
Metal-Complex Formation in Continuous-Flow Ligand-Exchange Reactors Studied by Electrospray Mass Spectrometry

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Electrospray ionization mass spectrometry was used to investigate complex formation of different metal complexes in a continuous-flow ligand-exchange reactor. A computer program was developed based on normal equilibrium calculations to predict the formation of various metal-ligand complexes. Corresponding to these calculations, infusion electrospray mass spectrometric experiments were performed to investigate the actual complex formation in solution. The data clearly show good correlation between the theoretically calculated formation of metal-ligand complexes and the experimental mass spectrometric data. Moreover, the approach demonstrates that the influence of the pH can be investigated using a similar approach. Indirectly, these infusion experiments provide information on relative binding constants of different ligands towards a metal-ion. To demonstrate this, a continuous-flow ligand-exchange detection system with mass spectrometric detection was developed. Injection of ligands, with different affinity for the metal-ion, into the reactor shows good correlation between binding constants and the response in the ligand-exchange detection system. Additional information on the introduced ligand, and the complexes formed after introduction of the ligand, can be obtained from interpretation of the mass spectra. (J Am Soc Mass Spectrom 2007, 18, 707–713) © 2007 American Society for Mass Spectrometry

Due to the increasing interest in metal complexes and metal-ligand interactions in several fields, e.g., drug research [1, 2] and catalyst design [3], novel detection methods are necessary. Conventional techniques to study these interactions and complexes are infrared spectroscopy, X-ray diffraction, and nuclear magnetic resonance spectroscopy (NMR) [4], but these techniques have several drawbacks. The use of electrospray ionization mass spectrometry (ESI-MS) for studying metal-ions, metal-ligand interactions and metal complexes is readily recognized [5]. Using MS as a detection technique offers the advantage of being, in general, more sensitive than NMR and X-ray diffraction and opens up the possibility to screen complex samples. Moreover, MS enables simultaneous monitoring the response of ligand-exchange reactions and additionally obtaining chemical information about the specific compound [6, 7].

Today, MS is often used in studying host-guest complexes [8–10], structural elucidation by collision-induced dissociation (CID) MS [11, 12], and studying relative binding constants of ligands to metal-ions [13,

14]. Additionally, due to the liquid-based ionization in the ESI process, it is suitable for studying liquid-phase reactions, e.g., ligand-exchange reactions [6, 7], which enables the study of complex systems.

In general, the assumption is made of a good correlation between complexes formed in solution and what is observed in the mass spectrum [15]. FAB-MS and ESI-MS have been used in the past to evaluate binding selectivities of various compounds, e.g., caged crown ethers to metal-ions [16]. An excellent correlation between obtained ESI mass spectral data and expected binding selectivities has been reported in several papers [13, 17, 18], although several parameters should be kept in mind. For a good correlation between complex formation in solution and what is observed in the mass spectrum [14], particularly the competing effect of the solvent, the conditions and ionization efficiencies of the different complexes, and molecules have to be taken into account.

This report describes a fundamental approach in studying metal complexes with ESI-MS. A computer program was developed to calculate which complexes are formed under predefined conditions, based on the affinities of the relevant ligands to the metal-ion and the experimental conditions. As an example, the theory describes the influence of increasing total ligand concentrations L_0 at different pH of the solution on the

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formation of different metal-ligand complexes. This theoretical approach is mimicked by monitoring the formation of the different complexes at increasing total ligand concentration with MS. Moreover, mass spectral data at different concentrations of ligand is evaluated to obtain a better insight about formed complexes at predefined conditions. The theory, as well as the mass spectral data, can aid in both the design of new ligand-exchange reactions for analytical purposes [7] and in the interpretation of these interactions studied by MS, since they are involved in all kinds of processes, e.g., in biological [19] and industrial processes [3]. Additionally, the mass spectral data aid in the identification of specific compounds, e.g., catalytic species or intermediates, responsible for certain processes. This fundamental approach was extended by studying several ligand-exchange reactions by MS. A continuous-flow ligand-exchange detection method was used to selectively detect certain ligands, and to correlate the response of the detector to the relative affinities of certain ligands of interest to the metal-ions.

