118. Application of Electrospray Mass Spectrometry for Characterizing Supramolecular Coordination Complexes

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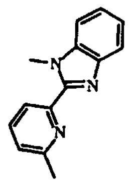
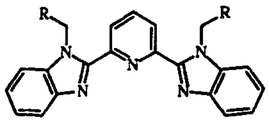
The Electrospray Mass Spectrometry (ES-MS) of eight different supramolecular complexes shows that the molecular peaks of the desolvated multiply charged cations can be recorded more easily than by Fast-Atom-Bombardment Mass Spectrometry (FAB-MS) measurements of similar compounds. The preliminary application of the ES-MS technique to self-assembled helical complexes obtained from ligands L^3 to L^8 with various metal ions (Cu, Co, Eu, Tb) indicates that ES mass spectra qualitatively reflect the species present in MeCN solution.

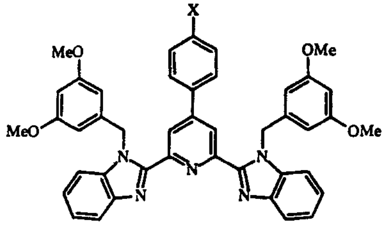
Introduction. – Progress in chemistry is frequently determined by the availability of suitable techniques for the characterization of compounds. In the field of supramolecular chemistry [1], where organized supramolecular units are constructed by the association of smaller molecules, the characterization of the resulting supermolecules is frequently difficult. Mass spectrometry which offers the possibility of observing compounds based on their mass-to-charge ratio in the gas phase, is obviously an attractive technique for proving the formation of supermolecules but, for many of the systems studied, the low volatility and the tendency of the complexes to undergo reduction during ionization process has limited the application of conventional techniques of mass spectrometry [2]. Fast-Atom-Bombardment Mass Spectrometry (FAB-MS) [3], a so-called 'soft' ionization technique, has been successfully applied to many cases as exemplified by the observation of molecular peaks for double-helical [4–6] and triple-helical complexes [6] [7], for a trefoil molecular knot [8], and for the first deoxyribonucleohelicates [9] with molecular weights around 7800 daltons. However, the restricted choice of matrices (*e.g.* nitrobenzyl alcool) for FAB-MS measurements [3] limits the scope of this technique to soluble complexes and often leads to matrix interferences, reduction, or demetalation of the complexes [10]. As far as supramolecular complexes are concerned, the use of a matrix, chemically very different from the solvents in which the studies in solution (NMR, UV/VIS) are performed, is a severe handicap for the correlation of the MS results with those from other techniques. More recently, Electrospray Ionization (ES-MS) has been used to produce multiply charged ions [11] from proteins [12], Ru^{II}, and other complexes [13], metallo[3]catenates and metallorotaxanes [14] in various solvents. ES-MS is an atmospheric-pressure ionization technique based on the ion-evaporation process where the ions are formed outside the vacuum region. It is particularly suitable for characterizing preformed ions in solution [12]. The wide scope of potential applications of ES-MS based



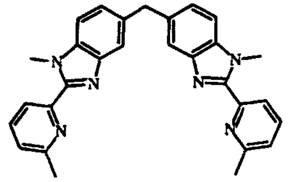
L^1



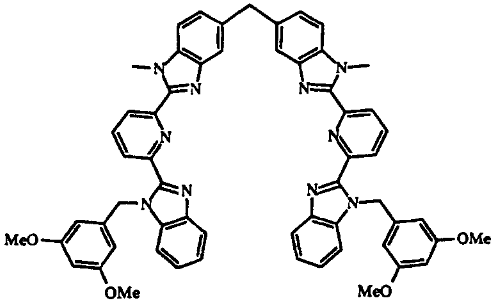
L^2: R = H
L^3: R = $(MeO)_2C_6H_3$



L^4: X = H
L^5: X = NO_2
L^6: X = NEt_2



L^7



L^8

on this technique leads us to consider the use of Ion Spray Mass Spectrometry (IS-MS, pneumatically-assisted electrospray) [15] for the characterization of coordination and supramolecular complexes that are obtained in solution from reactions between the ligands L^1–L^8 and various metal ions [5–7] [16].

