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Conference paper

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Counterion effect on sulfonatocalix[n]arene recognition

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Abstract: Sulfonatocalixarenes, like other ionic receptors, possess counterions that can affect the molecular recognition process. In the present review it is shown that the competitive effect of the alkaline cations frequently used as counterions determines not only the magnitude of the external guest association constant, but also the stoichiometry of the complexes. Experimental evidences are shown about the interaction of the counterions with sulfonatocalixarene, allowing to quantify its association equilibrium constants. The counterions recognition will be a competitive process that must be taken into account when investigating the interaction of calixarenes with an external guests. When the external guest is a neutral molecule it will be possible to form ternary complexes where the counterion shows a competitive and cooperative effect. By increasing the size of the receptor, sulfonatocalix[6] and sulfonatocalix[8]arene, the complexity of the system is increased due to the formation of counterion complexes with stoichiometries 1:1 and 1:2. In the presence of an external guest, the formation of heteroternary complexes with 1:1:1 stoichiometries including a counterion and an organic cation will be possible.

Keywords: calixarenes; host-guest chemistry; ICPOC-24; supramolecular chemistry.

Introduction

Calixarenes are a synthetically versatile class of macrocycles with high conformational flexibility that can accommodate a wide range of guest molecules [1, 2]. Applications of calixarenes were relatively limited due to their hydrophobic property and poor water solubility. Synthesis of water soluble calixarenes by sulfonation at the upper rims, esterification or etherification at the lower rim and functionalization of polar groups onto the edge of calixarenes were developed [3–5]. Sulfonatocalix[n]arenes (SCn) show many advantageous features as compared with other calixarene derivatives as (i) SCn can be prepared easily through the direct sulfonation of calixarenes at the upper rim; (ii) SCn are particularly attractive for the complexation of positively charged organic and inorganic guest molecules that can be stabilized by ionic, cation-π, CH–π, and π–π, hydrophobic and hydrogen bond interactions depending on the guest [6]; (iii) the sulfonate groups on the upper rim can provide the binding site towards cations through electrostatic interaction that further increase the binding interactions [7–10]; (iv) sulfonatocalixarenes also possess good water solubility and biocompatibility that

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are necessary properties for their potential biological and pharmaceutical applications. Besides being very useful models to investigate fundamental aspects of supramolecular chemistry, SCn have proven to be highly attractive building blocks for sensing and supramolecular self-assembly applications including displacement assays for monitoring enzymatic activity, supramolecular polymers, supra-amphiphiles, etc. [11–21]. Given the increasing interest and applications of these compounds, detailed knowledge of their biding mechanisms is mandatory for optimization and development of host:guest systems with increased binding affinity and selectivity. Knowledge of the absolute binding constants and its thermodynamics with salts, in particular alkali metal cations, is of exceptional practical importance because binding of alkali metal cations would interfere with any biological or environmental application.

The vast majority of molecular components that are designed to assemble into supramolecular complex [22] and mechanoestereochemical systems [23, 24] possess typically one or more formally charged atoms in either the host or the guest, or indeed in both. These charged species actively participate, with their associated counterions, in the assembly of higher ordered systems. Competition between the host and counterion for binding sites on cationic guests can also contribute to the observed counterion effects. If the counterions, such as PF_6^- are weakly coordinating, then rigorous quantitative studies have revealed [25] that host:guest complexation is relatively unaffected by the presence of the counterion, indicating that no competition exists between the host and counterion for the guest. Of course, these findings are specific to the host, guest, counterion, and the conditions of the investigation, and so each system needs to be studied individually to determine competitive effects, if any, of the counterion. If ion-pair dissociation must first take place prior to host:guest binding [26, 27], then a competition must exist between the host and the counterion for the guest species, which may then contribute significantly to the observed host:guest binding phenomena.

Sulfonatocalix[n]arenes have n acidic sulfonate groups as well as acidic phenolic ones. Phenolic hydroxyl groups in SCn form strong intramolecular hydrogen bonds [28–32] resulting in quite different acid dissociation constants for these OH groups in comparison to those of the corresponding noncyclic analogs. Consequently, SCn exist as polyions at neutral pH, so that any application and measurements require the presence of counterions, routinely sodium. Interaction of SCn with alkali metal cations has a rich and controversial scientific history. In this review we will focus on our recent studies providing irrefutable evidences of such interaction showing its influence on the recognition ability of SCn.

Evidence for counterion recognition

Alkali metal cations were shown to bind sulfonatocalix[4]arene (SC4) by fluorescence displacement assays in 2005 [33]. This complexation prevents measurement of the true binding constants with other guests, and this was suggested as a likely explanation for the observed large spread in binding constants for amino acids [34–39].

Direct evidence for SC4 counterion complexation was firstly reported in 2010 by ^{23}Na diffusion NMR experiments [40]. Observed Na^+ diffusion coefficients at different NaCl concentrations, D_{Na}^{NaCl}, are independent of sodium concentration as reflected by Fig. 1. The diffusion coefficients for the sulfonatocalix[4]arene, D_H^{SC4}, and sodium, D_{Na}^{SC4}, for different SC4 concentrations are reported in Fig. 1. Note that these diffusion coefficients, D_H^{SC4} and D_{Na}^{SC4}, were obtained in the absence of added NaCl. Calixarene diffusion coefficients are unaffected by SC4 concentration ruling out the possibility of self-aggregation [41–43]. It deserves to be highlighted that sodium cation diffusion coefficient in SC4 solutions, D_{Na}^{SC4}, are smaller than those obtained in NaCl solutions, D_{Na}^{NaCl}, and decreases on increasing the sulfonatocalixarene concentration supporting the complexation of Na^+ by the calixarene.

