

DIALKYLAMINOBENZONITRILES AS FLUORESCENCE POLARITY PROBES FOR AQUEOUS SOLUTIONS OF CYCLODEXTRINS

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Abstract—The dual fluorescence of 4-(N,N-dimethylamino)benzonitrile and 4-(N,N-diethylamino)benzonitrile (DMABN and DEABN, respectively), has been studied in aqueous solutions of cyclodextrins. Fluorescence parameters (peak maximum, lifetime, and relative intensity) have been measured and are found to be consistent with the formation of complexes of probe and cyclodextrin. Enhanced emission of the twisted internal charge transfer state (TICT) fluorescence is observed in cyclodextrin, with the greatest effect for the DMABN/ α -cyclodextrin system. Our results promote further understanding of both the photophysical behavior of DMABN and the complexation of probe with cyclodextrin. The use of dialkylaminobenzonitriles as polarity probes is discussed.

INTRODUCTION

Dimethylaminobenzonitrile (DMABN)[†] and diethylaminobenzonitrile (DEABN) exhibit dual fluorescence in a variety of organic solvents (Lippert *et al.*, 1962). The two emissions are generally believed to occur from two conformationally distinct excited singlet states—the short wavelength emission from a planar state, and the long wavelength emission from a twisted internal charge transfer (TICT) structure. These structural assignments are based on a range of experiments using both DMABN and appropriate analogues as models (Rettig and Banacic-Koutecky, 1979; Rotkiewicz and Rubaszewska, 1980; Grabowski and Dobkowski, 1933). The TICT excited state is believed to be formed from the planar excited state by twisting around the phenyl-nitrogen bond and appears to be induced by solvent-solute interaction in polar solvents (Wang and Eisenthal, 1982; Struve and Rentzepis, 1974).

The fluorescence of guest molecules has been used to establish complexation to cyclodextrins (CD) (Bender and Komiyama, 1978), and to determine the environmental polarity of a probe that is included in a cyclodextrin cavity, since environmental polarity can effect the nature of fluorescence (Turro *et al.*, 1980). Fluorescence polarity probes which have been employed include pyrene, 1-anilino-8-naphthalene sulfonate (1.8-ANS) and indole derivatives (Yorozu *et al.*, 1982; Cramer *et al.*, 1967; Turro *et al.*, 1982). In most cases, the polarity deduced for the cyclodextrin cavity depends on the fluorescent probe being used.

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[†]*Abbreviations:* DMABN, 4-(N,N-dimethylamino)benzonitrile; DEABN, 4-(N,N-diethylamino)benzonitrile; TICT, twisted internal charge transfer; CD, cyclodextrin; TNS, 6-*p*-toluidinylnaphthalene-2-sulfonate; 1.8-ANS, 1-anilino-8-naphthalene sulfonate; LE, long wavelength emission; SE, short wavelength emission.

This is not surprising since the actual geometry of complexation will determine which portions of the probe will be in polar or non-polar environments. Indeed, one may be able to infer from the inferred polarity the nature of the geometry in the cyclodextrin complex.

One set of fluorescent probes which has been extensively studied with cyclodextrins are the ANS derivatives and 6-*p*-toluidinylnaphthalene-2-sulfonate (TNS) (Cramer *et al.*, 1967; Seliskar and Brand, 1971; Kondo *et al.*, 1976). The quantum yield of fluorescence for these molecules increases significantly with decreasing solvent polarity. Therefore, upon complexation to β -cyclodextrin, the fluorescence quantum yield increases. Since 1.8-ANS and TNS have TICT excited states analogous to the dialkylaminobenzonitriles (Kosower, 1982), we have examined the effect of α - and β -cyclodextrin on the fluorescence parameters of DMABN and DEABN. We anticipated that DMABN and related molecules may be used as polarity probes of proteins and membrane-like environments. These probes possess an advantage because they are smaller and, therefore, potentially less perturbing of the native structure than pyrene derivatives (Lianos and Cremiel, 1980; Kano *et al.*, 1981), and ANS derivatives (Stryer, 1965; Turner and Brand, 1968). DMABN fluorescence has been previously examined in micellar media (Khalil and Sonnessa, 1977).

