Electrospray Mass Spectrometry of the Self-assembly of a Capped Polymetallic Complex

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Electrospray mass spectrometry gives direct insight into the metal complexes present in solutions of ligands **L**^{1}, **L**^{2} and Cu^I ions and into the self-assembly of the capped complex **1**; the results agree with UV–VIS spectrophotometric titration data.

A major task in coordination chemistry is to establish the nature and concentration of the species present in multicomponent equilibria in solution. This applies in particular to the spontaneous formation of polynuclear metal complexes by self-assembly of specifically designed ligands and metal ions, which has received much attention recently.[1–3] Studies on the thermodynamic characteristics of such systems are important in order to understand the basic principles of metal complex self-assembly and to design new systems of higher complexity.

The thermodynamic parameters of complexes displaying characteristic UV–VIS absorptions can in principle be obtained by spectrophotometric titration, if the system quickly reaches its thermodynamic equilibrium state after mixing the components. For example, the stability constants for a trinuclear Cu^I double helicate were determined.[2]

The investigation of the self-assembly thermodynamics is expected to be especially difficult in the case of multicomponent systems.[3] In order to be able to exploit the spectrophotometric titration data, it is important to obtain information about the nature of the species present during the titration. An analytical technique that would allow the determination of the metal complexes that are in thermodynamic equilibrium in (dilute) solutions would provide very valuable information.

Electrospray mass spectrometry (ESMS)[4] has been applied successfully to the characterization of cationic polynuclear metal complexes with a wide range of sizes and stabilities.[5] The spectra were usually clear and easy to interpret, often showing the exclusive presence of the complex cation peak and no fragmentation. Several pseudomolecular peaks were obtained by successive loss of counteranions.[5] Several complexes can be easily identified together.[6] Moreover, owing to the high sensitivity of this technique, the dilute solution used

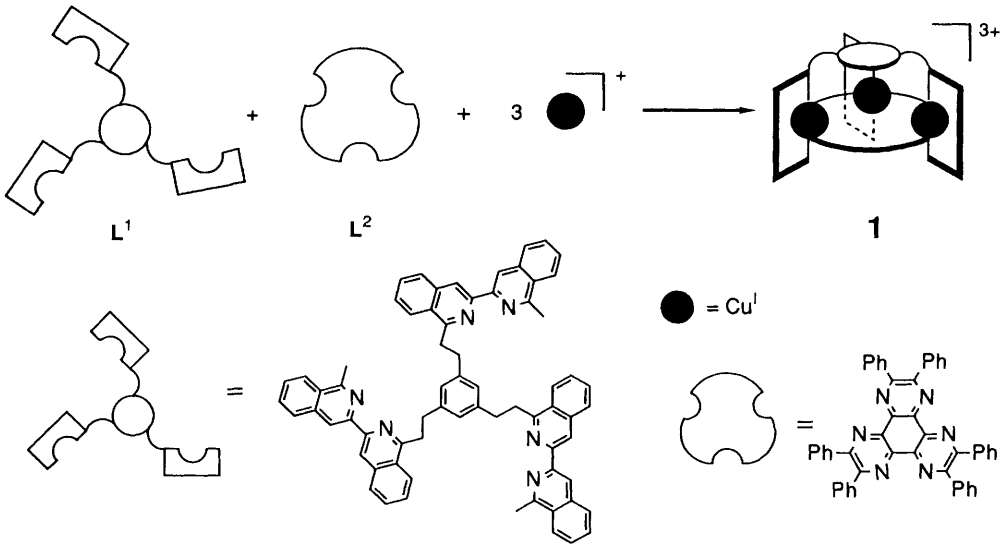

Fig. 1 Formation of the capped complex **1** $[Cu_3L^1L^2]^{3+}$ from L^1, L^2 and Cu^I

