Complexes of Macrocycles with γ-Cyclodextrin As Deduced from NMR Diffusion Measurements

Ayelet Gafni and Yoram Cohen*

School of Chemistry, The Sackler Faculty of Exact Sciences, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel

Received September 13, 1996[⊗]

The complexes of 12-crown-4 (12C4), cyclen (12N4), and 1,4,7,10-tetrathiacyclododecane (12S4) with γ-cyclodextrin (γ-CD) were studied by NMR diffusion measurements in the absence and in the presence of inorganic and organic salts. The 12-crown-4:γ-CD system was also used to evaluate the effect of the nature of the cation and the anion on the association between the macrocycle and the γ-CD. In addition, we have studied the solvent effect and the pH effect on the association constant of the γ-CD:12C4 complex. Based on these measurements, the following conclusions could be reached: (1) All three macrocycles form complexes of moderate stability with γ-CD, (2) the association constants of these complexes are much higher in the absence of the salts, (3) the decrease in the association due to addition of salts seems to be independent on the nature of the cation or the anion, and (4) in contrast to most γ-CD complexes, the association constants between 12-crown-4 and γ-CD are nearly identical in pure D_2O and in 80:20 (v/v) CD_3OD/D_2O and only slightly lower in pure DMSO-d_6, suggesting that the hydrophobic interaction is not the main driving force for complexation in these systems. The pH has only a nonsignificant effect on the association constant of the γ-CD:12C4 complex. Plausible explanations for the above observations and the advantages and disadvantages of NMR diffusion measurements for determination of association constants are discussed.

Introduction

Cyclodextrin (CD) complexes were studied extensively in recent years because of their theoretical and practical importance.[1,2] Since CD complexes are water soluble and chiral, they can be used as a good models for enzymes. Their water solubility, low toxicity, and relative low price made them suitable for a wide range of applications in food technology, as drug delivery systems, and as chemical sensors.[1,2] An intriguing class of cyclodextrin complexes are those in which the guests themselves have complexing capability and thus may potentially form multicomponent complexes like the macrocyclic complexes of CDs. Such complexes were first prepared by Vogtle and Muller[3] in 1979, and later a few examples were studied by X-ray crystallography by Hirotsu and his co-workers.[4] Surprisingly, there is very little information concerning these complexes in solution possibly due to the fact that these complexes have a moderate stability and since their formation is difficult to follow using conventional spectrometric methods such as UV and NMR spectroscopies. Recently, NMR relaxation measurements have been used to probe the association of γ-CD to the paramagnetic complex $TmDOTP^{5-}$ (DOTP-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(methylenephosphonic acid)).[5]

NMR parameters such as chemical shift and relaxation times have been used extensively to study inclusion complexes of CDs.[6] Since the changes in chemical shift upon formation of complexes between γ-CD and 12-crown-4 and its tetraaza and tetrathia analogues are small, we decided to investigate these complexes using NMR diffusion measurements. NMR diffusion measurement by the pulsed gradient spin echo (PGSE) technique[7] was used to study the diffusion characteristics of many different biological and chemical systems.[8] Stilbs and his co-workers have used this technique more than 10 years ago to probe the association of different alcohols to α-CD in aqueous solution,[9] but it was not until recently that this technique was used in the field of organic supramolecular chemistry. Recently, we demonstrated the utility of NMR diffusion measurements in calculating association constants of ammonium ions to macrocyclic systems[10] and in probing the structure of calix[*n*]arene complexes.[11] We have also used this technique to evaluate the interaction of macrocyclic systems with water molecules in chloroform.[12]

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1996.

(1) (a) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344. (b) Szejtli, J., Osa, T., Eds. *Cyclodextrins*. In *Comprehensive Supramolecular Chemistry;* Lehn, J.-M., Vogtle, F., MacNicol, D. D., Davies, J. E. D., Atwood, J. L., Eds.; Pergamon: New York, 1996; Vol. 3.

(2) (a) Szejtli, J. In *Topics in Inclusion Science*; Davies, J. E. D., Ed.; Kluwer: Dordrecht, 1988. (b) Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 803.

(3) Vogtle, F.; Muller, W. M. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 623.

(4) (a) Kamitori, S.; Hirotsu, K.; Higuchi, T. *J. Chem. Soc., Chem. Commun.* **1986**, 690. (b) Kamitori,S.; Hirotsu, K.; Higuchi, T. *J. Am. Chem. Soc.* **1987**, *109*, 2409. (c) Kamitori, S.; Hirotsu, K.; Higuchi, T. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3825.

(5) Sherry, A. D.; Zarzycki, R.; Geraldes, C. F. G. C. *Magn. Reson. Chem.* **1994**, *32*, 361.

(6) Inoue,Y. in *Annu. Rep. NMR Spectrosc.* **1993**, *27*, 59 and references cited therein.

(7) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 288.

