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Proparacaine complexation with β-cyclodextrin and *p*-sulfonic acid calix[6]arene, as evaluated by varied ^{1}H-NMR approaches

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This study focused on the use of NMR techniques as a tool for the investigation of complex formation between proparacaine and cyclodextrins (CDs) or *p*-sulfonic acid calix[6]arene. The pH dependence of the complexation of proparacaine with β-CD and *p*-sulfonic acid calix[6]arene was studied and binding constants were determined by ^{1}H NMR spectroscopy [diffusion-ordered spectroscopy (DOSY)] for the charged and uncharged forms of the local anesthetic in β-CD and *p*-sulfonic acid calix[6]arene. The stoichiometries of the complexes was determined and rotating frame Overhauser enhancement spectroscopy (ROESY) 1D experiments revealed details of the molecular insertion of proparacaine into the β-CD and *p*-sulfonic acid calix[6]arene cavities. The results unambiguously demonstrate that pH is an important factor for the development of supramolecular architectures based on β-CD and *p*-sulfonic acid calix[6]arene as the host molecules. Such host–guest complexes were investigated in view of their potential use as new therapeutic formulations, designed to increase the bioavailability and/or to decrease the systemic toxicity of proparacaine in anesthesia procedures.

Keywords: proparacaine; cyclodextrin; calixarenes; NMR

Introduction

The formulation of drugs is an important topic in pharmaceutical sciences. Many therapeutically active molecules are chemically and biologically fragile; so they need to be encapsulated in a drug carrying system to improve their physicochemical stability. The desirable features of drug carriers in drug-delivery systems include controlled release, targeting and absorption enhancing abilities. From the safety point of view, bio-adaptability is an important requirement for drug carrier systems, as well as high quality and low cost.[1,2] These carriers protect the loaded drug against degradation, the guest molecule being transported effectively in biological media.[3] The development of such systems include cyclodextrins (CDs),[4–9] calix[*n*]arenes,[10–15] micelles,[16] liposomes,[17,18] micro-[19] and nanoparticles[20] and solid lipid nanoparticles[21–23] as drug carriers.

CD was one of the first molecular receptors ('host' molecule) whose ability to bind organic (guest) compounds was recognized and extensively studied by various experimental techniques.[24–26] Consequently, CDs have pharmaceutical applications as high performance biomaterials in drug-delivery systems due to their ability to change physical, chemical and biological properties of *guest* molecules through the formation of inclusion complexes.[27–29]

Surprisingly, calix[*n*]arenes,[30] organic macrocyclic host molecules formed by the *ortho*-condensation of *para*-substituted phenols and formaldehyde, are well investigated in supramolecular chemistry but less studied with respect to their drug-delivery properties.[10]

The characterization of the delivery systems formed by complexation of the local anesthetic proparacaine **1**, in β-CDs **2** or *p*-sulfonic acid calix[6]arene **3** (Scheme 1) provides important information to optimize its possible future use, which requires a better knowledge of the molecular properties of the complexes.[31] Crucial information for the characterization of these complexes include the stoichiometry, the complexed population (%p_{bound}), the formation/dissociation constant (K_a) and the relative positioning of the carrier/guest inclusion complex, which can be determined from pulsed field gradient spin-echo(PGSE)[32–36] and nuclear Overhauser effect (NOE)[37] experiments, respectively.

The main objective of this investigation was the analysis of the complexation by β-CDs **2** or *p*-sulfonic acid calix[6]arene **3** (Scheme 1) of the local anesthetic proparacaine **1** in an aqueous medium under various experimental conditions (pH 5 and pH 10). Using the Job plot method, PGSE and NOE NMR approaches, we obtained large variety of information about the molecular interactions that drive the complexation process of proparacaine.

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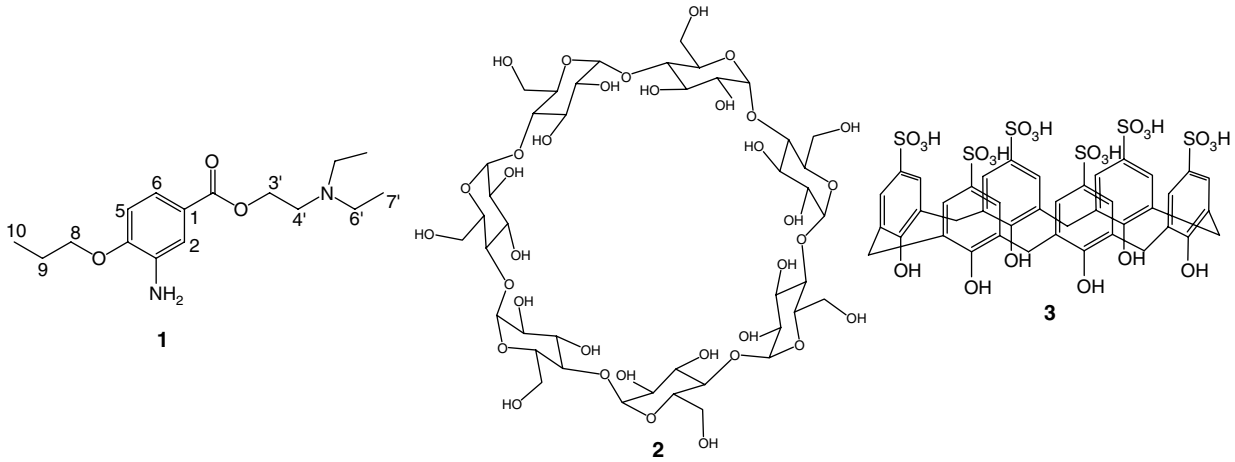

Scheme 1. Chemical structure of proparacaine (1), -cyclodextrin (2) and *p*-sulfonic acid calix[6]arene (3).