Experimental. – The ligands L^1–L^8 and the complexes $[Cu(L^1)_2](ClO_4)$, $[Co(L^1)_3](ClO_4)_2$, $[Eu(L^2)_3](ClO_4)_3$, $[Cu_2(L^n)_2](ClO_4)_2$ (n = 3–6), $[Cu_2(L^7)_2](ClO_4)_2$, $[Co_2(L^7)_3](ClO_4)_4$, $[Eu_2(L^8)_3](ClO_4)_6$, and $[Tb_2(L^8)_3](ClO_4)_6$ were prepared according to literature procedures [5–7] [16]. The ES mass spectra of the complexes were recorded at 10^{-4}–10^{-5} M from MeCN solns. and the free ligands at 10^{-6}–10^{-7} M from MeCN containing 0.1% HCOOH on a *TAGA 6000E* tandem triple quadrupole mass spectrometer upgraded to an *API-III* system (*Sciex*, Toronto, Ontario, Canada) and an in-house-modified *Hewlett-Packard HP-5985B* [17] by infusion at 4–10 μl/min. The spectra were recorded under low up-front declustering or Collision-Induced-Dissociation (CID) conditions, typically ΔV = 10–50 V between the orifice (Or) and the first quadrupole Q_0 of the Sciex and ΔV = 20–60 V between the heated capillary and the skimmer of the *HP-5985B* [17]. Sprayer potentials are typically in the range of 3–4 kV, and fused silica sprayers are preferred to stainless steel sprayers to avoid metal contamination.

Results and Discussion. – As a first step toward the characterization of supramolecular complexes, we have recorded the ES mass spectra of the free ligands L^1–L^8 from MeCN solutions containing traces of HCOOH (0.1%) as a source for protonation. The ES mass spectra of the ligands L^1–L^8 display the expected singly and doubly charged protonated species $[L^n + H]^+$ and $[L^n + 2H]^{2+}$. Structural information can be obtained by up-front CID or MS-MS, but are beyond the scope of this communication. As no decomplexation equilibria are involved for the free ligands, the ES mass spectra can be obtained from rather low concentrations (10^{-6}–10^{-7} M). However, the situation is completely different for the supramolecular species formed by the complexation of ligands L^1–L^8 with various metal ions, since the distribution of the complexes in solution strongly depends on the stability constants and the concentration. In this case, the charged complexes are already present in solution, and the ES mass spectra are recorded from pure MeCN, but higher concentration (10^{-4}–10^{-5} M) is necessary to avoid decomplexation. A detailed analysis of the ES mass spectra of the self-assembled triple-helical $[Co_2(L^7)_3]^{4+}$ at different concentrations has clearly established that decomplexation is responsible for the observation of peaks characteristic of the free ligands at low concentration. As expected from stability constant determination [6], a complex concentration of 10^{-4} M limits the decomplexation to *ca.* 5% and leads to the almost exclusive observation of the cation $[Co_2(L^7)_3]^{4+}$ (*m/z* 373, *Fig. 1*).

