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ORIGINAL CONTRIBUTION

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Vesicle formation of 1:1 cationic and anionic surfactant mixtures in nonaqueous polar solvents

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Abstract The vesicle formations of 1:1 cationic-anionic surfactants in various nonaqueous polar solvents and their aqueous mixtures were investigated. Outstanding vesicle-forming capability and stability of cationic and anionic surfactants were found in nonaqueous polar solvents and their aqueous mixtures except for in formamide. A small amount of formamide destroys the vesicles formed by cationic-anionic surfactants in aqueous solutions. These results could be very well explained based on the effect of the medium dielectric constant.

Key words Vesicle formation – cationic and anionic surfactant mixtures – nonaqueous polar solvent

Introduction

In the last two decades, there were many reports on vesicle formation from natural amphiphiles (mainly phospholipids) and synthetic surfactants [1, 2]. Synthetic vesicles have attracted more and more attention because they have been very useful for understanding the nature of biomembranes and for developing new techniques by biomimesis. Up to ten years ago, amphiphiles used to form vesicles were mainly double-chained compounds, including natural and synthesized amphiphiles. In 1989, Kaler et al. [3] reported vesicle formation from mixed cationic and anionic surfactants using cetyltrimethylammonium tosylate and sodium dodecylbenzene sulfonate. About the same time, similar work was done in our laboratory, using mixed surfactants of carboxylate and alkyltrimethylammonium compounds [4–5]. However, most work on vesicle formation has been involved with aqueous systems and little work has been done on nonaqueous systems. Some work on vesicle formation in aprotic solvent systems has been reported [6–7]; this concentrated on systems of double-chained fluorocarbon surfactants [8–11]. On the other hand, studies on vesicle formation in nonaqueous polar solvent and mixed polar solvent systems have mainly been on the liposome of natural phospholipids [12–14]. Compared with phospholipids and other double-chained amphiphiles, the cationic and anionic surfactants used as precusors have the characteristics of simple structure, excellent stability, convenience in production, and it is easy to form vesicles from them. Therefore, investigation of vesicle formation by cationic and anionic surfactants is of great significance for both theory and application. However, to our knowledge, investigations of vesicle formation of mixed cationic and anionic surfactants have mostly been in aqueous solutions [3–5, 15–16] and are scanty in nonaqueous polar solvents. In a previous paper [17], we first reported the vesicle formation of 1:1 cationic and anionic surfactant mixtures in pure ethanol, which is different from the early studies showing that ethanol addition deteriorates the molecular order in lipid bilayers [18–22]. An explanation based on the effect of the medium dielectric constant was suggested [17]. In this work, we have extended the investigation to various polar nonaqueous solvents. It was found that the cationic and anionic surfactants have outstanding vesicle-forming capability and that the vesicles show very strong stability in nonaqueous polar solvents of low dielectric constant, while addition of a small amount of polar solvent of higher dielectric constant destroyed the vesicle formed in aqueous solutions. These results support the explanation based on the effect of the medium dielectric constant [17] very well.

Experimental

Materials

Sodium alkylcarboxylates (C_nCOONa, $n = 9$, 11) were prepared by neutralizing the corresponding carboxylic acid (C_nCOOH) with NaOH in ethanol, then the solvent was removed and C_nCOONa was vacuum dried. C_9COOH was double-distilled and $C_{11}COOH$ was recrystallized 5 times from an ethanol-water mixed solvent (m.p. 43–44 °C). Alkyltrimethyl ammonium bromides (C_mNMBr $m = 8$, 10, 12) were synthesized from *n*-alkyl bromide and trimethyl amine. The products were recrystallized 5 times from an ethanol-acetone mixture [4]. The purities of all the surfactants were examined by measuring the surface tensions of aqueous solutions using the drop-volume method [23] and no surface tension minimum was found in their surface tension curves (γ–$\log C$). Ethanol, 1-propanol and isopropanol were refluxed with magnesium scraps and iodine for more than 2 h to remove traces of water and were then distilled. Dimethylsulfoxide (DMSO) was a product of Merck. Deionized water was treated with $KMnO_4$ and distilled before use. Other reagents and solvents (A.R. grade) were products of Beijing Chemical Co.