Results from Fig. 1 indicate that SC4 should not be fully dissociated, but rather a portion of the Na^+ counterions should be bound by the macrocycle neutralizing a fraction of the negative charge. Assuming the formation of the 1:1 complex between SC4 and Na^+ being fast in the NMR timescale, the observed diffusion coefficient can be expressed as a molar average of free and bound species. On the assumption that the

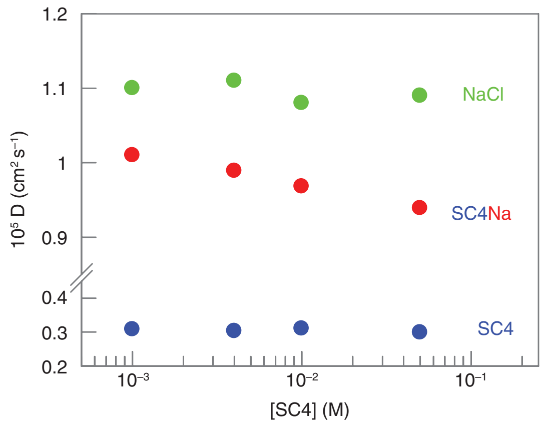


Fig. 1: Influence of SC4 concentration on the observed diffusion coefficient of (●) Na, D_{Na}^{SC4}, and (●) calixarene, D_{H}^{SC4}, in D_2O at 25 °C. For comparative purposes diffusion coefficients of Na^+, D_{Na}^{NaCl}, coming from NaCl solutions (●) are plotted.

diffusion coefficient for complexed Na^+ is the same as that for SC4, which is true when the hydrodynamic radius of the host is not changed by guest complexation, the fraction of bound sodium cations were obtained, allowing a binding constant, $K_{Na} = 100\ M^{-1}$ to be obtained.

True affinities of metal cations to SC4

Thermodynamic parameters for the complexation of Na^+, as well as other monovalent and divalent inorganic cations, were obtained by use of isothermal titration calorimetry (ITC). This technique provides [44] the complex stability constant as well as the changes in Gibbs free energy, enthalpy, and entropy in a single experiment. A solution of NaCl was sequentially injected into a SC4 solution into the sample cell of the calorimeter. Since the binding constants for all monovalent cations are significant but the related heat effects for complexation are very small, the experimental conditions must be carefully selected such that the total concentration of SC4 is sufficiently low to allow for a relatively high percentage of uncomplexed SC4, but sufficiently large to afford detectable absolute heats of complexation. Experimental conditions have been optimized for [SC4] = 0.5 mM where approximately 30 % of the host is occupied (see Fig. 2) affecting both the binding constant and the thermodynamic parameters.

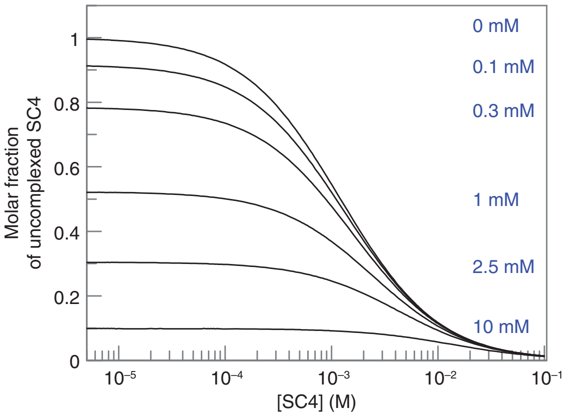


Fig. 2: Molar fraction of uncomplexed SC4 as a function of total sulfonatocalixarene concentration in the absence and presence of added Na^+ cations. Concentrations of added Na^+ are 0; 0.1 mM; 0.3 mM; 1 mM; 2.5 mM and 10 mM.

The unavoidable presence of counterions leads to a fraction of SC4 being already complexed by Na^+ even before addition of NaCl solution. Obviously, SC4 molecules already complexed with a sodium ion will not afford any heat effect on addition of sodium, so that the heat effect must be normalized for the fraction of uncomplexed calixarene (and uncomplexed sodium cations), which can be obtained from the following mass balance equations [45].

