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# Host–Guest Interactions in Aqueous Media with *p-tert.*-Butylcalix[4]arene Bearing Polyoxyethylene Chains

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**Abstract.** The interactions of *p-tert.*-butylcalix[4]arene bearing polyoxyethylene chains (C3) with pyrene (Py), 1-anilino-8-naphthalenesulfonate (ANS) and *N*-phenyl-naphthylamine (NPN) in aqueous solution were studied by absorption and fluorescence measurements. Absorption spectral changes and fluorescence enhancements reveal that C3, which has a hydrophobic cavity, can include organic molecules and ions in aqueous solution and form 1 : 1 host–guest complexes with ANS and NPN. C3 forms inclusion complexes with Py at different stoichiometries depending on the host : guest molar ratio. Binding constants of $2.2 \times 10^4$, $2.0 \times 10^4$ and $3.6 \times 10^5$ $dm^3$ $mol^{-1}$ were calculated for the C3 : Py, C3 : ANS and C3 : NPN complexes (1 : 1), respectively, based on the Benesi–Hildebrand equation.

**Key words:** *p-tert.*-Butylcalix[4]arene, polyoxyethylene chains, pyrene, 1-anilino-8-naphthalenesulfonate, *N*-phenylnaphthylamine, fluorescence spectroscopy.

## 1. Introduction

A great deal of interest has recently been shown in calixarenes [1,2] as they exhibit inclusion ability with organic molecules and ions. Water-soluble calixarenes are most interesting because of their potential to act as enzyme mimics. Several calixarene derivatives, such as *p-tert.*–butylcalix[4]arene tetracarboxylic acid [3], sulfonated calixarenes [4,5,6], *p*-(diallylaminomethyl)calixarenes and *p*-(2-carboxyethyl)calixarene [7,8] have been reported to be soluble in water. But all of them are ionic compounds and only dissolve in potassium carbonate or hydrochloric acid aqueous solutions. Many years ago Cornforth and coworkers [9,10] synthesized polyoxyethylated calixarenes and investigated their antituberculosis properties. Recently, we found that these kinds of water-soluble calixarenes which consist of a hydrophobic calixarene cavity and flexible hydrophilic chains, can provide a hydrophobic microenvironment and accept organic molecules and ions in aqueous solution [11,12]. This paper describes use of absorption and fluorescence emission to investigate the interactions of the calixarene bearing polyoxyethylene chains with Py, ANS and NPN.

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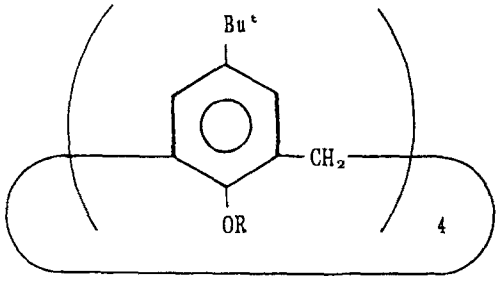



C1: R = H
C2: R = K
C3: R = $(CH_2CH_2O)_nH$

Scheme 1.

## 2. Experimental

### 2.1. Materials

Pyrene (Fluka, A.R.) was purified by vacuum sublimation and then recrystallized from ethanol. 8-Anilinonaphthalene-1-sulfonate(ANS) was kindly provided by Professor C. Tung. *N*-Phenyl-2-naphthylamine (NPN) was analytical grade from Beijing Chemicals and was recrystallized from ethanol. The water used was doubly distilled over potassium permanganate.

Compound C1, obtained according to Gutsche's methods [1], was treated with 4 equivalents of potassium *tert.*-butoxide in dry DMF and THF (1 : 4) and refluxed for 8 h, to afford C2 after removing the solvents. C2 was placed in a 250 mL stainless steel autoclave and purged with dry nitrogen. Ethylene oxide was then added, and the autoclave was heated at 170–180°C in an oil bath until the pressure fell to zero. Potassium ions in product (C3) were removed by addition of ion-exchange resins.

For comparison, Triton X–100 and polyoxyethylene *p-tert.*-butylphenol ether (C4) were used as references.

