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The Underestimated Role of Counter Ions in Electrostatic Self-Assembly: [1+1] Cavitand-Calix[4]arene Capsules Based on Azinium–Sulfonate Interactions

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The apparent K_a values of the formation of [1+1] cavitand-calix[4]arene capsules, based on azinium–sulfonate electrostatic interactions, obtained by direct titration experiments are concentration dependent due to the influence of the interaction of the charged capsule components with their initial counterions. ^{1}H NMR and UV dilution experiments, in which this ion-pairing is excluded, gave K_a-values larger than $2{\cdot}10^6$ M^{-1} in methanol and methanol/water (1:1) for the capsule formation. The capsule encapsulates small guests like methanol, ethanol, etc., as proven by ESI-MS.

Introduction

A large number of supramolecular architectures is based on the assembly of different components making use of electrostatic interactions.[1] In nearly all studies the interaction between cations and anions in the initial, charged components is neglected. The same holds for host-guest complexation studies[2] (unless an ion-pair recognition is involved[3]). It is assumed that the process of ion-pairing, or the formation of higher ionic aggregates, is weak and, hence, negligible. However, Smith et al.[4] and Gokel et al.[5] reported that anion binding by neutral hosts in organic solvents can be inhibited by the presence of alkali metal cations due to ion-pairing. Gibson et al.[6] found that the apparent K_a-values of pseudorotaxane formation between dibenzylammonium salts or paraquat and dibenzo-24-crown-8 are strongly concentration-dependent. This was explained by ion-paring of the charged guests with their anion. Anions influence cation recognition not only in non-competitive solvents like chloroform,[7] but also *in water*. Electrolytes (buffers or added salts) tend to depress apparent binding constants orders of magnitude (in the case of *n*-alkylpyridinium-containing receptor-guest systems).[8]

In this article it is demonstrated that for the determination of the binding strength of capsules based on electrostatic interactions it is essential to take into account the ion-pairing of the initial components. Capsules are spherical molecules that are formed by multiple interactions of two polyfunctionalized half-spheres.[9–11] Capsules based on multivalent electrostatic interactions have recently attracted much attention due to the good solubility and stability in polar competitive media like alcohols[12,13] or even water.[14] For this study capsules **1**×**2**, based on azinium–anion interactions were selected, since ion-pairing of azinium salts can be studied by UV/Vis spectroscopy due to the presence of (a) charge-transfer band(s) that correspond to a contact ion-pair.

Results and Discussion

Synthesis and Confirmation of the Capsule Structure

The capsules **1**×**2** are based on two halfspheres: cavitand **2**, functionalized with four azinium substituents, and calix[4]arene **1**, containing four sulfonate substituents. Four pyridinium–sulfonate interactions bring the two halfspheres together to form a capsule (Figure 1). Capsules **1**×**2a**–**d** were prepared in 89–97% yield by mixing aqueous solutions of equimolar amounts of azinium salts **2a**–**d**×4Br$^-$ with the known tetrasodium calix[4]arene tetrasulfonate **1**×4Na$^+$.[13,15]

Capsule components **2a**,[16] **2b**, **2c**,[17] and **2d**, as tetrabromide salts, were prepared by reaction of tetrakis(bromomethyl)tetramethylcavitand **3**[18] with the appropriate azine (Scheme 1). Heating of tetrabromide **3** in a 4-cyanopyridine melt furnished **2b** in 79% yield. The synthesis of **2d** (yield 83%) was carried out in solution due to the much higher reactivity of 4-picoline compared with pyrazine.[19]

The capsules **1**×**2** are very badly soluble in water. Their solubility in methanol is less than 2 mM, which is much lower than the solubility of the initial capsule components **2a**–**d** (> 100 mM).

Electrospray ionization mass spectrometry (ESI-MS) confirms the formation of the capsules **1**×**2**. For example, in the case of capsule **1**×**2a** the mass spectrum exclusively

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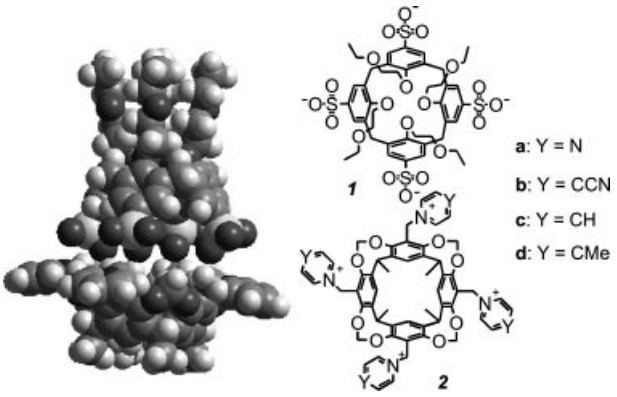
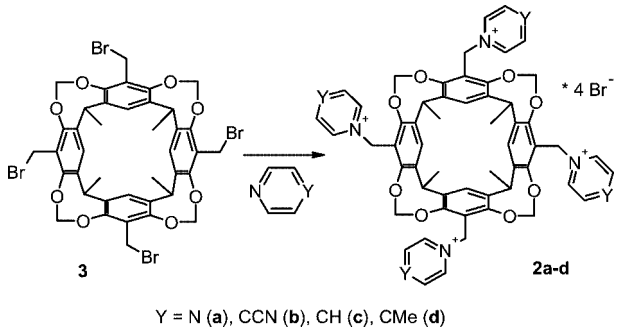


Figure 1. Molecular model representing capsule **1**×**2c** and structures of the capsule components **1** and **2a**–**d**.



