

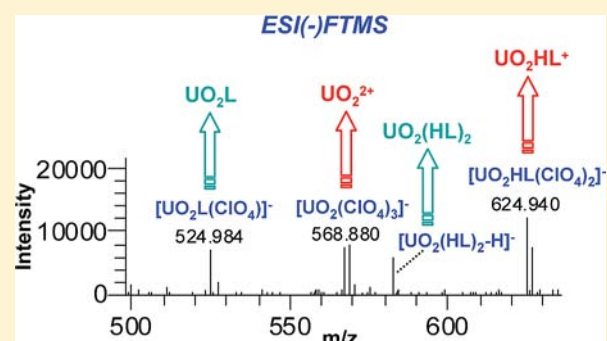
Trace Level Uranyl Complexation with Phenylphosphonic Acid in Aqueous Solution: Direct Speciation by High Resolution Mass Spectrometry

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S Supporting Information

ABSTRACT: The complexation of U(VI) by organic P-containing ligands in humic substances (HS) is an important issue of uranyl mobility in soil. We have investigated the complexation of uranyl by a model ligand for aromatic phosphorus functionalities in HS, phenylphosphonic acid, by using ultrahigh resolution electrospray ionization-mass spectrometry (ESI-MS). The high sensitivity permitted to investigate the complexation of trace level uranyl and to explore directly in the native aqueous solutions the nature of the uranyl-phenylphosphonate complexes. Positive identification of the complexes coexisting in solutions with low pH and varying ligand-to-metal ratio was achieved thanks to the high resolving power, high mass accuracy, and reliability of ion abundance of the technique. The positively charged and neutral uranyl species were detected simultaneously on negative ion mass spectra, evidencing formation of three types of U(VI)-phenylphosphonate complexes. Two complexes with a metal-to-ligand stoichiometry of 1:1 (in the monoprotonated and nonprotonated forms) existed in solutions at pH 3–5, and a 1:2 complex was additionally formed at relatively high ligand-to-metal ratio. A strategy based on the use of uranyl-phosphate solution complexes as internal standards was developed to determine from the ESI(-)MS results the stability constants of the complexes, which were calculated to be $\log K_{111} = 3.4 \pm 0.2$ for $\text{UO}_2(\text{HPhPO}_3)^+$, $\log K_{101} = 7.1 \pm 0.1$ for UO_2PhPO_3 , and $\log K_{112} = 7.2 \pm 0.2$ for $\text{UO}_2(\text{HPhPO}_3)_2$. The speciation model presented here suggests that organic P existing at low concentration in HS is involved significantly in binding by humic and fulvic acids of trace level uranyl in soil.



INTRODUCTION

Organic and inorganic phosphorus-containing ligands ubiquitous in soil systems are known to be strong ligands for various metals. The complexation of uranium(VI) by P-containing ligands is highly relevant to the biogeochemical cycle of uranyl in soils and the dissemination of uranyl in surface waters. Many field and spectroscopy studies have shown that competitive/cooperative processes between formation of uranyl complex with dissolved phosphoric acids and formation of U(VI)-phosphate complex/precipitate at surfaces of iron and aluminum (hydr)oxides play a major role in the mobility of U in phosphate-rich soil systems.^{1–4} In soils rich in organic matter, a high proportion of phosphorus exists in organic forms, as derivatives of phospholipids, phosphonic acids, phosphoric mono- and diesters.⁵ Although P content in humic substances (HS) is relatively low (800–1500 mg/kg), organic compounds containing P=O and P–OH functionalities show excellent ligand properties toward a wide range of metal centers^{6,7} and are expected to play a significant role in binding of uranyl by HS. Complexation of uranyl with carboxylic and phenolic ligands used as surrogates for HS functionalities has been extensively investigated.^{8–12} In contrast, few studies have explored the interactions existing between uranyl and model compounds with organic P=O and P–OH groups. In this

paper, we addressed the aqueous complexation of uranyl at trace level concentration relevant to the environment with a model ligand for aromatic phosphorus functional groups in HA.

A complete speciation model for a uranyl-organic ligand system requires joint information on the stoichiometry, structure, and stability of the complexes formed. Regarding organic complexes of uranyl, structural information has often been gained by means of X-ray Absorption Spectroscopy^{13,14} and Attenuated Total Reflectance Fourier Transform Infrared spectroscopy,^{14,15} which are powerful speciation techniques (because of their ability to probe the local structure around an atom and the chemical bonds, respectively) but are not applicable to poorly concentrated uranyl solutions. Thermodynamic stability data and constants for the uranyl-organic ligand systems have been obtained commonly by using potentiometry,^{16–24} UV–vis spectrophotometry,²⁵ and Time-Resolved laser-induced Fluorescence spectroscopy (TRLFS). Among all the techniques mentioned above, TRLFS is the only one that is applicable to solutions with very low uranyl concentrations relevant to the environment. TRLFS has been used to evidence, either directly or indirectly, the complexation of uranyl with a

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variety of aromatic carboxylic acids, glucose derivatives and amino acids,^{26–28} as well as orthophosphoric acid and phosphonic acids like HEDP and phenylphosphonic acid.^{29,30} However, the technique provides no structural information on the complexes. Moreover, two uranyl complexes differing in their structure by only one hydrogen ion show no difference in their emission spectra and lifetimes and are thus undistinguishable by TRLFS, as shown for $\text{UO}_2\text{H}_2\text{PO}_4^+$ and $\text{UO}_2\text{HPO}_4^{31}$. TRLFS data provide thus no qualitative information of metal–ligand systems and may leave some ambiguity in the identification of the speciation scheme. The technique has also some limitations such as quenching effects and possible photolysis of organic moieties induced by the laser pulse.

Electrospray ionization-mass spectrometry (ESI-MS) is a powerful speciation technique; the only technique that enables the positive identification of organo-metallic species at trace levels. ESI-MS has been used in the past years in speciation studies involving lanthanides and heavy metals, and the nature of many uranyl:organic ligand complexes existing over a range of environmentally relevant conditions has been clarified.^{29,32,33} ESI combined with (ultra) high resolution and high mass accuracy spectrometry has greatly improved the resolving power of mass spectrometry, providing high potentialities to the technique for identifying the molecular formula of complex molecules, such as natural organic matter,³⁴ or organo-metallic species present in solution at the trace level concentration.^{32,35,36}

ESI is a “soft” ionization method to transfer ions pre-existing in the solutions into the gas phase, in which ions are examined by the MS technique. In the ESI probe, species or molecules capable of acquiring charge through protonation, deprotonation, association with ionic solutes, or electron transfer are also transferred into the gas phase, so that much of the speciation information from the original solution can be retrieved. For example, Jacopin et al.²⁹ have shown that the uranyl dication UO_2^{2+} , which is the main chemical form of U(VI) in acidic perchlorate solutions, is preserved during negative ion mode analysis, and the UO_2^{2+} entity is detected in this mode upon its association in the ESI probe with the noncomplexing perchlorate ion, as $[\text{UO}_2+(\text{ClO}_4)_3]^-$. The authors also solved the identity and stoichiometry of U(VI)-HEDP complexes existing in acidic solutions. Both positively charged and neutral charge complexes appeared on ESI(-)MS spectra as the entity complexes associated with perchlorate ions and HEDP molecules. Samogyi et al.³⁷ showed that the ESI(+) ionization mode analysis preserved the speciation of uranyl in citrate-containing solutions with pH in the range 3.0–6.5, and confirmed the stoichiometry of the complexes determined by other conventional methods. Although many published ESI-MS studies have shown that interpretation of spectra is not always straightforward, as a variety of easily formed adducts between the entity complex and alkalis, water molecules, and molecules of acids may be generated during the ESI process, the technique appears unquestionably as the necessary technique to obtain definite information on chemical formulas and structures of trace organo-metallic complexes in aqueous solutions.³²

In this study, the interactions existing between uranyl and phenylphosphonic acid, H_2PhPO_3 (also noted H_2L), were studied by means of the highly sensitive, linear ion trap–orbitrap XL hybrid Fourier Transform mass spectrometer. The main objectives were to infer the stoichiometry and structure of the U(VI)-phenylphosphonate complexes formed in low-pH

solutions containing uranyl at the trace level concentration (3 μM), and to clarify the species distribution.

Advantage was taken of the high sensitivity, high resolution ($R = 100\,000$ at 400 m/z), and high mass accuracy (2–3 ppm) of the orbitrap analyzer to meet main challenges in uranyl complexation studies, that is, exploring directly in the native aqueous solutions the structure of the U(VI) species (without adding any cosolvent) and using trace levels of U. These aspects are important for the applicability of the results to the speciation of uranyl in soil systems. Positive identification of the uranyl-phenylphosphonate complexes existing in solutions at varying ligand-to-metal ratio was achieved.

Because of differences in ion response factors, an internal standard strategy was developed to derive the stability constants of the complexes from ESI-FTMS data recorded in the negative ionization mode. The orthophosphoric acid-uranyl system was used as the internal standard because (i) the system shows chemical analogies with the uranyl-phenylphosphonate system, (ii) each of the uranyl-phenylphosphonate complexes identified can be related to an uranyl phosphate complex (as its internal standard) exhibiting the same charge and same reliance in instrumental parameters, (iii) ESI-MS analysis provides an accurate picture of the well-established speciation of U(VI) in phosphate solutions before measurement, that is, the three complexes $\text{UO}_2\text{H}_2\text{PO}_4^+$, UO_2HPO_4 , and $\text{UO}_2(\text{H}_2\text{PO}_4)_2$ that are prevalent in acidic solutions, were definitely identified on ESI(-)MS spectra recorded for solutions at pH 3. The identity of species and stability constants determined here provide a comprehensive speciation model for the U(VI)-phenylphosphonate system, which is useful for assessing the role of aromatic phosphorus functional groups in binding of uranyl by HS, and thus, for improving predictive modeling of uranyl cycling in soil.