Experimental

Materials

Acetonitrile and methanol were purchased from Baker (Deventer, The Netherlands) and were purified over a 0.45 μm Millipore filter (Bedford, MA). The nitrate salts of Cu(II) and Zn(II) and all ligands were purchased from Sigma-Aldrich (Steinheim, Germany), except for nicotinamide, 2-(aminomethyl)pyridine, and 4-picoline, which were purchased from Acros Organics (Geel, Belgium).

Solution Preparation

Stock solutions of 10 mM ligands were prepared in methanol, except for 4,7-dimethyl-1,10-phenanthroline, which had a stock concentration of 4 mM. Cu(II) and Zn(II)-solutions were prepared in Milli-Q water (Bedford, MA). The reagent solution was prepared by adding a stock solution of metal-ion to a solution of ligand in ammonium formate-formic acid solution (20 mM; pH 6.5) containing 50% methanol. The actual conditions and concentrations of the reagent solutions used are indicated either in the text or in the figure legends.

Setup for Infusion Experiments

The setup for the preliminary infusion experiments on the on-line mixing of solutions of metal salts and ligands is shown in Figure 1.

The system consisted of a Shimadzu (Hertogenbosch, The Netherlands) LCMS-2010A, and a separate Shimadzu LC-10Ai pump (Pump 2). The LCMS-2010A consisted of two LC-10ADvp pumps (pump 1a and pump 1b), a SCL-10ADvp system controller, a SIL-10ADvp autosampler, a CTO-ACvp oven, and a single

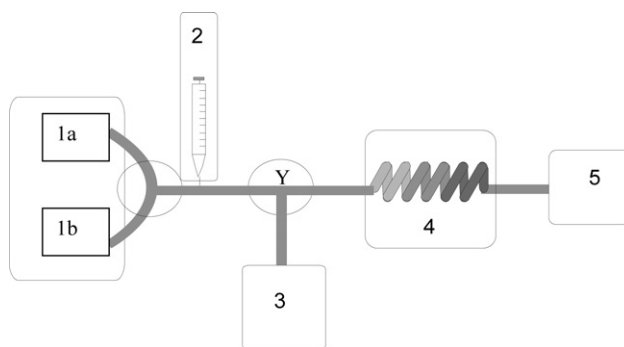


Figure 1. Schematic of the set-up used in infusion and direct-injection experiments. For the direct-infusion experiments, pump 1a and pump 1b generate a concentration gradient of either metal-ions or a ligand at a flow-rate of 50 $\mu\text{L}/\text{min}$ (unless otherwise reported), the second pump [3] continuously delivers the ligand or metal-ion of interest at 50 $\mu\text{L}/\text{min}$ (unless otherwise reported), a 10 μL thermostated reaction coil, and 5 the ESI-MS instrument. For the direct-injection experiments, additionally an autosampler [2] is added to the system. In these cases pumps 1a and 1b generate a sample carrier-flow (50 $\mu\text{L}/\text{min}$ running buffer unless otherwise reported); and 2, the reagent pump provides 50 $\mu\text{L}/\text{min}$ metal-ion-reporter-ligand solution (unless otherwise reported).

quadruple mass spectrometer equipped with an ESI probe. The binary pumps 1a and 1b were used to generate a concentration gradient of either a metal-ion or a ligand at 50 $\mu\text{L}/\text{min}$. Thus, pump 1a was pumping the running buffer consisting of methanol/5 mM ammonium formate (50/50 vol/vol) and pump 1b was pumping a certain concentration of either metal-ions or ligand in running buffer. The LC-10Ai pump (pump 3) delivered, at 50 $\mu\text{L}/\text{min}$, either the ligand or the metal-ion solution at a fixed concentration. The total flow into the home-made 40 μL , 0.18 mm i.d. knitted PEEK reaction coil and the ESI-MS was 100 $\mu\text{L}/\text{min}$. The reaction coil was thermostatted at 60° C by means of a water bath.

