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Study on formation and stability of azacage metal complexes using electrospray mass spectrometry

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Abstract

In this study electrospray mass spectrometry was used to investigate spherical azacages as host molecules for metal ions systems. Two different pyridino cryptands, [bis-tren(tris-pyridine)amine **L1** and bis-tren(tris-pyridine)phenylamide **L2**] were examined with regard to their affinity for coordinating metal ions such as Ag^+, Hg^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+}. The complex formation was performed in a water/methylene chloride system as well as in pure water, and subsequent electrospray mass spectrometry described the behavior and partition of the complex species. Generally, **L1** shows higher ability in the coordination of metal ions and the strongest binding for Ag^+. **L2** gives significant interactions only with Ag^+ and Co^{2+}. Collision activation experiments were used to study the stability of complex species and to distinguish between species formed in solution and during electrospray. (Int J Mass Spectrom 210/211 (2001) 327–339) © 2001 Elsevier Science B.V.

Keywords: Electrospray mass spectrometry; Azacage metal complexes; In-source collision activation

1. Introduction

In the last few years an increasing interest is dedicated to the study of different supramolecular structures in order to find novel efficient complexing agents for metal ions and to improve the selectivity of metal ion binding which can be used for both modeling molecular recognition in biological processes and developing methods with technical relevance [1]. Ligands have been designed by varying the size, including selected donor atoms and changing substitution to enhance selectivity for metal coordination. In this context, permanent effort has been made to design and synthesize spherical cage ligands capable to encapsulate one or more metal ions, anions or other moieties [2–4]. Among classical stability constant measurements, liquid–liquid extraction followed by nuclear magnetic resonance (NMR) spectrometry or electrochemical methods [1,5,6] mass spectrometry (MS) [7–9] is one of the most promising approaches to study metal complexation processes and the derived supramolecular complexes.

Intensive mass spectrometric investigations of intact metal complexes started with the development of soft ionization techniques, such as fast atom bombardment (FAB), matrix assisted laser desorption ioniza-

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Dedicated to Professor Nico Nibbering on the occasion of his retirement.

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tion (MALDI), and atmospheric pressure ionization (API) [10–14]. Binding constants of alkali and alkali earth metal/crown ether complexes determined in solution could be correlated with ion abundances obtained in FAB MS experiments [15]. Unfortunately, the interpretation of data from FAB spectra is often complicated because of interfering matrix adduct ions.

As a consequence of instrumental development and after comparison of different mass spectrometric ionization techniques such as FAB, atmospheric pressure chemical ionization (APCI), and electrospray (ES), [10,11,14], both spray techniques appear to be the methods of choice for studying coordination compounds and supramolecular complexes. Particularly, ES MS is preferred in the study of coordination compounds and supramolecular complexes [16–18]. ES mass spectra are simpler than FAB spectra, and mostly dominated by abundant, multiply charged complex ions.

Recently, a question of interest was whether ES MS is generally able to reflect accurately complexation processes in solution [19]. Extensive studies on, e.g. crown ether 18C6 and cryptate 222 1:1 coordinated to alkali metal chlorides proved the reliability of thermodynamic data, such as affinity constants predicted on the basis of ES MS measurements.

Several instrument parameters and solution compositions were examined as possible factors influencing the ionization process. It was demonstrated that at well-defined instrument conditions the ES spectra of the complexes were highly reproducible with respect to the relative abundance of the singly charged complex cations. Sensitive instrument parameters are the voltages within the ES interface because the application of potentials can modify the ratio of ion species produced in the gas phase, and therefore, the metal complex status in solution would not correctly reflected. Thus, careful optimization of the ES voltage parameters is needed to guarantee the spectra obtained are a real image of the complex solution at equilibrium. On the other hand, collisional-activated dissociation (CAD) experiments may provide information about the thermodynamic stability of complexes [20]. Finally, the relative affinity of the ligands to different alkali metal ions could be predicted from ES spectra [19].

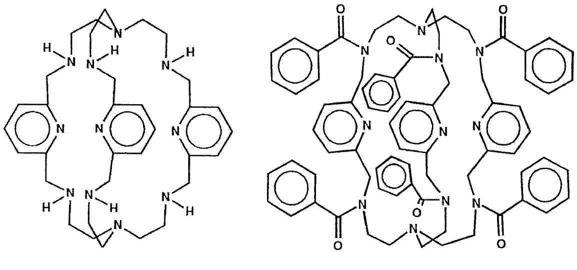
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Fig. 1. Structures of the azacage ligands bis-tren(tris-pyridine)amine **L1** and of bis-tren(tris-pyridine)phenylamide **L2**.

The ES spectrum of bi-cyclic hexamine cage compounds used for the encapsulation of, e.g. Co^{2+} showed mainly singly and doubly charged complex ions as well as ion pairs with appropriate counter ions. The investigations showed that at proper cone and skimmer potentials even ions were observed corresponding to complexes in which the metal ion has been reduced [14]. In ES spectra this process became visible in terms of proton abstraction. Besides the instrument parameters particularly the thermodynamic stability determines the mass spectrometric behavior of the macro-cyclic complexes. For example, sepulchrate (1,3,6,8,10,13,16,19-octaazabicyclo[6.6.6]icosane), is characterized by a high thermodynamic stability and tends rather to ion pair formation and redox reactions than to fragmentation at regular ES conditions (low skimmer potential) [14].

The dominance of (ligand + Me^{n+} + $H^+)^{(n+1)+}$ multiply charged ions has been observed for of open-chain ligands of the poly-N-heterocyclic type (pyrene and benzimidazol structures) which were studied in the presence of Cu, Co, Eu, and Tb as self-assembled helical complexes [16].

