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Spectroscopic Studies of Water-soluble Sulfonated Calix[6]arene

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Abstract. Fluorescence and absorption studies of water-soluble sulfonated calix[6]arene (SCX6) are reported. Water-soluble calixarenes are potentially useful as host molecules for luminophores, and studies of their spectroscopic characteristics are therefore crucial. The absorption and fluorescence spectra of these molecules in aqueous solution were collected, analyzed, and compared with 4-hydroxybenzene sulfonate at different pHs. A red shift in the absorption spectrum and a change in the fluorescence spectrum of the calixarenes are observed upon an increase in pH from 2.0 to 13.0. Some of these spectroscopic changes are attributed to intramolecular hydrogen bonding between adjacent hydroxyl groups of SCX6 after proton disassociation. The formation of excimers between phenolic groups in the calixarene molecule is proposed. In addition, inner-filter effects of SCX6 are discussed. These inner-filter effects prove to be a disadvantage for the use of SCX6 as a host molecule for complexation studies by use of fluorescence probes whose absorption spectra overlap with those of calixarenes.

Key words: Water-soluble calixarene, *p*-hydroxybenzene sulfonate, fluorescence, absorption, inner-filter effects, and excimer.

1. Introduction

The study of calixarene chemistry [1] is one of the fastest growing areas in supramolecular chemistry. Many chemists are using these molecules and their derivatives as artificial enzyme mimics for inclusion complexation of selected substrates [2]. Calixarenes are also very useful in the area of analytical chemistry. For example, calixarene derivatives have been used to efficiently purify C_{60} [3], to make highly sensitive and selective electrodes [4] for Na^+, K^+, and as sensors for Ca^{2+} [5]. However, the most common forms of calixarenes are not soluble in water. Therefore, much effort has focused on the synthesis of water-soluble derivatives such as the highly soluble sulfonated calixarenes [6, 7]. The calixarenes have also been used as additives to separate various phenol derivatives [8]. More recently, capillary electrophoresis has proved to be a viable technique for separation of sulfonated calixarenes [9].

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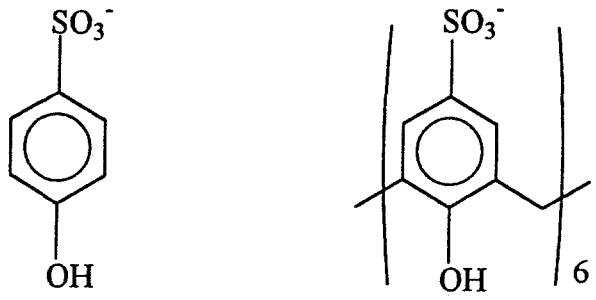

(a) (b)

Figure 1. Structures of (a) 4-hydroxybenzene sulfonate (monomer); (b) 4-sulfonated calix[6]arene (SCX6).

Water soluble sulfonated-calixarenes may selectively include various guest molecules according to their size and hydrophobicity in a manner similar to cyclodextrins [10, 11]. The properties of these two types of host molecules have been compared and summarized [12]. The proposed structure of sulfonated calixarene[6] is shown in Figure 1. One should note that the phenolic units of calixarenes are spectroscopically active in the UV region. This is significantly different from the oligosaccharide units of cyclodextrins. Therefore, the photophysics of calixarenes should be considered when using luminescence probes to study their inclusion complexes. The phenolic units in calixarene molecules are linked by —CH_2— groups. Thus, since the π -systems of the individual benzene rings are not coupled to each other, the spectroscopic properties of SCX6 should be similar to those of individual phenolic units.

The fluorescence and absorption of phenols and their derivatives have been well documented [13–17]. In particular, studies of excited state proton transfer and hydrogen bonding of phenols and naphthols have been extensively reported in the literature [13, 16]. The fundamentals of inter- and intramolecular reactions of such molecules have been reviewed [17]. The hydrogen-bonded complexes of phenol with proton-accepting molecules, such as ethers and alcohols, are known to shift the absorption and fluorescence spectra of phenols to longer wavelengths [14].

A red shift in the absorption of *p*-nitrocalix[6]arene in a mixture of water : tetrahydrofuran has been reported [18]. This shift is attributed to a deprotonation of the OH groups. The pK_as of sulfonated calix[6]arene obtained from titration calorimetry have also been reported [18–20]. However, complete fluorescence studies on SCX6 have not been reported. This paper presents the pH dependence of the fluorescence and absorption spectra of SCX6 in aqueous solution. Comparison

studies of SCX6 and *p*-hydroxybenzene sulfonate are also reported. In addition, potential problems due to inner-filter effects of SCX6 are discussed.

