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Counterion Exchange as a Decisive Factor in the Formation of Host:Guest Complexes by *p*-Sulfonatocalix[4]arene

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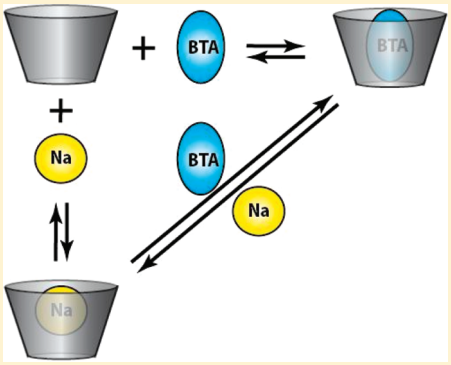
Supporting Information

ABSTRACT: Calorimetric and NMR titration experiments have been done to measure the binding constant between *p*-sulfonatocalix[4]arene and a quaternary ammonium ion. Our results show that the binding constants depend both on the calixarene concentration and on the presence of added Na^+. These results have been interpreted by considering the ion-exchange equilibrium between sulfonatocalixarene counterions and the added organic cation. Our results show that it is necessary to extrapolate the binding constants to zero calixarene concentration and zero added salts in order to get the true equilibrium constant.



INTRODUCTION

Calixarenes are host frameworks that belong to the most versatile building blocks in supramolecular chemistry. When they are functionalized at the upper rim with sulfonate groups, these calixarenes become water-soluble, which combined with a preorganized framework and biological compatibility[1,2] allow these calixarenes to have a variety of applications in fields such as molecular recognition/sensing,[3] crystal engineering,[4,5] catalysis,[6] enzyme mimics/enzyme assays,[7,8] and medicinal chemistry.[9,10] Consequently, *p*-sulfonatocalixarenes are one of the most studied derivatives in complexation of inorganic cations, organic ammonium cations, pyridiniums and viologens, neutral organic molecules, and others.[11]

Depending on the guest, different types of interactions can be involved (ionic, hydrophobic, van der Waals, $\pi-\pi$, cation$-\pi$, hydrogen bonding, ...), as demonstrated by the thermodynamic characterization of the binding process.[12] The binding affinities and the thermodynamics of *p*-sulfonatocalixarene upon metal ions were investigated by Bonal[13] and Morel-Desrosiers,[12] but according to the authors, significant heat effects were not detected for Na^+ and Ag^+, suggesting that these cations are not complexed.

Recently, evidence of Na^+ complexation by *p*-sulfonatocalix[4]arene (SC4) appeared in the bibliography. Nau et al.[14] monitored the binding of Na^+ among other cations by SC4 through competitive fluorophore displacement in aqueous solution. However, to discard potential problems with the incomplete release of the organic guest, which could lead to other stoichiometries, we confirm by ^{23}Na diffusion NMR measurements that SC4 fully binds an Na^+ counterion.[15]

Because of the anionic nature of SC4, Na^+ (or other counterions) are always present in solution and can introduce competitive binding equilibria in the presence of other guests. In addition to the complexation of inorganic cations by SC4, organic ammonium cations are another class of typical guests which has been extensively studied.[11] Most of these studies have been done by varying the concentration of the host or in the presence of a buffer solution.

Our hypothesis is that, if Na^+ binds to the SC4, then a competition between the guest and the counterions should be considered. As a result, the binding constants should be affected by the SC4 concentration. Since most of the binding studies involving SC4 and guest species have been done by varying the host concentration, it is important to evaluate the effect of this parameter on the binding constants. In the present work, we show that contrary to the traditional point of view the binding constant of benzyltrimethylammonium ion (BTA) decreases almost 10 times when the SC4 concentration is increased from 0.075 to 7 mM. NMR and isothermal titration calorimetry (ITC) experiments have been employed to measure the binding constant of the organic ammonium cation with SC4 at various concentrations.

EXPERIMENTAL SECTION

Materials. Benzyltrimethylammonium chloride (BTA) from Aldrich (97%) and NaCl from Fluka (assay ≥99.5%) were used as received.

p-Sulfonatocalix[4]arene (SC4) was prepared by *ipso*-sulfonation of *p-tert* butylcalixarene in H_2SO_4 at 80 °C. The pentasodium salt (SC4Na) of SC4 was obtained by

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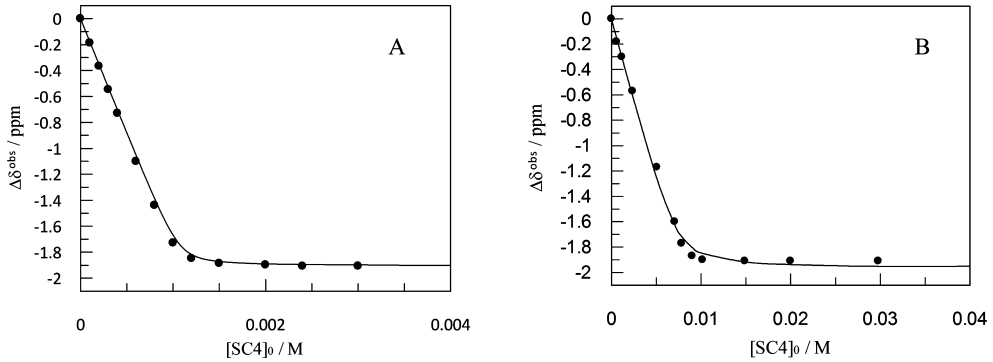

