Thermally Induced Variations in Polarity and Microviscosity of Phospholipid and Surfactant Vesicles Monitored with a Probe Forming an Intramolecular Charge-Transfer Complex

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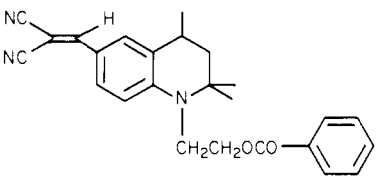
Abstract: The viscosity-dependent variations of the fluorescence yield Φ_F and the polarity induced shift of the emission band maximum $\lambda_{F(max)}$ of a derivative of (*p*-(dialkylamino)benzylidene)malononitrile (**1**) were used for the probing of phospholipid (DPPC, DSPC) and surfactant (DODAB, DHP) vesicles. The temperature-dependent behavior of **1** in the control phospholipid vesicles revealed that the phase transition can be detected through both the variation in Φ_F and the shift of $\lambda_{F(max)}$. However, the visibility of the transition by either parameter depends on the position of **1** in the bilayer. The ambiguity of the probe's response in terms of the change in Φ_F (a decrease with increasing temperature) is related to a limited degree of the probe–environment interaction as indicated by the failure of the reorientation dynamics of **1** in a model solvent (ethylene glycol) to fulfill a stick boundary condition of the hydrodynamic model. Therefore, the observed variation in the apparent microviscosities of the vesicles with temperature (30–3 cP) is regarded as a lower limit. The fluorescence emission of **1** as well as its thermally induced shift (the blue shift with increasing temperature) suggests significantly higher polarities of the surfactant bilayers than those of the phospholipids analogues. The red shift of $\lambda_{F(max)}$ is accounted for by a probe displacement in the vesicle bilayer induced by the variations in the chains' mobility.

Recently,[1] we have attempted to investigate surfactant vesicles in comparison with their phospholipid analogues by using an intramolecular excimer-forming probe. However, a conclusion on the differences in the molecular structures between these two kinds of molecular aggregates could not have been drawn mainly because of the dissimilar solubilization pattern of the probe.

The characterization of surfactant vesicles has become of increased importance after their being recognized[2] as a more stable model than phospholipid vesicles for mimicking the functions of biomembranes. The excellent colloidal stability of surfactant vesicles is attributed to the considerable surface potential[3] established at the interface of the vesicles as a consequence of the dissociation of the ionic head groups of surfactants. These molecular aggregates are very attractive for a number of applications[4] because of their significant solubilization power for various solutes, compartmentalization ability, and recently documented in-bilayer polymerization.[5]

The molecular organization in vesicles and its variations, especially resulting from a temperature change of the system, is one of the most important characteristics of vesicles which affects other properties such as the permeability of, and the lateral diffusion in the bilayer, the location of a solubilizate, and the solubilization power. A notion of the thermotropic phase transition between the gel (crystalline) and the liquid-crystalline phases, very well established in phospholipid vesicles,[6] has been adopted also for surfactant vesicles. However, the transition temperature, T_C, was rigorously determined only in positively charged vesicles made from double-chained alkylammonium salts.[1,7-10]

In this work, we have investigated thermally induced changes of microviscosity and polarity in surfactant (dioctadecyldimethylammonium bromide (DODAB) and dihexadecyl phosphate (DHP)) and phospholipid (dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC)) vesicles by taking advantage of a fluorescence probe, (*p*-(dialkylamino)benzylidene)malononitrile, (**1**). The phospholipid vesicles were



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studied with the intention of using them as control systems for the surfactant vesicles.

The S_1 state of the molecule **1** was attributed to an intramolecular charge transfer, π,π^* state.[11] The dominant deactivation process of this complex is nonradiative internal conversion strongly related to the internal rotation of **1**. Any motional restrictions imposed on the molecule of **1** result in an increased importance of radiative decay process at the expense of the nonradiative ones. Hence, an increase in, e.g., the viscosity of the probe's environment will be manifested through an augmentation of the fluorescence yield Φ_F, of **1**.[12]

A red shift of the fluorescence maximum, $\lambda_{F(max)}$, of **1** with solvent polarity was also observed.[11] It is presumably due to a lowering of the ionization potential of the donor group of **1** in more polar solvents. Importantly, the shift of $\lambda_{F(max)}$ was found[11] to be independent of the fluorescence yield. This enables the determination of the viscosity and polarity of the probe's site simultaneously. Such properties of **1** prompted us to use it for the probing of vesicular systems.

Experimental Section

1. Materials. DPPC and DSPC were obtained from Sigma and used without further purification. DODAB and DHP were purchased from

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Table I. Fluorescence Spectral Data of **1** in Vesicles

system	$\lambda_{F(max)}$, nm	$\Phi_F \times 10^3$	ϵ^a	η_a,[b] cP	η_a,[c] cP
ethylene glycol	500	9.6	47	18.5	18.9
DPPC	488	6.3	18	4.8	9.3
DSPC	492	14	25	48	32.5
DODAB	498	7.1	41	18	11
DHP (H_2O)	498	9.2	41	26	15.5
(buffer)	494	4.7	31	10	6.3

[a] Macroscopic dielectric constant; the estimation of ϵ based on Φ_F of **1** in straight-chain alcohols.[13] η_a = apparent vesicular microviscosity determined with the help of an empirical viscosity scale. [b] Straight-chain alcohols at room temperature.[13] [c] Ethylene glycol at different temperatures.

Fisher and ICN Pharmaceutical, respectively. These surfactants were recrystallized from acetone and methanol, respectively. Potassium dihydrogen orthophosphate was brought from Analar. The probe, **1**, was a gift from Dr. R. O. Loutfy (XRCC). Water used for the preparation of vesicles was purified by passage through a carbon black column and two columns of smaller grade ion-exchange resin (Millipore). Ethylene glycol (Fisher) was used without further purification.