Results and Discussion

We determined the ionization constant of proparacaine by the pH-titration method as 8.33. From that we designed experiments at pH 5 and 10 to evaluate the complexation of charged and uncharged proparacaine **1** species, with β-CDs **2** or *p*-sulfonic acid calix[6]arene **3**. Both anesthetic forms seem to be important for the anesthesia mechanism, with the uncharged species binding more strongly to the membranes while the protonated form has the necessary water solubility for the anesthetic to cross the biological compartments and reach the targeted axonal membranes in sufficient concentration.[38–40]

We started our investigation by analyzing the complexation-induced hydrogen chemical shifts ($\Delta\delta$) in the **1/2** and **1/3** complexes, relative to free **1**.

The hydrogens of **1** displayed discrete chemical shift variations ($\leq$0.04 ppm) in the presence of **2** complex (**1/2**) in pH 5 (Table 1). When the (**1/2**) complex was prepared in pH 10, the hydrogen chemical shift variations of **1** were more pronounced (up to 0.12 ppm, Table 2). This fact can be rationalized by the smaller water solubility of the uncharged proparacaine **1** species at pH 10, which favors the inclusion of **1** into the hydrophobic cavity of β-CD **2** at alkaline but not at acidic pH.

Complexation between **1** and **3** at acidic pH induced large shielding effects in all hydrogens of **1**, mainly H-**3**′, H-**4**′, H-**6**′ and H-**7**′ ($\Delta\delta$ = 0.74, 0.38, 0.84 and 0.42 ppm, respectively – Table 1), revealing interactions between the charged ammonium group of **1** (pH 5) and the SO_3H group of **3** (Table 1).[10,41]

As expected, smaller hydrogen chemical shifts changes were observed between free **1** and **1/3** complex at pH 10, when proparacaine is in its neutral form (Table 2) than at pH 5.

We further established the stoichiometries for the **1/2** and **1/3** complexes (pH 5 and pH 10), using the Job plot method.[42,43] The plots obtained from the NMR analyses indicated the predominant formation of 1 : 1 complexes, for **1/2** and **1/3**, at both pH. A representative Job plot experiment is illustrated in Fig. 1.

Diffusion-ordered spectroscopy (DOSY) NMR experiments were pivotal to demonstrate that **1/2** and **1/3** form stable complexes. These experiments allow distinguishing compounds or complexes by their differences in diffusion coefficients.[14] Representative spectra are given in Figs 2 and 3 for pure **1** and for the **1/2** complex (2 mmol l^{-1} samples, 298 K, respectively). The diffusion coefficients of pure **1**, **2** and **3** (at pH 5 and 10) were first determined ($D_{\mathbf{1}\,(\text{pH 5})} = 5.30 \times 10^{-10}$, $D_{\mathbf{2}\,(\text{pH 5})} = 3.27 \times 10^{-10}$, $D_{\mathbf{3}\,(\text{pH 5})} = 3.05 \times 10^{-10}$, $D_{\mathbf{1}\,(\text{pH 10})} = 4.72 \times 10^{-10}$, $D_{\mathbf{2}\,(\text{pH 10})} = 3.16 \times 10^{-10}$ and $D_{\mathbf{3}\,(\text{pH 10})} = 3.08 \times 10^{-10} m^2\,s^{-1}$, respectively, Table 3). In the presence of **2** or **3**, compound **1** showed a significant reduction in its diffusion rate ($D_{\mathbf{1}/\mathbf{2}\,(\text{pH 5})} = 4.73 \times 10^{-10}$, $D_{\mathbf{1}/\mathbf{3}\,(\text{pH 5})} = 3.32 \times 10^{-10}$, $D_{\mathbf{1}/\mathbf{2}\,(\text{pH 10})} = 4.13 \times 10^{-10}$ and $D_{\mathbf{1}/\mathbf{3}\,(\text{pH 10})} = 4.36 \times 10^{-10}\ m^2\,s^{-1}$, Table 3), indicating that **1** forms *host–guest* complexes with either **2** or **3**, at both pH. Moreover, the diffusion rate values (Table 3) of **1** in the **1/2** complex at pH 10 is in agreement with the assumption of a strong association. In the case of the **1/3** complex a more pronounced variation was observed in the diffusion coefficient of compound **1** at pH 5 (Table 3).

Taking into account that we are dealing with a system under fast equilibrium on the NMR scale, both chemical shifts and diffusion coefficients of **1** are the average values of the free and bound species. From these diffusion coefficients, and applying a well established methodology[44] we have calculated the complexed population (%p_{bound}) and apparent binding constants (K_a) of the complexes (Table 3). %p_{bound} was found to be 22 and 29% for **1/2,** and 73 and 14% for **1/3** at pH 5 and pH 10, respectively. The values of K_a at both conditions (181 and 288 M^{-1} for **1/2**, respectively) confirmed that more association took place between the neutral form of proparacaine (pH 10) and β-CD than between charged **1** and **2** (pH 5). In an earlier work, conducted at pH 7, an association constant (K_a = 208 M^{-1}) has been reported for the **1/2** complex, in good agreement with the values found in this study.[18] Studies conducted for **1/3** revealed a strong association between the charged proparacaine species **1** and *p*-sulfonic acid calix[6]arene **3** (K_a = 5007 M^{-1} at pH 5) and a diminished association between the uncharged proparacaine **1** and **3** (K_a = 11 M^{-1}). The results obtained from measurements in an acidic medium (pH 5) show that the protonated proparacaine molecule forms strong complexes with *p*-sulfonic acid calix[6]arene **3**, as opposed to the association of charged **1** with β-CD **2**.

