Behavior of 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine at the Surface of 3-Hydroxypropionitrile near the Critical Micelle Concentration

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The existence of a local minimum of surface tension near the critical micelle concentration has been reported for various surfactant solutions. In some cases, this is interpreted as an indication for the presence of impurities. Recently, this phenomenon has been interpreted as an inhibition of the aggregation for POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) in HPN (3-hydroxypropionitrile). This notion is supported by the present experiments on the phospholipid DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine) at the surface of HPN. Comparison of the results of both systems delivers improved understanding of the aggregation of surfactants in solution.

Introduction

The understanding of the self-organization of lipids at the surface of polar liquids (e.g., to form membranes) is of great interest in biochemical as well as in technical topics. We study monolayers on a liquid substrate which is suitable for investigation by several surface analytical techniques. Also, information about the relation between surface and bulk might be relevant to understand the relation between a membrane and the surrounding solvent.

In order to gain information about the surface structure of these systems, we have performed investigations using various particle spectroscopies, including, X-ray photoelectron spectroscopy (XPS),[1] ion scattering spectroscopy (ISS),[2] and metastable induced electron spectroscopy (MIES).[3,4] The method MIES utilizes a tunnel process and is therefore extremely sensitive for the investigation of the uppermost monolayer of a sample.

As the mentioned methods require high-vacuum conditions, water is not accessible in our present experimental setup due to its high vapor pressure. Therefore, we have dissolved the phospholipid 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) in hydroxypropionitrile (HPN), which has solution properties similar to water (Table 1).

The occurrence of a minimum of the surface tension of a solution of surfactants has been reported before. Lin et al. reported that this can be due to impurities.[5] Nevertheless, the same authors came to the conclusion that the presence of a local minimum in the surface tension cannot be taken as an unambiguous indicator for the presence of impurities.[5]

In addition, knowledge of the surface structure and surface excess allows drawing conclusions about the micellization of surfactants via evaluation of the chemical potential. For the system POPC in HPN, statistical treatment proposed the inhibition of aggregation at concentrations above the critical micelle concentration.[6] As the system DOPC in HPN delivers similar features in the surface tension data, we studied the

TABLE 1: Some Solvent Properties of Water and HPN[a]

property[14]	water	HPN
dipole moment μ (D)	1.85	~3.5
dielectric permittivity ε	80.1	~65
surface	72.1 (25 °C)	48 (20 °C)
tension σ (mN/m)	74.9 (5 °C)	50.6 (4 °C)

[a] The dielectric permittivity of HPN has been determined in the group of F. Kremer utilizing dielectric relaxation spectroscopy.[15]

surface structure in order to achieve additional information and to check whether the described model is also suitable for this system.

Experimental Section

The machine used for MIES has been described in detail elsewhere.[4] Therefore, only a brief description will be given here.

MIES utilizes metastable helium atoms in a 1s,2s triplet state which are produced by a gas discharge. As the gas pressure needed for this discharge is about 1 mbar, almost all metastable helium atoms will be quenched before they can reach the measuring chamber. Nevertheless, this first discharge enables to establish another discharge at lower pressures (approximately 1×10^{-4} mbar) right behind itself. Therefore, the projectiles used for measuring originate mainly from the second discharge.

The liquid surface is prepared by rotating a disk that is immersed into a liquid reservoir so that a film is established on the disk. A so-called "skimmer" scrapes the surface so as to ensure that fresh bulk material is exposed to the surface. Implied that the skimmer touches the liquid beside the rotation axis of the disk and the helium projectiles strike the surface vertical above the axis, the angle between the skimmer and the point of measurement is 90°. Therefore, the surface age can be defined as a quarter of the rotation time of the disk.

The analyzer used for recording the present data is a Phoibos 100 hemispherical energy analyzer supplied by SPECS GmbH Berlin.