(8) For excellent reviews concerning the application of the PGSE NMR technique to chemical systems see: Stilbs, P. *Prog. NMR Spectrosc.* **1987**, *19*, 1. Soderman, O.; Stilbs, P. *Prog. NMR Spectrosc.* **1994**, *26*, 445. Lindblom, G.; Oradd, G. *Prog. NMR Spectrosc.* **1994**, *26*, 483. Callaghan, P. T.; Coy, A. In *Nuclear Magnetic Resonance Probes of Molecular Dynamics*; Tycko, R., Ed.; Kluwer: Academic Press, 1994; p 489. For the importance of diffusion in medical neuroimaging see: *Diffusion and Perfusion Magnetic Resonance Imaging*, Le Bihan, D., Ed; Raven Press: New York, 1995.

(9) Rymden, R.; Carlfors, J.; Stilbs, P. *J. Incl. Phenom.* **1983**, *1*, 159.

(10) Mayzel, O.; Cohen, Y. *J. Chem. Soc., Chem. Commun.* **1994**, 1901.

(11) Mayzel, O.; Aleksiuk, O.; Grynszpan, F.; Biali, S. E.; Cohen, Y. *J. Chem. Soc., Chem. Commun.* **1995**, 1183.

(12) Mayzel, O.; Gafni, A.; Cohen, Y. *J. Chem. Soc., Chem. Commun.* **1996**, 916.

S0022-3263(96)01758-6 CCC: $14.00

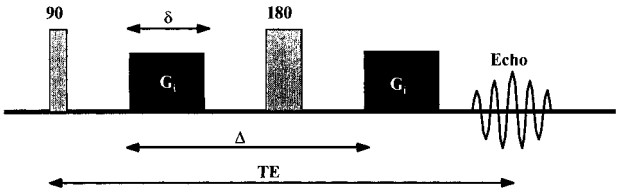

Figure 1. The pulse gradients spin echo (PGSE) NMR pulse sequence for diffusion measurements.[7]

Here we report the association constants of complexes of γ-CD with macrocyclic systems (i.e. 12-crown-4 (12C4), cyclen (12N4), and 1,4,7,10-tetrathiacyclododecane (12S4)) in the presence and absence of salts as obtained by NMR diffusion measurements. The effect of the nature of the cation and anion of the salts added and the solvent effect on the association constant of the γ-CD:12C4 complex was evaluated. These measurements clearly demonstrate that the complexes formed between γ-CD and the macrocycles are stronger in the absence of salts and that their association constants are nearly identical in aqueous solutions and in organic solvents. The decrease in the association constant between γ-CD and 12C4 due to the addition of salts is general and insensitive to the nature of the cation and/or the anion added. The pH has only a nonsignificant effect on the association constant of the γ-CD:12C4 complex. These results are used to discuss the driving force of the complexation process in this class of complexes. Finally, we discuss the advantages and pitfalls of NMR diffusion measurements, which surprisingly have seldom been used by organic chemists in studying organic supramolecular systems.

Experimental Section

All compounds, deuterated solvents, and salts were purchased from Aldrich (USA) and were used without further purification. NMR diffusion experiments were carried out on a ARX-500 NMR spectrometer (Bruker, Karlsruhe) equipped with a B-AFPA10 pulsed gradient unit capable of producing Z-gradient of nearly 50 G cm^{-1}. Experiments were carried out in a 5 mm high resolution inverse probe having self-shielded gradient coils. The pulsed gradients were incremented from 0 to ca. 40 G cm^{-1}. The pulsed gradients were typically of 2 ms duration separated by a 60 ms delay, and the total echo time was 124 ms.

Diffusion coefficients were obtained using the pulse sequence shown in Figure 1 according to which the ratio between the signal intensity in the presence and absence of pulse gradients (A_g and A_0, respectively) is given by eq 1[7]

$$\ln A_g/A_0 = -\gamma^2 g^2 \delta^2 (\Delta - \delta/3) D \qquad (1)$$

where γ is the gyromagnetic ratio (rad s g^{-1}), g is the strength of the diffusion gradients (gauss cm^{-1}), and D is the diffusion coefficient of the observed spins (cm^2 s^{-1}). δ is the length of the diffusion gradients (s) and Δ is the time separation between the leading edges of the two diffusion pulsed gradients (s).

NMR diffusion measurements were performed on the 1:1:1 solutions of γ-CD, macrocycles, and salts and on the 1:1 solutions of γ-CD and each of the macrocycles studied. In addition, we measured the diffusion coefficients of γ-CD, 12C4, 12N4, 12S4, and lithium acetate (LiOAc) and of the 1:1 solutions of 12C4:LiOAc, 12N4:LiOAc, and 12S4:LiOAc. The complexation of γ-CD with 12C4 and 12N4 was studied in aqueous solutions having a pD of 7.6 while the complexation of 12S4 was studied in DMSO-d_6 because of the low solubility of 12S4 in D_2O. In addition, the γ-CD:12C4 system was used to evaluate the effect of the nature of the of the cation and the anion added. To assess the solvent effect on the association constant of the γ-CD:12C4 complex, we performed all the diffusion measurements of this system in D_2O in a 80:20 (v/v) CD_3OD/D_2O mixture and in DMSO-d_6. We also measured the diffusion coefficients of all the components in the free state to be able to compare the diffusion of these molecular systems in the free and bound states. The changes in the diffusion coefficients of the macrocycles upon complex formation were used to calculate the fraction of bound macrocycles which, using the known concentrations of the samples, were translated to association constants as described before.[10−12] The concentration of the sample used for the NMR diffusion measurements were typically around 10 mmol. This concentration was selected after we have measured the diffusion coefficients of γ-CD samples having a concentration of 5, 10, 15, 20, and 40 mM. In these measurements we have found insignificant changes in the diffusion coefficient of γ-CD over the concentration range of 5 to 20 mM. pH measurements were performed using a Mettler Delta 320 pH meter (Essex, UK) in D_2O solutions, and the reported values are uncorrected.