Figure 1. Plots of $\Delta\delta^{obs}$ (ppm) for the trimethylammonium group signal of BTA vs SC4 concentration in D_2O at 25 °C: (A) [BTA] = 1 mM; (B) [BTA] = 7 mM. Solid lines were obtained by fitting the experimental data to eqs 4 and 2.

neutralization of the acid form of SC4 with Na_2CO_3 in H_2O. The product was then decolorized with activated charcoal and filtered through Celite. The final solution was placed in an open flask and allowed to crystallize by slow evaporation of water. The crystals were recollected by filtration, dissolved in a minimum quantity of water, and precipitated with methanol. The operation was repeated at least three times. Finally, the product was dried at 80 °C under high vacuum (by means of an oil diffusion pump) for three days. SC4 was characterized by nuclear magnetic resonance spectroscopy (NMR) and electrospray ionization-ion trap mass spectrometry (ESI-MS) in the negative mode (Figure S1, Supporting Information). ^{1}H NMR (300 MHz, D_2O, ppm) δ = 3.99 (br, 8H), 7.55 (s, 8H). ESI MS: m/z ($SC4^{5-}$ + $4Na^+$) 831.0; calcd: 830.91. The SC4 was also analyzed by thermal gravimetric analysis (TGA) to measure the lost weight.

The pK_a values of SC4 (3 mM) were determined by the pH-metric titration method and pK_{a1} = 3.6 and pK_{a2} = 11.9 were obtained (the data were analyzed using the program HyperQuad;[16] see the Supporting Information, Figure S2). These results are qualitatively in agreement with the results reported in the literature.[17]

Measurements of Acid-Dissociation Constant. Fully automated titrations were performed using a Crison microBU 2031 autoburet and a Crison 2002 pH meter. The program controlling the experiments incorporated certain restrictions to ensure reproducibility and to avoid stabilization problems and electrode drift (Radiometer GK2401C, Ag/AgCl reference). After addition of the titrant and sample homogenization, the potential value was read every 3 s until obtaining a set of six values that complied with the condition that the difference between any two consecutive measurements was less than 0.2 mV. The actual value of the potential was then calculated as the average of the set of six measurements whenever the standard error of the average did not exceed 0.2 mV. If the restrictions were not fulfilled, a new process for potential measurement was started. Aqueous solutions of SC4 (3 mM) and SC4 (3 mM) in the presence of BTA (3 mM) containing the definite concentration of HCl were placed in a thermostatted cell at 298.0 ± 0.1 K and titrated by adding small aliquots of KOH CO_2-free solution. Water-saturated N_2 was bubbled through the solution to maintain a CO_2-free atmosphere. Before each titration, the electrode was calibrated for H^+ ion concentration by titrating a strong acid (HCl) with a strong base (KOH).

Diffusion NMR. NMR spectra were recorded at 25 °C on a Varian Inova 500 spectrometer by using DSS as an external reference and equipped with a 5 mm 1H/X indirect probe with Z-shielded gradients. The NMR experiments were processed with MestreC v.3.9 software (Mestrelab Inc.). ^{1}H and ^{23}Na diffusion spectra were acquired with the Hahn spin−echo based PGSE pulse sequences.[18] In both cases, rectangular-shaped pulsed gradients (G) were applied with a power level linearly incremented from 4 to 65 G cm^{-1} in 32 steps. The duration of the pulse field gradients (δ) applied to encode and decode the diffusion was set to 1 ms for ^{1}H and 3 ms for ^{23}Na. The diffusion delay period Δ of the experiment was optimized to 100 ms for ^{1}H and 40 ms for ^{23}Na. Such an optimized Δ value provided a convenient sampling of the exponential decay of the signal intensity during the diffusion experiment, and this was essential to achieve accurate results for the determined diffusion coefficients.[19] Calibration of the absolute gradient strength was provided by the spectrometer, and the particular probe was calibrated with the actual diffusion pulse sequence by using a compound of known diffusion as a reference.[20] The reference sample for the ^{1}H diffusion experiments was 99% D_2O at 25 °C (D = 1.87 × 10^{-5} cm^2 s^{-1}) and for the ^{23}Na diffusion experiments was 2 M NaCl solution in 10% D_2O in H_2O at 25 °C (D = 1.14 × 10^{-5} cm^2 s^{-1}). As expected, both reference samples provided the same gradient strength with an error of less than 1%.

