

# Temperature-Controlled Vesicle Aggregation in the Mixed System of Sodium *n*-Dodecyl Sulfate/*n*-Dodecyltributylammonium Bromide

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Temperature-controlled vesicle aggregation was investigated in a cationic surfactant system of sodium *n*-dodecyl sulfate/*n*-dodecyltributylammonium bromide. Vesicle aggregation took place as the temperature reached the critical value ( $T_c$ ).  $T_c$  can be adjusted by the variations of the total surfactant concentration and the mixed molar ratio. It was also found that the temperature variation above  $T_c$  can greatly influence the vesicle aggregation rate. The vesicle aggregation process was irreversible as long as  $T \geq T_c$ , whereas the vesicle disaggregation process occurred only below  $T_c$ .

## Introduction

Vesicles are widely used as the models for cell membranes and drug delivery systems.<sup>1,2</sup> Living cells in a multicellular organism preserve their individuality. However, in certain physiological conditions their aggregation or fusion occurs. In the past few decades, the aggregation of vesicles has attracted special attention not only for its close relations to these biological processes as the analogue model,<sup>3,4</sup> but also for its practical importance in pharmacy and so on.<sup>5–7</sup> Normally, vesicle aggregation is induced by external additives. Salt-induced and polymer-induced vesicle aggregations have been well-investigated in numerous papers.<sup>8–12</sup> Recently, vesicle aggregation induced by the specific molecular recognition<sup>13</sup> (or referred to as the “host–guest” complexation)<sup>14</sup> also aroused much interest. However, no research on the temperature-induced vesicle aggregation has been reported so far, although temperature variation can provide quite a simple way to tailor assemblies.<sup>15,16</sup> In fact, in most vesicular

systems, phase transition or vesicle disruption will take place upon increasing the temperature.<sup>17,18</sup>

In this work, temperature-controlled vesicle aggregation is for the first time reported in a cationic surfactant system of sodium *n*-dodecyl sulfate (SDS)/*n*-dodecyltributylammonium bromide (DTBAB). Vesicle aggregation occurred as the temperature reached the critical value. The critical temperature showed a strong dependence on the total surfactant concentration and the surfactant mixed molar ratio. The aggregation rate can also be greatly influenced by the temperature variation above the critical temperature.

## Experimental Section

SDS was bought from ACROS ORGANICS Co., 99% purity. Quaternary ammonium bromide was prepared by reaction of 1-bromododecane and the corresponding trialkylamine. Turbidimetry was carried out with a Shimadzu UV-250 spectrophotometer at 514.5 nm. Dynamic light scattering (DLS) measurements were performed with a spectrometer (ALV-5000/E/WIN Multiple Tau Digital Correlator) and a Spectra-Physics 2017 200-mW Ar laser (514.5-nm wavelength). The intensity autocorrelation functions were analyzed using the *Contin* method, and the average hydrodynamic radius ( $R_h$ ) was deduced from the diffusion coefficient  $D$  by the Stokes–Einstein formula  $R_h = k_B T / (6\pi\eta D)$ . The temperature of turbidimetry and DLS measurements was controlled by an external thermostatic bath, and the time needed for the temperature changes was less than 2 min in the study. A negative-staining technique (with uranyl acetate water solution) was used, and a JEM-100CX electron microscope was employed in the microscopic observation. Differential scanning calorimetry (DSC) measurements were carried out (1 °C/min) using a Micro DSC III (Setaram-France) instrument. Zeta potentials were measured using a temperature-controlled ZetaPlus (Brook Heaven Co.) zeta potential analyzer.

## Results and Discussion

At 30 °C, the formation of microstructures in the mixed system of SDS/DTBAB was investigated at the total surfactant concentration ( $C_{\text{total}}$ ) of 10 mM and the mixed molar ratio of 2:1 (SDS/DTBAB). Separated spherical vesicles with the radius of 50–100 nm were observed by transmission electron microscopy (TEM) (Figure 1a), and

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(1) Fendler, J. H. *Membrane Mimetic Chemistry*; Wiley: New York, 1982.

(2) Rosoff, M., Ed. *Vesicles*; Marcel Dekker: New York, 1996.

(3) Poste, G.; Nicolson, G. L., Eds., *Membrane Fusion*; Elsevier-North-Holland Biomedical Press: Amsterdam, 1978.

(4) Buckingham, M. E.; Cohen, A.; Gross, F. J. *Mol. Biol.* **1976**, *103*, 611.