2. Experimental

2.1. Materials

Water-soluble sulfonated calix[6]arene (SCX6) was synthesized by use of previously reported procedures of Gutsche *et al.* and Shinkai *et al.* [6, 21, 22]. The synthesis used here is a three-step process to obtain the water-soluble sulfonated calix[6]arene. In the first step [21], *p*-tert-butylcalix[6]arene was prepared according to Gutsche's method. The second step involves debutylation of *p*-tert-butylcalix[6]arene [22]. The last step involves sulfonation of the calixarene in concentrated sulfuric acid (prepared with fuming sulfuric acid and 96% sulfuric acid) [6], producing the water-soluble sulfonated calix[6]arene (SCX6). Chemical analyses [$C_{42}H_{28}O_{24}S_6Na_8 \cdot 10H_2O$, C (32.78%), H (3.07%), S (12.74%)], produced values consistent with theory [C (33.47%), H (3.19%), S (12.70%)] within experimental error.

A stock solution of 1.04×10^{-3} M SCX6 was prepared in deionized water. Samples at pH = 2.0 were obtained by adjustment with HCl, whereas samples at pH = 13.0 were obtained by addition of NaOH. An aqueous KCl solution was used to adjust all samples to the same ionic strength. All other pHs were obtained by addition of buffers. The buffers used were as follows: pH = 3.0 & 4.0 (citric acid/citrate), pH = 5.0 & 6.0 [$NaOOCCH_3$ (NaAc)/$HOOCCH_3$ (HOAc)], pH = 7.0 & 8.0 (NaH_2PO_4/$NaHPO_4$), pH = 9.0 & 10.0 ($Na_2B_4O_7 \cdot 10H_2O$), pH = 11.0 & 12.0 (Na_2HPO_4) [23]. All compounds used in pH adjustment were purchased from Fisher Scientific Company and used without further purification.

2.2. Apparatus

Steady-state fluorescence measurements were acquired with a SPEX-Fluorolog Model F2T21I spectrofluorometer equipped with a cell compartment, thermostated by use of a VWR Model-1160 constant temperature circulator. The excitation and emission bandwidths were both set at 5 nm. The method employed to measure the fluorescence decays was time-correlated single photon counting (TCSPC) [24, 25]. The TCSPC instrumental setup used in our experiments has been described previously [25, 26]. The emission source is a Coherent 701-3 cavity-dumped dye laser, synchronously pumped by a frequency-doubled, mode-locked Quantronix 416 Nd-YAG laser. The output beam from the dye laser was frequency doubled by a BBO crystal and vertically polarized by a half-wave retarder. The excitation wavelength (at 286 nm) was selected with a three-plate birefringent filter. Emission (at 310 nm) was detected with a Hamamatsu R28090 Microchannel Plate Photomultiplier, which is wired for an instrumental response of 90 ps Instrumental Response Function (IRF). Data acquisition was controlled by a Macintosh IIcx