Scheme 1. Synthesis of tetrakis(aziniummethyl)tetramethylcavitands **2a**–**d**.

contains signals of the capsule (with one or two sodium cations) (Figure 2). In some cases, also signals of the capsule containing one or two solvent molecules are present (vide infra). Since no significant influence of suppression takes place at these concentrations,[20] it can be concluded that the absence of signals of the capsule components **1** and **2** is a strong indication of the almost complete association of the capsule at this low concentration (50 μM).

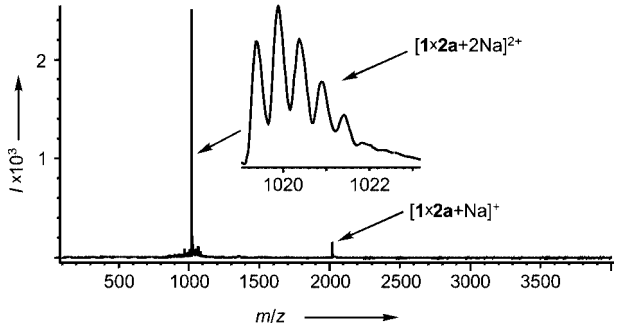


Figure 2. ESI-MS spectrum of a 50 μM solution of capsule **1**×**2a** in methanol (voltages, ring lens: 30 V, orifice 1: 2 V, orifice 2: 2 V).

The absence of signals of trimers, tetramers, etc. indicates that the signals correspond to a capsule and not to associates, in which only one or two azinium moieties are ion-paired and others are not complexed.[21]

Upon mixing the capsule half-spheres **1**×4Na$^+$ and **2**×4Br$^-$ significant changes in the ^{1}H NMR spectra are observed (for an example, see Figure 3), which is a clear indication of significant structural changes upon capsule formation. In the case of **1**×**2c**, the largest shift changes were observed for the γ- (≈ 1 ppm) and β-pyridinium hydrogen atoms (≈ 0.5 ppm).

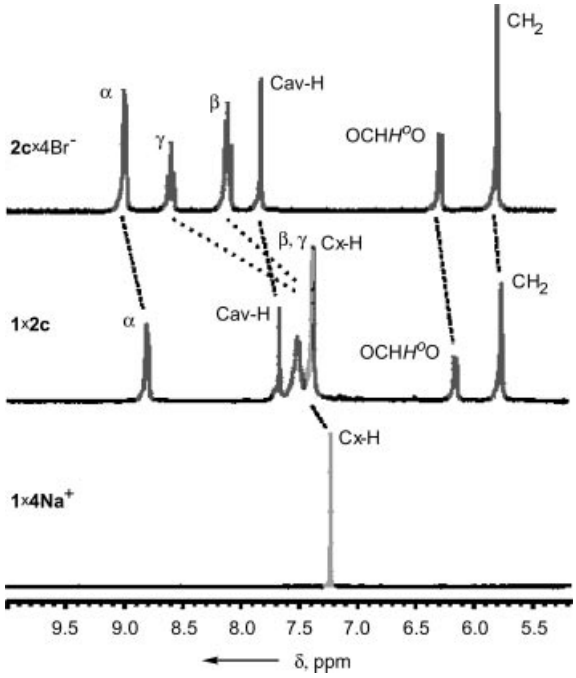


Figure 3. Spectra of 1 mM solutions of the initial compounds (**1**×4Na$^+$ and **2c**×4Br$^-$) and capsule **1**×**2c** in [D$_4$]methanol. The dashed lines indicate the changes in the ^{1}H NMR spectra upon capsule formation.

The α-pyridinium protons experience a smaller shift change (≈ 0.2 ppm). The considerable difference in shift changes between the pyridinium H atoms can be explained by the structural difference between the initial compound **2c** and capsule **1**×**2c**. In compounds **2**, all the pyridinium protons are influenced by electrostatic interactions (ion-pairing[22] and higher association[19]). In pyridinium–anion contact ion-pairs, the anion is located above the pyridinium plane.[23] In the case of capsules **1**×**2**, the rigidity of the calix[4]arene skeleton prevents the sulfonate substituent to be located above the pyridinium ring (Figure 1). Instead the sulfonate groups are positioned as such that they can only interact with the positively charged nitrogen center, and not with the whole pyridinium ring. Therefore the α-pyridinium hydrogens experience only a slight change, caused by the different influence of bromide and sulfonate. Protons in the β- and, especially, the γ-position of the pyridinium ring are located far away from the sulfonate group (Figure 1) and, consequently, a larger shift difference is observed for these protons upon capsule formation.

A small difference was also observed for the aryl hydrogens of the cavitand scaffold (≈ 0.1 ppm). Since cavitands form complexes with anions,[24] which can be monitored by ^{1}H NMR spectroscopy (vide infra), decomplexation of bromide from **2c** during the capsule formation leads to the shift change.

