Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

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Version of record first published: 24 Sep 2006.

To cite this article: A. A. Shahinian & V. V. Bagdasarian (1994): The Dynamic Structure of Micelles and Liposomes and Their Self-Fluorescence in Absence of Dye, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 241:1, 55-68

To link to this article: http://dx.doi.org/10.1080/10587259408029744

Mol. Cryst. Liq. Cryst., 1994, Vol. 241, pp. 55–68
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Printed in the United States of America

The Dynamic Structure of Micelles and Liposomes and Their Self-Fluorescence in Absence of Dye

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(Received March 18, 1991; in final form November 1, 1991)

A self-fluorescence of micelles and liposomes in the absence of dye and high conjugated groups was discovered in the investigation of absorption and fluorescence spectra changes of aqueous solution of amphiphiles with simple chromophores. We propose the existence of flickering "frozen" ordered structures in micelles and liposomes with exciton migration and emission inside them.

Keywords: micelle, liposome, dynamic, structure exciton, fluorescence

INTRODUCTION

It is well known that the spectral and luminescence properties of chemical compounds are depending not only from the character of their molecules but also from the aggregate state of compound. According to spectral and luminescence characteristics, the molecules may be grouped into two groups distinguished by their lowest electronically excited levels of different orbital nature.[1,2] Molecules containing high conjugated groups including aromatic compounds and dyes emitting from $\pi - \pi^*$ excited state belong to the first group. The rate constant of non-emission deactivation (K_1) is always considerably smaller than that of the emitting transition (K_2), $K_1 < K_2$. Therefore the fluorescence is relatively intense both in solid and liquid phases.

The emission of the molecules belonging to the second group occurs from the $n - \pi^*$ excited state. This group includes molecules with simple chromophores like C=O, S=O, P=O, N=O. The $S_{n\pi^*}$ state is subjected to non-emission deactivation stronger than $S_{\pi\pi^*}$. For this reason most molecules of the second group do not emit a luminescence ($K_1 > K_2$) in the liquid phase except in some rare cases.[1,3] On the other hand, when a rigid structure exist in the chromophoric part of the molecule as e.g. in the case of azoalkanes,[4] the rate constant of emission (K_2) may be increased and the molecule emits luminescence. Thus, the overwhelming ma-

jority of molecules of the second group shows no emission in liquid solutions in the absence of a rigid structure. This is a consequence of intermolecular low-frequency vibrations which increase the probability of nonradiative deactivation. The optical excitation energy is practically entirely converted into thermal and vibrational energy,[5] i.e. intrinsic quenching of fluorescence takes place.[6,7]

The amphiphiles (AM) with molecules containing simple, flexible chromophores as C=O, S=O, P=O and so on can be classified as substances, which do not emit a luminescence in liquid solutions.

On the other hand, it was established that micelles and liposomes of AM have a highly dynamic structure similar to liquids.[8-21] Investigations of the emission of micelles and liposomes, because of their liquid structure, were carried out only in the presence of chromophores with $\pi\pi^*$ transitions either dissolved within the micelles or liposomes or being inserted into the AM, or by using other chromophores with rigid structure having emission activity in the liquid phase.[4,15,22,23]

It is well known that the excitation energy in the molecular crystals, in contrast of liquid systems, can be localized both on impurities or defects of structure and be emitted as fluorescence.[24] Thus, in the case of flexible molecules with simple chromophores, the radiation of excitation energy has cooperative nature and can take place only in solid, ordered structures by the mechanism of intermolecular transfer of excitation energy.

The rigidity of molecules necessary for emission can be achieved by decreasing the degrees of freedom of interacting molecules,[7] i.e. when they are "frozen."

Basing on these considerations it may be assumed: the location of chromophore groups of the AM molecules on the surface of micelles and liposomes; sufficient relaxation times of micelles and liposomes (10^{-4}–10^{-8} sec)[15,25] compared with the fluorescence times (10^{-8}–10^{-9} sec),[5,24-26] the compressibility of micelles and liposomes[27-30] are to make possible the formation of rigidly "freezed" parts in them, on the fluctuations of inside density, with the lifetime sufficient for emission activation of molecules inside them.

The micellar dynamic structure is adequately investigated[31] using the methods and principles applied for condensed phases and molecular crystals based on changes of absorption and fluorescence spectra. If there is a resonance transfer of excitation energy between the excited and nonexcited molecules, new bands in absorption spectra arise.[32] According to Davidov theory,[26] the resonant interaction of chromophore groups of identical molecules must lead to the splitting of the optical levels of the isolated chromophores to sublevels giving rise to the new absorption bands.

The character of the fluorescence spectra in liquid and solid phases differ sharply.[26,32] The molecules in the liquid phase are unable to emit exciton energy except of rigid ones, but this is possible in solid systems, consisting of any molecules.

The fluorescence quenching in the liquid phase when complexes of dyes are formed is accompanied by the change of absorption frequency but there is no such change in solid-ordered systems.

If "frozen" parts are formed in micelles and liposomes if those are compressed,

and if they are of ordered structure and have sufficient lifetimes, excitation energy migration and a fluorescence typical for molecular crystals should be observed.