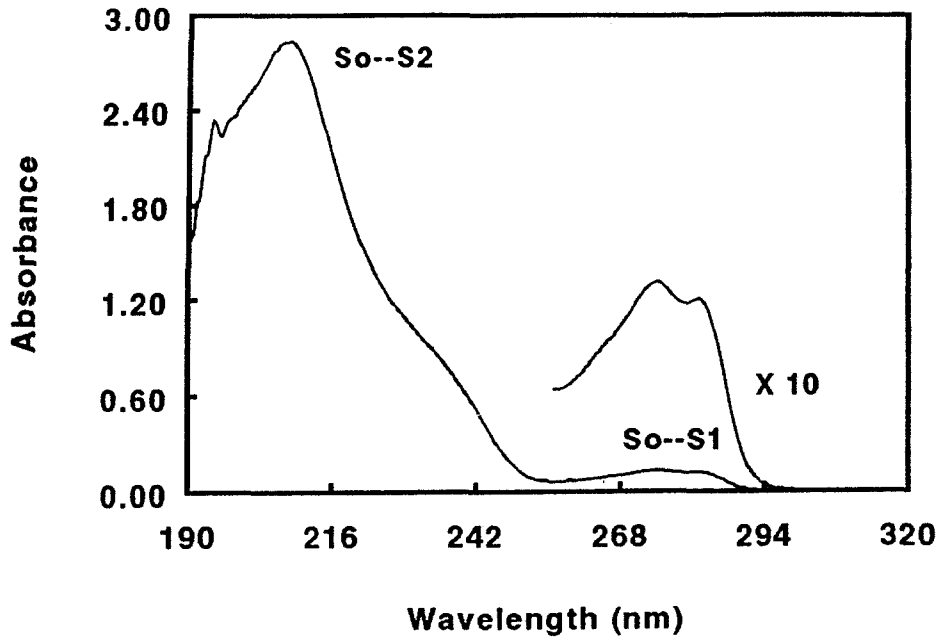

Figure 2. Absorption spectrum of SCX6 (2.08×10^{-5} M) at pH = 2.0.

computer using a program based on the LabVIEW software package [25]. Data were processed by use of TCSPC analysis software on a PTI LS-100 Luminescence Spectro-Photometer (Photo Technology International Inc. South Brunswick, NJ 08852) [27]. Absorption spectra were recorded on a Shimadzu UV-3101PC scanning spectrophotometer. All absorption spectroscopic analyses were conducted at room temperature.

3. Results and Discussions

3.1. Absorption Studies on Water-Soluble Calix[6]arene

The absorption spectrum of aqueous SCX6 is shown in Figure 2. There are two separate broad absorption bands which are associated with two excited electronic energy levels. The two absorption bands are attributed to the $S_0 \rightarrow S_2$ (λ_{max} at 210 nm) and $S_0 \rightarrow S_1$ (λ_{max} at 278 nm) transitions, respectively. The molar absorptivities were determined at pH = 2.0 from a calibration plot in the linear range of absorbance vs. SCX6 concentrations (Figure 3) by use of the Beer-Lambert law. The molar absorptivity at 275 nm is about 6750 L mol^{-1} cm^{-1} over the linear range of absorptivity up to 4.15×10^{-4} M SCX6.

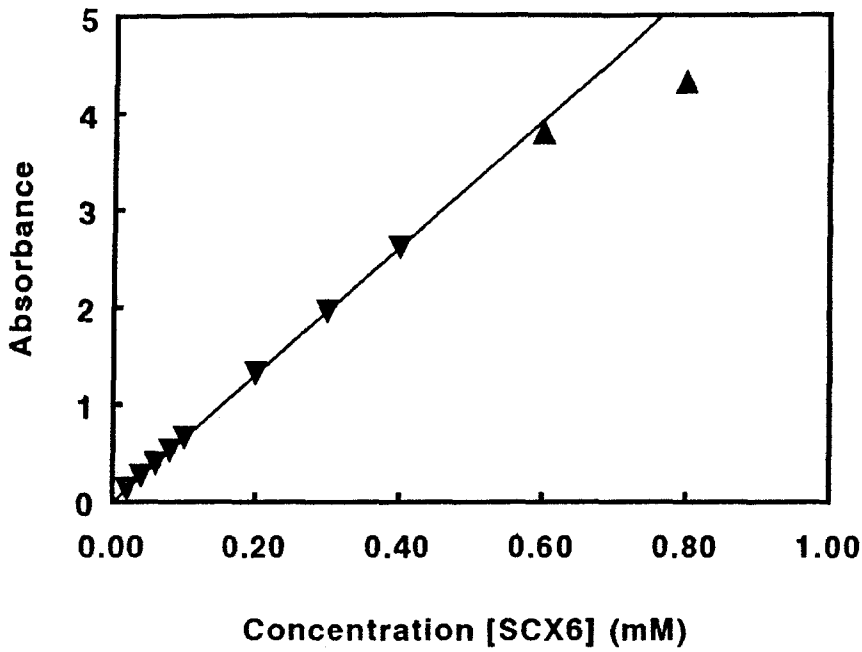

Figure 3. A calibration plot of SCX6 absorbance.

3.1.1. *The Effect of pH on the Absorption*

The absorption spectra of SCX6 (5.2×10^{-6} M) in aqueous solutions were collected at different pHs (Figure 4). As the pH increases from 2.0 to 13.0, the absorption band at 270–282 nm becomes structureless and an additional absorption band appears at about 295 nm. Shinkai *et al.* [18] have reported similar results for the absorption of *p*-nitrocalix[6]arene at different pHs in 70 : 30 (v/v) water : THF solution. They concluded that the red shift of the absorption is due to deprotonation of the hydroxyl groups.