■ EXPERIMENTAL SECTION

Materials. Uranium perchlorate hexahydrate and dihydrogenophosphoric acid disodium salt were purchased from Fluka, and phenylphosphonic acid disodium salt from Aldrich. Solutions were prepared by using reagent grade chemicals (HClO_4) and ultrapure water. Concentrated stock solutions of H_2PhPO_3 and H_3PO_4 were obtained by dissolving the required amount of salt in water adjusted to pH 3.0 (with HClO_4). Stock solution of uranyl was prepared by dissolving $\text{UO}_2(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ in 0.1 M HClO_4 . Solutions for the ESI-FTMS measurements were prepared by mixing 10 μL of UO_2^{2+} stock solution (final concentration: $3.00 \pm 0.03 \mu\text{M}$) with a precise volume of H_2PhPO_3 concentrated solution to give a ligand-to-metal ratio in the range 0–23. For experiments requiring the use of internal standards, H_2PhPO_3 –U(VI) solutions were doped with orthophosphoric acid, producing two sets of doped solutions with final H_3PO_4 concentration of 15 μM and 30 μM , respectively. The concentration of U(VI) in the daughter solutions was checked by ICP-MS analysis. No attempt was made to fix the ionic strength as addition of inert salts would induce formation of ionic aggregates and scatter the ESI-FTMS signal. Unless otherwise indicated, the experiments were conducted at pH 3.0.

ESI-FTMS Measurements. Solutions containing U and phenylphosphonic acid were systematically analyzed within 10 min following their preparation. Successive ESI-FTMS measurements performed on a time scale of 2 days have shown no degradation of the molecules (with the ESI-FTMS intensities remaining unchanged over time). ESI-MS spectra are generally obtained in organic solvents (methanol, acetonitrile) or in their aqueous mixtures, because pure water may cause discharges within the spray chamber leading to both signal instability and marked worsening of the signal-to-noise ratio. A previous study has shown that U has a higher affinity for alcohol molecules than for water,³⁸ so that question arises of the disturbance of

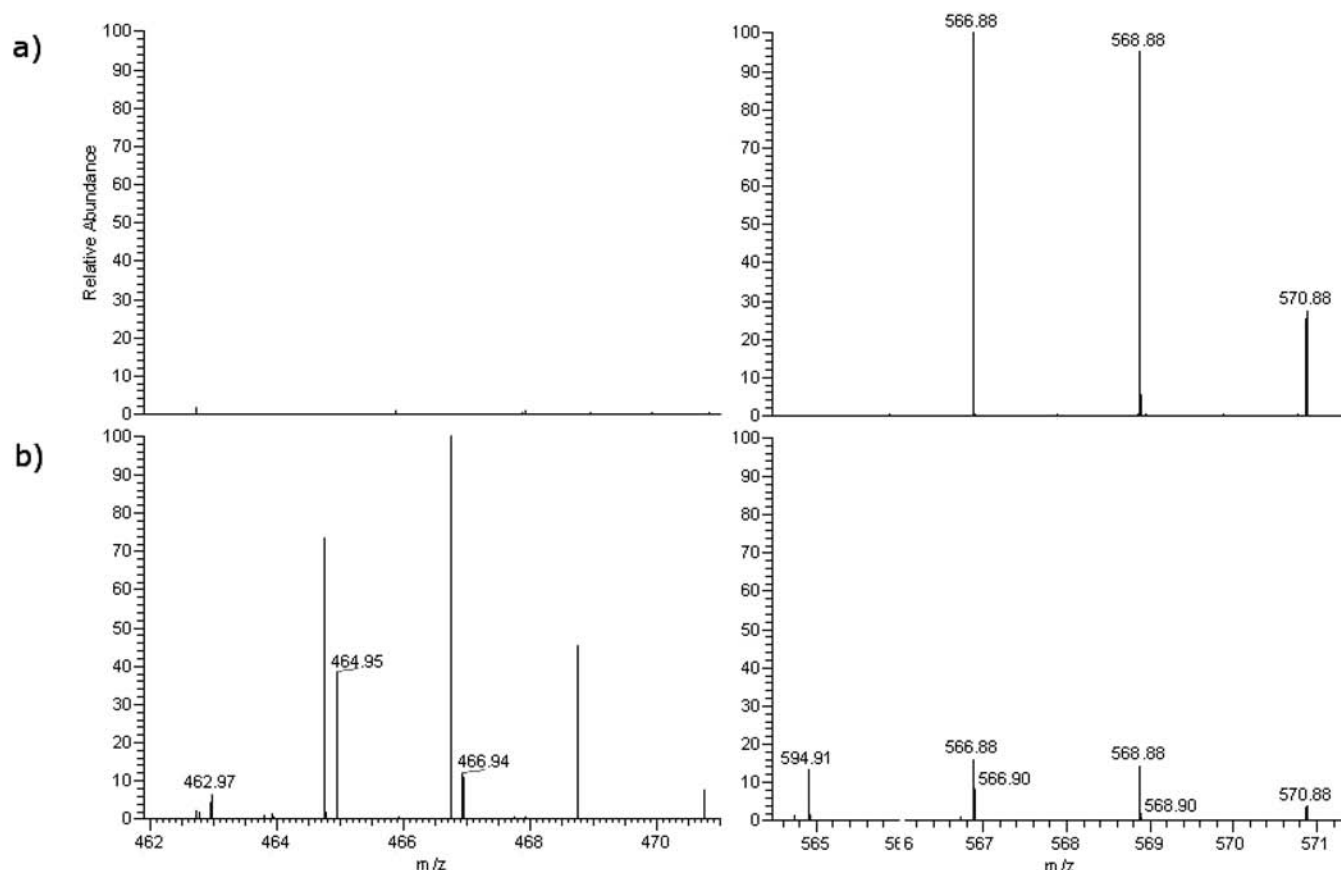


Figure 1. Zoom in the regions of interest for ESI(-)FTMS spectra of (a) a uranyl solution without ligand, and (b) a solution containing 3 μM U(VI) and a 10-fold molar ratio of phosphoric acid, at pH = 3.0 (ITL = 180 V) (The four peaks at 464.76, 466.76, 468.75, and 470.75 m/z correspond to the adduct $[\text{ClO}_4+(\text{NaClO}_4)_3]^-$).

metal speciation by addition of a cosolvent. We took up the challenge to work with pure aqueous solutions, furthermore with uranyl at trace level (3 μM), thanks to the high sensitivity of the hybrid LTQ Orbitrap XL analyzer. Particular attention was paid to avoid any pH shift during the electrospray process: a capillary in stainless steel was employed to partially substitute oxidation of water by oxidation of the capillary and solutions were infused at a high flow rate. Moreover, changes in pH induced by electrolytically produced protons could be neglected by the use of low-pH solutions.³²

ESI detection in the negative and positive modes was performed by using a linear ion trap-orbitrap mass spectrometer, LTQ Orbitrap XL (Thermo Electron). Solutions were introduced directly into the ESI probe with a syringe pump, at a flow rate of 10 $\mu\text{L min}^{-1}$. Nitrogen was used as the drying and spraying gas. The temperature of the transfer tube was fixed at 275 $^\circ\text{C}$. Spray voltages of 3 kV and 4.2 kV were applied in the negative and positive ionization modes, respectively. The voltage applied to the capillary and the multipoles was automatically tuned to obtain an optimized signal at 566.88 m/z (peak related to the adduct $[\text{UO}_2+(\text{ClO}_4)_3]^-$ generated, in the ESI probe, from the aqueous species UO_2^{2+}) in the negative ionization mode or 427.05 m/z (peaks related to the ion $\text{UO}_2\text{HPhPO}_3^+$) in the positive ionization mode. Analyses were performed at tube lens voltage (TL) values ranging from -10 V to -230 V in the negative ionization mode. Full MS spectra were recorded using the Orbitrap analyzer, by averaging 40 scans in the range 120–2000 m/z . Identification of each peak was made with the help of MS^n measurements. Precursor ion isolation and fragmentation by collision-induced dissociation (CID) were carried out in the linear ion trap. The daughter ions were transferred to the Orbitrap analyzer for acquisition of high-resolution, accurate-mass MS^n data. Acquisition and treatment of the data were done with the Xcalibur software.

RESULTS AND DISCUSSION

Regarding the positive ionization mode, the intrinsically positively charged uranyl species existing in the native solutions are clearly observable as such on the ESI(+)-MS spectra. Neutral solution species are identifiable on the ESI(+)-MS spectra, too, because they acquire positive charge in the ESI probe through formation of adducts with inorganic cations present in the solution (typically H^+ or Na^+).^{29,37} Water molecules also take part in the clustering process. However, it has long been reported in previous ESI-MS studies^{39,40} that uranyl undergoes a partial reduction taking place after the desolvation process and leading to UO_2^+ peaking at 270.04 m/z . We also observed this peak related to UO_2^+ at 270.04 m/z on the positive ionization mass spectra recorded for our native uranyl solutions at pH 3.

