-HG-AFS to check the valence state of the As in As-DOM complexes.

2.4. Complexation of As^{III} with HA

In order to demonstrate the application of the SEC-UV-ICP-MS method in studying As-DOM complexation, the SEC-UV-ICP-MS method was used to investigate the complexation of As^{III} with HA at neutral pH in the absence or presence of NS. Two sets of experiments were conducted. The first experiment investigated the direct complexation of As^{III} with DOM by simply mixing As^{III} with HA, while the second experiment studied the complexation of As^{III} with DOM in the presence of NS (acting as a solid phase). The NS used here contains no detectable water-extractable As [32]. The purpose of adding NS was to check whether the presence of a solid phase during the As^{III} complexation with DOM would interfere with the determination of As^{III}-DOM complexes using this SEC-UV-ICP-MS method. This experimental design was based on the consideration that in realistic aquatic environments DOM and solid particles often coexist and thus the complexation of As with DOM would occur in the presence of solid phases.

The complexation of As^{III} with DOM was conducted by adding different concentrations of As^{III} (0-1000 µg/L, covering the general range of As concentrations encountered in the environment) into 10 ml of HA solutions (diluted as in 2.3.) in HDPE centrifuge tubes without or with NS (1 g). The same procedures described in 2.3., including addition of sodium azide, shaking for 24 h, centrifugation, decantation and filtration of supernatants, were then followed. Centrifugation was also done with tubes without NS to keep the procedures identical during the experiment. After filtration the solutions were immediately analyzed by SEC-UV-ICP-MS for free and DOM-bound As^{III}.

All experiments (2.3. and 2.4.) were conducted in duplicate and the relative percent difference between duplicates was always lower than 15%. The average was reported unless otherwise stated. The pH values of the solutions were near neutral and remained unchanged over the course of experiment. Conversion of As^{III} to As^V during the course of the experiment was not observed, as evidenced by the absence of As^V peak (see Results and Discussion).

2.5. Calculation of Apparent Stability Constant of As^{III}-DOM Complex

After free and DOM-bound As^{III} concentrations were determined, an apparent stability constant (K_s , L/mol, Equation 1) was used to characterize the complexation of As^{III} with DOM:

$$K_s = \frac{C_b^{As}}{C_f^{As} \times C_{DOM}} \quad (1)$$

where C_f^{As} and C_b^{As} are free and HA-bound As^{III} concentrations in M; and C_{DOM} is the molar concentration of effective ligands contained in HA in M. K_s is a prime parameter to quantitatively describe interactions between metal and ligand in a stoichiometric way [33] and is useful for predicting the fate of As in aquatic environments using a speciation model [34]. Direct calculation of K_s from Equation 1 is difficult since C_{DOM} is usually unavailable due to the lack of accurate molecular structure for DOM and the unclear mechanism of DOM complexation with As. Therefore, the Scatchard plot, namely the plot of C_b^{As}/C_f^{As} versus C_b^{As} based on Equation 2 [35], was used to estimate K_s :

$$\frac{C_b^{As}}{C_f^{As}} = -K_s \times C_b^{As} + K_s \times B_{max} \quad (2)$$

where B_{max} is the maximum binding capability of DOM toward As^{III} in M. The Scatchard plot has not been used for As-DOM complexation, although it is widely used in protein chemistry and has found application to determination of metal humate stability constants [35-37]. In addition, K_s and B_{max} were also determined through nonlinear regression fitting of experimental data to ligand binding model:

$$C_b^{As} = \sum_i \frac{B_{maxi} \times C_f^{As}}{(1/K_{si}) + C_f^{As}} \quad (3)$$

where $i=1$ for uniform binding sites while $i \geq 2$ for heterogeneous binding sites.

