



Thermotropic phase behavior in aqueous mixtures of dioctadecyldimethylammonium bromide and alkyltrimethylammonium bromide surfactant series studied by differential scanning calorimetry

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ARTICLE INFO

Article history:

Received 24 November 2007
Received in revised form 5 March 2008
Accepted 10 March 2008
Available online 30 March 2008

Keywords:

DODAB
 C_n TAB
DSC
Melting temperature
Vesicles
Micelles

ABSTRACT

The effect of the micelle-forming surfactant series alkyltrimethylammonium bromide (C_n TAB, $n = 12, 14, 16$ and 18) on the thermotropic phase behavior of dioctadecyldimethylammonium bromide (DODAB) vesicles in water was investigated by differential scanning calorimetry at constant 5.0 mM total surfactant concentration and varying individual surfactant concentrations. The pre-, post- and main transition temperatures (T_s , T_p and T_m), melting enthalpy (ΔH) and peak width of the main transition ($\Delta T_{1/2}$) are reported as a function of the surfactant molar fraction. No clear dependence of these parameters on the C_n TAB chain length was found. At 5 mM, neat DODAB in water exhibits two transition temperatures, $T_s = 32.1$ and $T_m = 42.7$ °C, as obtained from the DSC upscans, but not a clear T_p . For every n , except $n = 12$, T_s vanishes as C_n TAB concentration increases and approaches CMC. T_m behaves differently for different n , the longer C_{14} TAB and C_{16} TAB decrease, while C_{18} TAB increases T_m with increasing concentration. The data indicate that changes in T_m , T_s , T_p and ΔH of the transition are related not only to the extent of C_n TAB affinity to DODAB but also to the surfactant chain length. Accordingly, C_{18} TAB yields a more compact bilayer, thus increasing T_m , while C_{14} TAB and C_{16} TAB yield a less organized bilayer and reduce T_m . C_{12} TAB does not much affect T_s and T_m , although it yields $T_p \approx 51.6$ °C.

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1. Introduction

Unilamellar vesicles consist of a lipid bilayer separating an aqueous solution from the bulk phase, forming roughly spherical structures with an inner aqueous core [1,2]. In general, the vesicle characteristics, such as size and stability, depend on the method of preparation. Vesicles can be prepared by sonication, extrusion, surfactant removal or spontaneously, that is, without sonication or extrusion [1,2]. Owing to their structural similarity to cell membranes, vesicles have attracted considerable interest as membrane models [1–4] and as drug delivery vehicles. Cationic vesicles are appropriate for DNA compaction [2]. On heating, the vesicle bilayer exhibits a transition temperature (T_m) characteristic of the system, ascribed to a gel to liquid crystalline state transition [3,4]. Vesicles may also present pre- and post-transition temperatures, T_s and T_p , respectively. These temperatures can be monitored to gain insight on the vesicle structure in different media and conditions.

In excess water, dioctadecyldimethylammonium bromide (DODAB) molecules assemble spontaneously above $T_m \approx 43$ °C and at low concentrations (typically 1.0 mM) as large unilamellar vesicles, that is, without sonication or extrusion [5,6]. At higher concentrations, in addition to unilamellar vesicles, they assemble as more complex structures such as multilamellar and multistructural vesicles yielding additional transitions [7,8]. The structural organization and properties of DODAB vesicles thus depend on the surfactant concentration, solvent and vesicle preparation method [5–10]. Up to 5 mM DODAB, however, the concentration has a minor effect on the transition temperatures [5].

Co-surfactants may have pronounced effects on the vesicle properties owing to their solubilization into the vesicle bilayer that changes the bilayer fluidity, thus affecting the transition temperatures. Anionic, nonionic and zwitterionic surfactants reduce the T_m of DODAB [11–13]. Cationic surfactants, however, may increase, decrease or leave constant DODAB T_m [14,15].