An ^{1}H NMR titration of calix[4]arene tetrasulfonate **1**×4Na$^+$ with tetrakis(pyridiniummethyl)tetramethyl cavitand **2c**×4Br$^-$ shows that till a 1:1 ratio is attained, all **2c** is bound. This is concluded from the negligible changes in the shift of the pyridinium protons; only addition of an excess of **2c** gives shift changes. This is a strong indication for a 1:1 binding stoichiometry.[25]

^{1}H NMR titration experiments of compound **2c**×4Br$^-$ with calix[4]arene tetrasulfonate **1**×4Na$^+$ in [D$_4$]methanol revealed that the apparent association constant is concentration dependent. K_a-values of $7\cdot10^4$ M^{-1} to $>2\cdot10^5$ M^{-1} were obtained varying the initial concentration of **2c**×4Br$^-$ from 0.5 mM to 0.05 mM. A plot of the ^{1}H NMR shift changes of the α-pyridinium protons during a titration of **2c**×4Br$^-$ with **1**×4Na$^+$ showed a systematic deviation from the best-fit data (Figure 4) indicating the presence of (an) additional process(es). This led us to study the influence of the ion-pairing of the initial components.

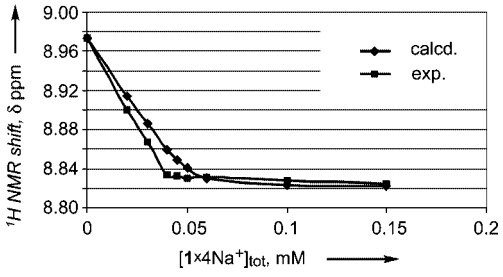


Figure 4. Plot of the shift of the α-pyridinium protons vs. the concentration of **1**×4Na$^+$ upon ^{1}H NMR titration of **2c**×4Br$^-$ (0.05 mM) with **1**×4Na$^+$ in [D$_4$]methanol:D$_2$O = 7:3 (v:v).

Ion-Pairing of the Initial Components

It is known that ion-pairing of pyridinium cations with anions causes concentration dependent ^{1}H NMR shifts of the pyridinium hydrogens.[22] ^{1}H NMR dilution experiments of the tetrakis(aziniummethyl)tetramethylcavitands **2a**–**d** exhibited changes of the shifts of the azinium protons, indicating ion-pairing of the pyridinium rings with bromide (for an example, see Figure 5). A large shift difference was also observed for the protons of the cavitand scaffold due to inclusion of the anion into the cavity (K_a-value > 30 M^{-1} in methanol[26]).

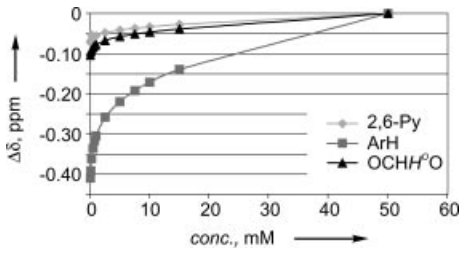


Figure 5. ^{1}H NMR shift changes upon dilution of **2c**×4Br$^-$ in [D$_4$]-methanol.

ESI-MS also shows ion-pair association of the azinium cavitands **2**×4Br$^-$. For example, the ESI-MS spectrum of a 100 μM solution of **2c**×4Br$^-$ in methanol contains signals of ion-pairs and triple ion capsules[19] (Figure 6).

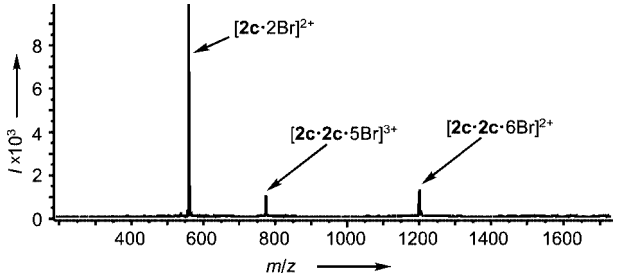


Figure 6. ESI-MS spectrum of a 100 μM solution of **2c**×4Br$^-$ in methanol.

From the literature it is known that UV/Vis spectroscopy allows the monitoring of the formation of contact ion-pairs from azinium cations with counterions in solution.[27–30] In the case of pyridinium salts, containing electron-withdrawing groups, such as cyano- or 4-pyridinium (viologen derivatives), the formation of ion-pairs can be easily followed by the appearance of (a) charge-transfer band(s) above 300 nm in the UV/Vis spectra.[30] The appearance of charge-transfer bands upon contact ion-pair formation has been observed for a variety of anions, such as iodide,[27,28,30] bromide,[31] sulfite,[31] sulfate,[32] hexacyanoferrate,[32,33] etc.

A freshly prepared methanolic solution (26 μM) of tetrakis(pyraziniummethyl)tetramethylcavitand tetrabromide **2a**×4Br$^-$ shows two maxima in its UV-spectrum: 276 nm and 317 nm (Figure 7). The high intensity of the charge-transfer band at 317 nm at such a low concentration indicates a significant ion-pairing of pyrazinium with bromide. The charge-transfer band can represent multiple equilibria, i.e. not only ion-pairs but also higher aggregates.[34] Upon addition of tetrasulfonate **1**×4Na$^+$, the intensity of the band at 317 nm was decreased, leaving a "pyrazinium shoulder"[35] covering the area of 300–350 nm (Figure 7). This change involves the replacement of a pyrazinium bromide for a pyrazinium sulfonate ion-pair. The absorption in the 300–350 nm area is not changed upon the addition of a larger amount of **1**×4Na$^+$ (Figure 7), indicating the complete replacement of bromide in the previous complex. Calix[4]arene tetrasulfonate **1**×4Na$^+$ has a band below 300 nm

