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DSC INVESTIGATIONS OF DDAB, DTAB AND DHAB VESICLE AQUEOUS SOLUTIONS IN PRESENCE OF SDS

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Abstract

Interactions of anionic surfactant sodium dodecyl sulphate (SDS) with vesicles formed by synthetic dialkyldimethylammonium bromides i.e. didodecyldimethylammonium bromide (DDAB), ditetradecyldimethylammonium bromide (DTAB), dihexadecyldimethylammonium bromide (DHAB) have been examined by using differential scanning microcalorimetry and electron transmission microscopy. The temperatures and standard enthalpies characterising gel to liquid-crystal transitions increase significantly with increase of SDS concentration for all investigated systems. It means that incorporation of SDS monomers in these vesicular bilayers significantly stabilises their gel states. Moreover, in case of DDAB and DTAB vesicle systems added SDS limits kinetic features of recorded isobaric heat capacity dependencies on temperature observed earlier for the pure vesicular solutions.

Keywords: didodecyldimethylammonium bromide vesicles, dihexadecyldimethylammonium bromide vesicles, ditetradecyldimethylammonium bromide vesicles, sodium dodecyl sulphate, surfactants

Introduction

In aqueous solutions the amphiphiles (lipids) i.e. dialkyldimethylammonium and dialkylphosphate salts form vesicles [1–8]. The magnitudes of vesicles depend on the method of their preparation and the concentration of lipid monomers. For DSC measurements the 'hot water' method for preparation of vesicle aqueous solution is recommended to obtain results fully reproducible [6, 8, 9]. It is important because the DSC data provide information about fluidity of the vesicular bilayers i.e. a gel (the ordered dialkyl chains state) to liquid-crystal (disordered dialkyl chains state) phase transition taking place within domains of bilayers. The gel to liquid-crystal transition can be described by the temperature of melting, T_m, the enthalpy of transition per monomer mol, $\Delta_m H^o$, and patch number, n [4–11].

Dialkyldimethylammonium bromides i.e. didodecyldimethylammonium (DDAB), ditetradecyldimethylammonium (DTAB), dihexadecyldimethylammonium (DHAB) and dioctadecyldimethylammonium (DOAB) bromides (0.002 (monomer mol) dm^{-3}) form spherical vesicles in aqueous solutions [4–8]. When the DSC results were compared for

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dialkyldimethylammonium bromide vesicle aqueous solution (0.002 (monomer mol) dm^{-3}) the patterns obtained for DDAB and DTAB appeared to be completely different than for DHAB and DOAB [4]. DSC scans recorded for freshly prepared DDAB and DTAB vesicle solutions showed no evidence of an extremum in isobaric heat capacity as a function of temperature associated with the gel to liquid-crystal state transition within vesicular bilayer at characteristic T_m. The extrema were observed at 15.8 and 29.3°C for DDAB and DTAB for the same vesicular solutions, respectively, when the solutions were held in the calorimeter cell at 5°C for periods up to 11 h [4]. The intensities of δC_p maxima were larger when the solutions were held longer in the calorimeter cell at low temperature before re-scanning. In the case of DHAB and DOAB vesicle solutions the recorded scans showed very intensive δC_p maxima at 28.1 and 45°C, respectively, for freshly prepared solutions as well as after keeping the solutions in the calorimeter cell before re-scanning for a few h. All scans had the same intensity and were reproducible independently of time of storing the solutions at low temperature. Time-dependence for the shape of recorded scans for DDAB and DTAB vesicle solutions can be explained in terms of kinetic control of dialkyl chains repacking requirements within bilayers of vesicles during cooling the systems [4]. Shorter alkyl chains in DDAB and DTAB monomers and resulting weaker van der Waals attraction forces between them within domains of bilayers are probably the reason why the rapid cooling (to 5°C) of vesicles being in the liquid crystal state (the disordered dialkyl chains packing) leads to supercooling of the bilayers - metastable states. The transition from the liquid crystal state to the gel state requires the ordering dialkyl chains within domains of bilayers. This process is not instantaneous at such low temperature and needs more time in the case of shorter alkyl chains.

