Extractive Electrospray Ionization Mass Spectrometry for Sensitive Detection of Uranyl Species in Natural Water Samples

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In the real world, detection of trace amounts of inorganic species, particularly with speciation information, is of great significance. Herein a method based on extractive electrospray ionization (EESI) tandem mass spectrometry (MS/MS) has been established for rapid detection of radioactive inorganic species in natural water samples. Negatively charged uranyl acetate undergoes characteristic fragmentation in the gas phase, providing the fundamental chemistry for specific detection of uranyl species in complex matrices without sample pretreatment. Under the optimized experimental conditions, uranyl species in various natural water samples were rapidly detected using multiple-stage EESI mass spectrometry. The mean time for each sample analysis was about 10 s. The limit of detection was about a few \(10^{-3}\) ng/L of uranium by utilizing the characteristic fragments obtained in the EESI-MS experiments. The typical relative standard deviation (RSD) of this method was 6.9–8.1% for 8 measurements (S/N = 3). The dynamic response range was \(10^{-1}\)–\(10^{3}\) ng/L for uranium in water samples. The isotope ratio of uranyl species was quantitatively detected using EESI-MS experiments. The results show that EESI-MS, a typical method initially developed for organic compound analysis, has promising perspectives for real-time, online monitoring of inorganic species such as uranyl species in natural water samples.

Driven by the practical needs of academic research and industrial production, an accelerating trend in modern analytical science is to perform trace analysis where the analyte concentration is of the order of parts per million or less. Serving as a powerful analytical platform for sensitive detection of analytes at trace levels, mass spectrometry, combined with other techniques if necessary, has been used as an irreplaceable tool for decades, due to the unparalleled sensitivity, premier specificity, and general applicability. Ambient mass spectrometry,¹⁻⁹ serving as a solution to the bottleneck of high-throughput mass spectrometric analysis, has dramatically improved the speed of mass spectrometric analysis of complex samples and thus successfully spread the mass spectrometric application across multiple disciplines. Typically, organic species involved in drug discovery,¹⁰⁻¹² organic chemistry,¹³⁻¹⁶ material science,¹⁷⁻¹⁹ and biology,²⁰⁻²⁴ etc. have been characterized at molecular levels using ambient mass spectrometry. Because of the complexity of the matrixes of practical samples, the next challenge is to perform trace analysis where the analyte concentration is of the order of parts per million or less.
energy technology,46,47 and life science.48–50 Solution chemistry of uranium is dominated by uranyl speciation, which involves in processes ranging from nuclear fuel processing51 and waste handling to mobility and fate in the geologic subsurface.52 Understanding uranyl speciation is fundamental to predicting radionuclide behavior in the environment and resulting in ultimately control of radionuclide in nuclear waste-storage facilities. Thus, uranyl species’ studies are certainly of great importance. Techniques including mass spectrometry,53–56 vibrational spectroscopy,47 and quantum calculations42,57 have been employed to study uranyl ions in the gas phase. Theoretically, these techniques can be used to obtain speciation information of uranium in the solution phase; in these cases, however, the result interpretation is sometimes problematic since the speciation information of uranium is not easily accessible in a straightforward way. Among ambient ionization techniques1–9 available, extractive electrospray ionization (EESI)55 was introduced in 2006 for direct, online analysis of liquid samples in real time. In EESI, neutral analytes present in raw samples are dispersed in a spatial cross section formed between a sample plume and an electrospray beam; thereby the analytes undergo interactions and collisions with the primary ions produced by electrospraying pure solvent (e.g., acetic acid/methanol—water solution) and then ionized for further mass analysis. Ion suppression is reduced in EESI by distributing the matrixes over a relatively wide section in a three-dimensional space. Another unique feature of EESI is that the neutral samples are safely isolated from any high voltage or direct bombardment by charged particles, thus EESI makes ionization of the analyte possible without subjecting it to a harsh environment, which minimizes the speciation changes caused by the chemical events such as redox reactions occurring in the pretreatment and/or