On the other hand, neutral molecules of proparacaine **1** (pH 10) showed a higher affinity for the β-CD cavity than those bearing formal charges. Moreover, the ester group of **1** became fully protected from nucleophilic attack of HO^-, the complexed drug being unreactive to alkaline hydrolysis (not shown) as observed earlier for another ester-type local anesthetic, tetracaine.[10] Further details on the **1/2** (pH 10) and **1/3** (pH 5) molecular complexation were obtained from rotating frame Overhauser enhancement spectroscopy (ROESY)-NMR experiments.

Table 1. ^{1}H NMR: Chemical shifts (δ) and chemical shift differences ($\Delta\delta = \delta_{1\ free} - \delta_{1\ complex}$) of pure **1** and its complexes with β-CD **1/2** or *p*-sulfonic acid calix[6]arene **1/3**, at pH 5 (2 mmol l^{-1} samples, 298 K)

	1	**1/2**	**1/2**	**1/3**	**1/3**
Hydrogen	δ	δ	$\Delta\delta = \delta_{\mathbf{1}\ free} - \delta_{\mathbf{1}\ complex}$	δ	$\Delta\delta = \delta_{\mathbf{1}\ free} - \delta_{\mathbf{1}\ complex}$
H-**10**	0.92	0.92	0.00	0.65	0.27
H-**7**′	1.24	1.24	0.00	0.83	0.42
H-**9**	1.73	1.73	0.00	1.61	0.12
H-**6**′	3.25	3.25	0.00	2.41	0.84
H-**3**′	3.54	3.55	0.01	2.80	0.74
H-**8**	4.02	4.02	0.00	3.87	0.15
H-**4**′	4.55	4.56	0.01	4.17	0.38
H-**5**	6.93	6.89	0.04	6.83	0.10
H-**2**	7.38	7.38	0.00	7.39	−0.01
H-**6**	7.46	7.44	0.02	7.46	0.00

Table 2. ^{1}H NMR: Chemical shifts (δ) and chemical shift differences ($\Delta\delta = \delta_{1\ free} - \delta_{1\ complex}$) of pure **1** and its complexes with β-CD **1/2** or *p*-sulfonic acid calix[6]arene **1/3**, at pH 10 (2 mmol l^{-1} samples, 298 K)

	1	**1/2**	**1/2**	**1/3**	**1/3**
Hydrogen	δ	δ	$\Delta\delta = \delta_{\mathbf{1}\ free} - \delta_{\mathbf{1}\ complex}$	δ	$\Delta\delta = \delta_{\mathbf{1}\ free} - \delta_{\mathbf{1}\ complex}$
H-**10**	0.91	0.92	−0.01	0.74	0.17
H-**7**′	1.01	1.02	−0.01	0.89	0.12
H-**9**	1.72	1.73	−0.01	1.68	0.04
H-**6**′	2.68	2.65	0.03	2.38	0.30
H-**3**′	2.95	2.91	0.04	2.75	0.20
H-**8**	4.01	4.02	−0.01	3.96	0.05
H-**4**′	4.35	4.35	−0.00	4.22	0.13
H-**5**	6.92	6.85	0.07	6.86	0.06
H-**2**	7.37	7.32	0.05	7.33	0.04
H-**6**	7.44	7.32	0.12	7.40	0.04

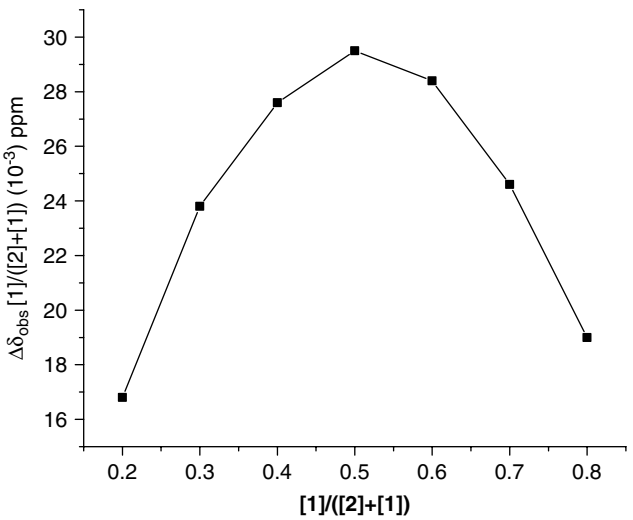

Figure 1. Representative Job plot for the complex formed between proparacaine **1** and β-CD **2**. Chemical Shifts were measured at 499.885 MHz in D_2O, at 298 K and pH 10, in 2 mmol l^{-1} samples).