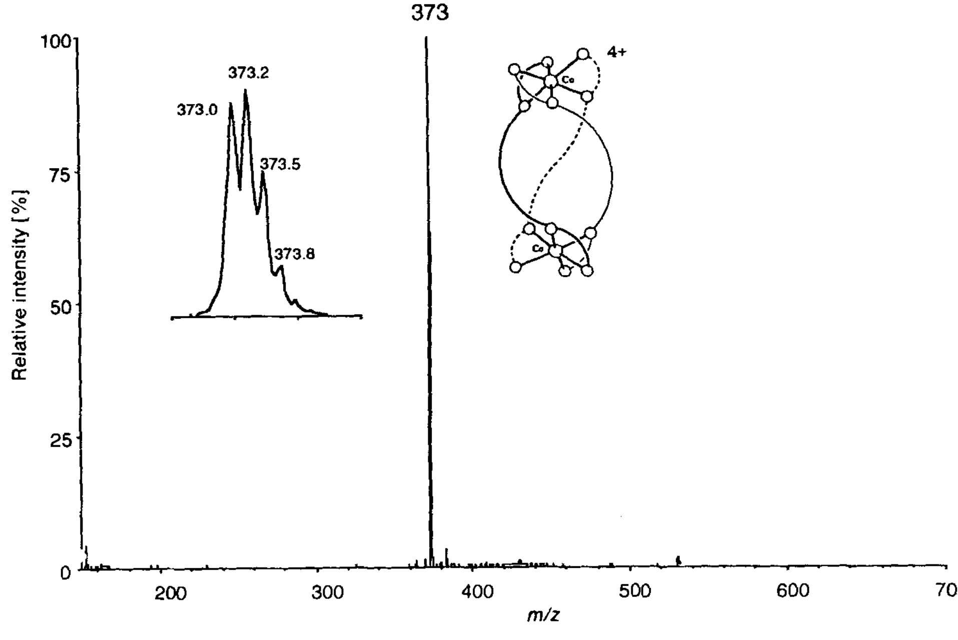


Fig. 1. *ES Mass spectrum of the complex* $[Co_2(L^7)_3](ClO_4)_4$

The bidentate ligand L^1, an analogue of 2,2′-bipyridine [6], reacts with $[Cu(MeCN)_4](ClO_4)$ and $Co(H_2O)_6(ClO_4)_2$ to give stable pseudo-tetrahedral $[Cu(L^1)_2]ClO_4$ and pseudo-octahedral $[Co(L^1)_3](ClO_4)_2$ complexes [6]. The ES mass spectra of these complexes show essentially only the peaks corresponding to the expected cations $[Cu(L^1)_2]^+$ and $[Co(L^1)_3]^{2+}$, without complications resulting from decomplexation, demetalation, or association with the ClO_4^- anions as usually observed for FAB-MS measurements [3–7]. A similar result is obtained for the pseudo-trigonal prismatic complex $[Eu(L^2)_3](ClO_4)_3$ whose ES mass spectrum displays a major peak corresponding to $[Eu(L^2)_3]^{3+}$ (*m/z* 390) and a small peak at *m/z* 367 ($[Fe(L^2)_2]^{2+}$) which results from contam-