Setup for Direct-Injection-Ligand-Exchange-ESI-MS

The general set-up applied in experiments involving the direct-injection combination with the continuous-flow ligand-exchange reaction coupled to ESI-MS was very similar to the direct-infusion system. An autosampler was placed between the binary gradient pump 1 and the Y-piece for the mixing of the solution from pump 2 (see Figure 1).

In the continuous-flow detection system, the metal/reporter-ligand solution (50 $\mu\text{L}/\text{min}$) was continuously mixed with a sample carrier flow (50 $\mu\text{L}/\text{min}$) into which the pure compounds were directly injected.

Mass Spectrometry Settings

MS detection was performed in positive-ion ESI. The probe voltage was 4 kV. Nitrogen (99.999% purity,

Praxair; Oevel, Belgium) gas flow was set at 1.5 L/min. A nitrogen counter gas flow was set at 0.06 MPA, which was applied to obtain better solvent evaporation. The curved desolvation line (CDL) temperature and the block temperature were set at 200 °C. ESI-MS data were acquired by switching between full-spectrum mode and selected-ion monitoring (SIM), detecting a number of specific m/z -values related to the reporter-ligand, the metal-reporter-ligand complex, the ligand of interest, and/or complexes of the metal-ion and the ligand of interest. In addition to the ligand and complex m/z -values, a system monitoring compound (SMC) was continuously detected in SIM during the direct injection to monitor the overall stability of the system and possible ion suppression effects.

MS/MS Settings

For structural elucidation and complex identification, a Micromass Q-TOF2 mass spectrometer (Wythenshawe, Manchester, UK) equipped with a Z-spray ESI source was used. The ESI source conditions were as follows: source temperature 80 °C, desolvation temperature 100 °C, capillary voltage 3 kV. The cone voltage was set at 30 V. Nitrogen (99.999% purity; Praxair) was used with flow rates of 20 L/h for nebulization, 50 L/h for cone gas, and 350 L/h for desolvation. Argon (99.9995% purity; Praxair) was used as collision gas in MS/MS experiments.

Results and Discussion

General Setup

The set-up used for both infusion experiments and flow-injection analysis is shown in Figure 1. Pump 3 continuously introduces a constant concentration of metal-ion. The binary gradient pump (pumps 1a and 1b) delivers the ligand to be studied at the concentration range defined by the gradient settings. In typical experiments, the binary pump was set to run a 0 to 100% gradient of the ligand of interest in 20 mM ammonium formate-formic acid buffer/methanol (50/50 vol/vol) at a total flow rate of 100 $\mu\text{L}/\text{min}$. ESI-MS was used to monitor both the free ligand and metal-ligand complex concentrations in the full scan mode. For flow-injection experiments, an autoinjector was placed between pump 1 and the mixing union Y, using pump 1 as a sample carrier flow and pump 3 to deliver the premixed metal-reporter-ligand solution.

Infusion Experiments (Zn(II) and 1,10-Phenanthroline)

In many papers describing ESI-MS detection of metal-ligand complexes, the assumption is made that ESI mass spectra reflect, to a large extent, the composition of the metal complexes formed in solution [9,10,15,18], although in-source fragmentation should be kept in