In order to better understand the effect of pH on the absorption of SCX6, variations in solution pH are presented in Figure 5 for *p*-hydroxybenzene sulfonate (the monomer of SCX6). The absorption of the monomer, at pH $\sim$ 2.0, has a maximum at about 230 nm accompanied by a broad band at 250–280 nm. The absorption spectrum appears to shift to the red at a pH of $\sim$ 13.0 with a peak at about 255 nm, which is also accompanied by a shoulder at 275–300 nm. The isosbestic point at $\sim$236 nm suggests a binary equilibrium between two molecular species. For the purpose of comparing the monomer with SCX6, it is sufficient to note that the protonated monomer is predominant at a pH of $\sim$2.0 while its deprotonated monomer is dominant at pH $\sim$13.0. This typical behavior of pH equilibrium between two forms was not observed for SCX6. Thus, no isosbestic point was observed for SCX6 (Figure 4).

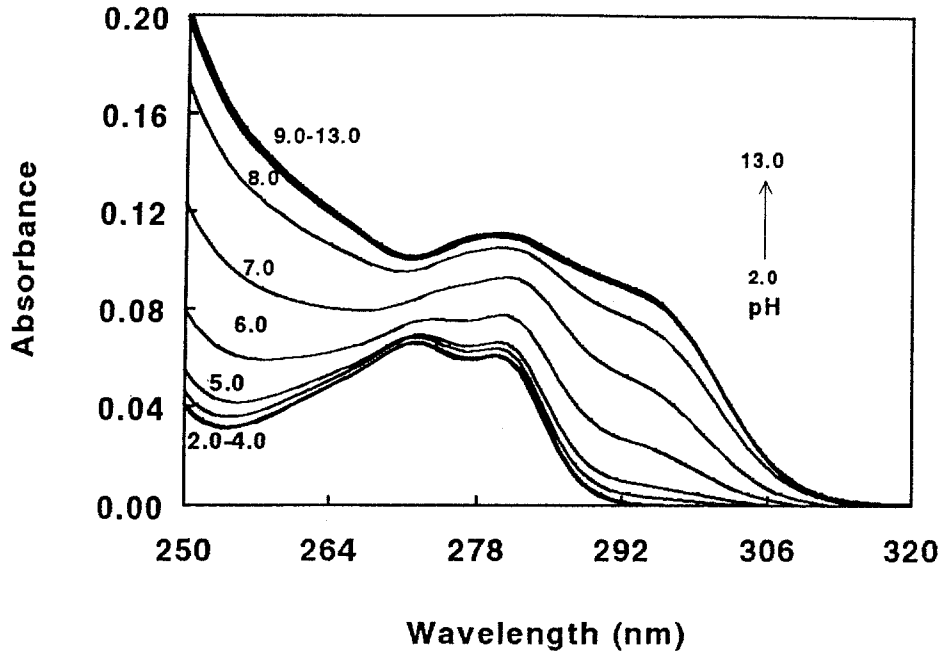

Figure 4. The absorption spectra of SCX6 at different pHs.

