Near-Infrared Spectroscopic Investigation of Inclusion Complexes between Cyclodextrins and Aromatic Compounds

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Near infrared (NIR) absorption in the 900−1800 nm region has been used to characterize and measure the association constants of the inclusion complexes between aromatic compounds (e.g., phenol, 4-chlorophenol, sodium 2-naphthalenesulfonate, and sodium 2-pyrenesulfonate) and α-, β-, and γ-cyclodextrin. It was found that the NIR method is particularly suited for this type of study because upon forming complexes, the oscillator strengths of the C−H stretching [$\Delta\nu = 2$ (at ~1650 nm) and 3 (at ~1120 nm)] overtone bands of the aromatic guest compounds were increased by at least 2−5-fold. The observed increases are rationalized in terms of the enhancement in the anharmonicity and the libration motions of the guest molecule in the CD cavity. This enhancement effect facilitates the calculation of the association constants of the inclusion complexes. As expected, the calculated values are in relatively good agreement with previously determined values.

Introduction

Inclusion of organic molecules into the cavity of the cyclic oligosaccharides (cyclodextrins) has been extensively exploited as models for enzyme catalysis. The popularity stems from the fact that inclusion complex formation offers many advantages, including reaction stereospecificity and stabilization of transition states.[1−3] The interiors of the cyclodextrins are relatively hydrophobic because of rings of C−H groups and glucosidic oxygens. The hydroxyl groups on the periphery supply the molecules the hydrophilicity. This special configuration provides the proper medium for inclusions of guest molecules including benzene, naphthalene, and anthracene within the cavity of α-, β-, and γ-cyclodextrins (CD), respectively.[3] Generally, investigations on the inclusion complex formation with CD's are based mainly on effects on the spectroelectronic properties of the guest molecule in the UV and visible region upon binding.[4−10] For instance, the fluorescence and phosphorescence quantum yields and lifetimes as well as the rotational relaxations of the guest molecules undergo significant increase upon inclusion complex formation.[4−10] A substantial change in the ratio of bands I and III of the fluorescence spectrum of pyrene has also been widely utilized as evidence of the complex formation. Spectral shifts induced by the complex formation not widely used because they are usually small.[11] Comparatively, fewer studies have been reported on the use of infrared (IR), near-infrared (NIR), and Raman techniques to analyze host−guest interactions with CD's.[4,12−14] This is rather unfortunate because the effect of the inclusion complex formation on the vibronic features of the guest molecule is expected to be large.[15]

The near-infrared technique has been widely used as the method for process analysis. Its use in the field of spectroscopy is rather limited.[16−18] The main drawback is probably due to the difficulty in assigning and interpreting the overtone and combination bands of mixtures having multiple components.[18,19] (This is partly due to the low resolution of the currently available NIR spectrophotometers.) Additionally, high concentration samples are usually required to bring the absorbances of these transitions into typical measurable range. However, the situation has changed significantly in the past few years with the development of low noise and highly sensitive InGaAs detectors and specially of the acoustooptic tunable filters (AOTF's).[20−24] AOTF is an all-solid-state, electronic dispersive device which is based on the diffraction of light by acoustic waves in an anisotropic crystal.[20−24] The wavelength of the diffracted light is dependent on the frequency of the acoustic wave; namely, only a monochromatic light will be diffracted from a crystal when a specific acoustic wave propagates through it. The scanning speed of the AOTF is, therefore, defined by the speed of the acoustic wave in the crystal which is on the order of microseconds. As a consequence, compared to conventional gratings, the AOTF's have such advantages as rapid scanning ability (microseconds), high resolution (few angstroms), and wide spectral tuning range.[20−24] The filters can also provide a unique means to maintain the intensity of the light source (by controlling either the frequency or the power of the applied radio-frequency (rf) signal through a feedback loop).[20,21,24] As a consequence of this development, a NIR spectrophotometer based on the AOTF is not only very sensitive but also very stable and has no drift. It provides, for the first time, a sensitive and accurate means for the spectroscopic study in the NIR region. The standard spectroscopic view for NIR, therefore, changes accordingly toward that of UV/vis spectra where the assignment of the electronic transitions is not as important as the spectral response as a function of the medium conditions.[17−19,25]