Specific rOe signals were observed between H-**2**/H-**5**, H-**6** and H-**8** of **1** (pH 10), and H-**3** (enhancement of 0.57, 1.36, 0.47%, respectively) and H-**5** (0.80, 1.73, 0.36% of signal enhancement respectively) of β-CD **2**. At pH 5, the same increments were observed in the rOe between the hydrogens of proparacaine **1** and β-CD **2**, but with smaller intensities. We therefore suggested the presence of two species, **A** and **B** (Fig. 4) on the basis of molecular models and the nuclear Overhauser enhancements. The proposed topologies are in agreement with a recent work published by Marsaioli and collaborators.[18]

The signal enhancements in **1/3** (rOe) between H-**6**′ and H-**7**′ of proparacaine **1** (1.27 and 0.52%, respectively, at pH 5) and H-Ar of the *p*-sulfonic acid calix[6]arene **3** indicate the binding of the ammonium group of **1** with the SO_3H group of **3**. This was confirmed by experiments performed at pH 10.0, which revealed that the association constant between **1** and **3** decreased for $K_a = 11\ M^{-1}$ in relation to that in pH 5 ($K_a = 5007\ M^{-1}$). The two proposed topologies, **C** and **D** (Fig. 5) for the **1/3** complexes at pH 5 were established using ROESY 1D.

Conclusion

Several noncovalent weak forces, including electrostatic, hydrophobic, π-π interaction and van der Waals, cooperatively contribute to the formation of the supramolecular complexes. Among these, electrostatic and hydrophobic interactions as well as structural matching effect are thought to play principal roles in forming the supramolecular systems **1/2** and **1/3**.

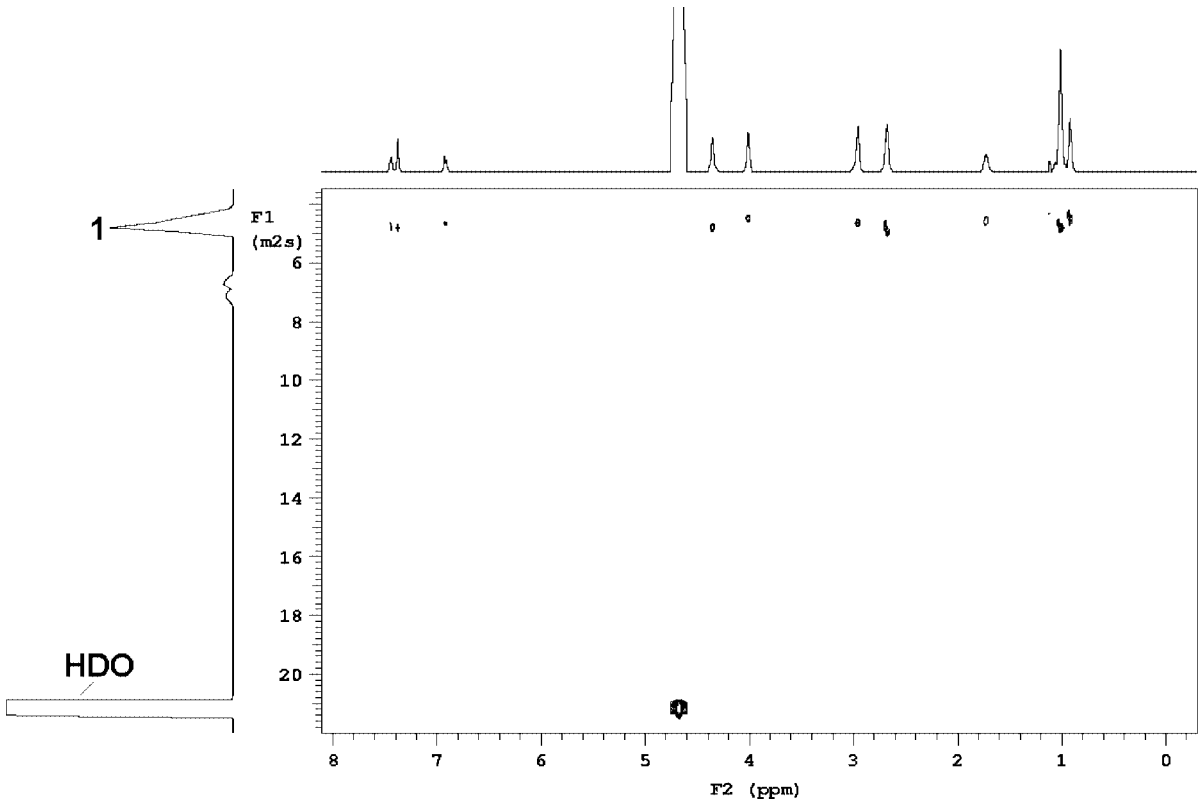

Figure 2. Representative ^{1}H DOSY NMR experiment of pure **1** (499.885 MHz, D_2O, 298 K, pH 10, 2 mmol l^{-1}).

