Journal of Inclusion Phenomena and Macrocyclic Chemistry (2005) 53:131–137
DOI 10.1007/s10847-005-1827-z

Original Research Article

Electrospray Ionization Mass Spectrometry (ESI-MS) as a Useful Tool for Fast Evaluation of Anion and Cation Complexation Abilities of a Cyclam Derivative

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(Received: 14 October 2004; in final form: 7 February 2005)

Key words: anion coordination, cyclam, electrospray ionization mass spectrometry, macrocycles, metal coordination, supramolecular chemistry

Abstract

Electrospray ionization mass espectrometry (ESI-MS) has been used for the study of a cyclam derivative noncovalent interactions. At acidic pH, diprotonated macrocycle bound to different anionic species were observed. The selectivity shown by competitive experiments is rationalized with the help of semiempirical theoretical calculations. At basic pH, the base peak corresponded to the macrocycle-alkaline metal complexes, and again competition experiments showed different binding strength. Finally, experiments carried out in the presence of transition metal salts allowed the detection of the complexes present in the mixture and revealed their different kinetic behavior.

Introduction

Cyclam derivatives are probably the most deeply studied macrocyclic tetraamines in several fields such as coordination chemistry, supramolecular chemistry, catalysis, biomimetic chemistry and medicine [1]. Most of their applications deal with their complexation abilities with different metal ions [2]. We have previously reported the synthesis of both enantiomers of a new C_2 symmetrical optically active cyclam derivative (**L1**, see Figure 1) [3]. We have also studied the structural changes produced by protonation in solid state (by X-ray diffraction), in solution (by NMR) and in gas phase (by semiempirical calculations) [4]. From that work, we initially concluded that this compound is able to interact with anions at very low pH by ionic and hydrogen bond interactions. Unfortunately, the low pHs required to detect these anion complexes (below 1) prevent from its study by potentiometric methods, which are considered the most useful techniques for this kind of measurements [5].

On the other hand, the study of noncovalently bound complexes by mass spectrometry has rapidly gained momentum due to the introduction of electrospray ionization (ESI-MS) as one of the softest ionization method to date [6]. In the last couple of years, many papers have come out showing the estimation of binding affinities by ESI-MS [7]. The advantages of this technique is fast performance and little sample consumption, which makes it very suitable for high throughput screening. As compound **L1** could interact with both cations and anions, depending on the pH of the medium, we considered this system for an ESI-MS study (Scheme 1).

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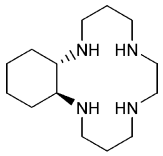
Experimental

General procedure

ESI mass spectra were acquired on a tandem mass spectrometer (Hewlett Packard HP 1100 Series LC/MSD system) equipped with an electrospray interface working in the positive mode (for detection of positive ions). The fragmentor was run at two fragmentor potentials of 40 and 80 V, respectively.

The samples were diluted to a concentration of approximately 10^{-5} M in $MeOH/H_2O$ (90/10) and the composition of the effluent used in the LC system was also $MeOH/H_2O$ (90/10). For acidic samples, known amounts of concentrated acid solutions (1 M) were added to the ligand sample to reach the desired pH (measured after the adition). In all the cases the final concentration of the acid was >10-fold the one for the ligand. For alkaline samples, 1 M stock solutions of metal hydroxide were used to fix the pH. The concentration of the metal was 10-fold of the ligand. For competition experiments, the concentrations of the



L1

Figure 1. Molecular structure of ligand **L1**.

