Relative Binding Affinities of Molecular Capsules Investigated by ESI-Mass Spectrometry

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Abstract: ESI-MS(/MS) has been used as a method which allows the fast, unambiguous and sensitive simultaneous detection and relative stability approximation of supramolecular assemblies in mixtures. In spite of the obvious fundamental differences between solution and gas phase, ESI-MS in the case of self-assembled molecular capsules has been shown to produce very similar results to single binding experiments monitored by NMR titrations as well as conformational searches performed by Monte-Carlo simulations. MS/MS experiments reveal the same relative order of gas phase stabilities as previously found in solution. Moreover, proton transfer reactions which lead to new molecular capsules, are not detectable in the time-averaged NMR spectrum. However, the newly produced species are found in the complex mixtures by ESI-MS and can be conveniently characterized by subsequent MS/MS experiments: in a collision-induced dissociation the single half-spheres are easily discovered and structurally assigned. Thus, ESI-MS has worked as a valuable tool for the rapid screening of complex supramolecular mixtures and in combination with MS/MS experiments elucidated both the path of unexpected side reactions as well as the thermodynamic gas-phase stabilities of all components in the mixture.

Keywords: binding affinities · mass spectrometry · molecular capsules · self-assembly · supramolecular chemistry

Introduction

The fast determination of relative stability constants of self-assembled architectures in polar solutions is problematic. In dynamically fast processes NMR spectra of mixtures show only one averaged set of signals and precludes the analysis of pairwise interactions.[1] The rapid development and expansion of application fields for electrospray ionization mass spectrometry (ESI-MS) especially during the last decade now offers soft ionization methods suitable for the detection of weakly bound non-covalent complexes.[2] Important advantages of MS over NMR are *speed*, *specificity* and *sensitivity*.[3]

However, since during the ionization process the investigated species is transferred from solution into the gas phase, it has been a question of much debate if ESI-MS really reflects the situation in solution. In this context, Daniel et al. distinguish between solution-phase methods with MS detection (ESI-MS) and pure gas-phase methods where the complex is dissociated in the mass spectrometer (ESI-MS/MS).[4] They stated that MS-based methods used for monitoring solution equilibria generally agree well with known solution-phase thermodynamic values. Though, a critical parameter, which has to be considered when applying mass spectrometry for semiquantitative analysis is the electrospray response factor, which is influenced by various parameters including the solvation energy, the ion size, the charge state, the solvent viscosity, and ionization conditions.[5] Thus, ion yields and intensities of mass signals may vary between different substances or non-covalently bound complexes even when measuring conditions are kept constant. For example, the "best fit" concept is widely accepted to explain the binding affinities between crown-ethers and alkali ions in solution. However, it was questioned by diverging selectivities observed in ESI-MS experiments. Thus, by far the most prominent signal in the electrospray mass spectrum of a LiCl solution containing [12]crown-4, [15]crown-5, and [18]crown-6 corresponds to the Li^+([18]crown-6) complex, and not, as

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author: Additional MS data (spectra and tables), molecular modeling data, RRK calculations (PDF). For all synthetic details about the syntheses of the calix[4]arene half-spheres, NMR titrations and Job plots, see Supporting Information: R. Zadmard, T. Schrader, T. Grawe, A. Kraft, *Org. Lett.* **2002**, *4*, 1687–1690.

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expected, to the Li^+([12]crown-4) adduct.[6] Similarly, for the other alkali ions, the [18]crown-6 complex was always observed to be the most abundant. These unexpected results were clarified when two reports on the importance of solvation energies for the electrospray response factors were published.[7,8] In light of these discrepancies, ESI-MS cannot be regarded as a useful tool for the determination of solution phase binding selectivities, unless these critical effects are kept constant or are thoroughly considered in the evaluation procedure.