In contrast, it has been shown that ESI(-)-MS analysis does not alter either the uranyl oxidation state (+6) nor the structure of the uranyl species existing in the ligand-containing solution.^{29,37} As will be shown later in the text, the uranyl ion and the U(VI)-ligand complexes that are positively charged or neutral in solution are identifiable on the ESI(-)-MS spectra mainly as perchlorate adducts. We purposely used sample preparation conditions favoring such a combination between the solution complex and the perchlorate anions in the ESI probe (i.e., HClO_4 was used to adjust the pH at a value of 3.0). At high ligand concentration, ESI induced additional formation of “mixed clusters” involving the solution complex, ClO_4^- ions, and one or two ligand molecules.

For all above-mentioned reasons, we preferred using the negative ionization mode to fully explore the uranyl-phenylphosphonate interactions.

1. Preliminary Studies. 1.1. Mass Spectrometry Analysis of Uranyl Solutions Containing No Ligand. Even in the absence of added organic or inorganic ligands, the aqueous speciation of uranyl may be very complex because of formation of numerous hydrolysis products and U(VI)-carbonate complexes. In this study, perchlorate was chosen to adjust the solution pH because of its well-known noncomplexing behavior toward uranyl.⁴¹ Moreover, we worked with trace level uranyl concentrations (3 μM) relevant to the environment, preventing thereby the formation of polynuclear species and true uranyl colloids. In a TRLFS study⁴ by our research team, the formation of such true uranyl colloids was detected since pH 3.3 in perchlorate solutions containing 10 μM U(VI). For poorly concentrated (3 μM) uranyl solutions with no ligand and with pH lower than 3.3, the uranyl speciation is well-established: U(VI) exists mainly in the form of UO_2^{2+} and the hydrolysis products represent less than 0.1% of total U (on basis of thermodynamic calculations using the updated thermodynamic database for uranyl⁴¹). Hence, the circumstances of the present study (pH = 3; $[\text{U(VI)}] = 3 \mu\text{M}$) represent the best scenario for assessing the effect of organic phosphate on uranyl speciation.

UO_2^{2+} is observable as such at 135.02 m/z on the ESI(+)FTMS spectra recorded for our native solutions at 3 μM of uranyl with pH 3 (The first hydrolysis products UO_2OH^+ and/or $(\text{UO}_2)_2(\text{OH})_2^{2+}$ are detected since pH 4; both hydrolysis products giving rise to a peak at 287.04 m/z and being indistinguishable). When analyzed in the negative ionization mode, the uranyl solutions with pH 3 yielded a series of peaks positioned at 566.88–572.88 m/z (Figure 1a), whose intensity increased linearly with U_T in the range 0.15 μM –3 μM (data not shown). The peaks are those of UO_2^{2+} , which undergoes attachment of ClO_4^- ions in the ESI probe and forms the adduct $[\text{UO}_2+(\text{ClO}_4)_3]^-$. The formation of such an adduct during negative ionization mode analysis has long been reported.²⁹ The identity assignment was moreover confirmed by isotopic adducts with the characteristic ^{35}Cl : ^{37}Cl intensity ratio (Table 1). Thus, the ESI(-)FTMS spectra showed predominance of UO_2^{2+} in aqueous uranyl solutions at pH 3.0 containing no ligand, in good accordance with thermodynamic calculations.

Table 1. Isotope Abundance Profiles for the $[\text{UO}_2+(\text{ClO}_4)_3]^-$ Cluster Detected on ESI(-)MS Spectra^a

aqueous species	m/z	peaks detected	relative isotopic abundance
UO_2^{2+}	566.88	$[\text{UO}_2+(^{35}\text{ClO}_4)_3]^-$	100 (100)
	568.88	$[\text{UO}_2+(^{35}\text{ClO}_4)_2(^{37}\text{ClO}_4)]^-$	96.2 (95.9)
	570.88	$[\text{UO}_2+(^{35}\text{ClO}_4)(^{37}\text{ClO}_4)_2]^-$	31.1 (30.7)
	572.88	$[\text{UO}_2+(^{37}\text{ClO}_4)_3]^-$	2.3 (3.3)

^aCalculated abundances in parentheses.

1.2. Speciation Analysis by Mass Spectrometry of a Well-Characterized System: The Uranyl-Orthophosphoric Acid System. Thermodynamic calculations of uranyl speciation using the OECD database⁴¹ indicate that UO_2^{2+} and the three complexes $\text{UO}_2\text{H}_2\text{PO}_4^+$, UO_2HPO_4 , and $\text{UO}_2(\text{H}_2\text{PO}_4)_2$ coexist in solutions with pH 3.0 containing 3 μM of uranyl and 35 μM of orthophosphoric acid. The free ligand exists mainly in the

form of H_3PO_4 and H_2PO_4^- . Figure 1b shows the ESI(-)MS spectrum obtained for this solution (recorded at value of tube lens voltage, $|TTL| = 180 \text{ V}$). During negative ionization mode, the solution complexes $\text{UO}_2\text{H}_2\text{PO}_4^+$ and UO_2HPO_4 acquire negative charge through association with perchlorate ions and form the adducts $[\text{UO}_2\text{H}_2\text{PO}_4+(\text{ClO}_4)_2]^-$, peaking at 564.91–568.90 m/z , and $[\text{UO}_2\text{HPO}_4+(\text{ClO}_4)]^-$ peaking at 464.95–466.95, respectively. The 1:2 complex undergoes deprotonation during the ESI process and is detected as the molecular ion $[\text{UO}_2\text{H}_2\text{PO}_4\text{HPO}_4]^-$ peaking at 462.97 m/z . The important conclusion of this preliminary study is that (i) ESI-MS is good at obtaining accurate description of the number and stoichiometry of complexes coexisting at trace level in native solutions, even for the two solution complexes $\text{UO}_2\text{H}_2\text{PO}_4^+$ and UO_2HPO_4 that only differ by one proton, and (ii) the ESI(-) analysis preserves the nature of the solution complexes.

Quantitative speciation analysis of uranyl from ESI-MS data is not straightforward. It is worth noting that the ESI-MS response of each analyte depends on the tube lens voltage (TL), as illustrated on Figure 2. Plotting the peak intensity

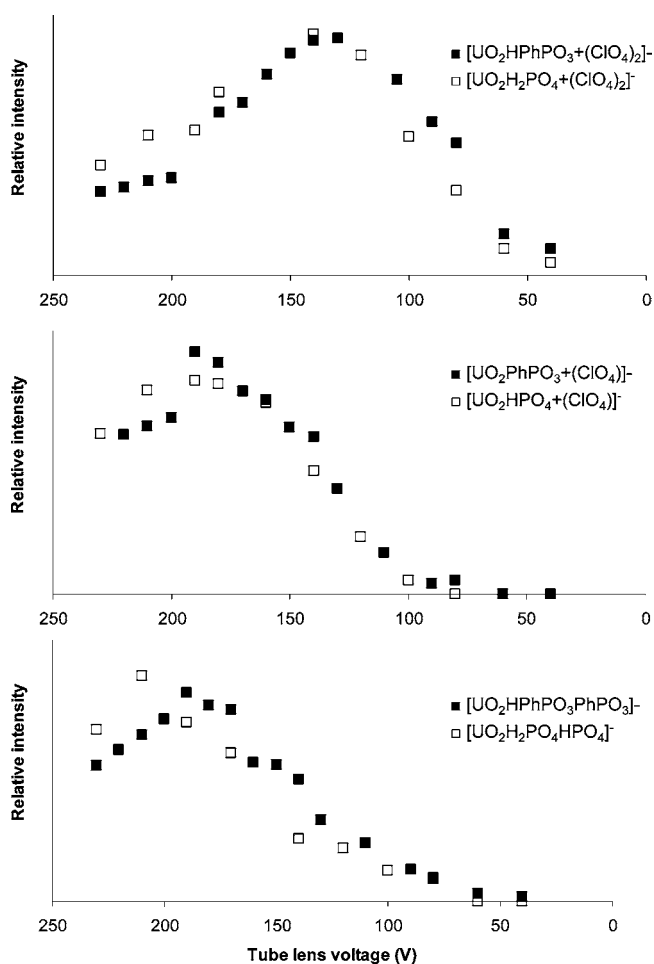


Figure 2. ESI(-)FTMS results showing dependence on tube lens voltage of the intensity of the base peak for species A (624.94–628.94 m/z), species B (524.98–526.94 m/z), and species C (583.04 m/z) identified in the U(VI)-phenylphosphonic acid system. Comparison with the TL-dependence of the three uranyl-phosphate solution complexes $\text{UO}_2\text{H}_2\text{PO}_4^+$ (564.91–568.90 m/z), UO_2HPO_4 (464.95–466.95 m/z), and $\text{UO}_2(\text{H}_2\text{PO}_4)_2$ (462.97 m/z). Experimental conditions: $[\text{U(VI)}]_T = 3 \mu\text{M}$, $[\text{H}_2\text{PhPO}_3]_T = 30 \mu\text{M}$ or $[\text{H}_3\text{PO}_4]_T = 30 \mu\text{M}$, pH = 3.0.