2. Preparation of Vesicles. The phospholipids (4 mg) and/or the surfactants (10 mg of DODAB and 5 mg of DHP) dissolved in dichloromethane or chloroform were placed into a small test tube (volume of about 4 mL). After addition of 5 μL of the 10^{-3} M dichloromethane solution of **1** (the final concentration of the probe was typically 2.5×10^{-6} M), the solvent was evaporated under N_2 stream leading to the formation of a thin film on the wall of the tube. Then 2 mL of phosphate buffer solution (5×10^{-3} M + 0.1 M NaCl) in the case of the phospholipids and 2 mL of water or 10^{-2} M phosphate buffer solution for the surfactants were added and the samples were sonicated by using a Sonicator 350 (Heat System Ultrasonic) set at about 40 W and equipped with a small probe. Sonication was carried out for 10–25 min at temperatures between 50 and 75 °C depending on amphiphile. After sonication, the vesicular dispersions were centrifuged for about 10 min with a laboratory centrifuge in order to remove Ti particles and undispersed phospholipids and/or surfactants. The concentration of the phospholipids was typically of 3×10^{-3} M. The concentration of the surfactants taken into the experiments was about 8×10^{-3} and 4.5×10^{-3} M for DODAB and DHP, respectively.

3. General Technique. The absorption spectra of **1** were recorded with a Cary spectrophotometer, Model 17, and the fluorescence measurements were carried out with a Perkin-Elmer MPF-4 spectrofluorimeter equipped with a differential corrected spectra unit (DCSU-2). The optical densities of all samples were ≤0.4. The fluorescence yields of **1** were obtained by comparison of the fluorescence emission spectra of **1** in vesicles with that in ethylene glycol where the yield was previously determined ($\Phi_F = 9.6 \times 10^{-3}$).[12] The corrections for the scattered light contributions to the emission spectra were made in all studied vesicular systems.

The temperature variation was achieved with the sample placed in a specially designed copper block which was fitted into the sample compartment of the spectrofluorimeter. Water from a thermostated water bath was allowed to circulate through the block. The temperature of the sample was monitored by means of a thermocouple immersed in the sample solution and connected to a Bailey digital meter.

Results

1. Room Temperature Experiments. Incorporation of the water-insoluble dye, **1**, into vesicles was manifested by the appearance of transparent yellowish solutions upon sonication of the amphiphiles and **1** in water. Another indication of a successful solubilization of the probe into vesicles should be seen in a significant increase (5–10 times) in Φ_F in the presence of vesicles as compared with Φ_F in low-viscosity alcohols.[12] The values of Φ_F at room temperature in all systems are summarized in Table I. The fluorescence yields can be correlated with the apparent microviscosities, η_a, at the site of **1** through an empirical scale relating Φ_F of **1** determined in various solvents with the solvents viscosities. Such a scale was recently established for **1** dissolved in alcohols and their mixtures.[13] By taking advantage of the viscosity scale, the microviscosities of the examined vesicles were derived and are presented in Table I. However, the rotational diffusion of a fluorescence probe governing the value of Φ_F may provide an inconsistent viscosity reading when various solvents constitute the viscosity scale. This may be due to a different degree of solute–solvent interaction and/or a change in solvation volumes.[14] Confirmed by experimental results, this phenomenon can be diminished by using only one solvent.[15,16] In such a case, a change in Φ_F can be related to the known variation of the solvent's viscosity with temperature. Ethylene glycol was used in this work as a reference solvent. This choice was made because of similarity between the value of Φ_F in ethylene glycol and those in vesicles. The microviscosities of the vesicular systems based on ethylene glycol are summarized in Table I. The thermally induced viscosity variations of ethylene glycol were taken from literature.[17] A systematic deviation between the values of η_a derived from the two empirical viscosity scales is evident from Table I.

The shift in $\lambda_{F(max)}$ of **1** attributed to the polarity change of its environment can be advantageously used for the monitoring of polarity at the solubilization site of **1** in the bilayers. The range of the shift with polarity has been established in various solvents including straight-chain alcohols.[13] The red shift from 480 to 500 nm corresponds to the variation in the macroscopic dielectric constant from about 10 to 50 when alcohols and/or ethanol–water mixtures are used. The fluorescence emission maxima and corresponding polarities (based on the alcohol scale) in the individual systems at room temperature are presented in Table I. It is evident from these data that the surfactant vesicles provide significantly more polar surroundings for **1** than do their phospholipid analogues. Interestingly, the polarity revealed by **1** in DODAB and DHP (H_2O) (the vesicles prepared by sonication of DHP surfactant in pure water) is the same but somewhat higher than in the case of DHP (buffer) (the vesicles prepared by sonication in a buffer solution; see Experimental Section, Table I). The presence of a buffer in the latter system was found[18] to significantly facilitate the formation of vesicles. Previously,[1] it was shown that the DHP (buffer) system manifested different solubilization power than DHP (H_2O) vesicles which may indicate unlike properties of these systems.