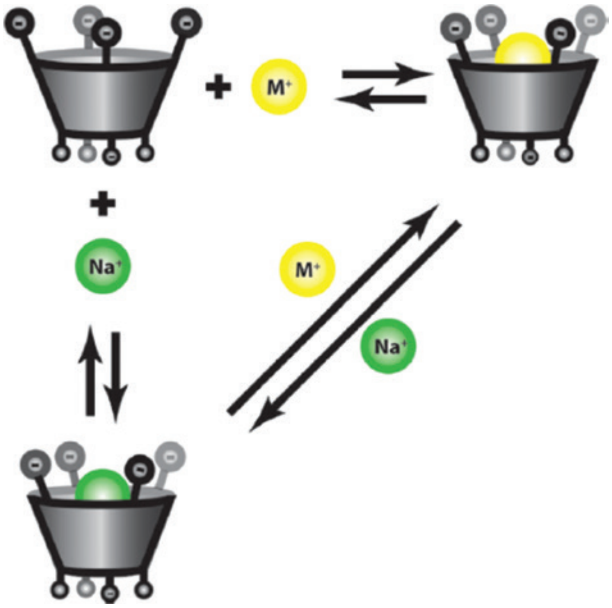
$$[\mathrm{SC4}]_\mathrm{T} = [\mathrm{SC4}]_\mathrm{Free} + [\mathrm{SC4@Na}] = [\mathrm{SC4}]_\mathrm{Free}(1 + K_\mathrm{Na}[\mathrm{Na}]_\mathrm{Free}) \tag{1}$$

$$[\mathrm{Na}]_\mathrm{T} = [\mathrm{Na}]_\mathrm{Free} + [\mathrm{SC4@Na}] = [\mathrm{Na}]_\mathrm{Free}(1 + K_\mathrm{Na}[\mathrm{SC4}]_\mathrm{Free}) \tag{2}$$

This nonlinear system of coupled equations was solved by using the Newton-Raphson algorithm allowing a value of $K_{Na} = 183\ M^{-1}$ as the true binding constant of SC4 with Na^+. Since the thermodynamic parameters obtained by the refined model afford positive values of $\Delta H° = 7.1$ kJ/mol and $T\Delta S° = 20.0$ kJ/mol, complex formation is entropically driven, and the binding constant is expected to increase with temperature. However K_{Na} values decrease from $K_{Na} = 275\ M^{-1}$ at 5 °C to $K_{Na} = 143\ M^{-1}$ at 65 °C with positive enthalpy and entropy contributions. The change in enthalpy and entropy is presumably related to the ordered water structure surrounding the cation and the sulfonate groups of SC4 that partially breaks down on increasing the temperature.

In contrast to the complexation of Na^+ by SC4 with Na^+ as counterion, the complexation of other cations by SC4 must take into account that the ions of the titrating solution compete with the Na^+ counterions in the binding to SC4 cavity, so that formally one ion is exchanged with or displaced by the other in the course of the titration. This displacement, and also the stoichiometric cation binding, is best rationalized through the involvement of an inclusion complex between the inorganic cations and SC4 (Scheme 1). An ion-exchange equilibrium between SC4 counterions and added alkali metal cations has also been demonstrated by self-diffusion NMR experiments [40].

Binding constants for SC4 complexation of mono- and divalent cations are reported in Table 1. The stronger binding of Cs^+ than Li^+ suggests that solvation and ionic radii of the cations influence the binding constant, since the smaller, better-solvated cations have a lower affinity than the bigger, less-solvated ones. The positive enthalpy and entropy changes for the association of the mono- and divalent metal cations



Scheme 1: Mechanism for counterion exchange in SC4 recognition. Reprinted with permission from Ref. [45]. Copyright 2013 Wiley-VCH.

Table 1: Binding constants for inorganic metal cations to SC4 at 25 °C and neutral pH.

Cation	Li^+	Na^+	K^+	Rb^+	Cs^+	Ag^+	Ni^{2+}	Zn^{2+}	Cu^{2+}
K_M/M^{-1}	139	183	217	253	760	271	5560	5580	5650

Error estimated for K_M being 5 %.

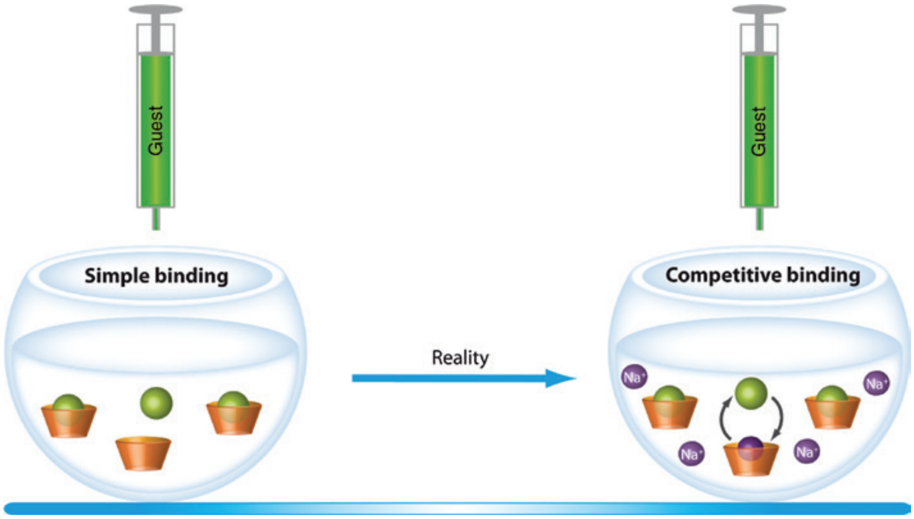
originate from the partial desolvation of M^+ and SO_3^- on interaction and from the concomitant release of water molecules.