The azacage ligands **L1** and **L2** (Fig. 1) used in this study were synthesized to find selective complexing agents for solvent extraction of transition metal ions. This structural type gives some interesting features to bind cations, anions or both together [4,21,22]. Previous solvent extraction studies [23] using **L1** and **L2** as ligands showed that the amino cage **L1** transfers preferably Ag^+ from the aqueous into the organic phase forming 1:1, 2:1, and 3:1 (metal:ligand) complexes. Molecular modeling of their possible structures favours an arrangement of the silver cations inside the cage molecule with two different binding modes, strongly by the nitrogene amine donors in a tetrahedral and weakly by the pyridine donor atoms in a trigonal planar coordination pattern. In contrast Co^{2+} was extracted only with 1:1 complex composition forming an octahedral arrangement. In all cases the picrate counterion supports the metal extraction. In comparison the extraction efficiency of amidocage **L2** has been investigated [23].

The differences in the ligand structures **1** and **2** are clearly reflected in their physico-chemical properties such as lipophilicity and basicity. Therefore, their partition in the two-phase system water/CH_2Cl_2 differs strongly, and influences the formation kinetics of derived complexes. The extraction behavior of **L1** and **L2** as well as similar cryptands for Na^+ and selected transition metal ions were investigated recently [23].

In the present study electrospray mass spectrometry was used to characterize the complexation processes of Ag^+, Hg^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} with **L1** and **L2** and the species formed in aqueous and organic solution.

2. Experimental

2.1. Materials and methods

Metal salts ($AgNO_3$, $HgCl_2$, $Ni(NO_3)_2$, $ZnCl_2$, $Co(NO_3)_2$) and solvents were purchased from Merck (Darmstadt, Germany). They were used as received.

Ligand **L1** was prepared by way of (2 + 3) Schiff base condensation and following reduction [4]. **L2** has been obtained by reaction of **L1** with benzoylchloride [24] Complex solutions were prepared by an extraction procedure using 1 mmol solution of each ligand in methylene chloride and a water solution containing 1 mmol of the appropriate metal salt and 5×10^{-3} M picric acid (HPic) or 1×10^{-2} M $NaNO_3$ (mediator salt). In general, mediator salt addition provides constant ion strength, and picrate addition has been approved to facilitate the transfer of the metal ions into the organic phase.

Aqueous and organic solutions were mixed in 1:1 phase ratio for 30 min. After shaking the phases were separated and analyzed individually by flow injection ES MS. Because the solubility of **L1** in water is relatively high, the complex formation was investigated also in water alone without two phase partition.

The concentration of the metal complexes was in the mmol range for **L1** complexes and below for the **L2** complexes (in CH_2Cl_2). The metal complex concentration varied in dependence on the extraction efficiency of the ligands. Besides several kinetic studies on the phase partition of the free ligands and the complexes were carried out, the main ES MS investigations were performed at partition equilibrium status of metal extraction.

Different silver salt concentrations (1, 2, and 3 mmol) were used in order to investigate the probability of polynuclear metal complex formation.

2.2. Instrumentation

All experiments were carried out as flow injection analysis using a six-port Rheyodyne injection valve (7725 Model) with an injection loop of 10 μL. Water was used as transport and spray medium at a flow of 10 μL/min (solvent delivery system from Thermo Separation Egelsbach, Germany). Water as eluent also for the analysis of methylene chloride solutions is only to see as a compromise needed in regard to solvent extraction experiments hold as reference.

A single quadrupole mass spectrometer Finnigan SSQ 7000 (San Jose, USA) equipped with a standard electrospray ionization interface was applied for mass analysis. Mass spectra were acquired in full scan mode in the mass range between 100 and 1700 u. Most of the mass spectra were recorded in profile mode to assign multiply charged ions.

The temperature of the spray capillary was maintained at 200 °C and the manifold temperature was set to 70 °C. The spray voltage was held at 4.5 kV. Nitrogen was used as sheath gas for drying and nebulization at a pressure of 460 kPa. The octapole potential (CAD offset) was varied to induce collision-activated dissociation.

The mass spectrometer was tuned and calibrated with a mixture of 5 pmol/μL apomyoglobin and 20 pmol/μL of a peptide, H-Met-Arg-Phe-Ala-OH (MRFA), delivered by Sigma (Deisenhofen, Germany) and Finnigan MAT (Bremen, Germany). Both standards were dissolved in methanol/water (1:1 v/v, 0.1% of acetic acid) and injected with a flow rate of 10 μL/min during calibration.

3. Results and discussion

3.1. ES spectra of azacage ligands used for complexation

The bis-tren(tris-pyridine)amine **L1** with a molecular mass of 601.4 g/mol is an amino cage based on two tren moieties which are connected by three pyridine spacer units. In contrast to this, the amido cage **L2**, bis-tren(tris-pyridine)phenylamide (1225.4 g/mol) is characterized by a weaker donor strength but a higher lipophilicity. So this ligand could be measured only in organic solvent.

In preliminary studies both cages **L1** and **L2** were analyzed separately. Both spectra detected in CH_2Cl_2 and shown in Fig. 2 are characterized by abundant pseudomolecular ions. In the case of **L1** one to four additional protons may be transferred to the cages during ionization. With increasing protonation degree the intensity of multiply charged ions decreases, surely caused by less stabilization of multiply charged ions in the gas phase. Although theoretically six protons could be localized on **L1,** in the ES spectrum of **L1** (in water phase) maximum fourfold charged molecular ions appeared, but in the CH_2Cl_2 fraction only triply charged ions were observed also in consequence of unfavorable proton donor properties of solvent.

The stable molecule structures **L1** and **L2** are reflected in their ES spectra. Apart from the pseudomolecular ions, only fragment ions are observed and those fragment ions are of low intensity. For **L1** ring opening and elimination of acetonitril, and ethyl amine leads to ions at *m/z* 560.4 $(M + H–C_2H_4N)^+$ and *m/z* 557.4 $(M + H–C_2H_7N)^+$. Further, loss of $CH_3CH_2NHCH_2$–pyridyl and $CH_3CH_2NHCH_2$–pyridyl–CH=NH from the molecular ion gives the very low abundance, ions at *m/z* 466.3 and 439.3, respectively. At CAD condition higher intensity of fragment ions such as the monocyclic $(C_{22}H_{36}N_8 + H)^+$ at *m/z* 413.3 and the subunit $(C_{11}H_{15}N_5 + H)^+$ at *m/z* 218.1 appear as well.