Results

Figure 2A−C shows the decay of the signal intensity of the 12C4 singlet as a function of the pulse gradient strength for 12C4 in a 1:1 solutions with γ-CD and LiOAc and in the free state, respectively. The signal intensity of 12C4 decays faster in the free state (2C) than in the 1:1 solutions of 12C4:LiOAc (2B) and γ-CD:12C4 (2A). The slowest decay is observed in the case of 2A. Figure 3 is a graphical representation of the dependency of ln A_g/A_0 on g^2 for the three components of a 1:1:1 D_2O solution of γ-CD:12C4:LiOAc, the two components of a 1:1 solution of γ-CD:12C4 and for each of the three components of the complexes in the free state.

Table 1 depicts the diffusion coefficients obtained for the different γ-CD:macrocycle systems studied as well as the diffusion coefficients of all the free components of these complexes along with the association constants calculated from these diffusion data. The data in Table 1 show, as expected, that the diffusion coefficient of the free γ-CD is much lower than the diffusion coefficients of the free macrocycles. It should be noted that the diffusion coefficient of γ-CD was constant in the concentration range of 5 to 20 mmol and was found to be 0.30 $\pm$ 0.01 $\times$ 10^{-5} cm^2 s^{-1}. Only at 40 mmol have we found about 10% reduction of the diffusion coefficient of the γ-CD, probably due to partial self aggregation.

In the presence of γ-CD there is a significant decrease in the diffusion coefficient of the macrocycles; however, surprisingly this decrease is much less pronounced in the presence of organic and inorganic salts. For example the diffusion coefficient of 12C4 decreases from 0.68 $\pm$ 0.02 $\times$ 10^{-5} cm^2 s^{-1} in the free state to 0.56 $\pm$ 0.01 $\times$ 10^{-5} cm^2 s^{-1} and 0.48 $\pm$ 0.01 $\times$ 10^{-5} cm^2 s^{-1} for the 1:1:1 solution of γ-CD:12C4:LiOAc and for the 1:1 solution of γ-CD:12C4, respectively, while the diffusion coefficient of 12C4 in a 1:1 solution of 12C4:LiOAc was found to be 0.60 $\pm$ 0.01 $\times$ 10^{-5} cm^2 s^{-1}. The values obtained for the γ-CD:12N4 system show the same trend and here again the diffusion coefficient of cyclen in the free state and the 1:1:1 γ-CD:12N4:LiOAc and the 1:1 γ-CD:12N4 solutions are 0.60 $\pm$ 0.02 $\times$ 10^{-5} cm^2 s^{-1}, 0.53 $\pm$ 0.01 $\times$ 10^{-5} cm^2 s^{-1}, and 0.42 $\pm$ 0.01 $\times$ 10^{-5} cm^2 s^{-1}, respectively. These data clearly indicate that the association constants between the macrocycles and the γ-cyclodextrin are considerably smaller in the presence of the salts.[13] To verify if these effects are specific for certain salts, we have repeated the experiments in the presence of various salts.

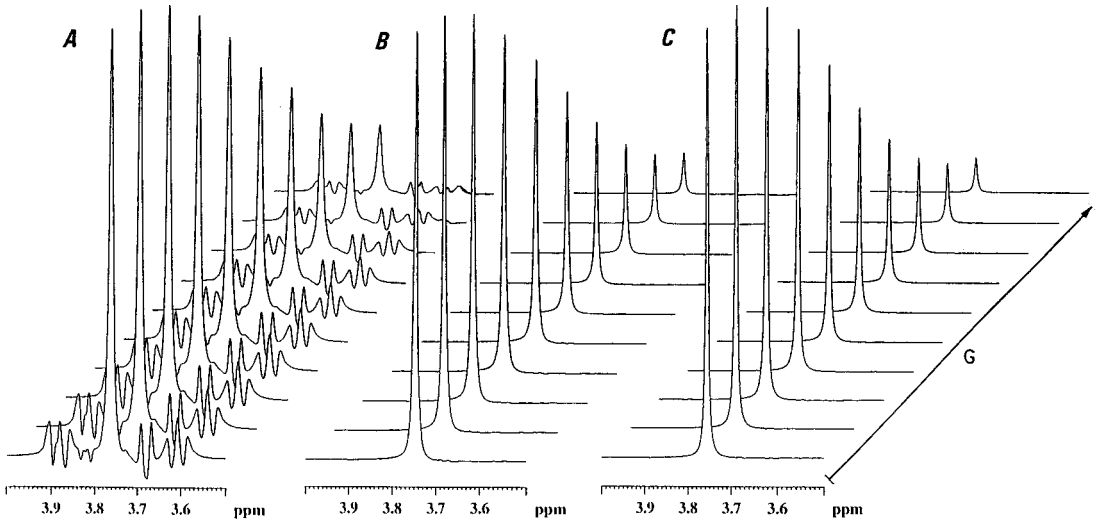

Figure 2. The signal intensity of the singlet of 12C4 in D_2O (pD = 7.6, 298 K) as a function of the pulse gradient strength (g) for (A) a 1:1 solution of γ-CD:12C4, (B) a 1:1 solution of 12C4:LiOAc, and (C) 12C4 in the free state. The rate of the decay in the signal of the singlet of 12C4 increases when going from case A to case C.

