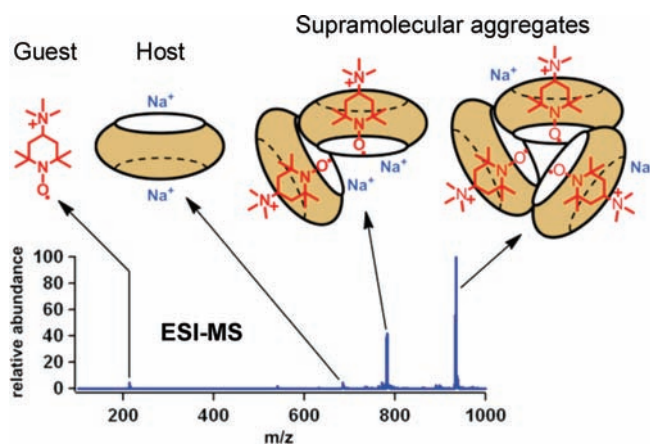


Aggregates of Cucurbituril Complexes in
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ABSTRACT



The self-organization of cucurbit[*n*]uril (*n* = 7 and 8, CBs) complexes was probed by electrospray mass spectrometry. The self-association of CB complexes is a general phenomenon but shows some dependence on the absence, presence, and type of included guest molecules.

Cucurbit[*n*]urils (CB_{*n*}, *n* = 5–10) are an emerging family of synthetic macrocyclic hosts that have the potential to encapsulate positively charged and neutral organic molecules (Scheme 1).¹ The CBs function as molecular containers forming 1:1 and 1:2 host–guest inclusion

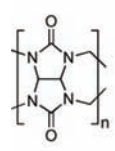
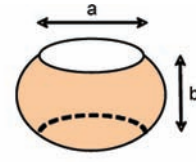
complexes (Scheme 1)¹ with association constants that are typically several orders of magnitude higher than those of cyclodextrins as hosts in aqueous solutions.² Several recent studies suggested the formation of CB6 (and CB7)

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Scheme 1. Structure and Dimensions (Atom to Atom Distance) of Cucurbit[7]uril (CB7) and of Cucurbit[8]uril (CB8)

			
$n = 7$ (CB7) $n = 8$ (CB8)			
	a (portal dia)	8.5 Å	10.3 Å
	b (height)	6.25 Å	6.25 Å
	MW (g/mol)	1162	1328

aggregates near the saturation concentrations and at low pH (0 to 2).³

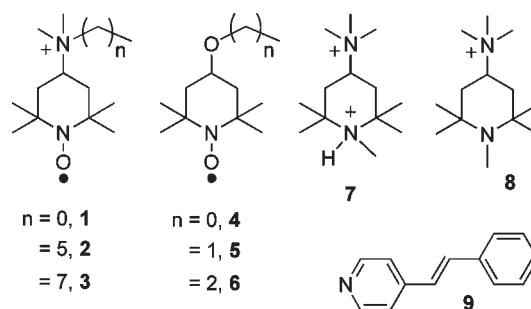
CB8 is a particularly interesting case. The supramolecules of nitroxides@CB8 associate as trimers ([nitroxide@CB8]₃) in aqueous solutions, as shown by the characteristic spin–spin coupled EPR signal.⁴ However, no such aggregation was detected for the corresponding CB7 complexes. The reasons for this difference are still not clear.

Electrospray ionization mass spectrometry (ESI-MS) has assumed an important role in investigations of host–guest complexes and other noncovalent systems.⁵ ESI-MS can transfer noncovalent complexes intact to the gas phase, allowing access to their intrinsic properties, i.e., without the influence of solvents. ESI-MS has been used to study complexes between CB_n and several ionic and neutral guests.^{4a,6–8} It provides valuable information regarding the nature and stoichiometry of the complexes,^{4a,6–8} their binding constants,⁷ and also their reactivity in the gas phase.⁸

In this work we demonstrate that ESI-MS is a powerful technique to directly observe and identify aggregates of CB_n complexes with several guests (Scheme 2). The results give new insights into the aggregation phenomenon.^{3,4}

We searched first for aggregates of CB7 and CB8 (CBs) in aqueous solutions containing H⁺ (HBr), Na⁺ (NaCl), and K⁺ (KCl). Both CBs are readily seen in the gas phase, mainly as double charged ions containing H⁺, Na⁺, or K⁺ (Figure 1 and S1–S3).⁹ A detailed investigation by tandem mass spectrometry (MSⁿ) revealed the formation of dimers and trimers of both CBs under a large diversity of solution chemistry and instrument conditions (Figures 1 and

Scheme 2. Structures of the Guests Examined in This Report



S1–S13).⁹ As an example, Figure 1 shows a mass spectrum for CB8.