### 2.2. Measurements

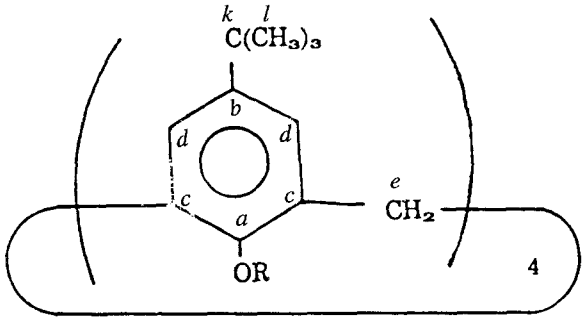
IR spectra were recorded on a Perkin-Elmer 983G spectrometer. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker WH-500 spectrometer. Chloroform-*d* was used as solvent and chemical shifts are reported as $\delta$ values in ppm relative to TMS ($\delta$ 0.0) as an internal standard. The absorption spectra and difference spectra were recorded using 1 cm pathlength cuvettes on a Hitachi UV-557 spectrophotometer at ambient temperature. The fluorescence spectra were measured with 1 × 1 cm quartz cuvettes on a Hitachi MPF-4 fluorescence-phosphorescence spectrophotometer at ambient temperature. Fluorescence yields were obtained from comparing the emission spectra areas on a Perkin-Elmer LS-5 fluorescence spectrophotometer

with data processing on the Perkin-Elmer 3600 data station. Samples were not deoxygenated.

## 3. Results and Discussion

### 3.1. CHARACTERIZATION OF C3

C3 is soluble in water and its molecular structure was confirmed by IR and NMR spectroscopy: IR(KBr) 3480 $cm^{-1}$ (OH stretch), 1604 $cm^{-1}$ (C=C of Ar stretch), 1130 $cm^{-1}$ (C–O–C stretch). $^1H$ NMR ($CDCl_3$)$\delta$ 6.50–7.28 (m, 8, ArH), 4.10 (d, 2, $ArCH_2Ar$, $J$ = 12Hz), 3.90 (s, 4, $ArCH_2Ar$), 3.63 (s, 150, $OCH_2CH_2O$), 3.40 (s, 4, $ArCH_2Ar$), 3.03 (4 lines, 2, $ArCH_2Ar$), 1.05–1.40 (m, 36, $C(CH_3)_3$); $^{13}C$ NMR ($CDCl_3$)$\delta$ 152.5–155.0 (4 lines, $C^a$), 143.5–145.5 (6 lines, $C^b$), 132.0–136.0 (5 lines, $C^c$), 125.0–128.0 (5 lines, $C^d$), 72.6 ($C^f$), 71.6 ($C^g$) 70.5, 70.2, 70.1 ($C^h$), 69.4 ($C^i$), 61.5 ($C^j$), 37.2 ($C^e$), 34.0 ($C^k$) 32.8 ($C^e$), 31.7, 31.6, 31.5 31.3 $C^l$).




$$R = \overset{f}{CH_2}\ \overset{g}{CH_2O}\overbrace{(CH_2CH_2O)_n}^{h}\ \overset{i}{CH_2}\ \overset{j}{CH_2OH}$$

The average length of the polyoxyethylene ether (POE) chain is about 9 carbon atoms. The $^{13}C$ NMR spectrum is very complex and suggests that C3 is a mixture of cone and partial-cone conformations. Whether the free phenolic groups of C2 have reacted with ethylene oxide completely can be detected by UV spectroscopy. In alcoholic potassium hydroxide any free phenolic group gives phenoxide ions, absorbing strongly at 300 nm, whereas the phenolic ethers show a characteristic double peak around 270–280 nm either in neutral or in alkaline solution [9]. The absorption spectra of C3 show that there is no free phenolic group (Figure 1).

The fluorescence emission and excitation spectra of C3 in aqueous solution are shown in Figure 2. C3 has a fluorescence emission peak at 305–312 nm and an excitation peak at 275–282 nm.

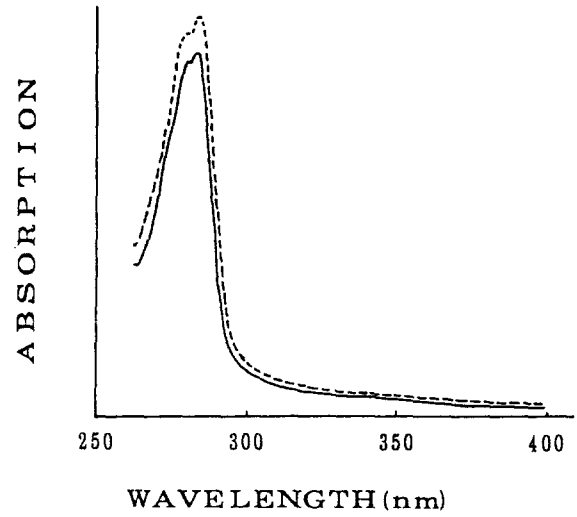



Fig. 1. Absorption spectra of C3 in water (—) and potassium hydroxide alcohol (– – –); [C3] = $1.0 \times 10^{-4}$ mol $dm^{-3}$; [KOH] = 1 mol $dm^{-3}$.