Except for C_{12} TAB, the CMC (critical micellar concentration) of C_n TAB ($n = 14, 16$ and 18) is below 5 mM, and these surfactants interact with DODAB vesicles either as monomers or micelles. The CMC of C_n TAB in water is $15.2, 3.98, 1.00$ and 0.35 mM, for $n = 12, 14, 16$ and 18 , respectively [16]. The Krafft temperature, on the other

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hand, increases almost linearly with the chain length [17]. DODAB exhibits a critical vesicle concentration (CVC) close to zero [6]. Thus, in the experiments the C_n TAB concentration is either below or above CMC, whereas DODAB concentration is always above CVC. In that way, the system is appropriate to investigate the effect of C_n TAB monomers and micelles on DODAB vesicles.

The aim of this study was to investigate the effect of the chain length of C_n TAB ($n = 12, 14, 16$ and 18) on the thermotropic phase behavior of DODAB vesicles at constant 5.0 mM total surfactant concentration and varying surfactant ratio.

2. Experimental

2.1. Materials

DODAB was purchased from Aldrich. Hexadecyltrimethylammonium bromide (C_{16} TAB) was supplied by Sigma, whereas dodecyl- (C_{12} TAB), tetradecyl- (C_{14} TAB) and octadecyltrimethylammonium (C_{18} TAB) bromides were obtained from Fluka. All surfactants were used as received. Ultrapure water of Milli-Q-Plus quality was used in sample preparations.

2.2. Sample preparation

DODAB and C_n TAB dispersions were prepared by simple dilution of 5.0 mM surfactant in water at room temperature (25°C). For complete dissolution DODAB and C_{18} TAB aqueous mixtures were warmed to 60 and 45°C , respectively, that is, above $T_m \approx 43^\circ\text{C}$ [6] and the Krafft temperatures ($T_k \approx 38^\circ\text{C}$) [18] of DODAB and C_{18} TAB. All the other dispersions of the C_n TAB surfactants were prepared by simply mixing the appropriate amount of the surfactant at room temperature (25°C), since at this temperature they are above T_k [17].

Mixed DODAB/ C_n TAB aqueous dispersions were prepared by mixing appropriate volumes of the 5.0 mM dispersions of these surfactants to have 5.0 mM total surfactant concentration and varying ratios of the individual surfactant concentrations. The data are presented as a function of the molar fraction of surfactant. For example, for the mixture of DODAB and C_n TAB, the molar fraction of DODAB is $x_{\text{DODAB}} = [\text{DODAB}]/[\text{TOTAL}]$, where brackets account for molar concentration and $[\text{TOTAL}] = 5.0$ mM.

2.3. Differential scanning calorimetry (DSC)

A VP-DSC (MicroCal, Northampton, MA) calorimeter with 0.542 ml twin cells for the reference and sample solutions was used to obtain the thermograms. Measurements were performed at a scan rate of $1^\circ\text{C}/\text{min}$ from 5 to 80°C allowing the system to be either below or above the Krafft temperature of the C_n TAB/DODAB/water system, especially for $n = 16$ and 18 . Prior the DSC experiments the samples were left standing at 25°C for at least 48 h to check for precipitate formation. Precipitate formation was observed only for the C_{18} TAB-containing system, as indicated by the phase diagram shown in Fig. 8 of the supplementary material. Those samples that formed precipitates were warmed to become clear before being transferred to the DSC cell. After the experiments the samples were left standing for months at 25°C to check for precipitate formation. Once again, only the C_{18} TAB-containing system exhibited precipitates. The baseline reference was obtained with both cells filled with water. The melting temperature (T_m , i.e., the temperature of the peak maximum), and melting enthalpy (ΔH) were determined from the peak area, and the cooperativity of the transition by the peak width ($\Delta T_{1/2}$) at the peak half height. For the pre- and post-transition peaks, only the transition temperatures T_s and T_p were obtained because they were not well defined. MicroCal Origin, v.5.0, was used for data acquisition and analyses. Further details on the DSC can be found in previous publications [5,8,13,19].