The ES spectrum of **L1** in water solution is similar to that in CH_2Cl_2 solution, only the ratio of singly and multiply charged ions varies because of different proton donor probabilities.

The partition equilibrium of **L1** in H_2O/CH_2Cl_2 is achieved after 6 h, and is about 3:1. This partition should be considered to be an estimate. It was calculated from the peak areas in the $[M + H]^+$ and $[M + 2H]^+$ fragmentograms taking the slightly different response of **L1** in methylene chloride and water solution (1:1.3, respectively) into account. The response was determined in separate experiments using defined solutions of **L1** in water and CH_2Cl_2.

In ligand **L2** the benzoyl substituents additionally implemented cause a higher mass spectrometric stability characterized by lower fragmentation (Fig. 2). Only low abundant fragmentation of the benzoyl groups mark the ES spectrum. The probability to incorporate a second proton during ionization is reduced by substituted amino positions, thus a doubly charged ion does not appear.

3.2. Formation of silver–L1 complexes

The formation of silver complexes by extraction of Ag^+ (1 mmol $AgNO_3$) from aqueous picrate solution (5 mmol HPic) with **L1** (1 mmol) in methylene chloride is described in more detail in the following.

Fig. 3 shows the appropriate ES spectra of the organic and aqueous phase after 30 min of mixing. In this time the equilibrium is achieved. The ES analysis of both reaction phases points to a partition equilibrium of the complex of about 35:1 (CH_2Cl_2/H_2O) summarizing the intensities of the silver containing ions. A comparable partition of the complexes was

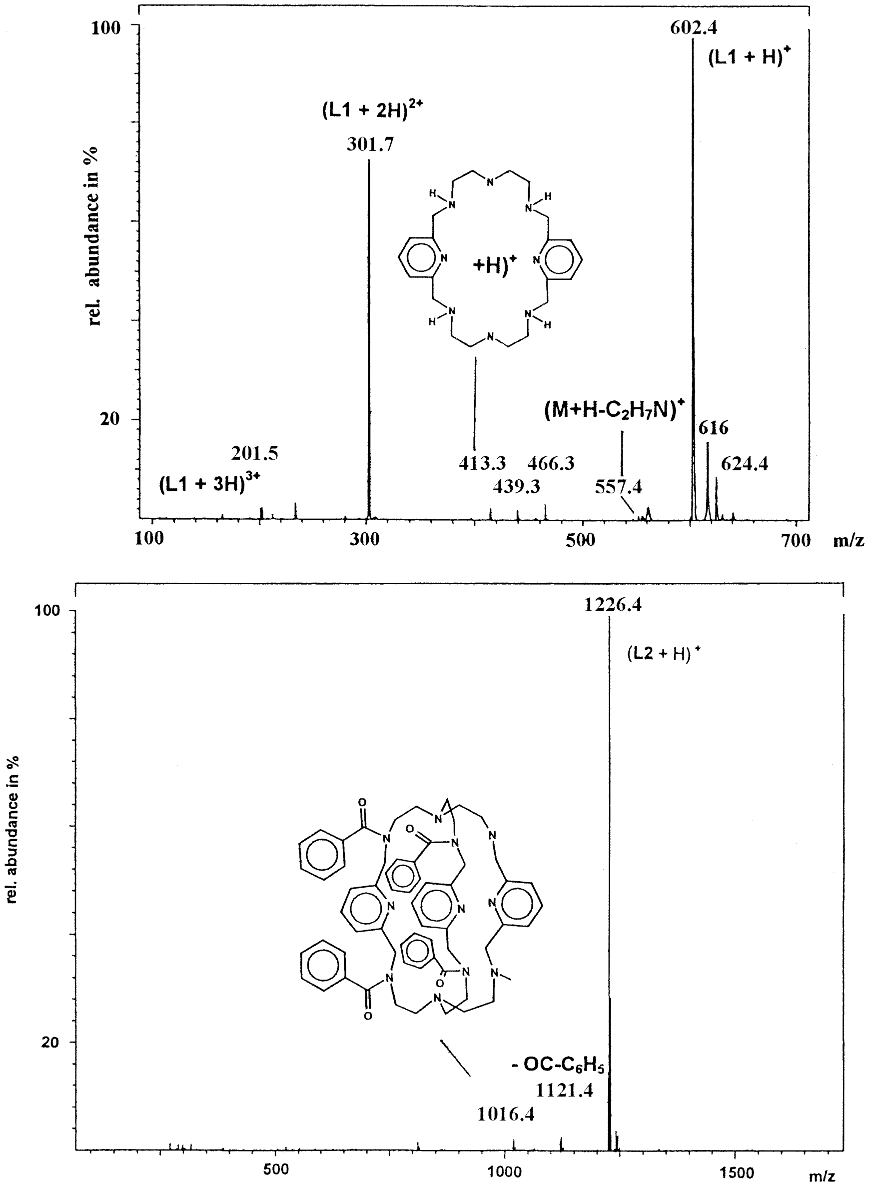

Fig. 2. ES spectra of **L1** (top) and **L2** (bottom) obtained at non-CAD conditions, both ligands dissolved in CH_2Cl_2. The structural proposal for fragment ion *m/z* 1016.4 is only one possibility to explain the two-fold loss of benzoyl substituents.

found in solvent extractions carried out previously [23].