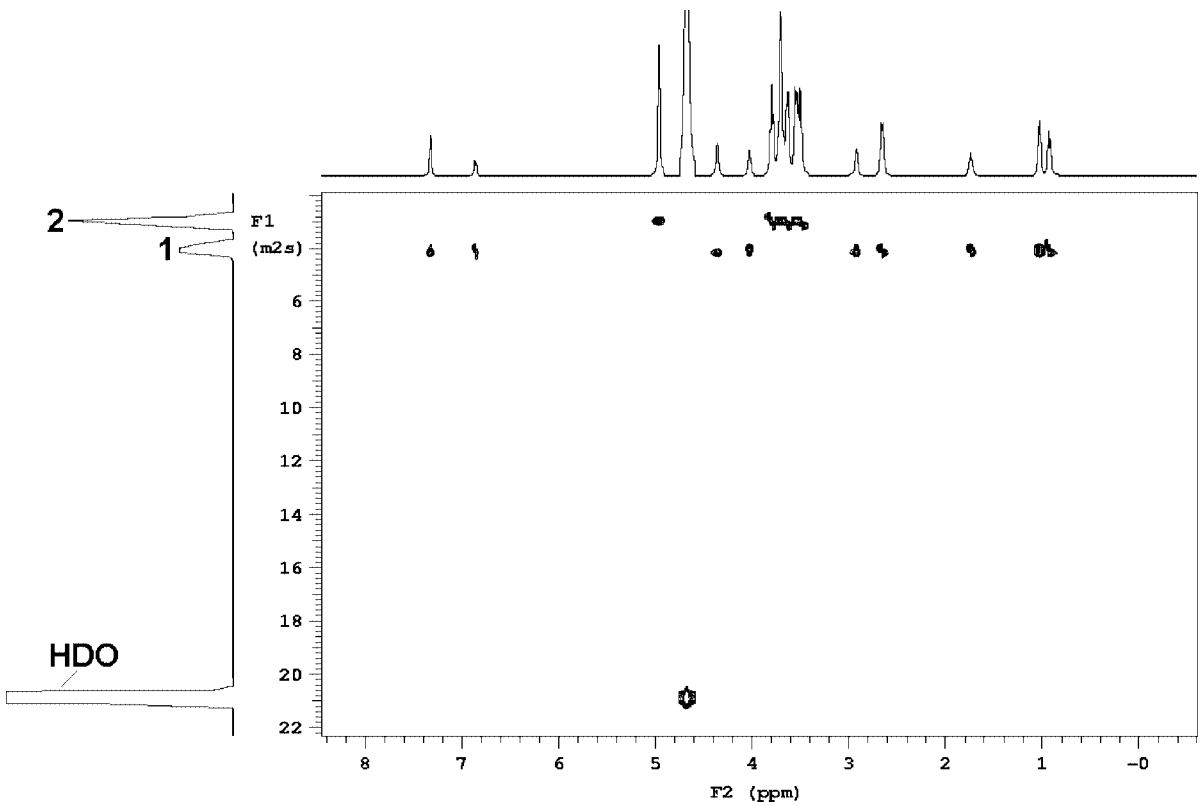

Figure 3. Representative ^{1}H DOSY NMR experiment for the **1/2** complex (499.885 MHz, D_2O, 298 K, pH 10, 2 mmol l^{-1}).

Table 3. Diffusion coefficients of pure **1**, **2**, **3** and **1/2** and **1/3** complexes in D_2O (2 mmol l^{-1} samples, 298 K) at pH 5 and 10

Complex	Compounds	Condition (pH)	D (10^{-10} m^2 s^{-1})	D/D^{H_2O}	%p	K_a (M^{-1})
–	**1**	5	5.30 ± 0.04	0.24	–	–
	1	10	4.72 ± 0.03	0.22	–	–
–	**2**	5	3.27 ± 0.03	0.16	–	–
	2	10	3.16 ± 0.02	0.15	–	–
–	**3**	5	3.05 ± 0.02	0.14	–	–
	3	10	3.08 ± 0.01	0.15	–	–
1/2	**1**	5	4.73 ± 0.05	0.22	22	181
	2		3.30 ± 0.01	0.15		
1/3	**1**	5	3.32 ± 0.03	0.16	73	5007
	3		2.85 ± 0.02	0.13		
1/2	**1**	10	4.13 ± 0.03	0.20	29	288
	2		3.08 ± 0.02	0.15		
1/3	**1**	10	4.36 ± 0.07	0.21	14	11
	3		3.13 ± 0.04	0.15		