MATERIALS AND METHODS

4-(N,N-Dimethylamino)benzonitrile (Aldrich) and 4-(N,N-diethylamino)benzonitrile (Pfaltz and Bauer) were purified twice by recrystallization from hexane (Nakashima and Mataga, 1973). α -, β -, and γ -Cyclodextrins (Aldrich) were used as received.

Spectroscopic measurements and techniques. Fluorescence spectra and lifetime measurements were obtained on

a SLM 4800S subnanosecond lifetime spectrofluorimeter. UV/VIS absorbance spectra were taken on a Perkin Elmer 559A UV/VIS spectrophotometer. A solution filter of potassium hydrogen phthalate was used in the quantum yield of fluorescence and emission energy maxima determinations. The excitation wavelength was 290 nm in all cases. The bandwidths for emission spectra were 4 nm for measurements in organic solvents, and 8 nm for measurements in aqueous solutions. In the λ_{\max} measurements, no corrections were made for overlap of the emission bands. A phase-modulation technique, with a glycogen/water light scattering reference, was used to generate lifetime measurements. Fluorescence lifetimes were measured by both the phase and the modulation methods. The modulation method yielded consistently higher values for the lifetimes. The phase method is generally considered to be the more accurate of the two methods so that the reported lifetimes are derived from measurements by this method. The differences in measured lifetimes is probably the result of the non-exponential decay of fluorescence, since the lifetimes of the bound and free probes are different. Lifetime measurements were made with a frequency of 30 MHz only. A cut-off filter was employed to eliminate emission from the short wavelength band. All aqueous solutions were Millipore filtered (0.5 μm cellulose filters, Rainin) before use. Temperature is 25°C unless otherwise indicated. Probe solutions were made by addition of a concentrated solution of probe in diethyl ether in a volumetric flask. After evaporation of the ether solvent, sufficient water was added to yield a final probe concentration of 2.5×10^{-5} M. The concentration of the cyclodextrins were designed to give at least 85% complexation of the probe.

RESULTS AND DISCUSSION

The dialkylaminobenzonitriles display a single broad fluorescence in non-polar solvents centered around 340 nm. In polar solvents, excitation of these molecules results in a second broad fluorescence emission, the long wavelength emission (LE). The position of the wavelength maxima (λ_{\max}) of the LE of 4-(N,N-dimethylamino)benzonitrile (DMABN) and 4-(N,N-diethylamino)benzonitrile (DEABN) has been monitored and is found to increase appreciably with solvent polarity (Table 1). This change is indicative of the twisted internal charge transfer (TICT) of the excited state (Grabowski and Dobkowski, 1983). A linear correlation is observed (Fig. 1) in plots of the emission energy of the wavelength maxima (in kcal/mol) vs the solvent polarity parameter $E_T(30)$, which are derived from the electronic charge transfer transition of a pyridinium phenol betaine (Reichardt and Dimroth, 1968; Kosower and Dodiuk, 1976) for a variety of solvents. Plots of emission energy of the λ_{\max} for the LE of DMABN vs dielectric constant (ϵ) also exhibits linearity at ϵ values above 10 (Fig. 2). DEABN exhibits very similar behavior (data not shown). Therefore, the λ_{\max} for the LE of DMABN and DEABN can be used as a polarity probe of micro-environments. There appears to be no correlation between the λ_{\max} for the short wavelength emission (SE) and solvent polarity (Table 1).