A first inspection shows that sodium cation, the most widely used for the preparation of buffers, has a small, but not negligible, complexation constant with SC4 ($K_{Na} = 183$ M^{-1} at neutral pH). This result should not be considered as a purely mechanistic peculiarity, but it has important repercussions when it comes to interpreting molecular recognition processes. This repercussion is determined by the fact that the binding of sodium ions to SC4 has generally been neglected and numerous association constants have been determined experimentally in the presence of buffers (in the presence of up to 0.20 M of Na^+). It is also important to note that sodium cations are generally present, at least as counterions, regardless of the use of buffer solutions. All of these binding constants for many different guests, which have been compiled and reviewed for SC4 and its homologues [6], are only apparent ones [46–51] and can only be compared in relative terms, and only when measured under identical concentrations of buffer, and, strictly speaking, at identical concentrations of SC4 (which itself supplies additional counterions). That the sodium concentration increases in the course of direct host:guest titrations due to the addition of SC4 is another complication. Small changes in salt concentrations have very large effects on the binding constants, and the comparison of binding constants of different guests may even be misleading if the same buffer, but in different concentrations (e.g. 10 vs. 100 mM) have been used.

It should be noted especially that the thermodynamic magnitudes corresponding to the processes of molecular recognition by SC4 are incorrect in absolute terms. The values presented in the bibliography for ΔH and ΔS do not correspond to the enthalpy and entropy variations associated with the recognition process, but must be interpreted as the values corresponding to the interaction of both the guest object of study and the calixaren counterion. Direct interpretations of the guest binding, for example, in terms of intermolecular interactions or desolvation, are not possible, and relative trends are only meaningful if the buffer concentration and also the SC4 concentration are both the same. The omnipresent binding of counterions reveals a pitfall of ITC measurements with SC4 as host: Instead of direct host:guest titrations, competitive ones are actually being performed but analyzed according to a direct binding model. The observation of negligible heat effects for a particular guest may actually indicate that the binding is endothermic, and the thermoneutral binding of a guest actually results in a net heat release.

Counterions influence on external guest recognition

Our hypothesis is that, if Na^+ binds to the SC4, then a competition between the guest and the counterions should be considered (Scheme 2). As a result, the binding constants should be affected by the SC4 concentration. Reported binding constants in the literature have been obtained by titration methods (calorimetric, NMR, fluorescence, UV-Vis absorption, etc.) where the host concentration is varied by keeping the guest concentration constant. As can be clearly observed from Fig. 2 this method is not suitable because the percentage of uncomplexed SC4 available to act as a receptor changes on changing its concentration. As a consequence, the obtained binding constants are underestimated. This situation is well reflected by Fig. 3 where benzyltrimethylammonium cation (BTA) recognition by SC4 was studied by using different experimental conditions: (i) SC4 titration of a 1 mM BTA solution; (ii) SC4 titration of 7 mM BTA solution and (iii) SC4 titration of 1 mM BTA solution in the presence of 1.0 M of added NaCl [52]. Titration plots yield binding constants of 9.5×10^4 M^{-1} and 1.7×10^4 M^{-1} for [BTA] = 1 mM and 7 mM, respectively. Note that SC4 concentrations for both titrations



Scheme 2: External cationic guest complexation by SC4 should be considered as a competitive process. Reprinted with permission from Ref. [45]. Copyright 2013 Wiley-VCH.

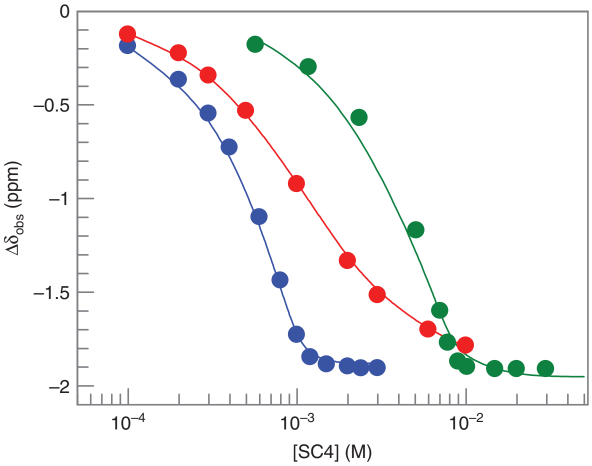


Fig. 3: Plots of $\Delta\delta_{obs}$ (ppm) for trimethylammonium group signal of BTA as a function of SC4 concentration in D_2O at 25.0 °C. (●) [BTA] = 1 mM; (●) [BTA] = 1 mM and [NaCl] = 1.0 M; (●) [BTA] = 7 mM.