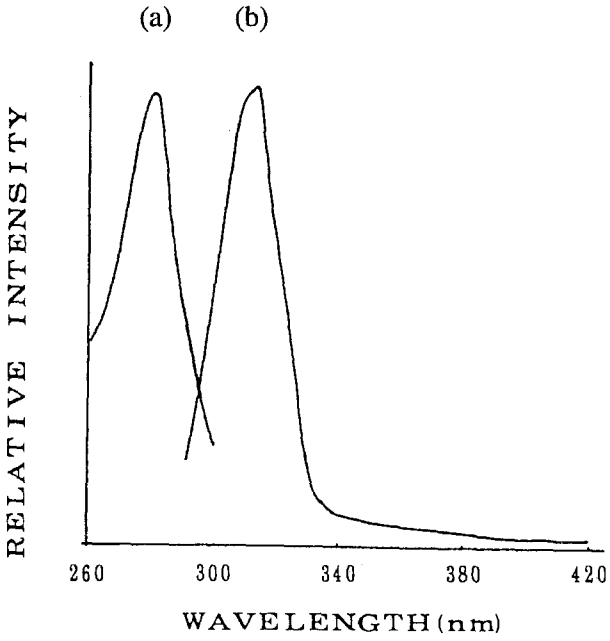



Fig. 2. Fluorescence emission and excitation spectra of C3 in aqueous solution; (a) excitation, $\lambda_{em}$ 310 nm; (b) emission, $\lambda_{ex}$ 280 nm.

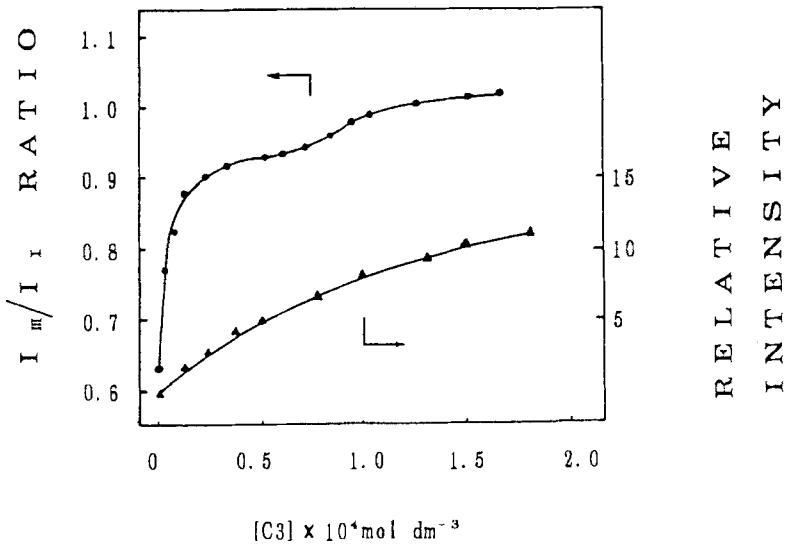



Fig. 3. Fluorescence intensity of $I_{III}/I_I$ ratios (•) and relative intensity (▲) for pyrene with [C3]; [pyrene] = $5 \times 10^{-7}$ mol dm$^{-3}$, $\lambda_{ex}$ 336 nm.