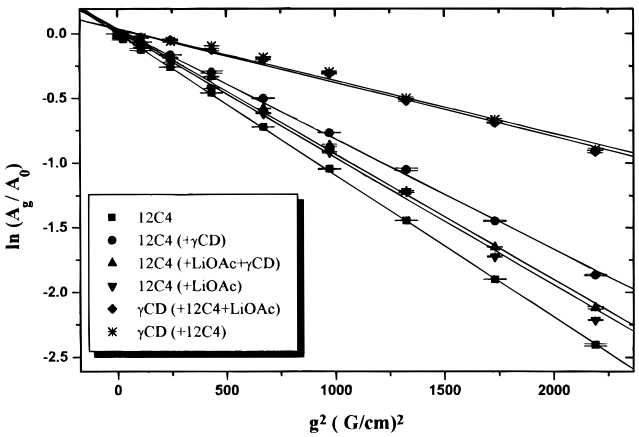

Figure 3. Normalized signal intensities (ln A_g/A_0) as a function of the gradient strength (g^2) for the free components and for the three components of the 1:1:1 solution of γ-CD: 12C4:LiOAc and the two components of a 1:1 solutions of γ-CD: 12C4. All measurements were performed at 298 K on solutions having a pD of 7.6.

Table 2 demonstrates that this reduction in the association constants is independent of the nature of the cation or the anion of the salt. Nearly the same results were obtained for the lithium acetate, lithium chloride, and lithium perchlorate, and only minor differences were observed when the cation was changed from lithium to potassium and to tetramethylammonium. Analysis of the data in Tables 1 and 2 show that both 12C4 and cyclen form complexes of moderate stability with γ-CD in the absence of salt as demonstrated by the calculated association constants (K_a) which were found to be 165 M^{-1} and 187 M^{-1} for the cyclen and 12C4 complexes, respectively. However, in the presence of salts the association constants between 12C4 or cyclen with γ-CD are significantly weaker and are in the range of 10−20 M^{-1}.[13]

The solvent effect on the diffusion coefficients and on the association constants derived from them for the γ-CD: 12C4 system is shown in Table 3. For example, we have found that the association constant between 12-crown-4 and γ-CD are 187 and 165 M^{-1} in water and in 80:20 (v/v) methanol/water, respectively. Even in DMSO-d_6 the calculated association constant for the γ-CD:12C4 system was found to be 111 M^{-1} which is only ∼30% lower than that in aqueous solution. These results are in contrast to the characteristics of most known CDs complexes.[2b,14] Interestingly, in all three solvents studied, addition of salts caused a pronounced decrease in the association between γ-CD and 12C4.

The effect of pH on the diffusion coefficients of the 1:1:1 solution of γ-CD:12C4:LiCl and the 1:1 solution of γ-CD: 12C4 and the free components along with the association constants calculated from these data are shown in Table 4. Experiments were performed in pure D_2O and in D_2O solutions having a pD of 2.0, 7.6, and 14. The association constant between γ-CD and 12C4 was similar in all pHs, and the calculated values were found to be 181, 187, and 167 for pD of 2.0, 7.6, and 14, respectively, although a decrease in the diffusion coefficients of the different components is observed when increasing the pH.

Discussion

As the main interest in CDs arises from their ability to form complexes with a wide range of organic molecules in aqueous solution, it is important to determine the association constant of such systems and even more so to determine the factors that govern the association between CD and organic guests. We chose to concentrate in this study on complexes in which the guests them-

(13) It should be noted that these values are only approximate values since these values were obtained by taking into account only the major interactions and by assuming that addition of γ-CD has only a minor effect on the interaction between the macrocycle and the salt. Therefore the diffusion coefficients of the macrocycle in the 1:1 solution of the macrocycle:salt and in the 1:1:1 γ-CD:macrocycle:salts were used to calculate the fraction of bound macrocycle. By doing so we ignored, for example, the competing reaction of the formation of a γ-CD: macrocycle complex; however, the diffusion coefficient of γ-CD in the absence and in the presence of salt is similar so our error can only be in the actual concentration that prevails in solution and therefore may have only a limited effect on the calculated K_as. Nevertheless one should note that in the cases of three- component solutions the reported values are only approximate values.

(14) (a) Only a few cases are known in which the addition of alcohol brings about an increase in the association constant of CD complexes in water and even there a decrease in the K_a was observed at high alcohol concentrations. See for example: Nelson, G.; Patonay, G.; Warner, I. M. *J. Incl. Phenom.* **1988**, *6*, 277. (b) Connors, K. A.; Mulski, M. J.; Paulson, A. *J. Org. Chem.* **1992**, *57*, 1974.

