

INTERACTIONS OF CATIONIC SURFACTANTS WITH DPPC

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The effects of different cationic surfactants (*n*-undecylammonium chloride, UDACl and dodecyldimethyl (dodecyloxymethyl) ammonium chloride, DDMDDACl) on fully hydrated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) vesicles have been studied. In the studied systems the molar ratio (*x*) of DPPC/surfactant ranged between $x=0.0164$ – 0.82 and from $x=0.0352$ – 1.76 for DPPC/DDMDDACl and DPPC/UDACl, respectively. For both systems, the enthalpy associated with the phase transition significantly decreases even at the lowest surfactant concentration. Also the main phase transition temperature is shifted towards lower temperatures.

The structural parameters of the phases have been characterised by small angle X-ray scattering (SAXS). The SAXS results have proved that UDACl at $x=0.0352$ molar ratio significantly influences the DPPC lamellar structure, while its total disappearance was observed for $x=0.176$. The presence of DDMDDACl causes a total disappearance of the DPPC lamellar structure already at the lowest molar ratio ($x=0.0352$). Each surfactant in the system with DPPC leads to a mixed micellar phase formation.

Keywords: cationic surfactants, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, DPPC, DSC, phase transition, phospholipids, SAXS

Introduction

Phospholipids are the main components of cell membranes and also participate in many biological processes taking place in the cells. In the hydrated phospholipids different phases can be formed depending on their concentration, pH and the length of the hydrophobic chains: lamellar with ordered chains, lamellar with disordered chains, rippled with partially ordered chains, micelles, hexagonal and cubic structures [1].

The fully hydrated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) vesicles feature three-phase transitions. The sub-transition from the lamellar crystalline orthorhombic (L_c) to lamellar orthorhombic ($L_{\beta'}$) is observed at 17°C [2]. The pre-transition, which is characteristic of that from the lamellar gel ($L_{\beta'}$) to the rippled gel phase (P_{β}), is observed at about 35°C. The main transition at 41°C, often called the ‘chain-melting’, is the transformation from the rippled gel (P_{β}) to the liquid-crystalline phase (L_{α}) [2–4].

The interactions between phospholipids and surfactants have been recently intensely studied [5, 6]. Surfactants make an attractive tool in investigation of phospholipid membranes structure and interactions [7] but membrane-perturbing surfactants are commonly used to lyse cells for DNA extraction [8], as well as to solubilize the membrane proteins [9], membrane proteins crystallization [10, 11] or in in-

vestigation of drug delivery systems [12] and in many other medical applications. The main mechanism observed in the interactions of this type is usually related to the mixed micellization among the surfactant and phospholipid monomers [7].

The aim of the study presented was to analyse the disturbing or stabilising effect of cationic surfactants on the structure of phases of hydrated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) by the differential scanning calorimetry (DSC) and the small angle X-ray scattering (SAXS) techniques. The study was performed for two surfactants: dodecyldimethyl(dodecyloxymethyl) ammonium chloride and *n*-undecylammonium chloride.

The choice of ternary ammonium salts is not accidental as recently this group of compounds has been intensely studied as prospective antibacterial and antifungal media [13–16].

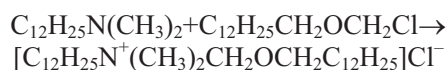
Experimental

Materials

Synthesis of the surfactants

Dodecyldimethyl(dodecyloxymethyl)ammonium chloride ($[(C_{12}H_{25}N^+(CH_3)_2CH_2OCH_2C_{12}H_{25})Cl^-]$; DDMDDACl) was obtained in the reaction of quaternization of *N,N*-dimethyldodecyl amine by chloromethyldodecyl ether:

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The reaction was performed at 90°C in heptane (5 h, on intense stirring). The product was obtained with the yield of 93.5%.

The *n*-undecylammonium chloride ($\text{C}_{11}\text{H}_{23}\text{NH}_3\text{Cl}$; UDACL) was prepared in our laboratory from undecylamine as described previously [17, 18]. *n*-Undecylamine (purity 99.9%) was purchased from Sigma-Aldrich.

