ЖЭТФ, 2012, том 142, вып. 6 (12), стр. 1258–1264 © 2012

PHASE BEHAVIOR OF DODAB AQUEOUS SOLUTION

V. P. Voronov, V. N. Kuryakov, A. R. Muratov*

Oil and Gas Research Institute, Russian Academy of Sciences
119991, Moscow, Russia

Received May 29, 2012

Phase behavior of DODAB aqueous solution, prepared without sonication, was studied by adiabatic scanning calorimetry. Measurements revealed four phase transitions with the temperatures 35.2, 39.6, 44.6, and 52.4 °C at heating and one transition at the temperature 40.4 °C at cooling. The first three transitions at heating occur in unilamellar vesicles. The first and third transitions correspond to the subgel–gel and gel–liquid phase transitions, corresponding enthalpy jumps are equal to 33 and 49 kJ/mol. The second transition appears after some aging and is similar to gel–ripple phase transition in a DPPC solution, with the enthalpy jump under the transition exceeding 7.4 kJ/mol. The transition occurs in unilamellar vesicles. The transition at the temperature 52.4 °C occurs in another subsystem of the solution, which we believe to be multilamellar vesicles. The enthalpy jump at this transition is equal to 97 kJ/mol, and data analysis suggests that this is a subgel–liquid transition. The phase transition at cooling is the liquid–gel transition in unilamellar vesicles. During the measurements, a slow evolution of the solution occurs, consisting in a change of concentrations of unilamellar and multilamellar vesicles. This transformation mainly occurs at low temperatures.

1. INTRODUCTION

Phase behavior of bilayer lipid membranes in water has been attracting significant attention in the last decades. The best studied systems are solutions of DPPC, DMPC, DOPC, and DODAB [1, 2]. Dioctadecyldimethylammonium bromide (DODAB) is a synthetic lipid having a polar head group and two tails consisting of 18 chains. In the concentration range 1–10 mM/L, it forms vesicular solution in water, which can be prepared by dissolving DODAB powder in hot water. A vesicle is a closed membrane consisting of one or several bilayers that surround a volume of solvent. Small vesicles are almost spherical due to the elastic energy. Various information about the bilayer structure was obtained experimentally by X-ray and neutron scattering, nuclear magnetic resonance, spectroscopy, and volumetry [1]. Although the details of transitions in various lipids are different, the behavior is largely similar for substances having close lengths of lipid tails. Despite a large number of papers, important questions concerning the phase behavior remain unclear.

Several phase transitions occur in the bilayer in the temperature range 0–55 °C. For example, four transitions can be observed at heating, they are referred to as sub- , pre-, main, and post-transitions. Such behavior is typical of the well-studied solution of DPPC, and we call the bilayer phases the same they are called in this solution. DPPC has two lipid tails with 16 chains and DODAB tails have 18 chains, and therefore temperatures of transitions in these substances are slightly different. In most experiments, two transitions with the temperatures 35° and 45 °C were observed in DODAB solution, a subgel–gel transition and a gel–liquid transition, which is called as main transition [2]. Bilayer phases have different ordering of molecules. In the high-temperature phase, the membrane is liquid; in gel, the phase membrane has a crystalline order close to hexagonal, and the low-temperature phase is a denser crystalline phase. We note that the structure of an isolated DODAB bilayer is not well studied.

In several experiments, an additional phase transition was observed at the temperature 52 °C [3–6] or only one transition at the temperature 45 °C was registered [7] (the main transition). The discrepancies in the experimental results appear because the state of the bilayer depends not only on the DODAB concentration and temperature but also on the solution preparation procedure and aging time at a given temperature. Recent measurements [8] demonstrated that

*E-mail: muratov@ogri.ru

there is no transition at 52 °C in the DODAB solution prepared without sonication if its concentration is less than 1 mM/L. This transition was observed in the solution with the concentration 10 mM/L, but it disappeared after sonication during 5 minutes [5] or extrusion of the solution through the filter with the channel size 400 nm [2, 7].

