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Interaction of sodium cholate with dioctadecyldimethylammonium chloride vesicles in aqueous dispersion

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Abstract

Mixtures of dioctadecyldimethylammonium chloride (DODAC) cationic vesicle dispersions with aqueous micelle solutions of the anionic sodium cholate (NaC) were investigated by differential scanning calorimetry, DSC, turbidity and light scattering. Within the concentration range investigated (constant 1.0 mM DODAC and varying NaC concentration up to 4 mM), vesicle → micelle → aggregate transitions were observed. The turbidity of DODAC/NaC/water depends on time and NaC/DODAB molar concentration ratio R. At equilibrium, turbidity initially decreases smoothly with R to a low value (owing to the vesicle–micelle transition) when $R = 0.5$–0.8 and then increases steeply to a high value (owing to the micelle–aggregate transition) when $R = 0.9$–1.0. DSC thermograms exhibit a single and sharp endothermic peak at $T_m \approx 49\,^{\circ}C$, characteristic of the melting temperature of neat DODAC vesicles in water. Upon addition of NaC, T_m initially decreases to vanish around $R = 0.5$, and the main transition peak broadens as R increases. For $R > 1.0$ two new (endo- and exothermic) peaks appear at lower temperatures indicating the formation of large aggregates since the dispersion is turbid. All samples are non-birefringent. Dynamic light scattering (DLS) data indicate that both DODAC and DODAC/NaC dispersions are highly polydisperse, and that the mean size of the aggregates tends to decrease as R increases.

Keywords: Vesicle; Micelle; Vesicle–micelle transition; Light scattering; Differential scanning calorimetry; Turbidity; DODAC; Bile salts; Sodium cholate

1. Introduction

A typical surfactant contains one or two hydrocarbon chains attached chemically to a polar group, for example, a quaternary ammonia, that favors the surfactants to assemble in solution as a number of structures such as micelles or vesicles [1]. Such a feature, however, is not characteristic of bile salts (BSs) that are natural anionic surfactants important as emulsifiers in the digestive tract and excretion of lecithin and cholesterol [2–4]. The BSs have planar molecular structure with the hydrophobic part being rigid nonpolar steroid rings that contain two or three $^{-}$OH as hydrophilic group. The BSs do not exhibit a well-defined critical micelle concentration (CMC) and their self-assembly has low cooperativity [5,6]. BSs have two CMC values. At the first CMC the BSs self-assemble as primary micelles of low aggregation number, typically 4–10 [2,3,7–10]. At the second CMC they form rather large micelles whose size increases with the BS concentration [9]. It has been reported values of 6.3 and 14.1 mM for the first and second CMC of sodium cholate (NaC) [10], although different values obtained by different methods can be found in the literature [11].

Dioctadecyldimethylammonium chloride (DODAC) is a long double chain surfactant that in excess water, self-assembles above the gel-to-liquid-crystalline phase transition temperature ($T_m \approx 49\,^{\circ}C$) as large unilamellar vesicles (LUV) [12–14]. At higher concentrations they tend to associate as more complex structures such as multistructural vesicles [14]. In addition to concentration and the chemical constitution of the cationic surfactant, the preparation method also determines the vesicles characteristics, including the size, stability and T_m [12,15,16].

Mixtures of micelle-forming with vesicle-forming surfactants yields formation of either micelle or vesicle structures as well as intermediate structures or phase separation depending on the surfactant architecture and concentration [17–20]. BSs can thus be used as additive (cosurfactant) to stabilize or destabilize vesicle structures, as well as to modify their properties. Aqueous mixtures of BS with DODAC give a rich variety of aggregate structures whose properties are not clearly understood.

Both vesicles and micelles are important in many applications and the capability of forming either vesicles or micelles from a mixture of surfactants in aqueous solution, by varying only the surfactant composition, is a very promising research

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field. In this work we report DSC, turbidity and light scattering results on the effect of adding the BS sodium cholate (NaC) to the cationic DODAC vesicles. The structural transitions vesicle → micelle → aggregate are reported. Since these structures are obtained by mixing a cationic and an anionic surfactant, the structures can be referred to as catanionic micelles, vesicles or aggregates.

