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Effects of pressure on inclusion complexation of methylene blue with water-soluble *p*-sulfonatocalix[*n*]arenes

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ABSTRACT: The effects of pressure were examined for the inclusion complexation of water-soluble *p*-sulfonated calix[*n*]arenes (Calix-S*n*: $n = 4$, Calix-S4; $n = 6$, Calix-S6; $n = 8$, Calix-S8) with methylene blue (MB). A differential pressure effect was found in the inclusion equilibria of Calix-S*n*. As the external pressure increases, the inclusion equilibrium of MB with Calix-S4 and Calix-S6 shifts to the dissociation side. However, with Calix-S8, the inclusion equilibrium shifts to the association side with increased pressure. From the pressure dependence of the inclusion equilibria of Calix-S*n*, the reaction volumes, which are changes in volume accompanied by inclusion, were estimated as $32.1\,cm^3\,mol^{-1}$ for Calix-S4, $30.1\,cm^3\,mol^{-1}$ for Calix-S6 and $-13.8\,cm^3\,mol^{-1}$ for Calix-S8. In addition, the structures of the inclusion complexes of Calix-S*n* with MB have been established by ^{1}H NMR measurements. Based on the results, the behavior of inclusion complexation with Calix-S*n* is discussed. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: pressure effect; inclusion complexation; NMR; calixarene

INTRODUCTION

Calixarenes are cyclic oligomers composed of phenyl units linked by methylene and they possess a cylindrical architecture similar to cyclodextrins (CD). Complexation of macrocyclic compounds with guest molecules in aqueous media has attracted much attention because of their potential as enzyme mimics.[1,2] The water-soluble *p*-sulfonatocalix[*n*]arenes, which were prepared by Shinkai *et al*.,[3] are powerful receptors for a variety of organic compounds and inorganic ions.[2] Recently, the conformation and inclusion complexation of water-soluble *p*-sulfonatocalix[*n*]arenes have been studied actively by several groups.[4–6]

Examination of the pressure effects on chemical reactions is instructive in clarifying the reaction mechanisms. The concept of volume is more direct than that of energy, and the results obtained from the pressure effects give useful information concerning reaction mechanisms.[7] In previous papers, to discuss the inclusion behavior of CD, we used spiropyran and azo dyes to study pressure effects on the inclusion complexation of CD.[8–10] Calixarene has a hydrophobic cavity similar to CD and it would be interesting to know the mechanisms involved in the change in volume during formation of the inclusion complex with calixarenes. However, no work has been reported in this field. This work was motivated by the expectation that the inclusion in the cavity of calixarenes might give rise to a large amount of volume change. In this study, we examined the pressure effects on the inclusion equilibria of three kinds of *p*-sulfonatocalix[*n*]arenes ($n = 4$, 6 and 8) with 3,7-bis(dimethylamino)-phenothiazin-5-ium chloride (methylene blue, MB), and undertook a volumetric study to clarify the inclusion behavior of *p*-sulfonatocalix[*n*]arenes.

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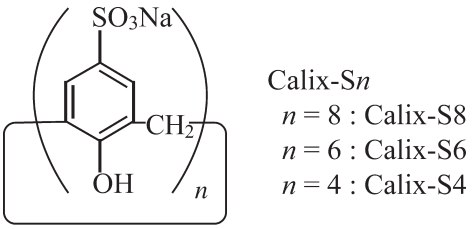
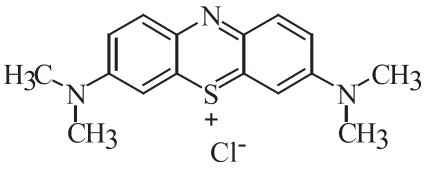
EXPERIMENTAL

The three kinds of water-soluble *p*-sulfonatocalix[*n*]arenes used in this study are shown in Fig. 1. Calix-S6 and Calix-S8 were purchased from Dojin Chemicals (Kumamoto, Japan) and Calix-S4 was obtained from Sugai Chemie Inc. (Wakayama, Japan). Methylene blue (MB) was purchased from Wako Pure Chemicals (Osaka, Japan). Calixarenes and MB were recrystallized from water–ethanol mixtures and dried under vacuum before use. Reagent-grade methanol was obtained commercially (Wako Pure Chemicals, Osaka, Japan). The methanol and water were purified by distillation.

A phosphate buffer (pH 6.9 and ionic strength = 0.1) was prepared from phosphate salts. To avoid the dimerization of MB,[11] we used a methanol–water mixture (1:1 v/v phosphate buffer) as solvent. The concentrations of the sample solutions were $[MB] \approx 1 \times 10^{-5}\,mol\,dm^{-3}$ and $[Calix\text{-}Sn] = 0\text{–}1(\times 10^{-3})\,mol\,dm^{-3}$. The spectral change of MB in the presence of Calix-S*n* at atmospheric



Calix-S*n*
n = 8 : Calix-S8
n = 6 : Calix-S6
n = 4 : Calix-S4



Methylene blue (MB)

Figure 1. Structures of MB and *p*-sulfonatocalix[*n*]arenes used in this study

pressure was monitored by using a Shimadzu MultiSpec-1500 spectrophotometer (Kyoto, Japan). The solution temperature was maintained to within ± 0.1 K at 298 K by means of an external circulating water bath.