(5) Hoffmann, H.; Munkert, U.; Thunig, C.; Valiente, M. *J. Colloid Polym. Sci.* **1994**, *163*, 217.

(6) Ravoo, B. J.; Kevelam, J.; Weringa, W. D.; Engberts, J. B. F. N. *J. Phys. Chem. B* **1998**, *102*, 11001–11006.

(7) Chiruvolu, S.; Walker, S.; Israelachvili, J.; Schmitt, F. J.; Leckband, D.; Zasadzinski, J. A. *Science* **1994**, *264*, 1753–1756.

(8) Walker, S. A.; Zasadzinski, J. A. *Langmuir* **1997**, *13*, 5076–5081.

(9) Minami, H.; Inoue, T. *Langmuir* **1999**, *15*, 6643–6651.

(10) Ravoo, B. J.; Engberts, J. B. F. N. *J. Chem. Soc., Perkin Trans. 2* **2001**, *10*, 1869–1886.

(11) Meyuhas, D.; Nir, S.; Lichtenberg, D. *Biophys.* **1996**, *71*, 2602–2612.

(12) Yang, Q. L.; Guo, Y. Q.; Li, L. H.; Hui, S. W. *Biophys.* **1997**, *73*, 277–282.

(13) Vermette, P.; Taylor, S.; Dunstan, D.; Meagher, L. *Langmuir* **2002**, *18*, 505–511.

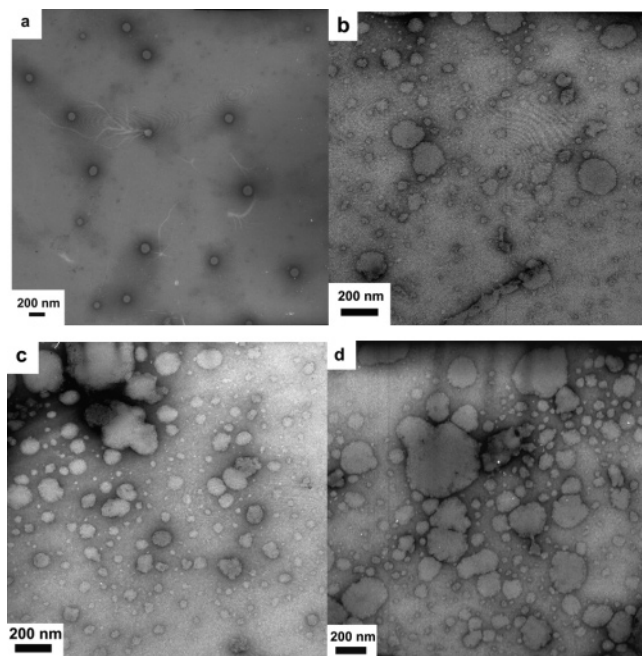
(14) Blokzijl, W.; Engberts, J. B. F. N. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1545–1579.

(15) Yin, H. Q.; Zhou, Z. K.; Huang, J. B.; Zheng, R.; Zhang, Y. Y. *Angew. Chem., Int. Ed.* **2003**, *42*, 2188–2191.

(16) Hoffmann, H.; Horbaschek, K.; Witte, F. *J. Colloid Interface Sci.* **2001**, *235*, 33–45.

(17) Chapman, D. In *Form and Function of Phospholipids*; Ansell, G. B., Hawthorne, J. N., Eds.; Elsevier: Amsterdam, 1973; p 117.

(18) Wikinson, D. A.; Nagle, J. F. In *Liposomes*; Knight, C. G., Ed.; Elsevier: Amsterdam, 1981; p 273.