Microcalorimetry. The microcalorimetric titrations were performed on an isothermal titration microcalorimeter (VP-ITC), purchased from Microcal Co. (Northampton, MA) at atmospheric pressure and 25 °C. In each run, a solution of host in a 0.270 mL syringe was sequentially injected with stirring at 459 rpm into a solution of guest in the sample cell (1.459 mL volume). Each solution was degassed and thermostatted using a ThermoVac accessory before titration. In each titration, the reference cell was filled with the same sample as that in the sample cell. The ORIGIN software (Microcal Inc.), which was used to compute the binding constant (K) from a single titration curve, gave a standard deviation based on the scatter of the data points in the titration curve. The net reaction heat in each run was calculated by the "one set of binding sites" model. Additionally, the first point was removed from the titration curve before doing the curve-fit, because of the probable leakage resulting from having the syringe stirring all the time in the cell a long time before the first injection, giving a smaller heat effect than it should have. To check the accuracy of the binding constant, two independent titration experiments were carried out and their average values were listed in the table.

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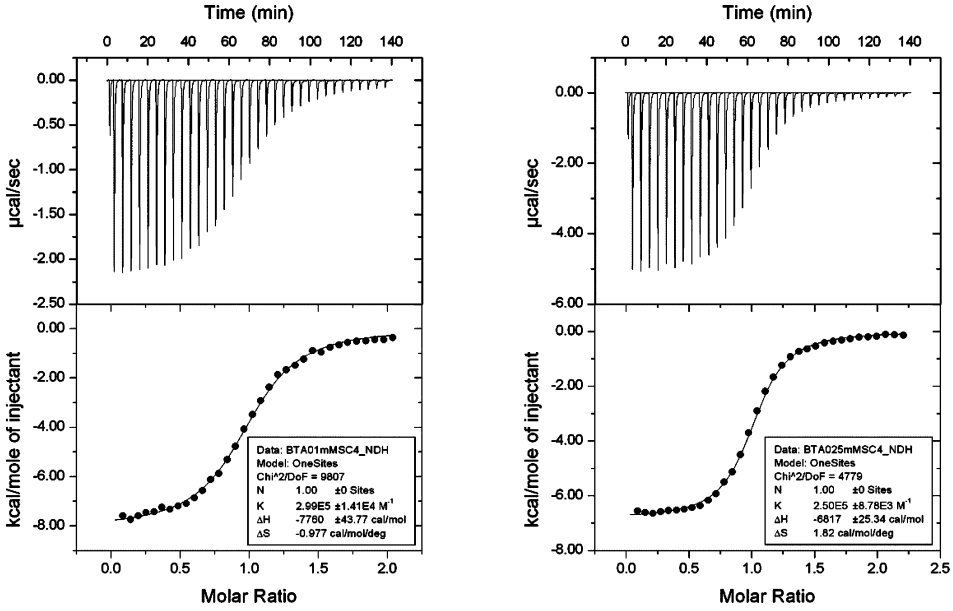

Figure 2. Microcalorimetric titration of BTA with SC4 at 25 °C: raw data for sequential 34 injections (8 μL per injection) of BTA solution (1 or 2.5 mM) injecting into SC4 solution (0.1 mM, left) and (0.25 mM, right): apparent reaction heat obtained from the integration of calorimetric traces.

RESULTS AND DISCUSSION

A set of ^{1}H NMR spectra were recorded keeping constant the BTA concentration, at 1 and 7 mM (Figure 1), at different SC4 concentrations.

In all cases, the guest protons were observed as a single resonance due to the fast exchange between a free guest and a complexed one on the NMR time scales;[20] therefore, the chemical shifts observed (δ_{obs}) for the BTA signals in the spectra are given by

$$\delta^{obs} = \chi^{l}\delta^{l} + \chi^{c}\delta^{c} = (1 - \chi^{c})\delta^{l} + \chi^{c}\delta^{c} \quad (1)$$

where χ^{f} and χ^{c} are the mole fraction of free and complexed BTA, respectively, and δ^{l} and δ^{c} are the chemical shifts for a specific signal of free and complexed BTA.

Defining chemical shift differences as $\Delta\delta^{obs} = \delta^{obs} - \delta^{l}$ and $\Delta\delta^{max} = \delta^{c} - \delta^{l}$ allows eq 1 to be expressed as

$$\Delta\delta^{obs} = \Delta\delta^{max}\chi^{c} \quad (2)$$

Since BTA forms a complex with 1:1 stoichiometry with SC4,[21] K_{obs} is defined as

$$K_{obs} = \frac{[BTA@SC4]}{[SC4][BTA]} \quad (3)$$

Solving the quadratic equation resulting from the insertion of the mass balance in eq 3, we can obtain the expression that depicts the complex concentration variation as a function of the total concentration of host and guest (eq 4)

$$[BTA@SC4] = \frac{K_{obs}([SC4]_0 + [BTA]_0 + 1)}{2K_{obs}} - \frac{\sqrt{\{(K_{obs}[SC4]_0 - K_{obs}[BTA]_0)^2 + 2K_{obs}([SC4]_0 + [BTA]_0) + 1\}}}{2K_{obs}} \quad (4)$$