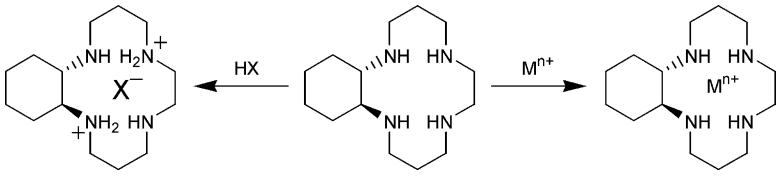
titrants were kept equal, being the total concentration of the alkaline hydroxides 10-fold of that of the ligand. Mass spectra were obtained by introducing the samples using FIA technique (flow injection) while effluent was introduced into the ESI-MS apparatus at a pump flow rate kept at 0.2 mL min^{-1}. The temperature of the nitrogen drying gas was set at 350 °C as it entered the electrospray chamber at a flow of 10 L min^{-1} to allow desolvation of the infused solution and the nebullizer was held at a constant pressure of 25 psi.

Results and discussion

In order to study the anion complexation abilities of **L1**, we prepared solutions of this macrocycle at pH = 1 and fixed the proton concentration with a series of acids (HCl, HBr, $HClO_4$, H_2SO_4, CF_3CO_2H, picric and *p*-TsOH). The ESI-MS spectra of these samples showed peaks corresponding to different species depending on the acid used (see Table 1). For example, clear peaks assigned to species of the type $[\mathbf{L1}H_2 + A]^+$, where A is the conjugated anion of the acid, were obtained in many cases (Table 1). Meaningful isotope patterns could be recorded for the complexes analyzed. It is of note the intensity obtained for the complex formed by diprotonated **L1** and the perchlorate anion, which appeared to be the base peak of the spectrum. The results shown in Table 1 suggest that tetrahedral anions are the most strongly bound [8]. To determine the relative binding affinities of the best-bound anions (perchlorate and hydrogenosulfate), competition experiments were performed. Equal amounts of the corresponding solutions with both anions were mixed, and the ESI-MS spectrum showed a peak for the sulfate complex $m/z = 353$), but not for the corresponding complex with the perchlorate anion. This result suggests a stronger interaction with the former anion.

In order to explain the observed binding selectivity of $\mathbf{L1}H_2$ towards different anions, some molecular modeling has been undertaken. We optimized the geometries of $[\mathbf{L1}H_2]^{2+}$ and $[\mathbf{L1}H_2 + A]^+$ species for two spherical (chloride and bromide) and two tetrahedral (perchlorate and hydrogenosulfate) monoanions at the semiempirical PM3 level of theory. Although sulfate anion is expected to be monoprotonated at the very low experimental pH, we initially considered two possibilities: $[\mathbf{L1}H_2 + HSO_4]^+$ and $[\mathbf{L1}H_3 + SO_4]^+$. Semiempirical PM3 calculations predicted that the most stable complex is formed between diprotonated ligand and monoanionic hydrogenosulfate. The obtained structures are shown in Figure 2. Some interesting features must be pointed out from these geometries. As expected, the presence of the counterion stabilizes the macrocyclic structure by both electrostatic and hydrogen bond interactions. The anionic species settle on top of one of the faces of the macrocycle, which has to suffer a conformational change to maximize the interaction with the guest. After comparison with the initial geometry of $[\mathbf{L1}H_2]^{2+}$, very distorted geometries were found for the spherical anions, suggesting that a reason for their weaker interaction could be a larger folding of the macrocycle upon anion complexation. Furthermore, if we consider the minima of energy obtained for complexes with ClO_4^- and HSO_4^-, again a larger folding of the receptor was obtained for perchlorate anion. Inspection of the hydrogen bonding pattern with the different anions revealed that the shortest average distances are obtained for HSO_4^-. Thus, this anion is the only one able to form H bonds with hydrogen atoms appended to the 4 nitrogens of **L1**, leading to a more efficient ion pairing stabilization. A combination of both facts: better geometrical preorganization and more effective electrostatic hydrogen bonding contacts could give rise to a good complementarity and, consequently, to a stronger binding with HSO_4^-. The stability of the complexes can be also compared by inspection of the PM3 energy of the optimized structures. Thus, the formation of $[\mathbf{L1}H_2 + HSO_4]^+$ from $[\mathbf{L1}H_2 + ClO_4]^+$ and HSO_4^- with the concomitant release of ClO_4^- renders a computed $\Delta H = -65.6$ kJ mol^{-1}. This result qualitatively agrees with the experimental observation from the ESI MS competition experiment.