The instructions and procedures for measurements under high pressures have been described elsewhere.[10] The sample solution was charged in a Teflon inner cell, and the pressure vessel was connected to a spectrophotometer through a Hitachi optical fiber. The spectral changes at high pressures were monitored by using a Hitachi U-3200 spectrophotometer (Tokyo, Japan). The reaction temperature was controlled at 298 K.

The ^{1}H NMR spectra were measured in 1:1 D_2O–CD_3OD mixtures with a Varian Inova AS600 instrument (600 MHz) at room temperature. Chemical shifts were reported as δ values relative to CHD_2OD (δ 3.31)[12] as internal standard. Methylene blue was maintained at a constant concentration of $\sim 1 \times 10^{-4}$ mol dm^{-3} and the concentration of Calix-S*n* was varied in the range 0–1.4($\times 10^{-3}$) mol dm^{-3}.

RESULTS AND DISCUSSION

Complexation of MB with Calix-S*n*

Figure 2 shows the UV–Vis absorption spectra of MB upon consecutive additions of excess Calix-S8. As the concentration of Calix-S8 increases, the intensity of the absorption peak at 663 nm due to MB decreases, and an absorption band caused by the complex of MB with Calix-S8 appeared in the vicinity of 690 nm accompanied by an isosbestic point at 673 nm. The existence of the isosbestic point is related to the following 1:1 inclusion equilibrium:

$$\mathrm{MB} + \mathrm{Calix} \rightleftharpoons \mathrm{MB\text{–}Calix}$$

$$K_{11} = \frac{[\mathrm{MB\text{–}Calix}]}{[\mathrm{MB}][\mathrm{Calix}]} \tag{1}$$

where MB–Calix denotes the inclusion complex. In the presence of excess Calix-S*n*, the K_{11} value can be expressed as:

$$K_{11} = \frac{[\mathrm{MB\text{–}Calix}]}{[\mathrm{MB}][\mathrm{Calix}]_0} = \frac{[\mathrm{MB\text{–}Calix}]}{([\mathrm{MB}]_0 - [\mathrm{MB\text{–}Calix}])[\mathrm{Calix}]_0} \tag{2}$$

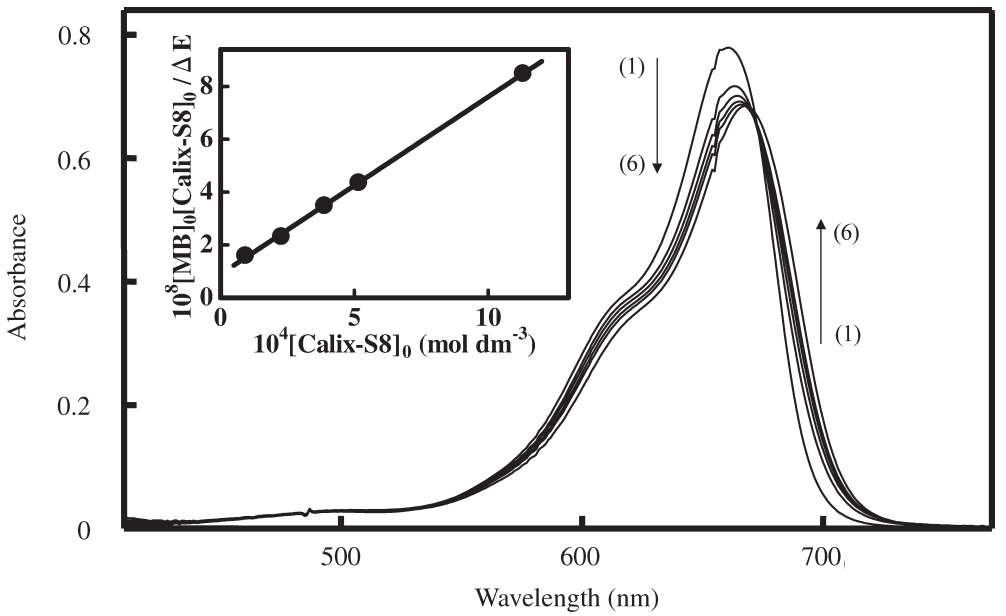


Figure 2. Absorption spectra of MB ($[\mathrm{MB}]_0 = 8.02 \times 10^{-6}$ mol dm^{-3}) containing different concentrations of Calix-S8 in a methanol–water mixture at 298 K: (1) 0, (2) 9.30×10^{-5}, (3) 2.27×10^{-4}, (4) 3.88×10^{-4}, (5) 5.19×10^{-4} and (6) 1.13×10^{-3} mol dm^{-3} of Calix-S8. (*Inset*) Determination of 1:1 inclusion equilibrium constant according to Eqn (3)

Table 1. The equilibrium constants (K_{11}) for inclusion complexation of MB with Calix-S6 and Calix-S8 in a mixture of methanol and water