EXPERIMENTAL SECTION

Instrumental Setup. Under the negative ion detection mode, experiments were carried out using a LTQ-XL mass spectrometer (Finnigan, San Jose, CA) equipped with a homemade EESI source. The principle and basic configuration of an ESI source were previously illustrated for rapid analysis of liquid samples. Briefly, the EESI source described here was developed for detection of radioactive species, with improved safety. As schematically shown in Figure 1a, the EESI assembly was air-tight enclosed to the LTQ mass spectrometer, so that no material can be released into the ambient air when the radioactive sample was infused into the EESI source. In the EESI source, the neutral analyte underwent numerous collisions with the charged particles generated by the electrospray using a negative high voltage (~4 kV). During the collisions, online droplet—droplet extraction occurred between the neutral sample droplets and the charged droplets (shown in Figure 1b), allowing continuous extraction of interesting compounds and subsequent ionization of neutral analytes. The analyte ions were then introduced into the LTQ mass analyzer for mass analysis through the ion guide system of the LTQ-MS instrument. The distance (a) between the end-tips of the two sprays and the distance (b) between the spray tips of the EESI source and the MS inlet was 2 and 5 mm, respectively. The angle (α) between the electrospray beam and the MS inlet of the LTQ-MS instrument and the angle (β) between the two spray beams was 150° and 60°, respectively. Thus, the angle (γ) was determined to be 150° as well. The LTQ-MS instrument was running in the negative ion detection mode. The temperature of the heated capillary of the LTQ-MS was maintained at 180 °C. The default values of voltages were used for the heating capillary, the tube lenses, the conversion dynodes, the detectors, etc. Further optimization was not performed.

All the full scan mass spectra were recorded using Xcalibur software of the LTQ-MS instrument with an average time of 30 s. The precursor ions of interest were isolated using a mass window of 1.4 mass/charge (m/z) unit. Collision-induce dissociation (CID) experiments were performed by applying 15–30% collision energy for 30 ms to the precursor ions. MS² spectra were collected with a recording time more than 30 s if necessary. Compounds of interest were identified using MS and CID data matching of the unknown compounds against authentic standards.

Materials and Reagents. Uranyl acetate samples (A.R. grade) were gifts from the China Institute of Atomic Energy (Beijing, China). Chemicals such as methanol (A.R. grade) and acetic acid (A.R. grade) were bought from Chinese Chemical Reagent Co. Ltd. (Shanghai, China). All chemicals were directly used without any pretreatment, unless dissolution and dilution with deionized water when it was necessary. Deionized water with 1.0% acetic acid (v/v) containing no uranium was used as the blank water sample. Uranyl acetate was precisely added into blank water samples to make a series of dilute uranyl acetate standards. For actual sample analysis, the natural water samples were obtained from three different sites including a well, lake, and river, in which upstream is a uranium ore. To ensure the acetic acid ligand (Ac⁻) was sufficient for the uranyl species in the natural water samples (5 mL, pH 6), 0.05 mL of pure acetic acid was added into each of the samples. After shaking for about 20 s, the resulting mixture was directly infused at a flow rate of 5 μL/min for EESI-MS analysis, without any further treatment. A methanol/water/acetic acid solution (40:40:20, v/v/v) was delivered at a flow rate of 5 μL/min to the ESI emitter, by using a syringe pump, for generation of the primary ions.

Safety Remarks. The handling of radioactive substances requires special permission. The complete EESI system had to be built as a ventilation hood coupled to the LTQ-MS instrument; and the waste, the exhaust of the LTQ-MS, was carefully collected for proper handling by another nuclear chemistry lab. One must be sure that no radionuclides are emitted into the laboratory atmosphere. Furthermore, the complete EESI system was equipped with an interlock switch in order to avoid contact with any high voltage by opening the ventilation hood.

RESULTS AND DISCUSSION

EESI-MS Spectrum of Uranyl Acetate. In the EESI mass spectrum recorded using a dilute uranyl acetate solution (≤0.01 μM), only one abundant peak is present at m/z 447 (Figure 2a),
which correspond to the deprotonated uranyl acetate complex (UO$_2$(Ac)$_3^-$, Ac = CH$_3$COO). A small peak at $m/z$ 119 ($\approx 3\%$) was also shown in the EESI mass spectrum (Figure 2a) due to the formation of deprotonated acetic acid dimers in the EESI process. When a uranyl acetate aqueous solution with a relatively high concentration (1 $\mu$M) was directly infused to the EESI source, a series of ionic uranyl acetate clusters formulated as $(UO_2(Ac)_2)_{n}Ac^-(n=1-5)$ were detected at $m/z$ 447, 835, 1223, 1611, and 1999 (Figure 2b), respectively. These peaks were not detected in the EESI mass spectrum recorded using low concentration solutions, probably because the initial concentration (e.g., 0.01 $\mu$M) was not high enough for the EESI process to form ionic clusters containing more than two uranyl species. When the methanol/water/acetic acid solution was electrosprayed, the spectral pattern (data not shown) did not vary along the concentrations of acetic acid in the sample solution because the acetic acid in the electrospray solution was enough for the complex formation.