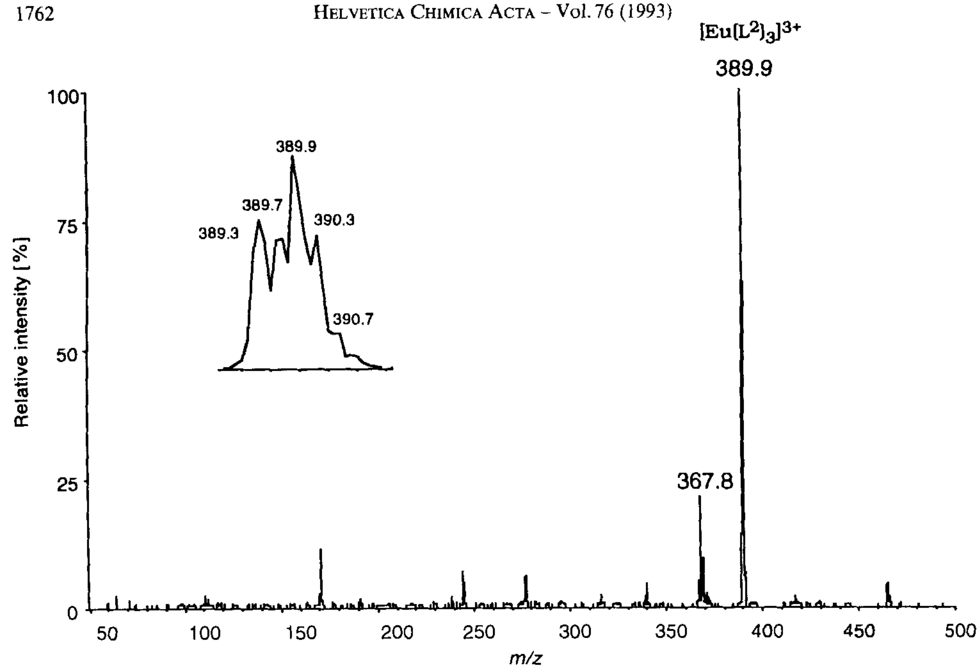


Fig. 2. *ES Mass spectrum of the complex* $[Eu(L^2)_3](ClO_4)_3$

ination with traces of iron in the infusion system. Under very low declustering conditions, some lower mass ions are observed which are tentatively attributed to solvent clusters. The isotopic pattern of the m/z 390 signal shows peaks separated by 0.3–0.4 dalton confirming the existence of a triply charged species (*Fig. 2*). Generally, multiply charged species are generated by ES-MS, and at least two different charge states are necessary to determine the molecular weight for proteins and metal complexes [13] but, in our case, only one multiply charged ion is observed, and an elegant alternative is the use of the isotopic pattern obtained by increasing the resolution as shown in *Fig. 2*.

The isotopic pattern was also used for the characterization of the self-assembled dinuclear double-helical complexes $[Cu_2(L^n)_2]^{2+}$ ($n = 3$–6) in MeCN [5] which represent typical cases of supramolecular complexes where the monomer $[CuL^n]^+$ and the dimer $[Cu_2(L^n)_2]^{2+}$ display the same m/z ratio. The FAB mass spectra only give a partial answer to this problem, since the peaks corresponding to the dimers $[Cu_2(L^n)_2(ClO_4)]^+$ are weak and are always observed together with intense peaks corresponding to the monomers $[Cu(L^n)]^+$. The dimeric nature of these complexes was unambiguously established by conductometric measurements [5] but ES-MS gives the same conclusion with less effort. The ES mass spectra of the complexes $[Cu_2(L^n)_2]^{2+}$ ($n = 3$–6) show separations of 0.5 dalton ($M/\Delta M = 1875$ at 10% peak height, *API-III*) between the components of the molecular peaks whose isotopic distributions (*Fig. 3a*) correspond to the dinuclear cations $[Cu_2(L^n)_2]^{2+}$ ($n = 3$–6, *Table*). Some low mass ions corresponding to partially solvated $[Cu(MeCN)]^+$ and $[Cu(MeCN)_2]^+$ (*Table*) are also observed in the mass spectra. The monomers $[CuL^n]^+$ ($n = 3$–6), with a separation of 1.0 dalton between the components of the molecular peaks (*Fig. 3b*), may be generated in the gas phase by the fragmen-