2. Experimental

2.1. Materials

Recrystallized DODAC was obtained from its homologous dioctadecyldimethylammonium bromide (DODAB) (Sigma) by counterion exchange, as described elsewhere [21]. Sodium cholate (NaC) was used as received from Sigma. Ultrapure water of Milli-Q-Plus quality was used for sample preparation. Fig. 1 shows the molecular structures of DODAC and the NaC.

2.2. Sample preparation

DODAC vesicles were prepared by warming a 10 mM DODAC/water mixture to 60 °C (above its $T_m \approx 49$ °C) to obtain a homogeneous solution by manual agitation and then cooling back to room temperature for storage, as described previously [12–14]. After being cooled DODAC vesicle dispersions remain stable for months at 5 °C [14]. NaC micelles were prepared by mixing 20 mM surfactant with water at room temperature. The DODAC dispersion was diluted to 1.0 mM to which aliquots of the NaC micelle solution were added to the desired concentration for the experimental investigation.

2.3. Turbidity

Turbidity was measured as a function of the NaC/DODAC molar ratio R using a Hitachi spectrophotometer (model U-2001) equipped with a quartz cell of 1.0 cm optic length. The measurements were made 5 min after each addition of the NaC solution into the vesicle dispersion; between the addition of NaC and the start of measurements the samples were mechanically mixed using a magnetic stirrer. Turbidity was also measured as a function of time after mixing the compounds, using a Perkin-Elmer UV/vis spectrophotometer model Lambda 14 with a quartz cell of 0.2 cm optic length. The measurements started immediately after addition of NaC into the vesicle dispersions.

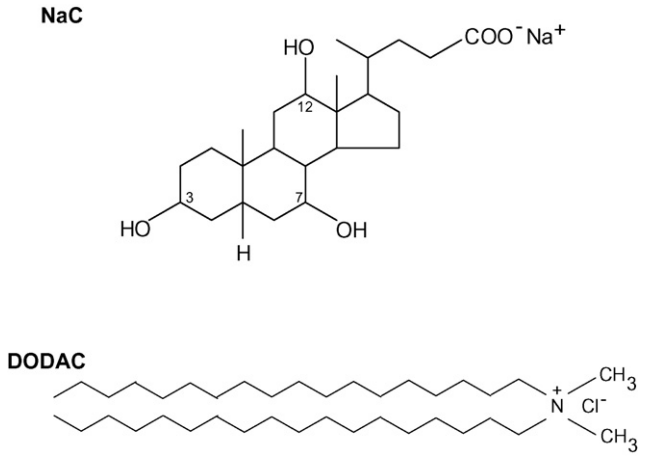

Fig. 1. Molecular structures of NaC and DODAC.

2.4. Differential scanning calorimetry (DSC)

DSC measurements were made using a MicroCal MC-2 (MicroCal, Northampton, MA) calorimeter equipped with twin cells of 1.2 ml for the reference and surfactant samples. A DSC thermogram indicates the power required to maintain the reference and sample at the same temperature and pressure during an upscanning of temperature at a constant rate (+1 °C/min in this work). The difference between the thermograms of the reference and sample is proportional to the change in the excess heat capacity (ΔC_p). The mean T_m of DODAC or DODAC/NaC dispersions is given by the position of the peak maximum in the thermogram, whereas the area under the peak gives the enthalpy change (ΔH) of this transition. The peak width at half-height ($\Delta T_{1/2}$) is inversely proportional to the cooperativity of the transition.

2.5. Dynamic light scattering (DLS)

DLS measurements give information about the vesicle hydrodynamic radius (R_h) and polydispersity of particles in solution. In this study we made DLS measurements to investigate the effect of NaC on the apparent size and polydispersity of DODAC vesicles in water. Since the dispersions are highly polydisperse, it is meaningless to obtain the actual R_h, but the trend of R_h to vary as R = NaC/DODAC is varied. The measurements were carried out using an ALV5000 scattering apparatus. The measurements were made 24 h after addition of NaC to DODAC. The scattering angle and temperature were 90° of 25 °C. The instrument measures the autocorrelation function of the light scattering intensity that exhibits a time exponential decay characteristic of the particle size. The data are usually presented as a distribution function of the characteristic or relaxation time [12].