MATERIALS AND METHODS

The following AM were used: sodium pentadecilsulphonate (SPD) - $C_{15}H_{31}SO_3Na$, a product of "VEB Leuna"; sodium dodecylsulphate (SDS) - $C_{12}H_{25}SO_4Na$, a product of "Sigma"; sodium stearate (SS) - $C_{17}H_{33}COONa$ and sodium oleat (SO) - $C_{17}H_{33}COONa$, products of "Reachim"; quaternary ammonium salt (QAS) - $C_{12}H_{25}(CH_2)_2HN^+Br^-$ and its alcohol derivative (AA) - $C_{12}H_{25}(CH_2)_2(C_2H_4OH)$-$N^+Br^-$ are synthetized in the Institute of Organic Chemistry Academy of Sciences of Armenia; ethylene oxide cetyl alcohol (OC-20) - $C_{16}H_{33}O(C_2H_4O)_{20}H$, a product of "VEB Leuna"; synthetic dimyristoyl phosphatydilcholine (DMPC) - a product of "Sigma". The atomic-absorption and EPR spectra showed practically absence of paramagnetic particles and metals of variable valency in aqueous micellar solutions. All of used AM were preliminary purified from water and oil solubilizing impurities by multirecrystallization from the aqueous solution and from the solution in CCl_4. The Ultra Violet (UV) spectra of aqueous solutions of micelles and liposomes measured with the spectrometer of "Pye Unicam" and spectrofluorimeter "Hitachi MPF-4". The spectra above and below the Critical Micelle Concentration (CMC) were obtained by the way mentioned in Reference 31.

RESULTS AND DISCUSSION

The absorption spectra of aqueous solutions for micelles of SPD, SDD, QAS, SS, SO and liposomes of DMPC are presented on the Figure 1a.

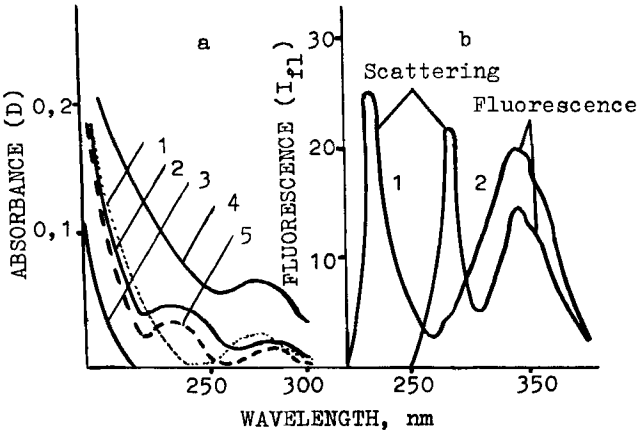

FIGURE 1 (a)-Absorption spectra of the aqueous solutions of SPD below CMC (1) and in the micellar state (2); QAS in the micellar state and the solution of AA (3); Stearic acid and $NaHSO_4$ (4); liposomes of DMPC (5). The absorption spectra of SDS, SO are identical to (2). (b)-Fluorescence spectra of SPD, SDS, SS micelles and DMPC liposomes, excited by 228 nm (1) and 287 nm (2).

As the figure shows, the absorption spectra of all AM containing unsaturated groups in molecules (SPD – S═O; SDS – O═S═O; SS and SO – C═O; DMPC – C═O and P═O) are similar and consist of strong $\lambda_1 \leqslant 200$ nm and weak $\lambda_2 \sim$ 287 nm bands and one medium intensity $\lambda_3 \sim 228$ nm band. The bands 287 nm and 228 nm are absent in the spectra of micellar solutions of QAS and AA which do not have unsaturated groups in molecules.

Besides the investigated substances some organic impurities may also absorb within the interval from 200 nm to 300 nm. In latter case the absorption spectra of aqueous-micellar solutions must undergo the corresponding changes when the AM is purified.

The absorption spectra of 15 wt % aqueous-micellar solution of SPD before and after the purification are represented on Figure 2 as an example. It is obvious that an increase of the whole spectrum take place while the purifying of AM. This indicates that the purification leads to the increase of the concentration of the substance absorbing at the wavelengths $\lambda < 200$ nm, 228 nm and 287 nm.

The absence of absorption at 228 nm and 287 nm in the micellar solutions of AM without unsaturated groups in molecules indicates that the mentioned absorption bands belong to the chromophores C═O, S═O, O═S═O, P═O.

The absorption in the region $\lambda < 200$ nm is due to the $n\pi^*$ transition in the groups ═N—, C—O for QAS and AA.

According to literature, the band at 287 nm can also belong to the chromophores of AM.[7,33] A weak absorption in the region of 280–300 nm corresponding to the spatially and symmetrically forbidden $n\pi^*$ transition and a strong absorption at $\lambda < 200$ nm corresponding to the $\pi\pi^*$ transition are typical for the chromophores C═O, S═O (maybe also for P═O) in aldehydes ketones and SO_2 groups.

By this it was shown that the increase of polarity of the micro-environment of

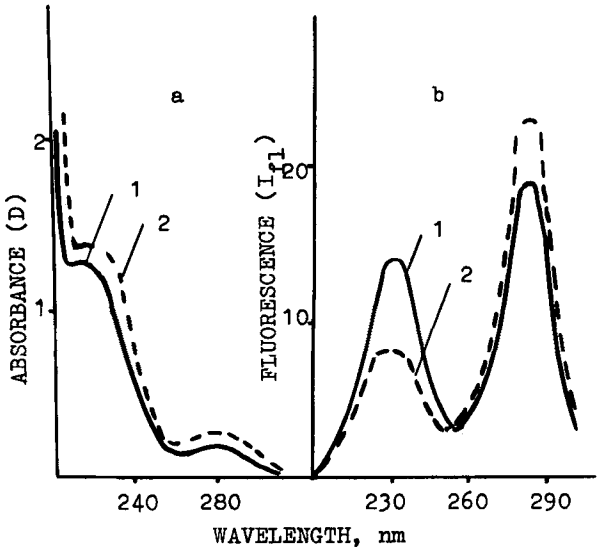

FIGURE 2 The spectra of absorption (a) and fluorescence excitation (b) of aqueous-micellar solution of SPD before (1) and after (2) the purification of the latter. Emission wavelength = 340 nm.

molecules leads to a shift of the $n\pi^*$ and $\pi\pi^*$ transition bands toward shorter and longer wavelengths, respectively.