Despite the two components are mixed in 1:1 ratio, the mass spectrum of the organic phase is dominated by a complex ion at *m/z* 407.6 $(\mathbf{L1} + 2Ag)^{2+}$. The monosilver complex appears at *m/z* 708.3. The ion of 354.6 u represents a complex of $(\mathbf{L1} + Ag + H)^{2+}$, and the doubly and singly charged molecular ions of **L1** were found at *m/z* 301.7 and 602.4, respectively. As recently described [14], the presence of ion pair structures is also probable, such as that observed at *m/z* 877.2 $(\mathbf{L1} + Ag + AgNO_3)^+$. In every case, the

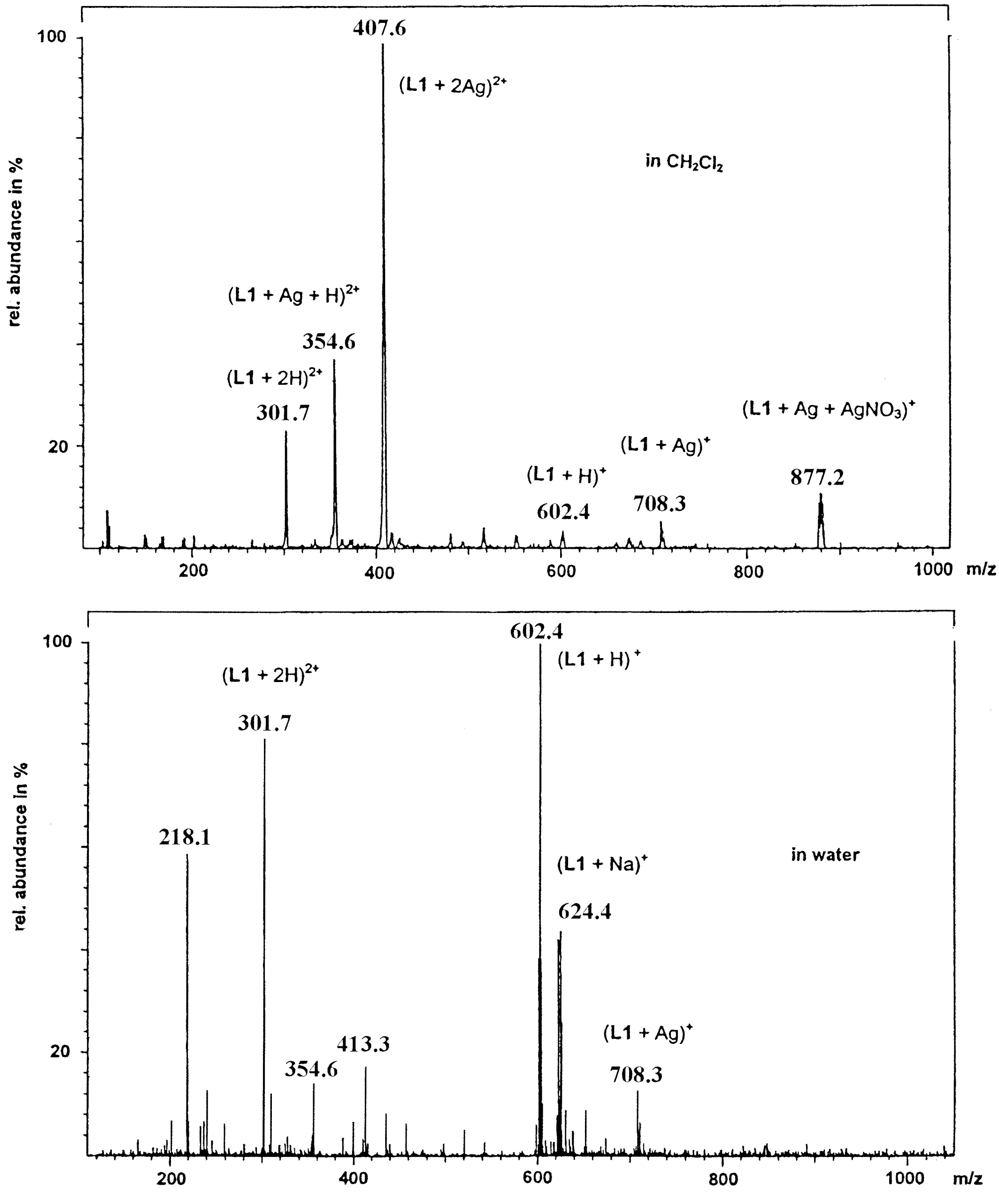

Fig. 3. ES spectra demonstrate the two phase partition of the 1:1 silver complex with **L1** using the extraction system $AgNO_3$–HPic–H_2O/**L1**–$CHCl_2$ ($[AgNO_3] = 1 \times 10^{-4}$ M; $[HPic] = 5 \times 10^{-3}$ M; [**L1**] $= 1 \times 10^{-3}$ M).

typical isotopic pattern of ions is helpful in the interpretation of the complex structures.

After an extraction period of 30 min the silver complexes had diffused into the water phase. The remaining portion of free **L1** in the organic phase is very small and proves the results obtained by liquid–liquid extractions achieving an extractability of about 98% of Ag^+ by **L1** [23].

In the water phase more **L1** was remained (Fig. 3). Additionally, competing complexation of sodium ions were observed. The ES spectrum of the water phase (Fig. 3) is predominated by (**L1** + H)$^+$ and (**L1** +

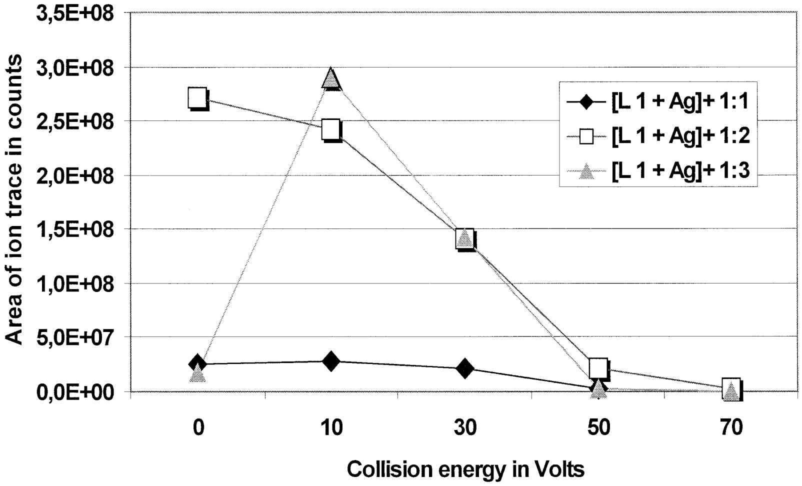

Fig. 4. Dependence of (**L1** + Ag)$^+$ ion abundance on the collision energy and $AgNO_3$ concentration used for complexation.

