Simultaneous Double Probing of the Microenvironment in Colloidal Systems and Molecular Assemblies by DPH Derivatives

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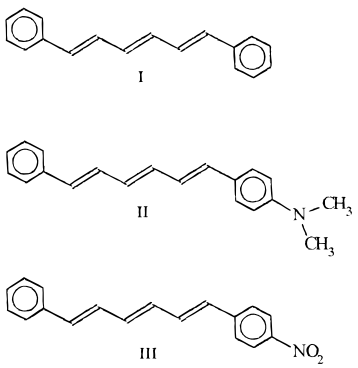
The spectroscopic properties of two derivatives of DPH, viz. DPH-N$(CH_3)_2$ and DPH-NO_2 (Chart 1), were examined as prospective fluorescence probes. It was found that, contrary to the parent molecule DPH, these two derivatives exhibit fluorescence spectra strongly dependent on the polarity of their immediate environment. It was also found that their fluorescence anisotropy r is as high as that of DPH ($r = 0.38$). Consequently, DPH-N$(CH_3)_2$ and DPH-NO_2 can be used for the simultaneous measurement of micropolarity and microviscosity (or any other property related to r, e.g. phase transition), at the site of their solubilization, in molecular assemblies and microheterogeneous media. Since probing by either one of these two DPH derivatives involves the use of the same probe for the determination of two different microproperties, it is assured that the two microproperties measured correspond to exactly the same microsite, viz. that of the solubilization of the one and only probe. Ordinarily, one uses different probes to determine different microproperties, in which case it is almost certain that these microproperties reflect different sites of the microenvironment. The suitability of these probes for the simultaneous determination of microviscosity and micropolarity has been demonstrated for a number of typical microheterogeneous systems. In all cases the microparameters obtained simultaneously by means of DPH-N$(CH_3)_2$ or DPH-NO_2 were at least as good as the ones determined separately by means of well established probes such as pyrene or DPH.

Introduction

The electronic spectroscopy of the various diphenyl-*n*-polyenes (Ph(−CH=CH−)$_n$Ph) has been the subject of continuous investigations for many years.[1] Among the numerous members of this class, 1,6-diphenyl-1,3,5-hexatriene (DPH) is the most extensively employed as a fluorescence probe for studies of molecular order, dynamic behavior, microfluidity, etc.,[2,3] because of its convenient spectroscopic properties. The most exceptional of these properties is the high and constant steady state fluorescence anisotropy ($r_{max} = 0.38$) across the lowest energy absorption band of the molecule.[4] This almost limiting value of r ($r_{lim} = 0.4$) is attributed to the fact that the absorption and fluorescence transition moments in DPH are nearly parallel to each other.[4] Since this common direction of the two transition moments is also parallel to the long molecular axis,[5] any change of r from its maximum value will indicate tumbling of the DPH molecule about its long axis, during the lifetime of its excited state. On the contrary, rotation around this axis will not have any effect on r. Moreover, the constancy of the fluorescence anisotropy across the lowest energy absorption band allows a choice of excitation wavelengths, so that undesirable absorption by other molecules than the probe can be avoided. It is therefore quite evident that, in association with the lifetime of the excited state of DPH, r can reflect either the viscosity of the medium in which DPH has been introduced or the size of a supramolecular system to which the probe has been affixed. Other interesting spectroscopic aspects of DPH which enhance its suitability as a fluorescent probe include the very strong absorption it exhibits in solutions ($\epsilon_{356} = 10^5$ M^{-1} cm^2 in hexane) and its good fluorescence quantum yield in most solvents (from ca. $\Phi = 0.25$ in acetonitrile to $\Phi = 0.8$ in benzene), except water, where it is practically insoluble.[6] Because of the above, the perturbation of the structure of the host medium caused by the addition of the probe can be kept at a minimum, since as little as 10^{-8} M of added DPH is adequate to obtain very good fluorescence spectra. Finally, the large Stokes' shift between the absorption and fluorescence bands prevents scattered excitation light from interfering with the fluorescence polarization spectra.

Chart 1



Contrary to its suitability for the determination of microproperties connected with r, DPH is not a good fluorescence probe for micropolarity, because its emission is totally insensitive to changes of the polarity of the environment.[7] Because of this DPH cannot be used for probing the micropolarity of its environment. This means that, although DPH measures all microparameters associated with r, nevertheless in order to measure micropolarity a different probe than DPH must be used, which usually is not expected to reside at exactly the same site as DPH. More generally, the problem concerning the ambiguity of the site of solubilization of a probe is always present in investigations of microenvironmental properties of organized or microheterogeneous systems. It becomes

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quite serious though when different probes are used to determine different microproperties of a microheterogeneous system. Indeed, because most of the probes are appropriate for the determination of only one parameter, one has to use two or more probes to determine different microparameters, but under these conditions it is not guarantied that all probes will reside at the same location in the system. The result will be that microparameters measured by different probes will give information about different sites. For example, if DPH is used to determine the microfluidity of a microheterogeneous system and pyrene to determine its micropolarity, there is no assurance that the two microparameters obtained will correspond to the same microenvironment, since it is very likely that each probe will reside at a different site.