A significant blue shift of the LE is observed upon addition of α - and β -cyclodextrin to aqueous solutions of DMABN and DEABN (Table 2). This result

Table 1. Emission λ_{\max} (nm) for the short wavelength (SE) and long wavelength (LE) emissions in various media*

Media	DMABN		DEABN	
	SE	LE	SE	LE
Dioxane	352	398	353	412
Methylene chloride	341	422	340	434
tert-butyl alcohol†	341	464	338	456
Acetonitrile	332	485	343	478
2-Propanol	349	479	350	472
n-Pentanol	339	479	334	470
n-Butanol	346	479	348	474
n-Propanol	348	483	349	477
Ethanol	330	488	346	480
Methanol	352	500	353	493
Ethylene glycol	341	518	339	511
Water	347	553	348	552

*All λ_{\max} are corrected for instrument response. †Measured at 30°C.

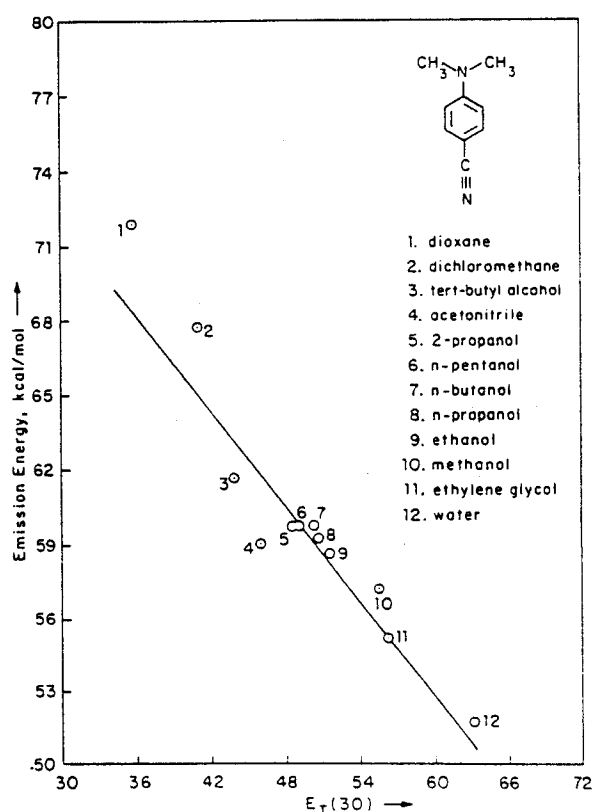


Figure 1. A plot of emission energy (kcal/mol) for the long wavelength emission (LE) of DMABN vs the solvent polarity parameter $E_T(30)$.

plex more strongly to β -CD than to α -CD. The values are reasonable in light of previously reported data (Saenger, 1980; Gelb *et al.*, 1981; Bergeron, 1977), although the constant for the DEABN β -CD complex is larger than most values for disubstituted benzene derivatives and β -CD.

The lifetimes for the probes in the different complexes (Table 5) parallel the quantum yield data.