2. Thermally Induced Changes. The very low fluorescence yield of **1** found in nonviscous solvents[12,19] is the result of a very fast nonradiative deactivation of the singlet excited state associated with the internal rotational relaxation of the dye molecule at the expense of the radiative deactivation[11] (fluorescence). Therefore, the fluorescence yield can be expressed as a competition between the rotation (reorientation) diffusion rate constant, k_{or}, and fluorescence rate constant, k_f:

$$\Phi_F = \frac{k_f}{k_f + k_{or}} \tag{1}$$

The intrinsic relaxation rate, k_{or}, of the S_1 state estimated in low-viscosity solvents for **1** is about 2×10^{11} s^{-1} [11] and the radiative decay rate of **1** was determined to be 2.8×10^8 s^{-1} at 77 K, using single photon counting spectroscopy.[20] Hence, any restriction in the intramolecular reorientation of **1** through interactions of **1** with its environment should result in an increase of Φ_F. Molecular reorientation affecting the rotational relaxation of the probe is, in general, a thermally activated process, and therefore, k_{or} can be expressed in terms of an Arrhenius equation:

$$k_{or} = A \exp\frac{-E_a}{RT} \tag{2}$$

E_a is an apparent activation energy representing all processes

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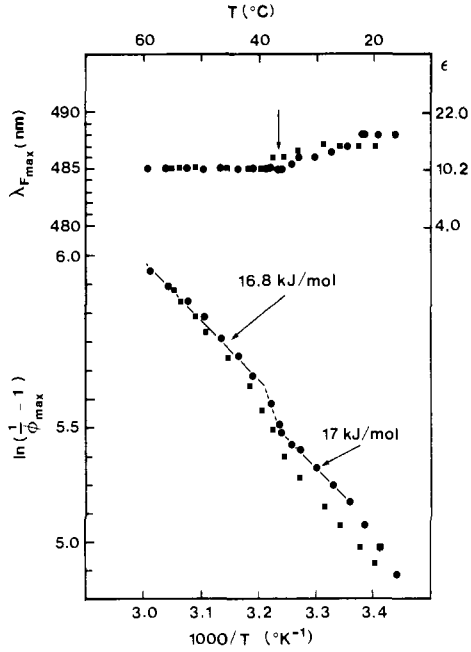

Figure 1. Variation of Φ_F and $\lambda_{F(max)}$ of **1** solubilized in sonicated DPPC vesicles as a function of temperature in (●) heating and (■) cooling cycles.

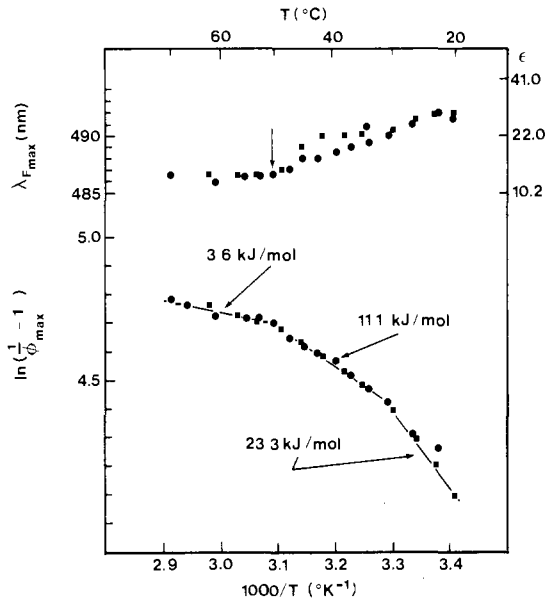

Figure 2. Variation of Φ_F and $\lambda_{F(max)}$ of **1** solubilized in sonicated DSPC vesicles as a function of temperature in (●) heating and (■) cooling cycles.

contributing to the hindrance of the probe's internal reorientation. When the expression for Φ_F (eq 1) is substituted into eq 2 and fluorescence is regarded as a temperature-independent process, the following relationship between Φ_F and temperature is obtained:

$$\frac{1}{\Phi_F} - 1 = \frac{A}{k_f} \exp\frac{-E_a}{RT} \qquad (3)$$

Thus a change, e.g., in the solvent viscosity with temperature, will result in a linear plot of $\ln((1/\Phi_F) - 1)$ vs. $1/T$ with a negative slope E_a/R. In solvents of different viscosity–temperature dependences (which also suggest different molecular mobilities), it is reasonable to expect different slopes of the plots. Thermally induced changes in the mobility of the chains of the bilayer should similarly affect the reorientational relaxation rate of the probe as in the case of the solvent viscosity variations. Around the transition temperature, T_C, corresponding to a modification of the molecular structure of the vesicular mesophase (i.e., from the

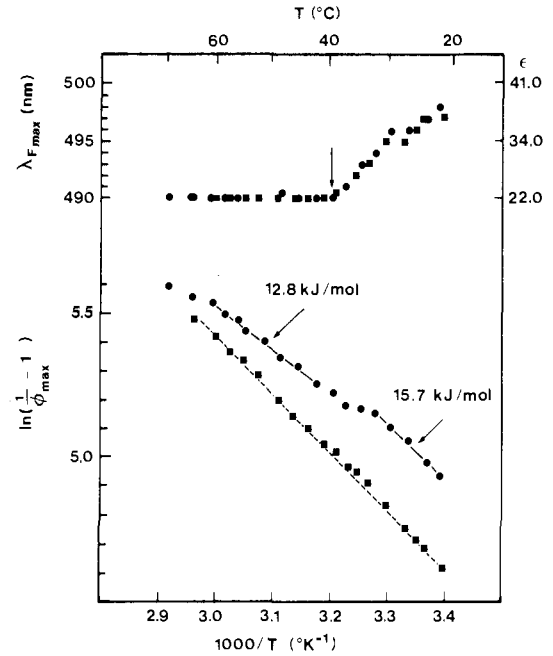

Figure 3. Variation of $\Phi_{F(max)}$ of **1** solubilized in DODAB vesicles as a function of temperature in (●) heating and (■) cooling cycles.