When switching to gas-phase experiments (ESI-MS/MS), the electrospray response factors become irrelevant. However, noncovalent interactions may change dramatically. On one hand, any interaction in noncovalent complexes which competes with the solvent (e.g., a hydrogen bond and electrostatic attraction) is greatly strengthened in the gas-phase.[4,9] On the other hand, interactions such as hydrophobic forces are weakened.[10] Therefore, with a few exceptions, no agreement exists between solution-phase and gas-phase binding energies.[4] One such exception are some complexes relying only on electrostatic interactions. Moreover, in the condensed phase dissociation and re-association processes are often in a rapid equilibrium. By contrast, in the gas-phase two or more partners are irreversibly separated from each other upon decomposition.[9]

Biomolecular investigations of protein–ligand complexes have demonstrated the above-mentioned marked difference between ESI-MS measurements from solution and MS/MS-experiments in the gas phase.[4,9,11] For example, Nesatyy recently investigated a noncovalent enzyme/inhibitor complex and reported "a reasonable agreement in relative binding order determined by ESI-MS with the known solution values", although there were large differences of 2–8 orders of magnitude between the absolute numbers. By contrast, MS/MS experiments even revealed a relative gas-phase binding order which did not agree to that in solution, suggesting that the complex-conformation was not preserved in the gas-phase.[11a]

Herein, we describe ESI-MS(/MS) as a suitable and fast method for the semiquantitative analysis of mixtures of molecular capsules[12] and show that the data obtained are in good agreement with NMR experiments as well as with theoretical calculations.

Results and Discussion

NMR spectroscopy: In extension of the well-established concept of hydrogen-bonded molecular capsules based on self-complementary calix[4]arenes,[13] we recently presented the self-assembly of ionic capsules from complementary oppositely charged half-spheres (Figure 1).[14] These are highly stable in methanol and even in aqueous solutions. Extensive investigations by various NMR techniques furnished evidence for the specific formation of capsules: Job plots produced discrete 1:1 stoichiometries; complexation-induced shifts were restricted to the ion-pairing region of the calixarenes. All NMR signals remained sharp at all half-sphere ratios. Very high binding constants were calculated from the 1:1 binding isotherms in highly competitive solvents such as methanol and water ($K_a = 10^4$–$10^5\,M^{-1}$).[15] Monocations strongly included in the anionic half-spheres were completely displaced by equimolar amounts of the cationic half-spheres. The specific ionic interactions were shown to be dependent on pK_a values,[16] cation/anion distances, solvation entropies and enthalpies (Table 1, column 2 (K_a [M^{-1}]).[14] In the cases of unfavorable pK_a differences a complete proton transfer led to solely hydrogen-bonded aggregates with lower association constants ($K_a \sim 10^3\,M^{-1}$). The best binders were benzylic phosphonate and ammonium half-spheres, followed by the rigid aromatic phosphonates and anilinium calixarenes. The worst combinations were found with carboxylates and pyrazolium groups. As Table 1 demonstrates, the rigid tetraphosphonate half-sphere **1** produces the most stable cage-like complexes, especially with the most basic tetraammonium half-sphere **3**. This pair is closely followed by the combination of two rigid half-spheres **1+4** (high preorganization) or two benzylic half-spheres **2+3** (high inter-

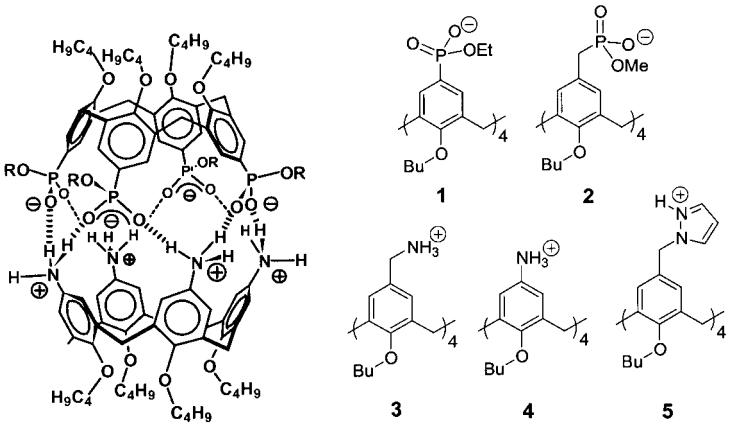

Figure 1. Left: Optimized structure of a representative rigid molecular capsule between the calix[4]arene tetraphosphonate **1** and the related calix[4]arene tetraanilinium half-sphere **4**. Right: Capsule components **1**–**5**.