2H)$^{2+}$, and the Na$^+$ complex (*m/z* 624.4) derived from the mediator salts added. The silver complex is of lower significance. The fragment ion *m/z* 218 represents the [monocycle of **L1** + Na + H]$^{2+}$ related from the sodium **L1** complex. The intensive ion of 622 u results probably from a decomposition of the silver complex. The mass difference of 86 u might be interpreted as $C_2H_{10}N_2$ unit which has to be questioned because a corresponding fragmentation is missed in the organic phase. In order to resolve the doubts MS/MS experiments needed.

Different mixtures 1:1, 1:2, and 1:3 (**L1**:Ag$^+$, respectively) were investigated to examine the possibility of inclusion of more than one silver ion in the cage. In the organic phase the maximum number of silver ions coordinated was three for the picrate system and two for the nitrate solution in agreement with the solvent extraction data [23].

The ES spectra of the corresponding water phases showed quite different results depending on the $AgNO_3$ concentration used and caused by adduct ion formation during electrospray. A typical feature of such weak noncovalent bonded solvent or salt adducts is their instability at collision activation conditions.

By application of an additional potential to initiate CAD it is possible to distinguish between stronger electrostatically based coordination of metal ions or weakly associated ions of adducts formed in the spray interface.

The results of CAD experiments support the suggestion of the existence of different **L1**/Ag species in solution and gas phase, and allow a distinction of both. For example, Fig. 4 presents the dependence of (**L1** + Ag)$^+$ ion abundance on the collision energy applied, and on the various $AgNO_3$ concentrations used for complexation. At 10 V CAD potential the relevance of different $AgNO_3$ concentrations is reflected by increasing [**L1** + Ag]$^+$ ion abundances, but not in the ratio 1:2:3. The probability that **L1** picks up one silver ion increases clearly with a twofold excess of Ag$^+$ whereas a larger extend of silver ions hardly influence the formation of [**L1** + Ag$^+$] products. The curves in Fig. 4 let one assume that most of the [**L1** + Ag$^+$] ions produced by 1:2 and 1:3 mixtures are not stable complexes but adduct ions of produced during spray ionization. The graphs representing the [**L1** + Ag$^+$] ion of 1:2 and 1:3 mixtures plunge down with increasing CAD potential whereas the 1:1 mixture produces a slightly sloped curve. This behavior points to a fairly stable species decomposes not until 50 V CAD potential. The **L1** + Ag$^+$ adducts formed in the gas phase decompose abundantly above 10 V CAD potential. The ratio of the adduct ions and the real complex ions present in

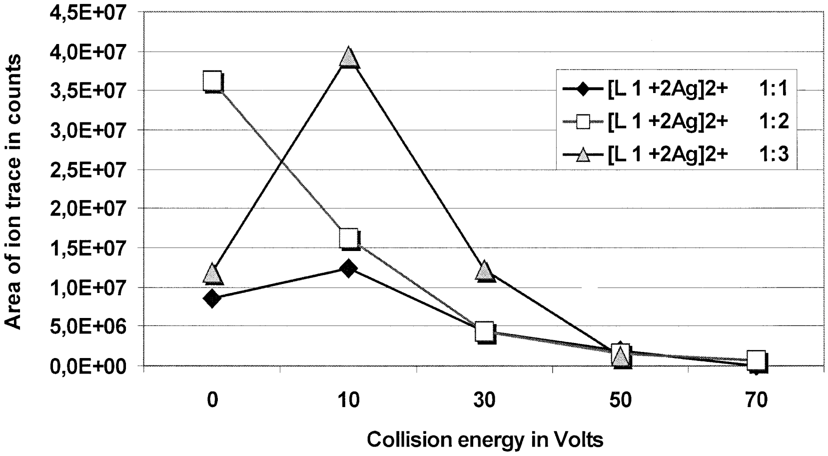

Fig. 5. CAD experiments showing the decomposition ability of (**L1** + 2Ag)$^{2+}$ ions in dependence on various $AgNO_3$ concentration.

the spray cannot be estimated on the basis of these data. More detailed CAD experiments coupled to MS/MS would be needed.

Consequently, the [**L1** + Ag$^+$] ions formed in a 1:1 mixture are mainly stable complex ions present in the liquid phase.

In a search for evidence for the inclusion of more than one silver ion in the azacage, the appropriate ions (**L1** + 2Ag)$^{2+}$ were located at *m/z* 407.6, 408.6, and 409.6. The graphs in Fig. 5 prove the smaller tendency to form doubly charged adduct ions. Apart from the 1:3 mixture the CAD behavior of (**L1** + 2Ag)$^{2+}$ ions is quite similar in all mixtures. Obviously, the stabilization of two charges per molecule is more difficult in gas phase and therefore, less probable. This means, already at 1:1 mixtures two silver ions are placed inside the cage, and the offer of higher Ag$^+$ amounts does not change this preference. As well cyclovoltametric analysis can confirm the particular stability of (**L1** + 2Ag) complexes in solution [25]. Maximum three Ag$^+$ can be coordinated to **L1** as previous solvent extraction experiments and related molecular modeling demonstrated [23]. Two Ag$^+$ ions are coordinated to the tren function at the top and the bottom of the cage. The third Ag$^+$ is coordinated to the centre of the cage by weaker interactions to the pyridine nitrogen donor atoms. The different stability of Ag$^+$ coordination (assumed as tetragonally orientated) is reflected also in ES experiments which verified maximum two silver ions per complex. Probably, the third Ag$^+$ is released immediately during ionization.