When the pH of the solution is around 13, the main species of this macrocyclic polyamine (**L1**) is the free base [4], which is able to form complexes with alkaline metal cations. Thus, the complexes detected in the ESI-MS spectra at a very basic pH should depend on the alkaline base used. We performed the mass spectrum experiments with a series of alkaline hydroxides (LiOH, NaOH and KOH). In all cases, the base peak of the spectrum was the $[\mathbf{L1} + M]^+$ species (Table 2), where M^+ corresponded to the alkali metal cation. Again for estimating **L1** binding selectivity, we carried out competition experiments[9] with all possible combinations of two alkali bases



Scheme 1.

Table 1. ESI-MS peaks observed for **L1** at acidic pH with different strong acids

pH	Acid	(*m*/*z*)	Asignation	Intensity (%)
6–7	–	255	$[\mathbf{L1H}]^+$	100
		128	$[\mathbf{L1H_2}]^{2+}$	22
1	HCl	255	$[\mathbf{L1H}]^+$	100
1	HBr	255	$[\mathbf{L1H}]^+$	100
		335	$[\mathbf{L1H}_2^{79}\ Br]^+$	< 1
		337	$[\mathbf{L1H}_2^{81}\ Br]^+$	< 1
1	$HClO_4$	255	$[\mathbf{L1H}]^+$	22
		355	$[\mathbf{L1H}_2^{35}\ ClO_4]^+$	100
		357	$[\mathbf{L1H}_2^{37}\ ClO_4]^+$	33
1	H_2SO_4	255	$[\mathbf{L1H}]^+$	< 5
		353	$[\mathbf{L1H}_3SO_4]^+$	8
1	CF_3CO_2H	255	$[\mathbf{L1H}]^+$	100
1	Picric acid	255	$[\mathbf{L1H}]^+$	100
1	*p*-TsOH	427	$[\mathbf{L1H} + p\text{-TsOH}]^+$	< 5
1	H_2SO_4 + $HClO_4$	255	$[\mathbf{L1H}]^+$	33
		353	$[\mathbf{L1H}_3SO_4]^+$	28