Methods

The mixed surfactant vesicles were prepared by simple 1:1 (molar ratio) mixing of the cationic and anionic surfactants in the solvent at room temperature (~30 °C) or by sonicating the mixed surfactant solution for 0.5 h at 50 °C (sonicator: Haitung CQ-250). Micrographs were obtained with an electron microscope (JEM-100CXII) using the freeze-fracture technique or the negative-staining technique for sample preparation. A post-staining method was adopted in the sample preparation using the latter technique: a few drops of the sample solution were applied to carbon-coated Cu grids and dried, then a drop of uranyl acetate-ethanol solution was added as the staining agent. The staining process was 1–2 min depending on the solvents. The phase-transition temperature T_c of the vesicle in the 1:1 mixed cationic and anionic surfactant systems was measured by use of a differential scanning calorimeter (DSC) (Dupont 1090; with a heating rate of 5 °C/min). The endothermic peak was determined to be the T_c value of the system.

Results and discussion

Vesicle formation of 1:1 cationic-anionic mixtures in nonaqueous polar solvents

Solutions of three pairs of cationic and anionic surfactants (1:1 C_9COONa-$C_{10}NMBr$, $C_{11}COONa$-$C_{12}NMBr$ and $C_{11}COONa$-C_8NMBr) in pure ethanol, 1-propanol, isopropanol, DMSO, formamide (FA) and in the aqueous mixtures of each were studied. At 30 °C, similar to the situation in the ethanol system [15], all the solutions of cationic and anionic surfactants in 1-propanol, isopropanol and in the mixed solvents of them with water, were clear. The electron microscopy (EM) results showed that globular vesicles formed with or without sonication in all these systems no matter whether the solvents were pure alcohol or their aqueous mixtures in various ratios (typical EM results are shown in Figs. 1–3 and Table 1). In particular, the EM images obtained by the negative-staining technique indicate the aggregates are vesicles, composed of enclosed surfactant membranes with uranyl acetate solution inside and outside. The sizes of the vesicles were about 20–40 nm as determined from the EM images obtained by the freeze-fracture technique. The DSC curves shown in Figs. 4–5 further confirmed the vesicle formation of 1:1 mixed cationic and anionic surfactants in alcohols. Their phase-transition temperature (T_c) are listed in Table 2. It is found that in the systems investigated, the T_c of 1:1 $C_{11}COONa$-$C_{12}NMBr$ are higher than those of the 1:1 C_9COONa-$C_{10}NMBr$ and $C_{11}COONa$-C_8NMBr systems. This may be attributed to the length of the CH chain in the $C_{11}COONa$-$C_{12}NMBr$ systems being longer than that of the CH chains in 1:1 C_9COONa-$C_{10}NMBr$ and $C_{11}COONa$-C_8NMBr systems, which induces a