Preparation of DPPC and DPPC-surfactant aqueous suspensions

The 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids Inc. (Alabaster, USA) as a solution in chloroform. Aqueous suspensions of pure DPPC and mixtures with surfactants (DDMDDACL and UDACL) were obtained by a modification of the method used by Csiszár *et al.* [19]. An appropriate volume of a chloroform solution of DPPC was placed in a glass vial and dried under vacuum. Then, a properly chosen volume of a 25 mM sodium phosphate buffer of pH 6.5 (reference sample) or a buffer with the surfactant was added into the vial. The suspensions were heated to 50°C and sonicated for 10 min. After sonication the sample was quenched to 4°C, then reheated to 50°C again and sonicated for 10 min. The process was repeated 10 times to achieve a homogeneous system. The samples before DSC and SAXS measurements were stored at 4°C. The reference solution of DPPC contained 0.136 mM (mass/mass) of phospholipid. The DPPC/surfactant molar ratios (*x*) studied were:

DPPC/DDMDDACL: *x*=0.0164, 0.082, 0.164 and 0.82, DPPC/UDACL: *x*=0.0352, 0.176, 0.352 and 1.76.

Methods

Differential scanning calorimetry (DSC)

The measurements were performed using a DSC-204 Phoenix Netzsch system. The samples of about 12-13 mg were sealed in hermetical aluminium crucibles. The measurements were performed in helium atmosphere in temperatures from 5 to 55°C scanned at the rate of 2°C min⁻¹. The results were processed and analysed using the TA (Netzsch) program. For determination of the enthalpy values of the representative phase transitions, linear or tangent-sigmoidal base lines were used.

Small angle X-ray scattering studies (SAXS)

The small angle X-ray scattering study of DPPC-surfactant mixtures were performed using the NanoSTAR system (Bruker AXS). The SAXS system had a pinhole collimation and a two-dimensional detector HiSTAR, mounted on an X-ray tube with a copper anode, and equipped with crossed Göbel mirrors. The sample-to-detector distance was 650 mm. The *s*-axis was calibrated by the observation of peaks from silver behenate diffraction pattern [20]. The exposure time for a single frame was 6000 s and the intensities were recorded within the range of 0.15 nm⁻¹ < *s* < 3.5 nm⁻¹, (where *s*=4πsinθ/λ and 2θ was the scattering angle and λ was the X-ray wavelength). The series of expositions starting from 5 to 70°C, in 5°C temperature step were recorded. The temperature of the samples in all experiments was controlled using a TCU-50 unit and monitored to an accuracy of 0.1°C. The peaks on the SAXS curves were fitted by the program TOPPAS (Bruker-AXS).

Results and discussion

The surfactants used in the study belong to the group of cationic surfactants but differ in the chemical structure.

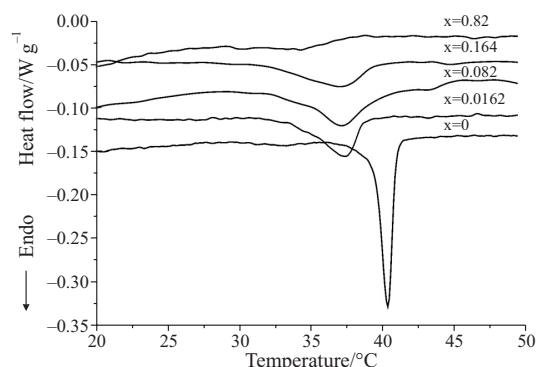


Fig. 1 DSC curves of DPPC and DDMDDACL mixtures

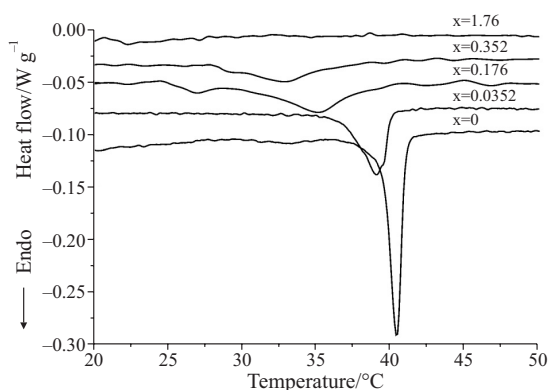


Fig. 2 DSC curves of DPPC and UDACL mixtures

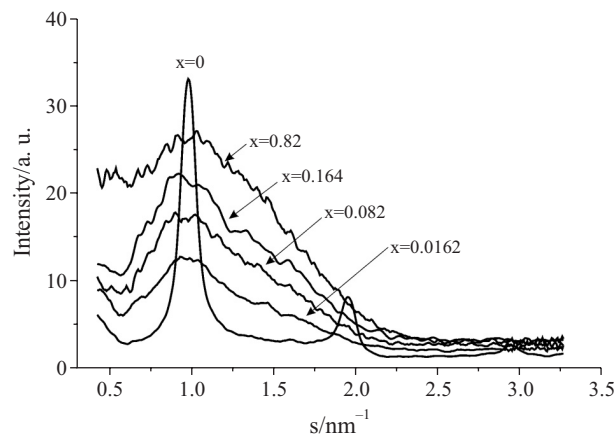
Table 1 Characteristic temperatures and phase transition enthalpies for the DPPC-surfactant systems