Such considerations prompted us to initiate this work which aims to use the recently developed AOTF based NIR spectrophotometer to investigate the inclusion complex formation between aromatic probes with α-, β-, and γ-CD's in D_2O in the 900−1800 nm region. Special attention will be paid to the effect of the inclusion complex formation on the oscillator constant force of the host molecules. Preliminary results which are reported in this paper show for the first time that molar absorptivities (ε) for the C−H second overtone stretching ($\Delta\upsilon = 2$)[19,26] of the guest molecules change significantly upon inclusion. This effect enables us to use the NIR technique to measure host−guest association constants for aromatic compounds including phenol, 4-chlorophenol, sodium 2-naphtha-

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lenesulfonate, and sodium 1-pyrenesulfonate with α-, β-, and γ-cyclodextrin.

Experimental Section

α-, β-, and γ-CD (Aldrich) were purified using the previously published method.[27] The crystals obtained were dried in vacuum for ~24 h. Stock solutions were freshly prepared in 99% D_2O (Cambridge). Concentrations of the stock solution were calculated based on the monohydrate form of CD's.

Phenol (loose crystals, 99%, Aldrich), 4-chlorophenol (4-ClP) (99%, Aldrich), sodium 2-naphthalenesulfonate (NSANa) (Aldrich), sodium 1-pyrenesulfonate (PSANa) (Molecular Probes), and sodium dodecyl sulfate (SDS) (Kodak, electrophoretic grade) were used as received. Stock solutions were prepared in D_2O , kept in well closed vials, and protected from light. The concentrations of stock solutions were determined by UV absorbance of appropriate aliquots (ϵ = 1600 (270 nm) , 1500 (280 nm) , 4900 (274 nm), and 34 600 (346 nm) M^{-1} cm^{-1} for phenol, 4-ClP, NSANa, and PSANa, respectively). Anions of phenol and of 4-ClP were prepared in situ by addition of few microliters of NaOH dissolved in D_2O to the corresponding solution of the neutral compounds. All other chemicals were the best available grade.

A home-built NIR spectrophotometer based on an acoustooptic tunable filter (AOTF) was used to measure NIR absorption spectra. In this spectrophotometer a 100 W, 12 V halogen tungsten lamp (Osram 64623) was used as the light source. The collimated incident white light from the light source was dispersed to monochromatic light and spectrally scanned by means of a noncollinear AOTF (Matsushita Electronics Model EFL-F20R2) which was fabricated from TeO_2. A driver constructed from a voltage control oscillator (VCO)[20,21,23] provided the rf signal. The rf signal from this driver was amplitude-modulated at 50 kHz by a home-built modulator and amplified by a rf power amplifier (Mini-Circuits Model ZHL 1-2 W) before being applied to the AOTF. The light diffracted from the AOTF was split into two beams (i.e., sample and reference beams) by means of a beam splitter. Intensity of the light in the sample and reference beams was detected by thermoelectrically cooled InGaAs detectors (Electro-Optical Systems Model IGA-020HS-E). The output signals (from the reference and sample detector) which were AM modulated at 50 kHz were connected to lock-in amplifiers (Stanford Research Systems Model SR 810) for demodulating and amplifying. The signals from the lock-in amplifiers were then connected to a microcomputer (Gateway 2000 DX2 50 MHz) through a 16-bit A/D interface board (National Instruments Model AT MIO 16X). A software written in C++ language was used to control the frequency and the power and to scan the applied rf signal. The same software also facilitated the data acquisition, analysis (e.g., to calculate the absorption spectra) and saved the data in ASCII format for the subsequent analysis using other software packages (e.g., Gram-386 and Slide-Write). Depending on the type of measurements and the information needed, the spectrophotometer was appropriately modified from a single beam to a double beam for a 2 or 10 mm path length cells. The collected spectra were subsequently treated by Grams/386 (Galactic software) for base line subtraction, spectra smoothing, and also spectra correction in cases when of one of the components was diluted during the titration of the inclusion complex. The reproducibility of the spectra was within 5−10%, independent of the experimental conditions.