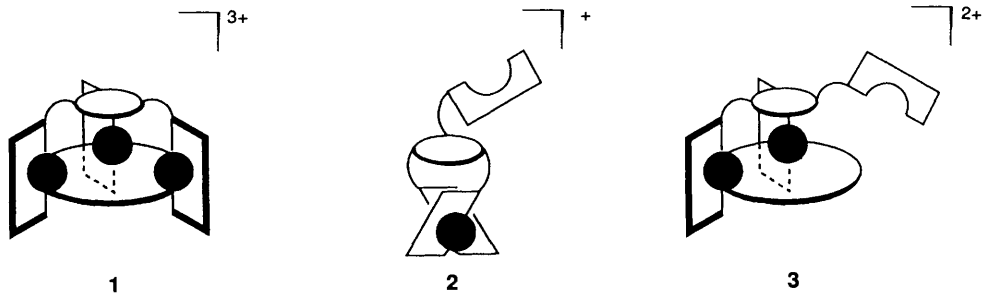

Fig. 2 Major species detected by ESMS in titration solutions of L^1, L^2 and Cu^I

for spectrophotometric titrations can be investigated directly, thus allowing a direct comparison of the data obtained by the two methods.

We describe here the use of electrospray mass spectrometry for the identification of the complexes **1–3** that appear in the course of the titration of ligands L^1 and L^2 with Cu^I (Figs. 1 and 2). The results are compared with the species distribution obtained by analysis of the spectrophotometric titration data.

An equimolar solution of the ligands L^1[7] and L^2[3,8] in $MeCN–CH_2Cl_2$ (1/1) was titrated spectrophotometrically with $CuPF_6$ in the same solvent.† The immediate colour change of the solution showed that the system rapidly reached thermodynamic equilibrium. The solution turned from yellow to brownish-green and finally to blue, indicating the presence of the yellow complex **2** (λ_{max} = 407 nm, ε = 9500 $dm^3\ mol^{-1}\ cm^{-1}$) and of the blue complex **1** (λ_{max} = 590 nm, ε = 13 800 $dm^3\ mol^{-1}\ cm^{-1}$). A simple chemical model was fed into the computer involving the formation of complexes **1** and **2** only.‡

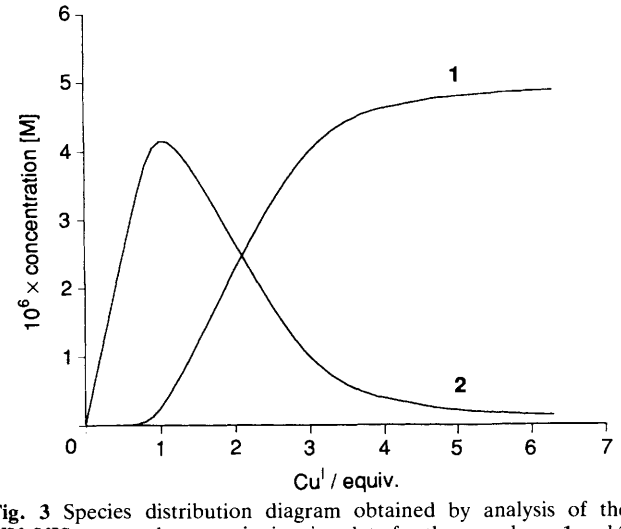

Fig. 3 Species distribution diagram obtained by analysis of the UV–VIS spectrophotometric titration data for the complexes **1** and **2** formed from ligands L^1, L^2 (5×10^{-6} mol dm^{-3} each) and $CuPF_6$ in $MeCN–CH_2Cl_2$ 1 : 1 at 20 °C

The formation constants for **1** and **2** were calculated and, based on these constants, a species distribution diagram was computed (Fig. 3). The distribution of **1** and **2** at 1.0, 2.5 and 6.0 equivalents of Cu^I is given in Table 1.

The titration is well described by exclusive formation of **2** up to addition of 1.0 equiv. Cu^I and subsequent conversion of **2** into **1** on addition of more Cu^I. Species like $[Cu_2L^1L^2]^{2+}$ **3** and

† CH_2Cl_2 and MeCN were freshly filtered over basic alumina (activity I) before use. The spectrophotometric titration was performed on an equimolar 5×10^{-6} mol dm^{-3} solution of L^1 and L^2 in $MeCN–CH_2Cl_2$ (1/1). $[Cu(MeCN)_4]PF_6$ was added as a 5×10^{-4} mol dm^{-3} solution in the same solvent. UV spectra were recorded on a Cary 3 UV–VIS spectrophotometer.