3. Results and discussion

At 5.0 mM $T_m \approx 43^\circ\text{C}$ of neat DODAB is the same as that of 1.0 mM often investigated [5]. At 5.0 mM DODAB, more pronounced pre- and post-transitions were expected, which could be used to investigate the effects of C_n TAB. However, the DSC traces in Figs. 1 and 2 reveal that DODAB concentration has little effect on the size of these peaks.

The melting temperature T_m , enthalpy ΔH and peak width $\Delta T_{1/2}$ are shown in Figs. 3–5, as functions of the C_n TAB molar fraction ($x_{C_n\text{TAB}}$). The neat DODAB dispersion ($x_{\text{DODAB}} = 1.0$) exhibits two endothermic transition temperatures, $T_s = 32.1^\circ\text{C}$ and $T_m = 42.7^\circ\text{C}$. The post-transition temperature (T_p) reported in previous publications [5,8,19] does not appear even in the enlarged curves, although it is induced by the presence of C_{12} TAB, as Fig. 9 in the supplementary material indicates. C_{12} TAB reduces slightly and linearly

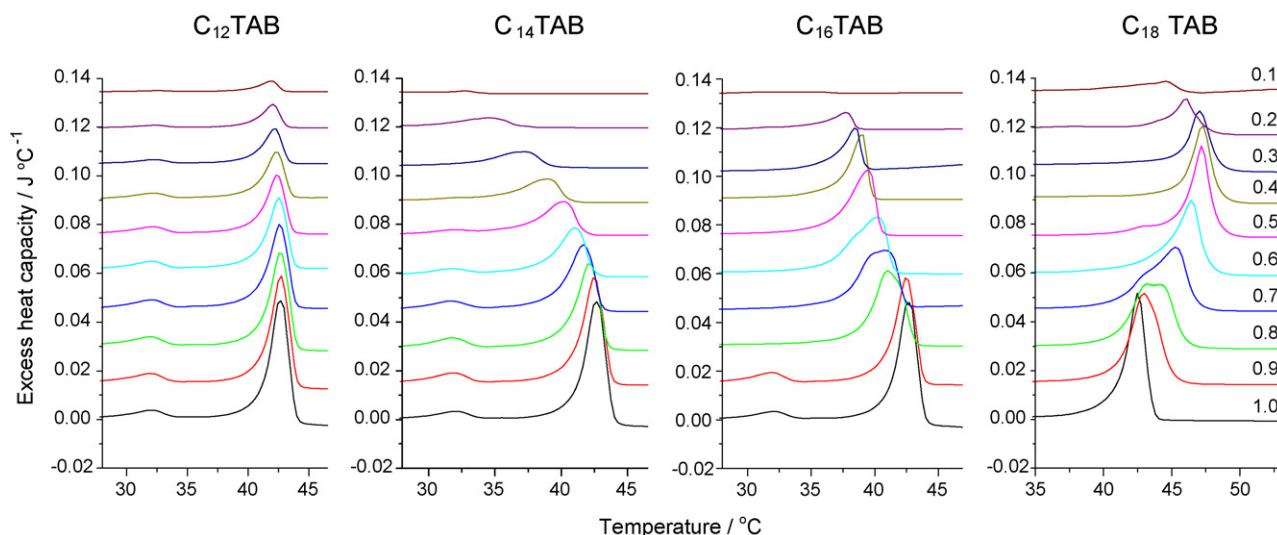


Fig. 1. DSC upscan thermograms from mixtures of DODAB/ C_n TAB/water, $n = 12, 14, 16$ and 18 , 5.0 mM total surfactant concentration, and $x_{\text{DODAB}} = 0.1, 0.2, 0.3, \dots, 1.0$, as indicated. To avoid overlap, the curves were offset by $0.015 \text{ J } ^\circ\text{C}^{-1}$ from each other.

