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Investigation of disulfonamide ligands derived from *o*-phenylenediamine and their Pb(II) complexes by electrospray ionization mass spectrometry

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Abstract

An electrospray ionization mass spectrometry (ESI-MS) method, in both positive and negative ion modes, was developed for characterization of disulfonamide ligands derived from *o*-phenylenediamine and their Pb(II) complexes. For the ligands, negative ion mode ESI-MS in methanolic solutions gave simple and easily interpretable mass spectra. However, the spectra of Pb complexes were not readily interpretable under the same conditions. Protonated ligands and their Pb (II) complexes were observed in methanolic solutions by ESI-MS in positive ion mode. The formation of Na⁺, K⁺, or NH₄⁺ adducts was also observed, complicating the mass spectra and decreasing the signal intensity. In order to optimize the detection of the ligands and the Pb complexes, a method was developed by adding NaOAc in the solutions. The presence of 0.2 mM NaOAc simplified the mass spectra of the ligands and the Pb complexes, and significantly increased sensitivity in both negative and positive ion modes. This modification makes ESI-MS in both modes suitable for characterization of sulfonamide ligands and their Pb complexes, thus providing a potentially powerful tool for evaluating formation of metal complexes and screening combinatorial ligand libraries.

Lead is highly toxic and lead poisoning is a threat to human health, especially for children. Development of selective Pb chelates is necessary for its extraction, sensing and detoxification in environmental and biological systems.¹ Numerous examples of coordination and extraction of Pb(II) by macrocycles have been reported.^{2–6} Ionizable chelates, which extract metals via ion exchange, offer potential advantages, such as versatility, synthetic ease, and favorable complexation-decomplexation kinetics. The recently reported disulfonamides, **1**,⁷ **2**,⁷ and **3**,¹ are practical Pb(II) ion-exchange extractants that take advantage of the preference of Pb(II) in lower coordination numbers for irregular ‘hemidirected’ coordination geometries with a stereochemically active lone pair. These sulfonamides are very effective in Pb(II) extraction, even at micromolar levels, from water into 1,2-dichloroethane, and **3** in particular is very selective against Zn(II) and Cu(II).^{1,7}

Electrospray ionization (ESI) is a soft ionization technique, and ESI-MS has been widely used to investigate non-covalent complexation, such as protein-protein interactions, enzyme-inhibitor complexes, nucleotides, drugs, and the formation of metal complexes.^{8,9} Although a number of cationic macrocycle–Pb complexes have been studied by ESI-MS,^{3–5,10} a method has not been established to investigate the neutral Pb complexes of ionizable chelators, such as disulfonamides, using MS. Recently, ESI-MS has been employed to measure stability constants for metal–crown ether complexes,^{11,12} to estimate relative stability of metal complexes,^{13,14} and to evaluate the binding selectivity of macrocycles.⁴ Therefore, ESI-MS

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could be potentially used to determine the stability of Pb–disulfonamide complexes, and a method based on ESI-MS could be eventually established for rapid screening of disulfonamide ligands for Pb complexation.

Understanding the ionization mechanisms of these ligands and their Pb complexes involved in the ESI-MS processes is critical in order to achieve our long-term goal of developing MS methods for rapid detection of metal–complex formation. In this paper, we present an ESI-MS method for characterization of the disulfonamide ligands **1–4** and their Pb(II) complexes in both positive and negative modes.

EXPERIMENTAL

Materials

All chemicals were purchased from Aldrich Chemical Co. or ACROS Organics, and used without further purification. HPLC-grade methanol, acetonitrile and isopropanol were used for all MS experiments. Ligands **1–4** and their Pb(II) complexes (PbL, L = **1–4**) were synthesized according to procedures reported elsewhere.^{1,7,15}