Sonication provides a simple way to obtain a reproducible initial state of lipid dispersion. The drawback of sonication is that the relation between the resulting state and the equilibrium state of dispersion is not clear. Moreover, according to our results, the average size of the DODAB vesicle after sonication at high temperatures is about 30 nm. This makes finite-size effects very important and, for example, prevents the study of the phase transition type, the near-transitional behavior, etc.

In this paper, phase behavior of a DODAB aqueous solution is studied by adiabatic scanning calorimetry. Such kind of research is rare recently (see [9]). In this method, unlike in popular differential scanning calorimetry, the temperature scan rate can be much lower, which allows studying the system near the equilibrium. Since the enthalpy is a measurable value, it is possible to determine the latent heat with a higher accuracy. In this paper, a series of cooling–heating cycles of the solution was done during 42 days. The solution was cooled to 3 °C and 25 °C, aged at these temperatures, and then heated to 55–60 °C. We classified phase transitions, determined the transformation of the system during our measurements, and estimated the enthalpy jumps at all transitions.

2. EXPERIMENT

The design of the adiabatic calorimeter setup and the method of measurements were described in detail in [10, 11]. The volume of a titanium calorimetric cell was about 6 mL, a steel magnetic stirrer was placed in the cell. The solution temperature was measured by a platinum resistor thermometer. The temperature scan rate during the measurements was 1.5–2 K/h. The solution was prepared by the "hot-water" method from DODAB produced by Acros Organics, Belgium, with the purity 99 %, and bidistillated water. DODAB was placed in the calorimetric cell with water, having the temperature about 60 °C, and was stirred slowly during 1.5 hours. Then the cell was placed in the calorimeter at room temperature. The volume of the prepared solution with the concentration 7.24 mM/L was 5.6 mL. Neither sonication nor extrusion through micro channels were used at the solution preparation. Before the measurements, the solution was held at the temperature 3 °C for several hours.

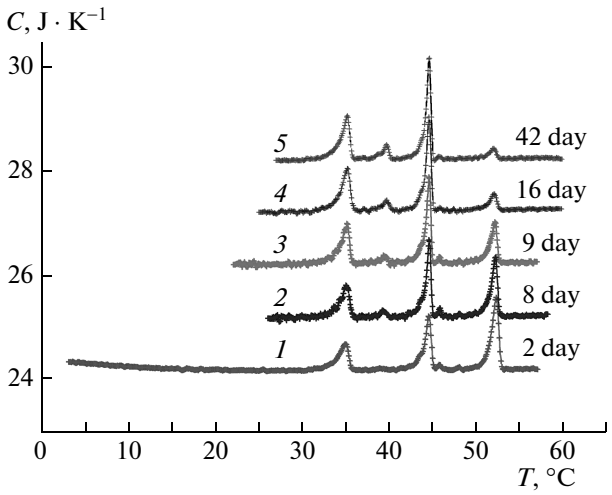

Fig. 1. Heat capacity of DODAB solution for five scans at heating from 3 °C to 60 °C

The presence of several phase transitions in a narrow temperature range makes the extraction of the singular part of enthalpy rather complicated. To simplify this procedure, the regular part of the enthalpy was calculated from the regular part of heat capacity, which is close to the heat capacity of water. The singular part of the enthalpy was determined as the integral of the difference between the complete heat capacity and its regular part.

Figures 1 and 2 present the results of measuring the heat capacity and the enthalpy of the DODAB solution at heating from 3 °C. For clarity, the heat capacity curves are shifted by 1 J/K with respect to each other; they are numbered in the order of measurement. The total measurement time was about 42 days, the date of measurement is shown on right of the curves.