As MIES receives information about the energy of orbitals with a significant density right above the surface, it is extremely surface sensitive. Furthermore, not only can the presence of a

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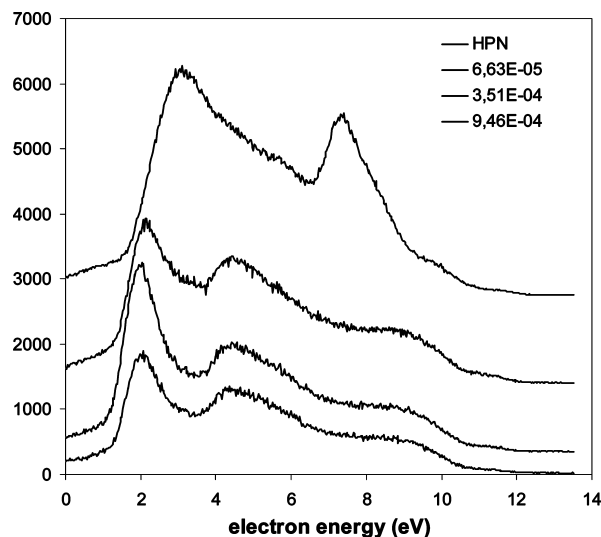
Figure 1. Spectra of DOPC in HPN for some concentrations of the lipid (concentrations given by the legend from top to bottom); the surface age is 1.53 s for all shown spectra.

Figure 2. Reference spectra (legend from top to bottom) for the evaluation of spectra of DOPC in HPN.

certain species at the surface be evaluated but also the spectral information will depend on the orientation of molecules at the surface due to different accessibility of the molecular orbitals. This has been shown very detailed in ref 3.

In order to evaluate the chemical potential of the system via the Gibbs equation, surface tension measurements were performed using the Wilhelmy plate method.

Results and Discussion

MIE spectra of DOPC in HPN have been recorded for concentrations of 3.36×10^{-5} to 9.46×10^{-4} mol/kg (Figure 1). The cmc in this solution has been determined as approximately 3.3×10^{-4} mol/kg via surface tension measurements using the Wilhelmy plate. The MIES data were evaluated utilizing singular value decomposition (SVD) yielding complex basis spectra.[3] The series of spectra is reconstructed in a next step as linear combinations of reference spectra produced from the base spectra by main axis conversion to a noncomplex matrix and a fit procedure.

Within a three-dimensional solution space for the series of spectra, we can represent all spectra S_i of the series by the expression

$$S_i = \alpha_i A + \beta_i B + \gamma_i C \qquad (1)$$

with S_i being measured spectra and A, B, and C the reference spectra. α_i, β_i, γ_i are coefficients, with $\alpha_i + \beta_i + \gamma_i = 1$.

It is found that the MIES series can be described as linear combination of three reference spectra, one of which is the solvent spectrum. This gives spectra of two distinguishable orientations of the surfactant (Figure 2). Those spectra can be connected to lying and standing gauche hydrocarbon chains of the hydrophilic fatty acid groups by comparison with the spectra in ref 3. The term "lying" refers to hydrocarbon chains oriented parallel to the liquid surface; "standing gauche" means chains pointing out of the liquid, possibly with a tilt vs the surface normal. The linear coefficients used for the reconstruction are plotted in Figure 3.