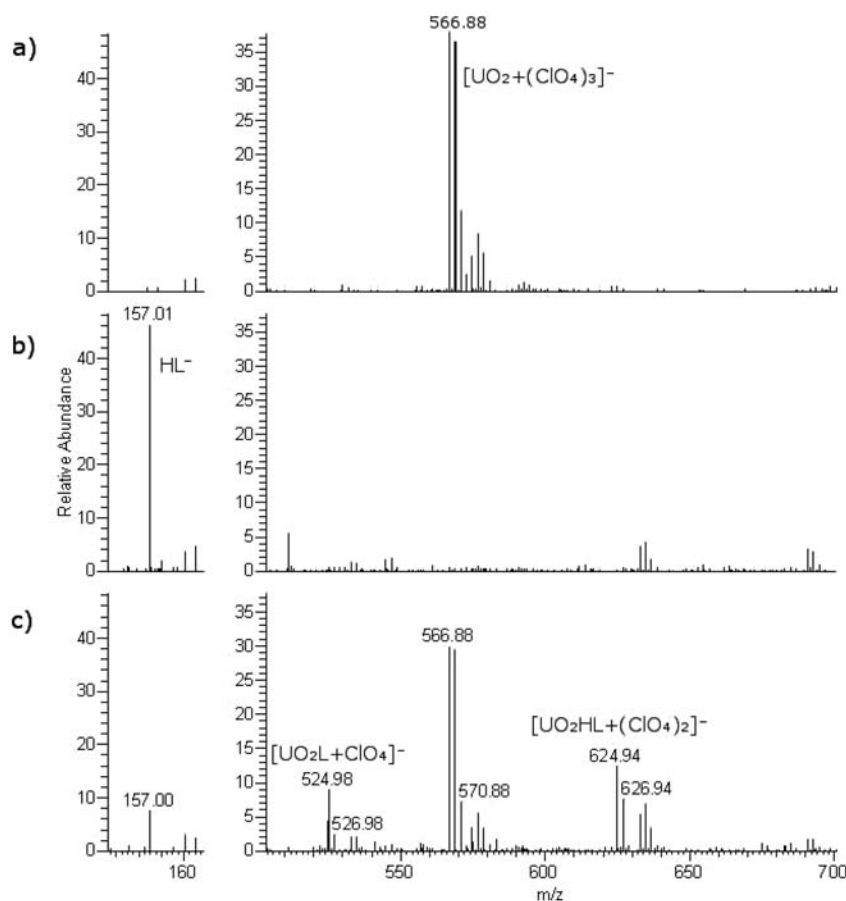


Figure 3. Zoom in the regions of interest for ESI(-)FTMS spectra of (a) uranyl solution without ligand, (b) phenylphosphonic acid solution without U, and (c) uranyl- H_2PhPO_3 solution at a ligand-to-metal ratio of 3 ($[\text{U(VI)}]_{\text{T}} = 3 \mu\text{M}$, $\text{pH} = 3.0$, $|\text{TLL}| = 180 \text{ V}$).

versus TL voltage shows a bell-shaped curve for $[\text{UO}_2\text{H}_2\text{PO}_4+(\text{ClO}_4)_2]^-$, as well as for $[\text{UO}_2\text{HPO}_4+(\text{ClO}_4)]^-$ and $[\text{UO}_2\text{H}_2\text{PO}_4\text{HPO}_4]^-$. The value of TL corresponding to the maximum of the curve differs from one adduct/molecular ion to another. The adduct formed from the aqueous species $\text{UO}_2\text{H}_2\text{PO}_4^+$ is transmitted with a relatively high efficiency over a broad range of tube lens voltage ($40 \text{ V} < |\text{TLL}| < 230 \text{ V}$). In contrast, the adduct and the molecular ion corresponding to the species UO_2HPO_4 and $\text{UO}_2(\text{H}_2\text{PO}_4)_2$, respectively, are observable on the ESI(-)MS spectra only at $|\text{TLL}|$ values higher than 80 V . Thus, the relative proportions of clusters/molecular ions observed on an ESI-MS spectrum do not reflect the relative proportions of the solution complexes. It is necessary to develop specific strategies if one wants to provide a quantitative description of the system using ESI(-)MS data.

2. Identification of the U(VI)-Phenylphosphonate Complexes Formed in Acidic Solutions. 2.1. Identity of Uranyl Complexes in Solutions with Low H_2PhPO_3 : U(VI) Molar Ratios (<3): ESI(-)MS Investigations at $|\text{TLL}| = 180 \text{ V}$. Uranyl speciation studies in the uranyl-phenylphosphonic acid system at $\text{pH} 3$ was started using solutions at low H_2PhPO_3 :U(VI) molar ratios (from 0.6 to 3) and analysis in the negative ionization mode at a tube lens voltage of 180 V (for which the ESI-MS analysis of the uranyl-phosphate system provided a good representation of the solution complexes).

Figure 3 shows as an example the mass spectra recorded for uranyl solutions (with no ligand), phenylphosphonic acid solutions (with no U), and uranyl- H_2PhPO_3 solutions at a ligand-to-metal ratio of 3 (with a zoom in the regions of

interest). The predominant form of the uncomplexed ligand in solutions at $\text{pH} 3$ is the HL^- ion, that is, HPhPO_3^- ($\text{p}K_{\text{a}1} < 2$ and $\text{p}K_{\text{a}2} = 7$), which has a value of 157.00 m/z observable for all the H_2PhPO_3 -containing solutions at $\text{pH} 3$ investigated in this study (for example in Figure 3b–c). For both in the absence and in the presence of the ligand, free uranyl was observed as the adduct $[\text{UO}_2+(\text{ClO}_4)_3]^-$. The mixed uranyl-organic molecule solutions exhibited peaks additional to those related to HL^- and UO_2^{2+} , which was indicative of the complexation of U(VI) with the organic ligand in the solution (Figure 3c). The additional peaks are observed at 624.94 – 628.94 m/z and 524.98 – 526.98 m/z . The isotopic distribution patterns and the exact masses are characteristics of the ClO_4^- adducts $[\text{UO}_2\text{HL}+(\text{ClO}_4)_2]^-$ and $[\text{UO}_2\text{L}+(\text{ClO}_4)]^-$, produced in the ESI probe from uranyl-phenylphosphonate complexes existing in solution with a uranyl:ligand stoichiometry of 1:1. An important point to consider for assignment of the adducts is that, for acidic solutions, deprotonation in the ESI probe is unlikely for compounds having $\text{p}K_{\text{a}}$ values in the range 5 – 28 .⁴² Therefore, the adduct having a base peak at 624.94 m/z is clearly derived from the protonated solution complex, UO_2HL^+ (noted as species A), and the adduct showing a base peak at 524.98 m/z can reasonably be attributed to the neutrally charged 1:1 complex UO_2L (noted as species B).

2.2. Identity of Uranyl Complexes in Solutions with High H_2PhPO_3 :U(VI) Molar Ratios (3–23): ESI(-)MS Investigations at $|\text{TLL}| = 180 \text{ V}$. At higher ligand concentration, the ESI(-)MS spectra (recorded at $|\text{TLL}| = 180 \text{ V}$) are more complicated, because of the tendency of the ligand molecules to associate

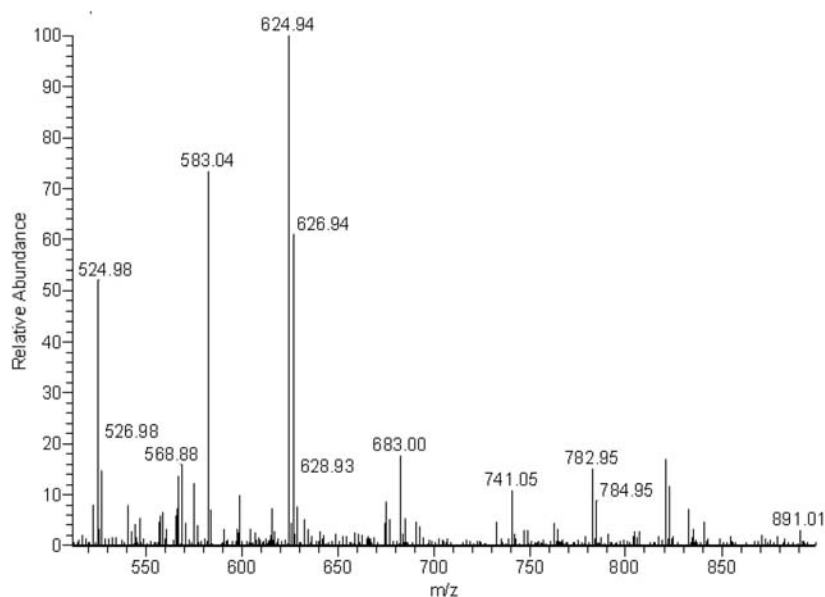


Figure 4. Zoom in the regions of interest for a ESI(-)FTMS spectrum of a uranyl- H_2PhPO_3 solution at a ligand-to-metal ratio of 10 ($[\text{U(VI)}]_{\text{T}} = 3 \mu\text{M}$, $\text{pH} = 3.0$, $|\text{TL}| = 180 \text{ V}$).