3. Results and Discussion

3.1. Detection of DOM-bound As^{III}

When SEC-UV-ICP-MS was used for As speciation, the retention time of DOM was clearly different from those of free As^{III}, As^V, MMA, and DMA (Fig. 1). From the chromatogram of HA solution without addition of As^{III} (Fig. 1a shows UV detection at 254 nm while Fig. 1b shows ICP-MS detection for ⁷⁵As), DOM in HA solutions eluted from the SB-802.5 HQ column at about 378 s under our experimental conditions. The SEC-UV-ICP-MS chromatogram of As standards indicated that the two As species commonly encountered in the environment, As^{III} and As^V, eluted at 678 and 444 s, respectively (Fig. 1c, no peaks were observed on UV detection for As standards, data not shown). MMA and DMA were observed to have the same retention time as As^V and not shown in Fig. 1. Based on the different retention times of DOM and free As on the SEC column used, this SEC-UV-ICP-MS technique seems to provide us with a useful tool for investigating the interactions between As^{III} and DOM.

For the As^{III}-DOM solutions prepared by adding As^{III} to HA, we observed an As peak at the retention time of DOM, in addition to the free As^{III} peak, for SEC-UV-ICP-MS analysis. Fig. 2a shows the chromatogram of As^{III}-HA solution at initial As^{III} concentration of 50 µg/L. This As peak was not caused by As inherently contained in the HA commercially purchased because no detectable As^{III} or As^V was present in the SEC-UV-ICP-MS chromatograms of the original HA solutions (Fig. 1b). Since As^V and DOM have close elution times (444 and 378 s, respectively), the observed As peak at the retention time of DOM might be caused by As^V which could be formed by oxidation of the spiked As^{III}. However, the results of speciation analysis of the As^{III}-HA solutions using AE-HPLC-HG-AFS revealed that, among all common As species (As^{III}, As^V, MMA, and DMA) that this technique is capable of detecting, As^{III} was the only As species detected (Fig. 2b). Therefore, the As peak observed at the retention time of DOM was not produced by commonly encountered environmental As species, such as As^V, MMA, and DMA, and should be a DOM-bound As species. Due to the fact that anion exchange column can retain DOM-bound As, AE-HPLC-HG-AFS failed to reveal the presence of As^{III}-DOM complexes, while SEC-UV-ICP-MS is able to detect this complex. In order to further confirm that the detection of As^{III}-DOM complex using SEC-UV-ICP-MS is independent of the interference of other As species, the 4 common arsenic species, As^{III}, As^V, MMA, and DMA, were spiked individually into this As^{III}-HA solution. No effect on the elution and area of the As^{III}-DOM complex peak was observed.

The next question regarding the DOM-bound As species is the valence state of As in this complex (As^{III} or As^V). The results of analysis of the As^{III}-HA solutions after acidification confirmed that the As species complexed by DOM is As^{III}, as evidenced by the appearance of only free As^{III} after acidification using HCl or HNO₃. Both analytical techniques employed in this study (SEC-UV-ICP-MS and AE-HPLC-HG-AFS) gave only one As^{III} peak for the acidified samples (Fig. 2c shows the SEC-UV-ICP-MS result of HNO₃ acidification only). It is unlikely that the As bound by DOM was As^V and reduced to As^{III} during acidification, in particular in the case of HNO₃ which is an oxidative acid and shown to be able to oxidize As^{III} [38]. In addition, acidification of As^{III} or As^V standard had no effect on their elution on the SEC column (data not shown). All these results suggest that the detected As peak along with HA elution was HA-As^{III} complex which was formed after mixing As^{III} standard with HA solution.