By analogy it is supposed that the bands $\lambda_1 \leqslant 200$ nm and $\lambda_2 \sim 287$ nm, respectively, in the spectra of micellar solutions correspond to the $\pi\pi^*$ and $n\pi^*$ transitions in chromophores C=O, S=O, O=S=O, P=O of the AM molecules.

If this assumption is correct, the absorption spectra of solutions must undergo changes according to the increase of the microenvironment polarity of AM molecules when the micellar state passes into the molecular solution.

This effect is clearly seen from the absorption spectra of the aqueous solutions of SPD below and above the CMC (Figure 1a), indicating that the bands $\lambda_1 < 200$ nm and $\lambda_2 \sim 287$ nm respectively, correspond to $\pi\pi^*$ and $n\pi^*$ transition in chromophores.

However, it is especially interesting that the absorption band $\lambda_3 \sim 228$ nm disappears when the micelles disappear. This indicates to the direct connection of that band with the existence of micelles.

The identity of the absorption spectra of the aqueous solutions of the substances which are unable from micelles—those are the salt $NaHSO_4$ and stearic acid (the curve 4 on the Figure 1a) and of corresponding AM molecules below the CMC (the curve 1 on the Figure 1a)—is also evidence suggesting that the absorptions $\lambda < 200$ nm and $\lambda_2 \sim 287$ nm are due to the $\pi\pi^*$ and $n\pi^*$ transitions of the chromophores of AM molecules.

Thus, in the absorption spectra of micellar solutions: $\lambda_1 < 200$ nm corresponds to the $\pi\pi^*$ transition, $\lambda_2 \sim 287$ nm is due to the $n\pi^*$ transition in chromophores C=O, S=O, O=S=O and $\lambda_3 \sim 228$ nm arises only upon micelle formation due to splitting of the band of the $\pi\pi^*$ transition (<200 nm), i.e. the Davidov splitting[26] takes place.

The investigation of aqueous solutions of AM showed the total absence of the fluorescence in molecular solutions (below the CMC) and its appearance upon micelle and liposome formation (above the CMC). The emission is totally absent in micellar solutions of AM without π-electrons in molecules. The fluorescence is absent in the case of SO.

It is necessary to point out that the fluorescence is excited only by the bands: 228 nm and 287 nm (Figure 3) and has maximum at ~340 nm (Figure 1b).

The appearance of the fluorescence simultaneously with splitting of the absorption spectra due to micelle formation (from the AM without emission activity in the liquid phase) provides evidence for a suppression of nonradiative deactivation of excited molecules in micelles and for excitation energy migration in "solid" structures.

Taking into account that the fluorescence is excited by the bands 228 nm and 287 nm specific to the AM molecules in micellar and liposomal states, it may be supposed that the chromophore groups of the AM molecules in micelles and liposomes act themselves as centres of emission.

The presence of some fluorescence at 287 nm (the $n\pi^*$ transition) excited by the band 228 nm (splitted $\pi\pi^*$) as well as the excitation of the fluorescence at 340 nm by the band of 287 nm are evidence for the existence of the excitation energy

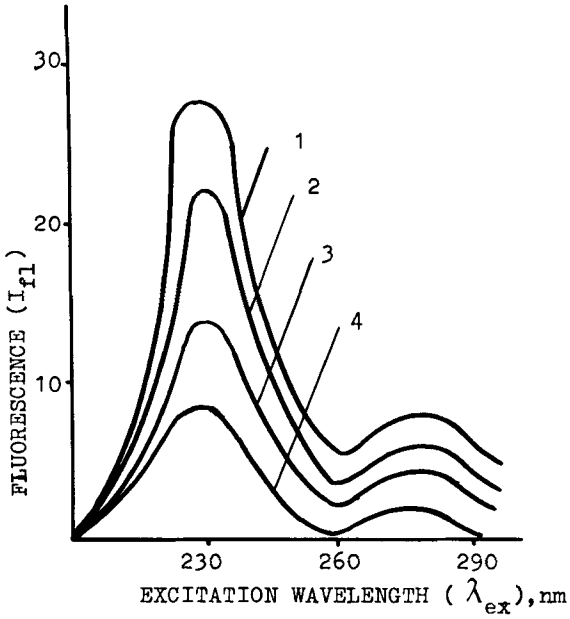

FIGURE 3 The fluorescence excitation spectra at 340 nm for micelles SPD (1), SDS (2), SS (3) and DMPC liposomes (4).

emission mechanism with passing of electron from the excited π^* to the ground n level which can take place only in the "frozen" state of the molecule.

The changes of spectra of the absorption and the excitation of the fluorescence with AM concentration were presented in Reference 31. It was shown that the fluorescence induced by the band 228 nm is subjected to concentration quenching, which is sensitive both to the micellar density (to the compactness of the molecular arrangement in micelle) and molecular mobility in a micelle. To elucidate this problem the intermolecular distances in a micelle were changed with the aid of electrolyte.