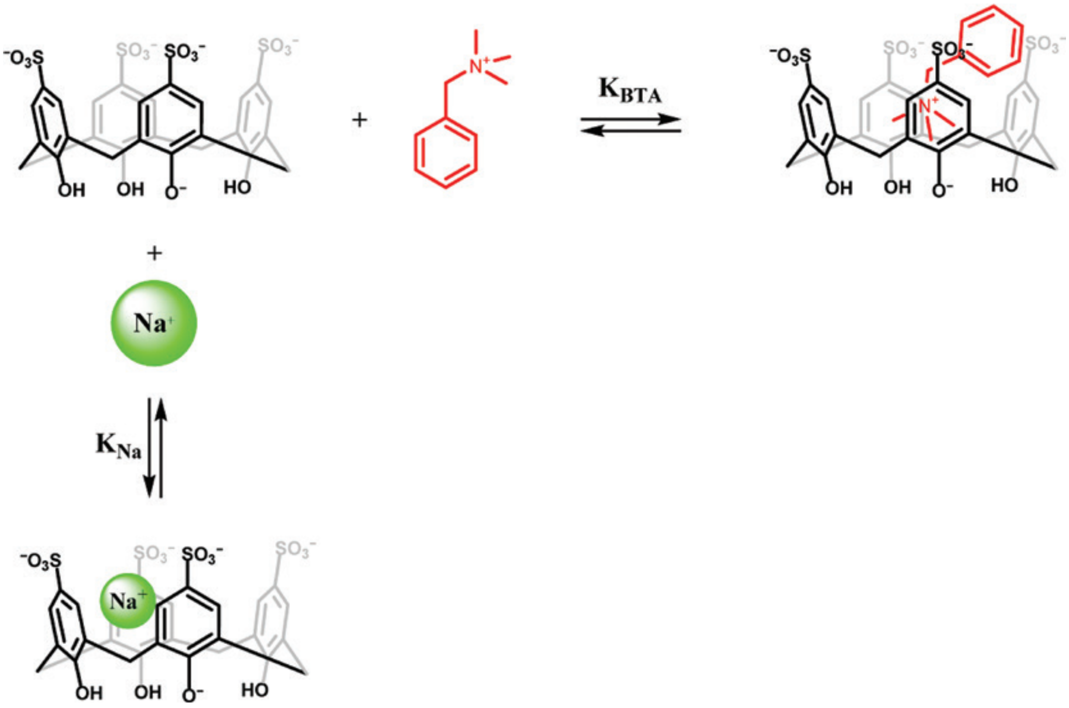
range from 0.1 mM to 10 mM in such a way that the percentage of uncomplexed calixarene decreases from 95 % to 15 %. This discrepancy in molar faction of calixarene available for complexation is the reason for the different BTA binding constants. Additionally, an NMR titration of the 1 mM BTA solution by SC4 was carried out in the presence of [NaCl] = 1.0 M yielding a binding constant of 1.86×10^3 M^{-1}. Note that in the presence of [NaCl] = 1.0 M all the SC4 is occupied by sodium counterions.

This situation can be avoided by carrying out experiments where the host concentration remains constant and the guest concentration is varied. Because the competitive counterion complexation, experiments should be carried out at different host concentrations and extrapolated to zero host concentration in order to obtain the true binding constants. This procedure has been applied by ITC experiments resulting in different binding constants for different calixarene concentrations (BTA binding constants decrease from 3.05×10^5 M^{-1} to 3.40×10^4 M^{-1} by changing the SC4 concentration from 0.075 mM to 7 mM). 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. In cases where experiments at different host concentrations can be performed and extrapolated to zero host concentration, the procedure is tedious because a large number of titrations should be necessary to report a true binding constant. Discrepancies between binding constants obtained under different experimental conditions are a consequence that the single complexation model does not take into account previous evidence of counterion complexation. In this way a competitive complexation scheme should be considered (Scheme 3) where both Na^+ and BTA compete for the SC4 cavity in a competitive way simultaneously [53].

An unique value of $K_{BTA} = (3.3 \pm 0.1) \times 10^5\ M^{-1}$ was obtained from a global fit of experiments in Fig. 3 by using a sodium cation binding constant of $K_{Na} = 183\ M^{-1}$. Note that the true binding constant can be obtained from just one titration by considering the competitive counterion complexation.

In addition to competitive effects, the formation of ternary complexes between SC4, selected organic guests and transition metal ions was observed in solution and in the solid state [54–56]. This behavior presents a parallelism with another feature of SC4: its ability to shift the pKa of complexed guest molecules [46]. While pKa shifts are expected due to the ability of this host molecule to preferentially stabilize cationic over neutral forms, the formation of ternary complexes with Lewis acids is subtler due to the larger size of the cation and geometric requirements for metal-ligand bond formation. In fact, it was demonstrated that the successful formation of such ternary complexes strongly depends on the structure of the guest and on the nature of the metal cation leading to a subtle and interesting interplay between cooperative and competitive binding. Such requirement imply that the formation of the ternary complexes should proceed with high selectivity and, therefore, present high potential to improve the binding ability of SC4 and its applications in catalysis, sensing, and supramolecular self-assembled materials and devices. Formation of ternary complexes should not be considered as an exception, but rather as a general behavior when neutral molecules bearing electrodonating groups are bounded to SC4 cavities.

Formation of 1:1:1 ternary complexes can be described by using 2-chloropyridine (2CP) as a guest. Figure 4 shows the tentative fitting of NMR titration data by using a competitive binding model with $K_{Na} = 183\ M^{-1}$. It should be noted that this model fails to explain the experimental behavior with the calculated induced chemical shifts, $\Delta\delta_{obs}$, being larger than the experimental ones.



Scheme 3: Competitive complexation of Na^+ and BTA with SC4.