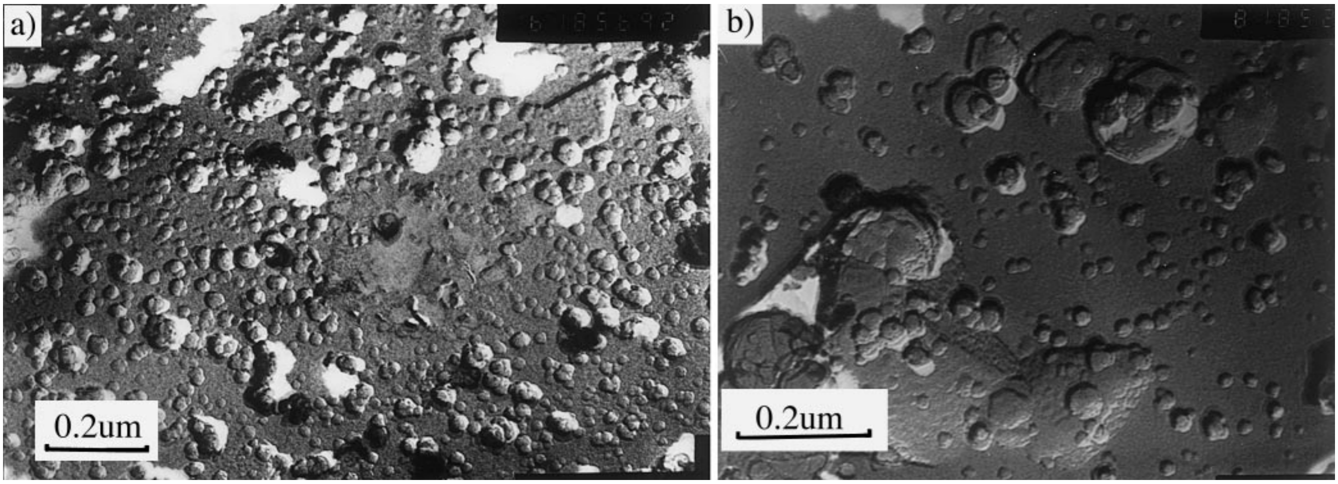

Fig. 1 Electron microscopic image in pure 1-propanol of **a**) 1:1 $C_{11}COONa$-$C_{12}NMBr$ ($c = 1.5 \times 10^{-2}$ mol dm^{-3}) and **b**) 1:1 $C_{11}COONa$-C_8NMBr ($c = 3.5 \times 10^{-2}$ mol dm^{-3}) systems, observed by the freeze-fracture technique

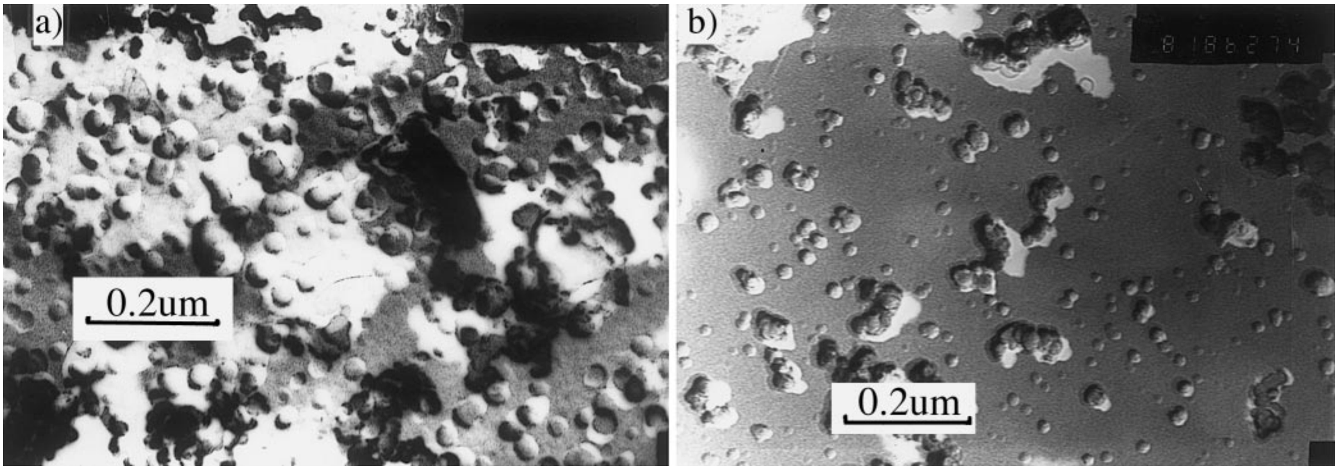

Fig. 2 Electron microscopic image in pure isopropanol of **a**) 1:1 $C_{11}COONa\text{-}C_{12}NMBr$ ($c = 8.0 \times 10^{-3}$mol dm^{-3}) and **b**) 1:1 $C_9COONa\text{-}C_{10}NMBr$ ($c = 1.6 \times 10^{-2}$ mol dm^{-3}) systems, observed by the freeze-fracture technique

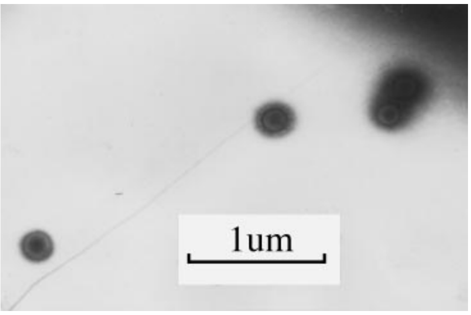

Fig. 3 Electron microscopic image in the 1:1 $C_{11}COONa\text{-}C_8NMBr$ isopropanol system observed by the negative-staining technique ($c = 1.0 \times 10^{-2}$ mol dm^{-3})