Results and Discussion

Due to its relative transparency in the NIR region, D_2O was selected as the solvent for this study (Figure 1). Two main

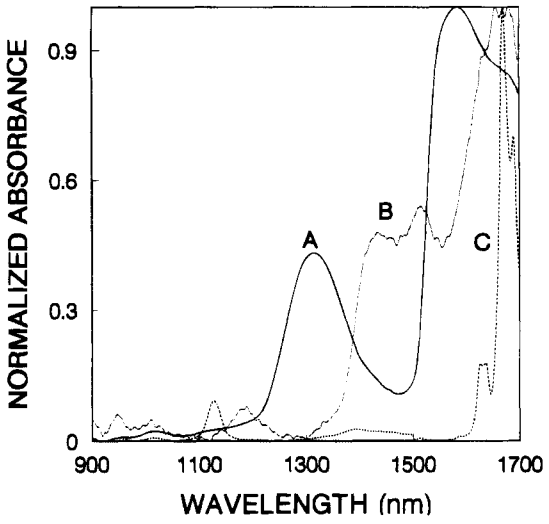

Figure 1. Normalized NIR absorption spectra of D_2O (A), benzene (B), and α-CD in D_2O (C). T = 23 °C (see Table 1).

TABLE 1: Molar Absorptivity (ϵ) of Selected NIR Transitions of D_2O, α-, β-, and γ-Cyclodextrins, Phenol, and 4-Chlorophenol (4-ClP)

compound/media	wavelength (nm)	molar absorptivity (M^{-1} cm^{-1})
D_2O	1315/1585	0.0024/0.0056333
α-CD/D2O	1430/1510/1670	1.410/1.474/2.917
β-CD/D2O	1430/1510/1670	1.864/1.859/3.571
γ-CD/D2O	1430/1510/1670	2.046/2.049/3.806
phenol/D_2O[a]	1123/1520/1653	0.042/0.085/0.519
phenol/benzene	1439/1450	1.297/1.316
phenol/α-CD/D_2O[a]	1123/1653	0.072/1.219
phenol/β-CD/D_2O[a]	1123/1653	0.086/1.940
4-ClP/D_2O[a]	1123/1520/1653	0.027/0.063/0.430
4-ClP/α-CD/D_2O[a]	1123/1653	0.115/1.783
4-ClP/β-CD/D_2O a	1123/1653	0.154/2.199

[a] Values for the respective anions are similar to the undissociated parent compound.

transitions at 1585 and 1315 nm with ϵ = 0.0056 and 0.0024, respectively (for ϱ_{D_2O} = 1.1047 g/cm^3, Table 1), agree well with those reported previously.[19,28,29] In the remaining data the absorbance of the solvent was subtracted out either by employing a double beam configuration or by recording the solvent signal in the computer and using it as the reference for the calculations of transmittances.

The spectra of α-CD in D_2O exhibits three transition regions at ~1650, 1450, and 1180 nm (Figure 1). These transitions can be assigned to the first and second overtone stretch vibrations and combination bands of C−H and O−H.[19,30] It is noteworthy to add that proton and deuterium exchange certainly occurs between D_2O and CD−hydroxyl groups. This type of exchange makes it cumbersome and less reliable to use the 1450 nm region for analysis. Values of ϵ for the selected transitions are summarized in Table 1. In this table (and also in Table 3) wavelength maxima and respective ϵ values are listed only for the most prominent transitions. As a consequence, data for the combination region for aromatic alcohols included in the cavity of α- and β-CD are not presented; for the other aromatic guests (i.e., NSANa and PSANa) only the first overtone region is shown.