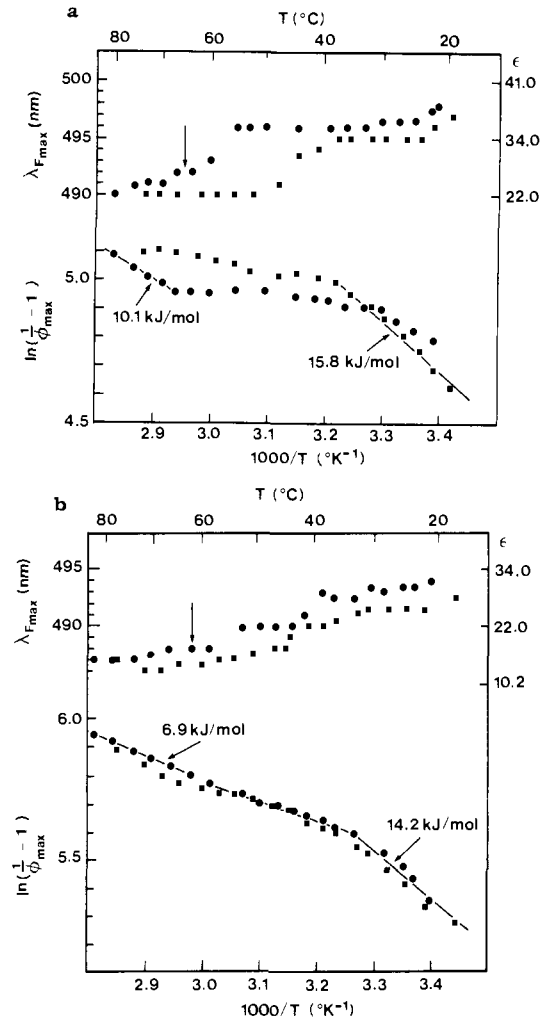

Figure 4. Variation of $\Phi_{F(max)}$ of **1** solubilized in DHP vesicles sonicated in (a) water and (b) a buffer as a function of temperature in (●) heating and (■) cooling cycles.

crystalline to the liquid-crystalline phase),[21] a change in the slope of the Arrhenius-type plots is expected. The changes of Φ_F in

phospholipid and surfactant vesicles due to the temperature variation are presented in terms of Arrhenius plots in Figures 1–4. A typical feature of all plots is the domination of the linear parts and the presence of discontinuities and/or nonlinearities. Apparent activation energies presented in the figures correspond to the slopes of the particular linear parts of the plot and were obtained by the least-square fit of the experimental points. The shifts in $\lambda_{F(max)}$ observed in both the heating and the cooling cycles simultaneously with the variation of fluorescence yield are plotted against temperature in the upper part of the figures.

Phospholipid Vesicles. Interestingly, the probe manifested an unlike behavior in the two control phospholipid vesicles. In DPPC vesicles, a discontinuity in the Arrhenius plot was observed in the vicinity of their transition temperature (37 °C),[22] and only a moderate shift of $\lambda_{F(max)}$ in DPPC is apparent from Figure 1. In contrast, in DSPC vesicles there is only a change in the slope of Arrhenius plot around T_C (50 °C)[22] visible with no significant discontinuity around this temperature (Figure 2). On the other hand, the fluorescence emission maximum was shifted from 492 to 486 nm during the temperature increase from room temperature to 50 °C. It should be stressed that no further shift in $\lambda_{F(max)}$ was found in all phospholipid vesicles above their T_C (Figures 1 and 2). The described changes of both Φ_F and $\lambda_{F(max)}$ of **1** were perfectly reproducible in the cooling cycles, indicating a high degree of stability of the probe–vesicle systems.

Surfactant Vesicles. The thermally induced changes in Φ_F and $\lambda_{F(max)}$ of **1** solubilized in surfactant vesicles are shown in Figures 3 and 4. Obviously, the character of the Arrhenius plots in DODAB vesicles (Figure 3) is quite different from that in DHP vesicles. Such a dissimilarity should not be totally unexpected keeping in mind that, unlike the phospholipids, DODAB and DHP surfactants are different in their chemical nature.

In DODAB vesicles, there was observed no significant change in the slope of the ln $((1/\Phi_F) - 1)$ vs. $1/T$ plots in the whole temperature range. However, a discontinuity between 35 and 40 °C (Figure 3) may well be correlated with T_C of these vesicles.[1,7-10] In the cooling cycle, a hysteresis is visible in this system. The recovery of Φ_F of **1** in DODAB vesicles with decreasing temperature is obviously faster than in the heating cycle. On the other hand, no hysteresis is visible in the $\lambda_{F(max)}$ shift with temperature. The shift was stopped around 40 °C, the temperature which is in good agreement with the published T_C.[7,8,18] An excellent reversibility of the $\lambda_{F(max)}$ shift in the cooling cycle proves that a leakage of the probe or other significant irreversible changes in its position did not take place in the sonicate DODAB vesicles.

In the two DHP vesicles significantly different thermally induced variations in Φ_F as well as $\lambda_{F(max)}$ were observed. The overall change of Φ_F within the experimental temperature range is obviously smaller in DHP (H_2O) vesicles than in the DHP (buffer) system (Figure 4). This is because of a plateau (a broad region of about 30 °C of no change in Φ_F (Figure 4a)) dominating the plot of the former system. A change in the slope of the ln $((1/\Phi_F) - 1)$ vs. $1/T$ plot in DHP (H_2O) occurred above 65 °C. In the cooling cycle, however, a retardation in the fluorescence yield recovery in the high-temperature region is evident. Interestingly, a similar hysteresis in the cooling cycle was found also in the thermally induced shift of $\lambda_{F(max)}$ in this system. While the major blue shift of $\lambda_{F(max)}$ is localized around 60 °C with no additional change in $\lambda_{F(max)}$ (491 nm) above 70 °C, the reverse shift of $\lambda_{F(max)}$ with decreasing temperature was obvious only below 50 °C. A very reasonable recuperation of the original room temperature values of both Φ_F and $\lambda_{F(max)}$ is evidenced in Figure 4a. In DHP (buffer) vesicles, on the other hand, no hysteresis in the Arrhenius plot of Φ_F is present. A slight increase in the slope of the plot above 60 °C is like in DHP (H_2O) vesicles, accompanied with an unchanged $\lambda_{F(max)}$ (487 nm).