Besides the formation of silver complexes also the behavior of the free ligand **L1** and of the sodium complex was taken into account. As demonstrated in Fig. 6, at non-CAD conditions the abundance of (**L1** + H)$^+$ ions depend conversely on the silver ion content of solution. The abundance of (**L1** + Na)$^+$ ions follows the same trend, the higher the $AgNO_3$ concentration the lower is the amount of sodium complexes formed. This fact points to a pronounced affinity of **L1** toward Ag$^+$ instead of Na$^+$, which agrees with solvent extraction experiments. When **L1**–Na complexes formed, they show high stability against CAD potentials because more than 50 V are needed for decomposition which is primarily marked by fragmentation of the intact complex rather than by simple decomplexation.

3.3. Formation of silver–L2 complexes

In comparison with **L1** the complex formation ability of **L2** toward Ag$^+$ is significantly weaker. Low abundance of (**L2** + Ag + H_2O)$^+$ at *m/z* 1350.3 proves the weak trend to coordinate silver ions. The organic phase shows primarily the free protonated ligand **L2** at *m/z* 1226.4 u. Even the transfer of a second proton is not significant as is the formation of

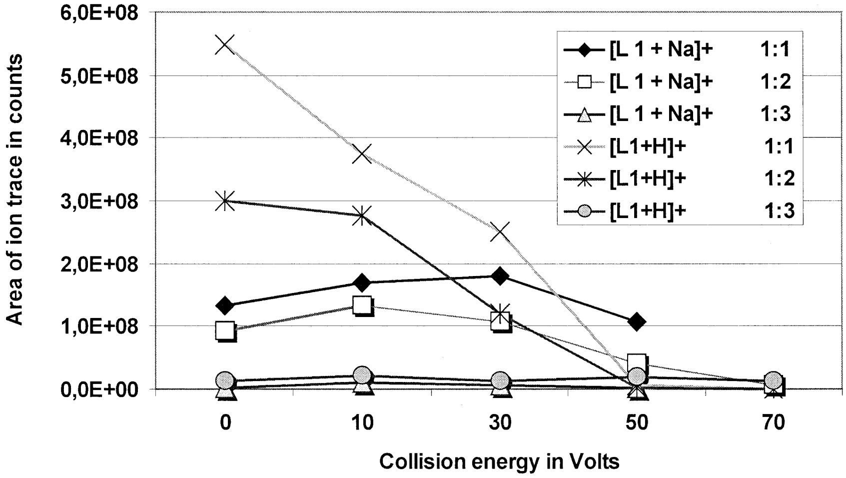

Fig. 6. CAD behavior of ligand **L1** and the Na^+ complex accompanying the silver complexation is shown as function of collision energy and $AgNO_3$ concentration.

sodium complexes. Obviously, the pyridine in **L2** is able to bind weakly silver. But in contrast to **L1** the silver selectivity is much higher. This result is comparable with preliminary extraction data.

In contrast to **L1**, ligand **L2** tends to easier fragmentation at CAD conditions starting with the elimination of benzoyl substituents. Further, the weak $(L2+Ag+H_2O)^+$ signal decreases when the CAD voltage is raised from 0 to 30 V. The water phase of this experiment did not contain any complex or ligand ions because of extremely low water solubility of **L2** and its silver complex.

3.4. Coordination of Zn, Co, Ni, and Hg by L1

In the case of doubly charged ions such as Zn, Co, Ni, and Hg the number of species formed with **L1** increased. A representative ES spectrum is shown in Fig. 7 for the [**L1**/Zn] complex. At first, complex formation was carried out in water as the sole reaction medium. Due to the simpler reaction system individual species can be studied without any distorting influence of the organic phase. The low water solubility of ligand **L2** limited these series of experiments to **L1** complexes.

The spectrum in Fig. 7 is dominated by the [**L1** + Zn]$^{2+}$ ion at *m/z* 332.7. The complex ions [**L1** + Zn + H]$^{3+}$ at *m/z* 222.1, [**L1** + Zn − H]$^+$ at *m/z* 664.3, and [**L1** + Zn + Cl]$^+$ at *m/z* 700.3 belong to typical species found also in the ES spectra of Co, Ni, and Hg complexes. The query whether the ion at *m/z* 602.4 [**L1** + H]$^+$ is caused by noncoordinated **L1** or by fragmentation of one of the complex species can be only resolved by MS/MS investigations. However, the results of collision activation experiments suggest that the high stability of the complexes prevents a simple release of the intact ligand. The CAD experiments showed that **L1** is destroyed to fairly small fragments such as *m/z* = 107.0 (represents an aminomethyl pyridinium cation) with increasing octapole potential. Considering these results, the present (**L1** + H)$^+$ ion is related to free **L1** in solution and leads to incomplete complexation in water. Consequently, an estimation of response factors of individual species is not conceivable.

The ion abundances of the typical complex species are strongly dependent on the collision potential applied. At noncollision conditions the most intensive ions are [**L1** + Zn]$^{2+}$ and [**L1** + Zn + H]$^{3+}$ (Fig. 8). Their ion abundances decrease drastically when applying a CAD potential of 30 V. At this condition the only ion with slightly increasing intensity is the species [**L1** + Zn − H]$^+$. Probably this increase is caused by decomposition of precursors enabling an