### 3.2. POLARITY OF THE CALIXARENE CAVITY

Pyrene monomer emission shows a strong solvent dependence and is widely used as a polarity probe in microheterogeneous media, which is expressed as a marked change of the ratio between the first (0–0) and third emission band ($I_{III}/I_I$ ratio) [13]. The peak ratio ($I_{III}/I_I$) increases with decreasing dipole moment of the solvent. The peak ratio $I_{III}/I_I$, which is observed to be small in high polarity solvents (0.63 in water), becomes markedly enhanced in low polarity or nonpolar solvents (1.65 in *n*-hexane). Here, pyrene was employed as a probe of polarity for the cavity of C3. It can be seen from Figure 3 that the $I_{III}/I_I$ ratio of pyrene in aqueous solution increases with increasing C3 concentration and reaches 0.92 when the C3 concentration is about $2.0 \times 10^{-5} \sim 5.0 \times 10^{-5}$ mol dm$^{-3}$. The results indicate that the hydrophobicity of the *p-tert.*-butylcalix[4]arene cavity is close to that of ethanol (0.91), and higher than that of a Triton X-100 micelle (0.76). The pyrene monomer fluorescence intensity also increases markedly on addition of C3 in aqueous solution. It suggests that the host eliminates quenchers such as water and oxygen from the surroundings of pyrene, as a result of encapsulation. The curve shows a sharp increase in the $I_{III}/I_I$ ratio from $5.0 \times 10^{-5}$ to $1.0 \times 10^{-4}$ mol dm$^{-3}$. This suggests that the C3–Py complex of the stoichiometry changed depending on the host : guest molar ratio.

The stoichiometry and the binding constant of the calixarene–pyrene complex were obtained according to the Benesi–Hildebrand equation, derived under an assumption that a 1 : 1 host–guest complex was formed [14].

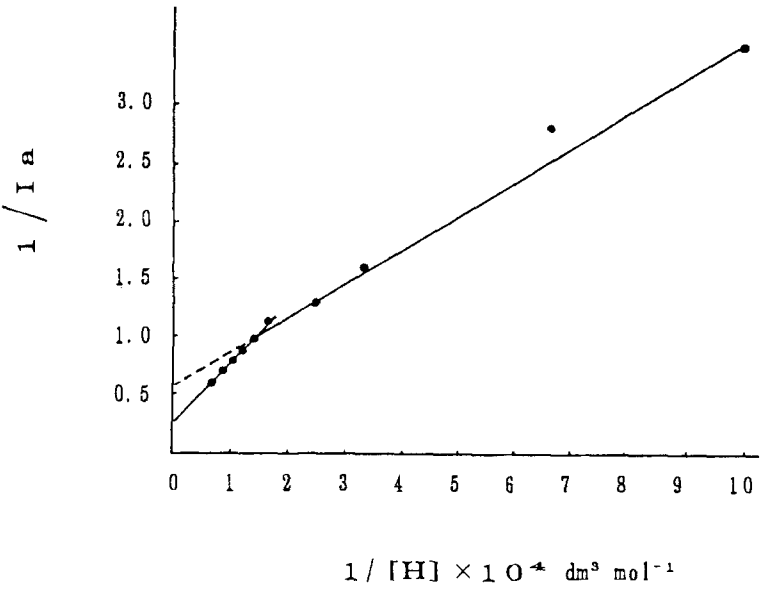



Fig. 4. Plot of $1/I_a$ vs. 1/[H] for the Py–C3 inclusion complex in aqueous solution from the Benesi–Hildebrand equation, $\lambda_{ex}$ 336 nm; $\lambda_{em}$ 392 nm.

$$\frac{1}{I_a} = \frac{1}{I_c[G]} + \frac{1}{I_c[G]} \cdot \frac{1}{K} \cdot \frac{1}{[H]}$$

Where, $I_a$ is the extent of the fluorescence intensity change upon addition of a host; $I_c$ stands for the difference in fluorescence intensity between complex and guest; [G] and [H] are the total concentrations of guest and host molecules, respectively.

Good linear correlations of $1/I_a$ vs. 1/[H] were obtained from the fluorescence spectra of pyrene with the change of C3 concentration from $1.0 \times 10^{-5}$ to $5.0 \times 10^{-5}$ mol dm$^{-3}$ (Figure 4). This shows that a 1 : 1 host–guest inclusion complex is formed between C3 and pyrene. From a consideration of the cavity size and the molecular size of pyrene, it can be deduced that pyrene was only partially included by the *p-tert.*-butylcalix[4]arene cavity, and the other part was embedded by the polyoxyethylene chains. The binding constant is about $2.2 \times 10^4$ dm$^3$ mol$^{-1}$ (correlation coefficient $r = 0.99$) calculated from the plot by using the least squares method. When the C3 concentration is raised, the plot of $1/I_a$ vs. 1/[H] deviates from linearity, suggesting that C3 and pyrene can form complexes with higher stoichiometric ratios besides a 1 : 1 host–guest complex.