The transition for the aromatic C−H stretching vibration overtone is expected to occur at slightly lower wavelengths, as observed for pure benzene (Figure 1).[19] Spectra of phenol dissolved in D_2O show, in addition to the usual O−H contribution at the combined region (~1450 nm), clear C−H first and second overtone stretching at 1650 and 1120 nm, respectively

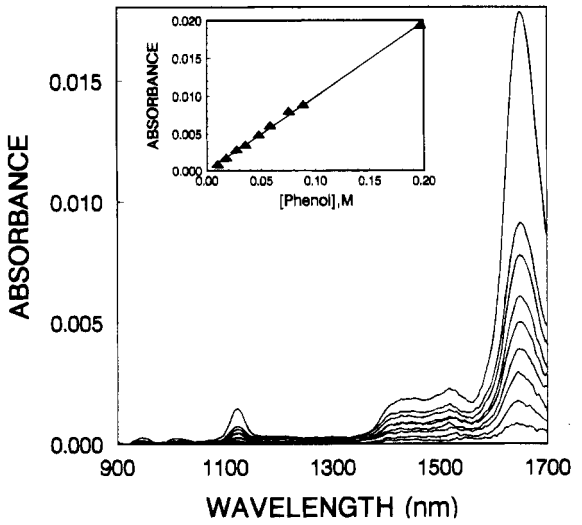

Figure 2. NIR absorption spectra of phenol in D_2O. Concentrations of phenol are (from bottom to top) 0.0094, 0.0179, 0.0272, 0.0353, 0.0477, 0.0582, 0.0754, 0.0887, 0.0887, and 0.1969 M. Path length = 2 mm, temperature = 23 °C. Insert: plot of absorbance maxima *versus* phenol concentration (see Table 1).

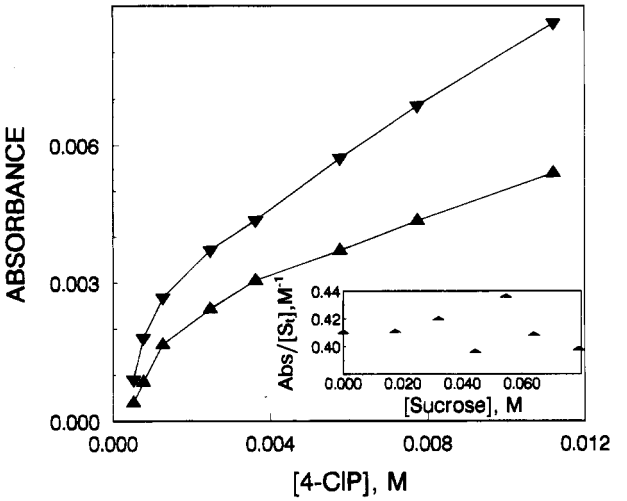

Figure 3. P lots of absorbances at ~1650 nm against concentration of 4-ClP for 7.07 mM β-CD (▼) and for 6.75 mM α-CD (▲). Path length = 10 mm, temperature = 23 °C. Insert: plot of absorbance per 4-ClP concentration (Abs/S_t) *versus* concentration of sucrose. Path length = 10 mm, T = 23 °C.

(Figure 2). To ascertain that these are not due to the phenol−OH group, spectra of phenol in benzene were also recorded (Table 1). It was found that the stretching of the O−H group of phenol occurs as a doublet with peaks at 1440 and 1460 nm.[25] An ϵ value for the 1650 nm band for phenol in D_2O was obtained by standard Beer-Lambert absorbance *versus* concentration curve. A good linear relationship was observed up to ~0.2 M concentration (Figure 2, insert).

In Table 1 ϵ values of the alcohols investigated are presented. Anions of phenol and 4-ClP were found to have the same ϵ values as their corresponding neutral compounds, and the substitution of hydrogen (in phenol) by chloride (to give 4-ClP) decreases the value of ϵ only by $\sim^1/_5$. This is as expected because the C−Cl group is practically transparent in this NIR region. Comparing ϵ values between phenol and 4-ClP and their anions reveals that the contribution per CH group is ~0.1 M^{-1} cm^1. Therefore, it seems that the C−H oscillators are essentially uncoupled.[31] The increase in ϵ value on going from α-CD to β-CD and to γ-CD can be considered as a direct addition of three (for β-CD) and seven (for γ-CD) extra O−H groups per one glucose ring moiety (one in β-CD and two in γ-CD) (Table 1). Take, for example, the 1430 and 1670 nm regions. In this region, changes in ϵ values are in fair agreement with 18/21/24 and 42/49/54 O−H and C−H ratios for α-, β-, and γ-CD, respectively (e.g., at 1430 nm $\epsilon_{\beta\text{-CD}}$ = 3.571 M^{-1} cm^{-1} and $\epsilon_{\alpha\text{-CD}}$ value was calculated based on the active groups proportional ratio of 3.061 M^{-1} cm^{-1}, see Table 1).