Assuming that the surfactant is only present in one layer at the surface, the surface excess can be estimated. For this purpose, the surface area of a gauche standing DOPC molecule
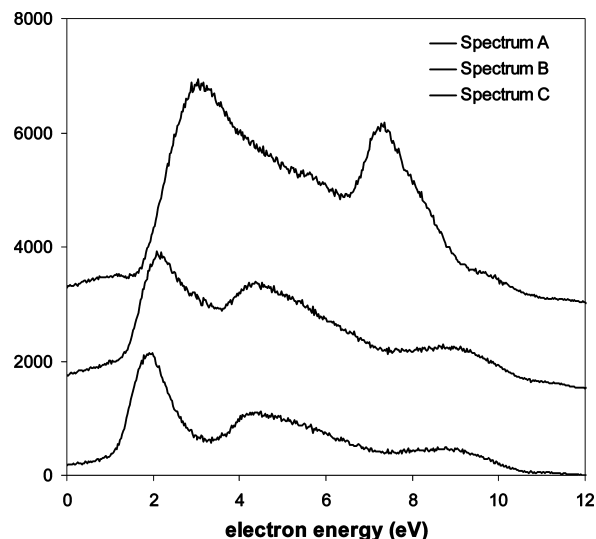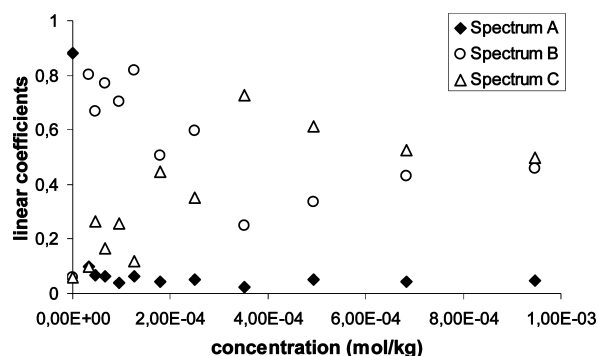
Figure 3. Linear coefficients of reference spectra related to the concentration of DOPC in HPN. Spectrum A is the HPN spectrum, and spectra B and C are interpreted as lying and standing hydrocarbon chains.
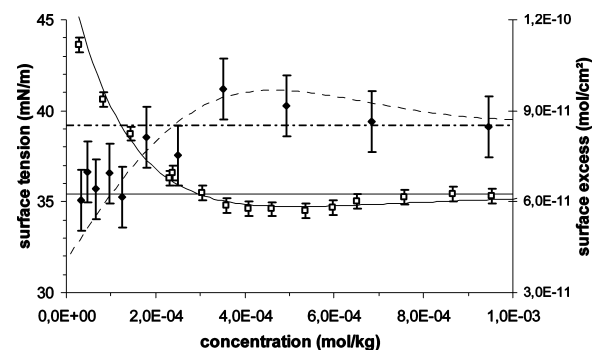
Figure 4. Surface tension (open squares) and surface excess (full diamonds) of solutions of DOPC in HPN related to the surfactant concentration.

is presumed as 70 Å[2] and that of a lying DOPC as 226 Å[2] (refs 7–9). The result is presented in Figure 4.

Obviously, the surface tension exhibits a minimum in a concentration range above the cmc. At these concentrations, also a maximum surface excess is found. This can be interpreted as a compression of the surface layer, resulting in the chains straightening up. This can presumably be ascribed to an inhibition of micelle building.[6]

Combination of surface tension and surface excess allows the evaluation of the chemical potential[10] μ via the Gibbs equation

$$d\sigma = -\Gamma^e \cdot d\mu \qquad (2)$$

where σ is the surface tension and Γ^e indicates the surface excess.

Equation 2 yields the relative chemical potential of the system against the bulk concentration (Figure 5).

Knowledge of the chemical potential curve allows conclusions about the aggregation of the surfactant. In ref 6 it is shown that the feature of a local maximum of the chemical potential can be evaluated as an inhibition of the aggregation due to statistical reasons. Application of the methods given in ref 6 results in a best fit with the following properties: $k_{thresh} = 113$; $V_{crit} = 3.04 \times 10^{-16}$ cm^3; $\mu_{mic} = 16\,880.54$ J/mol; $C_{crit} = 1.98 \times 10^{17}$ cm^{-3}, where k_{thresh} is the quantity of molecules involved in the aggregation, V_{crit} indicates the bulk volume in which one micelle is formed, μ_{mic} is the maximum of the chemical potential, and C_{crit} is the local concentration needed for successful aggregation. The number of molecules per micelle is given by the difference of the molecules involved in the aggregation and those needed for the chemical potential in the environment to be also equal to μ_{mic}, i.e.