Three peaks in the heat capacity corresponding to three phase transitions in the solution with the temperatures 35.2, 44.6, and 52.4 °C are present in all curves in Fig. 1. The values of transition temperatures were determined from the maximums of the heat capacity. Curve *1* was measured at permanent stirring of the solution. Comparison of the measurement results demonstrated that the stirring does not change the results, but leads to an increase in the noise level, and therefore subsequent measurements were done without mixing. Curves *2*–*5* in Fig. 1 present the heat capacity at temperatures above 25 °C. Phase transitions in the solution were not observed in the temperature range

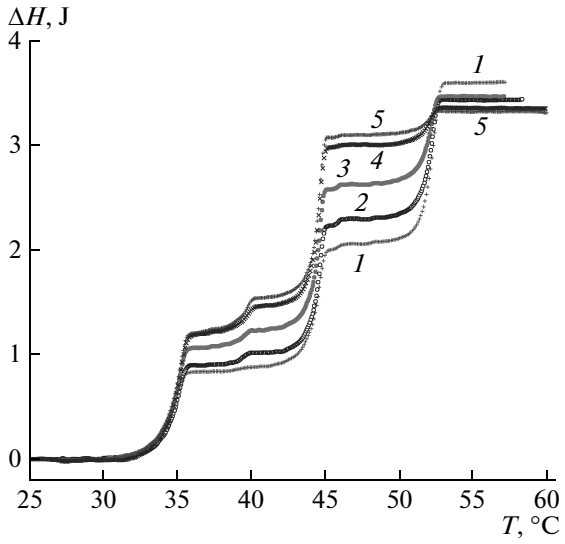

Fig. 2. Enthalpy of DODAB solution for scans at heating from 3 °C to 60 °C

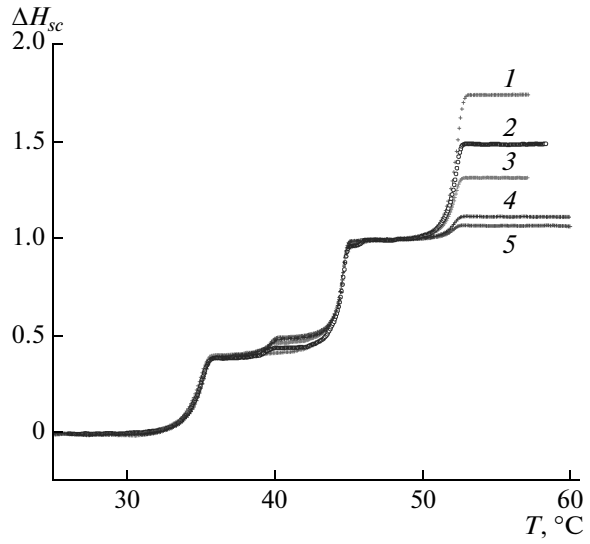

Fig. 3. Rescaled enthalpy ΔH_{sc} from the previous plot

3–25 °C (curve *1*), where the heat capacity is close to the heat capacity of water. Hence, this temperature region was passed with the scan rate 10 K/h without measurements at runs *2–5*.

We can see from Figs. 1 and 2 that the solution evolves during the 42 days of measurements. Anomalies of the heat capacity in the region below 50 °C increase, whereas the anomaly at 52.4 °C decreases. In addition, a new phase transition at the temperature 39.6 °C appears in curves *2–5*.

All anomalies of the heat capacity below 50 °C represent a sequence of phase transitions in one statistical subsystem. To prove this, it suffices to plot the rescaled enthalpy $\Delta H_{sc} = \Delta H_n / \Delta H_n (T = 47\,^{\circ}\mathrm{C})$, where ΔH_n is the enthalpy in curve n (see Fig. 3). Curves in this plot coincide below 50 °C (except in the temperature range 40–45 °C; see below) and diverge above 52.4 °C. According to known results [2], this subsystem is unilamellar vesicles (ULV). This means that the transitions at temperatures below 50 °C are successive transitions in unilamellar vesicles. The enthalpy of unilamellar vesicles varies proportionally to the amount of such vesicles in the solution. Analogously to transitions in DPPC, these transitions can be identified as subgel–gel and gel–liquid transitions (see [1, 2]).