As it is shown (Figure 4a), electrolytes compressing the micelles of SPD increase the intensity of absorption at 228 nm. The following series shows the ability of the used cations to increase the absorption intensity: $Ca^{++} > Mg^{++} >> K^+ > Cs^+ > Na^+ > Li^+$.

The same sequence of the ions appears by their influence on the fluorescence intensity of micelles (Figure 4b) excited by the band of $n\pi^*$ transition (287 nm).

In order to determine the influence of the intermolecular distance in the micelle on the absorption and fluorescence of micelle, the influence of the electrolyte concentration (KCl) on the degree of aggregation ($\bar{n}$) of SPD micelles was studied using the light scattering method.[34] The data of the influence of the electrolyte concentration on the shape and area of the micelle surface S,[34] was then used to calculate the average distance $\bar{r}$, between the chromophore groups of SPD molecules on the micelle surface. The following formula was used: $\bar{r} = (S/\pi \cdot \bar{n})^{0,5}$ ($\pi\bar{r}^2$ is the specific area occupied by the polar group of an AM molecule on the surface of a micelle).

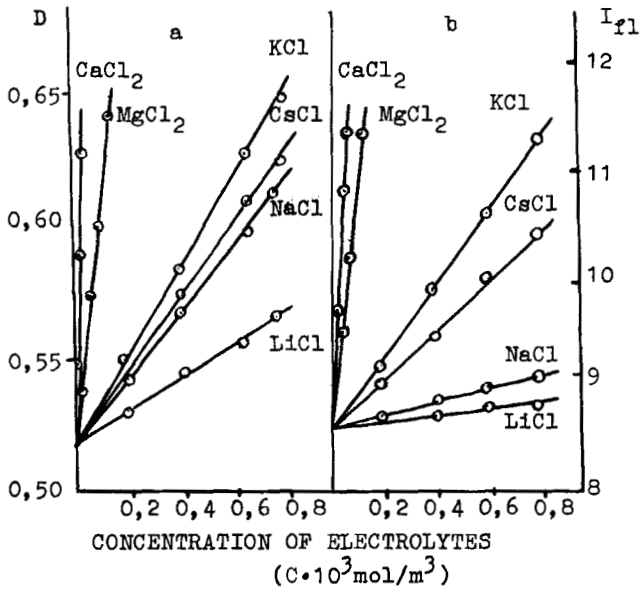

FIGURE 4 The influence of the electrolyte concentration (LiCl, NaCl, KCl, $MgCl_2$, $CaCl_2$) on the intensity of absorption at 228 nm (a) and on the intensity of the fluorescence at 340 nm (excited by the band 287 nm) (b) of the SPD micellar solution.

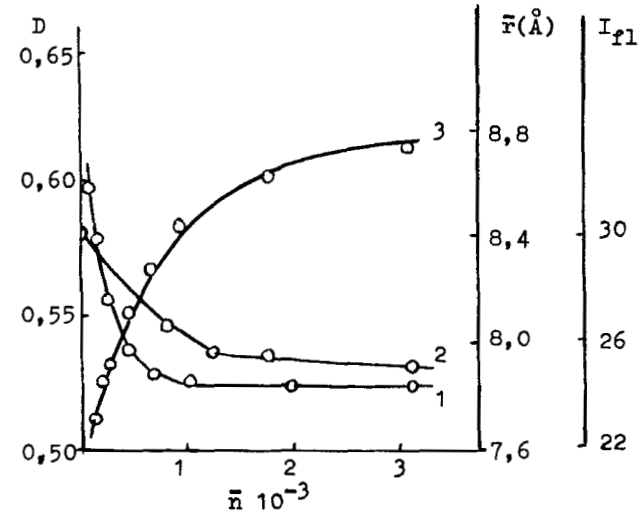

FIGURE 5 The influence of average degree of aggregation ($\bar{n}$) of SPD micelles on the average intermolecular distances ($\bar{r}$) in the micelle-1, the relative fluorescence intensity at 340 nm (I_n) excited by the band 228 nm-2, and the optical absorption density at 228 nm (D)-2 of system.

In Figure 5 the average intermolecular distance in the micelle, $\bar{r}$, the optical absorption density at 228 nm, D, and the relative fluorescence intensity of aqueous-micellar SPD solution at 340 nm, excited by the 228 nm band, I_F, are shown as a function of the micelle aggregation-number, $\bar{n}$. The aggregation number was varied by changing the KCl concentration in the solution.

As seen in the figure, an absolute correlation exists between the $\bar{r}$ and I_F curves. Where $\bar{r}$ does not depend on $\bar{n}$, neither does I_F, while D increases. This shows that the fluorescence quench results mainly from the change of the intermolecular distance in the micelle, while the absorption (228 nm) is mainly a consequence of the increase of the number of molecules taking part in the energy transfer process.

This data is a convincing argument in favour of the existence of a mechanism of excited energy migration in the "frozen", ordered structures in micelle, since it is known that the probability of the fluorescence quenching via excitation energy migration in a crystal increases much faster than the probability of migration without quenching.[5]

Unlike the fluorescence, excited by the band at 228 nm, the intensity of the fluorescence excited by the band of $n\pi^*$ transition (287 nm) continuously grows with the concentration of electrolyte (Figure 6).

The probable reason for this is that on excitation by the 287 nm band ($n\pi^*$-transition) the electron density of the C═O, S═O, O═S═O (or P═O) groups shifts from the oxygen atoms to carbon or sulphur (phosphorous) atoms.[34] The resulting decrease of the dipole moments of the molecules weakens the interaction of their chromophore groups. Thus the transfer probability diminishes and consequently the quench probability diminishes too.