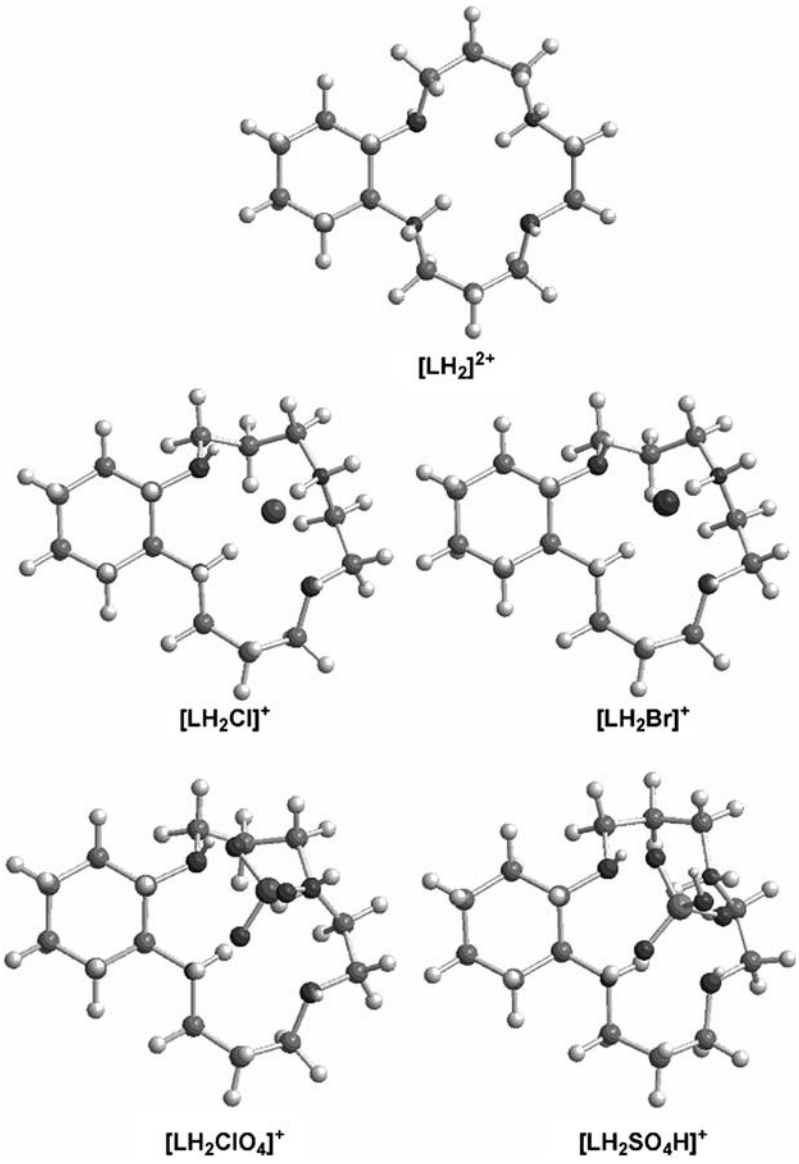


Figure 2. Optimized PM3 geometries for $[\mathbf{L1}H_2 + A]^+$ species.

Table 2. ESI-MS peaks observed for **L1** at basic pH with different alkaline bases

PH	Base	(*m*/*z*)	Asignation	Intensity (%)
13	LiOH	255	$[\mathbf{L1}H]^+$	<2
		261	$[L1Li]^+$	100
13	NaOH	255	$[\mathbf{L1}H]^+$	5
		277	$[\mathbf{L1}Na]^+$	100
13	KOH	255	$[\mathbf{L1}H]^+$	7
		293	$[\mathbf{L1}K]^+$	100
13	LiOH + NaOH	255	$[\mathbf{L1}H]^+$	<1
		261	$[\mathbf{L1}Li]^+$	100
		277	$[\mathbf{L1}Na]^+$	12
13	LiOH + KOH	255	$[\mathbf{L1}H]^+$	3
		261	$[\mathbf{L1}Li]^+$	9
		293	$[\mathbf{L1}K]^+$	17
13	NaOH + KOH	255	$[\mathbf{L1}H]^+$	5
		277	$[\mathbf{L1}Na]^+$	2
		293	$[\mathbf{L1}K]^+$	12
13	LiOH	255	$[\mathbf{L1}H]^+$	3
13	NaOH	261	$[\mathbf{L1}Li]^+$	5
13	KOH	277	$[\mathbf{L1}Na]^+$	2
		293	$[\mathbf{L1}K]^+$	24

(see Table 2). In the presence of a couple of different cations, the relative peak intensity of $[\mathbf{L1}+M]^+$ increased as $Li^+ > Na^+$; $K^+ > Na^+$; $K^+ > Li^+$. With the aim of assessing this order, we performed a similar experiment with the three bases mixed. Again the peak intensities confirm the same tendency in the complexation abilities, $K^+ > Li^+ > Na^+$. Assuming that ligand-cation peak intensity (ESI-MS) is proportional to the concentration ratio in solution [10], we can obtain an easy and rapid estimation of the binding selectivity exhibited by **L1** (see Table 2).