The heat capacity anomaly at 52.4 °C corresponds to a transition in another subsystem. It is known that multilamellar vesicles (MLV) appear in DODAB solution as the concentration increases above 10 mM/L [6]. Most probably, the transition at 52.4 °C is a phase transition in MLVs. It is not a transition of ULVs to MLVs, as it was supposed in [6], because the curves in Fig. 3 should then coincide everywhere. We note that the phase transition at 52.4 °C has not been observed or has been strongly suppressed in solutions prepared with ultrasonic mixing or extrusion [5, 7]. Apparently, intense mixing destroys the MLVs present in bare solution.

The type of polymorphism analogous to the one observed in DODAB was reported in [12]. In both cases, the solution consisted of two subsystems with different sequences of phase transitions. A transformation of the solution from one state to another occurs during annealing at low temperatures.

In our case, the bare solution contains both ULVs and MLVs. During the measurements in the solution with concentration 7.24 mM/L, the fraction of ULVs increases and the fraction of MLVs decreases, which results in the corresponding change of the solution enthalpy (see Fig. 4). Our experimental data can be successfully described under the assumption that DODAB is contained in solution in two subsystems. The fit of enthalpy data allows determining the concentrations of the ULV phase in solution and the values of the enthalpy jumps at all phase transitions. For five experimental runs, these concentrations are equal to 60, 68, 78, 90, and 94 %; the mean-square error of these values is 2.2 %. The enthalpy jumps at transitions with the temperatures 35.2, 44.6 and 52.4 °C at heating are equal to 33 ± 1.5, 49 ± 1.5, and 97 ± 4.4 kJ/mol.

A weak phase transition at the temperature 39.6 °C

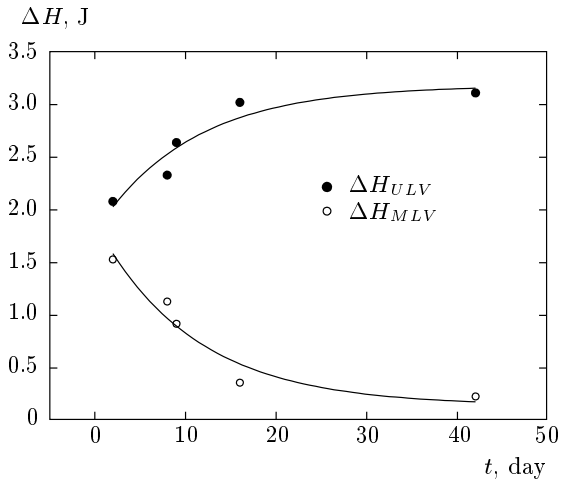

Fig. 4. Evolution of sum of the enthalpy jumps at three transitions in ULV and the jump at the transition in MLV. Curves are presented to demonstrate the tendency of variation