Table 1. Diffusion Coefficients and the Calculated Association Constants (K_a) of the Different γ-CD:Macrocycle and Macrocycle:Salt Systems Studied at 298 K[a,b]

system	$D_{\gamma-CD}$ ($\times10^{-5}$ cm^2/s)	$D_{macrocycle}$ ($\times10^{-5}$ cm^2/s)	D_{OAc^-} ($\times10^{-5}$ cm^2/s)	K_a M^{-1}
12C4+γ-CD+LiOAc	0.27 ± 0.01	0.56 ± 0.01	0.86 ± 0.02	11[c]
12C4+γ-CD	0.27 ± 0.01	0.48 ± 0.01	–	187
12C4	–	0.68 ± 0.02	–	–
γ-CD	0.32 ± 0.02	–	–	–
LiOAc	–	–	1.02 ± 0.01	–
12C4+LiOAc	–	0.60 ± 0.01	0.90 ± 0.01	40
12N4+γ-CD+LiOAc	0.30 ± 0.01	0.53 ± 0.01	0.96 ± 0.01	19[c]
12N4+γ-CD	0.29 ± 0.01	0.42 ± 0.01	–	165
12N4	–	0.60 ± 0.01	–	–
γ-CD	0.32 ± 0.02	–	–	–
LiOAc	–	–	1.09 ± 0.01	–
12N4+LiOAc	–	0.58 ± 0.01	0.96 ± 0.01	29
12S4+γ-CD+LiOAc	0.19 ± 0.02	0.36 ± 0.02	0.82 ± 0.01	21[c]
12S4+γ-CD	0.19 ± 0.01	0.34 ± 0.02	–	69
12S4	–	0.41 ± 0.02	–	–
γ-CD	0.24 ± 0.02	–	–	–
LiOAc	–	–	0.87 ± 0.02	–
12S4+LiOAc	–	0.39 ± 0.01	0.84 ± 0.01	10

[a] All experiments were performed three times and the reported values are means ± SD. [b] The 12C4 and the 12N4 systems were measured in D_2O solution having a pD of 7.6 while the 12S4 was measured in DMSO-d_6. [c] See footnote 13.

Table 2. The Diffusion Coefficients and the K_as of the γ-CD:12C4 Systems in the Absence and in the Presence of Various Salts[a,b]

system	$D_{\gamma-CD}$ ($\times10^{-5}$ cm^2/s)	D_{12C4} ($\times10^{-5}$ cm^2/s)	D_{anion} ($\times10^{-5}$ cm^2/s)	K_a M^{-1}
12C4	–	0.68 ± 0.02	–	–
12C4+γ-CD	0.27 ± 0.01	0.48 ± 0.01	–	187
12C4+LiOAc	–	0.60 ± 0.01	0.90 ± 0.01	40
12C4+LiOAc+γ-CD	0.27 ± 0.02	0.56 ± 0.01	0.86 ± 0.02	11[c]
12C4+LiCl	–	0.61 ± 0.02	–	–
12C4+LiCl+γ-CD	0.26 ± 0.01	0.56 ± 0.01	–	20[c]
12C4+$LiClO_4$	–	0.59 ± 0.01	–	–
12C4+$LiClO_4$+γ-CD	0.25 ± 0.01	0.57 ± 0.02	–	17[c]
12C4+KOAc	–	0.66 ± 0.02	0.96 ± 0.01	11[c]
12C4+KOAc+γ-CD	0.27 ± 0.01	0.61 ± 0.01	0.96 ± 0.01	10[c]
12C4+$(Me)_4NCl$	–	0.65 ± 0.01	1.10 ± 0.02	30[c]
12C4+$(Me)_4NCl$+γ-CD	0.26 ± 0.01	0.57 ± 0.01	1.01 ± 0.01	21[c]
$(Me)_4NCl$	–	–	1.23 ± 0.01	–

[a] All experiments were performed three times and the reported values are means ± SD. Experiments were performed at 298 K. [b] Values obtained for samples measured in buffered D_2O solution having a pD = 7.6. [c] See footnote 13.

Table 3. The Diffusion Coefficients and the Calculated Association Constants (K_a) of the γ-CD:12C4 Systems and γ-CD:12C4:LiOAc Systems in Different Solvents[a]