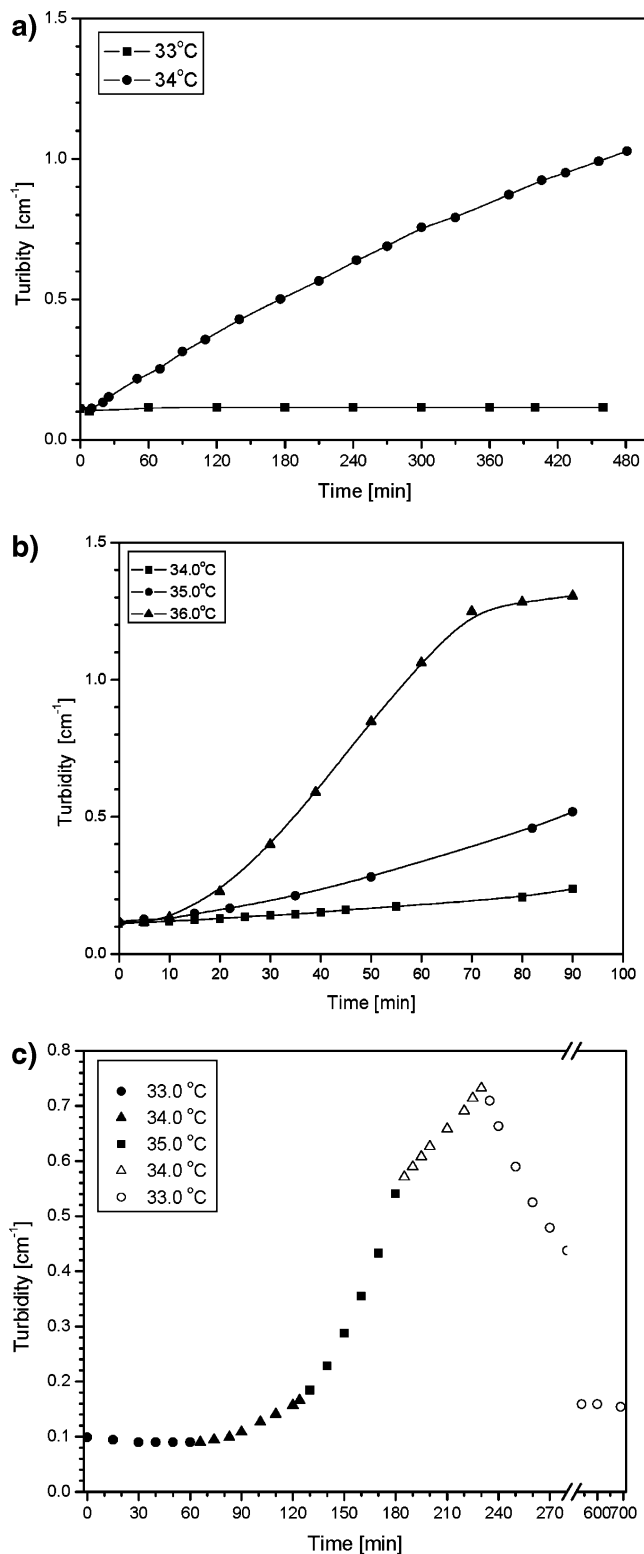


**Figure 1.** Micrographs by the negative-staining technique when the system SDS/DTBAB = 2:1,  $C_{\text{total}} = 10$  mM, was at 30 °C (a) and was heated to 34 °C for 2 h (b), 4 h (c), and 8 h (d), respectively.

vesicle aggregation was very scarce, if at all. Combined with the result of DLS (see Figure 1 in the Supporting Information), it can be demonstrated that spherical vesicles with the average hydrodynamic radius ( $\langle R_h \rangle$ ) of 60 nm were the dominating aggregates in the system. The zeta potential of the system was about  $-96$  mV at 30 °C, which indicates that the electrostatic repulsion between vesicles was relatively strong.<sup>19</sup> This kind of repulsive force together with the repulsive hydration force between the hydration layers of vesicles<sup>20</sup> may be responsible for the stability of the system.

As the temperature changed from 30 to 33 °C, the turbidity dropped a little and there was no obvious change in the system. However, when the temperature increased to 34 °C, the turbidity began to increase with time (Figure 2a). By TEM, gradual aggregation of vesicles was observed (Figure 1b–d), which reveals that the increase of the turbidity was attributed to the vesicle aggregation at the critical temperature, 34 °C (named as  $T_c$  hereafter). In addition, the formation of larger vesicles by vesicle fusion can also be noticed during the process of vesicle aggregation.

It is interesting to find that temperature variation above  $T_c$  had great influences on the vesicle aggregation rate. In fact, the turbidity still increased slowly after 12 h at 34 °C, indicating that the vesicle aggregation rate was relatively slow at this temperature. However, the increase of turbidity became much faster when further heating to 35 or 36 °C (Figure 2). It just took  $\sim 75$  min for the turbidity to reach the maximal value when the system was heated directly from 33 to 36 °C (Figure 2b), which suggests that the vesicle aggregation rate was remarkably accelerated by the increase of temperature. Further evidence was provided by the results of the light scattering intensity of the system measured at 90° ( $I_{90}$ ; Figure 3). The temperature effect on the kinetics of vesicle aggregation above