Compound	$\Delta H/\text{J g}^{-1}$	$T_{\text{onset}}/^\circ\text{C}$	$T_{\text{peak}}/^\circ\text{C}$
DPPC ($x=0$)	-6.48	39.8	40.7
DPPC/DDMDDACl ($x=0.0162$)	-5.81	34.8	37.5
DPPC/DDMDDACl ($x=0.081$)	-4.35	33.7	37.1
DPPC/DDMDDACl ($x=0.162$)	-3.21	29.3	36.7
DPPC/DDMDDACl ($x=0.81$)	-1.39	29.1	34.3
DPPC/UDACl ($x=0.0352$)	-3.82	36.9	39.0
DPPC/UDACl ($x=0.176$)	-3.05	28.3	34.8
DPPC/UDACl ($x=0.352$)	-2.63	27.6	32.6
DPPC/UDACl ($x=1.76$)	nd	nd	nd

UDACl is built of a straight linear hydrophobic hydrocarbon chain, while DDMDDACl makes two hydrophobic hydrocarbon chains. The DSC results obtained for the systems DPPC-UDACl and DPPC-DDMDDACl are shown in Figs 1 and 2, while the parameters characterising the phase transition obtained from these data are given in Table 1. For the reference system being a 10% DPPC solution, only one sharp main phase transition is observed corresponding to melting of the hydrocarbon chains and characterised by $T_{\text{onset}}=39.8^\circ\text{C}$ and $T_{\text{endset}}=41.3^\circ\text{C}$. These values are in good agreement with the corresponding ones obtained for pure hydrated 20% DPPC [21]. The pre-transition was poorly visible on the DSC curve in the range 33–35°C, which most probably was a consequence of a low sensitivity of the instruments used. A very small addition of a surfactant produces a clearly detectable change of main phase transition temperature and enthalpy. For the system containing DPPC/DDMDDACl at the molar ratio $x=0.0162$, the temperatures describing the main phase transition decrease ($\Delta T_{\text{onset}}=5^\circ\text{C}$, $\Delta T_{\text{peak}}=3.2^\circ\text{C}$) at a simultaneous decrease in enthalpy by 10.3% with respect to that of the reference system (10% DPPC). The differences noted for the DPPC/DDMDDACl systems with $x=0.081$ and $x=0.162$ are more pronounced. The T_{peak} temperature characterising the main phase transition are also shifted to lower temperatures ($\Delta T_{\text{peak}}=3.6$ and 4°C for $x=0.081$ and $x=0.162$, respectively). The enthalpy of the main phase transition is decreased by 32.9 and 50.5% for DPPC/DDMDDACl systems with $x=0.081$ and $x=0.162$ respectively. Moreover, for $x=0.162$ the range of the phase transition is significantly extended ($\Delta T_{\text{onset}}=10.5^\circ\text{C}$), while for $x=0.81$ a very weak and much broadened peak is observed ($\Delta T_{\text{onset}}=10.7^\circ\text{C}$, $\Delta H=78.5\%$). The changes observed in the DSC thermograms of a system of DPPC/UDACl are of similar character. For the DPPC/UDACl system with molar ratio of $x=0.0352$ the temperatures characterising the main phase transition have decreased by 10% ($\Delta T_{\text{onset}}=2.9^\circ\text{C}$, $\Delta T_{\text{peak}}=1.7^\circ\text{C}$) and the enthalpy

has decreased by $\Delta H=41\%$ relative to the corresponding values for the reference system. For the systems with $x=0.176$ and $x=0.352$, the main phase transition temperatures have further decreased with respect to those of the reference system ($\Delta T_{\text{onset}}=11.5$ and 12.2°C , $\Delta T_{\text{peak}}=5.9$ and 8.1°C for the DPPC/UDACl systems with molar ratio $x=0.176$ and $x=0.352$, respectively). This range of the temperature decrease differentiated this series of samples from the earlier studied one (DPPC/DDMDDACl), for which the shift of the phase transition temperature range towards lower temperatures was less pronounced. Also for both systems the phase transition enthalpy decreased ($\Delta H=52.9$ and 59.4% for the systems with $x=0.176$ and $x=0.352$, respectively). For the system with $x=1.76$ no phase transition was detected on the DSC curves.