system	solvent	$D_{\gamma-CD}$ ($\times10^{-5}$ cm^2/s)	D_{12C4} ($\times10^{-5}$ cm^2/s)	D_{anion} ($\times10^{-5}$ cm^2/s)	K_a M^{-1}
12C4+LiOAc+γ-CD	D_2O	0.27 ± 0.01	0.56 ± 0.01	0.86 ± 0.02	11[c]
12C4+LiOAc+γ-CD	$CD_3OD + D_2O$[b]	0.28 ± 0.02	0.89 ± 0.02	1.06 ± 0.02	10[c]
12C4+LiOAc+γ-CD	DMSO-d_6	0.19 ± 0.01	0.40 ± 0.01	0.82 ± 0.01	13[c]
12C4+γ-CD	D_2O	0.27 ± 0.01	0.48 ± 0.01	–	187
12C4+γ-CD	$CD_3OD + D_2O$[b]	0.26 ± 0.02	0.78 ± 0.02	–	165
12C4+γ-CD	DMSO-d_6	0.18 ± 0.02	0.36 ± 0.01	–	111
12C4+LiOAc	D_2O	–	0.60 ± 0.01	0.90 ± 0.01	40
12C4+LiOAc	$CD_3OD + D_2O$[b]	–	0.96 ± 0.03	1.09 ± 0.04	138
12C4+LiOAc	DMSO-d_6	–	0.38 ± 0.02	0.86 ± 0.02	10
12C4	D_2O	–	0.68 ± 0.02	–	–
12C4	$CD_3OD + D_2O$[b]	–	1.11 ± 0.04	–	–
12C4	DMSO-d_6	–	0.48 ± 0.02	–	–
LiOAc	D_2O	–	–	1.02 ± 0.01	–
LiOAc	$CD_3OD + D_2O$[b]	–	–	1.22 ± 0.06	–
LiOAc	DMSO-d_6	–	–	0.87 ± 0.02	–

[a] All experiments were performed three times and the reported values are means ± SD. Experiments were performed at 298 K. [b] A (80:20 v/v) CD_3OD:$D^{O\ solution.}$ [c] See footnote 13.

selves can serve as hosts because of the potential of such systems as drug delivery systems and as potential sensors.

The results clearly demonstrate that the three macrocycles studied form complexes of moderate stability with the γ-CD, although it is clear that 12S4 forms a somewhat weaker complex with γ-CD. The K_as for γ-CD:12C4 and γ-CD:12N4 in D_2O were nearly the same, while in DMSO-d_6 the K_as for the γ-CD:12C4 and γ-CD:12S4 complexes were found to be 111 and 69 M^{-1}, respectively. However, an interesting result is the fact that these complexes are much weaker in the presence of salts. Several plausible explanations can be suggested for the above observations. At first glance one might assume that when lithium salts are present in the solution a weak complex is formed between the macrocycle and the lithium salt, and consequently the macrocycle undergoes a conformational change and adopts a somewhat more rigid conformation which is less suited for optimal interaction with the γ-CD cavity. In contrast the free

Table 4. The Effects of pH on the Diffusion Coefficients and the Calculated Association Constants of the γ-CD:12C4 Systems Studied at 298 K[a]

		diffusion cofficients		
system	pH	$D_{\gamma-CD}$ ($\times10^{-5}$ cm^2/s)	D_{12C4} ($\times10^{-5}$ cm^2/s)	K_a M^{-1}
12C4+LiCl+γ-CD	2.0	0.27 ± 0.01	0.58 ± 0.02	21[b]
12C4+LiCl+γ-CD	7.6	0.26 ± 0.01	0.56 ± 0.01	20[b]
12C4+LiCl+γ-CD	14	0.24 ± 0.01	0.53 ± 0.01	31[b]
12C4+γ-CD	2.0	0.28 ± 0.01	0.49 ± 0.01	181
12C4+γ-CD	7.6	0.27 ± 0.02	0.48 ± 0.02	187
12C4+γ-CD	12	0.21 ± 0.02	0.44 ± 0.02	–
12C4+γ-CD	14	0.19 ± 0.01	0.41 ± 0.02	167
12C4+LiCl	2.0	–	0.61 ± 0.01	–
12C4+LiCl	7.6	–	0.61 ± 0.02	–
12C4+LiCl	14	–	0.58 ± 0.01	–
12C4	2.0	–	0.70 ± 0.01	–
12C4	7.6	–	0.68 ± 0.02	–
12C4	14	–	0.60 ± 0.02	–
γ-CD	2.0	0.34 ± 0.01	–	–
γ-CD	7.6	0.32 ± 0.02	–	–
γ-CD	14	0.26 ± 0.02	–	–

[a] All experiments were performed three times and the reported values are means ± SD. [b] See footnote 13.

macrocycles keep their conformational flexibility and are therefore totally free to adopt the optimal conformation which will result in the strongest interaction possible between the γ-CD cavity and the macrocycles. In order to accept the above explanation one must assume the formation of a complex between the lithium salts and 12C4 and cyclen in D_2O, and indeed the reduction in the diffusion coefficient of the acetate ion upon addition of 12C4 to the D_2O solution of lithium acetate may indicate the formation of such a weak complex. However, it should be noted that the value we obtained for the association constant between the lithium acetate and 12C4 in D_2O is relatively small (K_a of 40 M^{-1}) and was obtained by the indirect measurement of the change in the diffusion coefficient of the acetate anion and not of the lithium cation. Therefore caution should be exercised about this value especially in view of another report which claims that K_a of LiCl and 12C4 in H_2O is nearly 0.[15]

Another observation that seems to disfavor the explanation that the formation of a complex between the macrocycle and the salts is the major factor responsible for the low K_a between the γ-CD and the macrocycle is the fact that the reduction in the K_a is observed for different salts, some of which form only very weak complexes with 12C4 aqueous solution. In addition, in a control experiment we also found that in aqueous solution the diffusion coefficient of the acetate anion of the lithium acetate salt decreases upon addition of γ-CD. Therefore some other, nonspecific interaction between γ-CD and the salts, which is not mediated by the 12C4 or the cyclen, may also contribute to the decrease in the association between γ-CD and the macrocycles.