Many reagents can be used in the ESI spray to generate the primary ions for EESI purpose. In this study, for the uranyl standard solutions, no considerable difference was detected when the composition of the electrospray solution varied from pure methanol to methanol/water/acetic acid. For natural water samples, a relatively low level signal was observed using solvent without acetic acid, probably in the natural water sample there were other ligands which competed with the Ac$^-$ ligands from forming (UO$_2$(Ac)$_3$)$^-$ complex. Therefore, a mixture of methanol/water/acetic acid (40:40:20) was used for further experiments. It was also found that, in a relatively wide range, the variety of the acidity of the uranyl acetate solution (pH = 1–10) produced no considerable difference in the EESI mass spectrum.

The temperature of the heated capillary of the LTQ instrument affects the intensities of the $(UO_2(Ac)_2)_nAc^-(n=1-5)$ clusters. Below 180 $^\circ$C, the signal intensity ($m/z$ 447) increased slightly along with the rise of the temperature (Figure 3a), probably due to the improved desolvation of the ions. The abundances of the $(UO_2(Ac)_2)_nAc^-(n=2-5)$ clusters decreased notably when the temperature was beyond 200 $^\circ$C, due to the thermal dissociation of the clusters.

The effect of the high voltage for the ESI on the signal levels of $m/z$ 447 was plotted as shown in Figure 3b. Below –4 kV, the signal intensity ($m/z$ 447) increased obviously when the ESI voltage was increased, probably because more primary ions were created when the ESI voltage was increased. The abundances of the signals dropped down significantly when the ESI voltage was higher than –5 kV, probably because the ions were accelerated by the electric field more than necessary, resulting in more astray ions which could not be guided into the mass analyzer.

**Fragmentation of Uranyl Acetate in the Gas Phase.** Upon CID in the gas phase (1 $\times$ 10$^{-5}$ torr, 15% collision energy (CE)), the ions of $m/z$ 447 lose neutral radicals (CH$_3$COO$^*$), which results in a product of a radical anion of $m/z$ 388 (Figure 4a). The cleavage of the radical (CH$_3$COO$^*$) from the precursor ions indicates that the molecular residue (i.e., UO$_2$(Ac)$_2$) has a strong electron affinity. This is consistent with the observation that the radical anion ($m/z$ 388) can be retained in the gas...
phase for a long time (t ≥ 10 s). With relatively high collision energy (≥22%), the cleavage of CO₂ from the acetate group produced a small peak at m/z 403 in the CID mass spectrum of the precursor ions of m/z 447. The low relative abundance (∼1%) of the peak at m/z 403 suggested this pathway was not favored under the experimental conditions. The radical anion (m/z 388) loses CH₂dCcOdO and CO₂, respectively, to produce the fragments at m/z 346 or m/z 344 (Figure 4b) in the MS³ experiment. The major fragment of m/z 346 yields abundant product ions of m/z 304, 302, and 286 (Figure 4c) by the loss of CH₂dCcOdO and CO₂, respectively. Interestingly, only one fragment of m/z 286 (UO₃⁻) was observed, due to the loss of water or methane, in further CID experiments using either m/z 304 or 302 as the precursor ions. The final product of the CID experiments (i.e., UO₃⁻) gives no fragmentation in the CID experiment. Similar to the previous findings,⁶¹ these data show that the UO₃⁻ species is intrinsically stable with respect to electron autodetachment and thermal fragmentation in the gas phase. The detail mechanism of the dissociation will be explicitly illustrated elsewhere using quantum chemistry tools combining systematic gas-phase CID and ion/molecule reactions studies. Scheme 1 summarizes the dissociation pathways observed using the uranyl acetate complex (m/z 447).

Validation of Fragmentation Paths. Identical fragmentation patterns were obtained using the ²³⁵U isotope labeled uranyl acetate. As shown in Figure 4d–f, on each stage, the fragments observed were in good agreement with those obtained using ²³⁸U uranyl species, showing that the dissociation pathways summarized in Scheme 1 are correct.