3.2. Quantification of DOM-bound As^{III}

Because of the non-availability of DOM-As complex standards, As^{III}-DOM complexes cannot be directly quantified by comparing the peak areas of samples and standards. It is also not feasible to use HA solution as matrix to prepare As^{III} calibration curve and use this curve to quantify As^{III}-DOM complexes, due to the coexistence of free and DOM-bound As^{III} after adding As^{III} to HA solution. The concentrations of As contained in As^{III}-DOM complexes were quantified using the same calibration curve for free As^{III} which was prepared by dissolving As^{III} in DIW. This quantification method was primarily based on the fact that we did not observe significant influence of Aldrich HA used in this study on the response of As^{III} on ICP-MS. Fig. 3a shows the results of analysis of a series of As^{III} standards prepared in DIW and in HA solutions by using ICP-MS directly (without SEC or HPLC columns). The calculated As concentrations in DIW and in HA solutions (using calibration standards prepared with DIW) were shown on the *x* axis and *y* axis, respectively, in Fig. 3a. The proximity of As concentrations obtained for DIW and HA solutions (the relative percentage differences ranging from 3.3 to 7.7%) indicates that the matrix effect of HA on As determination is minimal in our experiments. Similar to our results, Buschmann et al. [10] observed that DOC from both Suwannee River and Aldrich humic acids had no significant influence on the As^{III} response on AFS. However, when this SEC-UV-ICP-MS technique is used for other As species and/or other types of DOM, the matrix effect of DOM on As response should be determined prior to using DIW-prepared As calibration curve for quantification and the response signals should be corrected for DOC content, if necessary.

The precision of the proposed SEC-UV-ICP-MS method for determining free As^{III} and As^{III}-DOM complexes was evaluated. A sample solution containing both free and DOM bound-As^{III} was analyzed five times and the relative standard deviations were 8.4 and 9.7% for free and DOM bound-As^{III}, respectively. The detection limit, calculated as three times standard deviation of the background divided by the slope of the calibration curve, was 0.3 µg/L for both As species. The recovery experiments were not carried out since no standard As^{III}-DOM complexes are available for spiking. However, we observed that, during the As^{III}-HA complexation experiments without NS, the sum of free and DOM-bound As determined by SEC-UV-ICP-MS agreed well with the initial As^{III} concentration spiked, with As^{III} mass balances being 100±10 % (Fig. 3b). This agreement provides additional support for the validity of this SEC-UV-ICP-MS method for determining free As^{III} and As^{III}-DOM complexes.

3.3. Complexation of As^{III} with HA

The suitability of SEC-UV-ICP-MS for studying As-DOM interactions was demonstrated by its capability of directly detecting As^{III}-DOM complexes, in addition to free As^{III}, in the absence or presence of NS. In both cases, it was observed that a relatively small fraction of As^{III} was present in the complexed form at the HA concentration used (14.4 mgC/L). As^{III}-DOM complexes account for about 1 to 10% of total As^{III} in solution, decreasing with initially spiked As^{III} concentrations.

Regardless of the absence or presence of NS, the Scatchard plots of C_b^{As}/C_f^{As} versus C_b^{As} deviated from linearity (Fig. 4), indicating the presence of different types of binding sites in HA. This is not unexpected, considering the heterogeneity in the chemical nature of the binding sites present in the DOM derived from HA. A curved Scatchard plot produces four independent parameters, two intercepts (one with the abscissa and the other with the ordinate) and two slopes, as shown in Fig. 4. The slopes and intercepts correspond to the parameter combinations of K_{si} and B_{maxi} (which are binding constant and capacity for each class of sites, $i=1, 2, \dots$), as denoted by Equations 4-7 [39-40]:

$$\text{Intercept } 1 = \sum_i (K_{si} \times B_{\max i}) \quad (4)$$

$$\text{Intercept } 2 = \sum_i B_{\max i} \quad (5)$$

$$\text{Slope } 1 = - \frac{\sum_i (K_{si}^2 \times B_{\max i})}{\sum_i (K_{si} \times B_{\max i})} \quad (6)$$

$$\text{Slope } 2 = - \frac{\sum_i B_{\max i}}{\sum_i \frac{B_{\max i}}{K_{si}}} \quad (7)$$

From these equations for the intercepts and slopes it is clear that the problem is determinate only for $i=2$ (where four variables are in a set of four equations). Therefore, only two types of complexes can be determined from such a plot. By simply classifying the binding sites present in HA into two types: strong (S1) and weak (S2) sites, the K_s and $B_{\max}$ for each class of sites were calculated by solving Equations 4-7 (Table 2). These binding parameters were also determined by nonlinear fitting of the experimental data using two-site ligand binding model (Table 2), for the sake of comparison.