It is interesting to point out that like the absorption spectrum splitting with the appearence of the band 228 nm, the fluorescence at 340 nm excited by 228 nm and 287 nm arise also when liposomes of phospholipid (DMPC) (chromophores C═O and P═O) are formed.

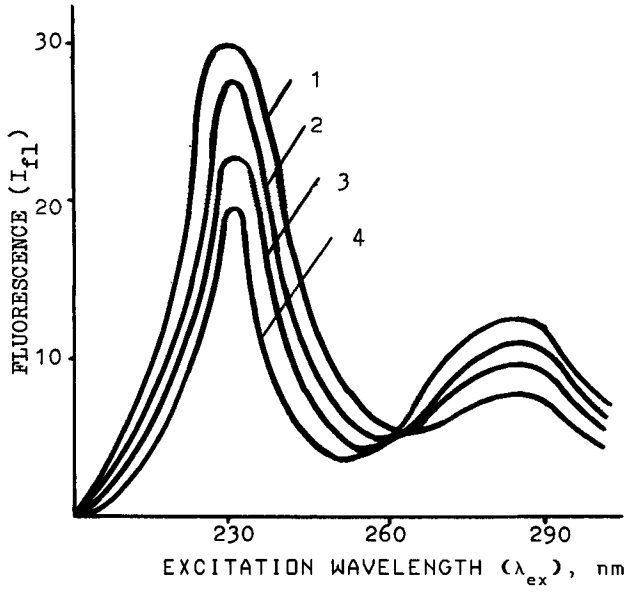

FIGURE 6 The influence of the concentration of KCl and cholesterol on the fluorescence excitation spectra (at 340 mm) of the 4 wt% aqueous-micellar solution of SPD: KCl (10^{-3} mol/m^3) 1 = 0; 2 = 0,4; 3 = 0,8 and 4 = 0,01 wt % (to SPD) of cholesterol.

It is supposed that the factors, which destroy the structure of micelle must suppress energy migration in contrast to the compressing factors which increase it.

Actually, a decrease of the intensity of the absorption band 228 nm takes place (Figure 7a) upon heating, which destroys the micellar structure. The influence of temperature on the intensity of the band 228 nm is a reversible process.

Further, a hypsochromic effect for the band 228 nm is observed during solution heating, which is connected most probably with the increasing of disorder in the region of the chromophore groups of the AM molecules in micelle. Besides temperature, the presence of kinks in the hydrocarbon chains of the AM molecules may increase the disorder in the micellar structure as e.g. in case of SO. Unlike the micelles of SPD, SDS as well as SS, the micelles of SO do not give rise to the fluorescence. However, in this case the splitting of the absorption spectra of micellar solution remain (Figure 1a).

This indicates that the interactions leading to the excitation energy migration take place in micelles of SO as well. However, the excitation energy totally dissipates on the internal conversions which probably may be connected with more "liquid" structure of SO micelle—the short lifetime of excited states of chromophores.

It's well known,[15] that cholesterol suppresses molecular mobility of micelles and phospholipid membranes. The role played by cholesterol in the stabilization of micellar structure (in the molecular mobility suppression) is seen by its influence both on the absorption and fluorescence excitation spectra of SPD micelles (Figure

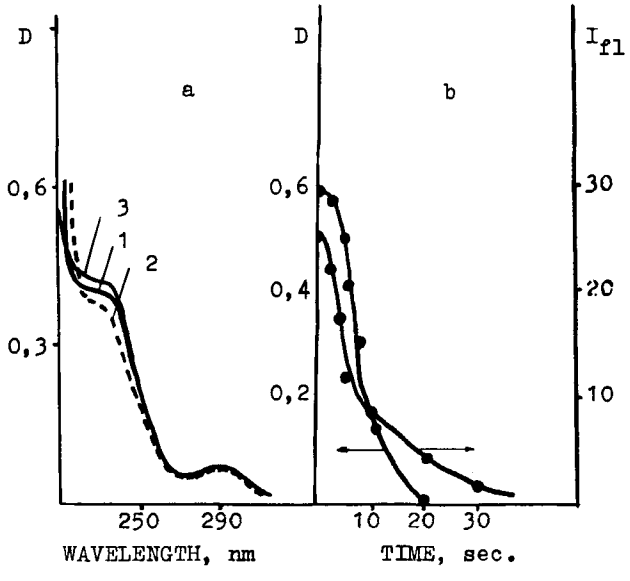

FIGURE 7 (a)-The influence of temperature (1 = 30°C, 2 = 60°C) and cholesterol at 30°C (1 = 0; 3 = 0,01 wt% to the SPD) on the absorption spectra of 4 wt% micellar solution of SPD. (b)-The influence of the duration of the UV-destruction on the absorption optical density at 228 nm (D) and on the relative intensity of the fluorescence (I_{fl}) at 340 nm of SPD aqueous-micellar solution excited by the band 287 nm. The concentration of SPD is 4 wt%, the temperature = 30°C.

6,·7a). Cholesterol quenches the fluorescence excited by the band 228 nm like the increase of the concentration of electrolyte.

The influence of micelle destruction by the hard UV-radiation on the absorption and fluorescence spectra of micelles can be regarded as convincing evidence for attributing the band at 228 nm and fluorescence to the micellar structure. The destruction of AM molecules in micelles leads to the decrease and, finally to the irreversible disappearance both of the emission and the absorption at 228 nm (Figure 7b). By this, the bands $\lambda_1 < 200$ nm and $\lambda_2 \sim 275$ nm in the absorption spectra remain.