Fragmentation of m/z 687 (MS²) leads to m/z 1351 (loss of Na⁺). Further fragmentation of m/z 1351 (MS³, 687 → 1351) leads to breaking of the CB ring (Figure S4).⁹ Ions with m/z 687 and 1351 were therefore assigned to [CB8 + 2•Na]²⁺ and to [CB8 + Na]⁺, respectively. Fragmentation of m/z 2016 from the full scan spectrum (Figures 1 and S5)⁹ (MS² 2016) gave m/z 1351 and 2681, a single charged CB8, [CB8 + Na]⁺, and a single charged dimer of CB8, [2•CB8 + Na]⁺, respectively. We therefore assign the ion at m/z 2016 to the trimer [3•CB8 + 2•Na]²⁺. Fragmentation of m/z 1351 from the full scan spectrum (MS² 1351) (Figure S6)⁹ shows the breaking of the CB8 ring together with the formation of m/z 2016, which then fragments (MS³, 1351 → 2016) into m/z 1351 and 2681. The ion at m/z 1351 in the full scan spectrum is a mixture of the single charged CB8, [CB8 + Na]⁺, whose fragmentation leads to ring breaking, and of the trimer [3•CB8 + 3•Na]³⁺, which, upon fragmentation, loses a Na⁺ ion and forms the trimer with m/z 2016. Trimers of CB8 can thus be seen as doubly and triply charged ions.

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(9) See Supporting Information for spectra.

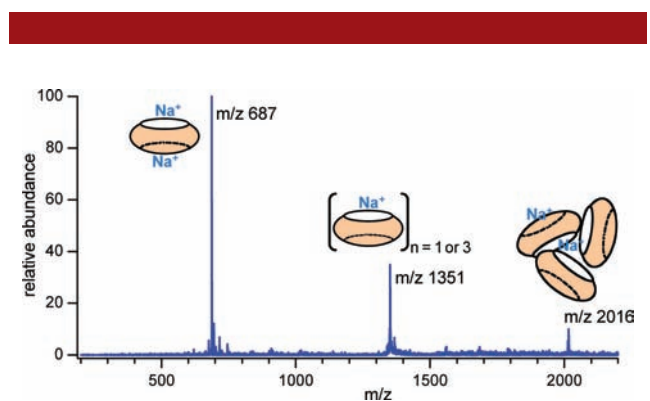


Figure 1. ESI-MS spectrum (full scan) of an aqueous solution of CB8 (25 μM) with NaCl (100 μM) (CE 300 V). m/z 687 [CB8 + 2•Na]²⁺; m/z 1351 [CB8 + Na]⁺ and [3•CB8 + 3•Na]³⁺; m/z 2016 [3•CB8 + 2•Na]²⁺. The illustration of the aggregates is only a schematic representation. Their actual configuration is not known.

The spectra shown so far were obtained under relatively high acceleration voltages (capillary exit, CE 300 V) in the medium pressure region of the mass spectrometer, conditions usually associated to source collisional induced dissociation.^{10,11} Under gentler conditions (CE 70 V) dimers of both CBs were also observed (Figures S2 and S3).⁹ Acceleration voltages CE > 150 V (Figure S1) induced dissociation of the dimers, indicating that these aggregates are less stable in the gas phase than trimers.

Similar spectral distributions were observed for concentrations from 10 to 100 μM of both CBs and using desolvation temperatures from 50 to 350 $^{\circ}\text{C}$. These results indicate that the trimers of CB8 are present in aqueous solution¹¹ and are desorbed intact into the gas phase. Aggregation seems to be a general phenomenon for CB8 and CB7.

The results discussed above were observed in the absence of guest molecules. The following discussion concerns how guest molecules (Scheme 2) included in CBs influence the aggregation. The mass spectrum of aqueous solutions of crystals of **1**@CB8 complexes under gentle desolvation conditions (CE 70 V) is shown in Figure 2. The strongest signal was assigned to trimers as discussed below. The low intensity signals at m/z 214 and 687 were assigned to the free guest (**1**) and free host ($[\text{CB8} + 2\text{Na}]^{2+}$), respectively.