Table 2. Assignment of Main Peaks Detected on ESI(+) Mass Spectra (Left Side) and ESI(-) Spectra (Right Side) of U(VI)- H_2PhPO_3 Solutions^a

<i>m/z</i>	assignment	<i>m/z</i>	assignment
Uranyl			
135.02	UO_2^{2+}	566.88→572.88	$[\text{UO}_2+(\text{ClO}_4)_3]^-$
270.04	UO_2^+	724.90→730.90	$[\text{UO}_2+(\text{ClO}_4)_3(\text{H}_2\text{PhPO}_3)]^-$
287.04	$\text{UO}_2(\text{OH})^+$	882.91→888.91 ^b	$[\text{UO}_2+(\text{ClO}_4)_3(\text{H}_2\text{PhPO}_3)_2]^-$
Free Phenylphosphonate Ligand			
159.02	$[\text{H}_2\text{PhPO}_3+\text{H}]^+$	157.01–158.01	HPhPO_3^-
181.00	$[\text{H}_2\text{PhPO}_3+\text{Na}]^+$		
317.03	$[\text{H}_2\text{PhPO}_3+\text{HH}_2\text{PhPO}_3]^+$		
339.02	$[\text{H}_2\text{PhPO}_3+\text{NaH}_2\text{PhPO}_3]^+$		
475.05	$[\text{H}_2\text{PhPO}_3+\text{H}(\text{H}_2\text{PhPO}_3)_2]^+$		
Species A, UO_2HL^+			
427.05	$\text{UO}_2(\text{HPhPO}_3)^+$	624.94→628.94	$[\text{UO}_2\text{HPhPO}_3+(\text{ClO}_4)_2]^-$
445.05	$[\text{UO}_2(\text{PhPO}_3)+(\text{H}_2\text{O})]^+$	782.95→786.95	$[\text{UO}_2\text{HPhPO}_3+(\text{ClO}_4)_2(\text{H}_2\text{PhPO}_3)]^-$
463.07	$[\text{UO}_2(\text{PhPO}_3)+(\text{H}_2\text{O})_2]^+$	940.96→944.96 ^b	$[\text{UO}_2\text{HPhPO}_3+(\text{ClO}_4)_2(\text{H}_2\text{PhPO}_3)_2]^-$
Species B, UO_2L			
427.05	$[\text{UO}_2(\text{PhPO}_3)+\text{H}]^+$	524.98→526.98	$[\text{UO}_2\text{PhPO}_3+(\text{ClO}_4)]^-$
445.05	$[\text{UO}_2(\text{HPhPO}_3)+\text{H}(\text{H}_2\text{O})]^+$	683.00→685.00	$[\text{UO}_2\text{PhPO}_3+(\text{ClO}_4)(\text{H}_2\text{PhPO}_3)]^-$
463.07	$[\text{UO}_2(\text{HPhPO}_3)+\text{H}(\text{H}_2\text{O})_2]^+$	841.00→843.01 ^b	$[\text{UO}_2\text{PhPO}_3+(\text{ClO}_4)(\text{H}_2\text{PhPO}_3)_2]^-$
Species C, $\text{UO}_2(\text{HL})_2$			
585.06	$[\text{UO}_2(\text{HPhPO}_3)_2+\text{H}]^+$	583.04–584.06	$[\text{UO}_2\text{PhPO}_3(\text{HPhPO}_3)]^-$
603.07	$[\text{UO}_2(\text{HPhPO}_3)_2+\text{H}(\text{H}_2\text{O})]^+$	741.06–742.06 ^b	$[\text{UO}_2\text{PhPO}_3(\text{HPhPO}_3)+(\text{H}_2\text{PhPO}_3)]^-$
743.07	$[\text{UO}_2(\text{HPhPO}_3)_2+\text{H}(\text{H}_2\text{PhPO}_3)]^+$	899.06–900.07 ^b	$[\text{UO}_2\text{PhPO}_3(\text{HPhPO}_3)+(\text{H}_2\text{PhPO}_3)_2]^-$
901.09	$[\text{UO}_2(\text{HPhPO}_3)_2+\text{H}(\text{H}_2\text{PhPO}_3)_2]^+$		

^aThe arrow connects the lower *m/z* value to the higher value of the isotopic profile of the ClO_4^- -adducts. ^bClusters observed only at tube lens voltage lower than -100 V .

with perchlorate adducts during ESI process. Main features of the ESI(-)MS spectra recorded for solutions at ligand-to-uranyl ratio ranging from 3 to 23 are illustrated in Figure 4, for a solution containing $3 \mu\text{M}$ U(VI) and a 10-fold molar ratio of H_2PhPO_3 . Species A and B were observable as the ClO_4^- adducts described in previous section (base peaks at 624.94 m/z and 524.98 m/z , respectively), and as additional mixed $\text{H}_2\text{L}-\text{ClO}_4^-$ clusters of minor intensity with base peaks at 782.95 m/z for species A and 683.00 m/z for species B (Table 2).

Assignment of the base peaks at 782.95 m/z and 683.00 m/z are ascertained by MSⁿ results. The MSⁿ measurements with collision-induced dissociation showed that the clusters at $782.95-786.95 \text{ m/z}$ and $683.00-685.00 \text{ m/z}$ readily dissociate by losing one H_2L molecule. This feature is the evidence that one H_2L molecule associates weakly with the solution complex during the ionization process, which rules out any participation of the readily removed H_2L molecule to the first coordination sphere of uranyl.

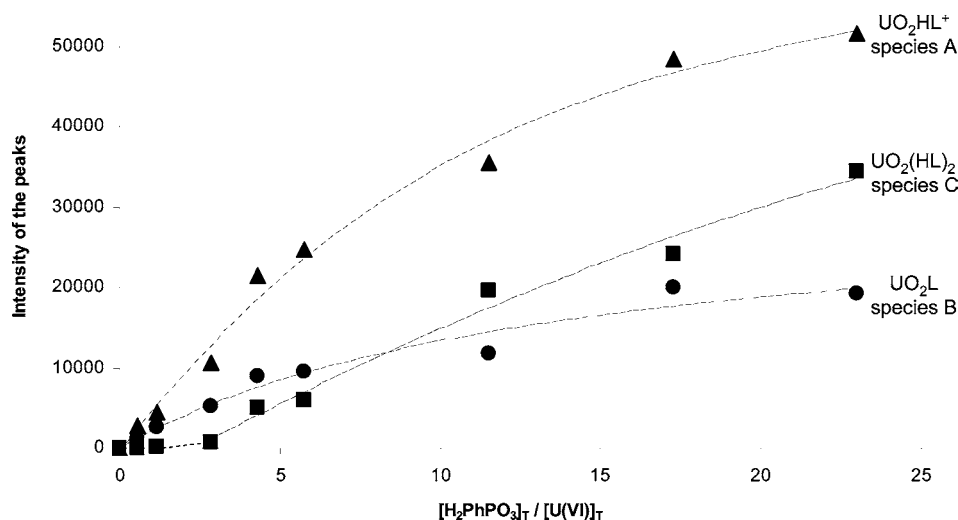


Figure 5. ESI(-)FTMS results showing changes with the ligand-to-metal ratio of the peak intensity of the complexes A, B, and C formed in U(VI)-phenylphosphonic acid solutions. Standard deviation is $\pm 10\%$. Lines are guides for the eyes. Experimental conditions: $[\text{H}_2\text{PhPO}_3]_{\text{T}}/[\text{U(VI)}]_{\text{T}} = 0\text{--}23$, $[\text{U(VI)}]_{\text{T}} = 3 \mu\text{M}$, $\text{pH} = 3.0$, $|\text{TL}| = 180 \text{ V}$.

The ESI(-)MS spectra recorded for the solutions at high ligand-to-uranyl ratio also display two additional sets of peaks (Figure 4). The most intense set of peaks is detected at $583.04\text{--}584.06 m/z$ and a minor one occurs at $741.06\text{--}742.06 m/z$. Their isotopic profiles correspond well to the theoretical isotopic distributions for the molecular ion $[\text{UO}_2\text{LHL}]^-$ and its cluster with one ligand molecule $[\text{UO}_2\text{LHL}+(\text{H}_2\text{L})]^-$, respectively. Appearance of these sets of peaks provides strong evidence for the formation, at high ligand concentration, of a third uranyl-phenylphosphonate solution complex with a 1:2 stoichiometry. This solution complex $\text{UO}_2(\text{HL})_2$ (noted as species C) involves two HL^- units, with one of the units undergoing deprotonation during ESI process.

A plot of the peak intensity of the main adducts of the three identified solution complexes A, B, and C versus the ligand-to-uranyl ratio (in the range 0.6 to 23) shows (i) typical convex curves, flattening out at ligand-to-metal ratio of 10–15 (Figure 5) for both species A and species B, (ii) appearance of the species C only at a ligand-to-metal ratio around 3, and (iii) an almost linear increase of the intensity of species C when increasing the $\text{H}_2\text{L}:\text{U(VI)}$ ratio of the solution from 3 to 23. The observed dependences on the ligand-to-metal ratio of the species A and B on the one hand and of the species C on the other hand are those expected for complexes with 1:1 and 1:2 stoichiometries, respectively.

2.3. Exploring Further Complex Identity: ESI(-)MS Investigations over a Broad TL Range (0–230 V). The speciation of uranyl in phenylphosphonate solutions at pH 3 was explored further by recording ESI(-)MS spectra of the solutions over a broad range of the tube lens voltage (from 0 to 230 V). The objective was to ensure that species A, B, and C are the only uranyl-phenylphosphonate complexes existing in the solutions, as the preliminary speciation study reported here (section 1.2) for the uranyl-phosphate system has shown that the value of TL controls the transmission rate of adducts/clusters.