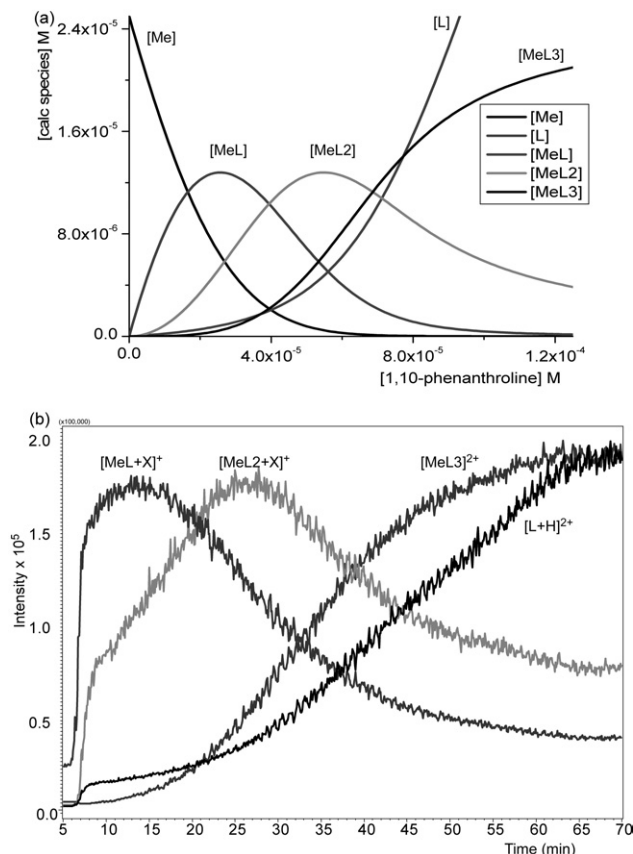


Figure 2. (a) Theoretical plot of the complex formation of Zn(II) with 1,10-phenanthroline at different concentrations of 1,10-phenanthroline at a pH^* 6.5. (b) Obtained mass chromatogram for experimental approach at a pH^* 6.5. A continuous flow of 50 μM Zn(II) was mixed with a gradient of the concentration of 1,10-phenanthroline in time. The gradient consisted of 0 μM to 250 μM in 60 min. The maximum concentration of 1,10-phenanthroline was 250 μM . The total concentration of Zn(II) and 1,10-phenanthroline that reached the MS was 25 μM and 125 μM , respectively.

mind [20, 21]. To evaluate the correlation between solution complex formation and the observed mass spectrum, a computer program was developed to calculate the type and concentration of metal complexes formed under predefined conditions as a function of experimental parameters. Since the presence of methanol alters the pH, the apparent pH^* , reflecting the actual pH after the addition of methanol (pH^* is measured with a pH-meter), is used in the calculations of the different complexes throughout this paper.

Figure 2a displays the theoretical plot of the concentration of the different metal complexes as a function of the concentrations of Zn(II), indicated as Me, and 1,10-phenanthroline ($\text{C}_{12}\text{H}_8\text{N}_2$, 180.1 Da), indicated as L. The Zn(II) concentration of 25 μM and the pH (pH^* 6.5) are kept constant, while the ligand concentration is increased in time from 0 to 125 μM . Zn(II) can bind three bidentate ligands such as 1,10-phenanthroline, resulting in the formation of 1:1 (MeL), 1:2 (MeL₂), and 1:3 (MeL₃) metal-ligand complexes. From these calculations, the ligand concentrations can be derived, for which max-

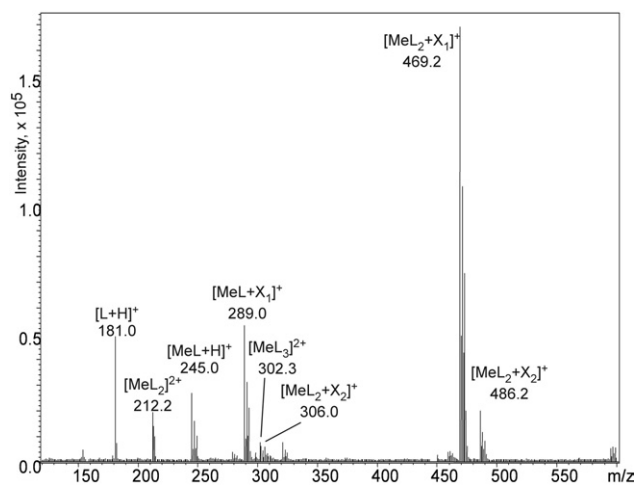


Figure 3. Average mass spectrum when the maximum concentration of 1:1 Zn:1,10-phenanthroline complex is formed (pH* 6.5); where M = Zn(II); L = 1,10-phenanthroline; X₁ = formate and X₂ = nitrate.

ima of the individual metal-ligand complexes can be observed.