3. Results and discussion

Turbidity and DLS measurements for the DODAC/NaC/water system were carried out as a function of the surfactant concentration and time in order to gain information on the structure changes of the aggregates upon addition of NaC to 1.0 mM DODAC vesicle dispersion. DSC measurements were as well performed to investigate structural changes in the DODAC vesicles, and the vesicle–micelle–bilayer aggregate transitions promoted by adding NaC to the dispersion.

3.1. Turbidity: kinetics of surfactant interaction

Fig. 2 shows the effect of time on the turbidity (220 nm) of the DODAC/NaC/water system measured immediately after the addition of NaC micelles into the dispersions of vesicles, for

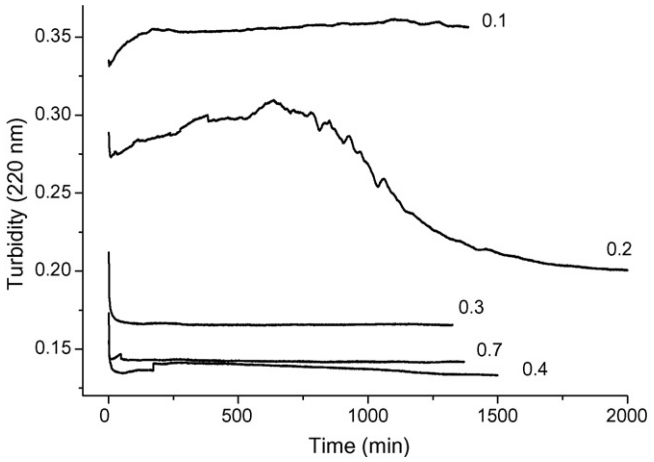

Fig. 2. The effect of time and NaC/DODAC molar ratio (numbers besides the curves) on turbidity for the NaC/DODAC/water system. Time $t = 0$ corresponds to the time when the surfactants were mixed (25 °C).

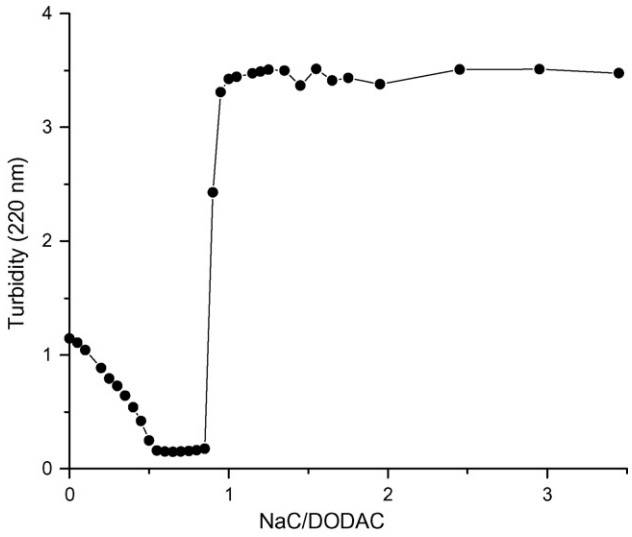

Fig. 3. Turbidity for the DODAC/NaC/water system as a function NaC/DODAC molar ratio, R, measured 5 min after mechanical mixing the solutions (25 °C).

constant 1.0 mM DODAC and varying NaC concentration. For neat DODAC ($R = 0$) in water the turbidity does not change with time (results not shown) and equals ca. 0.35. For $R = 0.1$ the turbidity initially decreases steeply and then it increases with time up to ca. 4 h (ca. 250 min) to attain a plateau value around 0.35, indicating mixing equilibrium.

For $R = 0.2$ there is an initial steep decrease in turbidity with time followed of a slightly increase up to about 12.5 h (ca. 750 min), when it attains a maximum and decreases to attain a plateau after about 33 h (ca. 2000 min). For this R, the system equilibrium is attained after a quite long time.