It is surprising that this order of complexation does not correlate with the ionic radii of the cations [11], but these effects could be explained by the formation of complexes in the inner or outer coordination sphere, or by the participation of $[\mathbf{L1}_2 + K_2]^{2+}$ species. In order to give some explanation to these binding trends, we performed *ab initio* quantum mechanics theoretical calculations. The geometries optimized at the HF/3-21G* level of theory for the Li^+, Na^+ and K^+ complexes (Figure 3) set the cation equidistant to the 4 nitrogen atoms of **L1**. While Li^+ cation nicely fit inside the macrocyclic cavity, K^+ is too big and is settled on top of it. In the case of Na^+, the ligand has to open its binding site to accommodate the guest metal. This situation produces a distortion in the structure which could be the explanation for the preference of **L1** for Li^+ over Na^+. Accordingly, the computed energy for the metal exchange in **L1** is about 129.9 kJ mol^{-1} favorable to the formation of the lithium complex. However, the explanation for the potassium selectivity seems to be more complicated. The optimized geometry shown in Figure 3 suggests that partial solvation of the cation could be important in this complex, as the ligand would leave the guest ion partially exposed to the medium. With that scenario, in a competition experiment, the other two cations (Na^+ and Li^+) would have to completely desolvate to fit inside the macrociclic cavity while K^+ could interact with **L1** with only partial desolvation.

Among the most exciting applications of cyclam derivatives are the use of their corresponding transition metal complexes for catalysis [12], ion sensing [13] or medicinal chemistry [14]. For example Ni(II)-cyclam complex efficiently catalyses the epoxidation of alkenes [12]. On the other hand, this cyclic polyamine has shown rapid Zn(II) uptake at physiological pH, an observation relevant to the anti-viral and co-receptor binding of anti-HIV cyclams [15]. The knowledge of the kinetic and thermodynamic properties of the complexes is essential for those purposes, but sometimes, the inertness of the cation prevents from the study of the complex formation. Different experimental techniques like UV, NMR or potentiometry have been used to study these reactions. We propose here an alternative method for a qualitative knowledge of the process. In order to study complexation properties of **L1**, we took into account those metals whose cyclam complexes have been deeply described (Cu^{2+}, Zn^{2+}, Ni^{2+}). ESI-MS spectra showed peaks at *m*/*z* values corresponding to the following species: $[\mathbf{L1} + M]^{2+}$, $[\mathbf{L1} + M + Cl]^+$, $[\mathbf{L1} + M + Br]^+$, $[(\mathbf{L1}-H) + M]^+$ (see Figure 4). The presence of bromide anion is due to the use of the **L1** · 4HBr salt and chloride anion comes from the metal salt (MCl_2). These cylam:metal:anion species have been previously described [16] and ESI-MS has allowed us its direct detection. The existence of these ternary complexes could be of great interest for future applications of our ligand in chiral anion recognition [17]. Again in this case, isotopic pattern was extremely useful for the unambiguous identification of the metal complex species.