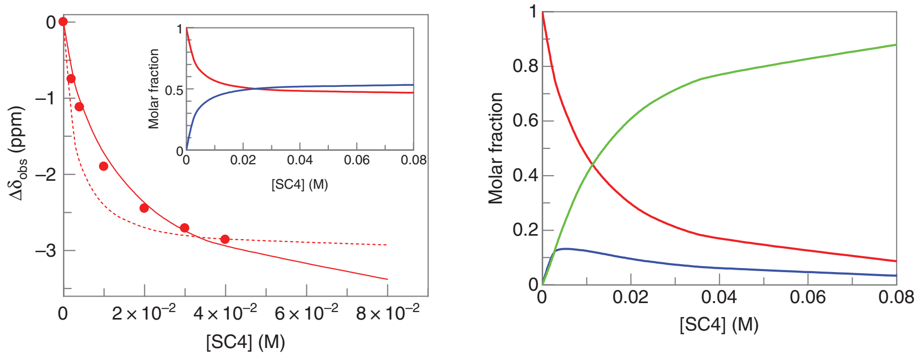


Fig. 4: (Left) Plot of $\Delta\delta_{obs}$ (ppm) for the H4 resonance signal of 2CP vs. SC4 concentration. Dotted and solid lines correspond to the competitive and competitive-cooperative binding models complexation. Insert shows the calculated molar fraction of uncomplexed 2CP (red) and host:guest complex (blue). (Right) Calculated molar fraction of uncomplexed 2CP (red); 1:1 complex (blue) and 1:1:1 complex (green).

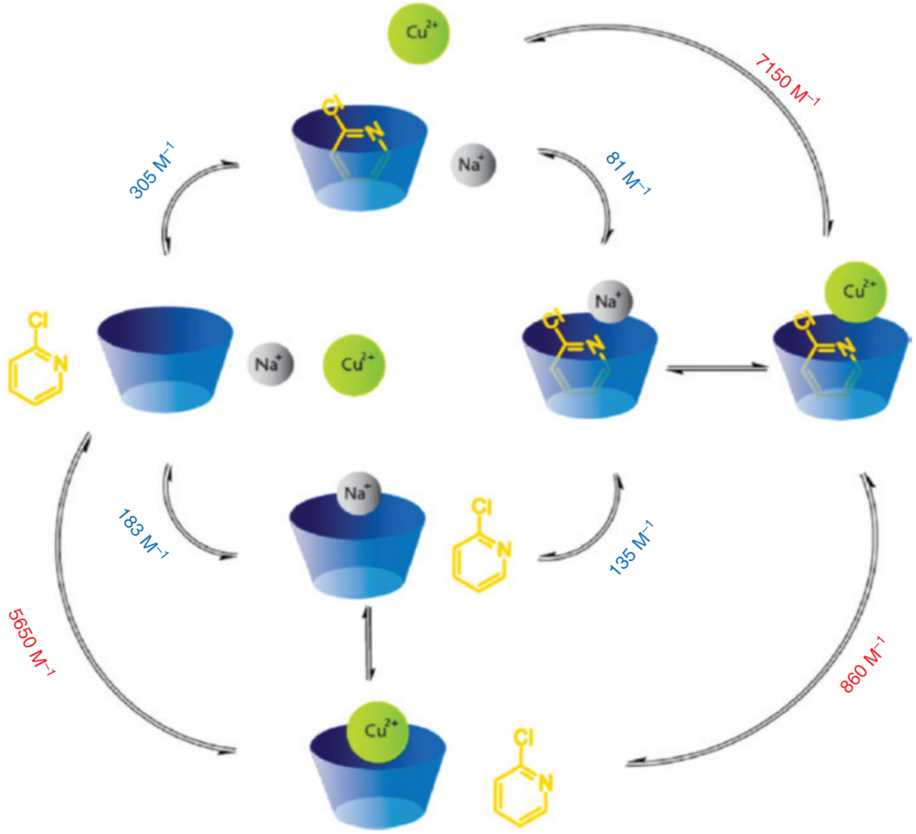
The calculated magnitude for the complexation induced upfield effect for H4 of 2CP should be close to a value of $\Delta\delta_{obs} = -3.30$ ppm contrasting with the calculated value of $\Delta\delta_{obs} = -5.50$ ppm according to the competitive binding model. The failure of this model is a consequence of the decreasing amount of 2CP reaching the SC4 cavity resulting from the competitive Na^+ complexation. According to a single binding model (an oversimplified model that ignores the role of the counterions) almost 90 % of 2CP is bound to the calixarene for [SC4] = 80 mM. The competitive binding model implies that the SC4 cavity can be filled both by Na^+ or 2CP in such a way that only 50 % of 2CP is bound for [SC4] = 80 mM. Failure of the competitive binding model has also been evidenced by ITC experiments where the 2CP complexation by SC4 has been studied at different NaCl concentrations [57].

Experimental results can be explained by considering the formation of a ternary complex between SC4, Na^+ and 2CP yielding binding constants for the binary, $K_{1:1} = 305\ M^{-1}$, and ternary, $K_{1:1:1} = 135\ M^{-1}$, complexes (see solid line in Fig. 4, left). Figure 4, right shows the molar fraction of 2CP present as uncomplexed, 1:1 and 1:1:1 complexes as a function of SC4 concentration. It should be noted that this model predicts that almost 85 % of pyridine is bound at [SC4] > 80 mM. Note that the ternary complex is the predominant one on increasing the calixarene concentration because the large increase in counterion concentration. Formation of ternary complexes by sulfonatocalix[4]arene is a widely unexplored behavior mainly because a large number of them have been considered as just 1:1 complexes in a single binding model ignoring the role of the counterion.