The above-developed representations elucidate the nature of the changes in the spectra of absorption and fluorescence of aqueous-micellar solution in the process of AM purifying (Figure 2). As is seen from the figure, the following processes take place: after the AM purifying the intensities of all the absorption bands grow up; the fluorescence excited by the band 228 nm is quenched; the intensity of the fluorescence excited by the band 287 nm grows up.

Comparing the absorption and fluorescence excitation spectra before and after the AM purification with analogous spectra depending on the AM concentration[31] we see that they are identical in nature. Thus, one can state that the process of AM purification is really equivalent to the increase of AM concentration. Therefore there is a correlation between the micellar (liposomal) structure and the laws governing the light absorption and emission.

The absence of the splitting of the band 287 nm ($n\pi^*$) indicates that the excitation energy, in this case, is not sufficiently large for the transfer to the other molecule. By this, the presence of the fluorescence excited by the same band (287 nm) and the absence of its quenching, testifies that the molecule excited by the band 287 nm, if it arranged in the rigidly "freezed" part of micelle becomes a emitting center itself.

The identical wavelength (340 nm) of the fluorescence excited by the band 228 nm (splitted $\pi\pi^*$) and 287 nm ($n\pi^*$), indicates that the emitting centers are the same in the both cases, namely, the emission takes place from the excited $n\pi^*$ level of the chromophores of AM molecules.

It is therefore possible to suppose that on $\pi\pi^*$ excitation and subsequent non-radiative losses of exciton energy due to jumping inside the "frozen" part of micelle and liposome, the exciton energy decreases to the $n\pi^*$ excitation energy. The exciton is then localized on one molecule. Subsequently fluorescence is excited by mechanism peculiar to the 287 nm band. The proposed mechanism is illustrated in the form of a diagram (Figure 8). The dotted arrow shows the process of non-radiative losses of the excitation energy caused by intermolecular migration within the micellar (liposomal) "freezed" part, waved arrows point to the non-radiative inner conversion.

Denoting the energy loss for one jump in "freezed" region of micelle (or liposome) by ε we obtain for the total energy loss on the transfer:

$$n \cdot \varepsilon = \Delta E_{228}^{\pi\pi^*} - \Delta E_{287}^{n\pi^*} \tag{1}$$

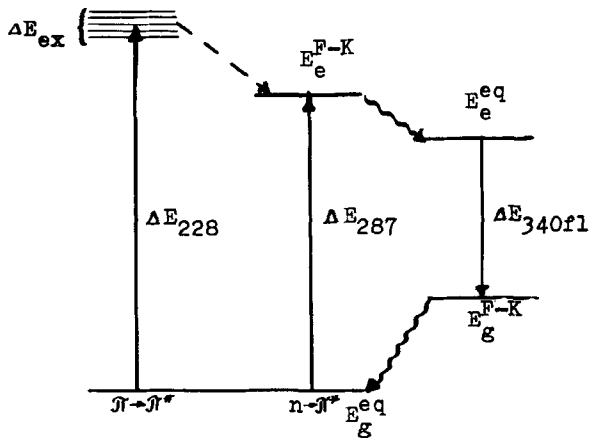

FIGURE 8 Diagram of the excitation of fluorescence in the "frozen" region of micelle or liposome, ΔE_{ex} = width of exciton zone; E_g^{eq} and E_e^{eq} = the equilibrium ground and excited energetic states; E_g^{F-K}, E_e^{F-K} = the ground and excited non-equilibrium Frank-Kondon energetic states; ΔE_{228} and ΔE_{287} = absorption energies at $\lambda_3 \sim 228$ nm and $\lambda_2 \sim 287$ nm; ΔE_{340} = fluorescence energy at 340 nm.

where

$$\begin{aligned} \Delta E_{228}^{\pi\pi^*} &= h\nu_{228} = 5{,}44 \text{ eV} \\ \Delta E_{287}^{n\pi^*} &= h\nu_{287} = 4{,}32 \text{ eV} \end{aligned} \tag{2}$$

n = number of jumps.

From (1) and (2) we can calculate the number of jumps (n) in one "frozen" region of micelle (liposome):

$$n = \frac{\Delta E_{228}^{\pi\pi^*} - \Delta E_{287}^{n\pi^*}}{\varepsilon} = 1{,}12 \text{ eV}/\varepsilon \tag{3}$$

Non-radiative losses of energy due to exciton jumps from one molecule to another may be caused by: a) the thermal vibration of molecules near the equilibrium positions with the energy ε_{therm}, b) changes in the intermolecular vibrational frequency ε_{vib}, and c) changes of the solvent film state of the excited molecule ε_{solv}.

In the considered case, ε_{solv} is taken to be the energy of a hydrogen bond in the aqueous surroundings of the chromophore of an AM molecule.

The above mentioned energies for S=O, C=O, P=O chromophores have the following values:

$$\left.\begin{aligned} \varepsilon_{therm} &= KT \approx 1{,}2\ 10^{-4} \text{ eV} \\ \varepsilon_{vib} &\approx 0{,}1 \text{ eV} \\ \varepsilon_{solv} &\approx 0{,}18 \text{ eV} \end{aligned}\right\} \varepsilon = \varepsilon_{therm} + \varepsilon_{vib} + \varepsilon_{solv} \tag{4}$$

The energy values show that $\varepsilon_{therm} \ll \varepsilon_{vib}, \varepsilon_{solv}$.

Substituting ε in (3) we obtain the number of jumps in a "frozen" region of micelle or liposome: $n \approx 5$.