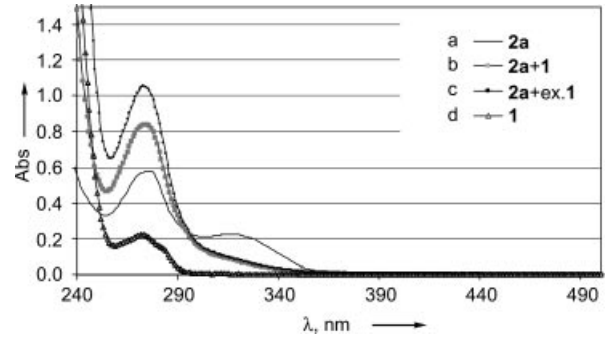


Figure 7. UV/Vis spectra of a) a solution of **2a** in methanol, b) solution a + **1**, c) solution a + excess of **1**, d) a solution of **1** in methanol.

in the UV-spectrum (Figure 7) that does not interfere with the charge-transfer band.

K_a-Value Determination of the Capsules 1×2

The K_a-values of capsule **1×2** formation were determined with dilution experiments. Upon dilution of capsules **1×2** in [D_4]methanol from 1 mM to 5 μM in the ^{1}H NMR spectrum a very small shift was observed for the α-azinium protons (up to ca. 0.005 ppm) due to some dissociation of the capsule at low concentrations. Attempts to fit the NMR dilution data resulted in K_a-values $> 10^6$ M^{-1}.

Upon dilution of methanolic solutions of the capsules **1×2** a systematic deviation from the Lambert-Beer law was observed in the UV-spectra. For example, in the case of capsule **1×2a** (Figure 8), an increase of the molar absorptivity of about 40–70 L·mol^{-1}·cm^{-1} was observed at 300 nm. This deviation is caused by the presence of a charge-transfer band between sulfonate and pyridinium (≈ 290–320 nm).[32] Non-linear fitting of the data yielded K_a-values of $> 2\cdot10^6$ M^{-1} for the capsules **1×2** in methanol and **1×2a**,**b** in methanol/water = 1:1 (band at 260–280 nm).[36]

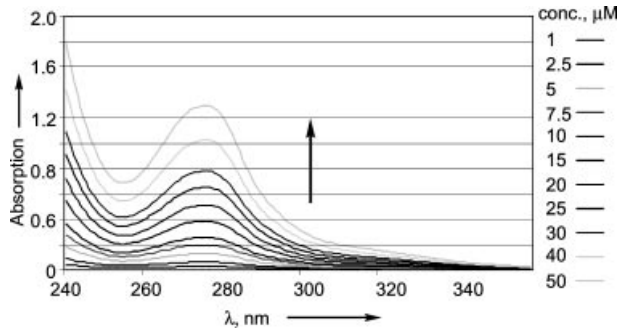


Figure 8. UV-dilution of **1×2a** in methanol.

The K_a-values are higher than those obtained via ^{1}H NMR titration experiments (vide supra). This is caused by the significant influence of ion-pairing on the titration data, which is excluded in the case of the dilution experiments.

The capsule is resistant to addition of an excess of bromide or iodide (as tetrabutylammonium salts). However, addition of tetrabutylammonium iodide to a solution of the capsule **1×2a** in methanol results in the appearance of a new charge-transfer band at 340 nm. This charge-transfer band does not disappear even upon addition of triphenylphosphane to remove traces of iodine and triiodide.[27] It could be caused by the formation of a complex of iodide with the pyrazinium sulfonate contact ion-pair at the outside of the capsule to give an iodide-pyrazinium sulfonate triple ion.[37]

Behavior of the Capsule in the Gas Phase (ESI-MS)

The high stability of capsules **1×2** allows studying both its gas-phase fragmentation and the behavior of ion-pairs at a high voltage. As examples, parts of the voltage-induced dissociation spectra of capsules **1×2a**,**c** are shown in Figure 9. It is striking, that the fragmentation begins with the loss of an ion-paired wall of the capsule: SO_3*pyrazine. Due to the loss of the wall, the capsule becomes very destabilized, and loses subsequently the other pyrazines (Figure 9, ***a***). It is the reason of the simultaneous presence in the spectrum of a high capsule signal and intensive signals of fragmentation products (Figure 9, ***a***). The other fragmentation pathway, the loss of azinium rings (without SO_3) from an ion-pair, is observed at higher voltages and is concluded from the appearance of the [**1×2c**+Na-3pyridine]$^+$ signal (Figure 9, ***b***). Qualitatively, the same fragmentation behavior is observed for all capsules **1×2**. The ratio of the intensities of the signals of the fragmentation products and those of the capsules is higher in the case of capsules based on ion-pairs of pyrazinium and 4-cyanopyridinium (**1×2a** and **1×2b**). The relative voltage stability of the –SO_3×CH_2-azinium contact ion-pairs, which form the walls of the capsules, follows the order 4-methylpyridinium > pyridinium > 4-cyanopyridinium > pyrazinium. Electron-accepting substituents are supposed to destabilize the CH_2-N bond within the -SO_3×CH_2-azinium contact ion-pairs.