The variations of the polarities of the probe's site with temperature monitored through the shift of $\lambda_{F(max)}$ of **1** in all studied systems are summarized in Figure 5 in terms of a macroscopic dielectric constant. The dielectric constant corresponding to a particular $\lambda_{F(max)}$ was taken from a plot of $\lambda_{F(max)}$ vs. ϵ constructed on the basis of the published $\lambda_{F(max)}$ of **1** in various alcohols and ethanol–water mixtures.[13] From Figure 5, it is clearly visible that the largest reversible polarity changes in the probe's solubilization site took place in the surfactant vesicles (about 20 units of ϵ). The lowest thermally induced polarity was detected in DPPC and DHP (buffer) vesicles.

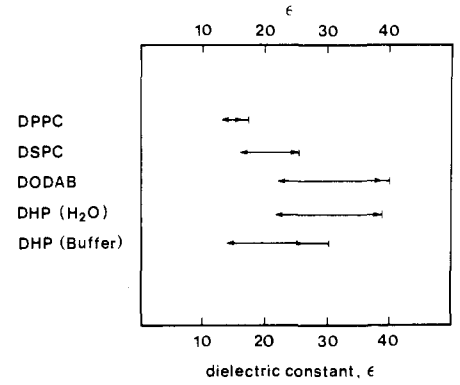

Figure 5. Variation in the polarity of the probe's environment in terms of macroscopic dielectric constant in all studied systems during heating (←) and cooling (→) cycles.

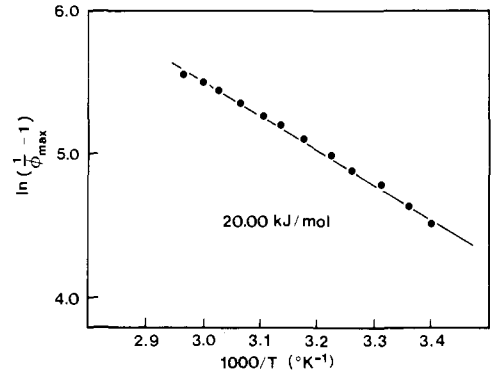

Figure 6. Variation of $\Phi_{F(max)}$ of **1** in ethylene glycol as a function of temperature.

Probe–Environmental Interaction. A considerable variation of Φ_F in the vesicles in a relatively narrow temperature range above room temperature (Figures 1–4) clearly demonstrates the responsiveness of **1** to the variations in the mobility of the amphiphilic molecules forming the bilayer. However, in order to estimate a part of the environmental shear viscosity, η, transferred upon the probe's reorientation relaxation rate through the probe–environment interaction, the validity of Debye–Stokes–Einstein (DSE) hydrodynamic theory[23] should be tested for a particular solute–solvent system. According to the theory, the reorientation relaxation time of a probe is proportional to η/T. In the case of vesicles, however, it is rather difficult, if not impossible, to perform such a test because of the lack of shear viscosity data for the bilayers. Therefore, as the first approximation, we decided to test DSE model for **1** in a solvent showing similarities with vesicles. As mentioned above, ethylene glycol seems to be the best practical choice.

The variation of Φ_F due to the thermally induced changes in the viscosity of ethylene glycol is presented in Figure 6. Unlike in the vesicles, the dependence of Φ_F on temperature is linear in the whole temperature range of our experiments and characterized with only one slope (20 kJ/mol). Recently, Waldeck and Fleming[15] derived the following expression relating the reorientation relaxation time, τ_{or}, of a fluorescent molecule in a solvent with the thermally induced changes in the solvent viscosity:

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$$\tau_{or}^{-1} = k_{or} = \frac{T}{C\eta_O} \exp\frac{-\Delta E_\eta}{RT} \quad (4)$$

Thus, a plot of ln (k_{or}/T) vs. $1/T$ should be linear. This equation is derived under the assumption that the probe molecule interacts with its environment under a stick boundary condition of DSE model and that solvent fluidity (or mobility $\equiv \eta^{-1}$) exhibits the Arrhenius-type behavior. The validity of the latter assumption for ethylene glycol was confirmed by the linearity of the plot of ln η^{-1} vs. $1/T$ over the temperature range of the experiments. The plot was constructed by using published viscosities for ethylene glycol[17] at different temperatures (the plot is not presented). From the slope obtained by the least-square fit of data (correlation coefficient 0.999) the resulting $\Delta E_\eta = 27$ kJ/mol was calculated.

The value of the reorientation activation energy of ethylene glycol, i.e., ΔE_η in eq 4, was calculated from the plot of ln (k_{or}/T) vs. $1/T$ (the plot is not presented). The reorientation relaxation time (and thus also k_{or}) of **1** was obtained from Φ_F by using the expression $\tau_{or} = \tau_f[\Phi_F/(\Phi_0 - \Phi_F)]$.[24] τ_f stands for the intrinsic radiative lifetime of the excited state of **1** and Φ_0 refers to the limiting fluorescence yield. Both values were previously determined[20] at 77 K to be 3.6×10^{-9} s and 1, respectively. The slope resulting from the plot yielded the value of ΔE_η to be 17 kJ/mol. A significant difference between ΔE_η obtained from the viscosity data (27 kJ/mol) and ΔE_{or}, i.e., ΔE_η determined according to eq 4 (17 kJ/mol), suggests that the viscous drag in ethylene glycol is not entirely transferred upon the rotating probe.