But if we suppose that the energy losses are mainly due to intermolecular vibrations ($\varepsilon = \varepsilon_{vib}$) we obtain $n \approx 10$.

Considering that exciton jumps take place between dimers, we can suppose that the number of molecules in the "frozen" region varies between 10 and 20.

The splitting value may be determined as being the sum of the resonance interaction energy of an arbitrary molecule with a neighbouring molecule.[26] Comparision with the experimental value of the splitting energy allows an estimate of the "frozen" region structure.

According to Reference 26 the value of the splitting energy at the moment of the formation of dimers from two differently orientated molecules, may be approximately represented by the following formula:

$$\Delta E = 2 \sum_n \frac{1}{R_{1f}^5} [(\mathbf{d}_{1f}\mathbf{d}_{nf})R_{1n}^2 - 3(\mathbf{d}_{1f}\mathbf{R}_{1n})\mathbf{d}_{nf}\mathbf{R}_{1n})]$$

where $\mathbf{d}_{1f}$, $\mathbf{d}_{nf}$ are the transition dipole moments for an arbitrary molecule (1) of one orientation and n molecules of another orientation, and $\mathbf{R}_{1n}$ is the radius vector connecting the centres of gravity of dipoles (1) and (n).

Because of the identity of the interacting molecules in micelles and liposomes:

$$|\mathbf{d}_{1f}| = |\mathbf{d}_{nf}|$$

On $\pi\pi^*$ excitation of —C═O, —S═O, and —P═O chromophores the electron cloud of the π-bond moves completely from the C, S and P atoms to the oxygen atom.[35] The value of their transition dipole moments is therefore:

$$d = 0{,}5 \cdot l \cdot e \tag{5}$$

where l is the length of the —C═O, —S═O and —P═O bond and e is the electron charge. Since $e = 4{,}8 \cdot 10^{-10}$ CGSE and 1 for —C═O, —S═O and —P═O has a value in the order of 1,4–1,5 Å, from (5) we get $d \approx 3{,}5$ D.

The distances between neighbouring molecules in the crystal are: $R \approx 4$–5 Å. Using the equation for ΔE we can now obtain splitting energies for the following structures of "frozen" regions of micelles and liposomes:

a. If the dimers of chromophore groups of AM molecules in "frozen" state are settled chaotically on the micelle or liposome surface (Figure 9a) the splitting energy will be approximately equal to the dimer energy $\Delta E \approx 0{,}2$ eV.
b. If the dimers are arranged one-dimensionally (Figure 9b), the splitting energy is mainly a sum of the interaction energy of dipole moments of a type-1 molecule with two other molecules of type-2, and has a value of $\Delta E \approx 0{,}4$ eV. The effect of incorporating more distant dipoles in the interaction energies is very small.
c. If the dimers are settled in a two-dimensional square lattice (Figure 9c), the

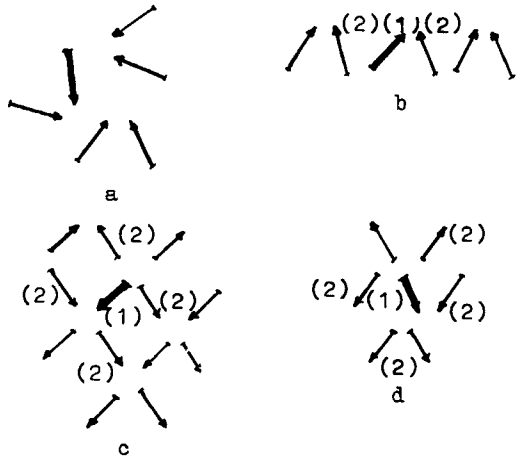

FIGURE 9 The hipothetic arrangement of transition dipole moments of AM molecules in the "frozen" region of micelle and liposome: (a)-Chaotic settlement of dimers; (b)-One-dimensional arrangement of dimers; (c)-Plane-square packing of molecules; (d)-Plane-hexagonal packing of molecules.

splitting energy is the sum of the interaction energy of the transition dipole moment 1 with the nearest neighbouring 4 dipole moments of different orientation and has a value in the order of $\Delta E \approx 0{,}8$ eV. This coincides with the value of the splitting energy calculated from the absorption spectra ($\Delta E \geqslant \Delta E_{200} - \Delta E_{228} = 0{,}8 - 1{,}0$ eV). Thus experimentally observed splitting may take place on formation of two-dimensional structure of square symmetry, in the "frozen" regions of micelles and liposomes, consisting of a minimum of three elementary celle with a total number of 12 molecules. This is the minimum number of molecules in a "frozen" region which was calculated from the energy balance.

d. The same splitting energy may be obtained when the dimers are arranged in a plane-hexagonal lattice (Figure 9d). In this case the minimum dimension of one "frozen" region is the elementary cell consisting of 7 molecules.

Thus the splitting obtained in the experiment may only take place when there are flickering "frozen" regions of well-ordered (specifically-plane square or plane-hexagonal) structure on the micelle or liposome surface.

It is interesting to note that, when preheated and highly concentrated AM solutions are cooled rapidly, "gel" and "coagel" phases appear, which show a rectangular or hexagonal packing of the molecules in the micelles of crystal structure.[36-38]

Thus the above-presented data allows us to contend that there exists the flickering "frozen" regions of ordered structure in micelles and liposomes which brought to their self fluorescence in absence of dye and high conjugated groups in AM molecules.