Procedure

All experiments were carried out on a single-quadrupole mass spectrometer (Finnigan ThermoQuest Navigator aQa). Experimental conditions were as follows: Capillary temperature, 100°C; capillary voltage, 4.5 kV; cone voltage, 30 V (positive ion mode ESI-MS) or –30 V (negative ion mode ESI-MS); scan time, 3.5 s. Stock solutions of ligands **1–4** (1 mM), their Pb(II) complexes (1 mM), NH_4NO_3 (1 mM), and NaOAc (1 mM) were prepared separately in methanol. Dilutions were carried out with methanol to give solutions of desired concentrations (0.02 mM of the ligands and the complexes). In order to improve signal intensity, 0.2 mM of NaOAc was added to all samples before analysis. In a separate experiment, 0.2 mM of NH_4NO_3 was added in order to confirm formation of ammonium adducts. Samples were directly infused into the mass spectrometer at an approximate rate of 10 $\mu\text{L}/\text{min}$.

RESULTS AND DISCUSSION

Atmospheric pressure chemical ionization (APCI)-MS and ESI-MS are soft ionization techniques that could be potentially used for the detection of the ligands and their Pb(II) complexes. Initially, APCI-MS was attempted in both positive and negative modes. Even

though signals for the ligands were observed in methanol, the complexes could not be detected by APCI in either mode. Therefore, we focused our efforts on ESI-MS.

Detection of sulfonamide ligands and Pb(II) complexes in negative ion mode

Diarylsulfonamides are weakly acidic compounds, and they readily lose a proton to form negative ions.¹⁶ Therefore, negative ion mode ESI-MS was used to detect the $[L-1]^-$ anions of these sulfonamides. Disulfonamide ligands and their Pb(II) complexes are soluble in methanol, isopropanol or acetonitrile. These solvents were used for ESI-MS analysis of the ligands and complexes, generating comparable mass spectra. Methanol was the preferred solvent in this study because the intensities of the sample peaks in methanol were the highest. Using negative ion mode ESI-MS, ligands **1–4** generated two kinds of ion species. The major ion species were deprotonated monomers $[L-H]^-$ (m/z 387, 499, 573 and 507, for **L = 1–4**, respectively), and the minor ion species were deprotonated dimers $[2L-H]^-$ (m/z 775, 999, 1147 and 1015, for **L = 1–4**, respectively) (Fig. 1).

Even though the mass spectra of the ligands recorded in negative ion mode ESI-MS had clear backgrounds and the results were readily interpreted, for the Pb complexes the negative ion mode did not yield analogous signals corresponding to $[PbL-H]^-$ (**L = 1–4**) anions. This finding can be explained, since the Pb(II) complexes do not possess ionizable N-H protons and therefore $[PbL-H]^-$ anions cannot be readily formed. Considering that Pb has three major stable isotopes [²⁰⁶Pb (24.1%), ²⁰⁷Pb (22.1%) and ²⁰⁸Pb (52.4%)], Pb complexes were expected to generate characteristic peak clusters with an intensity ratio of approximately 1:1:2. These characteristic Pb peak clusters were used to identify the presence of Pb complexes. Three peaks with weak intensities, $[PbL+1]^-$, $[PbL+42]^-$ and $[PbL+45]^-$, were determined as the product ions of the Pb(II) complexes according to the peak characteristic pattern of Pb (Fig. 2). $[PbL+45]^-$ could be attributed to the formate adducts of the Pb complexes. $[PbL+1]^-$ and $[PbL+42]^-$ ions could not be readily interpreted with the current data. Since no formate and other salts were used in the synthesis of the ligands and their Pb complexes, or in their analysis by ESI-MS, the formation of formate adducts of the Pb complexes could be due to impurities in reagents. Formation of formate–ligand adducts was not observed in negative ion mode ESI-MS (Fig. 1). Our results suggest that formate has some affinity to the Pb complexes. Previous results showed that complexation of additional ligands, such as methanol and dimethyl sulfoxide (DMSO), to Pb(II) via axial coordination can occur and formate could potentially bind in a similar fashion.¹⁵ The overall results suggest that negative ion mode ESI-MS in methanol without any additive is suitable for characterization of the ligands, but not of the Pb complexes.