Host	$10^{-3}K_{11}$ ($mol^{-1}\,dm^3$)				ΔV ($cm^3\,mol^{-1}$)
	1 bar	196 bar	490 bar	735 bar	
Calix-S6	1.92 ± 0.03	1.51 ± 0.02	1.05 ± 0.05	0.768 ± 0.030	30.1 ± 0.2
Calix-S8	7.53 ± 0.32	8.34 ± 0.06	9.62 ± 0.07	11.1 ± 0.2	-13.8 ± 0.2

Because the concentration of MB–Calix can be given by [MB–Calix] = $\Delta E/\Delta\varepsilon$, Eqn (2) can be rewritten as:

$$\frac{[\mathrm{MB}]_0[\mathrm{Calix}]_0}{\Delta E} = \frac{1}{K_{11}\Delta\varepsilon} + \frac{1}{\Delta\varepsilon}[\mathrm{Calix}]_0 \qquad (3)$$

where $[MB]_0$ and $[Calix]_0$ are the total concentration of MB and Calix-S*n*, respectively, $\Delta\varepsilon$ is the difference in the molar extinction coefficients for bound and unbound MB with Calix-S*n* and ΔE is the change in the absorption intensity of the MB solution as Calix-S*n* is added. Many investigators have used the Benesi–Hildebrand-type equation (Eqn (3)) to conduct extensive studies for the 1:1 complex formation.[13] The spectral data shown in Fig. 2 can be analyzed according to Eqn (3). As is seen in Fig. 2, a linear relationship between $[MB]_0[Calix]_0/\Delta E$ and $[Calix]_0$ can be obtained, indicating the 1:1 complexation. The association constants K_{11} for the complexation with Calix-S*n* can be obtained by dividing the slope by the intercept. Adding Calix-S6 produced a similar spectral change. The association constants of MB with Calix-S6 and Calix-S8 were determined by Eqn (3) and are given in Table 1. The K_{11} value for Calix-S8 is larger than that for Calix-S6. The stability of an inclusion complex may be determined by how the guest fits into the calixarene cavity. Therefore, the above results indicate that the fit of MB to the cavity space of Calix-S8 is better than to that of Calix-S6.

Figure 3 shows the spectral change of MB upon consecutive additions of Calix-S4. As the concentration of Calix-S4 increases, the intensity of the absorption peak at 663 nm due to MB decreases, which is different from the changes in intensity upon the addition of Calix-S6 and Calix-S8. When Calix-S4 exceeded MB ($[Calix\text{-}S4]_0/[MB]_0 > 13$–$124$), the plots of $[MB]_0[Calix]_0/\Delta E$ against $[Calix]_0$ for the 1:1 complexation of Eqn (3) did not give evidence of a linear relationship. There is a possibility that MB forms 1:2 inclusion complexes with Calix-S4. It can be assumed reasonably that 1:2 inclusion

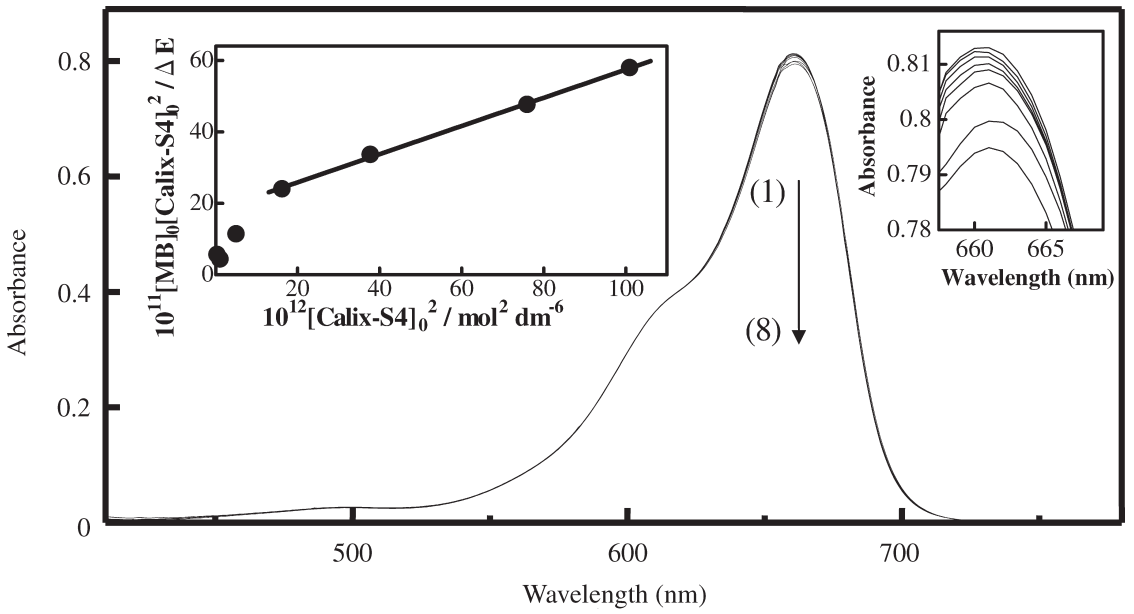


Figure 3. Absorption spectra of MB ($[MB]_0 = 8.02 \times 10^{-6}\,mol\,dm^{-3}$) containing different concentrations of Calix-S4 in a methanol–water mixture at 298 K: (1) 0, (2) 1.92×10^{-5}, (3) 1.08×10^{-4}, (4) 2.26×10^{-4}, (5) 4.04×10^{-4}, (6) 6.15×10^{-4}, (7) 8.72×10^{-4} and (8) $1.00 \times 10^{-3}\,mol\,dm^{-3}$ of Calix-S4. (*Inset*) Determination of 1:2 inclusion equilibrium constant according to Eqn (6)