Addition of Cu^{2+} to the complex formed between SC4 and 2CP indicate that chloropyridine is more effectively complexed by SC4 in the presence of Cu^{2+} due to the formation of the metal-ligand bond in the ternary complex [57], as was also observed for bicyclic azoalkanes [54, 55]. This is in contrast with behavior observed for Na^+, where the formation of the ternary complex is less favored in comparison with the respective binary complexes as a result of the very low $2CP{\bullet}Na^+$ interactions that are not effective enough to overcome the repulsive interactions associated with the formation of the ternary complex. An example of a small dynamic library of five different complexes that can be pushed toward the selective formation of one species by adjusting the concentration of the reactants is shown in Scheme 4.

Larger calix[n]arenes. A more complicated picture

Larger sulfonatocalixarenes, SC6 and SC8, form 1:1 and 1:2 host:guest complexes with the lucigenin fluorescence probe. This dye is particularly attractive for investigating host:guest complexation because its high fluorescence quantum yield enables the study of the association process in the μM concentration range [12]. Such conditions are ideal for investigating sulfonatocalixarene binding mechanisms at negligible concentration of non-spectator counterions. Formation of 1:1 and 1:2 host:guest complexes has been occasionally reported for systems comprising SC6 and SC8 receptors and cationic organic guests, but most of the studies



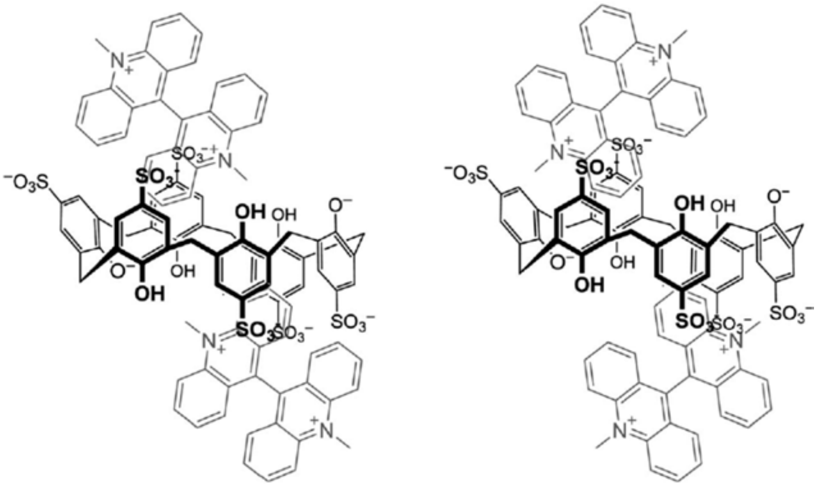
Scheme 4: Cooperative and anticooperative effect in cation recognition by SC4:Chloropyridine host:guest complexes. Adapted with permission from Ref. [57]. Copyright 2014 American Chemical Society.

report 1:1 stoichiometries. For both calixarenes, the value of the binding constant for the complexation of the second lucigenin molecule ($K_{1:2}$) is lower than $K_{1:1}/4$ suggesting negative cooperativity [58]. This observation can be rationalized by taking into account that the negative charge of the host is partially neutralized upon binding of the first guest molecule and the electrostatic repulsion between the two complexed molecules.

The ^{1}H NMR spectrum of SC6 in D_2O and in the absence of additives is composed by only two peaks: one corresponding to the aromatic protons and the other to the methylene protons. This is a result of the high flexibility of this molecule that exchanges rapidly between several possible conformations. In the presence of excess lucigenin, the signal corresponding to the calixarene aromatic protons splits into three 1:1:1 singlets and the signal corresponding to the methylene bridges splits into two doublets and one singlet (1:1:1). This pattern, only observed in the presence of the guest in excess, is assigned to the conformational freezing of the calixarene structure into the 1,2,3-alternate conformation upon the formation of the 1:2 host:guest complex (Scheme 5).

The host:guest binding pair formed between the fluorescent probe and anionic calixarenes can be used to investigate the complexation of other spectroscopically silent species by taking advantage of the lucigenin fluorescence regeneration upon competitive dissociation of the complex [59]. As already mentioned, this class of hosts can form complexes with a variety of metallic cations including sodium. Despite being already detected, the formation of complexes between alkaline metal cations and both SC6 and SC8 has not been properly quantified [40, 51].