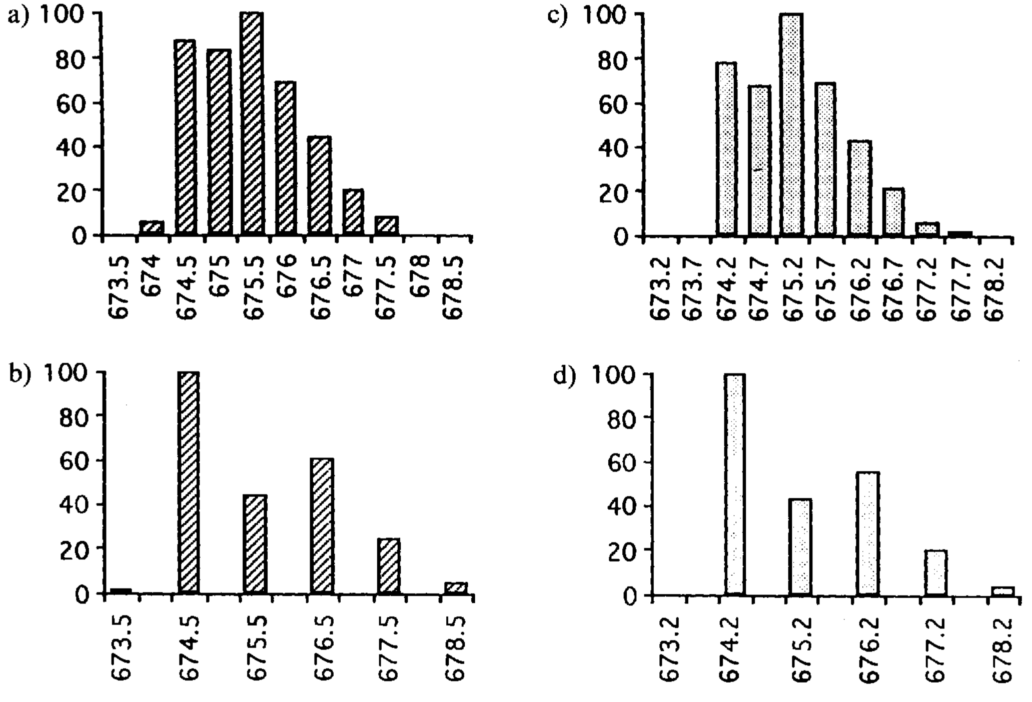


Fig. 3. a) *Isotopic pattern of* $[Cu_2(L^3)_2](ClO_4)_3$ *at* m/z = *675,* $[Cu_2(L^3)_2]^{2+}$; b) $[Cu(L^3)]^+$ *gas-phase product* ΔV = *108 V*; c) *calculated isotopic pattern for* $[Cu_2(L^3)_2]^{2+}$; d) *calculated isotopic pattern for* $[Cu(L^3)]^+$

tation of the dimers by up-front CID ($\Delta V = 108$ V between Or and Q_0 of the first quadrupole). The simulated isotopic patterns (*Figs. 3c* and *3d*) are in good agreement with those measured. However, the resolution of isotopic patterns with quadrupole mass spectrometers is limited to doubly or triply charged ions. For higher charge states, magnetic sector or ion cyclotron resonance (ICR) mass spectrometers are necessary.

Finally, we have used ES-MS to study the formation of dinuclear double- and triple-helical complexes obtained by selective self-assembly of the ligands L^7 and L^8 with different metal ions in MeCN solution [6] [7]. Spectrophotometric titrations [6] [7] have established that triple-helical $[Co_2(L^7)_3]^{4+}$ and $[Eu_2(L^8)_3]^{6+}$ complexes are produced by strict self-assembly [1], while the double-helical complex $[Cu_2(L^7)_2]^{2+}$ can be opened to give $[Cu(L^7)_2]^+$ which amounts to *ca.* 20% of the species for a Cu/L^7 ratio of 1.0 [6]. In this particular case, the ES mass spectrum of $[Cu_2(L^7)_2](ClO_4)_2$ confirms the spectrophotometric titrations and shows a major peak at *m/z* 521 ($[Cu_2(L^7)_2]^{2+}$) and a small peak at *m/z* 979 ($[Cu(L^7)_2]^+$), while the mass spectra of the strict self-assembled triple-helical complexes display only peaks corresponding to the multiply charged cations $[Co_2(L^7)_3]^{4+}$ (*m/z* 373) and $[Eu_2(L^8)_3]^{6+}$ (*m/z* 532) and the protonated ligands (*Table*, *Figs. 1* and *4*). The observation of peaks corresponding to the protonated free ligand in the ES mass spectrum of $[Eu_2(L^8)_3]^{6+}$ (*m/z* 482 $[L^8 + 2\ H]^{2+}$, 964 $[L^8 + H]^+$) is in complete agreement with the excellent ES-MS response of the ligand L^8 and with the stability constant of this complex [7] which predicts 10% of free L^8 for a 10^{-4} M concentration of complex in MeCN.