As can be seen from Table 2, the Scatchard plot and nonlinear regression produced K_s and $B_{\max}$ values at the comparable orders of magnitude. With or without NS, the $\log K_s$ for the strong (S1) sites (6.5–7.1 depending on the determination method) was apparently higher than that for the weak (S2) sites (4.7–5.0). Reversely, $B_{\max}$ for the strong complexation (0.011–0.027 μM) was lower by an order of magnitude, in comparison to the weak complexation (0.18 and 0.31 μM). These results, together with the observation that a larger fraction of As^{III} was complexed by HA at lower initial As^{III} concentrations, suggest that the number of S1 sites is limited, but has a higher affinity to form complexes with As, comparing with S2 sites. The S1 sites are occupied at lower As concentrations and would be exhausted at higher As concentrations, after which As^{III} would be complexed by S2 sites.

Humic acid contains various functional groups which can bind As through different mechanisms. It is possible that the S1 sites are moieties with chelating functional groups and can form bi-dentate (or multi-dentate) complexes with As^{III} , whereas the S2 sites contain no chelating groups and form monodentate complexes with As^{III} . Other mechanisms may be present for HA binding As [20]. For instance, the HA used here contained some metals such as Fe and Mn. These metals could act as cation bridges to facilitate the binding of As on HA, as metal-bridged complexation has been suggested as one of mechanisms for DOM binding As [20]. Probably also due the role of cation bridges, $B_{\max}$ for the S1 sites appears to be higher in the presence of NS than without NS (Table 2). This increased $B_{\max}$ could be related to the release of some metals (e.g., Fe and Mn) from NS, as previously observed [32].

The K_s values of As^{III} -DOM complexes for S2 sites are within the typical range of K_s for cation metal-humate and PO_4^{3-} -metal-humic complexes ($\log K_s = 4\text{--}6$) [33]. The K_s for S1 sites appears to be slightly higher than this range, indicating strong complexation could be formed between As^{III} and HA. The $B_{\max}$ values, compared to the free As^{III} in solution or potential binding sites in HA (e.g., expressed in terms of the proton exchange capacity,

PEC), were rather small, indicating that the effective complexation sites in HA are limited. Similarly, Guardado et al. [37] suggested that humic acid extracted from leonardite contains a relatively small fraction of sites that are involved in PO_4^{3-} complexation, even in the presence of Fe as the metal bridge. It is worth noting that in general the amount of potential binding sites in DOM is largely excessive, compared to As. Consequently, a small fraction of these binding sites could bind considerable As.

This study provided a useful technique for characterizing As-DOM complexation, through SEC-UV-ICP-MS analysis of DOM-bound As followed by Scatchard plot and nonlinear ligand binding model fitting to calculate K_s . Compared to previous methods for differentiating free and DOM-bound As, the SEC-UV-ICP-MS method developed in this study has some advantages. First, the SEC-UV-ICP-MS is a direct method for DOM-bound As determination by differentiating simultaneously free and DOM-bound As in one chromatographic run. Previous methods for analysis of DOM-bound As, including equilibrium dialysis and ion-exchange cartridge technique, determine DOM-bound As by calculating the difference between total and free As [18, 22]. The indirect determination of DOM-bound As could introduce large errors, in particular if the fraction of DOM-bound As against total As in solution is small (e.g., less than 10%) which has often been encountered. For example, assuming 5% of As is bound by DOM in an As-DOM solution with a total As of 100 $\mu\text{g/L}$, then the free and DOM-bound As are 95 and 5 $\mu\text{g/L}$, respectively. If a minus 10% error is introduced during the analysis of free As, the determined free As would be 85.5 $\mu\text{g/L}$ and the DOM-bound As would be calculated as 14.5 $\mu\text{g/L}$. The SEC-UV-ICP-MS direct method developed here has a detection limit of 0.3 $\mu\text{g/L}$ and a precision of 9.7% for determination of DOM-bound As and thus would provide more accurate results. Second, the current method can determine both free and DOM-bound As in less than 15 min (an analytical run). Previous equilibrium dialysis and ion-exchange cartridge methods take hours to days for sample treatment to separate free and DOM-bound As. Third, the method developed in this study uses a mild way to separate free and DOM-bound As, i.e. primarily through the differences in their sizes using a SEC column under neutral pH condition. For the previously used ion-exchange cartridge technique [22], the exchange of free As with ions on the resin could alter the equilibrium between free and DOM-bound As in solution and thus change the As-DOM complexation dynamics. However, this SEC-UV-ICP-MS method cannot be used for studying the complexation of DOM with As^{III} and As^{V} simultaneously. This is because DOM-bound As^{III} and DOM-bound As^{V} would be eluted out of the column at the same retention time, although this method is able to separate As^{III} , As^{V} , and DOM-bound As.