Detection of sulfonamide ligands and Pb(II) complexes in positive ion mode

In positive ion mode ESI-MS, ligands generated complex mass spectra. All four ligands produced molecular ions $[L+H]^+$. In addition to protonated ligand ions $[L+H]^+$, ligands **1**, **2**, and **4** produced $[L+NH_4]^+$, $[L+Na]^+$ and $[L+K]^+$ adducts (Fig. 3). Ligand **3** produced $[L+H]^+$ and $[L+Na]^+$, while $[L+NH_4]^+$ and $[L+K]^+$ were not found in the mass spectra (Fig. 3). The formation of these cationic adducts could be attributed to trace impurities of NH_4^+ , Na^+ and K^+ in the reagents. However, the Pb(II) complexes of the four ligands only produced the ions $[PbL+H]^+$ and $[PbL+MeOH+H]^+$. No NH_4^+ , Na^+ and K^+ adducts of these complexes were found in methanol (Fig. 4). The lack of formation of $[L+NH_4]^+$ for **3** was initially attributed to the possible weak binding of NH_4^+ to **3**, in comparison to **1**, **2** and **4**, or alternatively to the low ammonium concentration in the sample. To test this hypothesis, solutions of 0.02 mM of each ligand with the presence of 0.2 mM of NH_4NO_3 were analyzed in positive ion mode. The result showed that, even in this high ammonium concentration, the $[L+NH_4]^+$ adduct for **3** was not formed, suggesting that NH_4^+ could not bind to **3** under these experimental conditions (Fig. 5). It is possible that NH_4^+ cannot bind to the Pb(II) complex of **3**, because

the presence of ligand **3** in the coordination sphere of Pb is more sterically demanding, compared to ligands **1**, **2** and **4**. The fact that ligands **1** and **2** (unlike **3**) have been shown to readily form ternary PbL(bipy) complexes, solvent adducts, or coordination polymers, supports this hypothesis.¹⁵

There are several possible explanations for the formation of Na⁺ and K⁺ adducts with the ligands and complexes. The alkali metal cations could interact with the ligand either through the electronegative oxygen atoms or through cation- π interactions¹⁷ with the phenyl rings present in the ligands and their Pb complexes. However, Na⁺ or K⁺ adducts of the Pb complexes were not observed in methanol (Fig. 4), suggesting that the Pb complexes have lower affinity to Na⁺ and K⁺ than the ligands. Because some Pb complexes decomposed to ligands during the ionization process (Fig. 4), the produced ligands were shown to compete with the complexes, binding most of Na⁺ and K⁺ introduced as impurities from reagents. Na⁺ adducts of the Pb complexes were detected only when additional Na⁺ was introduced. When 0.2 mM of NH₄NO₃ was present, which could introduce more Na⁺ as impurity, or when 0.2 mM of NaOAc was added in 0.02 mM of the Pb complex solutions, the Na⁺ adducts of the ligands and the complexes were observed in the mass spectra (Figs. 5 and 7).

Detection of sulfonamide ligands and Pb(II) complexes in the presence of NaOAc

Since ESI-MS provided weak and not readily interpretable signals for the ligands and their Pb (II) complexes in methanol, modifications were necessary, so that the mass spectra could be interpreted easier, giving stronger signals for the adducts of the ligands and complexes. Based on the fact that the ligands and their Pb(II) complexes can form adducts with formate, addition of the analogous acetate would be expected to give improved signals. Experiments were conducted by adding 0.2 mM of NaOAc to the 0.02 mM ligand or complex methanol solutions. This modification significantly simplified the mass spectra of the ligands and Pb complexes and improved the signal intensities in both positive and negative mode ESI-MS (Figs. 6 and 7). In the negative ion mode (Fig. 6), NaOAc did not affect the product ion profiles for ligands **1-4**. The [L-H]⁻ anions were still detectable. However, the intensities of these ions increased by five- to ten-fold (results not shown) due to the fact that the weakly basic NaOAc facilitated formation of deprotonated ligands. On the other hand, addition of NaOAc changed the product ion profiles for the Pb complexes. As predicted, OAc⁻ adducts ([PbL+OAc]⁻) were generated in the presence of 0.2 mM of NaOAc (Fig. 6). [PbL+OAc]⁻ ions were the major product ions for the Pb complexes, and their intensities were at least four times higher than those of other Pb complex ions. These results clearly indicate that with addition of NaOAc in sample solutions, negative ion mode ESI-MS is suitable for characterizing both the ligands and their Pb(II) complexes. This modification greatly improved the signal intensities for the ligands and their Pb(II) complexes, and simplified the mass spectra for the Pb complexes.