Discussion

The published values of the apparent vesicle microviscosities obtained by using different probes vary within a rather broad range (20,[25] 40,[10] 65,[26] 140 cP[27]). Such a discrepancy may have its origin in different mechanisms of the response of various probes, inappropriate choice of empirical viscosity scales, dissimilar locations of different probes in the bilayer of vesicles and different degree of the interaction of probes with their environments. Therefore, the estimation of the extent to which our probe monitors the real situation in the bilayer with the help of an empirical viscosity scale should be attempted. A significant difference (30–40%) in the apparent microviscosities, η_a, of vesicles reported by **1** when two different viscosity scales were used (Table I) substantiates such a need. In both cases, i.e., log Φ_F vs. log η in straight-chain alcohols and ln $((1/\Phi_F) - 1)$ vs. $1/T$ in ethylene glycol, linear plots were found over the temperature range corresponding to our experiments. Hence, both plots can be used for the standardization purpose.

Higher values of η_a found in vesicles on the basis of the alcohol scale reflect the fact that **1** manifests lower fluorescence yields in an individual alcohol than in ethylene glycol at the temperature providing the same viscosity. This suggests that the molecule of **1** rotates faster in straight-chain alcohols than in ethylene glycol due obviously to a lower degree of the interaction of the probe with its environment.

The difference between the activation energy for viscous flow in ethylene glycol ($\Delta E_\eta = 27$ kJ/mol) and that for the viscosity-dependent reorientation relaxation of **1** ($\Delta E_{or} = 17$ kJ/mol) suggests that a stick boundary condition of DSE model is not fulfilled for this system inspite of the linearity of the plot of ln (k_{or}/T) vs. $1/T$ within the experimental temperature range. In other words, a molecule of **1** in ethylene glycol rotates faster than it is expected according to the theory. This conclusion is also corroborated by a smaller slope ($\alpha = 0.72$) of the plot of log k_{or} vs. log η (plot not presented) than $\alpha = 1$ which is expected for the solute–solvent interaction obeying stick hydrodynamic condition. In view of such observations, the probe in straight-chain alcohols should have an even lower degree of the solute–solvent interaction[16] than in ethylene glycol, leading to an overestimation of the apparent microviscosity when the former system is used as the reference scale. We are not in a position to discuss here in detail possible reasons for the observed deviation from DSE model. However, because of the absence of any saturation effect in ethylene glycol we believe that the deviation from a stick boundary condition is due mainly to a partial slip in the interaction of the rotating probe with the ethylene glycol molecules rather than because of the effect of the free volume of the solvent as reported in straight-chain alcohols.[12]

In cases like ours, where the mobility of molecules surrounding a probe is monitored through restrictions imposed by those molecules upon the probe's intramolecular rotation, the knowledge of the intramolecular barrier to such a rotation, ΔE_T, is of a significant importance. By applying a similar procedure as Saltiel and D'Agostino in their stilbene photoisomerization study,[28] we estimated ΔE_T of **1** to be between 2 and 3 kJ/mol. Such a low barrier for the rotation suggests that a prevailing part of the apparent activation energy observed from the change of Φ_F with temperature is related to the viscosity variation of the solvent.

In view of our uncertainity about the correct representation of the bilayer structure by ethylene glycol as well as possible changes in the short range interactions and the solubilization volumes of the probe due to the thermally induced structural changes in the bilayers, we regard our experimental microviscosities (Table I) as lower limits for a particular vesicular system. Our results indicate that η_a of bilayers varies with temperature between 30 and 3.5 cP.

Before we enter into the discussion of the individual systems, the question of the origin of the shift in $\lambda_{F(max)}$ of **1** should be addressed. It seems to be obvious that the observed shifts with temperature are due to the change in the polarity[11] of the local environment of the probe. Such a change, however, can have its origin in the thermally induced molecular reorganization around the probe without considering any probe relocation and/or in the thermally induced transport of the probe from the original site to a new location of a different polarity. Such a situation is quite expected in microheterogeneous systems like molecular aggregates. In the first case, the emission band shift is caused by the solvent–relaxation effect within the lifetime of excited state.[29] This phenomenon generally decreases the energy gap $S_0 \leftarrow S_1$, and therefore it is manifested by the red shift of $\lambda_{F(max)}$ with increasing temperature. Naturally, depending on the viscosity of the probe's environment it is operative within different temperature ranges in different solvents. In view of our results, i.e., no $\lambda_{F(max)}$ shift observed in ethylene glycol and the blue shifts with temperature found in vesicles, one should conclude that the thermally induced shifts in $\lambda_{F(max)}$ in vesicles cannot be attributed to the variations in the solvation of the excited probe.

Hence, the second mechanism should be operative. The displacement of the probe can be conceived in both directions along the chains, i.e., toward the interface and/or the center of the bilayer. When the diffusion of **1** toward the interface is considered, the red shift in $\lambda_{F(max)}$ is expected because of a higher polarity of that part of a bilayer as compared with that in the midst of the bilayer. The same argument is valid if we take into account the possibility of an increased water penetration into the bilayer due to loosening of the structure of the head group region in the course of the temperature change.

Therefore, the blue shifts with increasing temperature found in all systems is a strong indication that the probe diffuses into the center of the bilayer simultaneously with the chains "melting". The diffusion of the probe into a deeper region of the bilayer is not only in accord with the lowering of the polarity around the probe but also with a drop in Φ_F due to a higher chain mobility in the central part of the bilayer. Such conclusions are supported by published results. It was previously demonstrated, theoreti-

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cally[30] as well as by a variety of methods,[31-35] that the mobility of the acyl chains of phospholipids at room temperature augments from the head groups area to the terminal methyl group. In addition, the existence of a polarity gradient along the acyl chains in phospholipid vesicles was found[36] with decreasing polarity toward the center of the bilayer.