For ratios $R = 0.3$, 0.4 and 0.7 the turbidity exhibits similar behavior: a steep initial drop followed by a long plateau characteristic of stabilized structures, most probably mixed DODAC–NaC micelles because of the very low turbidity and solution transparency; at these ratios the stabilized structures (mixed micelles) are formed very quickly. The plateau turbidity is ca. 0.17 for $R = 0.3$ and ca. 0.14 for $R = 0.3$ and 0.7, indicating that the minimum plateau is attained when $R \approx 0.4$. These data suggest that vesicles and micelles coexist around $R = 0.2$ because of the intermediary turbidity values. For $R > 0.3$ the low turbidity indicate that mixed micelle structures dominate. According to Fig. 2, at the equilibrium plateau the turbidity drops with R, remaining constant for $R > 0.3$. The turbidity curve for $R = 0.2$ has a different profile from those for $R = 0.1$ and 0.3, possibly owing to the coexistence of mixed micelle and vesicle structures in the dispersion. The results thus indicate that NaC is an efficient solubilizer for DODAC molecules, yielding to a fast vesicle–micelle transition at a rather low NaC concentration.

Even though the CMC of DODAC is close to zero [12] and that for NaC is not well-defined in the range of 4–20 mM [11], the mixed surfactant system might have an intermediary CMC that favors micellization within appropriate surfactant concentrations, that is, the lower plateau is characteristic of mixed DODAC–NaC micelles instead of free DODAC and NaC monomers in solution.

3.2. Turbidity: the effect of concentration

Fig. 3 depicts the turbidity as a function of R = NaC/DODAC. The measurements were made 5 min after each addition of the NaC, at 25 °C and under mechanical agitation to accelerate the sample equilibrium. Due to structural changes in the dispersion promoted by the addition of NaC into the vesicle dispersion, the turbidity initially decreases progressively until $R \approx 0.5$, indicating that smaller structures are being formed in the DODAC/NaC/water mixture, probably mixed micelle and vesicle structures coexist within this transition region. For $R = 0.5$–0.9 the turbidity is constant equal to ca. 0.2, and is not affected by the addition of further NaC, indicating that the DODAC solubilization is complete and only NaC/DODAC mixed micelles are present in solution. The mechanism of DODAC solubilization by NaC to form mixed micelles resembles those for BS/lipid systems reported in the literature [7,22–25].

Around $R = 0.9$ the addition of a small amount of NaC induces a pronounced structure transition of mixed micelles to much larger structures, and the turbidity increases steeply to a high plateau value of around 3-fold the initial turbidity of pure DODAC vesicles or 22-fold the turbidity of mixed DODAC/NaC mixed micelles (Fig. 3). The architecture of these aggregates requires a detailed investigation and is beyond the scope of the present work.

3.3. The thermotropic phase behavior of the DODAC/NaC/water system

Fig. 4 shows the effect of NaC on the thermotropic phase behavior of DODAC vesicles in aqueous dispersions, according to the DSC thermograms. The notation R = NaC/DODAC = 0 represents pure 1.0 mM DODAC in water. Up to $R = 0.4$ the thermograms exhibit a well-defined peak characteristic of the gel-to-liquid-crystalline phase transition temperature T_m, or melting

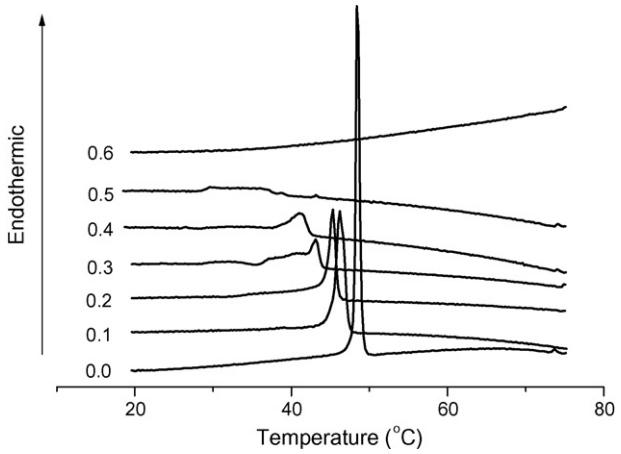

Fig. 4. The effect of NaC on the DSC (upscan) thermograms for 1.0 mM DODAC dispersions. The NaC/DODAC molar ratios, for $R < 1.0$, are indicated near the curves.