Table 1. Capsule stabilities as determined earlier by NMR titrations.[14]

Capsule	K_a [M^{-1}][a]
1+3	$(7 \pm 2.5) \times 10^5$
1+4	$(1 \pm 0.5) \times 10^4$[b]
1+5	$(2 \pm 0.6) \times 10^3$
2+3	$(4 \pm 0.4) \times 10^5$
2+4	$(7 \pm 1.0) \times 10^3$
2+5	$(4 \pm 0.1) \times 10^3$

[a] Determined by NMR titrations in methanol.[14] [b] H_2O/methanol 1:4.

nal mobility), indicating entropy terms as the dominating factors for strong affinity. Capsule **1+4** was only soluble in a 1:4 water/methanol mixture. For purely electrostatic interactions, K_a values are usually lowered ~50-fold in this solvent mixture. Thus, both complexes (**1+3** and **1+4**) can be regarded as equally stable.

Purely hydrogen-bonded capsules such as **1+5** and **2+5** are naturally much weaker in competitive highly polar solvents. Timmerman et al. recently reported about the formation of similar capsules with sulfonates and amidinium headgroups, a process which is mainly entropy-driven as determined by microcalorimetry.[17]

ESI mass spectrometry: ESI investigations on Rebek's hydrogen-bonded molecular capsules reflect the same preference for the formation of heterodimers as in solution and the same stability order of inclusion complexes between various half-spheres and one specific cationic guest, although no quantitative direct comparison was made.[18] In initial separate ESI-MS experiments, our ionic capsules, which are stable in methanol and water, gave molecular ion peaks for the 1:1 aggregates, but none for higher ones; this indicates again a specific interaction between the two half-spheres. The highest ionization efficiencies were obtained in the positive ion mode. Usually singly protonated, lithiated or sodiated M^+ peaks of the whole capsules were obtained, whereas the molecular ion peaks of the free half-spheres, which are highly charged in solution, were also only singly charged. Obviously, they avoid the mutual repulsion of four adjacent cations or anions on the upper rim without any solvent stabilization. Contrary to the ionic capsules, the weaker hydrogen-bonded ones involving the pyrazole half-sphere **5**, could not be detected by ESI-MS.

In order to check the suitability of mass spectrometry for determining relative binding affinities in ionic capsule mixtures, equimolar 1:1:1 mixtures (two cations **3** and **4**, one phosphonate **1** *or* **2**) were applied to ESI-MS. Both ionic capsules were observed as well as both cationic half-spheres (Figure 2). It has to be emphasized that the relative intensities for all molecular capsules in the ESI spectra closely parallels their relative free binding energies in methanol solution, which have been determined by NMR titration on the isolated capsules (Table 2). This is true for both mixtures—the one with the aromatic and the one with the benzylic tetraphosphonate. Moreover, a systematic increase or decrease of the phosphonate content in the parent solution did not significantly alter the relative capsule peak intensities (data not shown).

The situation changed when we moved to equimolar 1:1:1:1 (three cations, one phosphonate) mixtures. In the four component mixtures both ionic capsules were seen, but not the hydrogen-bonded third one. Again, all the three cationic half-spheres showed strong molecular ion peaks also in the mixture. However, three new significant molecular ion peaks occurred in both series, which could not be attributed to any of the expected molecular capsules. In addition, whereas the relative intensities of both ionic capsules remained the same in the 1:1:1:1 mixture with the benzylic tetraphosphonate (~5:1), their relative order was varying from one experiment to the next in the 1:1:1:1 mixture with the aromatic one (from 5:4 to 1:3).