The m/z traces shown in Figure 2b, reflect the experimentally measured responses for some of the observed metal-complex species during an infusion experiment. For clarification, the traces are normalized. Good correlation between the theoretical plot and the experimental data is achieved. Although a close relationship between the observed intensity of the mass spectrum and the calculated concentration of the formed complex was reported for 1:1 complexes of crown ethers with Na⁺ and K⁺ [18], in the present case it is more difficult to correlate intensities to concentrations of metal complexes because various complexes are formed (see below) and these complexes appear to have different ionization efficiencies. Despite this limitation, the measured optimum total ligand concentration for the maximum concentration of MeL ($\approx 23 \mu\text{M}$) and MeL₂ ($\approx 50 \mu\text{M}$) are in good agreement with the values predicted from the theoretical plot, 25 μM and 53 μM , respectively.

Since complexes of Zn(II) show specific isotope patterns (⁶⁴Zn:⁶⁶Zn:⁶⁷Zn:⁶⁸Zn; 49:28:4:19), they can easily be recognized in the mass spectrum. Figure 3 shows an averaged mass spectrum measured at pH* 6.5 at a ligand concentration of $\sim 23 \mu\text{M}$, at which the concentration of MeL is at a maximum. The spectrum shows the protonated 1,10-phenanthroline at m/z 181, various ions related to MeL as well as ions related to MeL₂ and MeL₃. Interestingly, the abundances of the peaks at m/z 289 and 469, corresponding to [MeL + formate]⁺ and [MeL₂ + formate]⁺, respectively, differ from what is predicted by the theoretical model. Under the conditions chosen, the MeL related signal is predicted to be similar to the MeL₂ signal, whereas in the experimental data the ratio of the MeL:MeL₂ signals is 1:4. This indicates that MeL₂ shows significantly higher ioniza-

tion efficiency than MeL. Despite the differences in ionization efficiency, the normalized patterns observed for MeL and MeL₂ closely coincide with those predicted by the theoretical model; moreover, all observed MeL_x complexes show a similar pattern.

Next to [MeL + formate]⁺, a number of other MeL-related ions are observed, e.g., peaks at m/z 245 and 321. While the latter can be explained as a methanol adduct of [MeL + formate]⁺, as frequently observed in spectra from this type of ESI source, the peak at m/z 245 is more difficult to explain. The isotope pattern reveals that one Zn metal-ion is present and that the molecular species is singly charged. When the intensity of the peak at m/z is plotted against the total concentration of ligand, it resembles the profile of [MeL₂ + formate]⁺. In-source CID and MS-MS experiments, however, demonstrate that the ion with m/z 245 may be a fragment of the ion with m/z 289, which could be formed by the loss of CO₂ from the [MeL + formate]⁺. Further fragmentation of the ion with m/z 245 in MS-MS results in protonated 1,10-phenanthroline (m/z 181), which in turn can be fragmented by two subsequent losses of HCN, similarly to protonated 1,10-phenanthroline itself. The fragmentation of the ion at m/z 289 is not yet fully understood: the loss of CO₂ implies that the singly-charged ion with m/z 245 would be a complex of Zn(II), 1,10-phenanthroline and a hydride anion, while further fragmentation to the ion with m/z 181 should involve the loss of neutral Zn.

Similar to MeL, a number of different ion species are observed for MeL₂, e.g., a doubly-charged [MeL₂]²⁺ complex with m/z 212 and a [MeL₂ + nitrate]⁺ complex with m/z 486.0. The latter complex is known from the literature [22], but under the present conditions the formate concentration from the ammonium formate buffer (5 mM) exceeds the nitrate concentration (50 μM), resulting from the zinc nitrate used. The formation of doubly charged ions implies that possibly a number of neutral complexes, e.g., [MeL + (formate)₂] or [MeL + formate + nitrate], are formed as well. These are obviously not observed under positive-ion ESI conditions, but to some extent they may obscure the actual agreement between the experimental and the theoretical plot (Figure 2).