### 3.3. Interaction of C3 with ANS

ANS shows a very weak fluorescence in water with a quantum yield as low as 0.004 and an emission maximum ($\lambda_{max}$) at 515 nm; while in organic solvents such as ethanol it exhibits a very strong fluorescence and the emission maximum is at

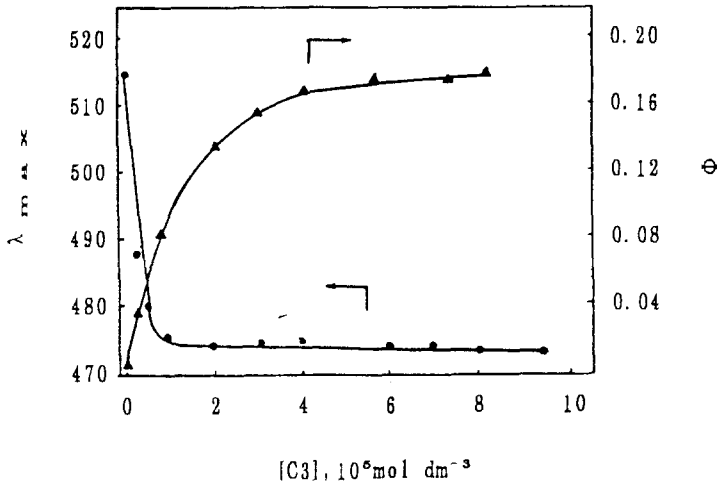



Fig. 5. Fluorescence emission maxima (•, nm) and quantum yields (▲) of ANS vs. C3 concentration in aqueous phosphate buffer solution (pH=7.0). [ANS] = $1.0 \times 10^{-5}$ mol $dm^{-3}$, $\lambda_{ex}$ 280 nm.

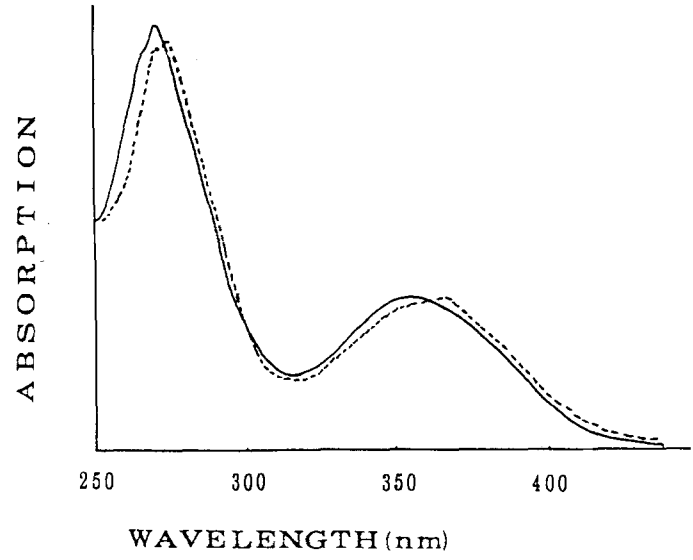



Fig. 6. Absorption spectra of $1.0 \times 10^{-5}$ mol $dm^{-3}$ of ANS (—) and ANS complexed by C3 (– – –). The absorption spectrum of ANS complexed with C3 was obtained as a difference spectrum (ANS–C3 complex vs. C3), [C3] = $1.0 \times 10^{-5}$ mol $dm^{-3}$.

468 nm [15]. Figure 5 shows that the fluorescence of ANS increases markedly on addition of C3. The quantum yield increases over forty fold and the $\lambda_{max}$ shifts from 515 nm to 473 nm. In the case of a C4 concentration $\sim 3.0 \times 10^{-4}$ mol $dm^{-3}$, the quantum yield only increased twofold and the $\lambda_{max}$ blue shifts from 515 nm