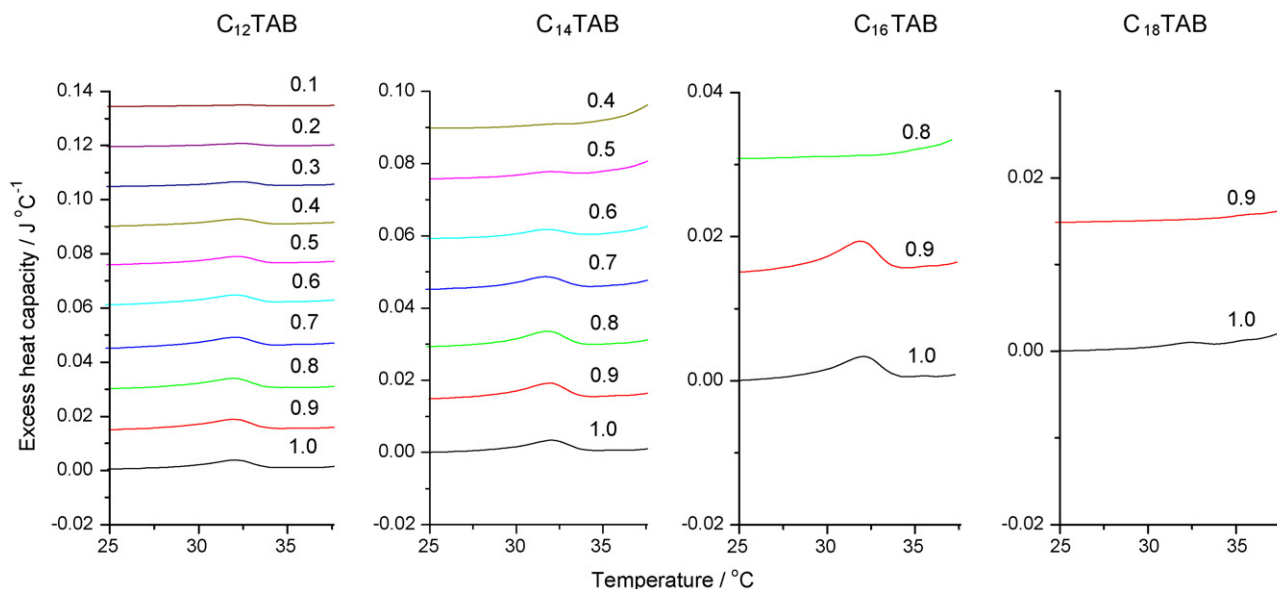


Fig. 2. Same as Fig. 1, except for the enlarged traces around the pre-transition temperature T_s .

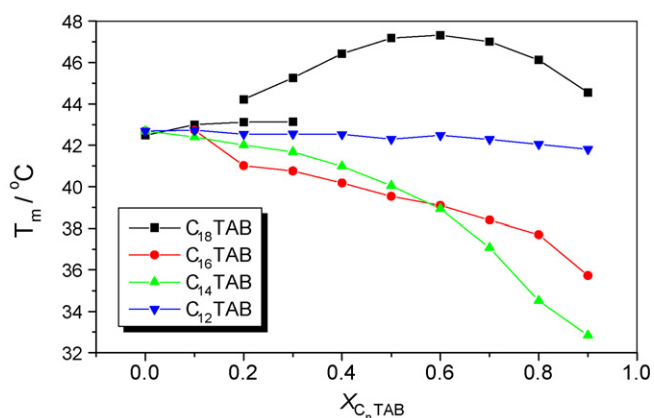


Fig. 3. The effect of C_n TAB molar fraction (x_{C_nTAB}) on the melting temperature T_m of the mixed cationic C_n TAB-DODAB vesicles in water.