complexation occurs with a large guest molecule such as MB, because the Calix-S4 cavity is small. The 1:2 inclusion equilibrium (K_{12}) of MB with Calix-S4 can be expressed as:

$$\mathrm{MB} + 2\mathrm{Calix} \rightleftharpoons \mathrm{MB\text{–}Calix_2}$$

$$K_{12} = \frac{[\mathrm{MB\text{–}Calix_2}]}{[\mathrm{MB}][\mathrm{Calix}]^2} \tag{4}$$

where MB–$Calix_2$ denotes the 1:2 inclusion complex. In excess of Calix-S4, the K_{12} value can be expressed as:

$$K_{12} = \frac{[\mathrm{MB\text{–}Calix_2}]}{[\mathrm{MB}][\mathrm{Calix}]_0^2} = \frac{[\mathrm{MB\text{–}Calix_2}]}{([\mathrm{MB}]_0 - [\mathrm{MB\text{–}Calix_2}])[\mathrm{Calix}]_0^2} \tag{5}$$

and because $[\mathrm{MB\text{–}Calix_2}] = \Delta E/\Delta\varepsilon$, Eqn (5) becomes:

$$\frac{[\mathrm{MB}]_0[\mathrm{Calix}]_0^2}{\Delta E} = \frac{1}{K_{11}\Delta\varepsilon} + \frac{1}{\Delta\varepsilon}[\mathrm{Calix}]_0^2 \tag{6}$$

The spectral data for the formation of the 1:2 complex can be analyzed according to Eqn (6). As shown in Fig. 3, a linear relationship between $[\mathrm{MB}]_0[\mathrm{Calix}]_0^2/\Delta E$ and $[\mathrm{Calix}]_0^2$ can be obtained in the presence of a great excess of Calix-S4 ($[\mathrm{Calix\text{-}S4}]_0/[\mathrm{MB}]_0 = 50$–$124$). This linear behavior is believed to indicate the existence of 1:2 inclusion complexes. The K_{12} value for the 1:2 inclusion complexation can be obtained by dividing the slope of Eqn (6) by the intercept, and is given in Table 2.

Under the condition of a low concentration of Calix-S4 ($[\mathrm{Calix\text{-}S4}]_0/[\mathrm{MB}]_0 = 0$–$28$), the spectral change of MB is very small and thus 1:1 complexation analysis by using a small spectral change has been unsuccessful. We consider that a part of the guest MB molecule is merely encapsulated by Calix-S4, which has a small cavity (upper-rim diameter = 3.8 Å[14]) resulting in a small spectral change.

The free energy changes for the complex formation of MB with Calix-S4, -S6 and -S8 can be estimated from:

$$\Delta G^\circ = -RT\ln K \tag{7}$$

The ΔG_{11}° values for the 1:1 complex formation of MB with Calix-S6 and Calix-S8 can be estimated to be −18.7 and −22.1 kJ mol^{-1}, respectively. The ΔG_{12}° value for the 1:2 complex formation with Calix-S4 can be estimated to be −34.5 kJ mol^{-1} according to Eqn (7). The absolute value of ΔG_{11}° for Calix-S6 is almost half that of ΔG_{12}° for Calix-S4, which is responsible for the difference in the number of host molecules upon complex formation.

Pressure effects on inclusion equilibria

The association constants for the inclusion equilibria of MB with Calix-S4, -S6 and -S8 at high pressures are given in Tables 1 and 2. The K_{11} and K_{12} values for Calix-S6 and Calix-S4 become smaller with increasing external pressure; conversely, the K_{11} value for Calix-S8 increases with increasing pressure. The equilibria of the inclusion complexation for Calix-S6 and Calix-S4 shift to the dissociation side with increased pressure, whereas the equilibrium for Calix-S8 shifts to the association side. The best straight lines for the plots of ln$K$ against pressure were drawn. The slopes then were used to evaluate the changes in volume (the reaction volume, ΔV) accompanied by inclusion of MB in the calixarene cavity according to the following equations:

$$\ln K = aP + b \tag{8}$$

$$\Delta V = -RT\left(\frac{\partial \ln K}{\partial P}\right)_T - \Delta n\kappa_T RT \tag{9}$$

where Δn is the difference between the numbers of species in the initial and final states, κ_T is the isothermal compressibility of the methanol–water mixture and was estimated to be 3.42×10^{-5} bar^{-1} at 298 K. The reaction volumes for inclusion complexation of MB with Calix-S4 and Calix-S6 have positive values, whereas that for Calix-S8 has a negative value (Tables 1 and 2).