One remarkable feature of multiple-stage mass spectrometric analysis is the capability to explicit molecular structures of analytes. False positive signals can be eliminated by comparison with the multiple-stage mass spectral data using authentic compounds. Tandem mass spectrometry is required when a raw sample is infused for ionization without cleaning up the complex matrices.⁶²–⁶⁵ In this work, the uranyl acetate clusters (m/z 447) was validated using ²³⁵U isotope labeled compounds: (a) MS² spectrum of m/z 447; (b) MS³ spectrum of m/z 388; (c) MS⁴ spectrum of m/z 346; (d) MS² spectrum of m/z 444; (e) MS³ spectrum of m/z 385; and (f) MS⁴ spectrum of 343.

Figure 4. The intrinsic fragmentation pathways of uranyl acetate (m/z 447) was validated using ²³⁵U isotope labeled compounds: (a) MS² spectrum of m/z 447; (b) MS³ spectrum of m/z 388; (c) MS⁴ spectrum of m/z 346; (d) MS² spectrum of m/z 444; (e) MS³ spectrum of m/z 385; and (f) MS⁴ spectrum of 343.

Scheme 1. Fragmentation Pathways and Fragments of [UO₂(CH₃COO)₃]⁻ in the Gas Phase

Note that the structures shown here are only postulated structures for the negatively charged ions, which should be validated using other methods such as theoretical calculations.

were dissociated when subjecting to collisions and produced the characteristic fragmentation patterns, which serve as a fundamental chemistry base for specific detection of uranyl species in mixtures with complex matrixes.

**Detection Limit and Reproducibility.** The characteristic signal of m/z 346 observed in the MS<sup>3</sup> spectrum was selected for quantitative measurement of uranyl species in water. Uranyl species are found widely in natural water at levels of about tens of parts per thousand. Using this method, we found uranyl signals from all the natural samples tested. Thus deionized water was used in our experiments to prepare the standard uranyl solutions for making the calibration curve. As shown in Figure 6a, a dynamic response was obtained between the signal of m/z 304 and the concentration of the uranyl acetate water solution using logarithmic scales. The linear equation was $y = 0.2664x + 3.8$ ($R^2 = 0.978$), providing a dynamic response range of 5 orders of magnitudes ($10^{-1} \sim 10^{3}$ ng/L). The signal
response was not directly linear to the concentration of the analyte, probably because the signal was obtained using MS3 experiments. The concentrations of the uranyl species in different natural water samples were quantitatively measured using this calibration curve. Table 1 summarizes the analytical results. As shown in Table 1, the recovery obtained using this method ranged 80%–110%, showing that this method is useful for quantitative detection of uranyl species in water. The limit of detection (LOD) of this method was found to be 2.33 × 10⁻³ ng/L, which is comparable to that reported with inductively coupled plasma mass spectrometry⁶⁶ and is much lower than the levels of uranium in natural water.

Measuring the Isotope Ratio of Uranyl Species. Uranium has three natural isotopes. The ²³⁵U, the most radioactive uranium isotope of 0.725% natural abundance, is used widely in nuclear power plants and might be abused by terrorists as massive weapons. Therefore, it is important to rapidly detect this species at ultralow levels. As shown in Figure 4, the ²³⁵U species provide the same fragmentation pattern and thus can be detected using this method with multiple-stage CID experiments. A ²³⁵U/²³⁸U ratio higher than 0.725% usually indicates that the ²³⁵U species has been artificially enriched intentionally. Therefore, the ²³⁵U/²³⁸U ratio can be a useful marker to identify the spots used for uranium separation and enrichment. Figure 7 shows mass spectra showing a natural ²³⁵U/²³⁸U isotope ratio (a) and unusually high ²³⁵U/²³⁸U ratio (b). The uranyl species for the isotopes of ²³⁴U, ²³⁵U, and ²³⁸U were detected simultaneously at m/z 443, 444, and 447 (Figure 7a), with the signal intensities of 12, 1040, and 144 000 cps, respectively. These isotope peaks were identified using multiple-stage CID experiments (MS³), which produced the same characteristic fragments as those shown in Figure 4 at the corresponding stage. The isotope abundance ratio detected from the nonenriched uranium sample was 100:0.725:0.005 (²³⁸U/²³⁵U/²³⁴U), which was reasonable for the natural uranium sample. For more precise measurement, it is desirable to eliminate background noise which might contribute to the signal abundances detected in the full scan mass spectra. Once a sample containing intentionally enriched ²³⁵U species was infused, the corresponding isotope ratio was