From the above discussion it becomes evident that probes capable of measuring simultaneously more than one physicochemical property of a microenvironment will be very helpful in studies of molecular assemblies and supramolecular systems. With this in mind and taking into account the established advantages of DPH, we have undertaken the task of finding appropriate DPH derivatives, which in addition to the aforementioned favorable properties of simple DPH, will also exhibit environment dependent fluorescence spectra. The two derivatives we have chosen for this study are the dimethylamino and the nitro derivatives of DPH (**I**), abbreviated as DPH-$N(CH_3)_2$ (**II**) and DPH-NO_2 (**III**), respectively. The drastic reduction of the molecular symmetry of DPH by the addition of a $N(CH_3)_2$ or NO_2 group at the para position of one of its phenyl rings will result in great changes in the arrangement of the excited states and the allowed/forbidden electronic transitions. Furthermore, the attachment of the electron donor/acceptor groups, $N(CH_3)_2$ and NO_2, is expected to affect the charge delocalization between the conjugated diphenylhexatriene system of DPH and these substituent groups, leading to a large increase of the dipole moment of the excited over the ground state and therefore causing strong solvent−solute interactions and sizable spectral shifts in fluorescence. If we can show that these two DPH derivatives have solvent dependent fluorescence, while at the same time the large r value of the mother molecule is kept intact, we will have obtained fluorescent probes appropriate for determination of more than one microproperty at the very same site of a microheterogeneous system. Noe that the synthesis and some preliminary fluorescence studies of molecules **II** and **III** have been published;[8,9] however, the reports were incomplete and did not elaborate either on the spectroscopic properties or the probing capabilities of these DPH derivatives.

Experimental Section

The all-trans 1,6-diphenyl-1,3,5-hexatriene (DPH) was purchased from Fluka, while the two DPH derivatives 1-(4-(dimethylamino)phenyl)-6-phenyl-1,3,5-hexatriene (DPH-$N(CH_3)_2$) and 1-(4-nitrophenyl)-6-phenyl-1,3,5-hexatriene (DPH-NO_2) were brought from "Lambda Probes & Diagnostics". They were of the highest purity available and therefore were used without further purification. The surfactants, dodecyl-, tetradecyl-, and octadecyltrimethylammonium chloride and dioctadecyldimethylammonium bromide, were purified by recrystallization from ethanol/ethyl acetate, and their purity was confirmed by the absence of any fluorescence emission in the range of interest to this study. The good quality and purity of all solvents used were confirmed by absorption and fluorescence spectroscopy. For absorption spectra the Perkin-Elmer Lambda-16 spectrophotometer was used, whereas fluorescence and excitation spectra were recorded on a Perkin-Elmer LS50-B fluorometer equipped with filter polarizers. The room temperature excitation spectra of the molecules studied here were found to be identical, in energy and vibrational structure, with their corresponding absorption counterparts, in all solvents; therefore, we have used absorption and excitation spectra interchangeably, viz. absorption for room temperature and excitation for lower temperatures. The emission spectra reported were corrected for the response of the instrument. Fluorescence quantum yields (Φ) were obtained from deoxygenated solutions, using as standard quinine sulfate in 1.0 N sulfuric acid, for which $\Phi_{st} = 0.55$.[10]

Results and Discussion

Since our objective here is to establish the probing suitability of the two DPH derivatives **II** and **III**, we should state the requirements of a fluorescent probe relevant to the present study. First, all probes must cause minimum perturbation to the structure under examination. Therefore, probes must absorb and emit light strongly, so that even a small amount of the probe will be sufficient for good fluorescence spectra. Also, when possible, the probe must be chosen to have a molecular structure similar to that of the molecules constituting the microheterogeneous phase; therefore, for the case of synthetic vesicles or biological membranes, rodlike molecules such as DPH and its derivatives are very appropriate. Micropolarity probes must, in addition to the above, exhibit fluorescence spectra sensitive to the solvent polarity, the sensitivity being expressed either as a shift of the energy or as a change of the spectral pattern upon change of the solvent. On the other hand, microviscosity probes, based on rotational depolarization of their fluorescence, must have high and constant fluorescence anisotropy over all the wavelength range of their lowest energy absorption band and large Stokes' shifts to avoid depolarization due to energy transfer in case the local concentration of the probe is high. With reference to the measurements of r, it must be mentioned that when a fluorophor exhibits good emission, modern fluorometers can measure accurately r values down to ca. 0.03 units. Therefore, in combination with the fluorescence lifetime of the probe one can measure with good accuracy a quite wide range of viscosities using the appropriate fluorophor. In the following we will discuss first the spectroscopic aspects of DPH-$N(CH_3)_2$ and DPH-NO_2 in solution, and in the second part we will present examples concerning the application of these derivatives as probing agents, for the study of some typical microheterogeneous media.