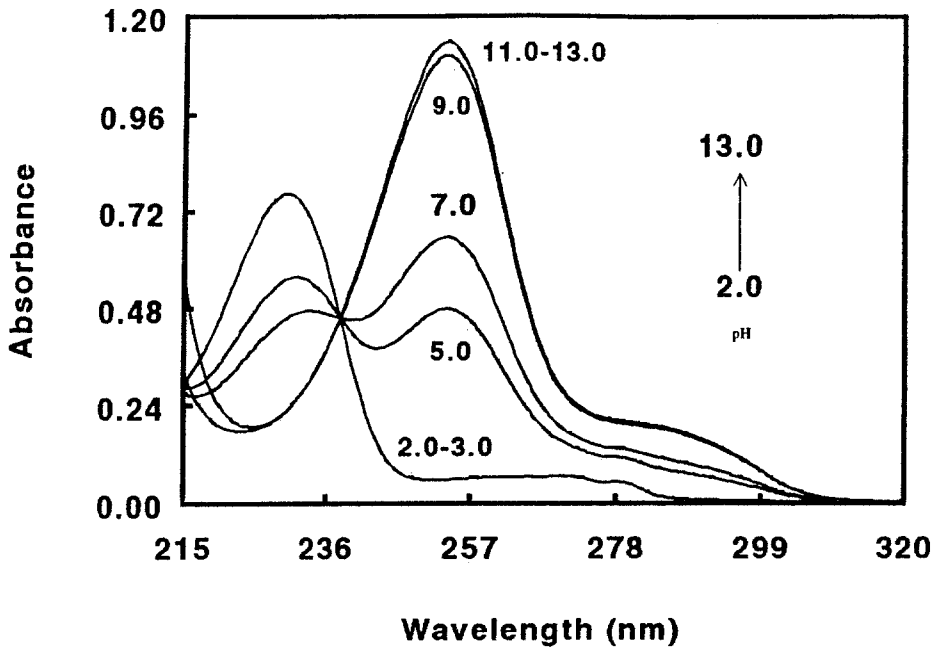

Figure 5. The absorption spectra of *p*-hydroxybenzene sulfonate (monomer) at various pHs.

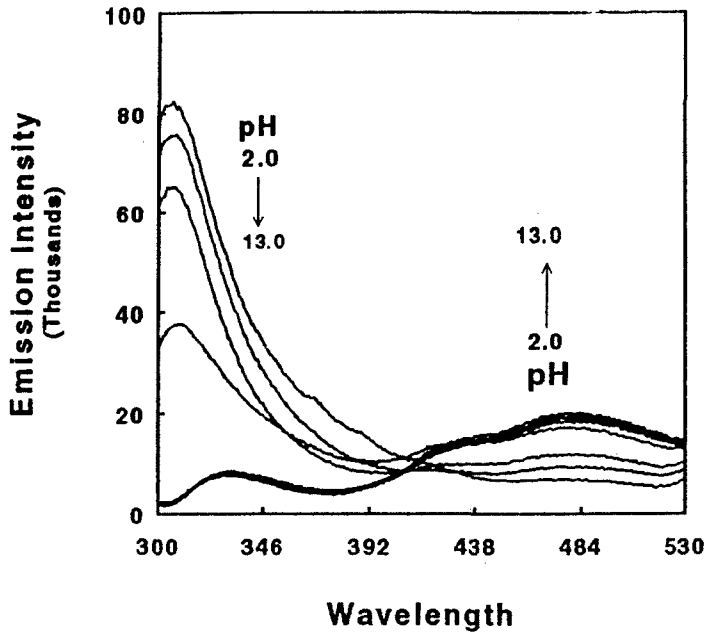

Figure 6. The fluorescence spectra of SCX6 excited at 270 nm (at various pHs).

The red shifts of the absorption spectra are observed for both SCX6 and its monomer, which suggests that SCX6 also undergoes deprotonation. If all protons on the rim of SCX6 are deprotonated, the shape of the absorption spectra should be similar to those of the monomer. However, we found that the absorption spectra of SCX6 are similar to those of the monomers only at low pHs. The absorption spectra of the two compounds at higher pH are distinctly different. This observation suggests that some of the phenolic hydroxyl groups of SCX6 are still protonated even at a pH as high as 13.0. This conclusion is consistent with the formation of strong hydrogen bonds between adjacent hydroxyl sites [6, 19, 20].

3.2. FLUORESCENCE STUDIES

3.2.1. *Fluorescence Measurements*

Our fluorescence studies on SCX6 suggest that the fluorescence of calixarene is sensitive to pH. The fluorescence spectra of SCX6 (1.04×10^{-4} M) at different pHs are displayed in Figure 6. At pH = 2.0, one emission band with a peak at 308 nm appears with a long tail. At pH $\cong$ 3.0 and up to 6.0, there are two emission bands with maxima at 308 nm and 480 nm. As the pH increases, the intensity at 308 nm decreases gradually, accompanied by an increase in intensity of the fluorescence band between 400–530 nm. At pH $\geq$6.0, the spectra do not change.