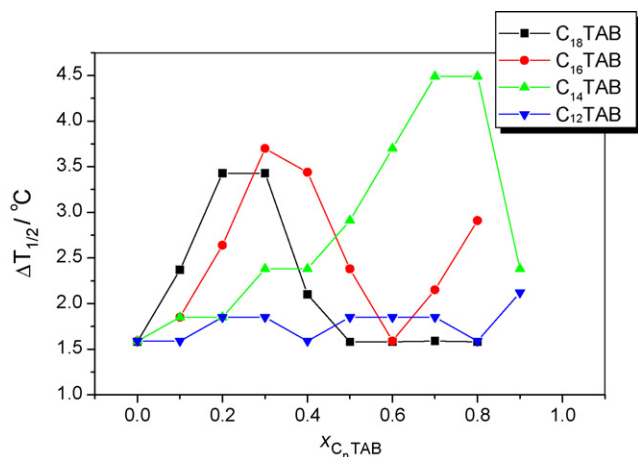


Fig. 4. The effect of C_n TAB molar fraction (x_{C_nTAB}) on the width peak at half-height $\Delta T_{1/2}$ of the mixed cationic C_n TAB-DODAB vesicles in water.

T_p , yielding $T_p = 51.6^\circ\text{C}$ upon extrapolation of the fitting curve to zero C_{12} TAB concentration (Fig. 10 in the supplementary material), in good agreement with previously reported $T_p \approx 53^\circ\text{C}$ [5,8,19].

The enlarged thermograms around the pre-transition temperature (Fig. 2) indicate that the longer the C_n TAB chain length, the stronger the inhibition of the pre-transition peak. C_{12} TAB thus exhibits minor effect on T_s (Fig. 6), probably because it is already in a more disordered state. The post-transition temperature (T_p) can only be poorly discerned for the thermograms of the C_{12} TAB-containing systems (Fig. 9 in supplementary material), but cannot be seen for $n = 14$ –18. Furthermore, T_p decreases only slightly and linearly with C_{12} TAB concentration (Fig. 10 in the supplementary materials).

The DSC curves (Figs. 1 and 2) indicate that T_s is rather sensitive to C_n TAB concentration and chain length. T_s vanishes when $x_{C_{18}TAB} < 0.1$, $x_{C_{16}TAB} \approx 0.2$ and $x_{C_{14}TAB} \approx 0.6$, whereas C_{12} TAB does not inhibit the pre-transition at all. Even though the T_s values are not well defined, because the peaks are very weak, they clearly indicate that the pre-transition is inhibited around the surfactant CMC.

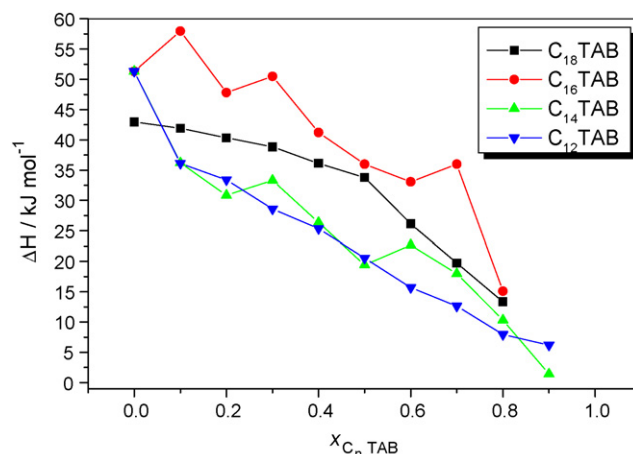


Fig. 5. The effect of C_n TAB molar fraction (x_{C_nTAB}) on the melting enthalpy ΔH of the mixed cationic C_n TAB-DODAB vesicles in water.