A. Spectroscopic Aspects. The main spectroscopic data concerning DPH and its two derivatives **II** and **III** are collected in Tables 1 and 2. They consist of the absorption and fluorescence spectral maxima, $\nu(\text{abs})_{max}$ and $\nu(\text{fluo})_{max}$, respectively, the fluorescence anisotropies, r, the fluorescence quantum yields, Φ, and the lifetimes of these molecules in various solvents. The absorbing and emitting behavior of **I**, **II**, and **III** will be briefly discussed in this section and only with reference to their use as fluorescence probes for the microproperties of colloidal systems.

The effect of the substitution by the $N(CH_3)_2$ and NO_2 groups on the absorption spectra of DPH is depicted in Figure 1, along with the effect of some solvents. Thus, upon substitution (i) the absorption of DPH undergoes a considerable red shift of up to ca. 3000 cm^{-1}, (ii) the maximum value of the extinction coefficient $\epsilon(\text{max})$ is reduced by as much as 40%, and (iii) the vibrational

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Table 1. Wavenumbers of the Maximum Absorption ($\nu(abs)_{max}$) and fluorescence ($\nu(fluo)_{max}$) Intensities of DPH and Its Derivatives in Various Solvents at 25 °C and the Kosower Parameter

			$\nu(abs)_{max}$ (cm^{-1})			$\nu(fluo)_{max}$ (cm^{-1})		
no.	solvent	Z	DPH	DPH-N(CH_3)$_2$	DPH-NO_2	DPH	DPH-N(CH_3)$_2$	DPH-NO_2
1	hexane		28 539	25 840	25 442	23 452	22 124	21 598
2	ethyl ether	55	28 490	25 773	25 556	23 419	21 008	19 724
3	pyridine	64		24 814			18 797	
4	acetonitrile	71.3	28 433	25 445	25 252	23 310	18 450	15 504
5	triethylamine			25 707			21 505	
6	ethyl acetate	61		25 575			19 960	
7	DMF	68.5		25 000	24 570		18 416	15 674
8	dioxane	58		25 380	25 000		20 661	19 084
9	CH_3Cl	63.2		25 252	24 630		19 920	16 806
10	MeOH	83.6		25 641	25 252		18 761	nf[a]
11	EtOH	79.6	28 425	25 707	25 125	23 419	19 455	nf
12	ButOH	77.7		25 840			19 960	nf
13	ethylene glycol	85.1	27 901	25 252	24 510	23 310	18 348	nf
14	benzene	54	27 902	25 125	24 679	23 272	20 724	19 608

[a] nf = nonfluorescent.

Table 2. Quantum Yields (Φ), Experimental Lifetimes (τ_{exp}), and Fluorescence Anisotropies (r) for DPH and Its Derivatives in Various Solvents at 25 °C

		Φ			τ_{exp} (ns)			r		
no.	solvent	DPH[a]	DPH-N(CH_3)$_2$	DPH-NO_2	DPH[a]	DPH-N(CH_3)$_2$[b]	DPH-NO_2[c]	DPH[e]	DPH-N(CH_3)$_2$	DPH-NO_2
1	hexane	0.63	0.265	0.002	15.9		2.4		0.037	0.268
2	ethyl ether									
3	pyridine									
4	acetonitrile	0.17	0.034	0.140	4.1	0.34	1.5		0.145	0.02
5	triethylamine									
6	ethyl acetate									
7	DMF		0.054	0.220		0.5			0.175	0.03
8	dioxane	0.68	0.120	0.46	9.2				0.171	0.045
9	CH_3Cl	0.57	0.063	0.35	6.2	0.8	1.6		0.152	0.024
10	MeOH	0.23	0.029	nf[d]	4.9	0.41	nf		0.207	
11	EtOH	0.26	0.037	nf	5.6	0.57	nf		0.214	
12	ButOH	0.37		nf	7.7		nf			
13	ethylene glycol		0.1	nf			nf	0.1	0.333	
14	benzene	0.71			6.1					

[a] Taken from ref 27, except the value for benzene (ref 17). [b] Taken from ref 9. [c] Taken from ref 8b. [d] nf = nonfluorescent. [e] All other values of r were below 0.01 due to the high τ_{exp} of DPH.

structure of the spectra is smeared out. More importantly, it can be seen from this figure that red shifts of comparable magnitude, viz. less than 800 cm^{-1}, are induced in the absorption spectra of all three molecules, as the polarity of the medium increases to its maximum value. The largest red shift, ca. 800−900 cm^{-1}, however, occurs when the solvent is benzene, which has the highest polarizability ($\alpha = 0.295$) among all the solvents used here. From the above discussion it becomes clear that the effect of the solvent on the absorption spectra of **I**, **II**, and **III** is rather small and unimportant, at least as far as the probing capabilities of these molecules are concerned.