Previously, we have discussed the contributions of different factors to volume changes for inclusion complex formation of cyclodextrins with spiropyran and substituted phenols.[8,10] Similarly, the volume changes for the inclusion complexation of MB with Calix-Sn can be expressed as:

$$\Delta V = \Delta V_{\mathrm{inclu}} + \Delta V_{\mathrm{desolv}} \tag{10}$$

where $\Delta V_{\mathrm{inclu}}$ and $\Delta V_{\mathrm{desolv}}$ are the volume changes related to inclusion of MB in the calixarene cavity ($\Delta V_{\mathrm{inclu}} < 0$) and to desolvation of the solvent around

Table 2. The equilibrium constants (K_{12}) for inclusion complexation of MB with Calix-S4 in a mixture of methanol and water

	$10^{-5}K_{12}$ (mol^{-2} dm^6)				ΔV
Host	1 bar	216 bar	510 bar	764 bar	(cm^3 mol^{-1})
Calix-S4	11.1 ± 0.3	8.40 ± 0.30	5.61 ± 0.20	3.64 ± 0.15	32.1 ± 0.5

the area of contact of MB and $—SO_3^-$ groups of Calix-S*n* ($\Delta V_{desolv} > 0$), respectively. The magnitude of the contributions of the ΔV_{inclu} and ΔV_{desolv} terms to the volume change is strongly related to the structure of the inclusion complex of MB with Calix-S*n*. In connection with the structure of the inclusion complex, details on the volume changes caused by the inclusion of MB into Calix-S*n* will be discussed later.

NMR study on the structure of inclusion complexes

^{1}H NMR is a valuable tool in the study of molecular interactions in solution, and ^{1}H NMR measurements give useful information on the complexation mode of the guest with the host. Figure 4(a) shows the 600 MHz ^{1}H NMR spectra of MB in a CD_3OD–D_2O mixture (1:1 v/v phosphate buffer). The assignments of the MB protons are given in Fig. 4(a). On adding Calix-S4 we can see that all peaks of the MB protons shift to a higher magnetic field. Figure 4(c) shows the chemical shifts for the N—CH_3, C^1—H, C^2—H and C^4—H protons of MB as a function of the $[Calix\text{-}S4]_0/[MB]_0$ ratios. The chemical shifts decrease along with increases in the concentration of Calix-S4. Table 3 shows the changes ($\Delta\delta$) in the upfield chemical shift of the MB protons in excess of Calix-S*n*. As suggested by Shinkai *et al.*,[6,14,15] the upfield shift of the guest protons indicates an inclusion of the proton moiety into the hydrophobic pocket of Calix-S*n*. From these changes in chemical shift we can make a few interesting observations:

Table 3. Changes ($\Delta\delta$)[a] in chemical shifts of MB in the presence of Calix-S*n*

Host	N—CH_3	C^1—H	C^2—H	C^4—H
Calix-S4[b]	−0.47	−0.12	−0.29	−0.24
Calix-S6[c]	−0.44	−0.08	−0.32	−0.32
Calix-S8[d]	−0.61	−0.18	−0.39	−0.54

[a] $\Delta\delta = \delta$(MB bound with Calix-S*n*) − δ(MB unbound with Calix-S*n*). Negative values indicate upfield shifts.
[b] $[Calix\text{-}S4]_0/[MB]_0 = 12$.
[c] $[Calix\text{-}S6]_0/[MB]_0 = 11$.
[d] $[Calix\text{-}S8]_0/[MB]_0 = 7.6$.

(i) The absolute values of $\Delta\delta$ for the N—CH_3 protons are large compared with those of other MB protons. This indicates that the MB molecule is encapsulated into the calixarene cavity from the $—N(CH_3)_2$ moiety.
(ii) The $|\Delta\delta|$ values of the C^2—H and C^4—H protons are large compared with those of the C^1—H protons. In the complexation of MB with Calix-S*n*, the C^2—H and C^4—H protons are included in the calixarene cavity, and the C^1—H protons may be located outside the cavity.