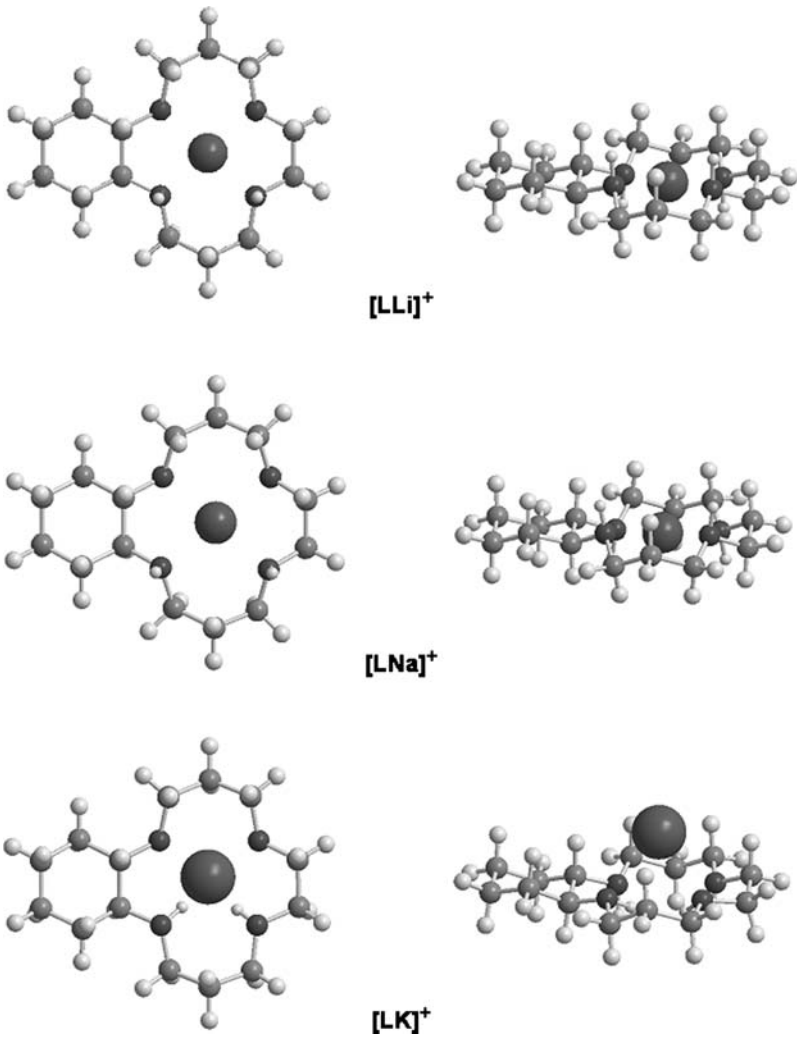


Figure 3. Upper and side views of the optimized geometries (HF/3-21G*) for $[\mathbf{L1} + M]^+$ species.

Comparison of results for these three metals showed that the largest differences emerge from their kinetic behavior. While formation of complexes with Zn^{2+} and Cu^{2+} is almost instantaneous, the analogous for Ni^{2+} requires longer reaction times. The inertness of Ni^{2+} in forming complexes has been previously reported [16a] and ESI-MS also reveals this feature. For this reason, we believed interesting to carry out the ESI mass experiments at different reaction time. Figure 5 shows a plot of the metal complex species and protonated ligand peak intensities as a function of time for 1:1 and 1:2 **L1**:Ni^{2+} molar ratio. The complexation rate seemed to be dependent on the metal concentration. Plotting of 1/[**L1**H] *versus* time corroborated this fact, and demonstrated a second-order kinetic behavior. In addition to this, it was possible to follow the time-dependent evolution of the different metal-complexes. Interestingly, an increase in the $NiCl_2$ molar ratio led to a relative increase in the peak intensities corresponding to $[\mathbf{L1} + Ni]^{2+}$ and $[\mathbf{L1} + Ni + Cl]^+$, as expected for the larger amount of both, metal and chloride ions. With this study, we described a fast alternative technique for studying the transition metal-ligand complexation abilities.

Conclusions

In conclusion, we have carried out an easy estimation of anion and cation complexation abilities of a cyclam derivative by ESI-MS. This technique offers an alternative way to estimate binding selectivities and provides rapid feedback with minimal sample consumption. Competition experiments with both anions and cations gave a qualitative estimation of the interaction preferences. Transition metal complexes were identified and their kinetic differences revealed.

Acknowledgements

Financial support from Principado de Asturias (Spain; Project GE-EXP01-03) and MCYT (Spain; Project