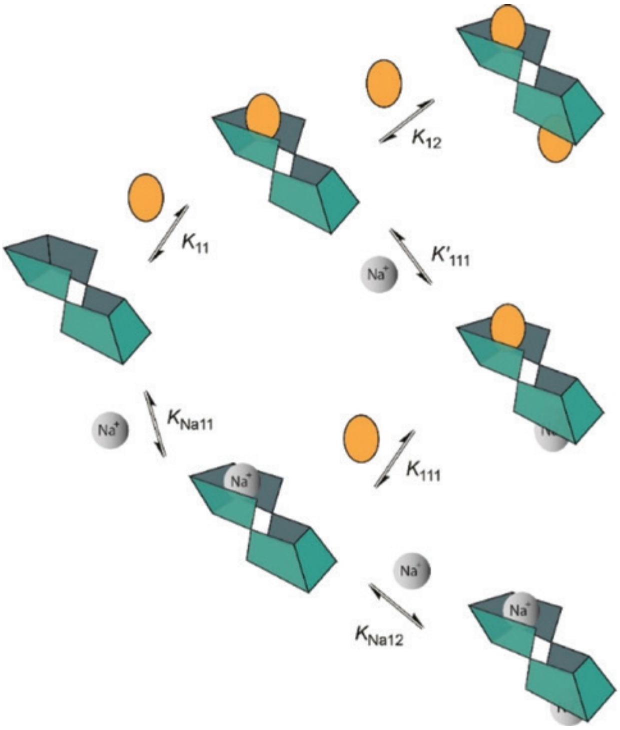
Previously ^{23}Na DOSY experiments provided evidence for the formation of 1:1 and 1:2 SC6:Na^+ complexes [40]. Because SC6 and SC8 have the ability to form 1:1 and 1:2 host:guest complexes with both lucigenin and metal cations, the formation of heteroternary 1:1:1 lucigenin:SC6:metal complexes cannot be discarded *a priori* and was experimentally confirmed [60]. It is worth noting that this stoichiometry was previously observed in solution in the case of SC4 with neutral organic molecules and metal cations [53–55, 57]. Conceptually, it



Scheme 5: Binding mode of lucigenin to SC6. Reproduced from Ref. [60] with permission from the PCCP Owner Societies.

is also reasonable to expect the formation of the heteroternary complex because, if the 1:1 and 1:2 complexes are formed with both lucigenin and the metal cations, in principle there are no structural or thermodynamic reasons to discard the formation of the 1:1:1 species. Owing to the structural features of SC6 which in the 1,2,3-alternate conformation can be assumed to display two independent binding sites for small cations, the proposed host:guest complexation mechanism is shown in Scheme 6.

The obtained binding constants for the complexation of sodium cation with SC6 and SC8 are reported in Table 2. Values obtained for SC8 are higher than those for SC6 which, in turn, are higher than those obtained for SC4. The higher stability of the complexes formed with a highly charged host is consistent with the role of Coulombic interactions as the main driving force for the formation of SCn:metal cation complexes. The



Scheme 6: Multiple complexation by larger Sulfonatocalixarenes. Reproduced from Ref. [60] with permission from the PCCP Owner Societies.

Table 2: Binding constants for sodium cation to SCn at 25 °C and neutral pH.

	$K_{SCn:Na}$/M^{-1}	$K_{SCN:2Na}$/M^{-1}
SC4	$(1.83\pm0.1)\times10^{2}$	–
SC6	$(2.5\pm1)\times10^{3}$	$(6.3\pm3)\times10^{2}$
SC8	$(7.3\pm2.5)\times10^{3}$	$(1.8\pm1.0)\times10^{3}$

Error estimated for binding constants being 5 %.

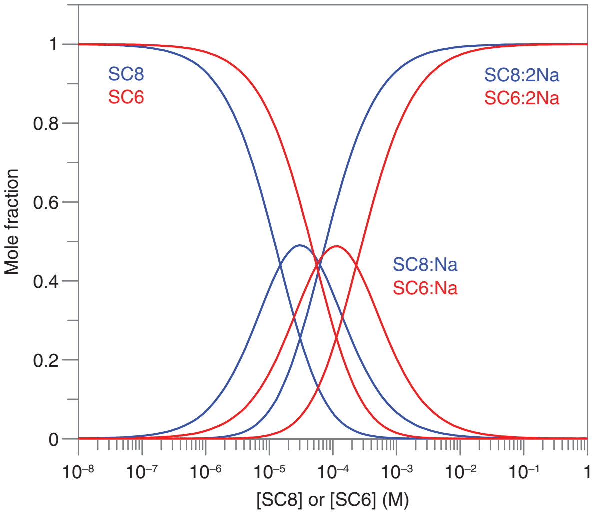


Fig. 5: Mole fraction distribution of sodium complexes formed by (—) SC6 and (—) SC8. The simulations were carried out assuming that SC6 is an octanion and SC8 is a decaion and Na^{+} counterions are the only source of metal cations.

mechanism revealed here for the complexation of metal cations with SC6 and SC8 has several implications for the host:guest chemistry of these water-soluble macrocyclic receptors. Figure 5 shows the mole fraction distribution of free calixarenes and their sodium complexes. The simulation was carried out using the binding constants of Table 2. As can be observed, the shape of the curves is similar for SC6 and SC8 but in the latter case they are displaced to lower concentrations owing to the higher binding constants and number of Na^{+} equivalents. Importantly, the concentration of 1:1 complexes becomes relevant in the µM concentration range and in the mM range the free receptor is practically null. This implies that for most of the complexation studies the replacement of sodium ions by the ligand or/and the formation of heteroternary complexes must be considered.

Formation of heteroternary complexes predominate at milimolar concentrations of metal cations [60]. This complexation mechanism has important implications for the host:guest chemistry of these receptors because the apparent binding stability of the complex is dependent on the concentration of metal cations in solution and, consequently, on the concentration of these anionic calixarenes. Furthermore, the nature of the host:guest complex is also centration dependent with simple 1:1 (and 1:2) complexes predominating at lower concentrations and heteroternary complexes at higher calixarene concentrations.

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