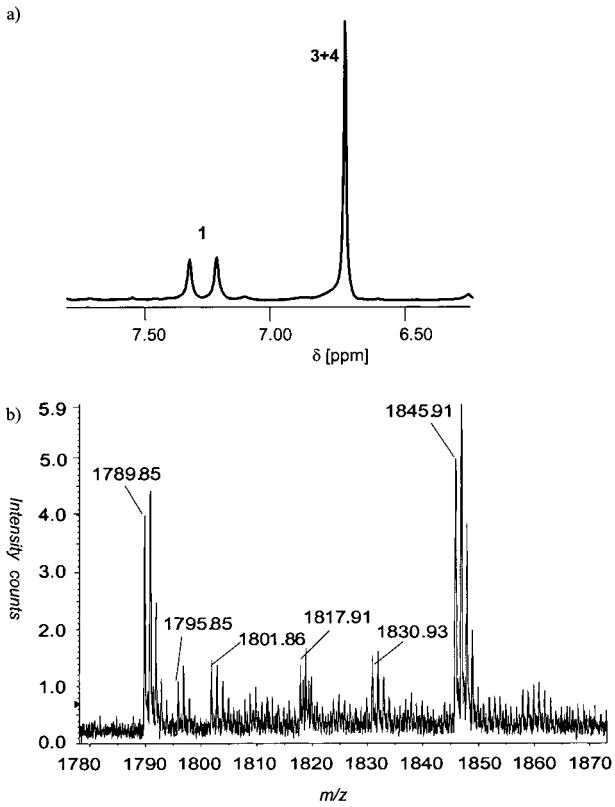

Figure 2. Comparison of the NMR and ESI-MS spectrum of the 1:1:1 mixture between **1**, **3** and **4** (expansion of the interesting regions). a) Note the simple averaged NMR signal of tetraphosphonate **1** (d, 7.3 ppm) for both capsules and the shift-isochronous signals for all species with **3** and **4** (s, 6.7 ppm). b) *m/z* 1789.85: [**1+4+**H]$^+$, 1795.85 and 1801.86: H$^+$/Li$^+$ exchange. *m/z* 1817.91: capsule heterodimer ([**1+4+**H][**1+3+**H])$^{2+}$. *m/z* 1845.91: [**1+3+**H]$^+$. The data shown represent an averaged mass spectrum over the time.

Table 2. Relative capsule stabilities as determined by two ESI-MS competition experiments from methanol (**1+3**, **4**, **5** and **2+3**, **4**, **5**).

Capsule	m/z_{calcd}	I_{ESI}[a]
1+3	1845.94	~1
1+4	1789.88	~1
1+5	2049.98	n.d.[b]
2+3	1845.94	4.8
2+4	1789.88	1.0
2+5	2049.98	n.d.[b]

[a] Relative intensities of the capsule (M^+ and M^{2+}) peaks in the 1:1:1 mixtures (two cations, one phosphonate); data represent two competition experiments, one with phosphonate **1**, the other one with **2**; errors for the ESI-MS intensities were estimated at $< \pm 13\%$. [b] Both phosphonium half-spheres containing capsules (**1+5** and **2+5**) were not detected by ESI-MS (proton transfer reaction).

At this point, MS/MS experiments proved to be a key instrument for the structural assignment of the new peaks, which in turn led to the discovery of a secondary reaction pathway, which had already occurred in solution. This technique, which will be described in more detail in the next paragraph, allowed isolation of the new singly positively

charged species and observation of their fragmentation patterns. To our surprise, the three new peaks represented 1:1 assemblies of all cationic half-spheres with the neutral tetrapyrazole half-sphere. Obviously, due to unfavorable pK_a relations in methanol, the pyrazolium half-sphere was deprotonated by the tetraphosphonate leading to a neutral tetrapyrazole. Its lone electron pair on the pyrazole nitrogen was now capable of forming strong ionic hydrogen bonds to the NH^+ groups in the ammonium-based half-spheres and also in its cationic pyrazolium counterpart (Figure 3).[19]