The Scatchard plot and nonlinear ligand binding model methods following sample analysis eliminate the necessity of knowing the exact concentrations of binding sites present in HA and calculate K_s by using only the determined free and DOM-bound As concentrations. It is worth noting that although the Scatchard plot bears the inherent disadvantage of potentially distorting the experimental error through linear transformation of nonlinear data, it produced compatible values for K_s and B_{max} as nonlinear regression did. The usefulness of Scatchard plot was further demonstrated by its ability of indicating whether or not single type (linear) or multiple types (nonlinear) of binding sites are present. Such information is usually difficult to obtain through direct nonlinear regression or by simply drawing a plot of C_b^{As} versus C_f^{As} . A combination of Scatchard plot and nonlinear regression using ligand binding model could provide supplementary information for the determination of binding parameters.

In addition to this study, very few studies have reported the calculation of conditional stability constants of As-DOM complexes [12, 20]. In separated studies, we investigated the

complexation of As with DOM under a variety of conditions (e.g., different pHs and varying As concentrations) by using the method developed here. It was observed that the log K_s of As-DOM complexes ranged from 4.5 to 7.0, depending on the types of binding sites within DOM [20-21]. If all sites within DOM are treated as one type, the log K_s would be around 4.8–5.2, agreeing well with the values calculated from the data of free and DOM-bound As concentrations reported in other studies [20]. Therefore, the K_s values of As-DOM complexes are relatively constant under environmentally relevant conditions, if all binding sites within DOM are classified into one group. However, the various binding sites within DOM are inherently heterogeneous. Under some unusual experimental conditions, different K_s could be observed. For example, Warwick et al. calculated K_s of As-HA complexes by using PEC as a surrogate for concentrations of binding sites in HA, following analysis of free As and derivation of DOM-bound As by the differences between free As and initially spiked As [12]. The log K_s of As^{III}-DOM complexes reported in that study ranged from 0.5 to 2.5 [12], which could be due to the extremely high As and DOM used there (up to 100 mg/L of As and 1500 mg/L of HA).

4. Conclusions

By using UV spectrometer to record DOM peak and ICP-MS to detect As, a SEC-UV-ICP-MS method was developed to analyze directly free and DOM-bound As^{III}. This SEC-UV-ICP-MS method was applied to study As^{III}-HA complexation at neutral pH in the absence or presence of NS. In both cases, multiple classes of binding sites were involved in As^{III}-HA complexation. The log K_s for the loosely classified strong (S1) and weak (S2) sites within HA are 6.5–7.1 and 4.7–5.0, respectively, with the Scatchard plot and nonlinear regression of ligand binding model producing compatible results. As demonstrated in the present study, the developed SEC-UV-ICP-MS technique is suitable for investigating As^{III}-DOM interactions because of its capability of simultaneous determination of both free and DOM-bound As^{III} through mild separation. The technique is now being applied to characterize As-DOM interactions under a variety of environmental conditions and a full account of characterization on As-DOM complexes will be reported elsewhere.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A novel SEC-UV-ICP-MS method was developed to directly determine DOM-bound As.
- This method was applied to characterize arsenite complexation with humic acid.
- Heterogeneous binding sites with varying stability for arsenic are present in DOM.
- Promising potential was demonstrated for this method in studying As-DOM interactions.