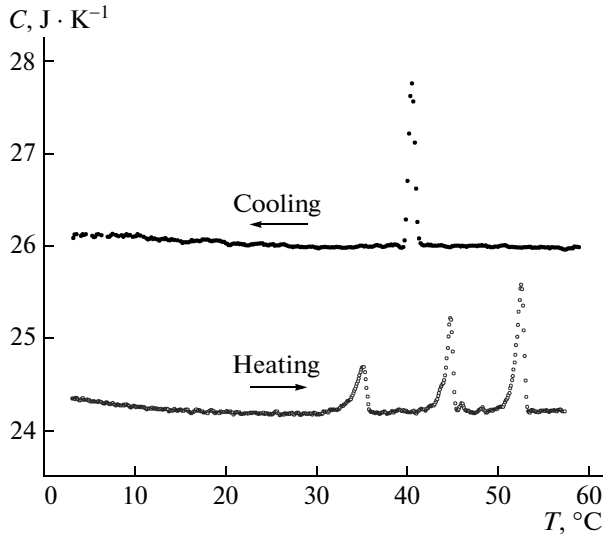

Fig. 5. Heat capacity of the solution at heating and cooling

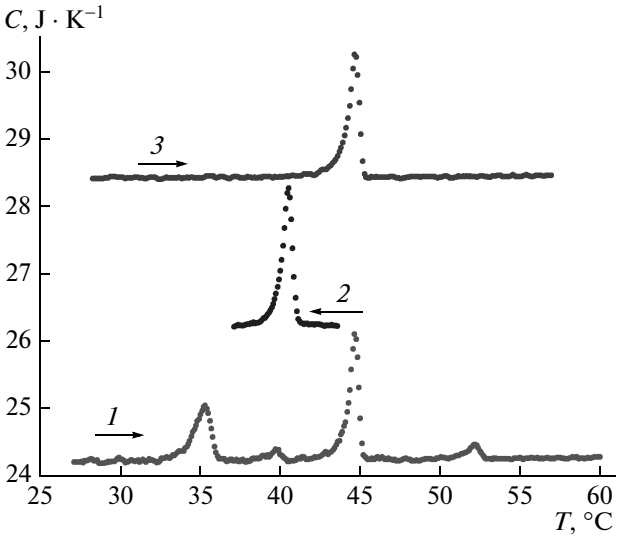

Fig. 6. Heat capacity of the solution at the additional scan with cooling to 25 °C

appears at heating in curves *2–5*. This transition strongly resembles the transition from gel to a ripple phase in DPPC. As shown in Fig. 2, the enthalpy jump at this transition increases with time, whereas the enthalpy jump at the temperature 52.4 °C decreases. In the last run, the enthalpy jump on the gel–ripple phase transition is equal to 0.3 J, whereas the enthalpy jump at the transition at 52.4 °C was 0.2 J, and therefore transition at the temperature 39.6 °C occurs in ULV subsystem. We can see that the enthalpy curves in Fig. 3 do not coincide in the temperature range of the ripple phase stability. This means that the gel–ripple phase transition occurs only in a part of ULVs. According to our data, the ratio of the enthalpy jump at this transition to the jump at the gel–liquid transition is equal to 0.15, which is close to the value of the enthalpy jumps ratio at gel–ripple phase and gel–liquid transitions for DPPC [9], equal to 0.14. If the observed phase transition in DODAB is a gel–ripple phase transition, the theories of this transition, based on taking the interaction between different bilayers into account are not applicable in this case.

At cooling of the solution starting from 60 °C, where the bilayer is in liquid phase, the only phase transition at the temperature 40.4 °C was always observed (Fig. 5). A close value of this transition temperature was obtained by other authors [4, 7], who used significantly higher rates of temperature variation. Since the ULV phase concentration was varying during the experimental cycle 3 °C–57 °C–3 °C, another experimental run was done to study the state of the system below 40.4 °C at cooling. This cycle included intermediate cooling of the solution from 60 °C to 25 °C and subsequent heating to the initial temperature (see Figs. 6 and 7).

Curve *1* in Fig. 6 coincides with curve *4* in Fig. 1. After cooling to 25 °C, the solution was aged at this temperature for 6 days. According to our observations, the main change in fractions of the ULV and MLV phases in the solution occurs at low temperatures. At heating from 25 °C to 60 °C (curve *3*), the only