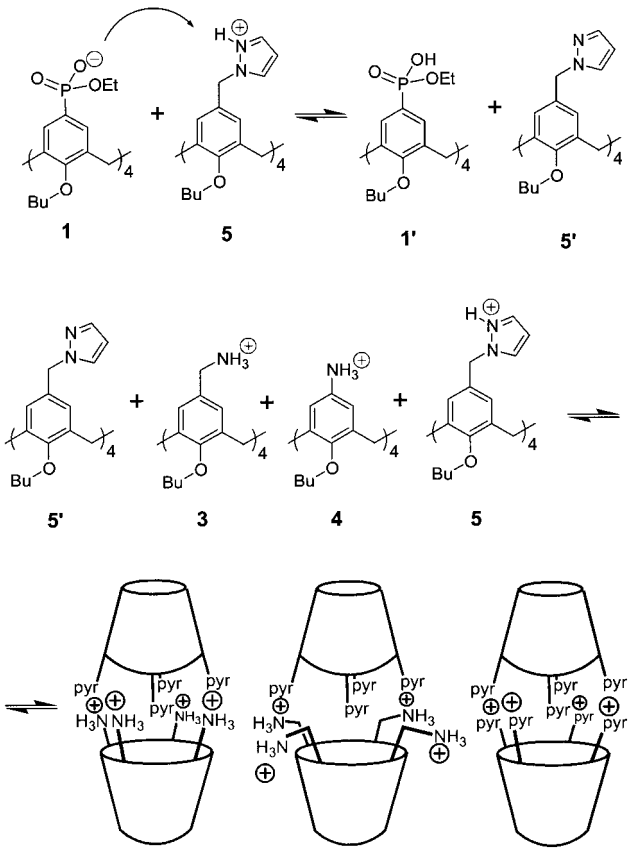

Figure 3. Unexpected side reaction of the pyrazolium calixarene half-sphere with the tetraphosphonate leading to three new molecular capsules in methanol solution. Loss of three protons explains the observed molecular ion peaks in the ESI-MS of the singly positively charged 1:1 assemblies in the four component mixtures.

Similar to the free cationic half-spheres, three protons were obviously stripped off the tetracationic complexes during the electrospray ionization process leading to singly positively charged assemblies in the gas phase. It is interesting that only the mixtures with the aromatic tetraphosphonate produced high amounts of these unwanted side products; this observation points to a higher pK_a value for those aromatic phosphonate anions over the benzylic derivatives.

MS/MS: Finally, to address the question of pure gas-phase stabilities of the molecular capsules, ESI-MS/MS experiments were carried out on various 1:1 mixtures of positive and negative half spheres.[11] In these experiments, the singly positively-charged capsule ion peaks were separated by their masses and accelerated into the collision cell of the mass spectrometer. In a series of experiments, this collision energy was varied and the relative amounts of intact capsules compared to the half-spheres and fragments thereof were estimated by subsequent TOF fragment analysis. The ESI-MS/MS results are illustrated exemplarily for capsule **1+3** in Figure 4. Based on the MS/MS data, dissociation curves of the ionic capsules were calculated, which are presented in Figure 5.

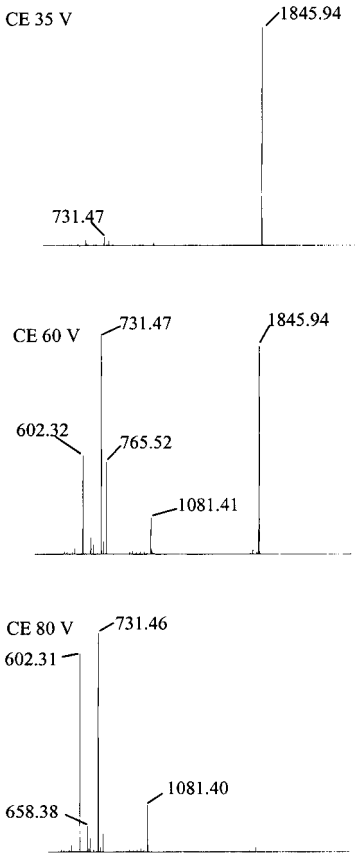

Figure 4. MS/MS experiment of capsule **1+3**. The ions with a *m*/*z* of 1846 were selected by mass (quadrupole **1** was fixed), and set to low resolution ensuring that all the isotopes of the capsule will be transmitted. Then they were accelerated into the collision cell (quadrupole **2** with nitrogen molecules) with varying collision energies and the resulting ions were analyzed in the TOF section of the instrument. With an increasing collision energy, the capsule peak decreases and the peaks corresponding to the half-spheres and fragments of them increase. Under experimental conditions applied (compare parameter settings), at a collision energy setting of 80 nearly all of the capsule amount is dissociated. Half-spheres: *m*/*z* 1081: $[\mathbf{1}+5H]^+$, 765: $[\mathbf{3}-3H]^+$. Fragment peaks: *m*/*z* 731: $[\mathbf{3}-3H-2NH_3]^+$, 602: $[\mathbf{3}-3H-3NH_3-2C_4H_8]^+$.