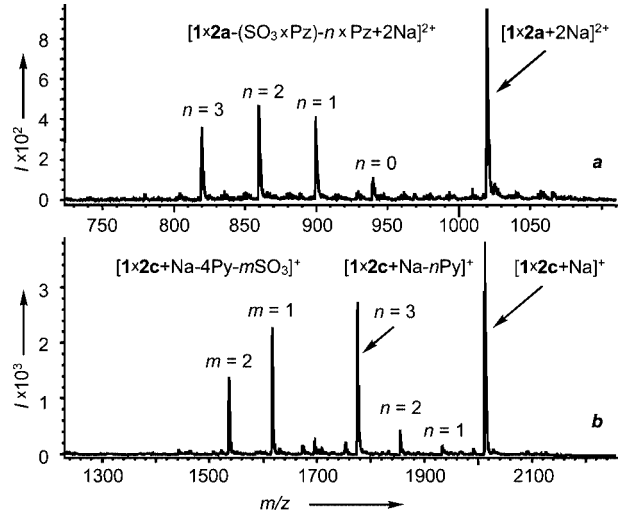


Figure 9. Parts of the ESI-MS spectra of 50 μM methanolic solutions of capsules **1×2a** (***a***: voltages, ring lens: 2 V, orifice 1: 75 V, orifice 2: 25 V), and **1×2c** (***b***: voltages, ring lens: 30 V, orifice 1: 200 V, orifice 2: 25 V); Pz = pyrazine, Py = pyridine.

Gas-Phase Guest Inclusion Complexes within the Capsules

Under low-voltage conditions the ESI-MS spectra of **1×2a**–**d** in methanol exhibit, in addition to the signal of the capsule, also peaks corresponding to the capsule and one or two methanol molecules. It should be noted that under these conditions desolvation takes place quite efficiently; the capsule components **1**×4Na^+ and **2a**×4Br^- do not show solvated species in the ESI-MS spectra. Therefore it can be concluded that in the case of the capsules **1×2** methanol is encapsulated. Using higher voltages, as in Figure 2, methanol encapsulation is not observed. The exception is capsule **1×2b**, which keeps methanol up to a very high voltage.

The influence of the size of the guest was studied by recording the ESI-MS spectra of 25 μM solutions of capsule **1**×**2a** in a 1:1 mixture of methanol and $CH_3(CH_2)_nOH$ (n = 1–5). In the case of ethanol, in addition to the complexes with either methanol or ethanol, also a capsule containing both alcohols is present (Figure 10). 1-Propanol, 1-butanol, and *n*-amyl alcohol form 1:1 complexes with the capsule. These complexes have a higher voltage stability than those with methanol. In the case of 1-hexanol (n = 5), a peak corresponding with the encapsulated guest in the capsule was not observed. This indicates that this alcohol is already too large to be accommodated within the cavity.

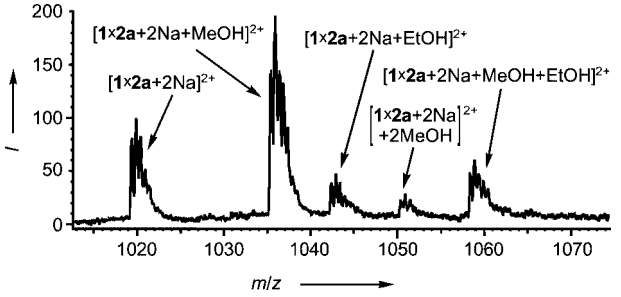


Figure 10. ESI-MS spectrum of a 25 μM solution of capsule **1**×**2a** in a 1:1 mixture of methanol/ethanol (v/v); voltages, ring lens: 20 V, orifice 1: 5 V, orifice 2: 5 V.

The capsule can also accommodate nitromethane, but experiments with ethyl acetate, toluene, and pyrazine in methanol showed no encapsulation. The inner volume of the capsule is smaller than that of the capsules of Rebek[10] and Böhmer,[11] nevertheless smaller molecules can be located inside as clearly shown by mass spectrometry.

Conclusions

In this paper it is clearly shown that it is essential to be aware of the presence of ion-pairing of the initial components in the formation of assemblies based on electrostatic interactions in polar media.

In the case of the formation of [1+1] cavitand-calix[4]-arene capsules, based on azinium–anion interactions, ^{1}H NMR titration experiment resulted in concentration-dependent apparent K_a-values ranging from $7\cdot10^4$ M^{-1} to $>2\cdot10^5$ M^{-1} in [D$_4$]methanol. However, ^{1}H NMR and UV/Vis dilution experiments, in which the influence of ion-pairing is excluded, in the latter case making use of the sulfonate–azinium contact ion-pair charge-transfer band, gave K_a-values of $> 2\cdot10^6$ M^{-1} in methanol.

In many supramolecular studies the effect of the ion-pairing of charged starting components has probably been overlooked. Therefore, in those studies where titration experiments have been used for K_a-value determination, it should be realized that the obtained data might be concentration dependent.