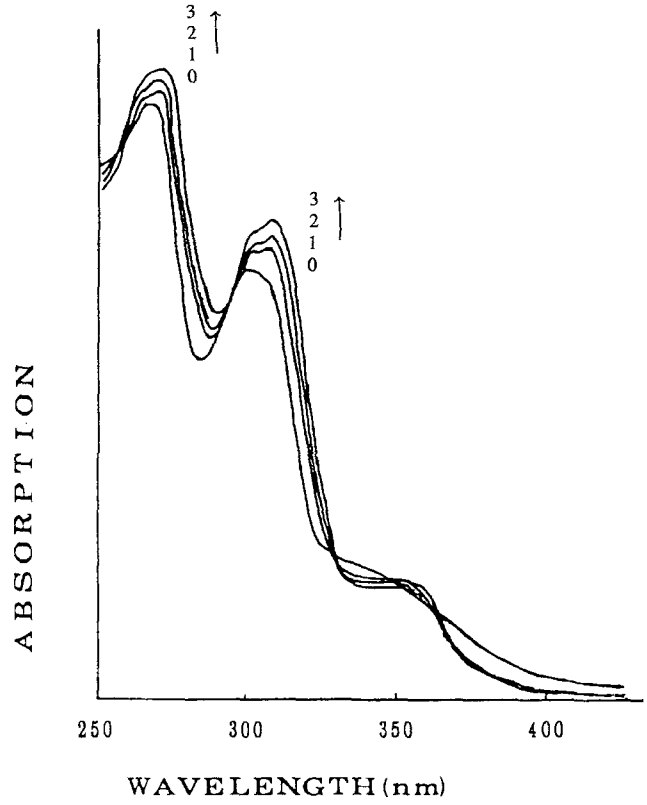



Fig. 7. Absorption spectra of $1.0 \times 10^{-5}$ mol dm$^{-3}$ of NPN at varying C3 concentrations: 0, $2.5 \times 10^{-6}$, $5.0 \times 10^{-6}$, $1.0 \times 10^{-5}$ mol dm$^{-3}$.

to 498 nm. This suggests that ANS is transported into the hydrophobic *p-tert.*-butylcalix[4]arene cavity and forms an inclusion complex. The excited states of ANS decay by rotation of the anilino group around the *N*-naphthyl bond toward an emissive 'twisted intramolecular charge-transfer' state. Due to the polar nature of the excited state, the $\lambda_{max}$ value depends strongly on the polarity of the medium. Since the microenvironment of the *p-tert.*-butylcalix[4]arene cavity is similar to that of ethanol, ANS was included by the cavity; the rotational freedom of the guest was restricted and energy loss caused by the molecular motion decreased; the radiative rate increased, and the quenching of water from the excited guest molecule surrounding was eliminated; the fluorescence efficiency of ANS was increased.

There is also a substantial change in the absorption spectrum of ANS on inclusion (Figure 6). The absorption peak at 265 nm shifts to 272 nm, while its extinction coefficient decreases slightly. In addition, the broad absorption maximum at 350 nm red shifts to 370 nm. The appearance of three isosbestic points at 270, 305 and 355 nm indicates that the C3–ANS inclusion complex has been formed in the

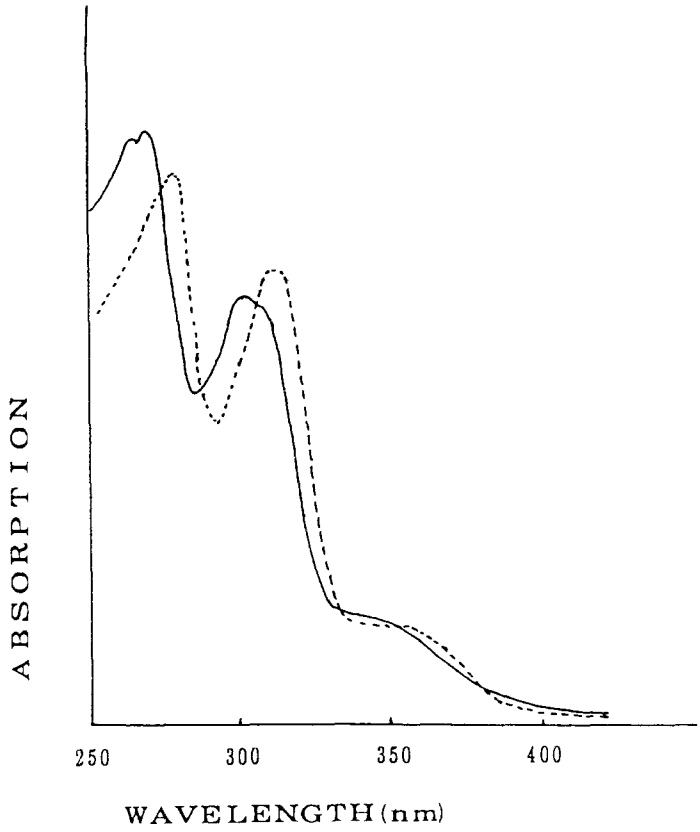



Fig. 8. Absorption spectra of $1.0 \times 10^{-5}$ mol dm$^{-3}$ of NPN (—) and NPN complexed by C3 (– – –). The absorption spectrum of NPN complexed with C3 was obtained as a difference spectrum (NPN–C3 complex vs. C3), [C3] = $1.0 \times 10^{-5}$ mol dm$^{-3}$.

ground state and the stoichiometry is 1 : 1. The equilibrium constant of ANS with C3 according to Benesi–Hildebrand is about $2.0 \times 10^4$ dm$^3$ mol$^{-1}$.