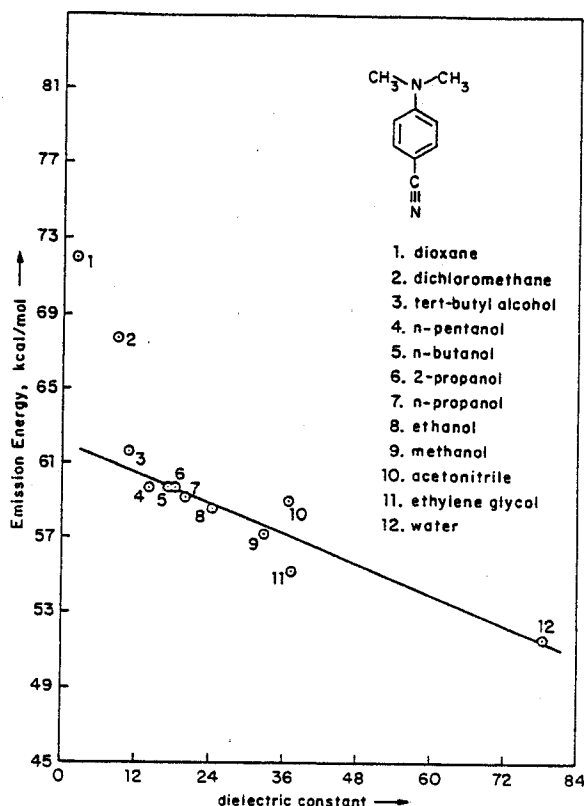


Table 2. Experimental $E_T(30)$ values* for DMABN and DEABN in aqueous cyclodextrin solutions

Media	DMABN		DEABN	
	LE	$E_T(30)$	LE	$E_T(30)$
α -Cyclodextrin	453	45	513	57
β -Cyclodextrin	520	57	517	58

*Values taken from the data in Fig. 1.

Table 3. Relative fluorescence quantum yields of DMABN and DEABN in α - and β -cyclodextrin solutions

Media	ϕ_n^*	
	DMABN	DEABN
α -Cyclodextrin	23.2	4.9
β -Cyclodextrin	3.5	4.2
H ₂ O	1.0	1.0

Figure 2. A plot of emission energy (kcal/mol) for the long wavelength emission (LE) of DMABN vs dielectric constant. Plot based on points three through twelve.

* ϕ_n is relative to the probe in water; error limit is 10% or less.

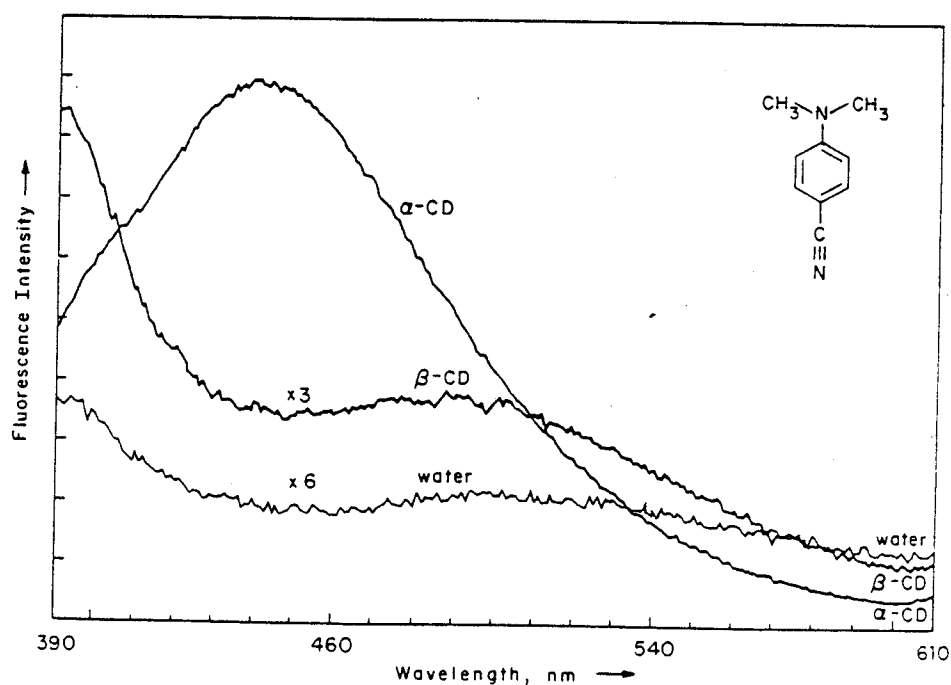


Figure 3. Fluorescence spectra for dimethylaminobenzonitrile with α -CD, β -CD, and water: Short wavelength emission not shown.