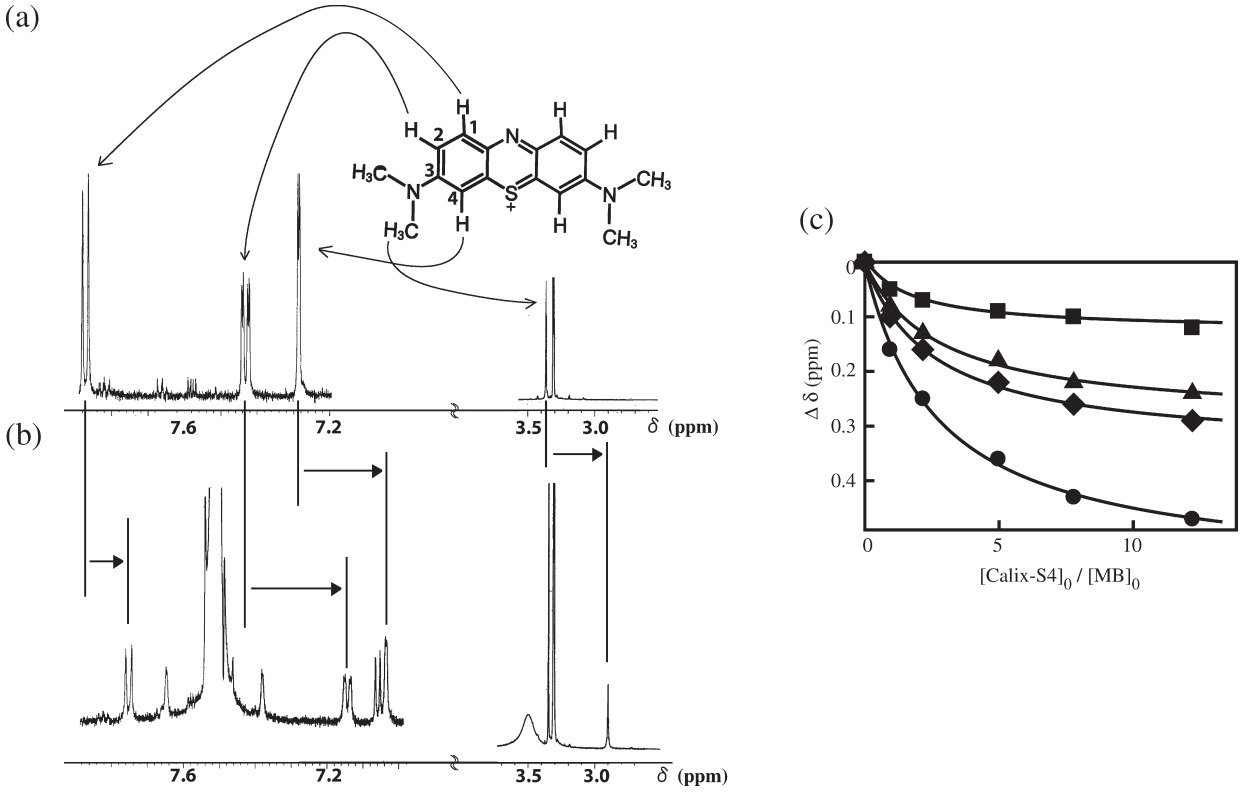


Figure 4. ^{1}H NMR spectra of MB ($[MB]_0 = 1.14 \times 10^{-4}$ mol dm^{-3}): (a) $[Calix\text{-}S4] = 0$ mol dm^{-3}; (b) $[Calix\text{-}S4]_0 = 1.39 \times 10^{-3}$ mol dm^{-3}. (c) Changes in chemical shifts of the MB protons at various concentrations of Calix-S4: (●) N—CH_3; (■) C^1—H; (◆) C^2—H; (▲) C^4—H

(iii) The $|\Delta\delta|$ values of the C^2—H and C^4—H protons for the inclusion complex with Calix-S6 are larger than those with Calix-S4. As reported by Shinkai *et al.*,[14] the diameter (5.0 Å) of the upper rim of Calix[6]-arene is larger than that (3.8 Å) of Calix[4]arene, which is responsible for the deep inclusion into the Calix-S6 cavity compared with that into the Calix-S4 cavity.

(iv) The $|\Delta\delta|$ values of the N—CH_3 and C^4—H protons for the inclusion complex with Calix-S8 are remarkably large compared with the others, indicating that the —$N(CH_3)_2$ and C^4—H moieties of both sides are included inside the Calix-S8 cavity.

Based on the above results, plausible structures of the inclusion complexes of MB with Calix-S*n* are depicted in Fig. 5. Judging from the study of the Corey–Pauling–Koltun (CPK) space-filling models, these structures of the inclusion complexes are the most plausible.

Incidentally, to obtain further information on the conformation of Calix-S*n* upon inclusion complex formation, the NMR measurements were carried out in the presence of excess guest MB over Calix-S*n* concentration. In the Calix-S4 and Calix-S6 inclusion complexes, the $ArCH_2Ar$ methylene protons of calixarenes showed split peaks at 3.50 and 4.38 ppm for Calix-S4 and at 3.72 and 4.15 ppm for Calix-S6. These results suggest a cone conformation of Calix-S4 and Calix-S6 in inclusion complexes. On the one hand, Calix-S8 gave only a singlet resonance, presumably due to a more flexible cyclic structure.[16] Using the CPK models, we examined the structure of the inclusion complexes of MB with Calix-S*n*. The CPK models show that a cone conformation is more suitable to the formation of inclusion complexes than an alternative conformation. This is in agreement with the results reported by Shinkai *et al.*,[17] who suggested that the conformation of water-soluble calixarenes is fixed to a cone upon inclusion of guest molecules based on the circular dichroism study of the inclusion complexes of chiral calixarenes.