The source of the vesicular bilayer stability is equilibrium between van der Waals attraction forces between alkyl chains within the bilayer and the repulsive forces between head-groups of lipids and electrostatic interactions between lipids and water molecules on the surface of the bilayer. For a given series of lipids with the same head-groups i.e. dialkyldimethylammonium bromides like for dialkylphosphate salts and phospholipids in nature [3, 4, 7, 12] the temperature of melting increases with the increase of alkyl chain length. The exceptions are DTAB and DHAB for which the observed temperatures of transitions are very close and in reverse order. Longer alkyl chains in DHAB than in DDAB and DTAB monomers cause that the van der Waals attractive forces between them in vesicular bilayers are stronger so their reorientation from disordered to ordered states is much easier at low temperature which was reflected on time-independent shapes of the recorded scans (4].

It is known that added solutes e.g. organic cosolvents, nonelectrolytes and surfactants are incorporated into the vesicle bilayers [5, 8, 15] and change the parameters of the gel to liquid-crystal state transitions. When ionic surfactants are added, the change in melting temperature, T_m, depends on the kind of head-groups, their charges (either similar or opposite signs) as well as the lengths of alkyl chains in both ionic surfactant and lipid monomers [9, 10, 14,15]. Changes of parameters describing the phase transition are much more significant when the charges on the head-groups of lipid and surfactant macroions are opposite. For example, when sodium dodecyl sulphate (SDS) at concentration 0.0015 mol dm^{-3} was added into DOAB vesicle aqueous

solution the melting temperature increased rapidly from 45°C observed for pure DOAB vesicles to about 65°C [9, 10] so, the added SDS stabilised the gel state. The aim of these studies is to probe the influence of SDS concentration on the gel to liquid-crystal transitions for DDAB, DTAB and DHAB vesicles in aqueous solutions (0.002 (monomer mol) dm^{-3}). Dialkyldimethylammonium bromides molecules contain positively charged head-groups $>N^{+}R_2$ and two symmetric alkyl chains with different length from 12 to 16 carbon atoms. The head-group of SDS is negatively charged and alkyl chain contain 12 carbon atoms.

Experimental

DDAB (Fluka) and DHAB (Fluka) with purity ≥97% and SDS (Sigma) with purity ≥99% were crystallised from acetone and dried under vacuum at 50°C for at least two days before use.

The DDAB, DTAB and DHAB vesicle aqueous solutions (0.002 (monomer mol) dm^{-3}) were prepared by 'hot water' method [6, 8, 9] for all measurements. Weighted amounts of lipid and water were heated with stirring and held at 50°C for 15 min. Then the solution was allowed to cool and stand at room temperature for one h. The required amount of SDS was added at room temperature and shaken until all the added surfactant dissolved. Then the solution was placed in the sample cell of the DSC microcalorimeter MicroCal MC2 (USA) held at 2°C for DDAB or 5°C for DTAB and DHAB vesicular solutions. The dependencies of δC_p on temperature were recorded from 2–60°C for DDAB and 5–80°C for DTAB and DHAB vesicular solutions. The scan rate was 60 degrees per hour from low to high temperatures. The volumes of sample and reference cells were approximately 1.2 cm^3. The reference, in all cases, was water. After the first scan the solution was cooled to 5°C and re-scanned to 80°C. The procedure was repeated two or three more times. Then, after cooling, the solution was kept for 3 h at 5°C before re-scanning. The last scan was recorded after cooling the DTAB vesicle solutions for 11 h and for DHAB vesicle solutions for 5 h at 5°C before re-scanning. In case of DDAB vesicular solutions containing SDS after the first scan the procedure was the same but the solutions were cooled to 2°C and re-scanned to 60°C and the last scan was recorded after cooling the solutions and held at 2°C for 3 h in the calorimeter cell before re-scanning. The scanning was started from 2°C because of very low melting temperature for DDAB. All data were storied on floppy disc and analysed using the ORIGIN software (MicroCal Ltd.) as previously described [6, 9].

The measurements were performed for DDAB, DTAB and DHAB vesicle solutions (0.002 (monomer mol) dm^{-3}) containing SDS at concentrations 0.0005, 0.001, 0.0015 for all systems and at 0.00173 and 0.0018 (monomer mol) dm^{-3} for DDAB and DHAB, respectively. In the case of DDAB and DTAB vesicle solutions adding SDS at concentration 0.0018 mol dm^{-3} caused precipitation of firstly gelatinous and then amorphous precipitate. Thus, the measurements for these solutions were impossible.