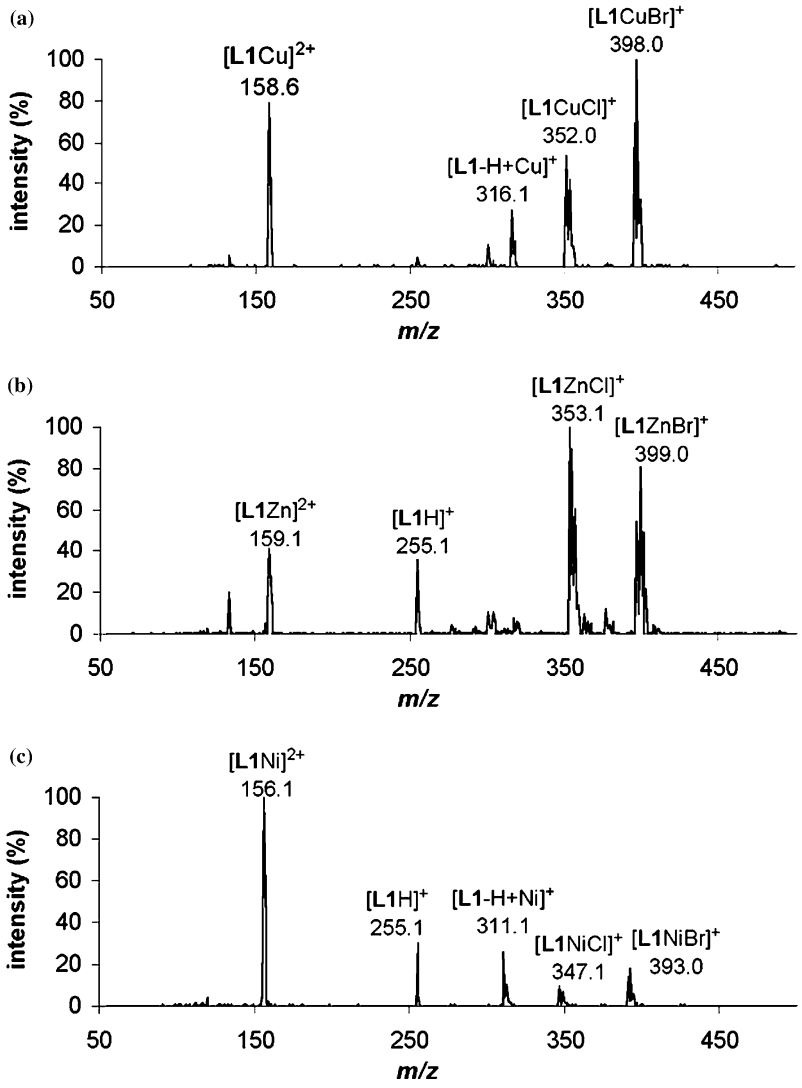


Figure 4. ESI-MS spectra of **L1** in the presence of MCl_2: (a) M = Cu, (b) M = Zn, (c) M = Ni (58 h after the addition).

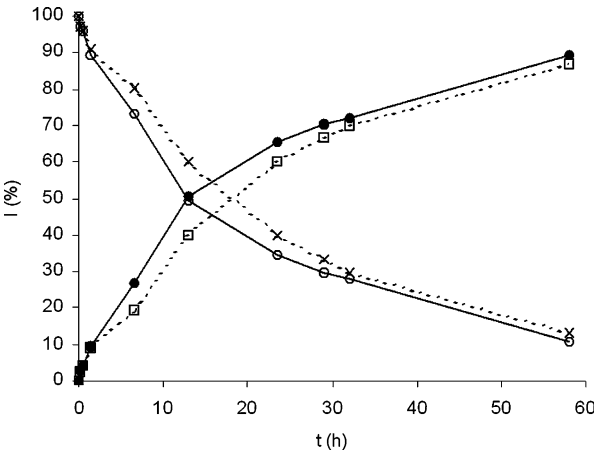


Figure 5. Time-dependent plot for the ESI-MS signal intensities of T[**L1**Ni] and $[\mathbf{L1H}]^+$ species for molar ratio of **L1**:Ni 1:1 and 1:2 (dashed lines and solid lines respectively).

PPQ-2001-2683) is gratefully acknowledged. I.A. also thanks MCYT (Spain) for financial support (Ramón y Cajal program).

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