The K_{obs} values have been determined through an iterative method (eqs 4 and 2), and the results have been fitted to the experimental values of δ^{obs}. The observed binding constants, K_{obs}, obtained for 1 and 7 mM were 3.25×10^5 and 1.7×10^4 M^{-1}, respectively. In order to corroborate these NMR data, we demonstrate by microcalorimetry that the observed binding constant of BTA by SC4 (K_{obs}) depends on the SC4 concentration. A representative titration curve is shown for 0.1 and 0.25 mM (Figure 2), and as can be seen, each titration of BTA into the sample cell gave an apparent reaction heat, caused by the formation of an inclusion complex between BTA and SC4.

The reaction heat decreases after each injection of BTA because less and less host molecules are available to form inclusion complexes. A control experiment was carried out in each run to determine the dilution heat by injecting BTA into Milli-Q water. The dilution heat determined for each run was subtracted from the apparent reaction heat measured in the titration experiments to give the net reaction heat. As can be seen in Figure 3, the K_{obs} values range from 3.05×10^5 to 3.40×10^4 M^{-1} by changing the SC4 concentration from 0.075 until 7 mM, and therefore corroborate the difference of K_{obs} obtained by NMR for the two selected concentrations of host.

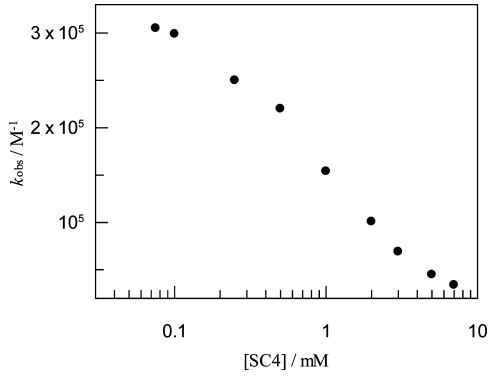

Figure 3. Influence of SC4 concentration on K_{obs} for the complex formation between BTA and SC4 at 25 °C. The K_{obs} values were obtained by microcalorimetric titrations and fitted to the "one set of binding sites" model.

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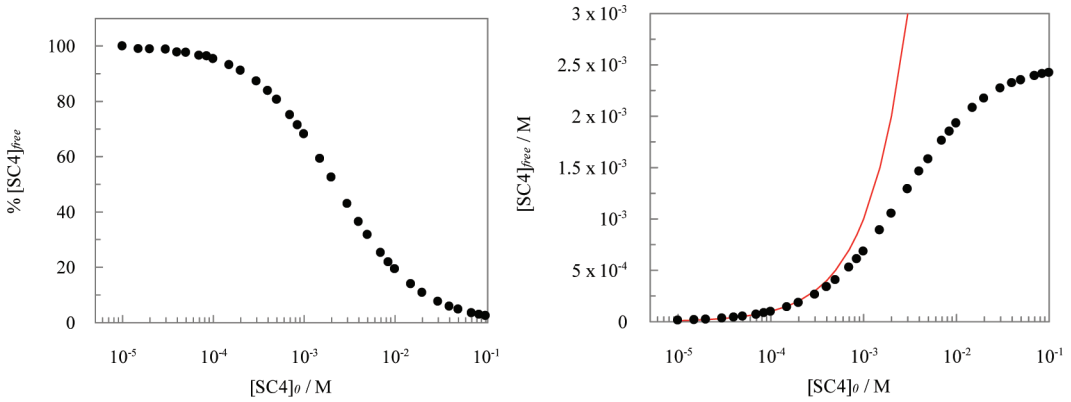

Figure 4. Percentage (left) and concentration of free SC4 (right) vs the total concentration of calixarene, $[SC4]_0$. The line represents the variation of the SC4 free vs the total concentration in the case where the complexation of Na^+ ions is neglected.

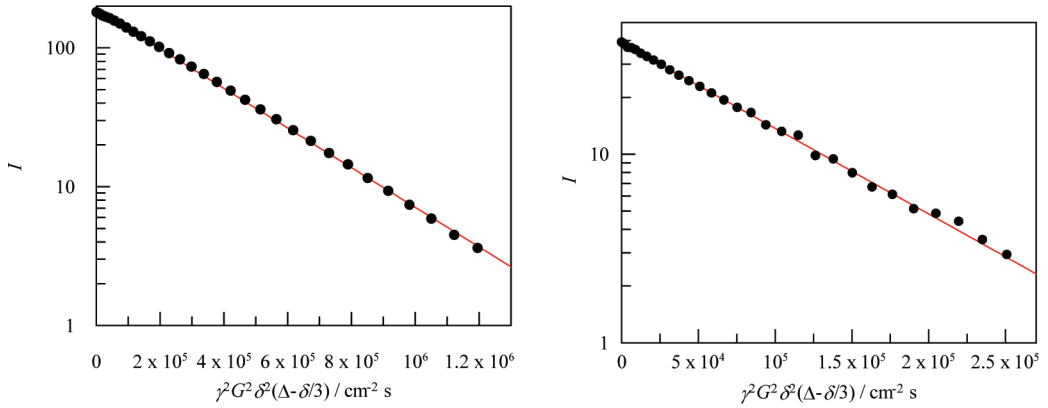

Figure 5. ^1H (left) and ^{23}Na (right) signal decay for a 10 mM SC4Na sample in D_2O at 25 °C. The solid line shows the nonlinear fit to the Stejskal–Tanner equation (eq 5).