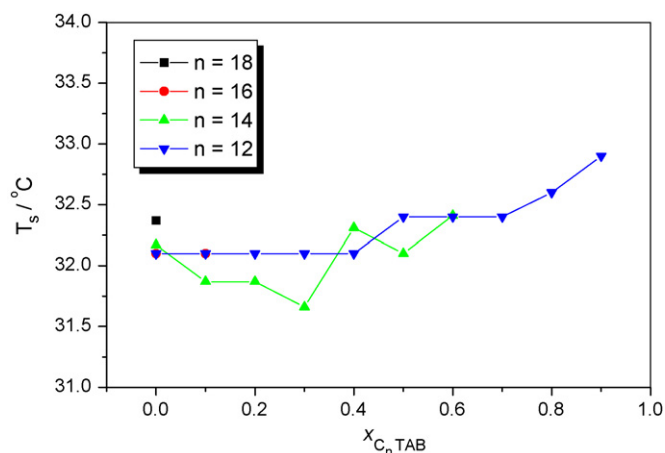


Fig. 6. The effect of C_n TAB molar fraction (x_{C_nTAB}) on the pre-transition temperature T_s of the mixed cationic C_n TAB-DODAB vesicles in water.

Since T_s exists only when C_n TAB concentration is below CMC, a fraction of monomers might be solubilized in the vesicle bilayer to form mixed C_n TAB-DODAB vesicles; above CMC the C_n TAB monomers have more affinity to the micelle, rather than the vesicle structure.

The main peak position in the thermograms gives the melting temperature (T_m), shown in Fig. 3 as a function of x_{C_nTAB} . Up to $x_{C_{18}TAB} \approx 0.6$, T_m increases to a maximum value about 47.2 °C, meaning that C_{18} TAB stabilizes the gel state of the DODAB-based vesicles, due to the solubilization of monomers into the DODAB bilayer, as reported for 1.0 mM total surfactant concentration [15]. Above this concentration, T_m decreases to values even higher than that for neat DODAB in water. Note that around 0.2–0.3 C_{18} TAB there are two melting temperatures due to solubilization of C_{18} TAB into two different vesicle populations, as reported [7,15]. The opposite effect was observed for C_{14} TAB and C_{16} TAB that shift T_m downward in temperature. C_{12} TAB does not much affect T_m of DODAB, indicating weaker interaction.

The main transition, which is related to the melting of the surfactant hydrocarbon chains, is influenced by perturbation caused by C_n TAB molecules solubilized into the vesicle bilayer. The bell-shaped variation of T_m with increasing x_{C_nTAB} (Fig. 3) indicates that the mixed C_{18} TAB-DODAB bilayer is more densely packed than that of neat DODAB. In presence of C_{14} TAB and C_{16} TAB the chains become more mobile, increasing the disorder of the surfactant chains, thus reducing T_m . The shorter C_{12} TAB molecules do not much affect T_m , T_s or T_p since they stay as monomers in solution below CMC = 15.2 mM [19] or, alternatively, the solubilized monomers are short enough to disturb the vesicle bilayers.

According to Fig. 3, the effect of C_n TAB chain length on T_m depends on the relative amount of the surfactants. First, C_{12} TAB does not affect T_m because its concentration is lower than CMC. Up to $x_{C_nTAB} \approx 0.1$ there is no effect of C_n TAB on T_m . Above this point and up to $x_{C_nTAB} \approx 0.6$ both C_{14} TAB and C_{16} TAB decrease T_m with a slightly stronger effect of C_{16} TAB, whereas C_{18} TAB increases T_m to a maximum value around 47 °C. Beyond $x_{C_nTAB} \approx 0.6$ C_{14} TAB exhibits a stronger decreasing effect on T_m relative to C_{16} TAB. C_{18} TAB also decreases T_m , however, to a value (45 °C) higher than that of neat DODAB (43 °C). These data indicate that there is no clear relationship between T_m and the C_n TAB chain length, with C_{18} TAB exhibiting the unusual behavior of increasing T_m . Overall, surfactants reduce T_m [11,13,14].