The CE_{50} values shown in Table 3 represent collision energies necessary for 50% capsule dissociation and therefore describe the relative order of their gas-phase stabilities. Intriguingly, they are again in the same relative order as the solution phase stabilities obtained from NMR and confirmed by ESI-MS experiments. However, now the MS experiments are independent of ionization efficiencies. With increasing collision energy, fragment ion peaks of both half-spheres appear, although most of the capsules are still

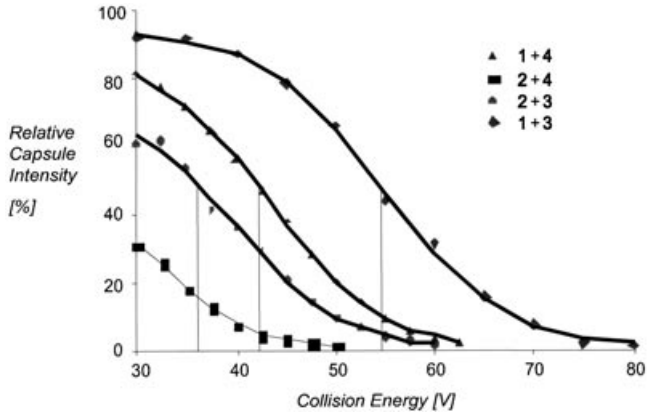

Figure 5. Overview of all collision-induced dissociation curves of the ionic capsules. Relative capsule intensity $= I_{capsule}/(I_{capsule} + I_{half\text{-}spheres} + \Sigma I_{fragments}) \times 100$ [%]; that is, the pure unfragmented capsule would furnish a relative peak intensity of 100%.

Table 3. Capsule stabilities in the gas phase as determined by MS/MS experiments.

Capsule	m/z_{calcd}	CE_{50}[a]
1+3	1845.94	54
1+4	1789.88	43
1+5	2049.98	n.d.[b]
2+3	1845.94	37
2+4	1789.88	<30
2+5	2049.98	n.d.[b]

[a] Threshold values for 50% capsule dissociation. [b] Both phosphonium half-spheres containing capsules (**1+5** and **2+5**) were not detected by ESI-MS (proton transfer reaction).

intact. These observations demonstrate again the high thermodynamic stability of the 1:1 assemblies.

Another factor should also be taken into account, that is, the rates of unimolecular dissociation, which may vary between capsules of a different number of internal degrees of freedom. Especially in the case of capsules **1+3** and **1+4** the higher CE_{50} value of the former (54 V vs 43 V) might simply reflect its extra methylene groups in the cationic benzylic half-sphere. In order to examine if kinetic shifts may significantly influence our varying CAD results, we performed Rice–Ramsperger–Kassel calculations (RRK). Capsule **1+3** has 273 atoms, while capsule **1+4** has only 261 atoms. This gives 813 versus 777 vibrational degrees of freedom with a difference of 36, so that the ratio of $k_{1+3}:k_{1+4} = [E-E_0/E]^{36}$. We conclude, that indeed, the potential difference in rates of unimolecular dissociation may be substantial. This is especially true, if E approaches E_0, so that the CE_{50} values may in part be determined by kinetic shifts. However, although we cannot exclude significant contributions of varying internal degrees of freedom, there are also results indicating that this is not the case: the CE_{50} value for **3+2** is lower than that for **1+4**, although the former (doubly benzylic half-spheres) has much more internal degrees of freedom than the latter.[20] In addition, for a direct comparison of CE_{50} values the collisional-to-internal energy deposition functions for both complexes must be equal; we assume that due to the similar structure this is the case for the complexes discussed here.

We also checked the relative stabilities of the new pyrazole-based 1:1 assemblies with MS/MS experiments. The calculated dissociation curves are shown in Figure 6. Interest-