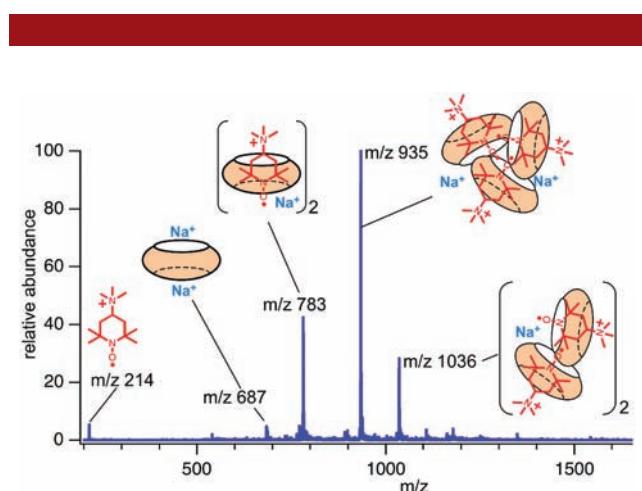


Figure 2. ESI-MS spectrum (full scan) of an aqueous solution of **1**@CB8 (50 μM) with NaCl (100 μM) (CE 70 V). Assignments: m/z 214 [**1**]⁺; 687 [CB8 + 2Na]²⁺; 783 [2•CB8 + 2•**1** + 2•Na]⁴⁺; 935 [3•CB8 + 3•**1** + 2•Na]⁵⁺; 1036 [4•CB8 + 4•**1** + 2Na]⁶⁺. The illustration of the dimer, trimer, and tetramer is only a schematic representation. Their actual configuration is not known.

Fragmentation of m/z 935 was used to assist with the peak assignment (Figure 3). In each fragmentation step a guest molecule (**1**) is lost (-214), giving at the end the ion of the CB8 trimer without guest molecules (m/z 2016). We assign the ion with m/z 935 to a trimer of **1**@CB8 complexes, $[3\bullet\text{CB8} + 3\bullet\mathbf{1} + 2\bullet\text{Na}]^{5+}$. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS)

studies confirmed that the peak at m/z 935 has a charge of 5+, because of the isotope pattern spacing of $\Delta m = 0.2$ amu (Figure S14).⁹ The ion at m/z 1036 (Figure 2) shows a fragmentation pattern consistent with a tetramer of the complex, $[4\bullet\text{CB8} + 4\bullet\mathbf{1} + 2\bullet\text{Na}]^{6+}$ (Figure S15).⁹ FT-ICR-MS spectra near m/z 1036 showed an isotope spacing of $\Delta m = 0.1665$ amu, which is consistent with an ion charge of 6+. The signal at m/z 783 (Figure 2) was assigned to a dimer $[2\bullet\text{CB8} + 2\bullet\mathbf{1} + 2\bullet\text{Na}]^{4+}$ based on consecutive fragmentation patterns (Figure S16)⁹ and on the FT-ICR-MS spectra ($\Delta m = 0.25$ amu).

The increase of CE to 300 V modifies dramatically the spectrum (Figures S17–S19).⁹ As a result, the intensity of the signals due to the free guest (m/z 214) and free host (m/z 687 and 1351) increased. Free complexes (m/z 783 and 1542) are present, but tetramers almost vanished. The trimers underwent fragmentation (loss of guest molecules) and gave rise to the structures observed after consecutive fragmentations of m/z 935 (m/z 1415 and 2016; Figure 3), indicating that in source collisional induced dissociation and fragmentation within the ion trap follow similar reaction channels. We conclude that the trimer structure composed of three CB8 molecules has special stability. Indeed, trimers were also observed in aqueous solutions with a 5:1 (host–guest) ratio, confirming the strong propensity for the formation of these aggregates (Figure S19).⁹ Unlike **1**@CB8 complexes, the MS of aqueous solutions of **1**@CB7 are dominated by monomers (1:1 complex) over a wide range of concentrations (5–500 μM) and acceleration conditions (CE 50–300 V). However, some aggregates are present (Figures S20–S22).⁹

Figure S20a,⁹ shows the formation of trimers (m/z 1033) and tetramers of CB7 (m/z 919), but their signals are much less intense than that observed for **1**@CB8 (Figure 2). The spectra at higher concentrations show that the preferred aggregate of **1**@CB7 is the tetramer (Figure S20b).⁹ Increasing the acceleration voltage (CE) induces collisional dissociation, but, unlike the case observed for **1**@CB8, the tetramer is much more stable in the gas phase (Figure S20c).⁹