Large and important spectroscopic differences between DPH and its derivatives **II** and **III** are however observed in the fluorescence spectra. As shown in Figure 2, the parent molecule DPH exhibits solvent insensitive emission, and this is the reason why this molecule cannot be used as a fluorescence probe for micropolarity. On the contrary, the spectra of the derivatives undergo considerable shifts of as much as 7000 cm^{-1}, depending on the medium, and this is what makes them good candidates as probing agents. In the following, and in order to examine the probing prospectives of the two DPH derivatives, we will discuss the effect of the medium on the fluorescence of DPH-N(CH_3)$_2$ and DPH-NO_2.

An empirical parameter which incorporates, albeit in a nonexact way, most of the factors involved in the term "solvent polarity" is the well-known Kosower Z-parameter.[11] Plots of the so-called solvatochromic shift (SS), which is the difference $\nu(abs)_{max} - \nu(fluo)_{max}$ between the maxima of absorption and fluorescence expressed in cm^{-1}, vs Z are shown in Figure 3a and demonstrate excellent linear relationships for the two derivatives of the present study as well as for the parent molecule DPH. This figure also shows very clearly that while in the case of DPH the solvatochromic shift is independent of Z and therefore DPH is not appropriate for the determination of the polarity of its environment, in its two derivatives, DPH-N(CH_3)$_2$ and DPH-NO_2, the SS is strongly dependent on Z, a fact which makes these molecules good fluorescent probes for micropolarity. Note also that while DPH-NO_2 does not fluoresce at all in protic media (see the tables), DPH-N(CH_3)$_2$ fluoresces in such media. The solvatochromic shifts associated with the Z values of protic solvents are howev er grouped separately from the other solvents, as shown in Figure 3a. Moreover, in all cases, the relationships between SS and Z are linear to a very good approximation. The separate grouping of protic and aprotic solvents in the case of DPH-N(CH_3)$_2$ raises an interesting point, viz. that for SS values between ca. 5500 and 7000 cm^{-1} there are two possible Z values, a lower Z value corresponding to nonprotic and a higher one corresponding to protic solvents. However, by using DPH-NO_2 as probing agent, one can distinguish between the two Z values, because if DPH-NO_2 fluoresces from the medium, this means that the environment is aprotic and therefore the smaller Z value—determined by DPH-N(CH_3)$_2$—is accepted. A similar ambiguity concerning Z values occurs also with the well established polarity probe

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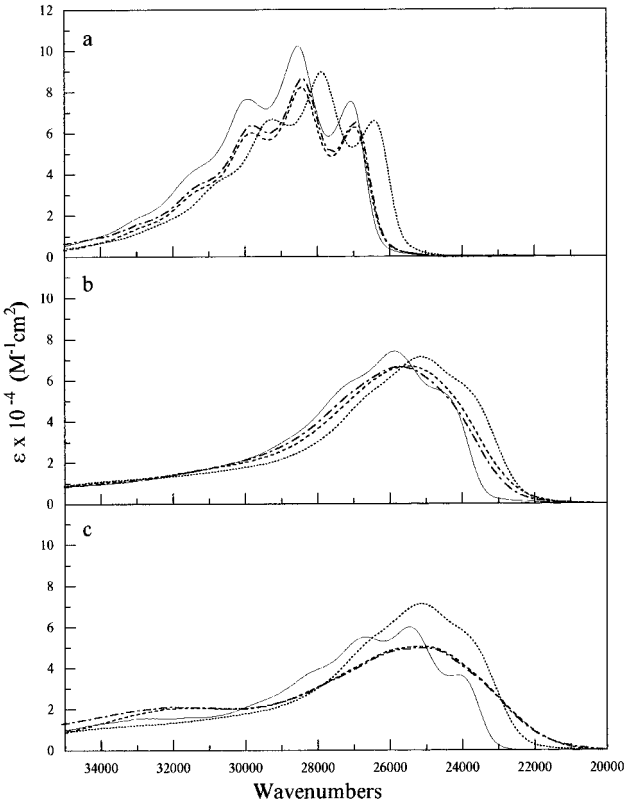

Figure 1. Absorption spectra of DPH (a), DPH-N$(CH_3)_2$ (b), and DPH-NO_2 (c) in various solvents: hexane, solid line; benzene, dotted line; acetonitrile, dashed line; ethanol, dashed-dotted line.