The effect of α- and β-CD's on phenol vibrational strength at the 1650 nm region is depicted in Figure 3. It is evident that ϵ values increase upon association. Table I lists ϵ values for included guests. These values were obtained either from the initial slopes of data as shown in Figure 3 or by taking the slopes of absorbance versus concentration plots under experimental conditions where [CD] ≫ [aromatic probe]. The enhancing effect is also observed in the second overtone stretching (Table 1). For phenol a 2−3-fold increase in ϵ value from D_2O to α- or β-CD is observed, whereas for 4-ClP the increase is in the order of 4−5 times. These changes is probably due to the fact that for 4-ClP the ortho and meta hydrogens are probably more tightly confined in the CD cavity than it is for phenol. A similar effect was also observed from the NMR studies for *p*-nitrophenol,[1] which can be attributed to the relatively high polarity of the C−Cl bond in the para position which, as for the axial O−H group, helps to settle the guest into the CD cavity in accordance with their association constants (see below). To ascertain that this effect is due to the inclusion complex formation, similar experiments were performed where CD was replaced by sucrose. As illustrated in the insert of Figure 3, sucrose, even when added at high concentration (8.0 × 10^2 M), exhibited no effect on the ϵ values of phenol (and also of other alcohols and their anions, not shown). It is, therefore, clearly evident that the change in ϵ value by CD's is due to their special cavity microenvironment.

The effect of confinement on the absorption intensity observed here can be related to the enhancement in the intensity of IR signals from liquid solution to pure crystal state[32] or from gas phase at low pressures to the condensed phase where intensities can be altered by factors of 10 or more.[33] Intensity changes between liquid and clathrate phases, however, are absent or even reduced.[31] For C−H stretchings local mode theory was shown to work satisfactorily;[31] rotational hindering and intermolecular coupling among oscillators have been argued as the cause for the enhancement of overtone oscillator strengths.[34] It is probable, therefore, that the observed intensity increase is related to the decrease in the hydration stress around the hydrophobic regions of the aromatic alcohols, and its inclusion provides a relatively more favorable microenvironment. This, in effect, increases the anharmonicity of the vibronic transition by intermolecular interactions, or through librational interactions as in liquid benzene[35] with C−H cavity groups, which, in turn, increases the local mode amplitude vibration leading to the enhanced transition probability.[31] Of course, it is possible that there was a change in the bandwidth of the absorption bands. However, changes in band frequency or in bandwidth (lower wavelengths and sharper bands, respectively) upon inclusion were too small compared with the resolution of this AOTF-based spectrophotometer (2 nm at 1000 nm and 4.2 nm at 1400 nm) to be observed.

Given the relatively low ϵ value of the aromatic alcohols in the NIR region studied (Table 1), host−guest association isotherms (at room temperature of 23 °C) can be obtained more easily by adding aliquots of α- or β-CD to the phenol solution which was initially recorded as the background reference signal. For each measurement the absorption by CD in the entire wavelength region was initially measured and then subtracted