stronger molecular interaction in the $C_{11}COONa\text{-}C_{12}NMBr$ systems than in the others, making the surfactant molecules in bilayers compact more closely. It is also interesting to note that the T_c of 1:1 cationic-anionic surfactant systems increase in the solvent order ethanol, isopropanol, 1-propanol. The nature of the solvent effect on the T_c change is still unclear. However, the assumption that solvent molecules participate in bilayers may explain the phenomenon. Alcohol, as a kind of amphiphile tends to participate in surfactant bilayers to a degree determined by its hydrophobic chain length and structure. Because the polar group of alcohol-hydroxide is small and neutral, its participation in oriented bimolecular layers will make the molecular packing closer and increase the T_c of the system. Among the alcohols investigated, 1-propanol has the longest CH chains and tends to participate in the surfactant bilayers more than the others. The CH chains of ethanol and isopropanol are shorter than that of 1-propanol and the branching of the CH chain in isopropanol results in the tendency for the chains to participate in surfactant

Table 1 Vesicle formation of cationic-anionic surfactant mixtures in alcohol

1:1 Mixed systems	Concentration c/mol dm^{-3}	Solvents	Electron microscopy observation[a]	
			Sonication	Without sonication
$C_9COONa\text{-}C_{10}NMBr$	2.4×10^{-2}	ethanol	+	+
$C_{11}COONa\text{-}C_{12}NMBr$	5.0×10^{-3}	ethanol	+	+
$C_{11}COONa\text{-}C_8NMBr$	3.5×10^{-2}	ethanol	+	+
$C_9COONa\text{-}C_{10}NMBr$	3.9×10^{-2}	1-propanol	+	+
$C_{11}COONa\text{-}C_{12}NMBr$	1.5×10^{-2}	1-propanol	+	+
$C_{11}COONa\text{-}C_8NMBr$	3.5×10^{-2}	1-propanol	+	+
$C_9COONa\text{-}C_{10}NMBr$	1.6×10^{-2}	isopropanol	+	+
$C_{11}COONa\text{-}C_{12}NMBr$	8.0×10^{-3}	isopropanol	+	+
$C_{11}COONa\text{-}C_8NMBr$	1.0×10^{-2}	isopropanol	+	+
$C_9COONa\text{-}C_{10}NMBr$	2.4×10^{-2}	water	+	−
$C_{11}COONa\text{-}C_{12}NMBr$	5.0×10^{-3}	water	+	+
$C_{11}COONa\text{-}C_8NMBr$	8.3×10^{-2}	water	+	+

[a] + = vesicle formed, − = no vesicle

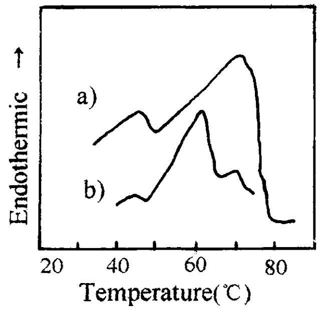

Fig. 4 Differential scanning calorimetry (*DSC*) and curves in 1-propanol of **a**) 1:1 $C_{11}COONa\text{-}C_{12}NMBr$ ($c = 1.5 \times 10^{-2}$ mol dm^{-3}) and **b**) 1:1 $C_9COONa\text{-}C_{10}NMBr$ ($c = 3.0 \times 10^{-2}$ mol dm^{-3}) systems

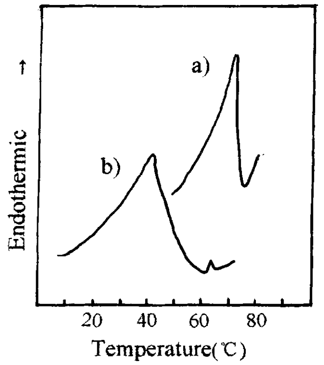

Fig. 5 DSC curves in isopropanol of **a**) 1:1 $C_{11}COONa\text{-}C_{12}NMBr$ ($c = 8.0 \times 10^{-3}$ mol dm^{-3}) and **b**) 1:1 $C_9COONa\text{-}C_{10}NMBr$ ($c = 1.7 \times 10^{-2}$ mol dm^{-3}) systems