Table. *Molecular Peaks and Main Fragments Observed by ES-MS for the Ligands L^1 to L^8 and Their Complexes with Metal Ions*

Compound	Cation	m/z[a])	M.wt.[b])
L^1	$[L^1 + H]^+$	224	223.3
L^2	$[L^2 + H]^+$	340	339.4
	$[L^2 + 2\,H]^{2+}$	170	
L^3	$[L^3 + H]^+$	612	611.7
	$[L^3 + 2\,H]^{2+}$	306	
L^4	$[L^4 + H]^+$	688	687.8
L^5	$[L^5 + H]^+$	733	732.8
L^6	$[L^6 + H]^+$	759	758.9
	$[L^6 + 2\,H]^{2+}$	380	
L^7	$[L^7 + H]^+$	459	458.6
	$[L^7 + 2\,H]^{2+}$	230	
L^8	$[L^8 + H]^+$	963	963.1
	$[L^8 + 2\,H]^{2+}$	482	
$[Cu(L^1)_2](ClO_4)$	$[Cu(L^1)_2]^+$	509	609.6
$[Co(L^1)_3](ClO_4)_2$	$[Co(L^1)_3]^{2+}$	364	927.7
$[Eu(L^2)_3](ClO_4)_3$	$[Eu(L_2)_3]^{3+}$	390	1468.5
$[Cu_2(L^3)_2](ClO_4)_2$	$[Cu_2(L^3)_2]^{2+}$	675	1549.4
	$[Cu(CH_3CN)_2]^+$	145	
	$[Cu(CH_3CN)]^+$	104	
$[Cu_2(L^4)_2](ClO_4]_2$	$[Cu_2(L^4)_2]^{2+}$	750	1701.6
	$[Cu(CH_3CN)_2]^+$	145	
	$[Cu(CH_3CN)]^+$	104	
$[Cu_2(L^5)_2](ClO_4)_2$	$[Cu_2(L^5)_2]^{2+}$	795	1791.6
	$[Cu(CH_3CN)_2]^+$	145	
	$[Cu(CH_3CN)]^+$	104	
$[Cu_2(L^6)_2](ClO_4)_2$	$[Cu_2(L^6)_2]^{2+}$	821	1843.8
	$[Cu(CH_3CN)_2]^+$	145	
	$[Cu(CH_3CN)]^+$	104	
$[Cu_2(L^7)_2](ClO_4)_2$	$[Cu_2(L^7)_2]^{2+}$	521	1243.1
	$[Cu(L^7)_2]^+$	979	
$[Co_2(L^7)_3](ClO_4)_4$	$[Co_2(L^7)_3]^{4+}$	373	1891.4
$[Eu_2(L^8)_3](ClO_4)_6$	$[Eu_2(L^8)_3]^{6+}$	532	3790.0
	$[Eu_2(L^8)_3(ClO_4)]^{5+}$	658	
	$[Eu_2(L^8)_3(ClO_4)_2]^{4+}$	848	
	$[Eu_2(L^8)_3(ClO_4)_3]^{3+}$	1164	
	$[L^8 + H]^+$	964	
	$[L^8 + 2\,H]^{2+}$	482	
$[Tb_2(L^8)_3](ClO_4)_6$	$[Tb_2(L^8)_3]^{6+}$	534	3803.9
	$[Tb_2(L^8)_3(ClO_4)]^{5+}$	661	
	$[Tb_2(L^8)_3(ClO_4)_2]^{4+}$	851	
	$[Tb_2(L^8)_3(ClO_4)_3]^{3+}$	1168	
	$[L^8 + H]^+$	964	
	$[L^8 + 2\,H]^{2+}$	482	

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[a]) Nominal m/z values given for the maximum of the peak.

[b]) Molecular mass of the ligands and complexes in daltons.