$$k_{mic} = k_{thresh} - V_{crit}C_{crit} \qquad (3)$$

Hence, micelles contain statistically ~52 DOPC molecules, which is approximately 46% of the 113 molecules involved in the aggregation. For the system POPC in HPN,[11] it was found that of 90 molecules involved in the aggregation, only 24% (~21 molecules) will be in the micelle.[6] Therefore, the inhibition of aggregation for DOPC is stronger than for POPC because the fraction of the involved molecules which are found in the established micelle is greater. The necessary local concentration in the environment of the micelle is larger and therefore it takes higher concentrations to reach an unhampered aggregation.

Also, the micelles are bigger so that the concentration in the environment of the micelles is more strongly affected by the aggregation. Nevertheless, the aggregates in HPN are much smaller than those found in aqueous systems so that no vesicle formation can occur. This is in accord with the results found by Wen et al.[12]

To get a handle on the reliability of the calculation, the importance of the precision of the surface excess has been checked. The hydrocarbon chains of the gauche standing molecules might be tilted or not totally stretched out. This will increase the surface area covered by these molecules.

Assuming the above-mentioned case of the gauche standing molecules covering up to 226 Å^2, the shown parameters vary so that the maximum of the chemical potential is even greater. In this limiting case, V_{crit} and k_{thresh} are slightly increased. Nevertheless, 46% of the molecules involved in micelle constitution are finally found in the micelle. As a conclusion, it is found that the assumption of an area of 70 Å^2 covered by each gauche standing molecule is the most secured one for the purpose of this evaluation. If the area is greater due to the chains being tilted, the general results are not affected.

It has been proposed that the "real" cmc is reached when the surface tension has reached its asymptotic value (c_3 in Figure 5). This has been concluded from conductivity measurements.[5] This concentration has been referred to as "micelle dissociation concentration".[13] However, the statistical evaluation of our data results in a critical concentration needed for the aggregation of DOPC of 1.98×10^{17} molecules per cm^3, equal to 3.29×10^{-4} mol/L (solid vertical line in Figure 5). This is below the
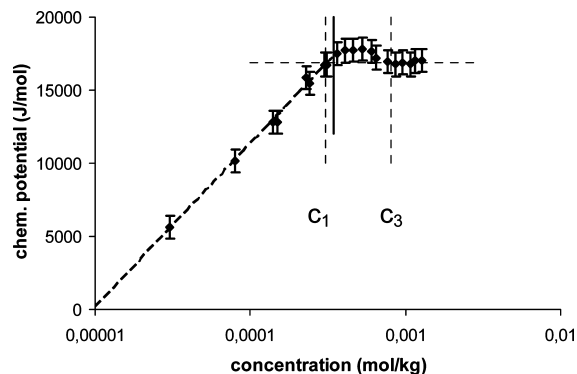
Figure 5. Chemical potential of DOPC in HPN. The cmc could in principle be considered equal to c_1 or c_3. c_3 is the cmc determined by conductivity measurements[5] and indicates the lowest concentration, where all added molecules instantly add to micelles.
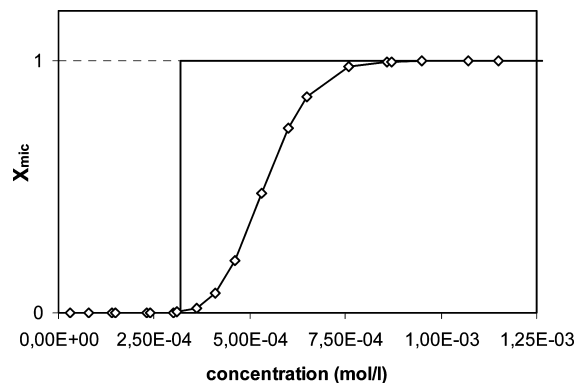
Figure 6. Fraction of DOPC molecules involved in micelle constitution. The solid line indicates ideal behavior.

concentration where the maximum of the chemical potential occurs. Obviously, the "real" cmc is reached when the asymptotic value is reached for the first time (c_1 in Figure 5).