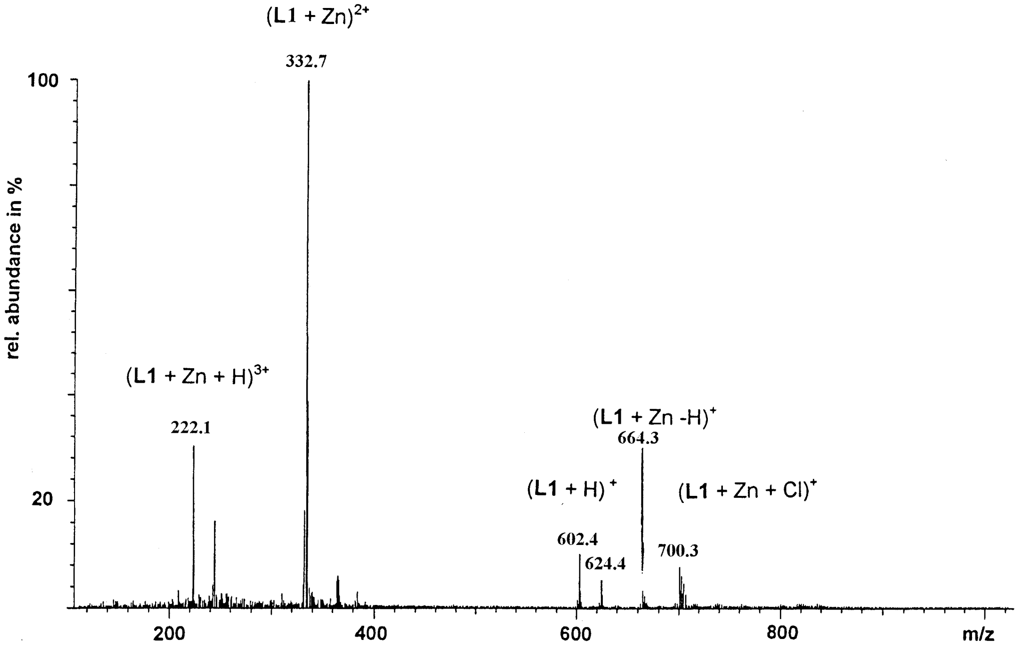

Fig. 7. ES spectrum of **L1**/Zn complexes formed in water by reaction of **L1** (1×10^{-4} M) with $ZnCl_2$ (1×10^{-4} M) dissolved in water.

elimination of a single proton or HCl. Only MS/MS experiments can provide evidence of this assumption. The formation of a double bond within the ligand system and additional stabilizing $\pi - d$ orbital relationships between Zn and ligand may support this decomposition.

Generally, all complex species as well as the **L1** itself fragment quickly under CAD conditions (Fig. 8). The omnipresent traces of sodium ions form fairly stable complexes, which survive even at 70 V. This observation is in accordance with the results obtained in CAD experiments with silver extraction (Fig. 6).

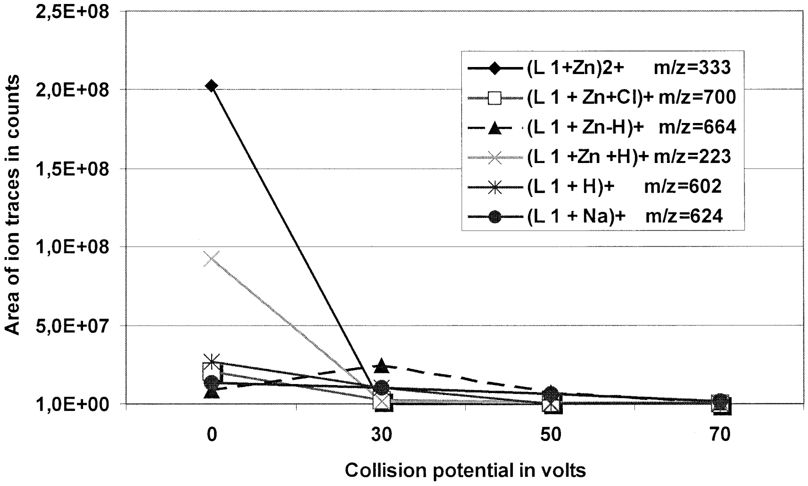

Fig. 8. Behavior of **L1**/Zn and **L1**/Na complexes in aqueous solution at different CAD potentials.

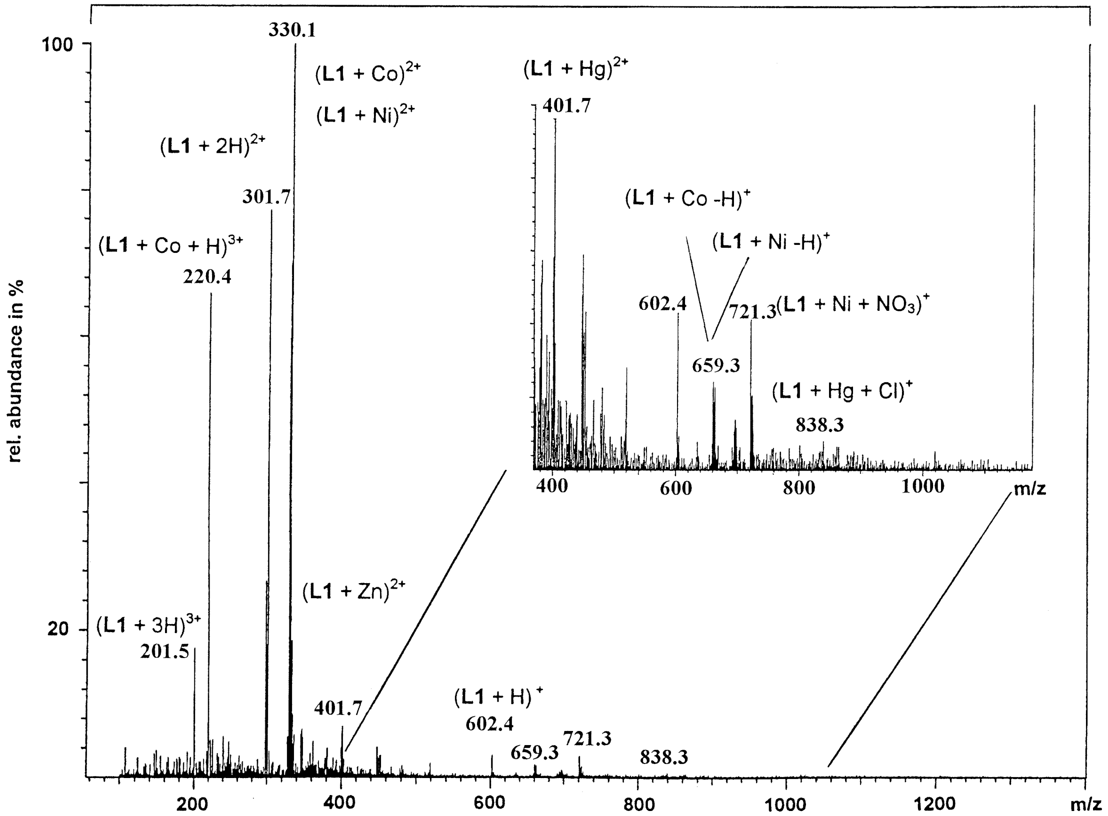

Fig. 9. ES spectrum of ligand **L1** (1×10^{-4} M) exposed to a mixture of $ZnCl_2$, $Ni(NO_3)_2$, $Co(NO_3)_2$, and $HgCl_2$ each of (1×10^{-4} M) dissolved in water.