The number and the nature of the clusters, adducts, and molecular ions detected on the spectra remain unchanged when varying the tube lens voltage from 140 V to 230 V. The solution species A, B, and C are observable on the spectra recorded at 140 V–230 V mainly as $[\text{UO}_2\text{HL}+(\text{ClO}_4)_2]^-$, $[\text{UO}_2\text{L}+(\text{ClO}_4)]^-$, and $[\text{UO}_2\text{LHL}]^-$, respectively, and as the additional

clusters involving one ligand molecule, as already described in details for a TL value of 180 V. For tube lens voltage lower than 140 V, there was observed on the spectra the appearance of additional three minor sets of peaks at $940.96\text{--}944.96$, $841.00\text{--}843.01$, and $899.06\text{--}900.07 m/z$. We firmly assigned these peaks to the clusters $[\text{UO}_2\text{HL}+(\text{ClO}_4)_2(\text{H}_2\text{L})_2]^-$, $[\text{UO}_2\text{L}+(\text{ClO}_4)(\text{H}_2\text{L})_2]^-$, and $[\text{UO}_2\text{LHL}+(\text{H}_2\text{L})_2]^-$, respectively, on the basis of the exact masses and MS^n measurements with CID (the MS^2 and MS^3 data showed that these clusters dissociated readily by losing two H_2L molecules). Thus, these three clusters are univocally generated during ESI process from the previously identified solution species A, B, and C, respectively. In summary, the m/z and MS^n results obtained over a broad range of TL strongly support that no uranyl-phenylphosphonate complex other than species A, B, and C exists in the solutions at pH 3. It is to be noticed that the uncomplexed uranyl was also subject to ligand-clustering at low tube lens voltage: it was actually observed on the spectra as the adduct $[\text{UO}_2+(\text{ClO}_4)_3]^-$ and as additional mixed clusters $[\text{UO}_2+(\text{ClO}_4)_3(\text{H}_2\text{L})]^-$ and $[\text{UO}_2+(\text{ClO}_4)_3(\text{H}_2\text{L})_2]^-$ (Supporting Information, Figure S1). For each solution complex A, B, and C, it can be observed that decreasing the tube lens voltage from 140 V to 0 V resulted in increasing the proportions of the clusters involving one or two ligand molecules relative to those of their precursor adduct/molecular ion ($[\text{UO}_2\text{HL}+(\text{ClO}_4)_2]^-$, $[\text{UO}_2\text{L}+(\text{ClO}_4)]^-$, or $[\text{UO}_2\text{LHL}]^-$, respectively). Such a trend is illustrated in Supporting Information, Figure S2 for the solution complex A. This feature can be depicted in term of energy of the collisions of the ions (perchlorate adducts, molecular ions, and/or clusters with ligand molecules) with the background gas in the ion optics. The tube lens voltage is actually applied at the output of the transfer tube to accelerate ions toward the skimmer with sufficient velocity to cause collision-induced dissociation of ions with background gas. Low values of TL voltage result in low energy collisions with the background gas and aid desolvation of ion. Under such conditions, the clusters containing more than one solution solute (typically, clusters involving perchlorate ions and one or two molecules of ligand) are preserved, and they are observable on the ESI(-)MS spectra. In contrast, high TL voltage yields high energy collisions with the

Scheme 1 Scheme Showing the Different Forms in Which the Uranyl Species Are Observed on ESI(-)MS Spectra, For the U(VI)-Phenylphosphonic Acid System

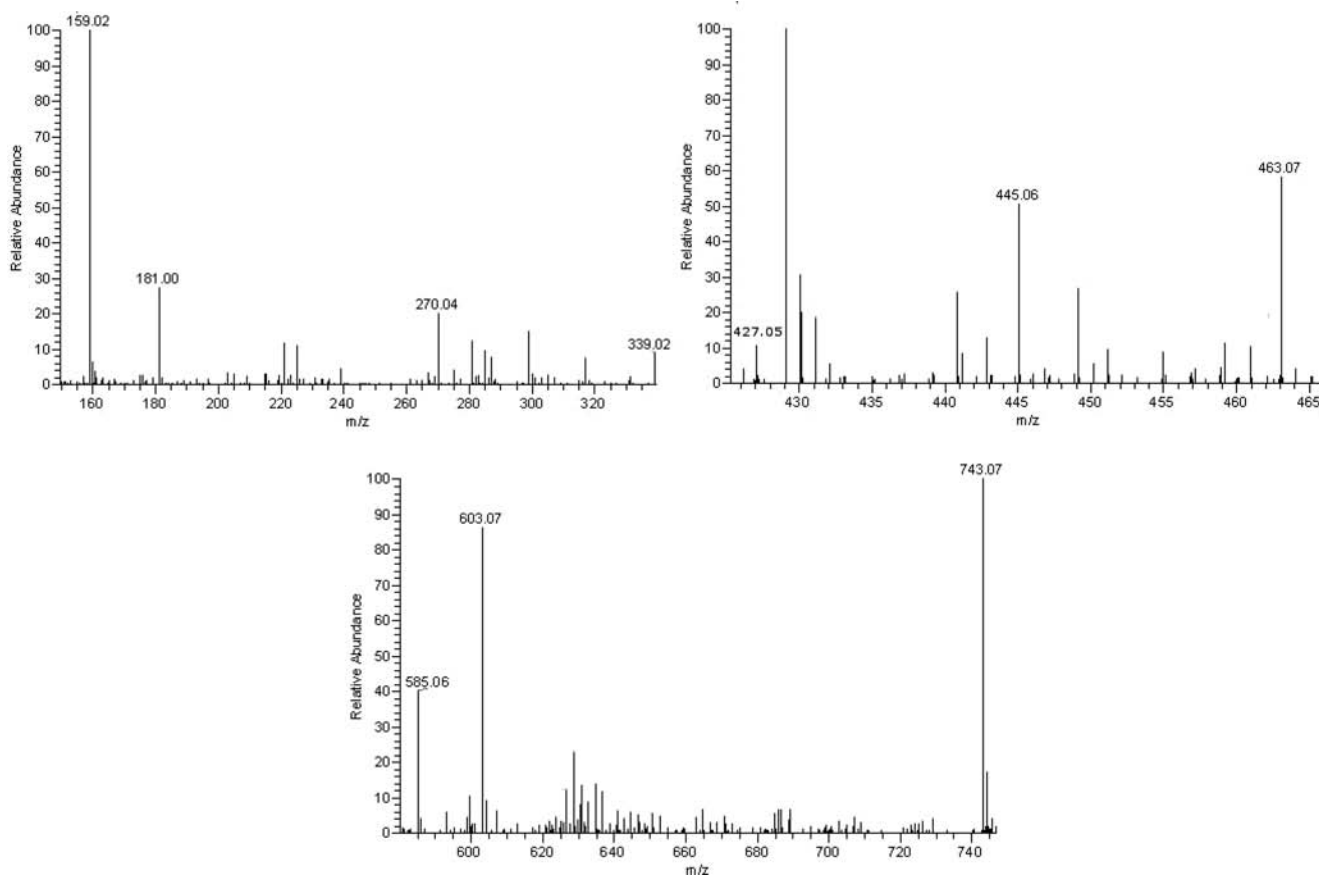
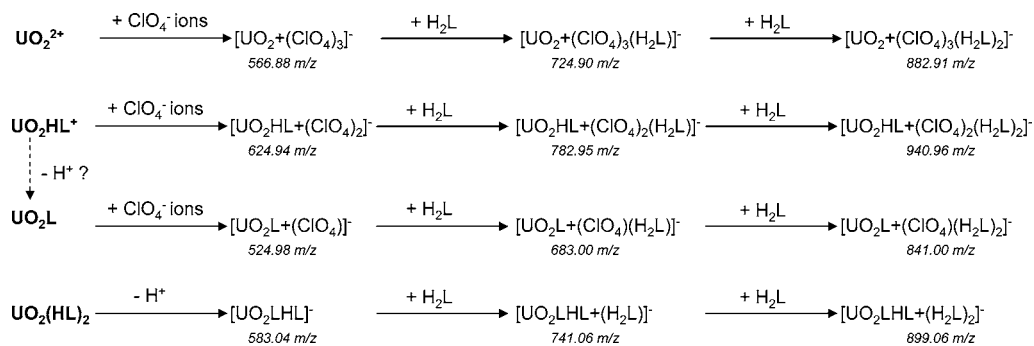


Figure 6. ESI(+)-FTMS spectrum recorded for a native solution containing 3 μM U(VI) and a 10-fold molar ratio of phenylphosphonic acid, at pH = 3.0 (ITL = 180 V).

background gas, favoring the fragmentation of these high molecular weight clusters. The TL-dependency of the clusters is thus consistent with current knowledge on the effect of tube lens voltage on the stability of the clusters/adducts/molecular ions. Finally, the TL-dependency of all clusters/adducts/molecular ions observed in present study strengthens the finding that only species A, B, and C coexist as uranyl-phenylphosphonate complexes in the solutions investigated.

In summary, from the extensive sets of data, that is, peak position, ion-clustering behavior, change in peak intensities as a function of the ligand-to-metal ratio and/or value of tube lens voltage, one can conclude that uranyl speciation in phenylphosphonic acid solutions at pH 3 is governed only by the species UO_2^{2+} , $\text{UO}_2\text{HPhPO}_3^+$, UO_2PhPO_3 , and

$\text{UO}_2(\text{HPhPO}_3)_2$, and a consistent scheme can be drawn showing the different forms in which these four uranyl species are observed on ESI(-)MS spectra (Scheme 1).