The presence of 0.2 mM of NaOAc also simplified the mass spectra of the ligands and their Pb(II) complexes, and increased signal intensities in positive ion mode ESI-MS (Fig. 7). The presence of NaOAc in the solutions changed product ion profiles of the ligands and their Pb (II) complexes. In methanol alone, the ligands generated [L+H]⁺, [L+NH₄]⁺, [L+Na]⁺ and [L+K]⁺, and the Pb complexes generated [PbL+H]⁺ and [PbL+MeOH+H]⁺ (Figs. 3 and 4). When 0.2 mM of NaOAc was present in the sample solutions, most of [L+H]⁺, [L+NH₄]⁺, or [L+K]⁺ were converted into [L+Na]⁺, and [PbL+H]⁺ and [PbL+MeOH+H]⁺ were converted into [PbL+Na]⁺ and [PbL+(NaOAc)_n+Na]⁺. These changes in the product ion profiles substantially improved the signal intensity and simplified the mass spectra of all ligands and their Pb(II) complexes, which made interpretation of the results much easier.

CONCLUSIONS

Both positive and negative modes of ESI-MS were used to characterize disulfonamide ligands and their Pb(II) complexes. In methanol, simple and easily interpretable mass spectra were obtained for the ligands using ESI-MS in negative ion mode, while mass spectra that were not readily interpretable were observed for the Pb(II) complexes under the same conditions. The molecular ions of the ligands and Pb complexes were generated in positive ion mode ESI-MS, but the NH_4^+ , Na^+ or K^+ adducts of the ligands and Na^+ adducts of the Pb(II) complexes were also produced, resulting in complicated mass spectra. The presence of NaOAc in the sample solutions significantly simplified mass spectra in both positive and negative modes and improved the signal intensities for the ligands and their Pb complexes. This modification makes both positive and negative ion modes of ESI-MS suitable for characterization of disulfonamide ligands and their Pb complexes, and provides a potentially powerful tool for characterizing metal complexation and screening ligand libraries for metal binding.

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Figure 1. Mass spectra of ligands **1–4** (L1–L4, 0.02 mM) in MeOH by negative ion mode ESI-MS. Capillary voltage: 4.5 kV; cone voltage: –30 V.

Figure 2.

Mass spectra of Pb(II) complexes 1–4 (PbL1–PbL4, 0.02 mM) in MeOH by negative ion mode ESI-MS. Capillary voltage: 4.5 kV; cone voltage: +30 V.

Figure 3. Mass spectra of ligands **1–4** (L1–L4, 0.02 mM) in MeOH by positive ion mode ESI-MS. Capillary voltage: 4.5 kV; cone voltage: 30 V.

Figure 4. Mass spectra of Pb(II) complexes **1–4** (PbL1–PbL4, 0.02 mM) in MeOH by positive ion mode ESI-MS. Capillary voltage: 4.5 kV; cone voltage: 30 V.

Figure 5. Mass spectra of Pb(II) complexes **1–4** (PbL1–PbL4, 0.02 mM) in MeOH with 0.2 mM of NH₄NO₃ by positive ion mode ESI-MS. Capillary voltage: 4.5 kV; cone voltage: 30 V.

Figure 6. Mass spectra of Pb(II) complexes **1–4** (PbL1–PbL4, 0.02 mM) in MeOH with 0.2 mM of NaOAc by negative ion mode ESI-MS. Capillary voltage: 4.5 kV; cone voltage: –30 V.

Figure 7. Mass spectra of Pb(II) complexes 1–4 (PbL1–PbL4, 0.02 mM) in MeOH with 0.2 mM of NaOAc by positive ion mode ESI-MS. Capillary voltage: 4.5 kV; cone voltage: 30 V.