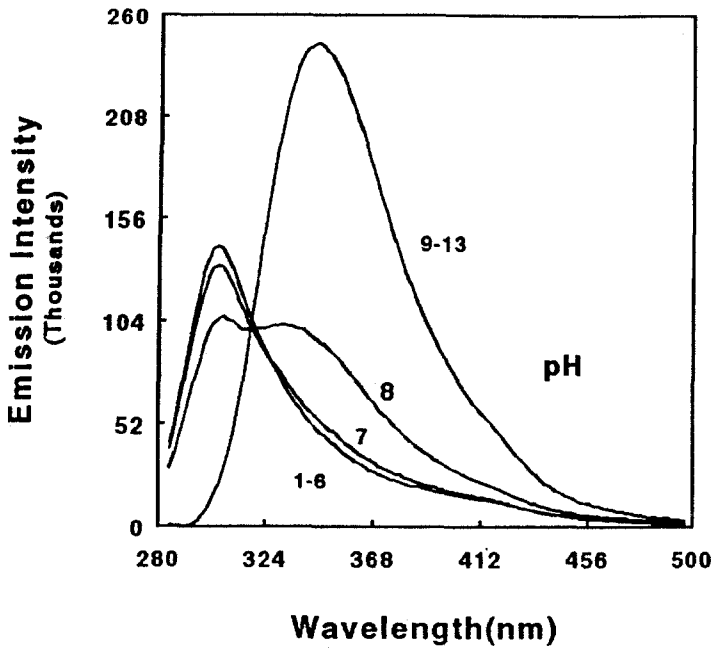

Figure 7. The fluorescence spectra of *p*-hydroxybenzene sulfonate (monomer) excited at 270 nm (at various pHs).

A study of the monomer unit (*p*-hydroxysulfonated phenol) was also performed in order to better assign the emission at various pHs to the corresponding SCX6 species. The emission spectrum of the monomer unit was acquired at different pHs (Figure 7). Two fluorescence bands appear in the spectra of the monomer unit, one at 306 nm and the other at 345 nm. At pH up to 7.0, only one fluorescence band appears with a maximum around 306 nm. However, at pH ≅8.0, a second emission appears and gradually shifts to a single long wavelength band at pH≅ 9.0. This observation is consistent with that of phenol which has a $pK_a \cong 9.0$, and a $pK_a^* \cong 4.0$ [28]. Ground state equilibrium and excited state proton transfer determine the relative intensities of the dual emission. These observations suggest that the sulfonate group has no significant effect on the absorbance of SCX6. Thus, the spectra of *p*-hydroxysulfonate phenol are similar to those of phenol.

The fluorescence changes of SCX6 and its monomer are reversible with changing pH, indicating an equilibrium process. It is reasonable, therefore, to assign the band at 306 nm to the protonated monomer (R—OH), while the deprotonated monomer ($R—O^-$) has its fluorescence band at 345 nm. This simple correspondence between the two forms of the monomer and the two fluorescence bands is not straight forward in the case of SCX6 since SCX6 has six hydroxyl groups. In the absence of interactions between the hydroxyl groups of SCX6, assuming a binary equilibrium, one would expect the spectra of SCX6 to be similar to those of the

monomer. However, our fluorescence observations of SCX6 cannot be interpreted according to a simple binary equilibrium.

The emission spectra of SCX6 and the monomer are similar at pH $\approx$ 2.0. The fluorescence of SCX6 has its maximum at 308 nm, and this band can be assigned to completely protonated SCX6. As the pH increases (pH $\geq$ 3.0), the emission spectra of calixarene deviate significantly from those of the monomer. To better interpret the spectra, we divide the spectrum into two regions: the band around 335 nm and the band between 400 and 530 nm (Figure 6). As the pH increases, more and more protons are removed from the rim of SCX6. Assuming that SCX6 is spectrally similar to the monomer, then the fluorescence peak of completely deprotonated SCX6 would be expected at 345 nm. However, even at pH = 13.0, the fluorescence of SCX6 does not have a single band, which would be expected from completely deprotonated SCX6. This observation suggests that completely deprotonated SCX6 is not possible over the pH range examined. The first band in the fluorescence spectrum of SCX6 at pH = 13.0 appears at 335 nm, a 27 nm red shift relative to its single fluorescence band at low pH. This band at 335 nm is, however, at a shorter wavelength than the fluorescence of the deprotonated monomer at 345 nm. We believe that this relatively smaller shift in the fluorescence of SCX6, as compared with that of the monomer, is likely due to intramolecular hydrogen bonding on the rim. This result also suggests that SCX6 is partially protonated in the excited state even at pH = 13.0, due to hydrogen bonding between the hydroxyl sites.