The pH-metric titration curve of SC4 was measured in the presence of an equimolar concentration of BTA (3 mM), and values of pK_{a1} = 3.1 and pK_{a2} = 12.5 were obtained (see the Supporting Information, Figure S2). If we compare with the values obtained for the SC4 without adding BTA, pK_{a1} and pK_{a2} have a change of −0.5 and 0.6, respectively. Since the experiments were performed at neutral pH, this confirms that in the complexation the calixarenes do not release another proton that could yield a heat effect that interferes with the binding process.

In a previous work, we confirm by ^{23}Na diffusion NMR measurements that SC4 fully binds a Na^+ counterion[15] with a binding constant of K_{Na} = 100 M^{-1}. Knowing the equilibrium constant of Na^+, we can calculate the fraction (%) of SC4 free of Na^+ cations that exists in solution as a function of the total calixarene concentration (Figure 4). In the figure, we show how the percentage and the concentration of sodium free SC4 varies compared to the total concentration of the calixarene. As can be observed, the percentage of sodium free macrocycle decreases on increasing the total concentration of SC4. The absolute concentration of uncomplexed calixarene increases with increasing total concentration but not in a linear way.

However, much of the studies between a guest and SC4 have been done by varying the concentration of host which implies a change in the state of complexation of SC4, or in the presence of a buffer solution. For example, at 0.1 mM SC4, ca. 95% is free of Na^+ cations but for 2 mM of calixarene only 52% of SC4 is uncomplexed (Figure 4). Our results show that the binding constant decreases on increasing the calixarene concentration, which is in agreement that, at lower SC4 concentration, the fraction of Na^+ associated with SC4 is smaller than that at higher SC4 concentration, and therefore, changing the SC4 concentration changes the fraction of cation associated to SC4. Thus, to determine the value of the binding constant of BTA to SC4, the K_{obs} values should be extrapolated at infinite dilution of calixarene.

In the literature, the stoichiometry for the BTA@SC4 complex shows that SC4 can only accommodate one BTA molecule in its hydrophobic cavity. However, since inorganic cations can be complexed by SC4, it is important to demonstrate if the complexation of BTA by SC4 is an exchange process or if another stoichiometry might exist such as ternary complexes. To corroborate this hypothesis, we performed ^1H and ^{23}Na DOSY experiments on 10 mM SC4 solution to which BTA is added successively. In a previous work,[15] we demonstrated that in 10 mM SC4 solution ca. 8 mM exists in the form of Na@SC4 complex; therefore, if only a 1:1 complex between BTA and SC4 is present in solution, the diffusion coefficient of Na^+ should increase to a level close to that observed for free Na^+ when BTA is successively added.

Quantitative analysis of the intensity of a relevant echo peak in the diffusion spectrum provided the respective translational diffusion coefficient of the corresponding molecule or ion. This is achieved by nonlinear fitting of the signal intensity to the Stejskal–Tanner equation (eq 5) (ref 20 and references therein):

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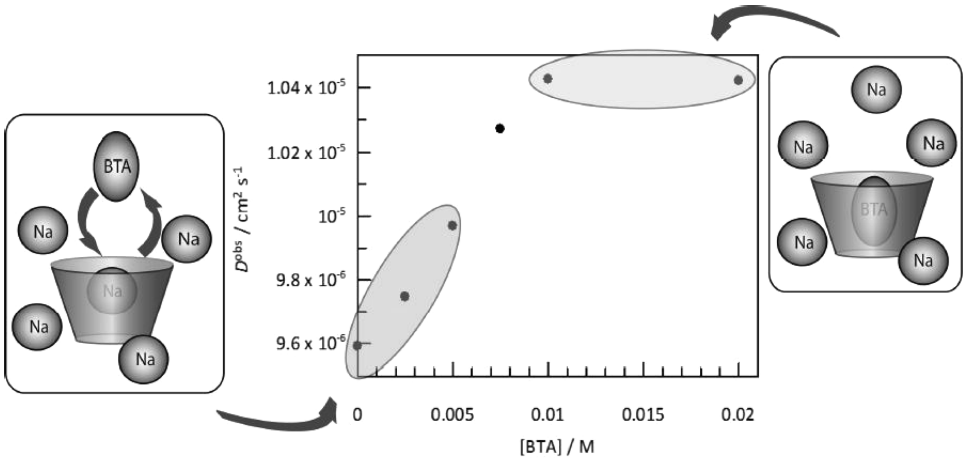