Table 1. Analytical Results of Uranyl Species Analysis Using EESI-MS

<table>
<thead>
<tr>
<th>sample</th>
<th>content measured a (ng/L)</th>
<th>values added (ng/L)</th>
<th>total content measured a (ng/L)</th>
<th>values found a (ng/L)</th>
<th>RSD b (%)</th>
<th>recovery (%)</th>
<th>RSD c (%)</th>
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<td>530</td>
<td>80</td>
<td>8.1</td>
<td>80</td>
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<td>74</td>
<td>11</td>
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<td>110</td>
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<tr>
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<td>10</td>
<td>91</td>
<td>9</td>
<td>7.4</td>
<td>90</td>
<td>7.4</td>
</tr>
</tbody>
</table>

a Mean values of eight measurements. b RSD for eight measurements. c Recovery was calculated using the formula: recovery = [(total content measured − value added)/content added] × 100%.

Figure 6. Signal responses of uranyl acetate obtained using EESI-MS. (a) The plots of the signal abundances of the fragment (m/z 346) obtained in MS³ vs sample concentration, showing a dynamic response range between 0.10 and 1.0 × 10³ ng/L. The bar symbols designate 5% of the mean values. The intercept of the calibration curve is not a zero, indicating that the limit of detection is much lower than the concentration of 0.10 ng/L. This is also in agreement with the LOD data; (b) single ion chromatogram of m/z 447 of 5 measurements.

Figure 7. Rapid detection of isotope ratios of uranyl species by EESI-MS: (a) mass spectra with the natural ²³⁵U/²³⁸U isotope ratio and an (b) unusually high ²³⁵U/²³⁸U ratio.

Clearly, the acceptable reproducibility was achieved using this method. The relatively standard deviation (RSD) was about 8% for multiple measurements (n = 5). These data show that the uranyl species can be directly quantified from natural samples with acceptable precision and accuracy.

rapidly detected (Figure 7b) and provided solid evidence to specify the activities of uranium enrichment. Extra experiments showed that for a sample containing a minimal amount of enriched 235U species (0.8%), the signal abundances of m/z 444 for 10 measurements were about 1152 ± 28 cps, which was differentiated from the signal levels (1010 ± 20 cps) of natural samples. These data show that EESI-MS is a convenient tool for rapid, sensitive detection of the isotope ratio of uranyl species. As demonstrated before, EESI can be implemented for in situ analysis without performance loss.67 It is evident that a miniature mass spectrometer68,69 installed with a specially designed EESI source for in situ analysis can be a good solution to screen the uranium enrichment spots on site, showing prospects for applications in homeland security and public safety programs.

Analysis Speed. Traditionally, multiple-step sample pretreatment is required prior to actual sample analysis using mass spectrometry-based techniques. Particularly, for trace detection of radioactive elements, sample pretreatment including extraction, separation, and preconcentration is usually required when using many techniques including mass spectrometry. With minimal sample pretreatment, ambient mass spectrometry has been successfully applied for rapid detection of organic compounds present in complex matrices. As demonstrated in this study, inorganic species such as uranyl acetate in natural water samples were directly analyzed without sample separation, extraction, or preconcentration. A single sample analysis was completed within 1.5 min using MS4. Currently, the throughput of this method was mainly limited by the sample loading and in-depth multiple-stage CID experiments. However, it takes only a few seconds to produce an EESI-MS spectrum of uranyl species in water with complex matrixes, showing that EESI-MS is a useful tool for high-throughput screening of uranyl species in liquids.

CONCLUSIONS

Extractive electrospray ionization mass spectrometry was successfully applied to characterize organic compounds in different complex matrixes without any sample pretreatment. In this work, an example of EESI-MS for rapid detection of radioactive inorganic species in natural water samples has been demonstrated. Under the experimental conditions, negatively charged uranyl acetate (m/z 447) undergoes characteristic fragmentation in the gas phase to produce UO3− as the final product, providing the fundamental chemistry for specific detection of uranyl acetate species in complex matrixes by EESI without sample pretreatment. Each single sample analysis was completed within 1.5 min using multiple stage CID experiments. The limit of detection was about a few 10−3 ng/L of uranium by utilizing the characteristic fragments obtained in the EESI-MS4 experiments. This method provides reasonable relative standard deviation (RSD = 6.9−8.1%) for 8 measurements (S/N = 3) and a linear dynamic range of 5 orders of magnitude. The isotope ratio of uranyl species can be rapidly detected, providing an alternative way to screen the spots used for uranium isotope separation and enrichment. Besides the fundamental importance, the results shown here establish a convenient method for fast detection of trace amounts of uranyl speciation, providing applications in analytical chemistry and technology development of uranium.

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