References

1. R. N. Nurmokhametov, V. G. Plotnikov and D. N. Shigorin, *J. Phys. Chem. (USSR)*, **40**, 1164 (1966).

2. D. N. Shigorin, *J. Phys. Chem. (USSR)*, **54**, 1905 (1980).
3. J. Barltrop and J. D. Coyle, *Excited States in Organic Chemistry*, John Wiley and Sons, London, New York, Sydney, Toronto (1975).
4. T. Wismontski-Knittl, R. Subramanian and L. K. Petterson, *J. Phys. Chem.*, **89**, 1367 (1985); Wu Shikang and Jiang Yong-cai, *Acta Chem. Sin.*, **40**, 939 (1982); M. Aikawa, A. Yeta and Jonq-Min Liu *et al.*, *Photochemistry and Photobiology*, **32**, 297 (1980).
5. M. D. Galanin, *J. Exper. and Theor. Phys. (USSR)*, **28**, 435 (1955).
6. T. Förster, *Fluoreszenz organisher Verbidungen*, Göttingen, Vandenhoeck Ruprecht (1951).
7. *Chemical Applications of Spectroscopy* (editor W. West), Interscience Publishers Ltd., London (1956).
8. Y. Chevalier and C. Chachaty, *J. Phys. Chem.*, **89**, 875 (1985).
9. A. S. Waggoner, O. H. Griffith and C. R. Christensen, *Proc. Nat. Acad. Sci. USA*, **57**, 1189 (1967); F. Reiss-Husson and V. Luzzati, *J. Colloid Interface Sci.*, **21**, 534 (1966).
10. H. Walderhaug, O. Soderman and P. Stibls, *J. Phys. Chem.*, **88**, 1655 (1984).
11. K. Shinoda, T. Nakagawa and B. Tamamushi *et al.*, *Colloidal Surfactants*, Academic Press, New York (1963).
12. P. Emerson and A. Holtzer, *J. Phys. Chem.*, **69**, 3718 (1965).
13. F. Reiss-Husson and V. Luzzati, *J. Phys. Chem.*, **68**, 3504 (1964).
14. T. Ahläs, O. Södermon and H. Walderhaus *et al.*, *Surfactants Solution, Vol. 1*, Proc. Int. Sym., Lund. June 27–July 2 (1982), New York, London (1984).
15. Ch. Tenford, *The Hydrophobic Effect*, John Wiley, New York (1973).
16. M. Shinitzky, A. C. Dionoux and C. Gitler *et al.*, *Biochemistry*, **10**, 2106 (1971).
17. P. Mukerjee, *J. Phys. Chem.*, **66**, 1733 (1962).
18. R. A. Parker and S. Wasik, *J. Phys. Chem.*, **62**, 967 (1958).
19. F. Booth, *J. Chem. Phys.*, **19**, 397 (1951).
20. H. H. Montcsh, V. B. Kartha and D. G. Cameron, *Surfactants Solution, Vol. 1*, Proc. Int. Sym., Lund, June 27–July 2 (1982), New York, London (1984).
21. D. W. R. Gruen, *Surfactant Solution, Vol. 1*, Proc. Int. Sym., Lund, June 27–July 2 (1982), New York, London (1984).
22. W. D. Turley and H. W. Offen, *J. Phys. Chem.*, **89**, 2933 (1985); S. Ikeda and G. D. Fasman, *J. Polym. Sci.*, **8**, 991 (1970).
23. K. Kalianasundram and J. K. Tomas, The Radiation Processes in the Nonionic Micelles, in: *Micellization, Solubilization and Microemulsion, Vol. 2* (Edited by K. L. Mittal). Plenum Press, New York and London (1977).
24. V. M. Agranovich and M. D. Galanin, *The Transfer of the Electron Excitation Energy in the Condensed Medium*. Nauka, Moscow (1978).
25. A. S. Davidov, *Theory of Light Absorption in the Molecular Crystalles*, Kiev (1951).
26. A. S. Davidov, *Theory of Molecular Excitons*, McGraw-Hill, New York (1962).
27. V. A. Zakharian, V. T. Karapetian, G. B. Badalian and A. A. Shahinian, *Biophysica (USSR)*, **31**, 37 (1986).
28. A. A. Shahinian, *Colloid Zurnal*, **40**, 297 (1978).
29. G. B. Badalian and A. A. Shahinian, *Biophysica (USSR)*, **33**, 92 (1988).
30. A. A. Shahinian, *Role of Ionic Micelles Structural Organization in the Mechanism of Macromolecular Formation*, Published by Academy of Sciences of the Armenian Republic, Yerevan (1985).
31. A. A. Shahinian and V. V. Bagdasarian, *Ber. Bunsenges. Phys. Chem.*, **91**, 670 (1987).
32. M. Vindsor, in: *Physics and Chemistry of the Organic Solid State, Vol. 2*, New York, London, Sydney (1965).
33. R. S. Drago, *Physical Methods in Chemistry*, W. B. Saunders Company, Philadelphia, London, Toronto (1979).
34. V. V. Bagdasarin, A. A. Shahinian and V. G. Barghudarian, *Colloid Zhurnal*, **45**, 295 (1983).
35. M. Orchin and H. H. Jaffe, *The Importance of Antibonding Orbitales*, Houghton Mifflin Company, Boston, New York (1967).
36. J. M. Vincent and A. E. Skoulios, *Acta Cryst.*, **20**, 432 (1966).
37. J. M. Vincent and A. E. Skoulios, *Acta Cryst.*, **20**, 441 (1966).
38. J. M. Vincent and A. E. Skoulios, *Acta Cryst.*, **20**, 447 (1966).