Conclusion and Outlook

The surface of solutions of the phospholipid DOPC in HPN has been studied by means of surface tension measurements and MIES. From the MIES data, the surface excess has been evaluated, enabling to evaluate also the chemical potential. The shape of the curve of the chemical potential shows a local maximum, interpreted as an inhibition of the aggregation of the surfactant. Statistical treatment results in the conclusion that the inhibition is stronger than for the system POPC in HPN. This is due to the fact that of the molecules involved in the aggregation a higher fraction is found in the established micelle. In other words, the quotient of k_{mic} (i.e., the number of molecules finally found in the micelle) and k_{thresh} is larger.

The characteristic point indicating the "real" cmc is sometimes referred to as c_1 and in some cases found equal to c_3 as given in Figure 5. Nevertheless, this seems to be due to the utilized method, i.e., whether the cmc has been evaluated from surface tension measurements or from conductivity measurements.

The occurrence of micelles is usually understood as a process that starts if the concentration of a surfactant exceeds the cmc. If the aggregation is not inhibited, micelle formation follows the shape indicated by the solid line in Figure 6.

In the present case, it is reasonable to assume that a linear correlation of the number of micelles and the nominal surfactant concentration is present only well above the cmc. Slightly above the cmc, the actual number of micelles should be smaller than

given by this linear relation; i.e., the inhibition results in a change of the shape of the slope. Also, the position of the slope is shifted toward higher concentrations. Therefore, close to and slightly above the cmc, fewer molecules are involved in micelle constitution than should nominally be involved in micelle constitution.

It might therefore be concluded that the finding of a "real" cmc is also dependent on the used method, i.e., on the sensitivity for the occurrence of a very low concentration of aggregates. This leads to the phenomenon described by Lin et al.[5] that in some cases the cmc determined by conductivity measurements is higher than the cmc from surface tension measurements. Especially in systems where the aggregation is inhibited, slightly above the cmc only very few micelles are constituted so that the conductivity is not influenced significantly, yet. The coverage of the surface, however, is already completed, and the surface tension does not change any more, or drops below the equilibrium value.
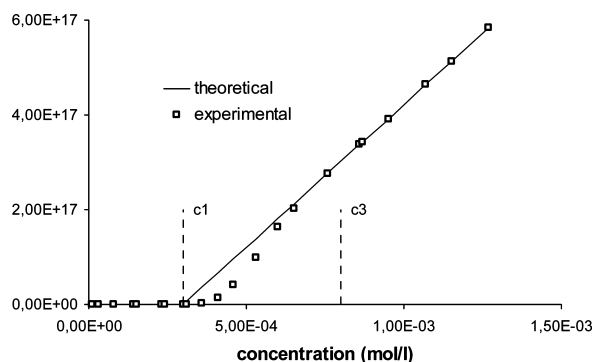
Figure 7. Number of DOPC molecules integrated in micelles. The cmc could in principle be considered equal to c_1 or c_3. c_3 is the cmc determined by conductivity measurements[5] and indicates the lowest concentration, where all added molecules instantly add to micelles.

It can also be seen that, at concentrations well above the cmc, all surfactant molecules are involved in aggregation. Figure 7 shows the number of molecules included in micelles vs the lipid concentration. For the transformation from Figure 6 to Figure 7, it is stated that the ratio of the molecules involved in micelle constitution and the available molecules is a measure for the inhibition of micelle constitution. Therefore, it is also used as the ratio between experimental backed data and the theoretical data in Figure 7.

In the future, the occurrence of micelles shall be studied directly, using elastic light scattering. It will be interesting to find out whether the predictions about micelle size and number of micelles can be verified experimentally.

References and Notes

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