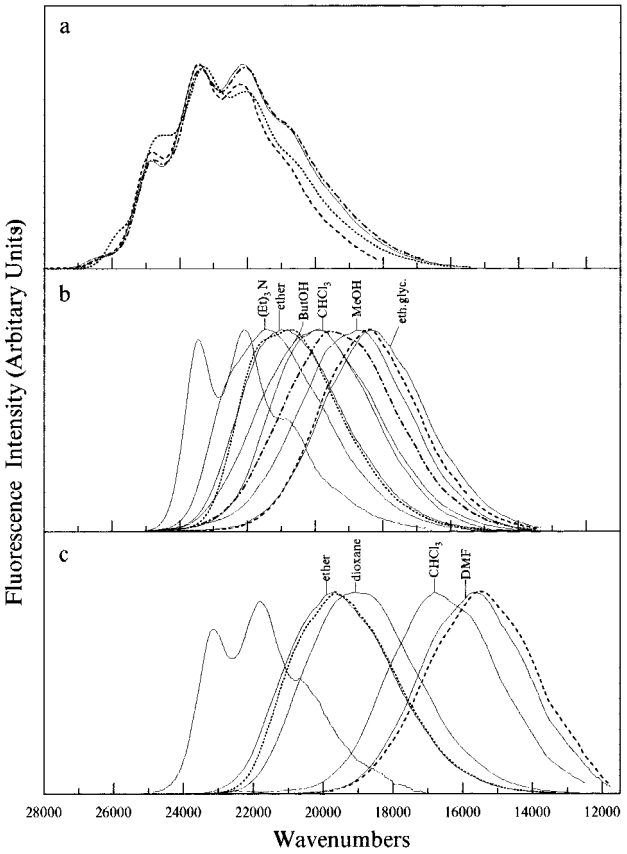

Figure 2. Fluorescence spectra of (a) DPH, (b) DPH-N$(CH_3)_2$, and (c) DPH-NO_2: Either the solvents are indicated by the same type of lines as in Figure 1 or they are written on the spectra.

pyrene. Thus the values of the intensity ratio I_1/I_3 of the first over the third fluorescence peak of pyrene, which is a good index of the solvent polarity,[12] are also grouped separately for protic and aprotic solvents, as shown in Figure 3b. From this figure it is also evident that in the case of pyrene there is no linear relationship between I_1/I_3 and Z; instead the data are scattered all over the plot of Figure 3b. From the above it becomes evident that the two DPH derivatives **II** and **III** are much more appropriate micropolarity probes than pyrene.

As far as the probing capabilities of **II** and **III** are concerned, their fluorescence anisotropy r is another parameter of interest. For pure DPH in optically transparent vitrified solutions the reported maximum value of r ranges between 0.36 and 0.39;[13,14] our own value determined in glycol at 218 K and in EPA at 77 K was $r_{max} = 0.38$, exactly the same value we found for the two derivatives in the same solutions and at the same temperatures. Moreover, we have found that the value of $r = 0.38$ is constant at all wavelengths above 360 nm, i.e. across the entire range of the lowest electronic transition. Values of r which we have measured in various solvents at room temperature are listed in Table 1. It is also interesting to mention that the fluorescence lifetimes of DPH are approximately one order of magnitude longer than those of DPH-NO_2, whereas DPH-N$(CH_3)_2$ has lifetimes with intermediate values (see Table 2). Since on the other hand, according to the well-known Perrin equation $r = r_{max}\tau_{corr}/(\tau_{corr} + \tau)$, the numerical value of r depends on the relationship between the fluorescence lifetime of the probe τ and its rotational correlation time τ_{corr}, the combination of DPH and its two derivatives can cover a wide range of viscosities; viz., DPH is appropriate for media with high viscosity, DPH-NO_2 for those with lower viscosity, and DPH-N$(CH_3)_2$ for intermediate values of viscosity. In every case, however, knowledge of τ is indispensable for such measurements. Another important fluorescence parameter, which was found to be sensitive to the viscosity of the environment, is the fluorescence quantum yield (Φ). Thus, we have found that while in DPH Φ increases only slightly when the viscosity of the solvent increases, in the two derivatives the rise of Φ with increasing solvent viscosity is dramatic, as shown in Figure 4.

B. Fluorescence Probing by DPH-N$(CH_3)_2$ and DPH-NO_2. In this section we will discuss examples of fluorescence probing in some typical colloids such as aqueous micelles and synthetic vesicles. The probing is based on the exceptional fluorescence properties of the two DPH derivatives introduced in the present study. Our point of interest is emphasized by comparing the probing capabilities of DPH-N$(CH_3)_2$ and DPH-NO_2 with those of the established fluorescent probes DPH and pyrene. It is clearly shown that the two DPH derivatives not only determine micropolarity and microviscosity more accurately than pyrene and DPH but also can measure them simultaneously; i.e., only one probe can determine both microparameters.