Fig. 4 shows the effect of the surfactant molar fraction on the width of the main transition peaks, $\Delta T_{1/2}$. $\Delta T_{1/2}$ does not vary much with $x_{C_{12}TAB}$, indicating that the transition is rather cooperative, that is, it takes place in a rather narrow range of temperature, between

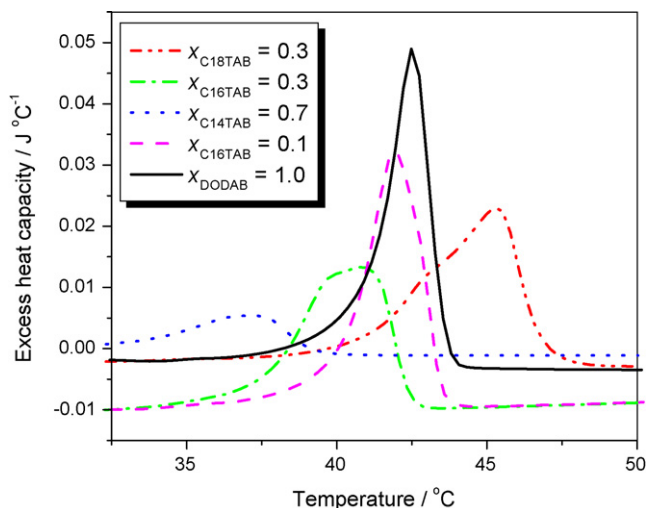


Fig. 7. DSC upscan thermograms from selected mixtures of C_n TAB-DODAB in water, indicating peak overlaps.

1.5 and 2.0 °C. The broadening of $\Delta T_{1/2}$ on addition of C_n TAB, for $n = 14$ –18, is due to peak overlaps. C_{18} TAB and C_{14} TAB broaden the peaks at low and high concentrations, respectively, while C_{16} TAB broaden the peaks both at low and high concentrations.

The peak overlap is due to sharp transitions that occur in a narrow range of temperature due to the vesicle polydispersity, since the vesicle curvature affects T_m [5]. The appearance of the extra peak at $x_{C_{18}TAB} = 0.5$ has been attributed to a vesicle population rich in C_{18} TAB in addition to the ordinary DODAB-rich vesicles [15]. A detailed analysis of the broader peaks clearly indicates peak overlaps (Fig. 7). Surprisingly this figure also indicates that even the narrower peaks for neat DODAB and the C_{12} TAB-DODAB vesicles are composed of peak overlaps. Such overlaps were attributed to different populations of DODAB vesicles differing mainly in size (rather than in structure) to explain the effect of C_{18} TAB on increasing T_m [15].

In the presence of a small amount of DODAB, broad (overlapped) peaks appear (Fig. 11, supplementary material) owing to multiple transitions in the C_{16} TAB- and C_{18} TAB-containing systems. This is the usual behavior of higher amount of cosurfactants, such as C_n TAB, since they exhibit specific transitions themselves [15]. Transitions for C_{18} TAB in water were previously reported indicating that this surfactant assembles as a bilayer in addition to micelle structures [15,18].

The enthalpy change (ΔH) associated to the main transition for neat DODAB in water lies between 43 and 51.5 kJ mol⁻¹ (Fig. 5) in good agreement with data previously reported for similar systems [8,11,19]. Accordingly, ΔH decreases with increasing x_{C_nTAB} , indicating that the addition of C_n TAB favors the main transition. The data, however, indicate no clear relationship between ΔH and the chain length.

4. Conclusions

The thermotropic phase behavior of the aqueous mixtures of DODAB and C_n TAB (for $n = 12$ –18) was investigated by DSC at 5.0 mM total surfactant concentration and varying surfactant ratios. The systems allow the investigation of the C_n TAB-DODAB vesicle interaction below and above CMC. The results indicate that not only the chain length, but also the relative concentration of C_n TAB influences the main-, pre- and post-transition temperatures (T_m , T_s and T_p). Accordingly, vesicles are formed even in the presence of very small amounts of DODAB (high amount of C_n TAB). C_{12} TAB does not