Experimental Section

General: The reagents were purchased from Aldrich or Acros Organics and used without further purification. All the reactions were performed under a dry argon atmosphere. All solvents were freshly distilled before use. Dry pyridine was obtained by distillation over calcium hydride. Melting points were measured using a Sanyo Gellenkamp Melting Point Apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova (300 MHz or 400 MHz for ^{1}H; 400 MHz for ^{13}C) spectrometer. Residual solvent protons were used as internal standard and chemical shifts are given relative to tetramethylsilane (TMS). UV/Vis spectra were measured on a Varian Cary 3E UV-spectrophotometer in 10 mm cuvettes. Compounds **1**,[13,15] **2a**,[16] **2c**,[17] and, **3**[18] were prepared in accordance with the literature procedures. The presence of water in the analytical samples was proven by ^{1}H NMR spectroscopy.

ESI-MS: The ESI-MS experiments were carried out with a Jeol AccuTOF instrument. In the standard mode the solutions were introduced at a flow rate of 6 μL/min. The data were accumulated in the mass range 230–4000 *m*/*z* for 2 min. The standard spray conditions, unless otherwise specified are: capillary voltage 2500 V, ring lens voltage: 30 V, orifice 1 voltage: 2 V (for compounds **2b**,**d**) and 100 V (for capsules **1**×**2**), orifice 2 voltage: 2 V, orifice 1 temperature: 60 °C, temperature in the desolvation chamber: 120 °C, drying gas flow: 0.5 L/min, nebulizing gas flow: 0.5 L/min. For the characterization of compounds **2b**,**d** and capsules **1**×**2** the most intensive signals of the isotopic pattern are shown.

Tetrakis(4-cyanopyridiniummethyl)tetramethylcavitand Tetrabromide (2b): Tetrakis(bromomethyl)tetramethyl cavitand **3**[18] (300 mg, 0.31 mmol) was added to a melt of 4-cyanopyridine (1.2 g, 11.5 mmol). The reaction mixture was stirred at 100 °C for 20 h. After cooling down the reaction mixture, diethyl ether (50 mL) was added and the reaction mixture was stirred for 1 h. The precipitate was filtered off and recrystallized from methanol/dichloromethane to give **2b** as a yellow powder. Yield: 340 mg (79%); m.p. > 300 °C. ^{1}H NMR (5 mM, CD_3OD, 300 MHz): δ = 9.24 (d, J = 6.6 Hz, 8 H; α-pyridinium-H), 8.49 (d, J = 6.2 Hz, 8 H; β-pyridinium-H), 7.95 (s, 4 H, CavArH), 6.32 (d, J = 7.3 Hz, 4 H; $O_2CH\mathit{H}^o$), 5.90 (s, 8 H, $ArCH_2Py$), 4.96 (q, J = 7.7 Hz, 4 H; Ar_2CH), 4.73–4.80 (bs; water + $O_2CH\mathit{H}^i$), 1.89 ppm (d, J = 7.3 Hz, 12 H; CH_3-Cav). UV/Vis (CH_3OH): ν = 278 nm. ESI-MS (200 μM, CH_3OH): *m*/*z* = [**2b**+2Br]$^{2+}$ 610.14 (calcd. 610.11); elemental analysis calcd. (%) for $C_{64}H_{52}Br_4N_8O_8$ + 3 H_2O (1434.8): C 53.57, H 4.07, Br 22.28, N 7.81; found: C 53.44, H 3.96, Br 21.95, N 7.60.

Tetrakis(4-methylpyridiniummethyl)tetramethylcavitand Tetrabromide (2d): To a stirred solution of tetrakis(bromomethyl)tetramethylcavitand **3** (1.0 g, 1.04 mmol) in chloroform (50 mL), 4-picoline (0.61 mL, 6.24 mmol) was added dropwise. The reaction mixture was stirred for 24 h. To the suspension formed, methanol (50 mL) was added and the resulting clear reaction mixture was stirred for an additional 24 h. The reaction mixture was evaporated to dryness under vacuum. The product was purified twice by reprecipitation with diethyl ether from methanol. Yield: 1.15 g (83%); m.p. > 300 °C. ^{1}H NMR (5 mM, CD_3OD, 300 MHz): δ = 8.78 (d, J = 6.6 Hz, 8 H; α-pyridinium-H), 7.91 (d, J = 6.2 Hz, 8 H; β-pyridinium-H), 7.86 (s, 4 H; CavArH), 6.28 (d, J = 7.3 Hz, 4 H; $O_2CH\mathit{H}^o$), 5.70 (s, 8 H; $ArCH_2Py$), 4.95 (q, J = 7.7 Hz, 4 H; Ar_2CH), 4.73 (d, J = 7.3 Hz, 4 H; $O_2CH\mathit{H}^i$), 2.67 (s, 12 H, CH_3-Py), 1.87 (d, J = 7.3 Hz, 12 H; CH_3-Cav). UV/Vis (CH_3OH): ν = 281 nm. ESI-MS (200 μM, CH_3OH): *m*/*z* = [**2d** + 2Br]$^{2+}$ 588.17 (calcd. 588.15), [**2d** + **2d** + 5Br]$^{3+}$ 811.22 (calcd. 811.18), [**2d** + **2d** + 6Br]$^{2+}$ 1257.28 (calcd. 1257.23); elemental analysis calcd. (%) for $C_{64}H_{64}Br_4N_4O_8$ + 4.5H_2O (1417.9): C 54.21, H 5.19, Br 22.54, N 3.95; found: C 54.20, H 5.10, Br 22.25, N 4.01.