A small peak attributable to [MeL₃]²⁺ is observed in Figure 3 as well. This peak becomes obviously far more abundant in spectra acquired at higher ligand concentrations (data not shown). No [MeL₃ + formate]⁺ ions were observed at m/z 649. This suggests that in the MeL and MeL₂ complexes, formate acts as inner-sphere ligands and not as outer-sphere anionic ligands.

pH* Variation

To study the influence of the pH on metal-complex formation, similar continuous-flow experiments were performed at a pH* below the pK_a of the ligand. At pH* of 3.4, the increased competition between the H⁺ and Mⁿ⁺ results in a shift of the total concentration of ligand

Table 1. Complexes of Cu(II) and 2,2'-bipyridyl formed at a 1:1 metal to ligand ratio

<i>m/z</i>	% Relative abundance	Interpretation
187.6	9	[MeL ₂] ²⁺
219.0 ^a		[MeL] ⁺
219.9	6	[MeL + H] ⁺
236.9 ^a	14	[MeL + H ₂ O] ⁺
251.0 ^a	15	[MeL + MeOH] ⁺
264.0	100	[MeL + formate] ⁺
280.9	6	[MeL + nitrate] ⁺
375.1 ^a	70	[MeL ₂] ⁺
420.1	45	[MeL ₂ + formate] ⁺
437.2	5	[MeL ₂ + nitrate] ⁺

^aIndicates complexes of Cu(I).

at which the maximum concentration of the relevant complex is observed. Again, there is good agreement between the experimental plot at a pH* of 3.4 and the theoretical plot (data not shown).

The averaged mass spectrum at the total ligand concentration for a maximum MeL complex at a pH* of 3.4 is similar to Figure 3, except for some changes in relative abundances of various ions, e.g., the [MeL₂]²⁺ (*m/z* 212) is more abundant than [MeL₂ + formate]⁺ (*m/z* 469).

Infusion Experiments (Cu(II) and 2,2'-Bipyridyl)

Similar infusion experiments and correlation with theoretical plots were carried out for other combinations of a metal-ion and a bidentate ligand. The results obtained with Cu(II) and 2,2'-bipyridyl (C₁₀H₈N₂, 156.1 Da) are briefly discussed here, especially with respect to the mass spectral interpretation (see Table 1). Good correlation between the theoretical plot and the experimental results at various pH* was achieved (data not shown). Table 1 shows the most abundant ions of averaged mass spectra observed at ~45 μM 2,2'-bipyridyl (pH* 6.5), that is the ligand concentration where the MeL complex concentration is at a maximum.

Reduction of Cu(II) to Cu(I) can occur upon dissociation of its complex with certain ligands such as bipyridyl [23] or upon reduction of Cu(II) complexes in the reaction with solvents, e.g., acetonitrile [24,25]. In the ESI process, the reduction of Cu(II) to Cu(I) is much faster than the process in solution. Cu(I) is a closed-shell ion [4], which may explain the ease of this reaction. Keeping this in mind, several of the observed ions, e.g., *m/z* 263.9, 251.0, and 375.0 are explained as [MeL + H₂O]⁺ and [MeL + CH₃OH]⁺ after reduction of Cu(II) to Cu(I). Again, a similar 1:1 complex (*m/z* 219.9) as in the experiments with Zn(II) and 1,10-phenanthroline (*m/z* 245.0) was observed. MeL₂ complexes show similar behavior where, next to [MeL₂ + formate]⁺, [MeL₂ + nitrate]⁺, and [MeL₂]²⁺, the most abundant complex is *m/z* 375, which can be interpreted as [MeL₂]⁺ after reduction of Cu(II) to Cu(I). The MeL₃ complexes expected are [MeL₃]⁺ with *m/z* 531 with Cu(I) (not observed) or [MeL₃]²⁺ with *m/z* 265.7 with Cu(II).

Direct-Injection Ligand-Exchange Detection

Next to the characterization of different metal-ligand species, the ability to readily detect metal-ligand complexes by ESI-MS also offers the possibility to study ligand-exchange reactions. We have demonstrated that ligand-exchange reactions can successfully be used as analytical tool [6,7].