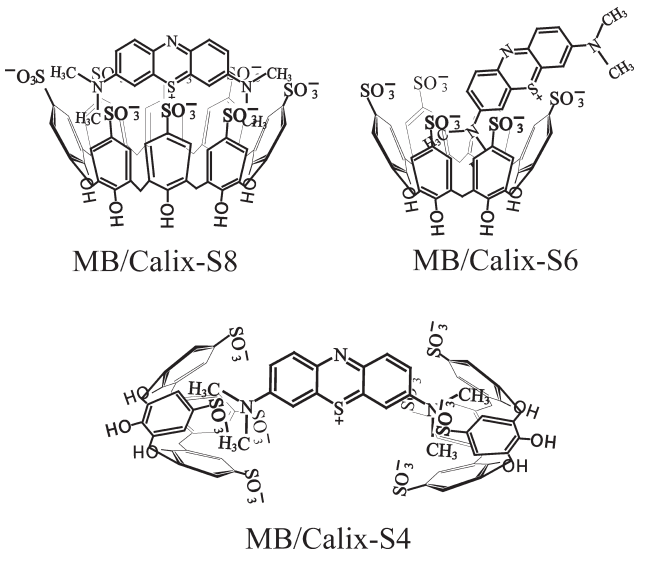


Figure 5. Plausible structures of inclusion complexes of MB with Calix-S*n*

Upon the addition of Calix-S*n* to the MB solution, large changes in chemical shifts for the MB protons are observed (Fig. 4). It is instructive to determine the association constants for the complexation of Calix-S*n* from analysis of the NMR shift changes. In the present NMR study, because the concentration of Calix-S*n* is not much higher than that of MB, we used the following approach for determining the association constants of the 1:1 inclusion complexation based on the Lang treatments.[18] The equilibrium constant for the 1:1 complex formation can be rewritten as follows:

$$K_{11} = \frac{[\text{MB–Calix}]}{[\text{MB}][\text{Calix}]} = \frac{[\text{MB–Calix}]}{([\text{MB}]_0 - [\text{MB–Calix}])([\text{Calix}]_0 - [\text{MB–Calix}])} \quad (11)$$

The observed chemical shifts ($\Delta\delta$) can be expressed as follows:[19]

$$[\text{MB–Calix}] = \alpha\Delta\delta \quad (12)$$

$$[\text{MB}]_0 = \alpha\Delta\delta^0 \quad (13)$$

where $\Delta\delta^0$ denotes the limiting chemical shift change, and α is a constant. By using Eqns (11–13), Eqn (14) for the 1:1 complex formation can be obtained and the association constants for the Calix-S8 and Calix-S6 inclusion complexations were estimated.

$$\frac{[\text{Calix}]_0}{\Delta\delta} = ([\text{Calix}]_0 + [\text{MB}]_0 - \alpha\Delta\delta)\frac{1}{\Delta\delta^0} + \frac{1}{K_{11}\Delta\delta^0} \quad (14)$$

Tentative values of α and $\Delta\delta^0$ were estimated from NMR chemical shifts and Eqn (13). Using this value of α, a plot of $([\text{Calix}]_0/\Delta\delta)$ against $([\text{Calix}]_0 + [\text{MB}]_0 - \alpha\Delta\delta)$ was made according to Eqn (14), producing a linear relationship between the two. A new value of $\Delta\delta^0$ can be determined with a new value of K_{11}. This procedure is repeated until a variation in the magnitude of the new K_{11} value converges within 5% relative to the K_{11} value from the previous plot for Eqn (14). Figure 6(a) shows the plot of $([\text{Calix}]_0/\Delta\delta)$ against $([\text{Calix}]_0 + [\text{MB}]_0 - \alpha\Delta\delta)$ for the Calix-S8 complexation according to Eqn (14), indicating the convergence of the plots relative to a previously drawn line. The first and last values of K_{11} were estimated as 7.66×10^3 and $7.84 \times 10^3\ \text{mol}^{-1}\,\text{dm}^3$, respectively. The K_{11} values were determined as $(7.84 \pm 0.19) \times 10^3\ \text{mol}^{-1}\,\text{dm}^3$ for Calix-S8 and $(2.53 \pm 0.21) \times$

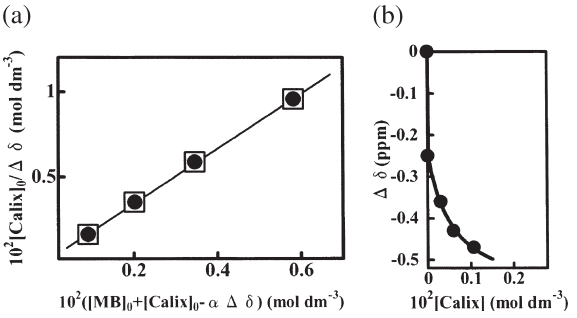


Figure 6. (a) Determination of the 1:1 inclusion equilibrium constant for the Calix-S8 complexation according to Eqn (14): (□) plot using the second tentative value of α; (•) plot using the third tentative value of α, $[MB]_0 = 3.25 \times 10^{-4}$ mol dm^{-3} and $[Calix\text{-}S8]_0 = (0.875\text{–}5.82) \times 10^{-3}$ mol dm^{-3}. (b) Plots of $\Delta\delta$(N—CH_3) against the Calix-S4 concentration: $[MB]_0 = 1.14 \times 10^{-4}$ mol dm^{-3} and $[Calix\text{-}S4]_0 = (0\text{–}1.39) \times 10^{-3}$ mol dm^{-3}. The solid line is the best-fit curve according to Eqn (17)

10^3 mol^{-1} dm^3 for Calix-S6. This linearity suggests the 1:1 inclusion complexation of MB with Calix-S8 and Calix-S6, and the association constants obtained are in good agreement with those estimated from the UV–Vis spectral analysis.

