Summary

Metal ions, metal complexes and metal-ligand exchange reactions are essential in a large variety of natural and industrial processes. Due to this importance and the related interest of researchers in metal complexes and metal-ligand interactions in several fields, e.g., drug research and catalyst design, novel detection methods are necessary for better understanding these compounds and their interactions as well as for obtaining quantitative information.

Mass spectrometry has taken a prominent role in the study of metal complexes and their interactions. This is due to several advantages of mass spectrometry such as better sensitivity and direct information about the formed complex as compared to conventional detection methods. This thesis describes the use of mass spectrometry in the study of complex formation as well as the application of ligand-exchange reactions coupled to mass spectrometry as an analytical tool, e.g., to obtain specific information on metal ions and ligands of interest or to develop specific and selective analyte detection strategies. In this context, mass spectrometry not only provides chemical information, but also information about the compound that is responsible for a change in the metal-ion reporter complex.

Chapter 1 provides a brief introduction about metal ions, metal complexes and ligand-exchange reactions. The chapter gives the reader insight in the detection of metal species and reactions by mass spectrometry, mainly focusing on the different applicable mass spectrometric techniques and strengths and weaknesses. Moreover, the behavior of metal species in the mass spectrometer is discussed.

Chapter 2 focuses on the metal-ligand interactions, and how these interactions can be monitored or detected. Moreover, this chapter provides a guideline how to develop continuous-flow ligand-exchange reactions as analytical tools for all kind of applications, thereby focusing on mass spectrometric detection of these reactions.

Chapter 3 deals with the fundamental study of metal complexes and metal-ligand reactions by electrospray ionization mass spectrometry. Electrospray ionization mass spectrometry was used to investigate complex formation of different metal-complexes in a continuous-flow ligand-exchange reactor. Normal equilibration calculations which are incorporated in a computer program, provide the prediction of the type and concentrations of metal complexes formed as a function of experimental conditions. These theoretical calculations were compared with mass spectral data using an approach which mimics the calculations, showing good correlation between the experimental data
obtained by MS and the theoretical calculations, even when experimental parameters such as pH of the solution vary. The usefulness of mass spectrometry is demonstrated by monitoring a ligand-exchange reaction by mass spectrometry, obtaining information about affinity properties of the introduced ligand to the metal ion as well as structural information about the ligand itself. The detection of this ligand-exchange reaction is based on the specific release of a reporter ligand from a metal-reporter ligand complex by a high affinity ligand in a continuous-flow system. The choice of reporter ligand mainly depends on the affinity of the ligand toward the metal ion and the ionization efficiency. Therefore, it is possible to obtain a highly selective and highly sensitive ligand-exchange detection method.

Chapter 4 describes the utilization of electrospray ionization mass spectrometry for the selective detection of metal ligands after a post-column continuous-flow ligand-exchange reaction. By applying a chromatographic separation prior to the ligand-exchange reactor, it is demonstrated that (complex) mixtures of ligands can be analyzed in one single run. By choosing the reporter ligand based on its affinity towards the metal ion, it was possible to tune the ligand-exchange reaction to either sensitivity or selectivity, thereby keeping the affinity of the ligand of interest in mind. A reporter ligand with a higher affinity provides better selectivity whereas a reporter ligand with a lower affinity may provide higher sensitivity.

Chapter 5 is divided in two parts, demonstrating two possible strategies for the application of ligand-exchange reactors as analytical tools for screening for specific ligands. Chapter 5a demonstrates application of a mass spectrometry based ligand-exchange reactor in the screening of phosphorylated peptides, separated by a reversed-phase liquid chromatography. The ligand-exchange reaction is directly monitored by mass spectrometry. A specific reporter trace indicates the presence of a phosphorylated compound, whereas mass spectral information can be obtained about the compound itself. In this way, it was possible to monitor specifically phosphorylated peptides as well as to obtain information on the phosphorylated ligands. The method proved to be linear in the tested range of 2 to 80 µmol/L with a limit of detection (S/N = 3) of 2 µmol/L. Chapter 5b uses the same ligand-exchange principle in a slightly different approach. After the chromatographic separation of the peptide mixture, the effluent is split in two parts. One part is directed to a fluorescence based ligand-exchange reactor, whereas the other part is directed to a mass spectrometer. By correlating the response in the fluorescence based ligand-exchange reactor, which selectively detects phosphorylated peptides, with the mass spectrometry chromatograms, phosphorylated peptides can be selectively subjected to tandem mass spectrometric analysis, for structural elucidation and
identification of the phosphorylated fragments of the protein. Moreover, using fluorescence a LOD of 0.5 µmol/L was obtained for mono-phosphorylated peptides. The number of phospho-groups influences the response in the ligand-exchange, which was demonstrated by the response of the mono-phosphorylated peptide which displayed a more or less three times lower response in the fluorescence trace than the tri-phosphorylated peptide. A common problem in metal affinity studies of phosphorylated compounds is that some non-phosphorylated compounds, especially highly acidic peptides, also possess high affinity to the metal ion and may show complexation. To overcome this problem, the sample was introduced twice into the LC-ligand-exchange detection system, once as it is and once after treatment with alkaline phosphatase to remove phosphate-groups. Comparison of the fluorescence chromatogram of the two runs demonstrated the selective removal of phospho-groups (by disappearance of the fluorescence response), thereby enabling the discrimination between phosphorylated compounds and non-phosphorylated interferences.

Summarizing, MS provides a tool to study metal-ligand interactions and metal complexes. The studied complexes showed a good correlation between theoretical calculations and mass spectral data. In theory, MS provides the possibility to study for instance (intermediate) (metal)-catalyst formation or protein-metal interactions. Ligand-exchange reactions coupled to mass spectrometry provides a selective analytical tool to obtain information (e.g., affinity and structure) about the ligand of interest, where the choice of reporter ligand provides either sensitivity or selectivity or even both.