2.4. ESI-MS Investigations of the Native Solutions Using the Positive Ionization Mode. Analyses were made to determine if the positive ionization mode allows detecting the peaks for species A, B, and C in native solutions (without adding any cosolvent). Species C was actually observable on the ESI(+) mass spectra as the adduct $[\text{UO}_2(\text{HL})_2+\text{H}]^+$ with a base peak at 585.04 m/z and as clusters with H^+ and H_2O molecules (at 603.07 m/z) or with H^+ and ligand molecules (at 743.07 m/z) (of higher peak intensity), for the solutions at pH 3.0 (Figure 6). Because species B underwent protonation in the ESI probe, both the protonated species A and the neutral

species B peaked at the same m/z value (i.e., 427.05 m/z) as UO_2HL^+ and $[\text{UO}_2\text{L}+\text{H}]^+$, respectively.

The positive ionization mode offered also the opportunity to study solutions at pH higher than 3 up to near neutral pH. This was not possible for the negative ionization mode because of experimental constraints. In fact, in the case of ESI(-)MS analysis, ClO_4^- ions must be added to the solutions at $\text{pH} > 3$ as NaClO_4 or NH_4ClO_4 (at 1 mM) to favor the formation of ClO_4^- -adducts with uranyl species. Now, these ClO_4^- ions combine preferentially during the ESI process with $\text{Na}^+/\text{NH}_4^+$ ions, hampering the detection of trace uranyl adducts at pH higher than 3.

Main features of the ESI(+)MS spectra recorded at pH 3–5.2 are in perfect agreement with typical pH-dependency of metal- H_2L complexes: a low pH is favorable to the involvement of the monodeprotonated ligand HL^- in the complexation reactions and an increase of pH promotes the formation of complexes involving the fully deprotonated ligand L^{2-} . As illustrated in Supporting Information, Figure S3, analysis by ESI(+)MS of U(VI)-phenylphosphonate solutions with pH 3–5.2 showed a decreasing contribution of species C to the uranyl-phenylphosphonate complexes formed, when increasing the solution pH. At pH 5.2, peaks related to species C are not detectable anymore. The species A, that is, the complex with a 1:1 stoichiometry involving the monodeprotonated ligand, is expected to exhibit similar pH-dependency and therefore to no longer contribute significantly to the ESI signal (at 427.05 m/z) at pH 5.2. Thus, the persistence of the peak at 427.05 m/z at pH 5.2 (and the slight increase of intensity of the ESI signal at 427.05 m/z observable when increasing the pH from 4.3. to 5.2) is consistent with a rise of the contribution of species B to uranyl speciation under less acidic conditions.

All the results obtained using the positive ionization mode are thus in good accordance with the qualitative uranyl speciation scheme drawn from the negative ion mode analysis, which is that uranyl interacts with phenylphosphonate to form solution complexes of identity $\text{UO}_2\text{HPhPO}_3^+$, UO_2PhPO_3 , and $\text{UO}_2(\text{HPhPO}_3)_2$.

2.5. Comparison of Our Qualitative ESI-MS Results on the U(VI)-Phenylphosphonate System with Other Reports. The nature of the uranyl-phenylphosphonate complexes identified in the present work is in general good agreement with previous studies of the complexation of uranyl with mono- and diphosphonic acids. Schmeide et al.³⁰ used TRIFS and observed, for solutions at pH 2 and 50 μM U(VI), a decrease in fluorescence of U upon addition of phenylphosphonic acid that provided indirect evidence for uranyl complexation. The authors obtained a good fit of their experimental data by assuming the existence of 1:1 and 1:2 complexes involving the monodeprotonated ligand. It is to be noted that the TRIFS technique does not allow discriminating among complexes differing by only one number of hydrogen,³¹ which prevented identification of both species UO_2HL^+ and UO_2L coexisting in the solutions with pH 2–4 investigated by Schmeide et al.³⁰ By combining analyses by ESI-FTMS and TRIFS, Jacopin et al.²⁹ have shown the existence of a complex of type 1:1 for the U(VI)-HEDP (1-hydroxyethane-1, 1'-diphosphonic) system at a uranyl concentration of 4×10^{-5} M and at ligand-to-metal ratios of 0–0.2. Bollinger and Roundhill⁴³ have studied by potentiometric titrations the complexation between U(VI) and the aminomethylenediphosphonate MAMDP and have provided a speciation model at pH 4 showing coexistence of two 1:1 complexes that differ in their protonation state (MH_2L and

MHL) and one 1:2 complex ($\text{M}(\text{H}_2\text{L})_2$). Our study results are also consistent with the ability of phosphonates to form monodentate and bidentate complexes with a variety of metals.⁷ Finally, the speciation scheme reported in the present study shows strong similarities with that reported for the U(VI)-phosphoric acid system. The species detected here for the U(VI)-phenylphosphonic acid system exhibit the same stoichiometry and the same states of charges than those of the species governing uranyl speciation in phosphate solutions at pH 3.0 (UO_2^{2+} , $\text{UO}_2\text{H}_2\text{PO}_4^+$, UO_2HPO_4 , and $\text{UO}_2(\text{H}_2\text{PO}_4)_2$).

3. Quantitative Description of the U(VI)-Phenylphosphonic Acid System. **3.1. Analytical Strategy Developed for Quantifying U(VI)-Phenylphosphonate Complexes.** To derive from their ESI-MS data the stability constants of uranyl complexes forming in a given U(VI)-ligand, several authors have made the assumption that all the uranyl solution species have the same ion response factors (whatever the state of charge of species).^{29,44,45} However, the ion response factor of a solution species depends on the tube lens voltage and on its characteristics: its charge, acid–base properties, solvation energy, and concentration. The former point is well illustrated by our preliminary ESI(-)MS investigations on the U(VI)-phosphate system (section 1.2).

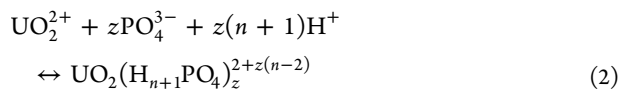
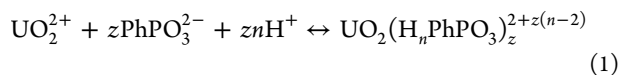
Di Marco et al.³² reviewed published analytical strategies developed to overcome this difficulty, including the use of an internal standard to correlate the intensity of the ESI-MS signals with species concentration. An important requirement is the chemical analogy between the internal standard and the species to be quantified. U(VI) exhibits a very rich and specific chemistry, so that it is unrealistic to find a chemical homologue for uranyl. In contrast, the U(VI)- H_3PO_4 system, for which the complex stability constants are known with good confidence,⁴¹ shows strong chemical similarities with the U(VI)- H_2PhPO_3 system. We used here the three uranyl phosphate complexes $\text{UO}_2\text{H}_2\text{PO}_4^+$, UO_2HPO_4 , and $\text{UO}_2(\text{H}_2\text{PO}_4)_2$ as internal standards for the three uranyl phosphonate complexes identified by ESI-MS analysis.

First, the three U(VI)-phenylphosphonate complexes have the same state of charge than their respective internal standard (i.e., $\text{UO}_2\text{H}_2\text{PO}_4^+$ is the standard for $\text{UO}_2\text{HPhPO}_3^+$, UO_2HPO_4 for UO_2PhPO_3 , and $\text{UO}_2(\text{H}_2\text{PO}_4)_2$ for $\text{UO}_2(\text{HPhPO}_3)_2$). Mass spectra show a same type of main adduct/molecular ion for a U(VI)-phenylphosphonate complex and its internal standard, that is, $\text{UO}_2\text{HPhPO}_3^+$ and $\text{UO}_2\text{H}_2\text{PO}_4^+$, are observable as the perchlorate adduct $[\text{UO}_2\text{HL}+(\text{ClO}_4)_2]^-$ on ESI(-)MS spectra. This point is of fundamental importance as the nature of the adduct dictates the ion response factor of the solution species. Second, the three uranyl-phenylphosphonate complexes, A, B, and C, identified from the ESI(-)MS analysis of the native solutions at pH 3 containing U(VI) and phenylphosphonic acid, follow same trends in TL-dependence than those found for the U(VI)-phosphate system (Figure 2). The phosphate complex of uranyl $\text{UO}_2\text{H}_2\text{PO}_4^+$ and the phenylphosphonate complex of uranyl $\text{UO}_2\text{HPhPO}_3^+$, in which the ligand appears in its monodeprotonated form, show the same TL-dependence. This is also true for the phosphate complex of uranyl UO_2HPO_4 and the phenylphosphonate complex of uranyl UO_2PhPO_3 , as well as for the 1:2 complexes $\text{UO}_2(\text{H}_2\text{PO}_4)_2$ and $\text{UO}_2(\text{HPhPO}_3)_2$. These features strongly support that the pairs $\text{UO}_2\text{HPhPO}_3^+ \cdot \text{UO}_2\text{H}_2\text{PO}_4^+$, $\text{UO}_2\text{PhPO}_3 \cdot \text{UO}_2\text{HPO}_4$, and $\text{UO}_2(\text{HPhPO}_3)_2 \cdot \text{UO}_2(\text{H}_2\text{PO}_4)_2$ exhibit the same reliance in the instrumental parameters. Third, the pK_a values of H_2PhPO_3

and H_3PO_4 are close.⁴⁶ Finally, differences in ion response factors arising from differences in solvation energy between a U(VI)-phenylphosphonate complex and its related U(VI)-phosphate complex can be handled from the difference measured in ion response factors between HPhPO_3^- and H_2PO_4^- . The ion response factor of HPhPO_3^- has been estimated in the present study to be 1.3 times higher than that of H_2PO_4^- , which is explained by the hydrophobic character of the Phenyl-P moiety. This factor of 1.3 has been applied to each pair "U(VI)-phenylphosphonate complex-internal standard" to account for difference in complex solvation energy. All the points explained above fully justify the choice of the internal standards.