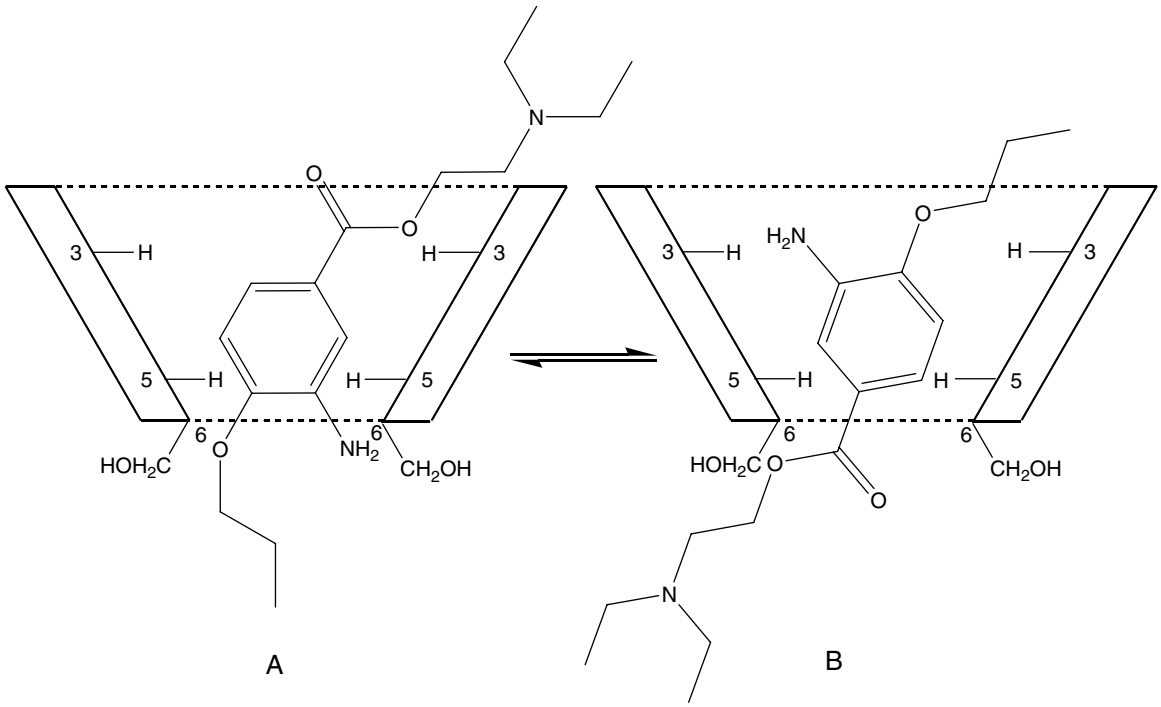

Figure 4. Proposed topology for the uncharged proparacaine/β-CD (**1/2** complex at pH 10), based on ^{1}H NMR evidences.

In summary, complexes possessing different degrees of compactness were constructed by the complexation of **2** and **3** with proparacaine **1,** at pH 5 and pH 10. Increasing the alkalinity of the medium with carbonate buffer favored the complexation of **1** in **2** ($K_a = 288$ M^{-1}) in relation to that in pH 5 ($K_a = 181$ M^{-1}) and prevented the alkaline hydrolysis of **1**. However the complexation of **1** and **3** at pH 10 ($K_a = 11$ M^{-1}) is weaker than that observed at pH 5 ($K_a = 5007$ M^{-1}), which is mainly a result of the protonation of the amine groups of **1**, and binding of such $^+NHR_2$ group of **1** to the SO_3H groups of **3**. These observations unambiguously demonstrate that the ionization state of the guest molecule is a crucial factor in the design of supramolecular architectures based on **2** and **3** as the host molecules.

The topology of the complexes (Figs 4 and 5) was determined from the 1D ROESY NMR results, revealing that the forces that govern complexation are different for **1/2** and **1/3**: hydrophobic interactions for the **1/2** complex and ionic-pair formation for the **1/3** complex.

Experimental

Chemicals and reagents

Proparacaine hydrochloride **1** (99%), β-CD **2** (99%), and D_2O (99.75%) were purchased from Aldrich, Acros Organics and Merck, respectively. All other reagents were of analytical grade. *p*-sulfonic acid calix[6]arene **3** was synthesized in our laboratory following literature procedures.[45–47]