In the Calix-S4 inclusion complexation, both 1:1 and 1:2 guest–host complexes are formed under the condition of $[Calix\text{-}S4]_0/[MB]_0 < 20$:

$$MB + Calix \overset{K_{11}}{\rightleftharpoons} MB\text{–}Calix$$

$$MB\text{–}Calix + Calix \overset{K_2}{\rightleftharpoons} MB\text{–}Calix_2$$

where K_{11} and $K_2 (= K_{12}/K_{11})$ denote the association constants. Under this condition, the concentration of Calix-S4 unbound with MB was estimated by solving the polynomial of Eqn (15):[20,21]

$$[Calix]^3 + \left(\frac{1}{K_2} - [Calix]_0 + 2[MB]_0\right)[Calix]^2 + \left(\frac{1}{K_{11}K_2} + \frac{[MB]_0}{K_2} - \frac{[Calix]_0}{K_2}\right)[Calix] - \frac{[Calix]_0}{K_{11}K_2} = 0 \quad (15)$$

The concentration of MB unbound with Calix-S4 is given by:[20]

$$[MB] = \frac{[MB]_0}{1 + K_{11}[Calix] + K_{11}K_2[Calix]^2} \quad (16)$$

The concentration of [MB–Calix] and [MB–$Calix_2$] is given by the equilibrium expressions for K_{11} and K_2. Using Eqn (16), the NMR chemical shift changes applying to the 1:1 and 1:2 binding models can be expressed by:

$$\Delta\delta = \frac{\Delta\delta_{CA}[MB\text{–}Calix] + \Delta\delta_{2CA}[MB\text{–}Calix_2]}{[MB]_0} = \frac{\Delta\delta_{CA}K_{11}[Calix] + \Delta\delta_{2CA}K_{11}K_2[Calix]^2}{1 + K_{11}[Calix] + K_{11}K_2[Calix]^2} \quad (17)$$

where $\Delta\delta_{CA}$ and $\Delta\delta_{2CA}$ are specific limiting chemical shifts for 1:1 and 1:2 complexes, respectively. From a simultaneous fit of the N—CH_3 proton resonance data to Eqns (15) and (17) (Fig. 6(b)), the association constants for the 1:1 and 1:2 inclusion complexations of MB with Calix-S4 were evaluated as $K_{11} = 8.80 \times 10^2$ mol^{-1} dm^3 and $K_{12} (= K_{11}K_2) = 1.52 \times 10^6$ mol^{-2} dm^6. The K_{12} value obtained is compared with the above result estimated from analysis of UV–Vis spectral data.

Equation (10) shows that the changes in volume accompanied by the inclusion of MB into Calix-S*n* are the sums of the two terms of $\Delta V_{inclu} (< 0)$ and $\Delta V_{desolv} (> 0)$. Calix-S4 and Calix-S6 encapsulate a part of the MB molecule into the cavity, as shown in Fig. 5. In this case, a part of the solvent molecules solvated around the MB and —SO_3^- groups of Calix-S*n* is released, as illustrated by the volume changes accompanied by the formation of ion-pairs. Volume changes for the formation of many ion-pairs are given in the review of Asano and LeNoble.[7] Upon ion-pair formation, the increase in reaction volume occurs, caused by exclusion of solvent around the area of contact of ions and desolvation due to the partial neutralization of charge. In the complexations of Calix-S4 and Calix-S6, the ΔV_{desolv} term in Eqn (10) overwhelms ΔV_{inclu}, resulting in the positive values of the reaction volume. Two Calix-S4 molecules form the 1:2 inclusion complex with the large MB molecule. Thus, the volume change accompanied by inclusion with one Calix-S4 molecule can be estimated as 16.1 cm^3 mol^{-1}. The large magnitude of desolvation caused by Calix-S6, which has a larger rim diameter, is responsible for the large pressure effect on the inclusion complexation of Calix-S6 ($\Delta V = 30.1$ cm^3 mol^{-1}). In contrast, as depicted in Fig. 5, Calix-S8 encapsulates the large moiety of the MB guest molecule. The inclusion of the MB molecule into the Calix-S8 cavity might cause the large decrease in the volume of ΔV_{inclu}, resulting in a negative value of the reaction volume.

In conclusion, we found that the external pressures either increase or decrease the inclusion equilibria of MB with Calix-S*n*, depending on the structure of the inclusion complexes. We believe that a high-pressure study might provide useful insights into the function of calix[*n*]arenes.

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