A crown ether in water should adopt an average conformation in which the hydrophilic lone pairs of the oxygens are pointing outside the macrocyclic ring.[1a] Therefore, in order to interact with the hydrophobic cavity of the CD, the macrocycle should undergo a conformational reorganization. However, if a cation is complexed by the crown ether it seems conceivable that the average conformation is one in which the oxygen lone pairs point out toward the cation in the center of the macrocyclic ring of the crown ether. Therefore, now the CH_2 groups of the crown ether point out from the center of the macrocyclic ring, and the outer part of the crown ether becomes more lipophilic. Consequently, it seems that the complexed macrocycle should be more preorganized toward complexation with the hydrophobic cavity of the CD, but our results show the contrary. On the basis of this argument, we came to the conclusion that the hydrophobic interaction is probably not the dominating driving force for these complexes. Therefore we anticipated that contrary to most CD complexes, addition of organic solvents should not have a devastating effect on the association constant of this particular type of complex and we hence decided to explore the effect of organic solvents on the association constant of these complexes.

The results (Table 3) clearly show that, as we expected and in contrast to the general trend observed for CD complexes,[14] there is nearly no difference between the association constants of the γ-CD:12C4 complex in D_2O and in 80:20 (v/v) CD_3OD/D_2O. Surprisingly, we have found that even in pure DMSO-d_6 K_a is smaller only by ca. 30% as compared to K_a in D_2O. In many CD complexes it has been shown that addition of less than 20% of methanol to an aqueous solution can bring about more than a 20-fold reduction in the association constant[14] of the CD complexes. Conners et al. have found also a 10-fold reduction in the association constant of the Methyl orange/α-CD system upon addition of less than 10% of DMSO to an aqueous solution.[14b] Several intramolecular interactions have been proposed as driving forces for the formation of CD inclusion complexes in water among which the hydrophobic interaction between the organic molecule and the cavity of the CD was found to play a major role.[16] If this is the case it is not surprising that addition of alcohol or other organic solvents reduce considerably the association constants of these complexes. Adding the alcohol to the aqueous solution reduces the polarity of the solvent and decreases the hydrophobicity difference between the solvent and the γ-CD cavity, and hence a decrease in K_a is to be expected. The fact that K_a for the γ-CD:12C4 complex is nearly the same in water and in 80% methanol and only slightly lower in DMSO may suggest that hydrophobic interactions are not the major factor responsible for complexation in the studied host/guest systems.

The additional interactions that can be the driving force for the formation of CD complexes in aqueous solutions are hydrogen bonding and van der Waals interaction.[16] Hydrogen bonds clearly depends on the pH. Therefore we repeated the measurements in different pHs, but the results show that although some changes in the diffusion coefficients are observed, the changes in the calculated association constants are not significant. The association constant nearly did not change in a pH ranging from 2 to 14, suggesting that hydrogen bonding is also not a dominant factor governing the association constant of the γ-CD:12C4 complex.

The many experimental methods used to study binding constants have been reviewed by Connors in his excellent monograph.[17] According to the literature, optical spectroscopy is the most widely used method for determining

(15) Smetana, A. J.; Popov, A. I. *J. Solution Chem.* **1980**, *9*, 182.
(16) Bortolus, P.; Monti, S. In *Advances in Photochemistry*; Neckers, D. C., Volman, D. H., Eds.; Wiley & Sons, Inc.: New York, 1996; Vol. 21, p 1.
(17) Connors, K. A. Binding Constants: The Measurements of Molecular Complex Stability; Wiley-Interscience: New York, 1987.

association constants, but recently the relative importance of NMR-based methods has increased.[18] When one refers to NMR methods, one generally has in mind NMR chemical shift- or relaxation time-based methods.[18,19] However, in the present study we have used NMR diffusion measurements to study the formation of macrocycle complexes of γ-CD. As stated previously, we have done so since the formation of the macrocycle:γ-CD complexes are accompanied by only negligible changes in the ^{1}H chemical shifts.