Electron macrographs for vesicular solutions were supplied by Electron Microscope Laboratory of School of Biological Sciences at the University of Leicester (England). Samples were prepared by negative staining with uranyl acetate solution before the water was evaporated from the vesicle solutions placed on the freshly prepared Pioloform/carbon coated grids. The grids were examined using JOEL JEM 100CX (Japan) transmission electron microscope. The investigations were performed for freshly prepared DDAB vesicle solution and freshly prepared DDAB vesicle solution containing SDS at concentration 0.00173 mol dm^{-3}. In the latter case the appropriate amount of SDS was added into DDAB vesicle solution and shaken until all added SDS dissolved. Then the solution was left for 1 h at room temperature. The solution prepared in such a way was pearly. Both investigated solutions were placed on the grids during 1 h after preparation.

Results and discussion

The patterns observed using the DSC for different concentrations of SDS for DDAB and DTAB are similar but differ from these observed for DHAB and DOAB vesicular systems as in the case of pure vesicle solutions [4]. For all investigated systems the first recorded scans are completely different from the re-scans, which means that incorporation of SDS molecules inside vesicular bilayers is not immediate at low temperature. SDS monomers can enter outer surfaces of the bilayers only because the flip-flop diffusion inside bilayer is generally a very slow process, especially at low temperatures [12].

All recorded extrema of δC_p dependencies on temperature are very broad in comparison with the ones obtained for pure vesicular solutions and much more complicated. Moreover, the complicated time-dependent patterns are recorded for DDAB and DTAB vesicle solutions with added SDS. The most complicated patterns are observed for DDAB.

Electron micrographs for pure DDAB aqueous solution confirmed that the vesicles were formed in the conditions described above (Fig. 1). The vesicles are spherical but their sizes are very diverse. Most of them have diameters lower than 500 nm but also a lot of giant multiwalled vesicles with diameters in the range 900–1500 nm are noticed. The DSC scans recorded for this solution exhibit no extremum of δC_p de-

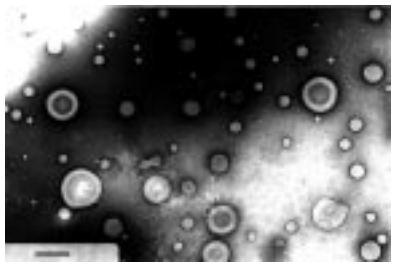

Fig. 1 Electron micrographs of freshly prepared DDAB vesicle aqueous solution (0.002 (monomer mol) dm^{-3}). The bar represents 1000 nm

pendence on temperature. The extremum with very low intensity at 15.8°C is observed for the same solution being held for 11 h at low temperature before re-scanning. When small amount of SDS (0.0005 mol dm^{-3}) is added to DDAB vesicle solution the observed very broad δC_p maximum with very low intensity is too small to analyse. For SDS at concentration 0.001 mol dm^{-3} maxima are spread over a wide range of temperatures and appear to extend to lower temperatures. The recorded plots are difficult to analyse but the phase transitions are noticed at different melting temperatures even higher than 25°C and what is more different for all the recorded scans. Even the scans recorded immediately after heat-cool-..... cycles are not reproducible. For higher concentration of SDS i.e. 0.0015 mol dm^{-3} the δC_p maxima are much broadened at lower temperatures and their intensities change slightly with time. The mostly reproducible δC_p plots are observed for the solutions containing 0.00173 mol SDS dm^{-3}.

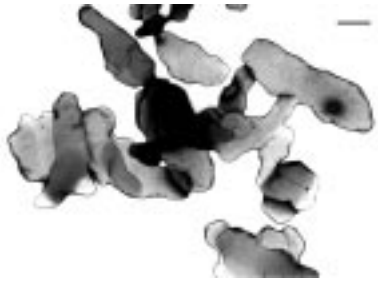

Fig. 2 Electron micrographs of freshly prepared DDAB vesicle aqueous solution (0.002 (monomer mol) dm^{-3}) in presence of SDS at concentration 0.00173 mol dm^{-3}. The bar represents 1000 nm