General Procedure for the Synthesis of Capsules 1×2: An aqueous solution (6 mL) of **1** (15.7 mg, 14 μM) was added dropwise to an

aqueous solution (6 mL) of **2** (14 μM). The suspension formed was stirred for 1 h and allowed to stay for an additional 1 h. The product was filtered, washed with water, diethyl ether and dried in vacuo.

Tetrakis(1-pyraziniummethyl)tetramethylcavitand Tetrakis(ethoxyethyl)calix[4]arenetetrasulfonate 1×2a: Yield: 24.9 mg (89%); m.p. > 250 °C. ^{1}H NMR (CD_3OD, 400 MHz): δ = 9.33 (br. s, 8 H, α-pyrazinium-H), 9.02 (d, J = 4.4 Hz, 8 H; β-pyrazinium-H), 7.66 (s, 4 H; cavArH), 7.45 (s, 8 H; calixArH), 6.17 (d, J = 7.7 Hz, 4 H; OCHH^oO), 5.91 (s, 8 H; $ArCH_2Py$), 4.96 (q, J = 7.3 Hz, 4 H; Ar_2CH), 4.73 (d, J = 7.7 Hz, 4 H; OCHH^iO), 4.72 (d, J = 12.8 Hz, 4 H; ArCH$^{eq}H^{ax}$Ar), 4.27 (t, J = 5.5 Hz, 8 H; $ArOCH_2$), 3.91 (t, J = 5.5 Hz, 8 H; $ArOCH_2CH_2$), 3.54 (q, J = 7.3 Hz, 8 H; CH_2CH_3), 3.29–3.38 (m; CHD_2OD + ArCH^{eq}H^{ax}Ar), 1.81 (d, J = 7.3 Hz, 12 H; CH_3), 1.22 ppm (t, J = 7.3 Hz, 12 H; CH_2CH_3). ^{13}C NMR (CD_3OD): δ = 159.1, 155.2, 152.5, 141.0, 140.8, 138.5, 136.0, 128.0, 124.9, 121.1, 101.9, 75.1, 70.9, 67.6, 57.5, 32.9, 32.7, 16.2, 15.9 ppm. UV/Vis (CH_3OH): ν = 275 nm. ESI-MS (50 μM, CH_3OH): m/z = $[\mathbf{1}\times\mathbf{2a}+2Na]^{2+}$ 1019.82 (calcd. 1019.78), $[\mathbf{1}\times\mathbf{2a}+Na]^+$ 2016.61 (calcd. 2016.57); elemental analysis calcd. (%) for $C_{100}H_{104}N_8O_{28}S_4$ + 6 H_2O (2102.3): C 57.13, H 5.56, N 5.33; found: C 56.98, H 5.50, N 5.14.

Tetrakis[1-(4-cyanopyridinium)methyl]tetramethylcavitand Tetrakis(ethoxyethyl)calix[4]arenetetrasulfonate 1×2b: Yield: 28.4 mg (97%); m.p. > 250 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ = 9.24 (d, J = 7.0 Hz, 8 H; α-pyridinium-H), 8.46 (d, J = 7.0 Hz, 8 H; β-pyridinium-H), 7.59 (s, 4 H; cavArH), 7.45 (s, 8 H; calixArH), 6.18 (d, J = 7.5 Hz, 4 H; OCHH^oO), 5.92 (s, 8 H; $ArCH_2Py$), 4.93 (q, J = 7.3 Hz, 4 H; Ar_2CH), 4.65–4.84 (water + ArCH$^{eq}H^{ax}$Ar + OCHH^iO), 4.26 (t, J = 5.5 Hz, 8 H; $ArOCH_2$), 3.89 (t, J = 5.5 Hz, 8 H; $ArOCH_2CH_2$), 3.55 (q, J = 7.0 Hz, 8 H; CH_2CH_3), 3.29–3.33 (CHD_2OH + ArCH^{eq}H^{ax}Ar), 1.78 (d, J = 7.7 Hz, 12 H; CH_3), 1.21 ppm (t, J = 7.0 Hz, 12 H; CH_2CH_3). UV/Vis (CH_3OH): ν = 278 nm. ESI-MS (50 μM, CH_3OH): m/z = $[\mathbf{1}\times\mathbf{2b}+2Na]^{2+}$ 1067.79 (calcd. 1067.78), $[\mathbf{1}\times\mathbf{2b}+2Na+CH_3OH]^{2+}$ 1083.91 (calcd. 1083.80), $[\mathbf{1}\times\mathbf{2b}+Na]^+$ 2112.65 (calcd. 2112.57), $[\mathbf{1}\times\mathbf{2b}+Na+CH_3OH]^+$ 2144.71 (calcd. 2144.60); elemental analysis calcd. (%) for $C_{108}H_{104}N_8O_{28}S_4$ + 5.5H_2O (2189.4): C 59.25, H 5.29, N 5.12; found: C 59.32, H 5.12, N 5.15.