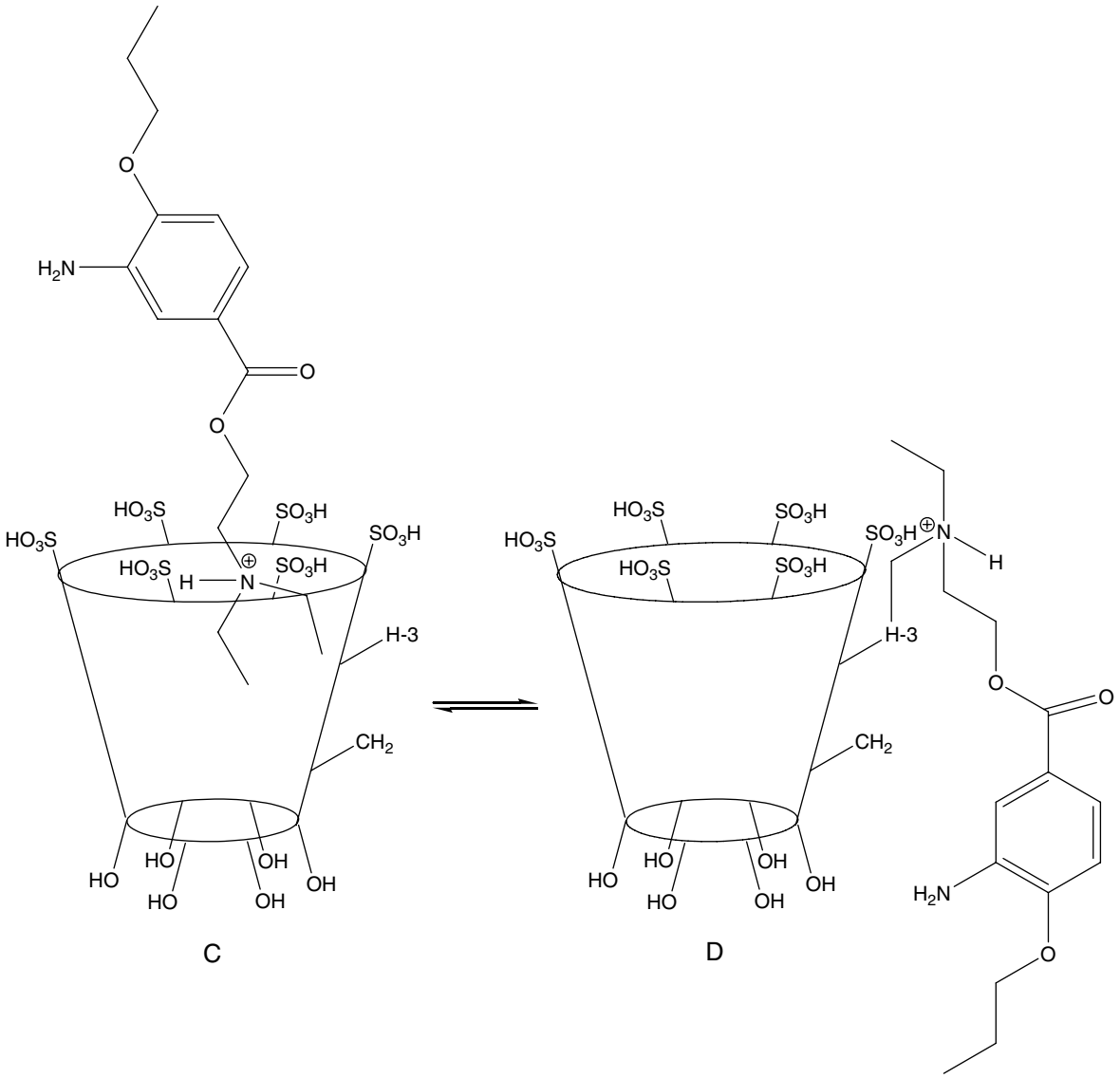

Figure 5. Proposed topologies for the charged proparacaine/*p*-sulfonic acid calix[6]arene (**1/3** complex, pH 5) in fast equilibrium, according to ^{1}H NMR evidences.

Preparation of solid inclusion complexes

Inclusion complexes (**1/2** or **1/3**) with 1 : 1 molar ratios were prepared by shaking appropriate amounts of **1** and **2** or **3**, e.g. 2 mmol l^{-1}, in deionized water at room temperature (298 ± 1 K) for 1 h. Kinetic experiments revealed that equilibrium was reached after 40 min (data not shown).

After reaching equilibrium, the solution was freeze-dried in a Labconco Freeze-dry system (Freezone 4.5) and stored at 253 K until further use.

NMR Spectroscopy

All experiments were performed at 298 K in D_2O. For the experiments with uncharged **1**, the pH value of the solutions was adjusted by addition of lyophilized 0.02 mol l^{-1} carbonate buffer resuspended in D_2O.

Routine 1D

^{1}H experiments were acquired either with an INOVA-500 Varian spectrometer operating at 499.885 MHz for ^{1}H (64 k data points, 30° excitation pulse with duration of 2.2 μs, spectral width of 6 kHz, acquisition time of 3.3 s and relaxation delay of 10 ms) in a 5-mm probe with inverse detection mode at room temperature unless stated otherwise.

NOE measurements

The ROESY 1D experiments were obtained with a selective 180° and a nonselective 90° pulse, with a mixing time of 0.5 s during the spin-lock. The selective pulses were generated by a waveform generator which automatically attenuates the shape, power, and pulse duration to obtain the required selectivity. The subtraction of the on- and off-resonance acquisition furnished the ROESY 1D experiment. All spectra were acquired with a 5-mm inverse probe at 298 K in 5-mm tubes.

HR-DOSY experiments were carried out by carefully choosing the correct pulse sequence and gradients for the experiments. The measurements were made using: (i) 5 mm inverse probe with z-gradient coil; (ii) the Gradient Compensated Stimulated Echo Spin Lock (GCSTESL) HR-DOSY sequence; (iii) amplitudes of the gradient pulses ranging from 0.000685 to 0.003427 T cm^{-1}, where an approximately 90–95% decrease in the resonance intensity was achieved at the largest gradient amplitudes. For all experiments, 25 different gradient amplitudes were used. The baselines of all spectra were corrected prior to data processing. The processing program (the DOSY macro in a Varian instrument) involves the determination of the peak heights of all signals above a pre-established threshold and the fitting of the decay curve for each peak to an exponential decay. The DOSY macro was run with data transformed using fn = 64 K. Very crowded spectra were processed in sections due to the limitation of handling only 512 lines at a time. The results of the DOSY method of analysis are pseudo two-dimensional spectra with NMR chemical shifts along one axis and calculated diffusion coefficients ($\times$ 10^{-10} m^2 s^{-1}) along the other.

Determination the stoichiometry of complexation

Job plots were prepared with 2 mmol l^{-1} stock solutions of **1** and **2** or **1** and **3**.[48]