Fig. 1. SEC-UV-ICP-MS chromatogram of HA solution (without spiking As, DOC=14.4 mg/L) on (a) UV detection at 254 nm and (b) ICP-MS detection for ^{75}As , and (c) SEC-ICP-MS chromatogram of 50 $\mu\text{g/L}$ of As^{III} and As^{V} standard in DIW (MMA and DMA have the same retention time as As^{V} and are not shown).

Fig. 2. Chromatograms of (a) As^{III}-HA solution on SEC-UV-ICP-MS; (b) As^{III}-HA solution on AE-HPLC-HG-AFS; (c) acidified As^{III}-HA solution (final HNO₃ = 10%, v:v) on SEC-UV-ICP-MS. For As^{III}-HA solution, DOC = 14.4 mg/L and initial As^{III} = 50 µg/L).

Fig. 3.

Minimal matrix effect of HA on quantification of As^{III} -DOM complex using SEC-UV-ICP-MS as shown by (a) proximal response of As^{III} standards in DIW and HA solutions on ICP-MS and (b) agreement between the sum of free and DOM-bound As^{III} and the initial As^{III} concentration added in the complexation experiment.

Fig. 4. Scatchard plot for As^{III} complexation with humic acid in the (a) absence and (b) presence of natural sand. The nonlinearity of the plot suggests the presence of multiple classes of binding sites in humic acid. The two intercepts and two slopes produced by the plot are the parameter combinations of K_s and B_{max} for each class of sites.

Table 1
Instrument operating conditions

SEC (HPLC: Thermo SpectraSYSTEM P4000)	
Analytical column	Shodex OHpak SB-802.5 HQ
Mobile phase	0.02 M sodium nitrate
Flow rate	1.0 ml/min
Elution mode	Isocratic
Injection volume	100 µL
UV detector wavelength	254 nm
Anion exchange (HPLC: Thermo SpectraSYSTEM P4000)	
Analytical column	Hamilton PRP-X100
Mobile phase	0.015 M phosphate buffer (pH=5.8)
Flow rate	1.0 ml/min
Elution mode	Isocratic
ICP-MS (Hewlett-Packard HP4500 PLUS)	
Forward RF power	1300 W
Plasma gas (Ar)	15 L/min
Auxiliary gas (Ar)	1.1 L/min
Nebulizer gas (Ar)	1.0 L/min
Spray chamber	Glass, double pass
Integration time/point	0.6 s
Integration time/mass	1.8 s

Table 2

Apparent stability constants (K_s , L/mol) and maximum binding capabilities (B_{max} , $\mu\text{mol/L}$) of As^{III} to Aldrich humic acid (HA). DOC concentration was 14.4 mg/L. The binding sites in HA were loosely categorized into two classes: strong (S1) and weak (S2) sites. Scatchard plot and nonlinear regression fitting of ligand binding model were used to calculate these parameters. The numbers in parentheses are the 95% confidence intervals.

	Binding site	$\text{Log } K_s$				B_{max}	
		Scatchard plot		Nonlinear fitting		Scatchard plot	Nonlinear fitting
Without NS	S1	7.1	6.5 (6.2-7.1)	0.012	0.011 (0.008-0.014)		
	S2	4.7	4.8 (4.7-4.9)	0.18	0.31 (0.28-0.34)		
With NS	S1	6.6	7.0 (6.8-7.1)	0.027	0.021 (0.012-0.033)		
	S2	4.8	5.0 (4.4-5.3)	0.22	0.25 (0.14-0.36)		