Hence, simultaneous variations of temperature and the probe's position make unambiguous correlation of the determined microviscosities with a specific part of the bilayer rather difficult, and therefore a considerable change in the apparent microviscosity of vesicles cannot be related to only one particular site of the bilayer.

It is obvious, from the observations presented above, that the solubilization site of **1** in a bilayer strongly affects the character of the probe's response. Such a position effect is the most probable explanation for the different results found in our control systems, DPPC and DSPC vesicles. A rather small shift of $\lambda_{F(max)}$ in DPPC as compared with a notable one in DSPC vesicles can be rationalized by the solubilization of the probe in a central part of the bilayer of DPPC vesicles whereas in DSPC systems, **1** is localized in the peripheral region of the vesicles. Such a situation may well account also for different viscosities at room temperature reported by **1** in the two phospholipid vesicles because of the proven difference in the chain mobility in the middle of the bilayer and close to the interface.[30-35] The unlike solubilization sites of **1** in DPPC and DSPC vesicles is also supported by a dramatic change in Φ_F around T_C in the former vesicles in contrary with the situation in DSPC vesicles. Spin-label experiments in phospholipid vesicles[33] revealed that both the pretransition as well as the transition temperatures were much more distinctly manifested through the labels located close to the end of the acyl chains than those in the vicinity of the head groups due to a greater mobility of the chain ends.

Dissimilar solubilization sites of **1** in DPPC and DSPC vesicles as evidenced by our results may be caused by a different hydrophobic–hydrophilic balance of these two phospholipids. A more difficult vesicle formation in the case of DSPC as compared with DPPC observed in our experiments should be associated with the longer acyl chains in the former phospholipid. This, in turn, may affect the site of incorporation of **1** in the bilayers.

A comparison of the thermally induced changes of Φ_F and $\lambda_{F(max)}$ in the control phospholipid systems revealed that the shift in $\lambda_{F(max)}$ of **1** served as a more indicative means for the determination of T_C than the variation in Φ_F. The preference of the shift of $\lambda_{F(max)}$ over the variation in Φ_F is also supported by a possible variation in the probe–bilayer interaction because such a variation inclines to affect mainly the fluorescence yield of the probe. A relevant example illustrating justification of such a conclusion may be seen in the reversible change in the slope of Arrhenius plots in the low-temperature region for DSPC vesicle where, however, no structural changes have been reported.[22,37,38] Therefore, the observed change of the slope should be attributed only to a probe displacement as it is indicated in the upper plot for $\lambda_{F(max)}$ (Figure 2).

In view of our previous discussion, the unlike variation of Φ_F as well as $\lambda_{F(max)}$ with temperature in DHP (H_2O) and DHP (buffer) vesicles can be rationalized by different characters of solubilization sites of 1 in the two systems. The blue-shifted emission band of **1** in DHP (buffer) vesicles as compared with that in DHP (H_2O) system suggests a deeper location of the probe in the bilayer of the former system if we assume the identical polarity gradient in both DHP vesicles. A significant difference in the molecular packings at these unlike solubilization sites is evident from the dissimilar variations of Φ_F in the two DHP vesicles. The hysteresis observed in DHP (H_2O) vesicles simultaneously for Φ_F and $\lambda_{F(max)}$ together with both invariant parameters below 65 °C (probably T_C) (Figure 4a), suggesting a polar and rather rigid environment of the probe indicates that the peripheral region of DHP vesicles respond differently to a temperature change than an area closer to the center of the bilayer. The reasons for the different solubilization sites of the probe in the two DHP vesicles are not known at the present time. It should be pointed out that the presented results cannot be accounted for by introducing the possibility of an unequal distribution of the probe molecules in the two systems. The emission bands were of the same width in all studied systems.

The above discussion is presented with an implicit assumption that both DHP vesicles are principally the same as far as their molecular organizations are concerned. However, because of the lack of a decisive argument for such an assumption the possibility of significantly different organizations of DHP surfactants in the two aggregates cannot be completely disregarded. Evidently, more experimental work is needed.

A comparison of Arrhenius plots in DODAB vesicles in both cycles with the shift in $\lambda_{F(max)}$ suggests that the probe–bilayer interaction in this system is the least effective for the T_C determination. A broad discontinuity in the Φ_F change in the heating cycle which, however, disappears in the cooling cycle and practically no change in the slopes of both plots (Figure 3) make the determination of T_C uncertain. On the other hand, the highly reproducible plot of $\lambda_{F(max)}$ vs. T in both cycles defines the phase transition very precisely at 40 °C in agreement with published values.[7,8,10]

The thermally induced variations in Φ_F and $\lambda_{F(max)}$ of **1** revealed significant differences among the individual systems. As discussed above, more reliable results were obtained through the shifts in $\lambda_{F(max)}$ than from the variations in Φ_F. This is, obviously, the result of an inadequate "probe–bilayer interaction". An improvement of such an interaction may be achieved by modifying the molecular structure of the dye. The importance of one and/or two long hydrocarbon chains in the molecule of a probe for the responsiveness of the probe in the phospholipid vesicular bilayers has been recently pointed out.[39]

Considerably higher polarities in both surfactant vesicles than those in the control phospholipid systems, one of the most interesting observations of this work, should be viewed as a direct consequence of the small ionic head groups of the surfactants. The character of the head groups may determine both the solubilization site of the probe as well as the polarity of the site. However, it is rather difficult to distinguish between these two effects.

Our conclusion about the different polarities in the bilayers of surfactant and phospholipid vesicles is strongly supported by the observation of unlike limiting polarities, i.e., the polarities reported in those systems above T_C. The probe diffuses into the central part of a bilayer during the "chains melting" period, probably due to the solubility reason and advantageous entropy of mixing,[30] reaching the center of the bilayer around T_C.