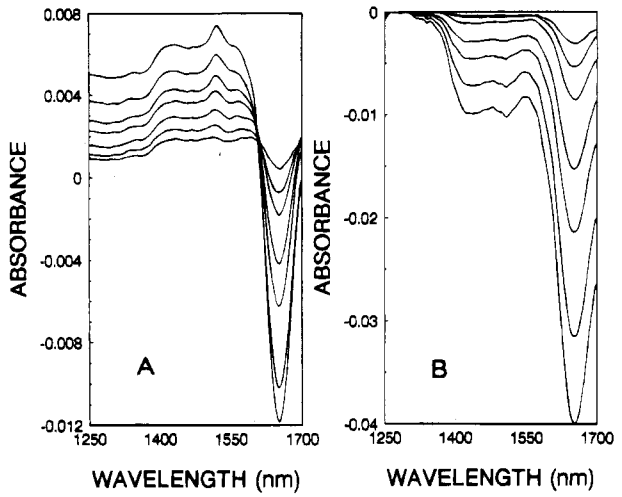

Figure 4. NIR absorption spectra of phenol in β-CD. (A) Raw spectra, concentrations of phenol (from bottom to top) are 0.1367, 0.1304, 0.1248, 0.1148, 0.1063, 0.0957, and 0.0870 M; concentrations of β-CD were 0.000 31, 0.000 59, 0.000 85, 0.001 30, 0.001 69, 0.002 17, and 0.002 56 M. Path length = 10 mm, T = 23 °C. (B) Corrected spectra obtained by subtracting out the contribution of β-CD. Spectra shown correspond to dilution effect by phenol; absorbances values are equal to ΔA (see text for detailed information).

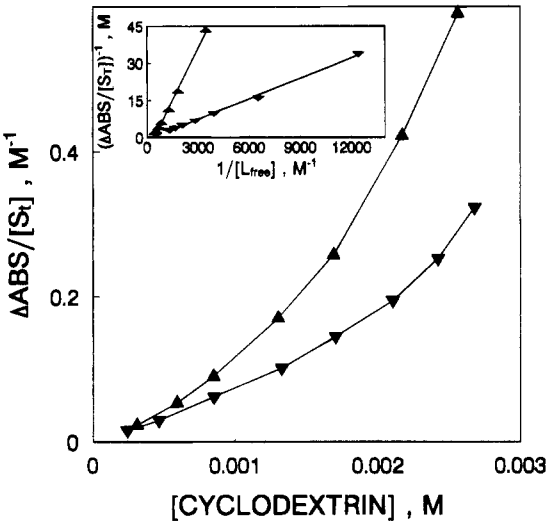

Figure 5. Plots of absorbance variation (ΔABS) per total phenol concentration (S_t) *versus* total cyclodextrin concentration for β-CD (▲) and for α-CD (▼). Path length = 10 mm, temperature = 23 °C. Insert: plot of $(\Delta ABS/[S_t])^{-1}$ *versus* reciprocal of free CD concentration ($[L_{free}]$) (see text for detailed information).

out using the GRAMS/386 program. A typical result is presented in Figure 4 a,b. It should be noted that the final absorbance value being measured is that of absorbance change (ΔABS) as a function of added host concentration and of the dilution effect by the host. It is also important to point out that the dilution spectral correction exemplified in Figure 4 is unique; namely, if incorrect subtraction factors were applied to the raw spectra, the resulting spectrum would not represent that of the pure alcohol. Also, the subtracted spectra do not account for changes in ϵ value of the guest compound. Also, because of the overlap between the spectra of the guests and those of CD's (see Table 1), it may be possible that some of the observed enhancement is due to the contribution of C−H's of the host molecule.

In Figure 5 plots of ΔA over the analytical guest concentration ($[S_t]$) ($\Delta ABS/[S_t]$) *versus* analytical host concentration ($[L_t]$) for α- and β-CD for 4-ClP are presented. It is evident that binding into β-CD is stronger. Association constants (K_s) were

TABLE 2: Association Binding Constants (K_s, in M^{-1}) of Phenol, 4-ClP, Their Anions, and NSANa and PSANa with α-, β-, and γ-CD (T = 23 °C)

compound	α-CD	β-CD	γ-CD
phenol	87 (37)[a]	214 (94)[a]	
phenolate	43	187	
4-ClP	324	371	
4-ClP$^-$	150 (292)[a]	223 (410)[a]	
NSANa		205	425
PSANa			100

[a] Values in parentheses were taken from ref 11.