The principle of the ligand-exchange detection method relies on the exchange of a reporter-ligand with a competing ligand of interest. The extent of the exchange reaction depends on the concentration and the affinity of the ligand L for the metal-ion relative to the reporter-ligand R. Indirectly, such infusion experiments can provide information on relative binding constants of ligands towards a metal-ion. Since the concentration of R is continuously monitored by ESI-MS, a positive response in the MS-trace can be related a ligand with a high affinity (relative to the reporter-ligand). By changing the nature of R, and therefore the binding strength of MeR, the method can be tuned to achieve either higher or lower selectivity [7]. Furthermore, using MS as detection method, both R and MeR can be monitored to measure the extent of the ligand-exchange reaction. Typically, the species with the highest ionization efficiency are finally selected as reporter molecule.

Next to the ionization efficiency, the binding constant of MeR and the number of available binding sites have to be considered. Weak MeR complexes result in an easy dissociation of the complex and, therefore, a decrease of the MeR signal. Moreover, when all available coordination sites are occupied, a decrease of the reporter complex relies only on the exchange itself. Figure 4 displays the ligand-exchange detection method for different concentrations of ligands with a known affinity for Cu(II) using 2,2'-bipyridyl as reporter-ligand R. The upper trace corresponds to the protonated molecule of the free 2,2'-bipyridyl. A peak in the 2,2'-bipyridyl trace indicates that ligand exchange has occurred upon injection of a ligand of interest. Additional proof that ligand exchange occurred is obtained by analyzing the MS trace corresponding to one or more copper-2,2'-bipyridyl complex species. In the present example, *m/z* 264.0, corresponding to [MeR + formate]⁺, was used and a ligand-exchange reaction results in a negative peak. The ESI-MS spectrum provides additional information on identity of the analyte ligand and the metal-analyte complexes formed. This is especially interesting when dealing with unknown ligands or when information about specific metal complexes is required.

The response in the free 2,2'-bipyridyl trace is related to the relative binding constants of the Cu(II)-analyte complexes. EDTA, which forms strong complexes with Cu(II), shows the highest response. Increasing EDTA concentrations result in increasing positive peak heights in the free 2,2'-bipyridyl trace and increasing negative peak heights in the Cu(II)-2,2'-bipyridyl trace. The molecular structure of the injected ligand also influ-

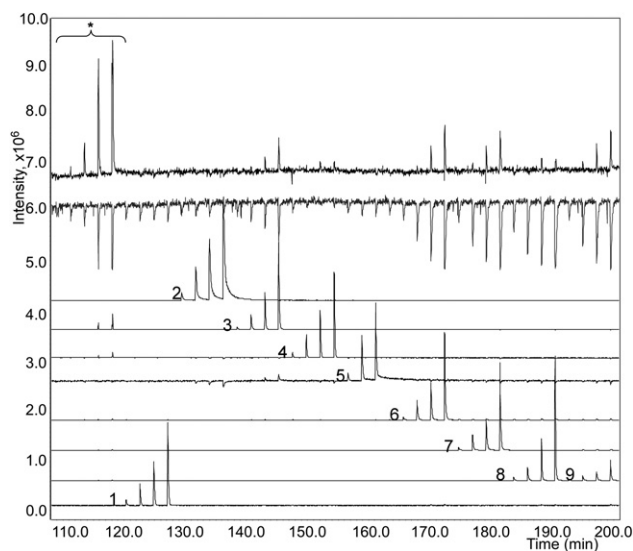


Figure 4. Ligand-exchange detection of different ligands. Reagent conditions: Cu(II)-(2,2'-bipyridyl)₂ in 5 mM NH₄HCO₂/methanol (50/50 vol/vol) pH* 6.5. The reporter-ligand trace (R) is 2,2'-bipyridyl ([M + H]⁺; *m/z* 157) and the complex related trace (C) is [Cu(II) + 2,2'-bipyridyl + formate]⁺ (*m/z* 264). 10, 50, 100, and 200 μ M injections (10 μ L) are performed of the ligands of interest. 1, glycinamide (*m/z* 75); 2, 2-aminomethyl-pyridine (*m/z* 109); 4, benzimidazole (*m/z* 119); 5, nicotinamide (*m/z* 123); 6, 1,10-phenanthroline (*m/z* 181); 7, 5-methyl-1,10-phenanthroline (*m/z* 195); 8, 2,9-dimethyl-1,10-phenanthroline (*m/z* 209); 9 4,7-dimethyl-1,10-phenanthroline (*m/z* 209). * 10, 50, 100, and 200 μ M injections (10 μ L) of EDTA, which is not observed as a ligand in positive electrospray mass spectrometry, since it has an overall negative charge.