1. Aqueous Micelles. With respect to micellar systems, the main questions concerning fluorescence probing involve micropolarity and microviscosity at the site of solubilization of the particular probe. Ordinarily one uses one probe to determine micropolarity, e.g. pyrene, and

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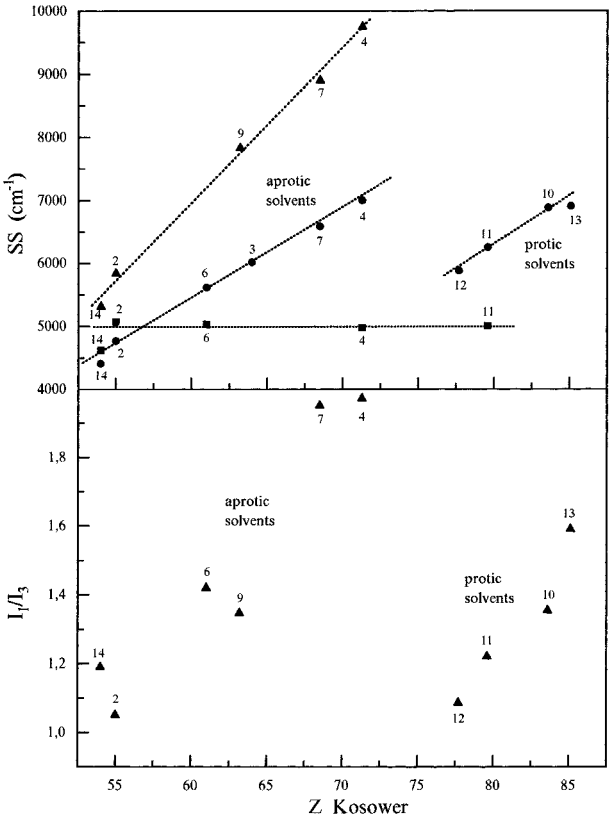

Figure 3. Plots of solvatochromic shifts (SS) vs the Kosower *Z* parameter for DPH (squares), DPH-N$(CH_3)_2$ (circles), and DPH-NO_2 (triangles). Numbers as in tables.

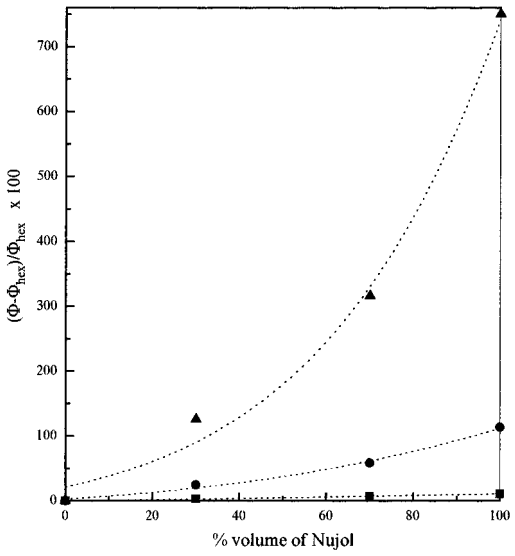

Figure 4. Percent of fluorescence quantum yield (Φ) increase of DPH (squares), DPH-N$(CH_3)_2$ (circles), and DPH-NO_2 (triangles) vs solvent viscosity, expressed as volume percentage of Nujol (viscosity n > 100 cP) in hexane (n = 0.259 cP) at 20 °C.

another, e.g. DPH, to determine microviscosity. However, it is more than certain that these two probes, and any other two different probes for that matter, will occupy different sites in the micellar structure; therefore, the microproperties determined by different probes will correspond to different microlocations and, consequently, any attempts to correlate them will be meaningless. On the other hand, using one of the two proposed probes DPH-N$(CH_3)_2$ or DPH-NO_2, both micropolarity and microviscosity can be determined simultaneously at exactly the same microlocation, viz. the site of solubilization of the