‡ The synthesis and characterisation of complex **1** will be described elsewhere.[7] Complex **1** is isolated in almost quantitative yield by three-component self-assembly of the tris-bisisoquinoline ligand L^1, hexaphenylhexaazatriphenylene L^2 and Cu^I ions (Fig. 2). **2** could be obtained from L^1 and Cu^I ions and was also characterized by FAB^+ (*m/z* = 1029.2).

Table 1 Comparison of relative species distribution obtained by ESMS and by UV–VIS spectrophotometry for the titration of solutions containing an equimolar mixture of L^1 and L^2 with $CuPF_6$

Cu^I/ equiv.	ESMS[a] 1	2	3	UV–VIS[b] 1	2	3
1.0	0.02	1	<0.05	0.06	1	0
2.5	1	0.50	0.17	1	0.53	0
6.0	1	0.04	0.02	1	0.03	0

[a] Relative ESMS peak intensities. [b] Relative abundance obtained by analysis of the spectrophotometric titration data.

$[CuL^1L^2]^+$ do not seem to be present in detectable amounts. Chemical models that include these compounds are rejected by the computer analysis and do not allow a calculation of association constants. This might indicate that the formation of **1** occurs with positive cooperativity.

Three samples corresponding to titration solutions after addition of 1.0, 2.5 and 6.0 equiv. $CuPF_6$ were prepared and analysed by ESMS.§ The spectra (Fig. 4) were reproducible with respect to relative peak intensities.

For the sample containing 1.0 equiv. Cu^I, the major peak corresponds to the doubly-charged ion (isotopical peaks separated by 0.5 *m/z* unit) of **2** (*m/z* = 516.1). The charge results from the loss of the counterion PF_6^- and from the protonation of the uncoordinated bisisoquinoline. A small peak was also observed for unprotonated **2** (*m/z* = 1031.0), which corroborated the mass measured for **2**. Other peaks of low intensity (<5%), but highly reproducible, were found at *m/z* = 323.2 ($[L^1 + 3H]^{3+}$), *m/z* = 346.1 ($[L^2 + 2H]^{2+}$), *m/z* = 595.5 ($[3 + H]^{3+}$), *m/z* = 691.5 ($[L^2 + H]^+$) and *m/z* = 861.2 ($[L^1L^2Cu + H]^{2+}$).

The solution containing 2.5 equiv. of Cu^I showed two intense peaks: the triply-charged ion **1** (isotopical peaks separated by 0.33 *m/z* unit; loss of all PF_6^-: *m/z* = 616.3) and the doubly charged ion of **2** (*m/z* = 516.0), still present. A small peak corresponding to protonated **3** (*m/z* = 595.5) as well as a series of very small (<5%) but very reproducible peaks were also visible in this spectrum. They can all be interpreted as combinations of L^1, L^2, protons and Cu^I: $[L^2 + 2H]^{2+}$ (*m/z* = 346.2), $[L^2 + H]^+$ (*m/z* = 691.5), $[L^1L^2_2Cu_3]^{3+}$ (*m/z* = 846.4), **3** (*m/z* = 891.5).

The spectrum of the solution containing 6.0 equiv. Cu^I was dominated by the fully-assembled complex **1** at *m/z* = 616.3, which was also observed at *m/z* = 996.7 with one PF_6-attached ($[L^1L^2Cu_3PF_6]^{2+}$). Very small peaks (< 5%) corresponding to **2** (*m/z* = 516.1) and **3** (*m/z* = 595.5) were also detected. The MS-MS[4] spectrum of the parent ion **1** (*m/z* = 616.2) showed only fragments at *m/z* = 546.9, corresponding to $[L^1Cu_2]^{2+}$ and at *m/z* = 753.2 corresponding to $[L^2Cu]^+$. This latter result confirms that some of the peaks observed in the ESMS spectra are not produced by MS fragmentation of **1**, but correspond indeed to species preexisting in the solution before MS analysis.

The intensity of the peaks corresponding in each ESMS spectrum to species **1**, **2** and **3** were used for tentative quantification. The results were compared with those obtained from the UV–VIS study (Table 1). It appeared that ESMS gave a clear picture of the different species formed in the solution during the titration. ESMS revealed the presence of a small amount of **3**, that was not detectable by the spectrophotometric investigations; however it cannot be ruled out that **3** is generated by the electrospray ionisation conditions. The relative amounts of **1** and **2** obtained from ESMS fitted closely with those deduced from the UV–VIS study. In all cases the ESMS response factor was the same for **1**, **2** and **3** probably because they were all three ionized by a similar process (loss of all PF_6^- counterions and protonation of the uncoordinated bisisoquinoline).