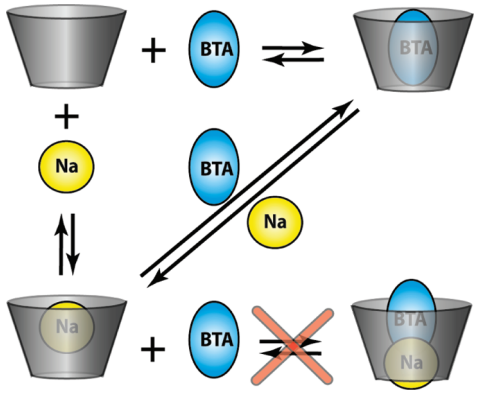
Figure 6. Influence of BTA concentration in the diffusion coefficient of Na^+ for a 10 mM sulfonatocalix[4]arene solution in D_2O at 25 °C.

$$I = I_0 \exp[-D\gamma^2G^2\delta^2(\Delta - \delta/3)] \quad (5)$$

where I is the measured signal intensity, I_0 is the signal intensity at the lowest gradient pulse power, γ is the magnetogyric ratio of the observed nucleus, and the rest of the parameters are defined above in the Experimental Section. In all experiments, the intensity decay of the signals gave good fits to the monoexponential eq 5, which shows that they represent a single self-diffusion coefficient. As an example, the experimental intensities and the respective nonlinear fit to eq 5 of a ^1H and ^{23}Na signal are shown in Figure 5.

The diffusion coefficient obtained for SC4 does not vary significantly when adding BTA; however, as shown in Figure 6, the observed diffusion coefficient, D_{obs}, for the Na^+ cation increases to a value close to that of free Na^+ in bulk solution, suggesting that Na^+ is replaced by BTA molecules inside the calixarene. This excludes the possibility of formation of ternary complexes and confirms both the formation of 1:1 complexes between BTA and SC4 and the presence of an exchange process (Scheme 1).

Scheme 1



Opposite experiments, i.e., BTA displacement from the cavity of the calixarene on adding sodium counterions, have been carried out by using the ^1H NMR signals of the trimethylammoniun group and confirm the hypothesis of an ionic exchange complexation (see below).

In an effort to gain further insights into the influence of Na^+ cation in the complexation of BTA by SC4, ^1H NMR and ITC experiments were performed to determine the influence of the cation concentration on the binding constants (Table 1). In the NMR experiments, different concentrations of NaCl were added to the experiments in which SC4 was successively added to a constant concentration of BTA. We chose to keep the BTA concentration constant instead of the SC4 concentration because despite changing the SC4 concentration we change the fraction of Na^+ concentration inside the calixarene; this is the titration method employed by other authors.[21] The K_{obs} values (Table 1, right) have been determined through an iterative method as explained above.

Table 1. Binding Constants (K_{obs}) Determined by ITC and NMR Experiments for 1:1 Intermolecular Complexation of BTA with SC4 at Different Added Na^+ Concentrations[a]

$[Na^+]_{add}$ (mM)	K_{obs} (M^{-1}) (ITC)	$[Na^+]_{add}$ (mM)	K_{obs} (M^{-1}) (NMR)
	220000 ± 780	10	185000
10	81700 ± 309	20	95000
20	54600 ± 184	50	45000
40	31000 ± 196	100	24500
80	17100 ± 253	200	11500
150	11000 ± 141	400	5200
300	5711 ± 89	700	2650
833	2060 ± 55	1000	1850
1666	1007 ± 67		

[a]ITC binding constants were obtained in experiments keeping constant the SC4 concentration, 0.5 mM, and varying the BTA concentration. NMR binding constants were obtained in experiments keeping constant the BTA concentration, 1 mM, and varying the SC4 concentration.

When we compare the K_{obs} obtained by NMR with ITC experiments, we can observe a discrepancy in the higher values of the binding constants obtained at a small concentration of added sodium cations (typically binding constants higher than 10^4 M^{-1}). This can be explained with the reliability of the K_{obs} determined by NMR experiments for the concentration of guest chosen, since for 1 mM of BTA the limit for direct NMR titration is about 10^4 M^{-1} and therefore the K_{obs} obtained above this limit can only be estimated. The problem with measuring large K_{obs} (>10^4 M^{-1}) is that there is no curvature in the $\Delta\delta$ versus $[H]_0/[G]_0$ plot at realistic reagent concentrations. The guest is completely complexed by any available host, and the graph therefore rises linearly with increasing $[H]_0$ until $\Delta\delta_{max}$ is reached at the 1:1 stoichiometry.[22,23]