affect T_m and T_s since its concentration is much lower than CMC. It yields, however, a $T_p \approx 51.6^\circ\text{C}$ that cannot be viewed in the DSC traces (Fig. 1) for neat DODAB. For $n = 14$ –18, upon increasing C_n TAB concentration the pre-transition is completely inhibited when concentration approaches CMC. C_{14} TAB and C_{16} TAB decrease, whereas C_{18} TAB increases T_m . According to the peak width, for all C_{12} TAB-DODAB mixtures the main transition is cooperative even though it is composed of overlapped narrow peaks, for larger n the peaks are broader owing as well to peak overlaps. The melting enthalpy is lowered on increasing C_n TAB concentration, indicating that the surfactant favors the melting transition. The C_n TAB-DODAB systems thus offer excellent opportunity to deal with mixed cationic vesicles with well-defined characteristics and high potential application as membrane mimetic systems as well as vehicle for drug delivery.

Acknowledgements

FRA and EF acknowledge CNPq for PhD and research (Grant 304543/2006-3) grants, respectively. Dr. W. Loh (UNICAMP) is acknowledged for kindly supplying the DSC equipment.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tca.2008.03.017.

References

- [1] J.H. Fendler, *Membrane Mimetic Chemistry*, Wiley-Interscience, New York, 1982.
- [2] D.D. Lasic, *Liposomes. From Physics to Applications*, Elsevier, Amsterdam, 1993.
- [3] G. Ceveç, D. Marsh, *Phospholipid Bilayers-Physical Principles and Models*, v. 5, New York, 1987.
- [4] J.B.F.N. Engberts, D. Hoeskstra, *Biochim. Biophys. Acta* 1241 (1995) 323–340.
- [5] E. Feitosa, P.C.A. Barreleiro, G. Olofsson, *Chem. Phys. Lipids* 105 (2000) 201–213.
- [6] E. Feitosa, W. Brown, *Langmuir* 13 (1997) 4810–4816.
- [7] E. Feitosa, G. Karlsson, K. Edwards, *Chem. Phys. Lipids* 140 (2006) 66–74.
- [8] E. Feitosa, P.C.A. Barreleiro, *Progr. Colloid Polym. Sci.* 128 (2004) 163–168.
- [9] C.R. Benatti, E. Feitosa, R.M. Fernandez, M.T. Lamy-Freund, *Chem. Phys. Lipids* 111 (2001) 93–104.
- [10] R.O. Brito, E.F. Marques, *Chem. Phys. Lipids* 137 (2005) 18–28.
- [11] E. Feitosa, N.M. Bonassi, W. Loh, *Langmuir* 22 (2006) 4512–4517.
- [12] J. Cocquyt, U. Olsson, G. Olofsson, P. Van der Meeren, *Langmuir* 20 (2004) 3906–3912.
- [13] P.C.A. Barreleiro, G. Olofsson, W. Brown, K. Edwards, N.M. Bonassi, E. Feitosa, *Langmuir* 18 (2002) 1024–1029.
- [14] A. Kacperska, *J. Thermochem. Anal.* 45 (1995) 703–714.
- [15] F.R. Alves, M.E.D. Zaniquelli, W. Loh, E.M.S. Castanheira, M.E.C.D. Real Oliveira, E. Feitosa, *J. Colloid Interf. Sci.* 316 (2007) 132–139.
- [16] J. Mata, D. Varade, P. Bahadur, *Thermochim. Acta* 428 (2005) 147–155.
- [17] T.W. Davey, W.A. Ducker, A.R. Hayman, J. Simpson, *Langmuir* 14 (1998) 3210–3213.
- [18] M. Swanson-Vethamuthu, E. Feitosa, W. Brown, *Langmuir* 14 (1998) 1590–1596.
- [19] E. Feitosa, F.R. Alves, A. Niemiec, M.E.C.D. Real Oliveira, E.M.S. Castanheira, A.L.F. Baptista, *Langmuir* 22 (2006) 3579–3585.