Figures 3 and 4 present exemplary series of SAXS data recorded for the two systems studied of different concentrations, and for the reference system. Some correlation with the DSC data is visible but only for the samples with a small admixture of surfactants. For the reference system (DPPC) the data indicate a distinct phase transition at 40–45°C. At 40°C and below, the data imply the presence of a lamellar gel phase characterised by $d_{001}=6.41$ – 6.43 nm. The values

**Fig. 3** SAXS curves of DPPC and DDMDDACl mixtures at 20°C

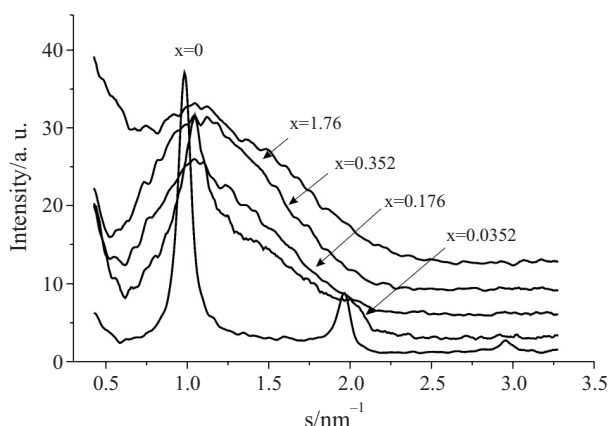


Fig. 4 SAXS curves of DPPC and UDACL mixtures at 20°C

are in agreement with the results reported by Rappolt *et al.* [22] at 45°C and above, the hydrophobic chains get 'molten' and a phase characterised by $d_{001}=6.88$ to 7.41 nm appears. Even a small ($x=0.0162$) addition of DDMDDACL the lamellar phase disappears. The diffraction pattern shows only a broad peak characterised by $d_{001}=6.51$ –6.55 nm in all the temperature range studied. The phase transition is poorly manifested at about 40°C. A similar situation is noted for the other DPPC/DMPC systems with $x=0.081$, 0.162 and 0.81, for which d_{001} took the values from 6.55 to 6.67 nm. For these systems it was difficult to determine the range of the phase transition on the basis of the SAXS data. The DSC curves recorded for these systems also indicated a considerable broadening of the phase transition range. The results for the systems DPPC/UDACL are similar. For the system DPPC/UDACL at the molar ratio $x=0.0352$ a small contribution of the lamellar phase characteristic of pure DPPC ($d_{001}=6.45$ nm) and a phase transition above 40°C are detectable, whereas for the systems at $x=0.176$ and more the lamellar phase is undetectable. In the SAXS curve for this system the peak position significantly differs from the positions obtained for the pure phases of UDACL [23]. For the system containing DPPC/UDACL with $x=1.76$ at temperatures above 40°C the mixed micellar phase also partly disappears, which is manifested by a reduction of the peak intensity in the diffraction pattern. Most probably in these conditions a transition to the isotropic solution can occur, which could be observed as an increase in the clarity of the system and a decrease in its viscosity. A greater disturbing effect of DDMDDACL can be interpreted as a result of a greater structural similarity of this compound to DPPC. As DDMDDACL has two hydrocarbon chains its interactions with DPPC are more effective and probably the mixed micellar systems formed by it are more stable than those formed with UDACL. The SAXS and DSC results obtained for the system DPPC/DDMDDACL are in good agreement

with our earlier FTIR study of the effect of similar surfactants: dodecyldimethyl (hexyloxymethyl) ammonium chloride, HMDDACL (decyloxymethyl) dodecyldimethyl ammonium chloride, DMDDACL on fully hydrated DPPC [24]. For these systems the interactions of surfactants with the polar group of the phospholipids via hydrogen bonds formation have been observed. Also a lowering of the temperature corresponding to the main phase transition of the systems surfactant/DPPC has been detected as a result of disordering of the phospholipids structure by the surfactants and phospholipids interaction.

Conclusions

The surfactants studied (DDMDDACL and UDACL) added to DPPC cause a disturbance to or disappearance (depending on their concentration) of the lamellar phase characteristic of DPPC vesicles. Their disturbing effect is manifested by a decrease in the phase transition temperature, broadening of the temperature range of the phase transition and a decrease in the phase transition enthalpy. Their presence most probably stimulates the appearance of a mixed micellar phase. The disturbing effect of DDMDDACL is greater as its presence in the lowest concentration ($x=0.0162$) in the system resulted in a total disappearance of the lamellar phase.

Acknowledgements

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