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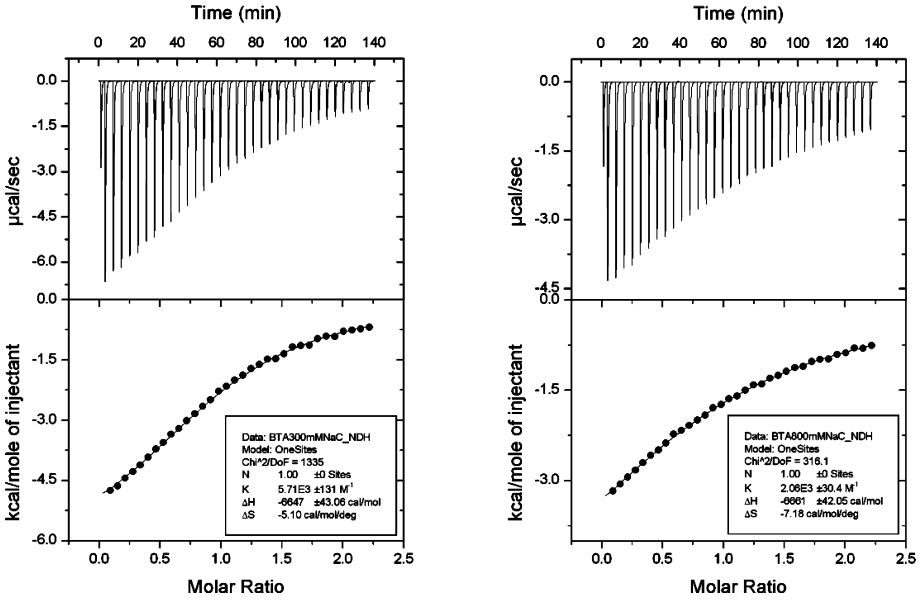

Figure 7. Microcalorimetric titration of BTA with SC4 at 25 °C: raw data for sequential 34 injections (8 μL per injection) of BTA solution (5.2 mM) injecting into SC4 solution (0.5 mM) in the presence of 300 mM NaCl (left) and 833 mM NaCl (right): apparent reaction heat obtained from the integration of calorimetric traces.

A representative titration curve obtained by ITC for the complexation of BTA by SC4 at different NaCl concentration is shown in Figure 7. We noted that at 150 mM of added NaCl (ITC experiments) the constant is similar to the literature when comparing the results obtained by other groups through NMR[21] and calorimetric[24] techniques using buffer solutions. We also performed titration in the presence of two different concentrations of phosphate buffer solution at pH 7.2. By changing the buffer concentration from 50 to 100 mM, the binding constant for the complex between SC4 and BTA is reduced by half (see the Supporting Information, Table S1 and Figure S3). These values are comparable with the change in the binding constant obtained when the experiments were performed in the presence of different NaCl concentrations.

In order to analyze the data in Table 1, we propose a complexation model where the Na^+ ions compete with BTA to be complexed by SC4 (Scheme 2).

Scheme 2

$$\text{BTA} + \text{SC4} \overset{K_{BTA}}{\rightleftharpoons} \text{BTA@SC4} \qquad (a)$$

$$Na^+ + \text{SC4} \overset{K_{Na}}{\rightleftharpoons} \text{Na@SC4} \qquad (b)$$

$$\text{BTA} + \text{Na@SC4} \overset{K_E}{\rightleftharpoons} \text{BTA@SC4} + Na^+ \qquad (c)$$

In this scheme, the exchange constant that measures the affinity of Na@SC4 for BTA, K_E, can be defined as K_{BTA}/K_{Na}. Moreover, according to the above scheme, the observed binding constant can be defined by eq 6.

$$K_{obs} = \frac{[\text{BTA@SC4}]}{[\text{BTA}]([\text{SC4}] + [\text{Na@SC4}])} \qquad (6)$$

Combining this equation with equations *a* and *b* from Scheme 2 and assuming that the added amount of sodium cation, $[Na^+]_0$, is much higher than the sodium cations supplied by the SC4, we can deduce the equation that defines the dependence of K_{obs} with Na^+ concentration:

$$K_{obs} = \frac{K_{BTA}}{(1 + K_{Na}[Na^+]_0)} \qquad (7)$$

$$\frac{1}{K_{obs}} = \frac{1}{K_{BTA}} + \frac{K_{Na^+}}{K_{BTA}}[Na^+]_0 \qquad (8)$$

Figure 8 (left) shows the influence of sodium concentration on the observed binding constant, K_{obs}, for BTA by SC4 for experiments carried out by ITC. Figure 8 (right) shows a plot of the inverse of K_{obs} against Na^+ concentration according to eq 8. From the linear fit of the experimental data to eq 8, we get $K_{Na} = 138\ M^{-1}$, which is compatible with the value reported in the literature[15] and obtained from ^{23}Na DOSY experiments. The value obtained for K_{BTA} was $3.29 \times 10^5\ M^{-1}$. The curve-fitting analysis of K_{obs} obtained by NMR experiments at different Na^+ concentrations (see the Supporting Information, Figure S5) is comparable with the results obtained by ITC, since the association constant obtained for K_{BTA} was 3.28×10^5 and 100 M^{-1} for K_{Na} despite the accuracy of the K_{obs} obtained by this technique.