TABLE 3: Molar Absorptivities (ϵ) of Selected NIR Transitions of NSANa, PSANa, and SDS

compound/media	wavelength (nm)	molar absorptivity (M^{-1} cm^{-1})
NSANa / D_2O	1650	1.123
NSANa/β-CD/D_2O	1650	2.014
NSANa/γ-CD/D_2O	1650	2.825
PSANa / D_2O	1650	2.195
SDS / D_2O	1188/1411/1700	0.137/0.092/0.331

calculated by linearization of the absorbance/ligand data by the standard Benesi−Hildebrand double-reciprocal plot[5] (Figure 5, insert). Concentration of free CD ($[L_{free}]$) was initially approximated from the determined change in ϵ (Tables 1 and 3) and subsequently calculated K_s (slope/intercept from plots as in Figure 5, insert). After several reruns and convergence of values, the most probable K_s (Table 2) and ϵ change (Tables 1 and 3) were selected. For comparison, reported K_s values for phenol and 4-ClP with α- and β-CD in H_2O were also listed (in parentheses) in the Table 2.[11] It seems that the K_s values obtained in the present work are somewhat higher than the reported values. This is hardly surprising considering the fact that the association constants in H_2O are known to be about 70−80% of that in D_2O.[36] In fact, when the present K_s values were corrected taking into account the effect of D_2O, they are found to be in relatively good agreement with reported values. K_s values for the aromatic alcohol anions are consistently lower than the respective neutral parent compound. This may be due to the relatively higher hydrophilicity of the anions (Table 2).

To further confirm the enhancement effect presently observed, effects of β- and γ-CD on the first overtone stretching intensity of relatively water soluble 2-naphthalenesulfonate (NSANa) and 1-pyrenesulfonate (PSANa) probes were studied. The spectra of NSANa and of PSANa (not shown) in D_2O are similar to those of phenol (Figure 2) and of naphthalene[30] with $\Delta\upsilon_{C-H}$ = 2 and 3 overtone regions at ~1650 and 1100 nm, respectively, and a combination band at ~1450 nm. For NSANa the absorbance was linearly proportional to concentrations up to ~8 mM, whereas for PSANa absorbance/concentration plots deviates from linearity at relatively lower concentrations. This behavior may be related to the previously reported dimerization and self-aggregation of PSANa.[38]

For comparison absorbances of a micelle forming compound below and above the cmc were recorded. Absorbances of SDS in D_2O (see last entry in Table 3) were found to be linear up to ~0.1 M (spectra not shown). Since there is no apparent difference in the absorptivity of (surfactant) monomer exposed to bulk media and of micellized C−H groups (cmc of SDS in H_2O ~8 mM[39]), the solvation−stress argument used to explain the molar absorptivity enhancement of aromatic probes in CD's needs additional supporting experimental data.

Transitions of aliphatic C−H (SDS) are as expected more energetic than that of the aromatic probes (Tables 1 and 3).[19,40] It is clear also that oscillator strengths per C−H group increases in the order CH_3 < CH_2 < phenol < NSANa < PSANa,

indicating clearly the effect of the electronic mixing on ϵ values (Tables 1 and 3).[37,40] The observed enhancement in $\Delta v = 2$ upon inclusion for the aromatic probes can be related to an enhanced anharmonicity from the electronic mixing in a confined region.[34]

Binding of NSANa and PSANa into the CD cavity was obtained in the same manner as for the aromatic alcohols (Table 2). As expected, the binding constant for NSANa with γ-CD is more than twice that with β-CD. The binding constant between PSANa and γ-CD which was found to be 100 and is comparable with those reported by Warner et al.[41-43] (on the order of 80−300 M^{-1}).

It has been demonstrated that the near infrared technique can be successfully used to study the inclusion complex formation between aromatic compounds and CD's. The method is particularly suited for this type of study because it was found that the oscillator strength of the $\Delta v = 2$ (at ~1650 nm) and 3 (at ~1120 nm) C−H stretching overtone bands of the aromatic guest compounds were increased by at least 2−5-fold when they formed inclusion complexes with α-, β-, or γ-CD. The observed increases were rationalized in terms of the enhancement in the anharmonicity and the libration motions of the guest molecule in the CD cavity. This enhancement effect facilitates the calculation of the association constants of the inclusion complexes. As expected, the calculated values are in relatively good agreement with previously determined values. Because the enhancement was observed for C−H groups, it is expected that this method is of applicable not only for aromatic compounds but also for aliphatic compounds as well. Consequently, the method is particular importance because it will enable the study of inclusion complex formation between aliphatic guest and a variety of host including crown ethers, cryptates, calixarenes, and amyloses. Such studies are currently under progress in our laboratory.

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