Acknowledgments

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References

[1] K. Uekama, *Adv. Drug Delivery. Rev.* **1999**, *36*, 1.
[2] K. Uekama, *Chem. Pharm. Bull.* **2004**, *52*, 900.
[3] P. Shahgaldian, L. Quattrocchi, J. Gualbert, A. W. Coleman, P. Goreloff, *Eur. J. Pharm. Biopharmaceut.* **2003**, *55*, 107.
[4] M. E. Davis, M. E. Brewster, *Nat. Rev. Drug Discovery.* **2004**, *3*, 1023.
[5] M. J. Kirchmeier, T. Ishida, J. Chevrette, T. M. Allen, *J. Lipid Res.* **2001**, *11*, 15.
[6] F. Veiga, C. Fernandes, F. Teixeira, *Int. J. Pharm.* **2000**, *202*, 165.
[7] M. E. Dalmora, S. L. Dalmora, A. G. Oliveira, *Int. J. Pharm.* **2001**, *222*, 45.
[8] N. M. Zaki, N. D. Mortada, G. A. S. Awad, S. S. A. ElHady, *Int. J. Pharm.* **2006**, *327*, 97.
[9] K. Uekama, F. Hirayama, H. Arima, *J. Inclusion Phenom. Macrocyclic Chem.* **2006**, *56*, 3.
[10] S. A. Fernandes, L. F. Cabeça, A. J. Marsaioli, E. de Paula, *J. Inclusion Phenom. Macrocyclic Chem.* **2007**, *57*, 395.
[11] J. S. Millership, *J. Inclusion Phenom. Macrocyclic Chem.* **2001**, *39*, 327.
[12] E. da Silva, C. Valmalle, M. Becchi, C. Y. Cuilleron, A. W. Coleman, *J. Inclusion Phenom. Macrocyclic Chem.* **2003**, *46*, 65.
[13] W. Z. Yang, M. M. de Villiers, *Eur. J. Pharm. Biopharm.* **2004**, *58*, 629.
[14] W. Z. Yang, M. M. de Villiers, *J. Pharm. Pharmacol.* **2004**, *56*, 703.
[15] W. Yang, D. P. Otto, W. Liebenberg, M. M. de Villiers, *Current Drug Discov. Techn.* **2008**, *5*, 129.
[16] K. Kataoka, A. Harada, Y. Nagasaki, *Adv. Drug Delivery Rev.* **2001**, *47*, 113.
[17] Y.-P. Zhang, B. Ceh, D. D. Lasic, Liposomes in drug delivery, in *Polymeric Biomaterials* (2nd edn) (Ed.: D. Severian), Marcel Dekker: New York, NY, **2002**, 783.
[18] L. F. Cabeça, S. A. Fernandes, E. de Paula, A. J. Marsaioli, *Magn. Res. Chem.* **2008**, *46*, 832.
[19] H. Kawaguchi, *Prog. Polym. Sci.* **2000**, *25*, 1171.
[20] R. Alvarez-Roman, G. Barre, R. H. Guy, H. Fessi, *Eur. J. Pharmacol. Biopharmacol.* **2001**, *52*, 191.
[21] W. Mehnert, K. Mader, *Adv. Drug Delivery Rev.* **2001**, *47*, 165.
[22] R. H. Muller, W. Mehnert, J. S. Lucks, C. Schwarz, A. zur Muhlen, H. Weyhers, C. Freitas, D. Ruhl, *Eur. J. Pharmacol. Biopharmacol.* **1995**, *41*, 62.
[23] R. H. Muller, K. Mader, S. Gohla, *Eur. J. Pharmacol. Biopharmacol.* **2000**, *50*, 161.
[24] J. Szejtli, *Cyclodextrin Technology*, Kluwer Academic Publishers: City Netherlands, **1988**, Chapter 2.
[25] K. A. Connors, in *Comprehensive Supramolecular Chemistry*, vol. 3, (Eds: J. Szejtli, T. Osa), Pergamon: New York, **1996**, pp 205.
[26] M. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875.
[27] J. Szejtli, *J. Inclusion Phenom. Macrocyclic Chem.* **2005**, *52*, 1.
[28] K. Uekama, F. Hirayama, T. Irie, *Chem. Rev.* **1998**, *98*, 2045.
[29] C. Merino, E. Junquera, J. Jiménez-Barbero, E. Aicart, *Langmuir* **2000**, *16*, 1557.
[30] C. D. Gutsche, *Calixarenes*, Royal Society of Chemistry: Cambridge, **1989**.
[31] A. V. Davis, R. M. Yeh, K. N. Raymond, *Proc. Natl. Acad. Sci.* **2002**, *99*, 4793.
[32] C. S. Johnson Jr., *Prog. NMR Spectrosc.* **1999**, *34*, 203.
[33] W. S. Price, *Concepts Magn. Res.* **1997**, *9*, 299.
[34] W. S. Price, *Concepts Magn. Res.* **1998**, *10*, 197.
[35] P. Stilbs, *Prog. NMR Spectrosc.* **1987**, *19*, 1.
[36] E. O. Stejskal, J. E. Tanner, *J. Chem. Phys.* **1965**, *42*, 288.
[37] H. Gunther, *NMR Spectroscopy* (2nd edn), John Wiley & Sons: Chichester, **1994**, Chapt. 6 and 10.
[38] E. de Paula, S. Schreier, *Braz. J. Med. Biol. Res.* **1996**, *29*, 877.
[39] G. R. Strichartz, V. Sanchez, R. Arthue, R. Chafetz, D. Martin, *Anesth. Analg.* **1990**, *71*, 158.
[40] F. Yanagidate, G. R. Strichartz, *Handb. Exp. Pharmacol.* **2006**, *177*, 91.
[41] A. Specht, F. Ziarelli, P. Bernad, M. Goeldner, L. Peng, *Helv. Chim. Acta* **2005**, *88*, 2641.
[42] P. Job, *Ann. Chim.* **1928**, *9*, 113.
[43] L. Fielding, *Tetrahedron* **2000**, *56*, 6151.
[44] R. Rymdém, J. Carlfors, P. J. Stilbs, *J. Incl. Phenom. Macro.* **1983**, *1*, 159.
[45] S. Shinkai, S. Mori, T. Tsubaki, *Tetrahedron Lett.* **1984**, *25*, 5315.
[46] C. D. Gutsche, L. G. Lin, *Tetrahedron* **1986**, *42*, 1633.
[47] C. D. Gutsche, M. Iqbal, *Org. Synth.* **1989**, *68*, 234.
[48] Y. L. Loukas, V. Vraka, G. Gregoriadis, *Int. J. Pharm.* **1998**, *162*, 137.