temperature, of the surfactant system. For $R = 0$ (neat DODAC vesicles), $T_m = 48.4\,^{\circ}C$, in good agreement with data previously reported [13,18]. Upon addition of NaC, T_m decreases and the peak broadens and becomes smaller to vanish around $R \approx 0.5$; at this ratio the peak is not well defined. That also reflects in the transition enthalpy that decreases with increasing R, from 11.6 to 0.2 kcal/mol when R increases from 0 (neat DODAC) to $R = 0.4$ (Table 1). Similar behavior was reported for the effect of nonionic surfactants to DODAC (and to the homologous bromide surfactant, DODAB) vesicles [18]. The DSC results clearly indicate that the complete vesicle–micelle transition for the DODAC/NaC/water system takes place around $R \approx 0.4$–0.5 when the transition peak vanishes, in good agreement with the turbidity data shown above. Relative to nonionic surfactants [13,18], the NaC is a better solubilizer of DODAC (or DODAB), most probably because of the strong electrostatic interaction between the oppositely charged surfactants.

The well-defined peak for $R = 0.1$ and 0.2 indicates the existence of NaC–DODAC mixed vesicles with smaller T_m relative to the neat DODAC vesicles. The peak broadens when R increases, indicating that the mixed bilayer structures exhibit a fairly cooperative transition around T_m, indicative of a smooth vesicle–micelle transition. Beyond $R \approx 0.5$ there is no sign of bilayer structures in solution, but only micelles and the solution is clear. As discussed above, probably a mixture of bilayer and micelle structures coexist in the intermediary region between the neat DODAC vesicles and mixed DODAC–NaC micelles.

Table 1
Values of T_m, $T_{1/2}$ and ΔH for the DODAC/NaC/water system obtained from DSC experiments

NaC/DODAC	T_c (°C)	$T_{1/2}$ (°C)	ΔH (kcal/mol)
0.0	48.4	0.7	11.6
0.1	46.2	1.3	7.3
0.2	45.3	0.9	3.8
0.3	43.1	0.9	0.8
0.4	41.2	2.2	0.2

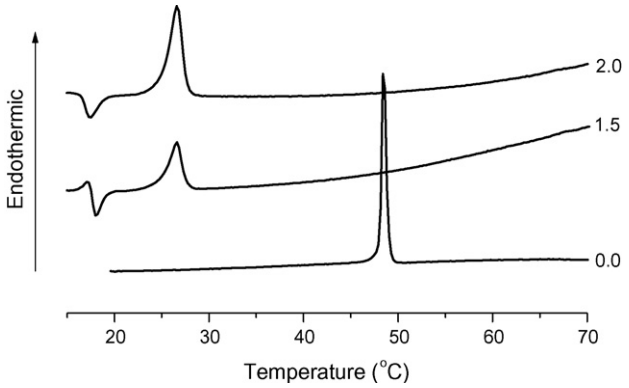

Fig. 5. The effect of NaC on the DSC (upscan) thermograms for 1.0 mM DODAC dispersions. The NaC/DODAC molar ratios, for $R > 1.0$, are indicated near the curves.

The thermograms for $R > 0.5$ exhibit no peak indicating that there are only small mixed micelles in solution, that is, the vesicle–micelle transition is complete. A second (micelle–aggregate) structure sharp transition occurs when $R > 0.9$ (Fig. 3). Beyond $R \approx 0.9$ the dispersion is turbid and the DSC thermograms exhibit two peaks, one exothermic centered at 18.2 °C and the other endothermic around 26.5 °C for $R > 1.5$ (Fig. 5). The origin of these transition peaks is not clear, but is probably related to changes in structure and phase that are sensitive to temperature. Table 1 summarizes the values of T_m, $\Delta T_{1/2}$ (peak width) and ΔH (change in transition enthalpy) for different R up to 0.4. Accordingly, $\Delta T_{1/2}$ increases from 0.7 to 2.2 °C (the transition becomes less cooperative) and ΔH decreases from ca. 12–0.2 kJ/mol when R increases from 0 to 0.4 (the gel-liquid crystalline transition becomes less energetic), that is, NaC stabilizes the liquid-crystalline state of the surfactant chains.