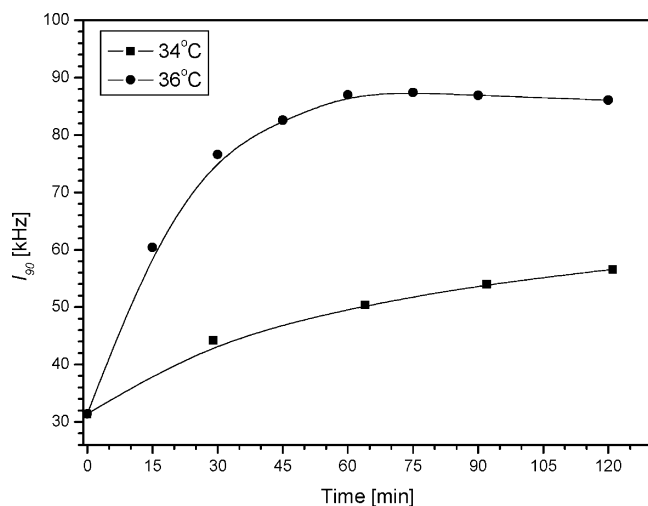


**Figure 2.** (a) Turbidity variations as a function of time at 33 and 34 °C for the system SDS/DTBAB = 2:1,  $C_{\text{total}} = 10$  mM. (b) Plots of turbidity versus time when the system was heated from 33 to 34, 35, and 36 °C for the system SDS/DTBAB = 2:1,  $C_{\text{total}} = 10$  mM. (c) Turbidity curve of a heating–cooling circulation as a function of time for the system SDS/DTBAB = 2:1,  $C_{\text{total}} = 10$  mM.

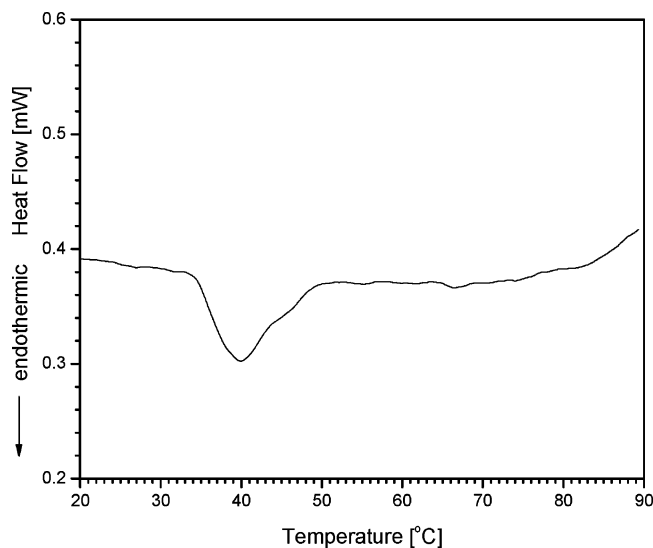
$T_c$  is clearly shown in Figure 3, which is coincident with the turbidimetrical results. From Figure 2c, it is also noteworthy that, even as the temperature decreased but still remained above 34 °C, the turbidity still went on increasing but with a slower rate. Only as the temperature

(19) Kawasaki, H.; Imahayashi, R.; Tanaka, S.; Almgren, M. *J. Phys. Chem. B* **2003**, *107*, 8661–8668.

(20) Tanford, C. *The Hydrophobic Effect: Formation of Micelles & Biological Membranes*; John Wiley: New York, 1980.



**Figure 3.** Variations of the light scattering intensity at 90° ( $I_{90}$ ) as a function of time when the system SDS/DTBAB = 2:1,  $C_{\text{total}} = 10$  mM, was heated from 33 °C to 34 and 36 °C.



**Figure 4.** DSC heating curve of the system SDS/DTBAB = 2:1,  $C_{\text{total}} = 10$  mM.

fell down to 33 °C did the turbidity begin to decrease, and finally it fell back close to the original value. Thus, the conclusion can be drawn that the vesicle aggregation process was irreversible above  $T_c$  while the vesicle disaggregation took place only below  $T_c$ .

DSC measurements were employed to monitor the heat effect of the temperature-induced vesicle aggregation in this system. An obvious endothermic peak with the outset temperature of 34 °C is shown in the DSC heating curve (Figure 4), suggesting that the transfer of enthalpy during the transition was positive. Considering the spontaneity of this transition, it can also be deduced that the vesicle aggregation was accompanied by an increase of entropy.