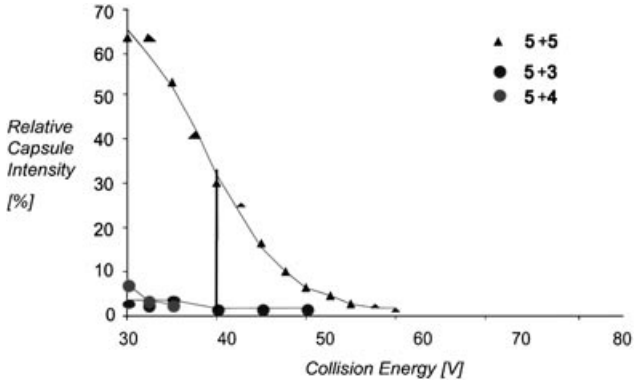

Figure 6. Overview of all collision-induced dissociation curves for the assemblies with the free tetrapyrazole half-sphere. For a definition of "relative capsule intensity" see caption Figure 5.

ingly enough, the pyrazolium/pyrazole assembly turned out to be the most stable one with a CE_{50} value of 40; the other monocationic assemblies are much weaker ($CE_{50} < 30$). Modeling experiments reach intriguing complex geometries for the assumed tetracationic assemblies in polar solution. Unfortunately, a control experiment of the free tetrapyrazole with the tetraanilinium calixarene did not produce any chemical shift changes during an NMR titration. However, the same mixture produced a clean molecular ion peak for the 1:1 assembly in the ESI spectrum. We assume that all three new molecular capsules with the neutral pyrazole half-sphere were already formed in solution. Although in water the pK_a difference between pyrazole (2.5) and phosphonate (1.8) is still in favor of the ion pair, this may change when shifting to methanol. Unfortunately, the time-averaged NMR spectrum does not allow a direct observation of this discrete species in solution, since the chemical shifts of the neutral and protonated pyrazole halfsphere are almost identical. The absence of any chemical shift changes during the NMR titration of **4** with **5′** could either be a consequence of the capsule's low thermodynamic stability, as demonstrated by MS/MS, or indicate its "forced" formation in a shrinking droplet. However, the rising pH during the ESI process cannot be the source of these new capsules, because it would prevent the initial protonation of the pyrazolium half-sphere.

Molecular modeling: The thermodynamic stabilities of the capsules in the gas phase were additionally calculated by force-field methods using Monte-Carlo simulations or molecular dynamics calculations.[21] The capsule structures were characterized by the total difference in enthalpy calculated between the capsule and its single components. Table 4 shows these results for all possible combinations. Again, the pyrazolium-phosphonate capsules are calculated to be much weaker than the respective ammonium-based ones. In addition, the relative stabilities in each series determined by

Table 4. Capsule stabilities as determined by theoretical calculations.

Capsule	ΔH [kJ mol^{-1}][a]
1+3	−860
1+4	−830
1+5	−80
2+3	−810
2+4	−790
2+5	−30

[a] Calculated gas phase stabilities (MacroModel 7.0, OPLS-AA, 1000 steps).

NMR and ESI are roughly reflected in the calculations. Only the considerable difference in stabilities between capsules **2+3** and **2+4** found by NMR and ESI-MS is levelled out in the minimization. This minor discrepancy may be due to the neglection of entropy contributions in the calculation.

Conclusion

The above-described experiments demonstrate the elegance of ESI-MS and ESI-MS/MS as opposed to NMR titrations for the determination of the relative stabilities of the described self-assembled architectures in polar solutions. When investigating mixtures, NMR was found to be totally inapplicable, since it gave only one averaged complex signal, while ESI-MS gave concise spectra for each complex species (Figure 2). Most interestingly, the results obtained by ESI-MS were in the same relative order as previously judged by time-consuming NMR experiments. Complexes relying mainly on electrostatic attraction obviously show the same relative stabilities in solution (NMR) and gas phase (ESI-MS), if solvent effects are comparable for every species. In our case, all the capsules are formed from very similar calixarene-based building blocks, each adorned with four small cationic or anionic groups; this design probably levels out all solvation enthalpy and entropy effects. In such a scenario, ESI-MS as well as MS/MS stabilities become comparable to solution stabilities.

Care has to be taken when generalizing these results, because the solution-phase (NMR) and gas-phase (ESI-MS/MS) binding affinities of the above-described self-assembling molecular capsules were in the same relative orders. If supramolecular systems are investigated where this is not the case, the results from NMR and ESI-MS experiments may be different. In our case, however, ESI-MS was proven to be a superior method for the fast, sensitive and selective screening of complex mixtures of self-assembled non-covalent architectures. Moreover, ESI-MS/MS experiments were successfully applied to determine their gas-phase stabilities and new capsule species were discovered.