3.4. Dynamic light scattering (DLS) results

Fig. 6 shows that at equilibrium the light scattering intensity varies with R = NaC/DODAC in a similar fashion as the turbidity (Fig. 3), thus corroborating the vesicle–micelle transition. The distribution functions of relaxation time (τ) for different

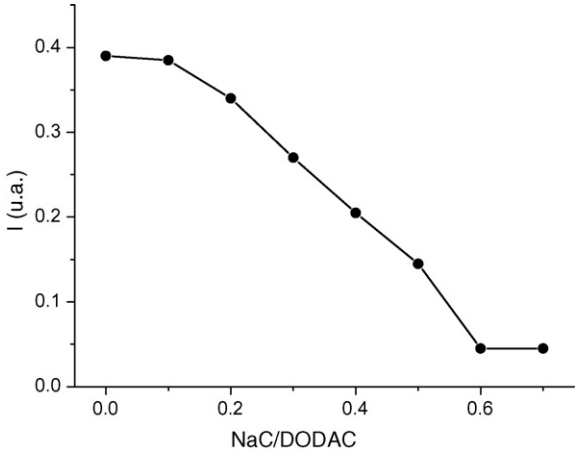

Fig. 6. Mean light scattered intensity as a function of the NaC/DODAC molar ratio R, for the NaC/DODAC/water system. $\theta = 90^{\circ}$ and 25 °C.

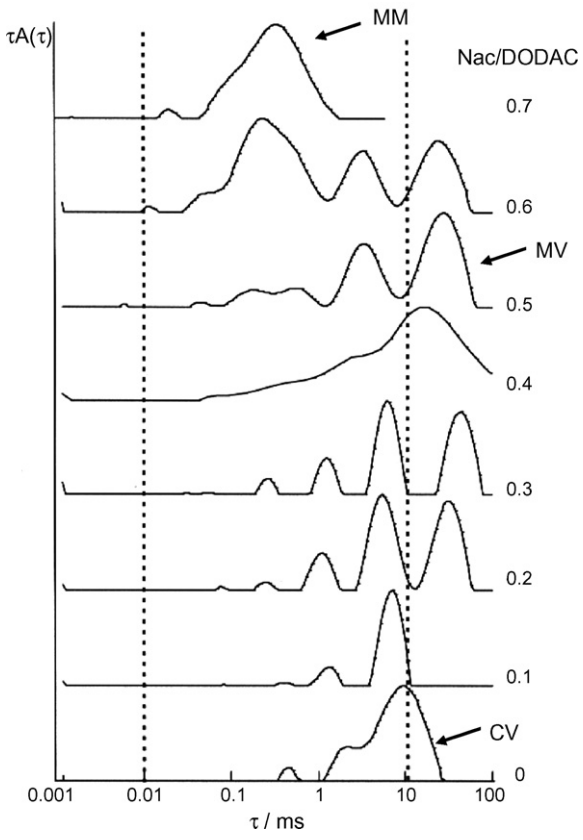

Fig. 7. Relaxation time distribution functions for the NaC/DODAC/water system, and selected NaC/DODAC molar ratios R. $\theta = 90°$ and 25 °C. Arrows indicate the peaks related to mixed micelles (MM), mixed vesicles (MV) and neat DODAB cationic vesicles (CV).

values of R are shown in Fig. 7. These curves indicate that the dispersions of 1.0 mM DODAC vesicles are quite polydisperse, as reported before [14] and the added NaC tends to shift the peaks to the left, i.e., the modes of the relaxation time distribution tend to become faster, indicating the formation of smaller aggregates. Note that the relaxation time distributions exhibit multi-peaks, that is, populations of structures having different size. Each peak represents a population of vesicle and/or micelle structures. It is also indicates that not only the vesicles but also the mixed micelles are large and polydisperse. Grossly R_h of the neat DODAC vesicles ranges from 1000 to 1100 nm whereas R_h of the mixed NaC–DODAC micelles, for $R = 0.7$ (Fig. 7) ranges from 15 to 20 nm, since R_h is inversely proportional to the diffusion coefficient which is in turn proportional to $\Gamma = 1/\tau$, where τ is the relaxation time [12].