Further studies in this kind of system revealed that  $T_c$  was strongly dependent on the total surfactant concentration and the surfactant mixed molar ratio (Tables 1 and 2). Thus,  $T_c$  can be easily adjusted to the expected value by controlling one or both of these two factors. For instance, we can adjust  $T_c$  to 37 °C, which is close to the temperature of the human body, by changing the surfactant mixed molar ratio from 2:1 to 2.1:1 (SDS/DTBAB) at 10 mM (Table 2).

**Table 1.**  $T_c$  of Several SDS/DTBAB = 2:1 Mixed Systems with Different Total Surfactant Concentrations

	$C_{\text{total}} = 8.7$ mM	$C_{\text{total}} = 10$ mM	$C_{\text{total}} = 15$ mM	$C_{\text{total}} = 17$ mM
$T_c$	30 °C	34 °C	47 °C	49 °C

**Table 2.**  $T_c$  of Several  $C_{\text{total}} = 10$  mM SDS/DTBAB Mixed Systems with Different Molar Ratios (SDS/DTBAB)

	1.8:1	2.1:1	2.3:1	2.5:1
$T_c$	29 °C	37 °C	44 °C	49 °C

It is worth noting that the zeta potential of the solution was almost constant from 30 to 34 °C, indicating that there was no significant change of the electrostatic repulsion during the vesicle aggregation process.<sup>19</sup> Usually the repulsive hydration force is thought to be weakened upon heating, which may facilitate the approaching of vesicles.<sup>21,22</sup> However, the variation of the hydration force is insufficient to induce the aggregation of charged vesicles in ionic systems. Therefore, another kind of inter-vesicular attraction may exist in the system which will overcome the repulsive forces to induce the vesicle aggregation above  $T_c$ . It was found that no such temperature-controlled vesicle aggregation occurred in the mixed systems of SDS/*n*-dodecyltrimethylammonium bromide (DTMAB), SDS/*n*-dodecyltriethylammonium bromide (DTEAB), or SDS/*n*-dodecyltripropylammonium bromide (DTPAB) (see Figure 2 in the Supporting Information), indicating that the relatively large and hydrophobic headgroup (tributylammonium) of DTBAB may play an important role in this transition. In fact, owing to the steric restriction, it is not feasible for all three of the butyl groups to bend toward the inner hydrophobic region of the vesicle bilayer, and some butyl chains will stretch out to contact with water. Previous studies revealed that the cross-link of the exposed butyl groups due to the hydrophobic interaction among them was considered to account for the heating-induced micelle aggregation in some ionic surfactant systems using tetrabutylammonium as the counterion.<sup>23–25</sup> Therefore, it is reasonable to think that the hydrophobic interaction among the exposed butyl groups also make a significant contribution to the vesicle aggregation in our case. When the temperature reaches  $T_c$ , the exposed butyl groups of adjacent vesicles may link together to induce the aggregation of vesicles. As a result, the hydrated water molecules previously surrounding these hydrophobic groups will be released,<sup>10,19</sup> which may be responsible for the increase of entropy during the transition. Further study is still going on in our lab.

It is for the first time reported that a temperature-controlled vesicle aggregation occurred in a surfactant system. The critical temperature can be adjusted by the variations of the total surfactant concentration and the surfactant mixed ratio. Moreover, the temperature variation above  $T_c$  can remarkably alter the kinetics of vesicle aggregation. This work provided a simple and effective way for the controlling of vesicle aggregation by temperature variation. We hope it may also advance the understanding on the inter-aggregate interactions and promote corresponding applications in other fields.

(21) Hayter, J. B.; Zulauf, M. *Colloid Polym. Sci.* **1982**, *260*, 1023–1028.

(22) Metcalf, D. J.; Tiddy, G. J.; Waring, L.; Bostock, T.; MacDonald, J. *J. Chem. Soc., Faraday Trans. 1* **1983**, *79*, 975–1000.

(23) Kumar, S.; Sharma, D.; Din, K. U. *Langmuir* **2000**, *16*, 6821–6824.

(24) Buckingham, S. A.; Garvey, C. J.; Warr, G. G. *J. Phys. Chem.* **1993**, *97*, 10236–10244.

(25) Bales, B. L.; Zana, R. *Langmuir* **2004**, *20*, 1579–1581.

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**Supporting Information Available:**  $R_h$  distribution of the mixed system (SDS/DTBAB = 2:1,  $C_{\text{total}} = 10$  mM) at 30 °C measured by DLS and turbidity heating curves of the vesicular systems of SDS/DTEAB and SDS/DTPAB. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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