In principle, similar products are formed during fast reactions of $Co(NO_3)_2$ or $Ni(NO_3)_2$ and **L1.** The reaction yields are high, thus only few (**L1** + H)$^+$ ions appear in ES spectra. Both complexes are mainly characterized by (**L1** + Me)$^{2+}$ as well (**L1** + Me − H)$^+$ ions. The complexation of Hg^{2+} is not so successful in water solution. (**L1** + Hg + Cl)$^+$ is the major complex ion detected in the ES spectrum. Although no response factors of the various metal complexes are available and slightly different product species distributions were observed, an estimation of complexation affinity was made. On the basis of the ratio (L1 + Me)$^{2+}$/(L1 + H)$^+$ the transition metals showed decreasing affinity for inclusion by cage **L1** in pure water solution: Co $\geq$ Ni $>$ Zn $\gg$ Hg {related to (L1 + Hg + Cl)$^+$/(L1 + H)$^+$}.

3.5. *Selectivity of coordination*

Is this graduation of coordination affinity also reflected when the metal ions offered as mixture to **L1**? This question could be answered by appropriate experiments, first in pure water. A mixture of $ZnCl_2$, $HgCl_2$, $Ni(NO_3)_2$, and $Co(NO_3)_2$ (each of those of 1 mmol) was exposed to 1mmol **L1**. Co^{2+} and Ni^{2+} won the competition if the number and abundance of complex species formed is taken into account. The ES spectrum in Fig. 9 indicates the preference of **L1** to coordinate Co^{2+} and Ni^{2+}. The predominating ion of 330.4 u represents probably both doubly charged ions (**L1** + Co/Ni)$^{2+}$ because the nominal mass separation of the quad can not quite separate the interfering signals of the theoretically calculated masses of

330.15 and 329.65 u, respectively. However, the singly charged ions at *m/z* 659.3 and 658.3, their interfered isotope pattern as well as selective ion monitoring provide evidence for the presence of (**L1** + Me − H)$^+$ for both metals. The ion of 220.4 u is also caused by (**L1** + Co + H)$^{3+}$ and 722.3 u formed by (**L1** + Co + NO$_3$)$^+$. Only a few small signals of (**L1** + Zn)$^{2+}$ at *m/z* 332.7 and of (**L1** + Hg + Cl)$^+$ at *m/z* 838.3 were detected.

Assuming that no significant differences exist in the response of the different metal complexes the selectivity of the cage **L1** for Ni and Co coordination in water solution was proven.

In two-phase extraction experiments **L1** shows less selectivity toward the various metal ions. In order to minimize the differences of phase transfer rates of the metal ions picrate was added. It forces the interphase transfer of metal ions and possibly, it equalizes coordination preferences.

Generally, all complex species detected in the organic layer were of lower abundance. Sodium ions enter the competition around **L1** and show comparable complexation tendency like the transition metals. The spectrum of the water phase contained only some **L1** ions and fragments. Thus, it was not informative regarding the selectivity of complex species.

The same reaction was repeated with **L2** as ligand. The ES spectrum of the organic phase was dominated by **L2** ions and fragment ions of the cage. Only (**L2** + Na)$^+$ and (**L2** + Co + NO$_3$)$^+$ ions were of noticeable intensity. Nevertheless, the extraction yield of Co^{2+} ions is very small compared to **L1**. No hints were obtained regarding the formation of Ni, Zn, and Hg complexes. Obviously, the donor ability in the molecule changed by introduction of the amide function and a sterical hinderance of the donor sites limit the variety of complex species for **L2**. Corresponding CAD experiments as in the case of **L1** complexes were not performed because of only low abundant complex ions signals.

4. Conclusion

The results presented prove the suitability of electrospray mass spectrometry to investigate processes of metal coordination by azacage compounds. Two cryptands, **L1** and **L2**, have been used as ligands to coordinate transition metal ions such as Ag$^+$, Hg^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+}.

The different properties of both cages define their ability for metal complexation. Generally, ligand **L1** showed a higher ability for coordination of the metal ions selected. Most abundant complex ions in the ES spectra are (**L1** + Me), (**L1** + Me−H), (**L1** + Me + H), and (**L1** + Me + anion). Particularly, the amino and pyridine donors of **L1** are capable to coordinate at least two Ag$^+$ ions or one of the two-valence transition metals. **L1** shows a stronger binding to Co^{2+} and Ni^{2+}, lower to Zn^{2+} and Hg^{2+}. The coordination capability of cage **L2** is limited. Only low abundant complex ions of Ag$^+$ and Co^{2+} were observed. The lower complexation ability of **L2** is obviously caused by steric hindrance for entering the donor sites and by changed donor strength of the ligand. The ES results could prove the higher extraction selectivity of **L2** compared to **L1**. The mass spectrometric behavior of both ligands was in agreement with liquid–liquid extractions and cyclovoltametric analysis performed previously.

CAD experiments provided evidence for the presence of different stable ion species in solution and gas phase. Typical complex or adduct ions were characterized regarding their stability toward collision activation. The adduct species decomposed between 30 and 50 V whereas complex ions disappeared not until 50 V. CAD experiments allowed a distinction between adduct ions formed during spray and stable complex ions also present in solution.

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