We are, of course, aware that the similar experimental results could be obtained also with an immobile probe and the expulsion of the bilayer water as a result of the increasing mobility of the chains. Clearly, we are unable to distinguish between these two models in this work. An alternative model which would account for the observed polarity differences in the vesicles, e.g., by placing the probe at different sites of similar bilayers as far as their polarity gradients are concerned ought, however, to provide an explanation why the diffusion of **1** is restricted above T_C. The higher bilayer polarities in the surfactant vesicles should be explained through a greater water penetration into the hydrophobic region of the bilayer, a generally accepted mechanism, which is probably en-

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abled by the mutual repulsion of the ionic head groups. This leads to the conclusion that the central part of the surfactant vesicle bilayers is significantly more polar than that of the phospholipid vesicles.

Regardless of our limited understanding of the mechanism of the thermally induced diffusion of the probe, the mere discovery of such a phenomenon in vesicles may have significant implications in various applications of vesicles. A variation of the polarity around a solubilizate (a reagent) with temperature may profoundly affect the results of photochemical, electrochemical, and other processes catalyzed or retarded in the heterogeneous vesicular media.

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Registry No. 1, 74677-08-0; DPPC, 2644-64-6; DSPC, 4539-70-2; DODAB, 3700-67-2; DHP, 2197-63-9; ethylene glycol, 107-21-1.

Studies of Siloxane Oligomers by Depolarized Rayleigh Scattering

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Abstract: We have studied the depolarized Rayleigh scattering spectra and the total scattering intensities of four siloxane oligomers: (A) 1,3-diphenyl-1,1,3,3-tetramethyldisiloxane; (B) 1,1,3,3-tetraphenyl-1,3-dimethyldisiloxane; (C) 1,1,5,5-tetraphenyl-1,3,3,5-tetramethyltrisiloxane; (D) 1,1,3,5,5-pentaphenyl-1,3,5-trimethyltrisiloxane. Our objective was to examine the applicability of depolarized Rayleigh spectroscopy of nonrigid molecules with low symmetry. The shear viscosity and the Rayleigh relaxation time for each of the four oligomers have been determined as a function of temperature. The Arrhenius equation is found to be inadequate to describe the temperature dependence, but the Vogel–Fulcher–Tamman (VFT) equation fits well to the experimental data. The mechanism for the depolarized Rayleigh scattering spectrum of each oligomer is shown not due to the overall rotation of the molecule but to the rotation of the end groups. Concentration-dependent studies of oligomer A in CCl_4 have also been carried out. The results show that in these siloxane oligomers the intramolecular pair orientation correlation is operative but the intermolecular pair orientational correlation is absent, in contrast to the result of poly(phenylmethylsiloxane) polymer in which the depolarized Rayleigh spectrum is affected by the intra- and intermolecular pair orientational correlation.

Introduction

Depolarized Rayleigh scattering is a valuable technique for the study of molecular reorientation in the liquid state. When a molecular liquid consisting of optically anisotropic molecules is illuminated with monochromatic laser radiation, the depolarized component of the light Rayleigh scattered from the medium consists of a distribution of frequencies. The width of the frequency distribution directly reflects the rate of the molecular reorientation.

It is now well established that, for a system of symmetric top molecules, the characteristic feature of the depolarized Rayleigh spectrum, $I_{VH}(\omega)$, is a sharp central component of Lorentzian shape superimposed on a broad background. For molecular fluids of low viscosity (<1 cP), the width of the sharp component can be measured with a Fabry–Perot interferometer. For a system of rigid asymmetric top molecules, the sharp depolarized Rayleigh spectrum may consist of from two to five Lorentzians, depending on the symmetry of the asymmetric top. For example, the $I_{VH}(\omega)$ spectrum is predicted to consist of two Lorentzians if the principal symmetry axis of the asymmetric top molecule coincides with the rotational diffusion constant tensor.[1] However, the separation of Lorentzian components is difficult using the Fabry–Perot interferometer, unless the widths of the Lorentzians differ at least by a factor of 10. Thus, the line width of the depolarized Rayleigh spectrum of a system of asymmetric top molecules reflects the average rate of molecular reorientations about different principal axes.[2,3]

For polymeric liquids, Bauer et al.[4] have found that the $I_{VH}(\omega)$ spectra of polystyrene in solutions consist of a narrow molecular weight dependent and a broad molecular weight independent component. They interpret the narrow component to be associated with the long-wavelength mode of the Rouse–Zimm chain, and the broad component to be associated with the localized crankshaft type of motion involving the phenyl groups. Using a Fabry–Perot interferometer, Jones and Wang have studied the $I_{VH}(\omega)$ spectra of poly(propylene glycol) of varying molecular weights.[5] They have found only the molecular weight independent component, the narrow molecular weight dependent component being absent in the $I_{VH}(\omega)$ spectrum. They show the molecular weight independent component to be due to the localized segmental motion. Fabry–Perot interferometry has also been used to study the motion of the phenyl group in poly(methylphenylsiloxane) by Lin et al.[6]

The main objective of this paper is to examine the applicability of depolarized Rayleigh scattering spectroscopy as a probe of the orientational dynamics of nonrigid molecules with low symmetry. We have studied the depolarized Rayleigh scattering spectra of four siloxane oligomers: (A) 1,3-diphenyl-1,1,3,3-tetramethyldisiloxane (B) 1,1,3,3-tetraphenyl-1,3-dimethyldisiloxane; (C) 1,1,5,5-tetraphenyl-1,3,3,5-tetramethyltrisiloxane; (D) 1,1,3,5,5-pentaphenyl-1,3,5-trimethyltrisiloxane. The depolarized Rayleigh scattering study of these four oligomers in the liquid state as a function of temperature has been carried out using a Fabry–Perot interferometer. In order to evaluate the molecular weight independent static properties such as the optical anisotropy and the

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