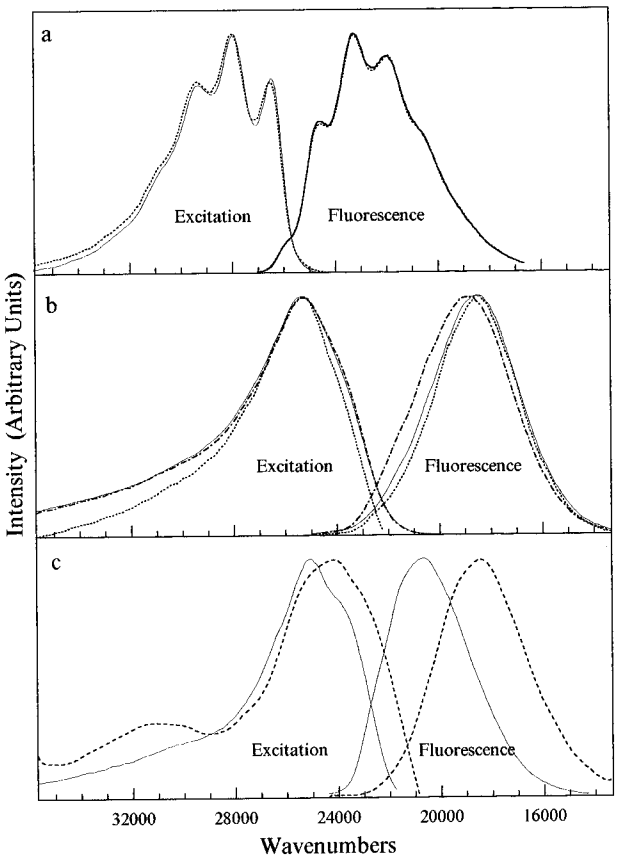

Figure 5. Excitation and fluorescence spectra of DPH (a) and DPH-N$(CH_3)_2$ (b), solubilized in aqueous micelles of $CH_3(CH_2)_{11}N(CH_3)_3^+Cl^-$ (solid lines), $CH_3(CH_2)_{13}N(CH_3)_3^+Cl^-$ (dotted lines), and $CH_3(CH_2)_{17}N(CH_3)_3^+Cl^-$ (dashed-dotted lines). (c) Excitation fluorescence spectra of DPH-N$(CH_3)_2$ (solid lines) and DPH-NO_2 (dashed lines) solubilized in vesicles of $(CH_3(CH_2)_{17})_2N(CH_3)_2^+Br^-$.

probe. Here we have used the solvatochromic shift (see Figure 5b) of DPH-N$(CH_3)_2$ to determine the micropolarity in aqueous micelles of $CH_3(CH_2)_nN(CH_3)_3^+Cl^-$, where n = 11, 13 and 17. The numerical values of these solvatochromic shifts are SS = 6839, 6764, and 6441 cm^{-1} for the micelles with n = 11, 13, and 17, respectively; then, introducing these SS values in the plot of DPH-N$(CH_3)_2$ of Figure 3a, we find the corresponding polarities, expressed in terms of the Kosower parameter *Z*, to be either *Z* = 71, 70, and 67 (if the environment is aprotic) or *Z* = 83, 82.5, and 80 (if the environment is protic). However, we have also found that the other probe, DPH-NO_2, which solubilizes at the same site as its diamino homologue, does not emit from these micelles, which indicates that it senses a protic environment. Therefore the latter of the above two sets of *Z* values, viz. 80−83, reflects the polarities of the three micelles. These *Z* values correspond to an environment similar to that of ethanol and methanol. For comparison we have also measured the micellar micropolarities by means of the I_1/I_3 intensity ratio of the fluorescence of pyrene solubilized in the same three micelles. The numbers obtained were the same within experimental accuracy, for all three micelles, viz. 1.38−1.43, corresponding to *Z* values equal to either around 80 or around 65 (see Figure 3b). It is obvious that the DPH-N$(CH_3)_2$ probe is much more sensitive to the micropolarity of the environment than pyrene, since the former can differentiate between the larger and the smaller micelles, as shown by the different positions of the fluorescence spectra in Figure 5b. Moreover the magnitude of the I_3/I_3 ratio suggests two possible values for *Z*, whereas in the case of the DPH derivatives

combination of DPH-N$(CH_3)_2$ with DPH-NO_2 allows assignment of the correct Z value. Note that DPH-NO_2 cannot be combined with pyrene, because it does not solubilize at the same site with it. Furthermore, the same probe DPH-N$(CH_3)_2$ was used to determine the relative microviscosities in the above three micelles. Thus, the fluorescence anisotropy r of DPH-N$(CH_3)_2$ was found equal to 0.25, 0.27, and 0.28 in the micelles with $n = 11$, 13, and 17, respectively, indicating similar microviscosities. The corresponding r values of DPH, which is one of the established microviscosity probes, were 0.06, 0.06, and 0.07, which also indicate similar viscosities in these micelles. Recall that, in order to find numerical values of the microviscosities, one must relate the data to a calibration curve, but it is not our objective here to determine the absolute microviscosity; we simply want to show that this probe can determine microviscosity as accurately as DPH. In conclusion, we have shown that the proposed probe DPH-N$(CH_3)_2$ can determine simultaneously two microparameters, polarity and viscosity, with better accuracy for the former and with at least the same accuracy for the latter as the established probes DPH and pyrene, which being different, solubilize at different sites.