In order to test the validity of the ion-exchange model for BTA complexation by SC4, the influence of adding NaCl on the ^1H NMR signals of BTA was studied. For this experiment, we add NaCl to an equimolar solution of BTA and SC4 (4 mM) and a downfield shift of the signals is observed. The chemical shift of the trimethylammoniun group increases on increasing the concentration of sodium counterions due to its expulsion to the bulk water (Figure 9).

This behavior is the opposite to that shown in Figure 1 and is consistent with an ionic exchange where sodium complexation by the calixarene compels BTA out of the cavity. Without adding NaCl, the δ value of the $N(CH_3)_3$ headgroup of BTA has an appreciably upfield shift after complexation ($|\Delta\delta| = 1.73$ ppm), but adding NaCl to the equimolar mixture of SC4 and BTA, the δ value of $N(CH_3)_3$ shows a smaller upfield shift ($|\Delta\delta| = 1.19$ ppm at 1.8 M of NaCl added). From a quantitative

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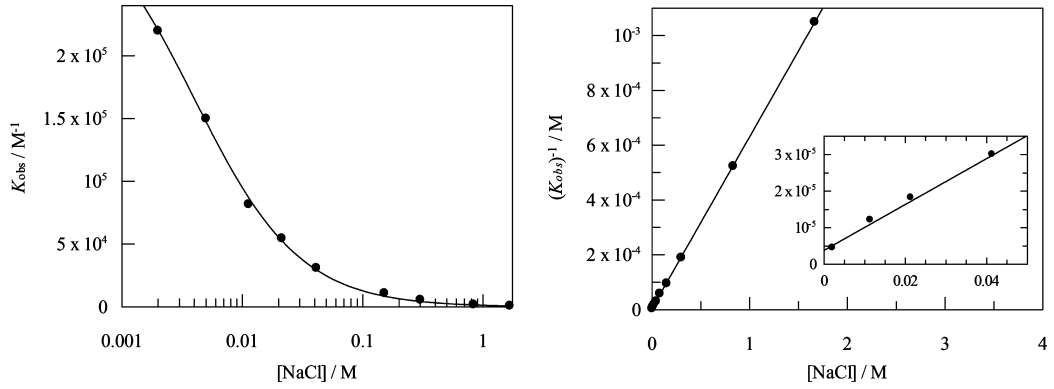

Figure 8. Influence of NaCl concentration in K_{obs} (left) and $1/K_{obs}$ (right) for the complex formation between BTA and SC4. The K_{obs} was obtained by microcalorimetric titration and fitted to the "one set of binding sites" model.

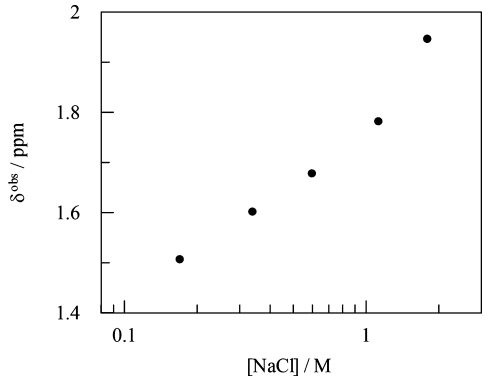

Figure 9. Chemical shift changes for the trimethylammonium group signal of BTA (4 mM) in the presence of SC4 (4 mM) vs NaCl concentration in D_2O at 25 °C. A coaxial tube with DMSO-d_6 was used as the external standard.

point of view and by using the BTA and sodium binding constants, $K_{BTA} = 3.28 \times 10^5$ M^{-1} and $K_{Na} = 100$ M^{-1}, and the total concentration of SC4 and BTA (4 mM), we can calculate that the 30% of BTA is displaced from the calixarene in the presence of 1.8 M NaCl. For this displacement, a chemical shift for the trimethylammonium group of BTA close to $\delta^{obs} \approx 2$ should be expected, as is observed in Figure 9. This indicates that BTA is being expelled from the SC4 cavity by exchange with Na^+ cation.

CONCLUSIONS

Our results show that complexation of organic cations by sulfonatocalixarenes should be considered as an ion-exchange equilibrium with the counterions of SC4. Increasing the calixarene concentration leads to an increment of the counterions present in solution, and consequently, the fraction of cation-free calixarene decreases. As a consequence, the observed binding constant of organic cations by SC4 decreases both on increasing SC4 or added Na^+ concentration. According to these findings, the binding constants of cations to sulfonatocalixarenes obtained by varying the host concentration or in the presence of buffers or other salt sources should be revised. In order to get the true binding constants, experimental results should be extrapolated to zero sulfonatocalixarene concentration.

ASSOCIATED CONTENT

Supporting Information

ESI-MS spectrum for SC4, potentiometric titration in the presence and absence of BTA, and binding constants obtained by ITC at different calixarene concentrations and different salt concentrations (obtained by NMR). This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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