It is noticed that all vesicle solutions formed by dialkyldimethylammonium bromides with SDS concentration 0.001 mol dm^{-3} and higher removed from the sample calorimeter cell were pearly. Electron micrographs obtained for DDAB vesicle solution at SDS concentration 0.00173 mol dm^{-3} show planar lamellar giant aggregates with irregular shapes (Fig. 2). The magnitudes and shapes of the giant aggregates account for the pearly appearance of the solutions. The results of EM examinations confirm that SDS monomers are incorporated into vesicle bilayers and change their structure. The appearance of pearly solutions change dramatically when heated to 50°C. The solutions became turbid like pure vesicular solutions but their turbidity was markedly higher. The solutions became pearly again when left at room temperature. It seems that in case of all the systems investigated here the aggregates have completely different structure in the gel and liquid-crystal states that can be concluded on the basis of the appearance of solutions at room temperature and heated. After heating the solution to high temperature during the first scan and then cooling it to low temperature the bilayers of vesicle crack and the penetration of the inner part of the vesicle bilayers become possible. At high temperatures the bilayers probably cluster together to form closed much larger vesicular aggregates and probably with

different shapes than for the pure ones. Then the process is repeated in the heat-cool-heat-cool... cycles.

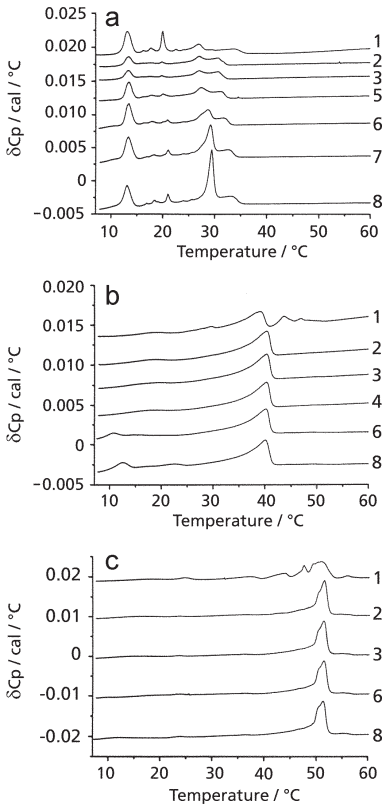

Fig. 3 Dependences on temperature of the differential heat capacities for aqueous solutions of DTAB vesicles (0.002 (monomer mol) dm^{-3}) with added SDS at concentrations: a – 0.0005, b – 0.001, c – 0.0015 mol dm^{-3}. Scans recorded for freshly prepared solution (1), after cooling the solution to 5°C and re-scanned immediately (2)–(4), after being held at 5°C for (5) 1, (6) 3, (7) 5, (8) 11 h before re-scanning. The scans have been displaced on the heat capacity axis for clarity

EM for DTAB aqueous solution prepared by 'hot water' method confirmed the lipid formed spherical vesicles with diameters about 700 nm but δC_p dependence on temperature for the freshly prepared vesicle solution exhibited no maxima and for re-scans the time dependent intensities of recorded plots were noticed [4]. The DSC results obtained for DTAB vesicle solution with added SDS at different concentrations are shown in Fig. 3. It is characteristic that adding even small amounts of SDS i.e. 0.0005 mol dm^{-3} to DTAB vesicle solution causes that maxima of the dependencies of δC_p on temperature are recorded for freshly prepared solution. The scans are

very complicated, a lot of δC_p maxima are observed in a very wide temperature range what is presumably connected with local concentration of SDS in DTAB vesicle bilayers. Moreover, time-dependence of recorded plots intensity is noticed. When the concentration of SDS increases, scans recorded for freshly prepared solutions are complicated but after heating the solution to 80°C, cooling to 5°C and immediately re-scanning only one maximum is observed but the plot is distinctly asymmetric. Generally maxima are much broadened at lower temperatures and the melting temperatures significantly shift towards higher temperature with the increase of SDS concentrations. When the concentration of SDS is 0.001 mol dm^{-3} the re-scans very slightly depend on time of the solution being held at 5°C before re-scanning. The positions and intensities of high temperature maximum at 40.1°C are the same but much smaller maximum appears at 10.8 and 12.5°C for the solution held for 3 and 11 h at