2. Synthetic Vesicles. The synthetic vesicle dioctadecyldimethylammonium bromide is known to undergo a phase transition between 30 and 40 °C,[15] which is detectable, among other techniques, by means of the variation of the fluorescence anisotropy r of DPH.[16] Since the phase transition involves a change of the viscosity of the interior of the vesicular bilayer and since the fluorescence quantum yields of both DPH-N$(CH_3)_2$ and DPH-NO_2 increase with increasing viscosity of the medium (see Figure 4), it is expected that the phase transition will be evident by the change in the fluorescence intensity of these two probes, as well as by the variation of r. Figure 6 shows the changes of both r and the fluorescence intensity vs temperature for DPH, DPH-N$(CH_3)_2$, and DPH-NO_2 solubilized in the above vesicles. The evidence for a phase transition is quite clear from the variation of r in all three probes; it is however even more evident from the variation of the fluorescence intensity of the two DPH derivatives but not DPH itself. These results show that a membrane phase transition can be determined by simply recording the fluorescence intensity of DPH-N$(CH_3)_2$ or DPH-NO_2 solubilized in the membrane vs temperature. Note that the differences in the r values for the three probes must be attributed, as we have mentioned, to their different lifetimes. Furthermore, from the solvatochromic shifts of DPH-N$(CH_3)_2$ and DPH-NO_2 solubilized in the vesicles of dioctadecyldimethylammonium bromide (Figure 5c) and the corresponding plots of Figure 3a we have estimated equal micropolarities with both probes, viz. 53−55 units of the Z scale. This value shows that the two probes sense the same environment, which is less poalr than that in the micelles discussed above, where Z was found equal to 80−83. On the contrary the I_1/I_3 ratio of the fluorescence of pyrene has the same value in the vesicle and the micelles, ca. 1.36. This value of the I_1/I_3 ratio corresponds (from Figure 3b) to a micropolarity equal to either 85 or 60−63 units of the Z scale. Again the superiority of the DPH derivatives **II** and **III** over pyrene is obvious. In conclusion these derivatives are better polarity probes than pyrene and at least as good viscosity probes as DPH itself, but the important fact is that these derivatives can determine simultaneously both microparameters, which is impossible to do with either the pyrene or the DPH probe alone.

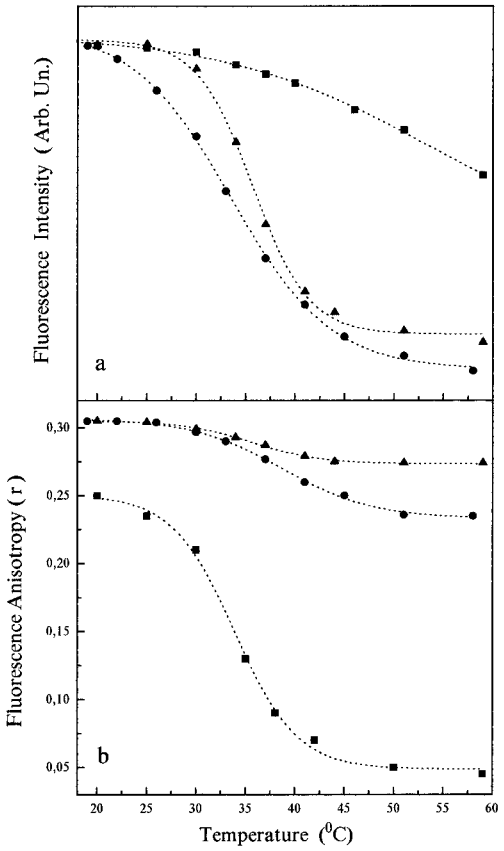

Figure 6. Fluorescence intensity (a) and r (b), of DPH (squares), DPH-N$(CH_3)_2$ (circles), and DPH-NO_2 (triangles), solubilized in vesicles of $(CH_3(CH_2)_{17})_2N(CH_3)_2{}^+Br^-$.

Conclusions

The main conclusions of this work are the following. (1) Contrary to DPH, its two derivatives DPH-N$(CH_3)_2$ and DPH-NO_2 exhibit fluorescence spectra strongly dependent on the solvent polarity. Therefore these derivatives can be employed as micropolarity probes in aqueous colloidal systems. (2) The fluorescence quantum yields of the derivatives increase sharply with increasing viscosity of the solvent, the increase being more pronounced in the case of the NO_2 derivative, while the parent molecule DPH is practically insensitive to this parameter. Therefore **II** and **III** can be used as fluorescence probes in molecular aggregates, for the determination of order−disorder phase transitions, which are followed by changes in the viscosity. (3) The fluorescence anisotropy r of all three probes can also be used to determine microviscosities. In addition, because of the different fluorescence lifetimes of **I**, **II**, and **III**, these probes can be used complementarily to each other for the determination of a very large range of viscosities. (4) A most important conclusion is that DPH-N$(CH_3)_2$ or DPH-NO_2 can be used, each separately, for the simultaneous determination of both micropolarity and microviscosity in colloids and microaggregates in aqueous media.

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