### 3.4. Complex of C3 with NPN

A completely hydrophobic version of ANS is *N*-phenyl-2-naphthylamine (NPN). NPN exhibits a spectral shift of about 10 nm in aqueous solution on adding C3 (Figure 8). Figure 7 shows the spectrum of NPN at varying C3 concentrations. The isosbestic points at 262, 300 and 332 nm indicate a 1 : 1 equilibrium.

NPN also shows high fluorescence enhancements and blue shifts in hydrophobic environments and is used widely in fluorescence polarization studies [16]. It can be seen from Figure 9 that the emission maximum of NPN in aqueous solution shifts from 446 nm to 412 nm accompanying the addition of C3, and the fluorescence increases as much as 10.6 times at $4.0 \times 10^{-5}$ mol dm$^{-3}$ of C3. However, the emission maximum of NPN shows a smaller shift (about 10 nm) and the intensity

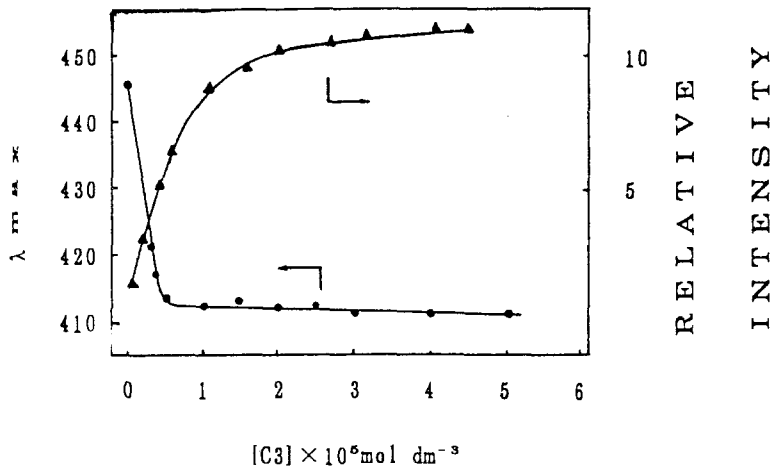



Fig. 9. Fluorescence emission maxima (•, nm) and relative intensity (▲) of NPN at different concentrations of C3 in aqueous solution (phosphate buffer, PH = 7.0); [NPN] = $5.0 \times 10^{-6}$ mol $dm^{-3}$; $\lambda_{ex}$ 310 nm; $\lambda_{em}$ 410 nm.

shows no detectable change upon addition of C4 (concentration $\sim 1.0 \times 10^{-3}$ mol $dm^{-3}$). These data imply that NPN is well shielded from the surrounding water molecules and thus it must be included by the hydrophobic cavity of C3. In view of the concentration ratio of host and guest at the break point in curve (a), it is assumed that one host molecule includes a NPN molecule. A Benesi–Hildebrand plot based on 1 : 1 host–guest complexation was found to be linear; this also demonstrates that the stoichiometry ratio of host : guest is 1 : 1, and the formation constant for inclusion of NPN by C3 is about $3.6 \times 10^5$ $dm^3$ $mol^{-1}$.

ANS and NPN are of similar molecular structure and size, but NPN is more hydrophobic than ANS. The binding constant for the inclusion complex of C3 with NPN is one order of magnitude larger than that for the complex of ANS with the same host. It demonstrates that C3 is more effective at binding hydrophobic molecules than hydrophilic molecules in aqueous solution.

## 4. Conclusions

*p-tert.*-Butylcalix[4]arene bearing polyoxyethylene chains can provide a hydrophobic microenvironment and form host–guest complexes with organic molecules and ions in aqueous solution. The fluorescence intensity of ANS and NPN increases markedly on inclusion. This host molecule has potential application for enhancement of fluorescence sensitivity in aqueous solution.

## Acknowledgement

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