We have employed picosecond (ps) lifetime measurements for further studies of this molecule. The lifetimes obtained for SCX6 are about 35 ps and 350 ps, whereas the lifetimes of the monomer are about 120 ps and 950 ps. It is worth noting that short bi-exponential lifetime decays are observed for both SCX6 and the monomer. Such short decay times may reduce the excited state proton transfer. However, these fast fluorescence decays do not seem to be the reason for the lack of observation of a single fluorescence band of SCX6 in alkaline pH solutions because such a single fluorescence band around 345 nm is achievable in the fluorescence of the monomer. This again suggests that the hydrogen bonds between the hydroxyl groups of calixarene appear to play a major role in stabilizing the protons of the SCX6, resulting in different pK_a values for the six protons [18–20]. Otherwise, it would be expected that all six protons should have the same pK_a-values. As a result, the steady state and time resolved fluorescence should be similar to that of the monomer.

The second emission of SCX6 at 400–530 nm has no comparable emission in the monomer spectrum. In addition, this band appears only at higher pH. Apparently, this fluorescence is a result of interaction between the individual phenols in SCX6. One should keep in mind that SCX6 is a cyclic supramolecule which is composed of sulfonated phenols linked by —CH_2— groups (Figure 1). Those phenolic units can form intramolecular exciplexes or ground state dimers. Since the absorption spectra do not show any evidence of ground state dimers, we hypothesize that

exciplexes are formed in the excited state. In general, it should also be noted that all calixarene molecules may have different conformers [1, 12] such as cone, pinched cone, partial cone, chair, and alternate conformers. Earlier studies showed that at high pH the phenolate groups form stronger hydrogen bonds leading to a fixed cone conformation for sulfonated calix[6]arene [18]. In the solid state, X-ray data suggest that the double partial cone is stabilized by intramolecular hydrogen bonding [19], where two protons are up and two down on the rims of a double partial cone conformer. In other words, two protons are deprotonated from the SCX6 molecule. Thus, if the emission between 400–530 nm is due to excimer formation, the excimers in SCX6 molecule should be affected by changes in the pH of the solution. This hypothesis stems from our reasoning that the formation of an intramolecular exciplex depends on intramolecular orientational motion [29]. In fact, observation of the band between 400 and 530 nm only at higher pH supports this hypothesis.

Lifetime measurements of SCX6 were employed to test the above hypothesis. The lifetime of SCX6 was measured to be as short as 0.3 ns. As discussed earlier, hydrogen bonding between the hydroxyl groups is expected to be very weak at low pH since the phenolic units are more flexible and randomly orientated [18]. The short lifetime of SCX6 does not allow the phenolic units to reach the required geometry for excimer formation. Therefore, the band around 400 and 530 nm is not clearly shown at low pH. In contrast, SCX6 at higher pH will likely have a rigid cone or partial cone conformation in basic solution due to stronger hydrogen bonding. The orientation of the cone conformers would allow excimer formation. No re-orientation movement would be required.

3.2.2. *The effects of buffer components on fluorescence and absorption spectra*

The pH in these experiments has been controlled by the addition of buffers. The samples of calixarenes at different pHs include one or more buffer components. Since calixarenes may form complexes with some of these species [1, 12], it is crucial to examine the effects of buffers. Thus, the fluorescence and absorption of the samples with different components at pH = 5.0 are tested, in order to be certain that the observed fluorescence and absorption changes at different pHs are not induced by buffer components. At pH = 5.0, both the absorption and fluorescence spectra of calixarene are sensitive to pH. Thus, any effects of different buffer components on the spectra should be easier to observe at pH = 5.0. The intensities of the absorption and fluorescence are collected from the samples composed of the same calixarene concentration and various buffer components. The data obtained show that the effects of the components are less than 5.0%, which is within instrumental error. These small changes in the presence of different buffers would not account for the observed spectral changes at different pHs. The observed effects are, therefore likely due to pH equilibria.