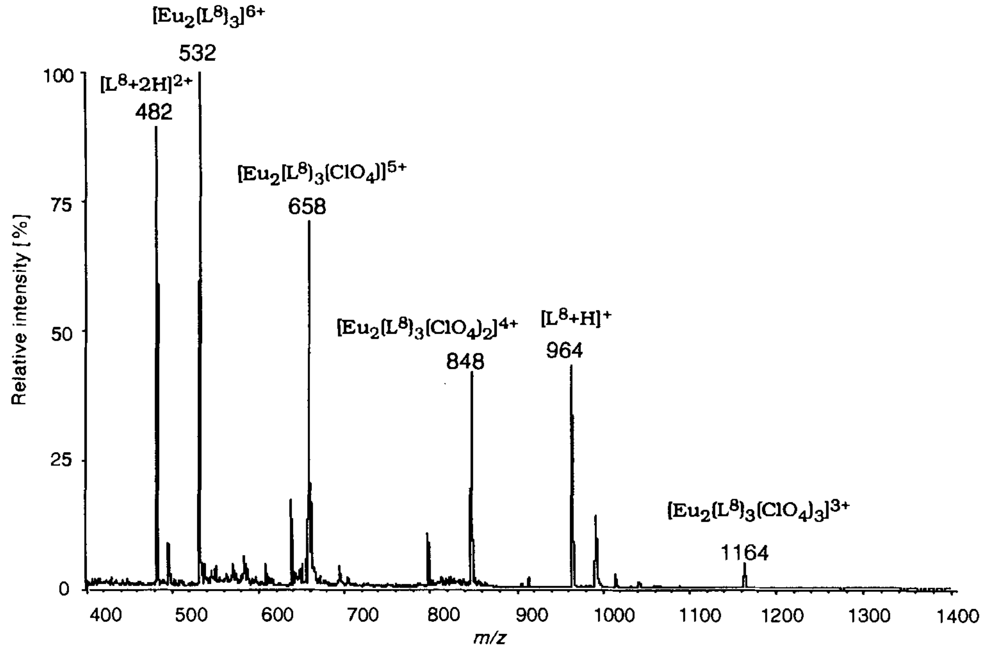

Fig. 4. *ES Mass spectrum of the complex* $[Eu_2(L^8)_3](ClO_4)_6$

However, the resulting free metal is particularly difficult to observe under low declustering conditions, since various clusters with the solvent are normally obtained [18]. In the mass spectra of $[Eu_2(L^8)_3](ClO_4)_6$ (*Fig. 4*) and analogous complexes $[Ln_2(L^8)_3](ClO_4)_6$ (Ln = La, Gd, Tb, Lu), we also observe some smaller peaks corresponding to the species $[Ln_2(L^8)_3(ClO_4)_i]^{(6-i)+}$ ($i = 1$–3) where ClO_4^- anions are associated with the multiply charged cations. Although this effect is not observed for $[Eu(L^2)_3]^{3+}$, associations between cations and ClO_4^- in solution [19] or in nitrobenzyl alcool matrices [3–7] are well-documented, and we tentatively attribute this effect to the higher charge of the dinuclear cations $[Ln_2(L^8)_3]^{6+}$ (Ln = La, Eu, Gd, Tb, Lu). ES-MS was also used to characterize the complexes obtained by mixing stoichiometric quantities of $Eu(ClO_4)_3$, $Tb(ClO_4)_3$ with 3 equiv. of ligand L^8 in MeCN. We observe, for the pseudo-molecular peak, three components separated by 1.2 dalton and corresponding to the three expected complexes $[Eu_2(L^8)_3]^{6+}$ (*m/z* 532.0), $[TbEu(L^8)_3]^{6+}$ (*m/z* 533.2), and $[Tb_2(L^8)_3]^{6+}$ (*m/z* 534.4) whose relative intensities are in qualitative agreement with the distribution of these complexes determined by ^{1}H-NMR in the same solvent.

In conclusion, the molecular weights of all the supramolecular cationic complexes studied are determined easily from ES mass spectra at rather low *m/z* values as a result of the multiply charged nature of the cations. This preliminary systematic application of this technique to the study of self-assembly processes strongly suggests that the ES mass spectra qualitatively reflect the nature and the distribution of the different species present in solution.

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