Table 2 T_c of cationic-anionic surfactant mixtures in short-chained alcohols

1:1 Mixed systems	c/mol dm^{-3}	Solvents	T_c
$C_9COONa\text{-}C_{10}NMBr^a$	5.2×10^{-2}	water	33.1
$C_{11}COONa\text{-}C_{12}NMBr$	1.0×10^{-2}	water	54.2
$C_{11}COONa\text{-}C_8NMBr$	8.3×10^{-2}	water	25.6
$C_9COONa\text{-}C_{10}NMBr$	2.4×10^{-2}	ethanol	27.9
$C_{11}COONa\text{-}C_{12}NMBr$	5.0×10^{-3}	ethanol	43.0
$C_{11}COONa\text{-}C_8NMBr$	3.5×10^{-2}	ethanol	42.4
$C_9COONa\text{-}C_{10}NMBr$	3.0×10^{-2}	1-propanol	62.2
$C_{11}COONa\text{-}C_{12}NMBr$	1.5×10^{-2}	1-propanol	71.9
$C_{11}COONa\text{-}C_8NMBr$	3.5×10^{-2}	1-propanol	62.8
$C_9COONa\text{-}C_{10}NMBr$	1.7×10^{-2}	isopropanol	42.9
$C_{11}COONa\text{-}C_{12}NMBr$	8.0×10^{-3}	isopropanol	73.6
$C_{11}COONa\text{-}C_8NMBr$	1.0×10^{-2}	isopropanol	48.9

[a] Sonication

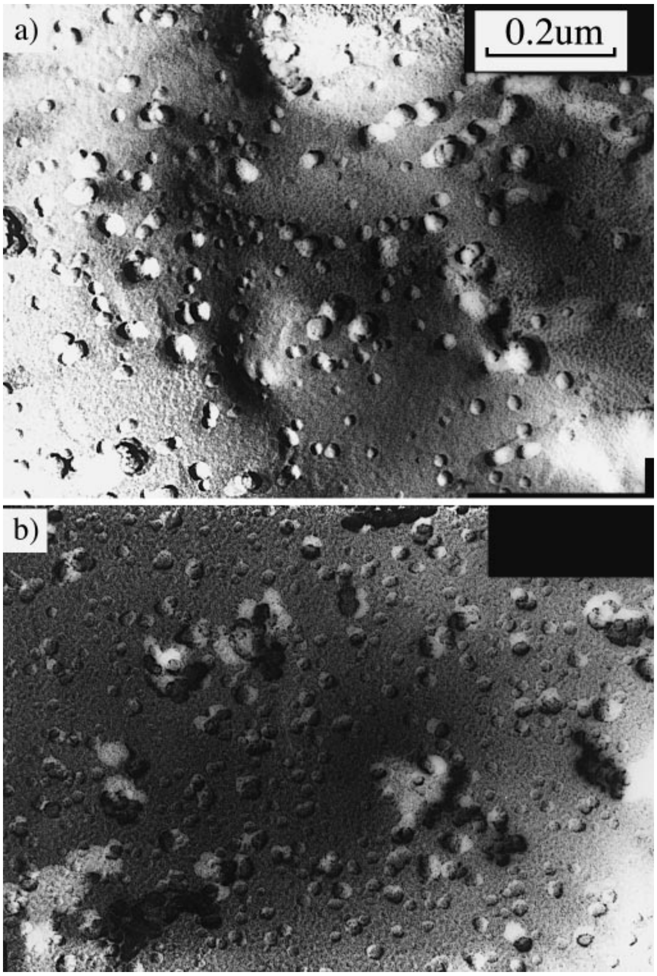

Fig. 6 Electron microscopic image of **a**) 1:1 $C_{11}COONa\text{-}C_{12}NMBr$ ($\alpha_D = 0.9$, $c = 8.0 \times 10^{-3}$ mol dm^{-3}) and **b**) 1:1 $C_{11}COONa\text{-}C_8NMBr$ ($\alpha_D = 0.75$, $c = 1.0 \times 10^{-2}$ mol dm^{-3}) systems, observed by the freeze-fracture technique

bilayers and the effects to make molecular compacting closer in bilayers are weaker. Thus, it is reasonable that the T_c in ethanol and isopropanol systems is generally lower than those in 1-propanol.