3.2. Complex Stability Constants: Determination and Discussion. Two sets of phosphate-doped uranyl solutions at different $\text{H}_2\text{PhPO}_3:\text{U(VI)}$ molar ratios (5–23) and at final H_3PO_4 concentration of 15 μM and 30 μM , respectively, were analyzed in the negative ionization mode. As expected, the addition of the phosphate ions to the solutions resulted in the appearance on the ESI(-)FTMS spectrum of peaks related to $\text{UO}_2\text{H}_2\text{PO}_4^+$, UO_2HPO_4 , and $\text{UO}_2(\text{H}_2\text{PO}_4)_2$. Because of competitive complexation of uranyl by H_2PhPO_3 and H_3PO_4 , the intensity of the peaks associated with the uranyl-phenylphosphonate species A, B, and C were lower in the doped-solutions than in the phosphate-free solutions. This feature is illustrated in Supporting Information, Figure S4.

For the ternary system, the formation of U(VI)-phenylphosphonate complexes and U(VI)-phosphate complexes is described by eq 1 and eq 2, respectively.



The equilibrium constants are given by eq 3 for the U(VI)-phenylphosphonate complexes and eq 4 for the U(VI)-phosphate complexes.

$$K_{\text{Ph},1nz} = \frac{[\text{UO}_2(\text{H}_n\text{PhPO}_3)_z^{2+z(n-2)}]}{[\text{UO}_2^{2+}][\text{PhPO}_3^{2-}]^z[\text{H}^+]^{nz}} \quad (3)$$

$$K_{\text{P},1nz} = \frac{[\text{UO}_2(\text{H}_{n+1}\text{PO}_4)_z^{2+z(n-2)}]}{[\text{UO}_2^{2+}][\text{PO}_4^{3-}]^z[\text{H}^+]^{z(n+1)}} \quad (4)$$

with $n = 0$ or 1 and $z = 1$ or 2 .

Brackets in equations stand for activities. For every U(VI)-ligand ratio, the intensity measured by ESI-FTMS is expressed as follows:

$$I_X = \alpha_X[X] \quad (5)$$

where X is a U(VI)-bearing species and α_X the response factor of species X.

The stability constants for the complexes involving phenylphosphonate ions are obtained by substituting eq 4 and eq 5 into eq 3, which leads to eq 6.

$$K_{\text{Ph},1nz} = K_{\text{P},1nz} \frac{I_{\text{UO}_2(\text{H}_n\text{PhPO}_3)_z^{2+z(n-2)}} \alpha_{\text{UO}_2(\text{H}_{n+1}\text{PO}_4)_z^{2+z(n-2)}}}{I_{\text{UO}_2(\text{H}_{n+1}\text{PO}_4)_z^{2+z(n-2)}} \alpha_{\text{UO}_2(\text{H}_n\text{PhPO}_3)_z^{2+z(n-2)}}} \frac{[\text{PO}_4^{3-}]^z[\text{H}^+]^z}{[\text{PhPO}_3^{2-}]^z} \quad (6)$$

$[\text{PhPO}_3^{2-}]$ and $[\text{PO}_4^{3-}]$ are obtained by eq 7 and eq 8, respectively, in which the concentrations of the U(VI)-phenylphosphonate and U(VI)-phosphate complexes were neglected with respect to those of the corresponding free ligand, as ligands were in large excess relative to uranyl.

$$[\text{PhPO}_3^{2-}] = \frac{[\text{H}_2\text{PhPO}_3]_T}{\left(\frac{[\text{H}^+]^2}{K_{\text{a}_{\text{ph}1}}K_{\text{a}_{\text{ph}2}}} + \frac{[\text{H}^+]}{K_{\text{a}_{\text{ph}2}}} + 1 \right)} \quad (7)$$

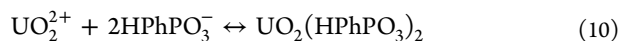
$$[\text{PO}_4^{3-}] = \frac{[\text{H}_3\text{PO}_4]_T}{\left(\frac{[\text{H}^+]^3}{K_{\text{a}_{\text{p}1}}K_{\text{a}_{\text{p}2}}K_{\text{a}_{\text{p}3}}} + \frac{[\text{H}^+]^2}{K_{\text{a}_{\text{p}2}}K_{\text{a}_{\text{p}3}}} + \frac{[\text{H}^+]}{K_{\text{a}_{\text{p}3}}} + 1 \right)} \quad (8)$$

where $K_{\text{a}_{\text{ph}1}}$, $K_{\text{a}_{\text{ph}2}}$ are the K_{a} values for the pairs $\text{HPhPO}_3^-/\text{PhPO}_3^-$ and $\text{H}_2\text{PhPO}_3/\text{HPhPO}_3^-$, respectively; $K_{\text{a}_{\text{p}1}}$, $K_{\text{a}_{\text{p}2}}$, $K_{\text{a}_{\text{p}3}}$ are the K_{a} values for the pairs $\text{H}_3\text{PO}_4/\text{H}_2\text{PO}_4^-$, $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$, and $\text{HPO}_4^{2-}/\text{PO}_4^{3-}$, respectively.

The values of the stability constants at infinite dilution for the U(VI)-phenylphosphonate complexes with a 1:1 and a 1:2 stoichiometry were calculated by substituting in eq 6 the terms $[\text{PhPO}_3^{2-}]$ and $[\text{PO}_4^{3-}]$ by their expressions given by eqs 7 and 8, respectively. Activity corrections were made by using the Davies equation. Calculation of the stability constants was done for each of the investigated uranyl-phenylphosphonic acid solution doped with phosphate ions (Supporting Information, Figure S5). For the reactions of complexation written as follows:



and



the average values of the stability constants are found to be $\log K_{\text{Ph},111} = 3.4 \pm 0.2$ and $\log K_{\text{Ph},112} = 7.2 \pm 0.2$, respectively. The only data set of stability constants published previously for this system (i.e., $\log K_{111} = 3.61 \pm 0.14$ and $\log K_{122} = 6.95 \pm 0.22$ at $I = 0.1 \text{ M NaClO}_4$) was obtained by fitting TRLFS data obtained at pH 2 and $[\text{U(VI)}] = 50 \mu\text{M}$, and by assuming in the fit the existence of 1:1 and 1:2 complexes involving the monodeprotonated phenylphosphonate ligand.³⁰ In the present study, the stability constant for the species UO_2PhPO_3 was assessed to be $\log K_{\text{Ph},101} = 7.1 \pm 0.1$. The calculated speciation of uranyl in phenylphosphonic acid solutions at pH 3–5 using the stability constants determined in this work is reported in Supporting Information, Figure S6. The figure illustrates that UO_2^{2+} and UO_2HL^+ are predominant uranyl species in very acidic solutions, whereas UO_2L governs the U speciation at pH 5 (at a ligand-to-metal ratio of 10).

The affinity of uranyl for phenylphosphonic acid is, within the experimental uncertainties, similar to that reported for phosphoric acid. Compared with diphosphonic acids, such as 1-hydroxyethane-1,1'-diphosphonic acid (HEDPA) and aminomethylenediphosphonate (AMDP), the stability constant obtained for the $\text{UO}_2\text{HPhPO}_3^+$ species is some orders of magnitude lower than that reported at low pH values for the protonated U(VI)-HEDPA ($\log K_{111} = 6.5$, $\text{pH} < 5.5$ ⁴³) and U(VI)-AMDP complexes ($\log K_{111} = 6.7$ ⁴³). The affinity of uranyl for phenylphosphonic acid is stronger than that reported for the isostructural carboxylic acid.²⁷ A higher stability of the phosphonate complexes of the uranyl ion with regard to the corresponding carboxylate complexes has already been out-

lined, and has been explained in terms of differences in electrostatic net charge between phosphonate and carboxylate ligand and to an entropic effect of the phosphonate unit having more potentially binding oxygen atoms than has carboxylate ions.⁴⁷ The greater ability of phosphonate dianion with respect to the carboxylate group to coordinate cations has been also related to the difference in basicity of both ligands.⁴⁸ Transferring to the uranyl-natural organic matter system, our results imply that the complexation reactions involving P-containing functional groups existing at low concentration in HS are involved significantly in the binding by humic and fulvic acids of trace levels of uranyl in the soil. These complexation reactions have to be considered to improve the modeling of U geocycling in organic-rich geochemical systems.

■ ASSOCIATED CONTENT

● Supporting Information

Figure S1 presents the ESI(-)FTMS spectrum recorded at low tube lens voltage for a solution containing U(VI) and phosphoric acid at pH = 3.0. Figure S2 presents the effect of tube lens voltage on the relative abundances of adducts and clusters formed in the ESI probe (using negative ionization mode) for the uranyl-phenylphosphonate complex A. Figures S3 and S4 provide additional FTMS data showing the pH-dependency of the ESI(+)FTMS spectra of uranyl-phenylphosphonic acid solution, and changes in peak intensity on ESI(-)FTMS spectrum for complexes A, B, and C induced by addition of phosphate ions to uranyl-phenylphosphonic acid solutions at various H₂L:U(VI) ratio. Figure S5 reports the discrete values of stability constants of the U(VI)-phenylphosphonate complexes derived from ESI(-)FTMS data of uranyl-phenylphosphonic solutions at various H₂L:U(VI) ratios and doped with phosphate ions. Figure S6 presents the calculated speciation of uranyl in phenylphosphonic acid solutions at pH 3–5 using the stability constants determined in the present work. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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