Tetrakis(pyridiniummethyl)tetramethylcavitand Tetrakis(ethoxyethyl)calix[4]arenetetrasulfonate 1×2c: Yield: 25.6 mg (92%); m.p. > 250 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ = 8.82 (d, J = 5.5 Hz, 8 H; α-pyridinium-H), 7.5–7.7 (m, 16 H; β,γ-pyridinium-H and cavArH), 7.47 (s, 8 H; calixArH), 6.14 (d, J = 7.7 Hz, 4 H; OCHH^oO), 5.79 (s, 8 H; $ArCH_2Py$), 4.96 (q, J = 7.3 Hz, 4 H; Ar_2CH), 4.7–4.87 (water + ArCH$^{eq}H^{ax}$Ar), 4.60 (d, J = 7.7 Hz, 4 H; OCHH^iO), 4.31 (t, J = 5.3 Hz, 8 H; $ArOCH_2$), 3.93 (t, J = 5.3 Hz, 8 H; $ArOCH_2CH_2$), 3.58 (q, J = 7.0 Hz, 8 H; CH_2CH_3), 3.37 (d, J = 12.1 Hz, 4 H; ArCH^{eq}H^{ax}Ar), 1.80 (d, J = 7.3 Hz, 12 H; CH_3), 1.23 ppm (t, J = 7.0 Hz, 12 H; CH_2CH_3). ^{13}C NMR (CD_3OD): δ = 159.2, 155.2, 140.9, 136.2, 128.1, 75.3, 70.8, 32.9 ppm. UV/Vis (CH_3OH): ν = 279 nm. ESI-MS (50 μM, CH_3OH): m/z = $[\mathbf{1}\times\mathbf{2c}+2Na]^{2+}$ 1017.84 (calcd. 1017.79), $[\mathbf{1}\times\mathbf{2c}+Na]^+$ 2012.67 (calcd. 2012.60); elemental analysis calcd. (%) for $C_{104}H_{108}N_4O_{28}S_4$ + 7 H_2O (2116.4): C 59.02, H 5.81, N 2.65; found: C 59.09, H 5.78, N 2.54.

Tetrakis(4-methylpyridiniummethyl)tetramethylcavitand Tetrakis(ethoxyethyl)calix[4]arenetetrasulfonate 1×2d: Yield: 26.7 mg (93%); m.p. > 250 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ = 8.65 (d, J = 5.1 Hz, 8 H; α-pyridinium-H), 7.62 (s, 4 H; cavArH), 7.54, 7.50 (s, 12 H; β-pyridinium-H, calixArH), 6.07 (d, J = 7.7 Hz, 4 H; OCHH^oO), 5.72 (s, 8 H; $ArCH_2Py$), 4.95 (q, J = 7.3 Hz, 4 H; Ar_2CH), 4.65–4.85 (water + ArCH$^{eq}H^{ax}$Ar), 4.61 (d, J = 7.7 Hz, 4 H; OCHH^iO), 4.30 (t, J = 5.5 Hz, 8 H; $ArOCH_2$), 3.91 (t, J = 5.5 Hz, 8 H; $ArOCH_2CH_2$), 3.58 (q, J = 7.3 Hz, 8 H; CH_2CH_3), 3.38 (d, J = 12.8 Hz, 4 H; ArCH^{eq}H^{ax}Ar), 1.90 (s, 12 H, CH_3-Py), 1.80 (d, J = 7.3 Hz, 12 H; CH_3), 1.23 ppm (t, J = 7.0 Hz, 12 H; CH_2CH_3). ^{13}C NMR (CD_3OD): δ = 158.8, 155.2, 146.1, 141.5, 140.9, 136.6, 129.3, 128.0, 124.5, 121.8, 101.9, 75.3, 70.8, 67.6, 56.4, 32.9, 16.3, 15.8 ppm. UV/Vis (CH_3OH): ν = 280 nm. ESI-MS (50 μM, CH_3OH): m/z = $[\mathbf{1}\times\mathbf{2d}+2Na]^{2+}$ 1045.82 (calcd. 1045.82), $[\mathbf{1}\times\mathbf{2d}+Na]^+$ 2068.70 (calcd. 2068.66); elemental analysis calcd. (%) for $C_{108}H_{116}N_4O_{28}S_4$ + 8 H_2O (2190.5): C 59.22, H 6.07, N 2.56; found: C 59.26, H 6.07, N 2.40.

K_a-Value Determination: K_a-values were determined from ^{1}H NMR and UV-titration and dilution data by non-linear fitting. Capsule formation was described by standard equations for 1+1 stoichiometry.[38] For the fitting of the NMR titration and dilution data standard relationships between the shift of the α-pyridinium protons and the concentrations of the capsule and capsule components were used.[39] The error in the K_a-value determinations is about 5%. For the fitting of the UV dilution data, the K_a-value of the capsule formation and the molar absorptivity of the scaffold (ε_c) and the charge-transfer band (ε_{c-t}) were varied to obtain a minimal difference between calculated and experimental data using the least square method (an approximate maximal value of ε'_{obs} was initially determined at a higher concentration to limit the sum of ε_c and ε_{c-t}). The observed molar absorptivity ε'_{obs} at the wavelength, where a charge-transfer band is appearing, includes the molar absorptivity of the scaffold ε_c and that of the second component ε'_{c-t}, which is proportional to the degree of association of the capsule [Equations (1) and (2)].

$$\varepsilon'_{obs} = \varepsilon_c + \varepsilon'_{c-t} \quad (1)$$

$$\varepsilon'_{c-t} = \varepsilon_{c-t}\,([\mathbf{1}\times\mathbf{2}]/[\mathbf{1}\times\mathbf{2}]_{max}) \quad (2)$$

Supporting Information (see also the footnote on the first page of this article): Differentiation between ion-paired and free azinium cations using ESI mass spectrometry.

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