It is shown that the three pairs of cationic-anionic surfactants can form vesicles in all the pure short-chained alcohols investigated. The vesicles are quite stable. It was shown by EM observation that in $C_{11}COONa\text{-}C_{12}NMBr$-ethanol, $C_{11}COONa\text{-}C_{12}NMBr$-1-propanol and $C_{11}COONa\text{-}C_{12}NMBr$-isopropanol vesicles can be stable after 6 weeks aging. Nevertheless, in these systems there was still vesicle existence through a very large temperature variation – heating to 50 °C (aging for 15 min) or cooling to −20 °C (aging for 1 h) then returning to about 30 °C. All these results demonstrate that the mixed cationic and anionic surfactants display superior vesicle-forming capability and stability in short-chained alcohols. It is worth noting that for the 1:1 $C_9COONa\text{-}C_{10}NMBr$ system no vesicles were found in water without sonication, whereas vesicles form spontaneously in all the alcohol solvents investigated (Table 1). It is implied that the vesicle-forming ability of cationic and anionic surfactants in media containing alcohols is better than that in pure water. This is in contrast to the situtaion in the systems of phospholipids.

The 1:1 cationic and anionic surfactant mixtures also show strong capability to form vesicles in DMSO-water solutions. Since the three pairs of cationic and anionic surfactants (1:1 C_9COONa-$C_{10}NMBr$, $C_{11}COONa$-$C_{12}NMBr$ and $C_{11}COONa$-C_8NMBr) cannot be solved in pure DMSO (gels formation), it is impossible to study the vesicle formation of the 1:1 cationic and anionic surfactant systems in it. The maximum DMSO-water mixed volume ratios studied were 90% or 75% DMSO for various cationic and anionic surfactants. The EM and DSC results of the 1:1 cationic and anionic surfactant systems in DMSO-water mixtures are shown in Figs. 6–8 and in Table 3. It can be seen that 1:1 cationic and anionic surfactants can form globular vesicles in mixtures of almost every DMSO/water ratio.

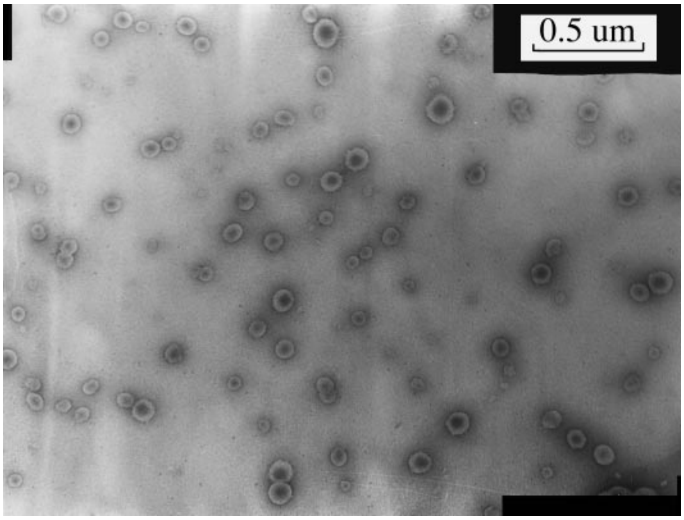

Fig. 7 Electron microscopic image in the 1:1 C_9COONa-$C_{10}NMBr$ system observed by the negative-staining technique ($\alpha_D = 0.9$, $c = 3.0 \times 10^{-2}$ mol dm^{-3})

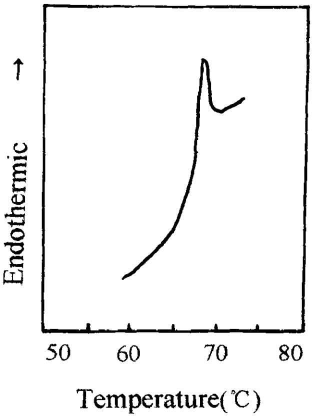

Fig. 8 DSC curve of the 1:1 $C_{11}COONa$-C_8NMBr ($\alpha_D = 0.75$, $c = 1.0 \times 10^{-2}$ mol dm^{-3}) system

However, the situation of the 1:1 cationic and anionic surfactants in FA is very different. A very small amount of FA destroys the vesicles formed in aqueous solutions (see Table 4). Such great difference between the 1:1 cationic and anionic surfactants in FA and in the other polar organic solvents investigated can be attributed to dielectric effects on the interactions of surfactant molecules of these polar organic solvents: this will be discussed in detail in the following section.