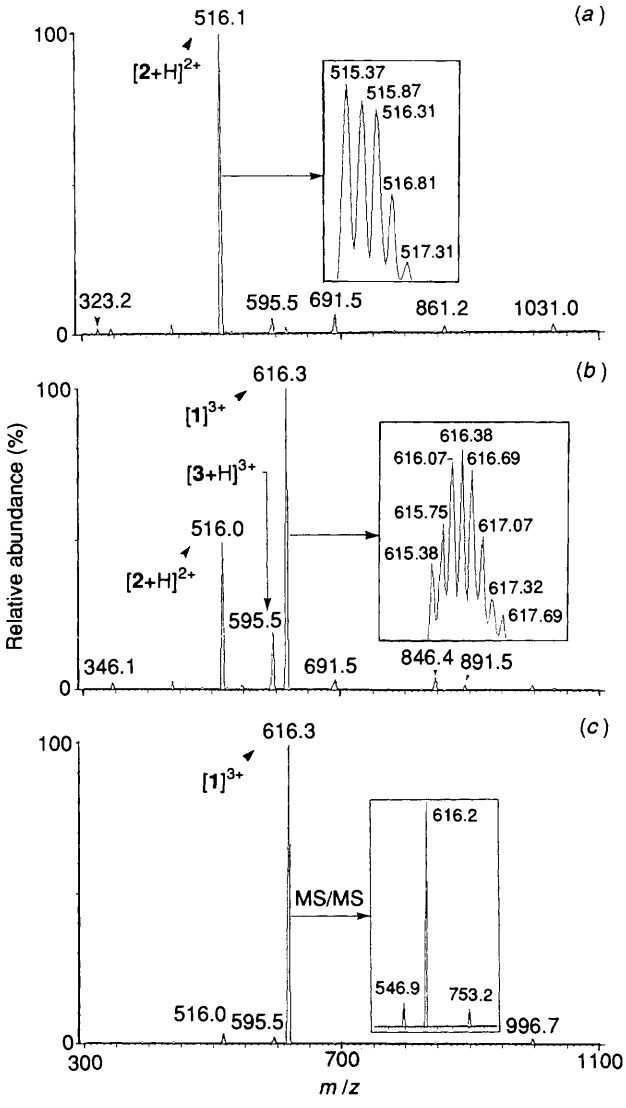

Fig. 4 ESMS spectra of the three titration solutions containing 1 equiv. L^1, 1 equiv. L^2 and either (*a*) 1.0, (*b*) 2.5 or 6.0 equiv $CuPF_6$

In conclusion the spontaneous formation of **1** by self-assembly has been unambiguously demonstrated by both methods, UV–VIS spectrophotometry and ESMS. ESMS appears to be a very valuable technique for the analysis of metal complex mixtures in highly diluted solutions, even if the complexes are in thermodynamic equilibrium. The general applicability of ESMS has obviously to be verified by investigation of various other systems. For the study described here, the mass spectra seem to really reflect a 'frozen' thermodynamic equilibrium state of the injected solution. However, new species may be generated or species distribution may be altered by ESMS conditions, especially at high cone voltage.

ESMS may become a very important and sensitive analytical tool for the study of multicomponent mixtures in the case

§ Electrospray mass spectra were obtained on a VG BioQ triple quadrupole with a mass to charge (*m/z*) range of 4000 (VGBioTech Ltd, Altrincham, UK). The electrospray interface was heated to 50 °C. The sampling cone voltage (Vc)[4] was at 30 V. This low voltage avoids any fragmentation processes; at Vc > 70 V, some fragmentations were detected. Titration solutions containing L^1, L^2 (5×10^{-6} mol dm^{-3} each) and 1.0, 2.5 and 6.0 equiv. $CuPF_6$, respectively, in $MeCN–CH_2Cl_2$ (1/1) were injected into the spectrometer with a syringe (10 μl). The flow rate was about 4 μl min^{-1}. Aqueous MeCN 1/1 was used as eluent.

of metal complexes as well as of other supramolecular systems, in particular when self-assembly is involved.

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