The NMR diffusion measurement is an NMR method and as such has the advantage over UV and fluorescence spectroscopy because NMR is not subject to misinterpretations that can be caused by minor impurities and provides better structural information on the investigated species. In addition the use of NMR diffusion measurements prevents the possibility of confusing acid−base chemistry with binding phenomena, one of the main drawbacks of other NMR methods.[18] In contrast to chemical shifts, which can be effected by proton transfer, diffusion is relatively insensitive to such processes. Therefore, NMR diffusion measurement was found to be a useful method for studying the complexation of methyl ammonium salts to cryptands[10] which are known to be partially protonated by ammonium salts.[20] The main disadvantage of NMR diffusion measurements is that they are relatively time consuming as compared to the NMR chemical shift method for example. However, NMR diffusion measurements are less time consuming than relaxation time measurements. An additional advantage of NMR diffusion measurements is that the diffusion coefficient is a parameter which is intuitively related to the formation of complexes,[21] in contrast to chemical shift or relaxation time. For example, one would expect the diffusion coefficient of a small guest to decrease upon formation of a complex with a much larger host and to approach that of the host in the case of total binding. In other words one can predict the diffusion coefficient of a small guest which is entirely bound to a large host, while the chemical shift (or the relaxation time) of the small guest in the complexed form cannot be predicted a priori and can be obtained only after lengthy titration. Therefore one should perform less measurements in order to feel confident with the data when using diffusion measurements. In our experience when the host and the guest are both organic species then NMR diffusion measurement is an attractive method for studying complexation. The larger the difference in size between the guest and the host, the more accurate are the values obtained from NMR diffusion measurements. It should be noted, however, that host/guest systems in which the components of the complex are of comparable size may be difficult to study as they may have very similar diffusion coefficients in the free state. Another limitation of the PGSE technique is that this technique is based on spin echo and as such is difficult to apply on systems which are characterized by short spin−spin relaxation time (T_2). Therefore the PGSE may not be the method of choice for studying the association between cation and macrocycles, for example, especially in a medium in which one or more components of the complex are highly solvated. In such systems one has to measure the diffusion coefficient of the components of the complex by two different experiments which is time consuming especially in view of the fact that diffusion measurements of cations are usually more demanding because of their unfavorable NMR characteristics as compared to protons (low sensitivity and short T_2). In addition, in water cations may be strongly solvated and as such their diffusion coefficients may also reflect their solvation state. For example, it is conceivable that the replacement of the solvating water molecules of the lithium cation by a macrocycle, for example, may lead to a much smaller decrease in the diffusion coefficient of the cation upon complexation which will complicate the extraction of the association constant out of these data.[22] However, all these problems are not crucial limitations for organic host/guest systems since most of them are characterized by relatively long T_2 and are only weakly solvated. It should be noted that some large organic supramolecular systems comprising CDs have been prepared recently which should be characterized by a short T_2.[23] However, the progress in pulse gradients technology made in recent years enables us to study most organic CDs host/guest systems known with the available technology. In addition, if host/guest systems having a short T_2 are the focus of the study, one can use the stimulated echo diffusion technique[24] which is less sensitive to T_2 relaxation.

Since modern commercial NMR spectrometers are equipped with gradient units which enable easy and accurate simultaneous determination of diffusion coefficients of complexed systems and in view of the recent advance in diffusion-ordered 2D techniques,[25] we anticipate that NMR diffusion measurements will find more applications in organic supramolecular chemistry in the near future.

JO961758X

(18) Wilcox, C. S. In *Frontiers of Supramolecular Chemistry and Photochemistry;* Schneider, H.-J., Durr, H., Eds.; VCH: Weinheim, 1991; p 123.

(19) See for example: (a) Khazaeli, S.; Dye, J. L.; Popov, A. I. *J. Phys. Chem.* **1983**, *87*, 1830. (b) Strover, H. D. H.; Delville, A.; Detellier, C. *J. Am. Chem. Soc.* **1985**, *107*, 4167. (c) Wang, T.; Bradshaw, J. S.; Izatt, R. M., *J. Heterocycl. Chem.* **1994**, *31*, 1097 and references cited therein.

(20) Smith, P. B.; Dye, J. L.; Cheney, J.; Lehn, J.-M. *J. Am. Chem. Soc.* **1981**, *103*, 6044.

(21) Recently, fluorescence was used to calculate the association constant of 5,10,15,20 tetrakis(4-carboxyphenyl)porphyrin to β-CD and the discrepancy was not only in the values obtained but even the sign of the changes in the fluorescence that should be observed upon complex formation. In one report, Zhao, S.; Luong, J. H. T. *J. Chem. Soc. Chem. Commun.* **1994**, 2307, an increase in fluorescence was reported while in another Venema, F.; Rowan, A. E.; Nolte, R. J. M. *J. Am. Chem. Soc.* **1996**, *118*, 257 a decrease in fluorescence was reported.

(22) The complexation of Cs^+ to 18-crown-6 in D_2O has been studied previously using ^{133}Cs and ^{1}H NMR diffusion measurements, see: Geringer, M.; Sterk, H., *Magn. Reson. Chem.* **1989**, *27*, 1148. In addition diffusion coefficients obtained by electrochemical methods have been used to calculated association constants between CDs and ferrocene derivatives: Isnin, R.; Salam, C.; Kaifer, A. E. *J. Org. Chem.* **1991**, *56*, 35.

(23) (a) Harada, A.; Li, J.; Kamachi, M. *Nature* **1992**, *356*, 325. (b) Li, G.; McGown, L. B. *Science* **1994**, *264*, 249.

(24) Tanner, J. E. *J. Chem. Phys.* **1970**, *52*, 2523.

(25) Very recently some diffusion ordered 2D-NMR pulse sequences were presented which are likely to increase our ability to study diffusion of more complexed systems, see for example: Wu, D.; Chen, A.; Johnson, C. S., Jr. *J. Magn. Reson. A* **1996**, *121*, 88. Gozansky, E. K.; Gorenstein, D. G. *J. Magn. Reson. B* **1996**, *111*, 94.