The dielectric effect on vesicle formation of 1:1 cationic and anionic mixtures in polar nonaqueous solvents

In the previous section the cationic and anionic surfactant vesicle formation in some nonaqueous solvents and their aqueous mixtures was reported. Albeit, all these nonaqueous solvents are polar organic solvents and can be mixed with water very well: the influences of their addition into aqueous solutions of cationic and anionic surfactants are so different: some of them are helpful to vesicle formation, such as short-chained alcohols, while some can destroy the vesicles formed in aqueous cationic and anionic surfactant solutions easily, such as FA. Different solvent effects on vesicle formation must be caused by different influences of various solvents on molecular interactions.

In previous work [17], we suggested an explanation based on the medium dielectric effect on vesicle formation in ethanol solutions. According to Coulomb's law, the relative dielectric constant of a medium will affect

Table 3 Cationic and anionic surfactant vesicle formation in dimethyl sulfoxide (*DMSO*)-water systems

1:1 Mixed systems	Concentration c/mol dm^{-3}	$\alpha_D{}^a = 0.3$		$\alpha_D = 0.5$		$\alpha_D = 0.75$		$\alpha_D = 0.9$	
		NS[b]	S[c]	NS	S	NS	S	NS	S
C_9COONa-$C_{10}NMBr$	3.0×10^{-2}	+	+	+	+	+	+	+	+
$C_{11}COONa$-$C_{12}NMBr$	8.0×10^{-3}	+	+	+	+	+	+	+	+
$C_{11}COONa$-C_8NMBr	1.0×10^{-2}	+	+	+	+	+	+		

[a] α_D = volume ratio of DMSO in total solvents
[b] NS = no sonication
[c] S = sonication

Table 4 Vesicle formation in formamide (FA)-water solutions of cationic and anionic surfactant systems

1:1 Mixed systems	Concentration c/mol dm^{-3}	$\alpha_F{}^a = 0.05$	$\alpha_F = 0.1$	$\alpha_F = 0.15$
C_9COONa-$C_{10}NMBr^b$	2.5×10^{-2}	$+-^c$	$-$	$-$
$C_{11}COONa$-$C_{12}NMBr^d$	5.0×10^{-3}	$+-$	$+-$	$-$

[a] α_F = volume ratio of FA in total solvents

[b] Sonication

[c] $+-$ = organized assemblies, but not certain if it is vesicle by electron microscopy observation

[d] No sonication

the electrostatic force between two ionic surfactants directly. Thus, ethanol addition will enlarge the electrostatic interaction between the cationic and anionic surfactants since the dielectric constant of ethanol is much lower than that of water. This effect is of advantage in energy requirement and the molecular packing geometry [24] for vesicle formation. In a suitable case, it may overcome the disadvantageous influence of ethanol addition on the hydrophobic effect [25] and result in more favorable energy and geometric conditions for vesicle formation.

According to this explanation, it can be predicted that vesicle formation of cationic and anionic surfactants in nonaqueous polar solvents with lower dielectric constant are possible. The results of this work agree with the prediction and strongly support our previous explanation [15]. The relative dielectric constants of ethanol, 1-propanol, isopropanol and DMSO (24.6, 20.3, 18.3 and 46.6, respectively) are lower than that of water (78.5) [26], and of the cationic and anionic surfactant vesicles formed in these systems. The situation of the cationic and anionic surfactants in FA/water solution is different. The dielectric constant of FA is 111.0 [24], being much larger than that of water. Thus, the attractive interaction between the polar groups of 1:1 mixed cationic-anionic surfactants and the solvophobic effect will be reduced by FA addition. Therefore, FA addition is disadvantageous for vesicle formation whether from the point of energy requirement or from that of molecular packing geometry. Thus, it is unstandable that FA addition can destroy the cationic and anionic surfactant easily, behaving so differently from the other polar nonaqueous solvents investigated.

Conclusion

The vesicle formations of three pairs of 1:1 cationic-anionic surfactants in various polar nonaqueous solvents were investigated. The cationic and anionic surfactants can form vesicles in mixtures of DMSO-water, alcohol-water and in pure alcohol, spontaneously; however, a small amount of FA will destroy the vesicles formed in aqueous solutions. All these results strongly support the explanation previously suggested based on the medium dielectric effect. The outstanding vesicle-forming capability and stability of the simple cationic and anionic surfactants in alcohol and other nonaqueous solvents may open a vast vista for enlarging the application scope of the functions of vesicular systems, such as entrapment, transport and compartmental reaction media etc., beyond the limit of aqueous solutions and water-soluble material.

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