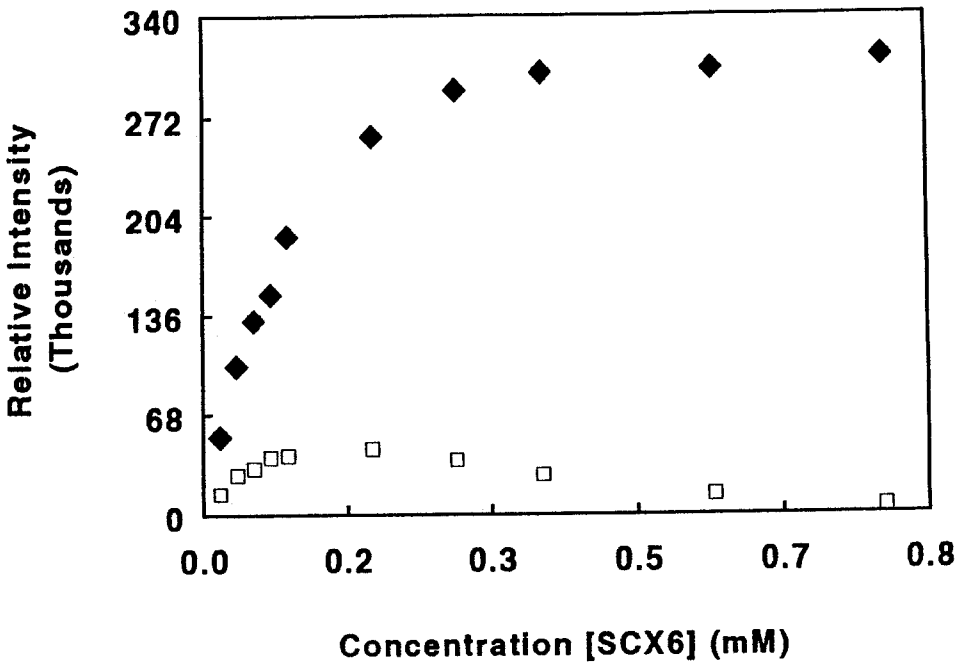

Figure 8. Plots of front face and right angle fluorescence measurements (excited at 270 nm) against the concentrations of SCX6. ♦ Front face fluorescence. □ Right angle fluorescence.

3.2.3. *Inner-filter effects studies*

Calixarenes are potentially useful as host molecules for organic guest compounds. When the absorption of SCX6 overlaps that of the monitored guest, one must be cautious of the influence of inner-filter effects. Small polyaromatic compounds such as naphthalene have absorption spectra at about 270 nm. The absorptivity of SCX6 at this wavelength is not negligible, reaching 6750 L M^{-1} cm^{-1} at pH = 2.0.

Comparison studies were conducted using front face fluorescence measurement and right angle fluorescence measurement of SCX6. The front face fluorescence intensity of SCX6 was obtained by use of a triangular fluorescence cell. Figure 8 portrays the front and right angle fluorescence intensities at different concentrations of SCX6. The front-face fluorescence intensity of SCX6 is consistently higher than the right-angle fluorescence, and increases as a function of SCX6 concentrations up to 4.15×10^{-4} M, where it levels off. In comparison, right- angle fluorescence is proportional to SCX6 concentrations up to 1.04×10^{-4} M and decreases to zero at higher concentrations. This observation indicates that inner-filter effects of SCX6 cannot be ignored in fluorescence measurements.

From a study of the inner-filter effects, we have concluded that the fluorescence intensities of some probes which absorb between 250 and 320 nm can be significantly decreased by the addition of SCX6 when excited at these wavelengths. The

probe molecules studied include several polyaromatic hydrocarbon molecules such as β-naphthol, naphthalene, and pyrene. The observed decreases in the fluorescence intensity upon excitation at 270 nm are a result of SCX6 absorption. Therefore, the inner filter effect of SCX6 in the range of 250–320 nm must be considered in studies of complexation between host SCX6 and guest fluorescence probes. Fluorescence probes which can be excited above 320 nm, where SCX6 does not absorb strongly, are more appropriate for studying SCX6. Otherwise, correction for inner filter effects is crucial.

4. Conclusions

A comparison study of the spectroscopy of SCX6 and its monomer reveals that the spectroscopy of the monomer is similar to the spectroscopy of phenol, while SCX6 does not follow either. The sulfonate group of the monomer does not significantly influence its spectroscopy. However, hydrogen bonding and excimer formation of SCX6 produce a more complicated spectra than those of the monomer. We conclude that the red shifts of the fluorescence and the absorption of SCX6 are due to disassociation of protons from the rim of SCX6. Moreover, the relatively smaller red shift of the fluorescence of SCX6, as compared with that of the monomer, suggests that intramolecular hydrogen bonding occurs in the SCX6 molecule. Strong hydrogen bonding causes retention of some protons on the rim of SCX6, even at pH = 13.0. The emission in the range 400–530 nm is apparently due to excimer formation between two phenol groups.

Inner-filter effects are a serious disadvantage of SCX6 as a host molecule for studying the complexation of certain fluorescence probes. A simple method for correction of the inner filter effect is currently under study in our laboratory. However, as an alternative, one can use probes which can be excited at longer wavelengths than 320 nm where the SCX6 does not absorb strongly.

Acknowledgments

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