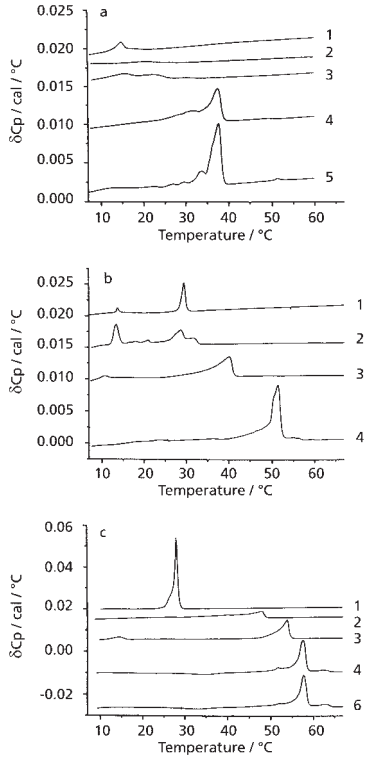

Fig. 4 Dependencies on temperature of the differential heat capacities for aqueous vesicular solutions (0.002 (monomer mol) dm^{-3}) of a – DDAB, b – DTAB, c – DHAB with added SDS. Scans recorded following heating the solutions to 60°C for DDAB and to 80°C for DTAB and DHAB cooling to and storing at 2°C and to 5°C in the calorimeter cell for 3 h before re-scanning (1) for pure vesicle solution; with SDS at concentration (2) 0.0005, (3) 0.001, (4) 0.0015, (5) 0.00173, (6) 0.0018 mol dm^{-3}. The scans have been displaced on the heat capacity axis for clarity

5°C before re-scanning, respectively. For the system with SDS concentration 0.0015 mol dm^{-3} all re-scans are fully reproducible with one asymmetric maximum at 51.3°C on δC_p plots.

In the case of DHAB vesicle solutions with added SDS the patterns resulting from DSC results are simpler. Only the first scans differ from re-scans and the latter are fully reproducible. DSC plots for all re-scans show one asymmetric maximum which shifts significantly with the increase of SDS concentration to 57.8°C for the system containing 0.0018 mol SDS dm^{-3} (Fig. 4c). The fact that the scans are reproducible can mean the scanning of equilibrium states and recording the transitions which are reversible in the thermodynamic sense.

The influence of SDS concentration on the temperature of gel to liquid-crystal state transitions for all investigated systems is compared in Fig. 4. The presented δC_p plots are recorded for the solutions being held at low temperature for 3 h before re-scanning. Generally, despite the complexity of the scans recorded for all vesicular systems which can be associated with local concentrations of SDS in domains of vesicular bilayers the overall effect is to raise the melting temperature with the increase of SDS concentration (Fig. 4, Table 1). Parameters describing the gel to liquid-crystal transitions for systems with one broad extremum are presented in Table l. SDS incorporation into vesicle bilayers formed by dialkyldimethylammonium bromides stabilises the state with ordered dialkyl chains i.e. the gel state what is reflected in the increase of average temperatures and standard enthalpies of transitions with increase of SDS concentration.

Table 1 Parameters describing the gel to liquid-crystal phase transitions in vesicle formed by dialkyldimethylammonium bromides (0.002 (monomer mol) dm^{-3}) with added SDS at concentration, c_{SDS} (mol dm^{-3}); average melting temperatures, T_m in °C and average standard enthalpy of melting, $\Delta_m H^o$ (kJ (monomer mol)$^{-1}$)

	c_{SDS}	T_m	$\Delta_m H^o$
DDAB	0	15.8	
	0.0015	37.4	34.6
	0.00173	37.7	45.0
DTAB	0	29.3 [4]	
	0.001	40.1	36.2
	0.0015	51.3	57.7
DHAB	0	28.1	64.0
	0.0005	47.9	54.0
	0.001	53.8	43.5
	0.0015	57.7	81.2
	0.0018	57.8	86.6

It is known that the formation of vesicles is a self-assembly process in water and the hydrophobic interactions are the major driving force for the formation of the bilayer. The reason for the stability of vesicles in aqueous solutions is the equilibrium between the

electrostatic and hydrogen bonding interactions between the head-groups on the surface of the bilayer and with water molecules and the van der Waals attractive forces which favour close packing of the alkyl chains inside of the bilayers. In the case of pure vesicles formed by dialkyldimethylammonium bromides the head-groups $>N^{+}R_2$ are the same, so the electrostatic interactions between them and with water molecules are the same but the van der Waals attraction forces between the dialkyl chains depend on the alkyl chains length and they are stronger for longer alkyl chains. Thus, in our case these interactions are the strongest for DHAB and the weakest for DDAB. That is why the vesicles formed by dialkyldimethylammonium bromides have different magnitudes, the smallest for DDAB (with diameters about 500 nm) and the largest for DHAB (diameters about 900 nm [16]). The vesicles are spherical for all reported systems therefore, the distances between DDAB head groups are larger than for DHAB head-groups on the surfaces of their bilayers and the alkyl chains inside DHAB are closer than inside DDAB vesicles.