Table 4. Equilibrium constants for probe-cyclodextrin complexation*

Media	Probe	
	DMABN	DEABN
α -Cyclodextrin	$160 \pm 30 M^{-1}$	$575 \pm 100 M^{-1}$
β -Cyclodextrin	$440 \pm 110 M^{-1}$	$2500 \pm 250 M^{-1}$

*Values determined using the Benesi-Hildebrand equation, with fluorescence intensity monitored at the wavelength maximum of the long wavelength emission of the most concentrated solution. †Error based on standard deviation for both the slope and intercept.

Table 5. Fluorescence lifetimes of DMABN and DEABN probes in water, cyclodextrin, and organic solvents

Media	τ (ns)*	
	DMABN	DEABN
H ₂ O	1.82 ± 0.21	0.56 ± 0.10
β -Cyclodextrin	2.43 ± 0.19	1.68 ± 0.09
α -Cyclodextrin	3.26 ± 0.19	1.91 ± 0.11
Ethanol	2.46 ± 0.22	2.88 ± 0.19
Acetonitrile	$3.26 \pm 0.13^\dagger$	
Dichloromethane	3.96 ± 0.19	3.76 ± 0.24

*Error limits based on a range of 18 measurements. †Huppert *et al.* (1981) list a value of 3.7 ns.

indicates that these dialkylaminobenzonitrile probes form complexes with both α - and β -cyclodextrin and, when complexed, experience an environment less polar than water. In fact, using the data in Fig. 1, the environmental polarity experienced by DMABN and DEABN in cyclodextrin solutions can be calculated (Table 2). The solvents nearest to the cyclodextrin environments are tert-butyl alcohol for DMABN in α -CD and ethylene glycol for all other complexes.

The quantum yields for the LE of DMABN and DEABN increase in the presence of cyclodextrins compared to aqueous solutions (Table 3, Fig. 3). The most dramatic effect (a factor greater than twenty) occurs with DMABN in α -CD, greater than even the ten-fold increase observed with 1,8-ANS in β -CD (Cramer *et al.*, 1967)! The increase in quantum yield results from the lower polarity, the quantum yield of the LE decreases with increasing polarity (Kosower and Dodiuk, 1976). The much lower polarity observed in the DMABN/ α -CD system compared to the other samples is clearly reflected in the quantum yield and λ_{max} data. The significant increases in intensity upon addition of α - or β -CD to aqueous solutions of probe is not observed upon addition of γ -CD. These results are consistent with the postulate that the DMABN probe may complex to α -CD in a different geometry from the other complexes. For example, inspection of molecular models reveals that

the cyano protrusion into the α -CD cavity is possible for DMABN, but that a second conformation, with partial entry of the dimethyl group, is conceivable (Harata *et al.*, 1982). For DEABN, this latter conformation appears to be unfavorable.

Equilibrium constants for complexation can be determined based on the changes in quantum yield by monitoring fluorescence intensity at a fixed wavelength as a function of cyclodextrin concentration (Benesi and Hildebrand 1949; Kinoshita *et al.*, 1974). For a given cyclodextrin, DEABN complexes more strongly than DMABN (Table 4). This difference in K_{eq} may be due to the fact that DEABN is more hydrophobic. Both probes complexed. Both sets of measurements indicate that, at high polarity, the quantum yield of the LE state decreases due to an increase in the non-radiative rate with increase in polarity. This phenomenon is observed for many excited states which have large dipole moments (Mataga and Ottolenghi, 1979).

CONCLUSION

In summary, the results presented here clearly demonstrate that DMABN and DEABN form complexes with α - and β -cyclodextrin. An important observation from these experiments is that polarity experienced by a probe in a cyclodextrin complex does not correlate with the size of the equilibrium constant. The results support the large dipole of the TICT state but no statement can yet be made as to the effect of cyclodextrin on the initial rotation to the non-planar form. Further studies to address the rotation phenomenon should be of interest in light of the evidence supporting solvent-probe interaction as an important aspect for the formation of the TICT state. In cyclodextrin, this interaction may involve the hydroxyl groups facing the interior of the cavity.

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