4. Conclusion

The results indicate that the aggregates formed in the mixture of up to 2.0 mM NaC with 1.0 mM DODAC vesicles depend on the NaC concentration. Within the range of surfactant concentration investigated, a vesicle → micelle → aggregate transition was observed, with the vesicle–micelle transition being smooth while the micelle–aggregate transition being steep. By "aggregate" we mean large unknown structures in turbid dispersions, that exhibit two transition peaks for the DSC thermograms, one exothermic at 17.5–18 °C and the other at 26.6 °C (Fig. 5). Mixed NaC/DODAC micelles are formed for intermediary values of $R \approx 0.5$–0.8. According to the DLS data, these mixed micelles are ca. 100-fold smaller than the vesicles and the solution is clear. For smaller R (<0.5) there is most probably a mixture of mixed vesicles and micelles in solution, since the vesicle–micelle transition is smooth. For larger R (>0.8), a mixed micelle–aggregate cooperative transition takes place. Dynamic light scattering results also indicate that 1.0 mM DODAC vesicles in aqueous dispersion are large and polydisperse, either in the absence or presence of NaC.

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References

[1] J.H. Fendler, Membrane Mimetic Chemistry, Wiley–Interscience, New York, 1982.
[2] M.C. Carey, D.M. Small, Am. J. Med. 49 (1970) 590–608.
[3] D.M. Small, P.P. Nair, D. Kritchevsky (Eds.), The Bile Acids, Plenum Press, Nova Iorque, 1971.
[4] B. Borgström, Int. Rev. Physiol. 12 (1977) 305–323.
[5] B. Lindman, N. Kamenka, B. Brun, J. Colloid Interf. Sci. 56 (1976) 238–336.
[6] K.J. Mysels, Hepatology 4 (1984) 80S–84S.
[7] N.A. Mazer, M.C. Carey, R.F. Kwasnick, G.B. Benedek, Biochemistry 18 (1979) 3064–3075.
[8] J.P. Kratohvil, J. Colloid Interf. Sci. 26 (1986) 131–154.
[9] K. Matsuoka, M. Maeda, Y. Moroi, Colloids Surf. B 32 (2003) 87–95.
[10] K. Matsuoka, Y. Moroi, Biochim. Biophys. Acta 1580 (2002) 189–199.
[11] A. Coello, F. Meijide, E.R. Nunez, J.V. Tato, Aggregation behavior of bile salts in aqueous solution, J. Phys. Chem. 97 (1993) 10186–10191; A. Coello, F. Meijide, E.R. Nunez, J.V. Tato, Aggregation behavior of bile salts in aqueous solution, J. Pharm. Sci. 85 (1996) 9–15.
[12] E. Feitosa, W. Brown, Langmuir 13 (1997) 4810–4816.
[13] E. Feitosa, P.C.A. Barreleiro, G. Olofsson, Chem. Phys. Lipids 105 (2000) 201–213.
[14] E. Feitosa, G. Karlsson, K. Edwards, Chem. Phys. Lipids 140 (2006) 66–74.
[15] J. Cocquyt, U. Olsson, G. Olofsson, P. Van der Meeren, Colloid Polym. Sci. 283 (2005) 1376–1381.
[16] C.R. Benatti, M.J. Tiera, E. Feitosa, G. Olofsson, Thermochim. Acta 328 (1999) 137–142.
[17] E.F. Marques, A. Khan, B. Lindman, Thermochim. Acta 394 (2002) 31–37.
[18] P.C.A. Barreleiro, G. Olofsson, W. Brown, K. Edwards, N. Bonassi, E. Feitosa, Langmuir 18 (2002) 1024–1029.
[19] E.F. Marques, O. Regev, A. Khan, M.G. Miguel, B. Lindman, J. Phys. Chem. B 102 (1998) 6746–6758.
[20] E.F. Marques, O. Regev, A. Khan, M.G. Miguel, B. Lindman, J. Phys. Chem. B 103 (1999) 8353–8363.
[21] I.M. Cuccovia, A. Sesso, E. Abuin, P.F. Okino, P.G. Tavares, J.F.S. Campos, F.H. Florenzano, H. Chaimovich, J. Mol. Liquids 72 (1997) 323–336.
[22] R. Schubert, K.H. Schmidt, Biochemistry 27 (1988) 8787–8794.
[23] R.C. Malloy, J.S. Binford Jr., J. Phys. Chem. 94 (1990) 337–345.
[24] A. Walter, P.K. Vinson, A. Kaplun, Y. Talmon, Biophys. J. 60 (1991) 1315–1325.
[25] D. Meyuhas, A. Bor, I. Pinchuk, A. Kaplun, Y. Talmon, M.M. Kozlov, D. Lichtenberg, J. Colloid Interf. Sci. 188 (1997) 351–362.