To explore the generality of the CB aggregation we examined two more charged (**2** and **3**) and three neutral (**4**, **5**, and **6**) nitroxides, diamagnetic doubly (**7**) and single charged (**8**) guests, and also a noncharged diamagnetic guest (**9**) (Scheme 2). Trimers were observed for all nitroxides@CB8 (Figures S23–S26).⁹ The trimers of **4**, **5**, and **6**@CB8 are however much less stable in the gas phase than those of charged nitroxides, **1**–**3**. In fact, under collisional induced dissociation conditions (CE 300 V), only the empty trimer of CB8 is seen for these systems. As observed for **1**@CB7, the complexes of the nitroxides **2**–**6**@CB7 are much less prone to aggregate. Guest **7** forms 1:1 host–guest complexes with CB7 and CB8, but no trimers were detected (Figure S27),⁹ although complexes of **8** do form trimers with CB8 as observed by MS (Figure S28).⁹ 4-Stilbazole (**9**) is known to form 1:1 and 1:2 host–guest complexes with CB7 and CB8, respectively.¹²

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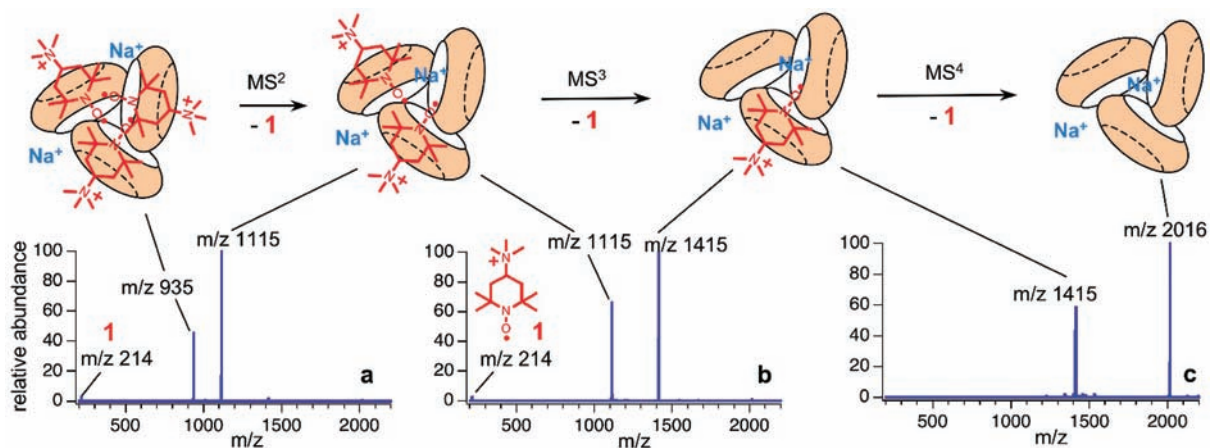


Figure 3. Consecutive fragmentations of m/z 935: (a) MS^2 935; (b) MS^3 935 \rightarrow 1115; (c) MS^4 935 \rightarrow 1115 \rightarrow 1415. Assignments: m/z 935 $[3\bullet\text{CB8} + 3\bullet\mathbf{1} + 2\bullet\text{Na}]^{5+}$; m/z 1115 $[3\bullet\text{CB8} + 2\bullet\mathbf{1} + 2\bullet\text{Na}]^{4+}$; m/z 1415 $[3\bullet\text{CB8} + \mathbf{1} + 2\bullet\text{Na}]^{3+}$; m/z 2016 $[3\bullet\text{CB8} + 2\bullet\text{Na}]^{2+}$. The illustration of the trimer is only a schematic representation. The actual configuration of the trimer is not known.

Mass spectra showed the formation of trimers for both $9@CB7$ and $9@CB8$ 1:1 complexes (Figures S29–S32).⁹

The above mass spectral studies are in full agreement with previously reported EPR studies of guest@host complexes for nitroxides with CB8 and CB7 in aqueous solutions.^{4a} Previous EPR studies in aqueous solutions showed aggregate formation of three units of nitroxides@CB8.⁴ In the current study, we observed these trimers directly by ESI-MS (Figure 2) and FT-ICR-MS (Figure S14).⁹ The above MS studies in the gas phase qualitatively reflect solution-phase properties quite well.

ESI-MS revealed that aggregation of CBs is a general phenomenon forming dimers, trimers, and tetramers. All studied nitroxides (**1**–**6**) form 1:1 host–guest complexes with CBs, leaving one of the CB portals free for interaction and thus aggregation. However, with the dicationic guest **7**, where both CB portals are occupied, no trimers of $7@CBs$ were observed by MS. The formation of trimers for $8@CB8$ but not for $7@CB8$ suggests the need for a free CB portal for aggregation. We are currently pursuing the

generality of aggregation of complexes using other organic guests, larger CBs, and computational methods.

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Supporting Information Available. Sample preparation procedures and additional MS data. This material is available free of charge via the Internet at <http://pubs.acs.org>.