ences the response of the ligand-exchange detection. EDTA is a hexadentate ligand, which, in principle, can displace or exchange three bidentate reporter-ligands from MeR, whereas upon injection of a bidentate ligand, e.g., 1,10-phenanthroline, only one reporter-ligand is exchanged. This effect is substantiated by the results^o(Figure^o4)^owhere^oEDTA^ogives^oa^ohigher^oresponse in the reporter-ligand trace than 1,10-phenanthroline. At the same time, the decrease in the metal-reporter-ligand complex trace is similar for the injection of EDTA or 1,10-phenanthroline. One molecule of EDTA exchanges three reporter-ligand molecules from one metal-reporter-ligand complex molecule, but affects only one metal-reporter-ligand complex, whereas a 1,10-phenanthroline molecule only exchanges one reporter-ligand molecule from that complex.

In some applications, it may be of interest to study the complexes formed with the analyte ligands in detail. Next to homogeneous complexes, the formation of heterogeneous, mixed-ligand complexes has to be considered. By injecting 5-methyl-1,10-phenanthroline (C₁₃H₁₀N₂, 194.1 Da) in the Cu(II)-2,2'-bipyridyl ligand-exchange detection system, all three types of metal complexes may be formed, i.e., complexes of the analyte ligand (MeL), mixed complexes with both the analyte ligand and reporter-ligand (MeLR), and complexes with the reporter-ligand (MeR) (data not shown).

Correlation of Response in Ligand-Exchange Detection and Affinity of the Injected Ligands

As expected, an increase in the concentration of the injected ligand results in an increase in the response in the reporter-ligand trace. For EDTA, 1,10-phenanthroline, 5-methyl-1,10-phenanthroline, and 4,7-dimethyl-1,10-phenanthroline, the increase of the response shows a linear relationship with the increase in concentration (correlation coefficient of >0.99), while such a linear correlation is not observed for 2,9-dimethyl-1,10-phenanthroline. The latter may be caused by steric hindrance (which is also related to the equilibration constant) and/or ion suppression.

As expected, the type of MeL complex formed depends on the concentration of the ligand of interest. At low concentrations, the MeL complex species are relatively more abundant, while at higher injected concentrations a shift to the MeL₂ and MeL₃ complexes is observed. At the same time, data show that the response due to MeL₃ complexes starts decreasing at lower ligand concentration, while the decrease of MeL₂ and MeL starts at higher ligand concentrations.

Conclusions

Continuous-flow ligand-exchange detection systems coupled to ESI-MS are useful tools in studying interactions between metal-ions and a variety of ligands. In principle, monitoring the dissociation and formation of metal-ligand complexes by means of ESI-MS provides a wealth of information. However, a proper picture on the liquid-phase complexation processes can only be obtained if the mass spectral data adequately reflect the metal-ligand complexes formed in solution. This study demonstrates that for several model systems good correlation is found between experimental data obtained by ESI-MS and theoretically predicted metal-ligand concentrations, even when experimental parameters like solution pH vary. Infusion experiments were also found to be a fast method to obtain a proper insight in the complexes formed before the development of an actual ligand-exchange detection system. The various complexes formed may well differ in ionization efficiency. In addition, these experiments show the complexity of the liquid phase constituents during complex formation and ligand-exchange reactions.

Injection of several ligands with different affinity for the metal-ion in a continuous-flow ligand-exchange reaction clearly shows that there is a good correlation between binding constants and the ligand-exchange detection response. Additional information of the introduced ligand and the complexes formed after introduction of the ligand can be obtained from interpretation of the mass spectrum.

This fundamental approach may aid in the future design of a customized ligand-exchange reaction, e.g., as a selective detection method, to study relative binding constants or to study the ligand-metal interactions

in general. The applicability of these ligand-exchange reactions is not restricted to the metal-ions Cu(II) and Zn(II), but can be expanded to other metal-ions of interest or ligands of interest.

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