Undoubtedly, the stabilisation of the gel state by SDS is mainly caused by electrostatic interactions between oppositely charged head-groups of lipids and SDS monomers but also depends on the van der Waals interactions connected with the length of alkyl chains in both the lipid and the added surfactant monomers. The latter is confirmed by the most significant raise of the mean temperature of melting expressed in the case of DHAB. The influence of alkyl chain length in the lipid monomers is seen the best when DSC results are compared for investigated vesicular systems with added SDS at the same concentration for example at 0.0015 mol dm^{-3}. The average temperatures of transitions increase by about 21.6, 20.0 and 29.6 for DDAB, DTAB and DHAB, respectively, in comparison with observed for the pure vesicular systems.

The incorporation of SDS monomers inside of vesicular bilayers disturbs the balance between the van der Waals interactions of alkyl chains inside bilayers of DDAB, DTAB and DHAB vesicles and decreases lipids head-groups repulsion on the surfaces in all investigated vesicular systems. The latter is connected with appearing of the attraction forces between the lipids and SDS oppositely charged head-groups but these effects are the same for all the investigated systems. The influence of the latter effects on the thermal stability of the gel state is clearly reflected for the DDAB vesicle – SDS system because of the same length of alkyl chains. All effects depend on the local concentration of SDS in the domains of the bilayers so, in general on the SDS concentration in the investigated systems.

The raise of SDS concentration inside the bilayers dramatically changes the electrostatic interactions between the lipids head-groups and water and the lipids head-groups on the surface of the bilayer-decreases the repulsion forces between lipids head-groups and increases the attractive forces between the lipids and SDS head-groups. Therefore, it causes the decrease of intermolecular distances in the bilayers and favours the ordering of alkyl chains, so stabilises the gel state, which is reflected in the increase of the temperature and the standard enthalpy of melting (Table 1). These effects are also reflected in the change of spherical shape of vesicles confirmed by EM examinations (Fig. 2). They produce the surfaces of the bilayers flattening at high enough SDS concentration (about 0.001 mol dm^{-3}) and cracking the

bilayers that induces the formation of planar aggregates. Then the inner side of the bilayers which were hidden inside of vesicles can be penetrated by SDS molecules what decreases the asymmetry of the bilayers.

Furthermore, changes of electrostatic interactions with SDS concentration affect the rate of reorganisation of disordered alkyl chains packing (the liquid crystal state) to the ordered (the gel state) in the domains of DDAB and DTAB vesicular bilayers, which is confirmed by DSC results. The recorded dependencies of δC_p on temperature are fully reproducible when the SDS concentration exceeds 0.0015 or 0.001 mol dm^{-3} for DDAB and DTAB vesicular systems, respectively. Thus, for these systems the reorganisation of alkyl chains from the liquid-crystal state to the gel state in their domains at low temperature is remarkably faster than observed for pure DDAB and DTAB vesicles.

The DSC and EM results seem to provide convincing evidences that the raise of SDS concentration in the domains of vesicular bilayers formed by dialkyldimethylammonium bromides affect significantiy for the ordering of alkyl chains inside of the bilayers, so stabilise their gel state. It is expressed in the increase of the melting temperatures and standard enthalpies of transitions for all investigated systems and limiting the kinetic control of the ordered state in case of DDAB and DTAB vesicular systems. The changes of structural features of the bilayers connected with electrostatic interactions between oppositely charged head-groups of lipid and SDS monomers facilitate the ordered packing of alkyl chains inside of the bilayers and influences the van der Waals interactions between the alkyl tails. On the other hand the van der Waals attractive forces decrease with increase of local concentration in the domains of bilayers formed by DTAB and DHAB because of the shorter alkyl chains in SDS monomers but the changes of the van der Waals interactions are relatively small in comparison with long range electrostatic interactions.

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