Experimental Section

All MS and MS/MS spectra were recorded with a Qstar ESI-q-TOF mass spectrometer (Applied Biosystems, Germany). The standard electrospray ion (ESI) source supplied with the instrument was used to generate the ions. Samples with concentrations for each component ranging from 1 μM to 1 mM were injected using a constant flow (50 μL min^{-1}) of methanol, which was either supplied by the machines Harvard Syringe Pump or alternatively by a quarternary Agilent 1100 HPLC pump (Agilent, Germany). Values of 35 for the nebulizer gas and 25 for curtain gas (both nitrogen) as well as an ion spray voltage of 5400 V were set as ion source parameters for both, ESI-MS and ESI-MS/MS experiments. The TOF mass range was 250–2500 for all experiments. The mass accuracy of the QStar is specified to be better than 25 ppm with external calibration.

ESI-MS experiments: For ESI-MS experiments in the positive mode the following settings were applied: declustering potential (DP1) 100, focusing potential (FP) 320 and declustering potential two (DP2) 25. As CAD (collision activated dissociation) gas nitrogen was used with the standard setting of 3. ESI-MS experiments were done with all 1:1 mixtures, with all 1:1:1 mixtures as well as with both 1:1:1:1 mixtures. The relative amounts of capsules in the mixtures were quantified by peak height, implying that the ionization efficiencies of the different capsules, which are structurally similar to each other, will be in the same order of magnitude. The mixture experiments, which were used for the semi-quantitative analysis of capsule stabilities in solution, were at least repeated three times with separately and freshly prepared solutions and each sample was injected twice. Additional control experiments, where the phosphonate amount was systematically increased (1:1:1:2 and 1:1:1:3 as well as 1:1:2 mixtures) showed an increase of capsule-peak intensities, but no significant influence on the relative capsule intensities compared to each other. In contrast, an increase of the amount of only one positive half-sphere in the mixtures lead to an increase of the corresponding capsule signals compared to the others. Therefore, for semi-quantitative analysis it is important to have exactly the same concentrations of the positive half-spheres. Especially in the more complex 1:1:1:1 mixtures sample preparation seems to be critical, with respect to exact molar ratios. In contrast to the mixtures' composition, the results gained with the same samples when injected again were almost identical, indicating that the mass spectrometrical method works very reliable.

ESI-MS/MS experiments (gas-phase experiments): The applied parameter settings for the gas-phase experiments (ESI-MS/MS) were slightly different compared to the ESI-MS experiments. Here, the DP1 setting was 85, the FP setting 230, and the CAD gas setting 9, while the collision energy was varied from 30 to 80.

In contrast to ESI-MS experiments, the ionization efficiencies of the different capsules do not have an effect on this kind of experiment, because only one distinct kind of capsule is selected by its m/z, accelerated into the collision cell and the corresponding half-spheres or fragments of them are analyzed in the TOF-section of the instrument. Following, the relative amount of intact capsule was calculated by comparing its intensity (peak height) with that of the half-spheres and fragments of them. Thereby, the total amount of capsule molecules accelerated into the collision cell does only have an effect on the absolute, not on the relative signal intensities of capsule and half-spheres. To compare the gas-phase stabilities of the different capsules, CE_{50} values were estimated which represent the collision energy needed to dissociate half of the capsules amount in the gas-phase. MS/MS experiments were done with all capsules (**1+3**, **1+4**, **2+3**, and **2+4** as well as the free pyrazole calixarene with **2**, **3** and **4**) which had given a stable capsule peak in previously done ESI-MS experiments. Thereby, each experiment was repeated separately at least two times. The margin of error was determined to be less than 5%.

Acknowledgments

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[20] In fact, the argument based on RRK calculations would seem to grossly exaggerate the possible differences in kinetic shifts as the number of effective oscillators that should be used in the RRK expression is probably less than half of the number of vibrational modes. If we assume, that at CE_{50} the collisional energy E reaches about one half of the binding energy E_0 needed for the total dissociation of the capsules, the ratio in k converges to 1.
[21] MacroModel 7.0, Schrödinger Inc. 2000, Force-field: OPLS-AA, water, Monte-Carlo: 1000 steps.

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