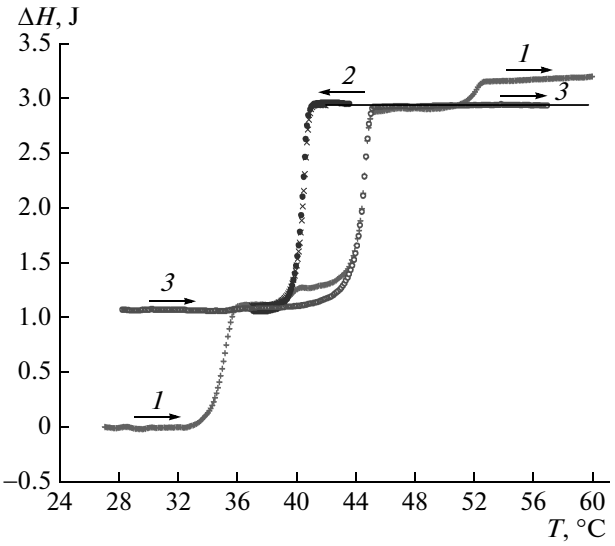

Fig. 7. Enthalpy of the solution at the additional scan with cooling to 25 °C

phase transition at the temperature 44.6 °C, coinciding with the temperature of ripple phase–liquid transition in curve *1* was observed. The heat capacity anomaly at 52.4 °C was not observed, and therefore the crystalline phase did not appear in MLVs at the cooling of the solution to 25 °C. Most probably, the reason is that the corresponding transition in the MLV phase has very slow kinetics, analogously to the transition gel–subgel in ULV at cooling [2, 6, 9].

The absolute value of the enthalpy jump at cooling (curve *2* in Fig. 7) coincides with the enthalpy jump at the subsequent heating of the solution from 25 °C (curve *3*) and is equal to the sum of the enthalpy jumps at the gel–ripple phase and ripple phase–liquid transitions at heating from 3 °C (curve *1*). The temperature remained above 25 °C during the cycle and the ULV phase concentration did not change. Thus, we see that the transformation of the ULV to the MLV phase occurs mainly at low temperatures.

We conclude that the transition at 40.4 °C at cooling is a phase transition in ULVs from a liquid phase to gel. The value of the transition temperature was the same and practically independent of the cooling rate, and therefore this temperature is close to the temperature of stability loss of the bilayer liquid phase in ULVs. Thus, the liquid phase in ULVs can be overcooled by 4.2 °C. We note that the observed transition temperature 40.4 °C at cooling is close to the temperature of the phase transition occurring at heating in ULVs, which is equal to 39.6 °C. In all measurement cycles, these temperatures were constant.

3. LIGHT SCATTERING

To verify some of the above statements, an additional light scattering study of the DODAB aqueous solution was fulfilled. The solution with the same concentration 7 mM/L was prepared in the same way as for the calorimetric study. Before the measurements, the solution was cooled to 0 degrees and aged at this temperature for one hour.

Figure 8 presents the scattered light intensity of the solution as a function of temperature at the heating and subsequent cooling of the system. The measured scattered light intensity jumps at the temperatures corresponding to phase transitions. In principle, interpreting the light scattering data is difficult because the scattered intensity depends on numerous details. In general, the intensity of scattered light is a decreasing function of the temperature. We believe that this behavior is a result of a decrease in the difference between the refractive indices of DODAB and water at heating. The refractive index of water can be written approximately as $n_w \approx 1.332 - 0.000167\,(T - 20)$ [13]. The DODAB refractive index is equal to 1.42 at 20 °C and its derivative with respect to the temperature is ~ -0.00055.

The refractive index of DODAB in crystalline phases is larger than in the liquid phase. This results in drops of the scattered light intensity at the temperatures corresponding to phase transitions. At cooling, the phase transition at 40 °C appears as an increase in intensity. We note that the observed behavior at heating is well reproducible, but the peculiarity at the phase transition at cooling can look like a drop with a subsequent increase in intensity. Thus, the subgel–gel and gel–liquid phase transitions in ULV and the subgel–liquid transition in MLV at heating as well as the phase transition at cooling are easily observed by light scattering measurements. The transition at 40 °C at heating is weak and is not clearly seen. The value of the intensity drop at 52.4 °C is approximately equal to 70 % of the total intensity decrease in the relevant temperature range. The fraction of the MLV subsystem in the solution is therefore large.

Curve *3* in Fig. 8 presents the scattered light intensity of the same sample, obtained after the ultrasonic stirring of the solution at the temperature 75 °C during 5 minutes. The total intensity is significantly lower. The phase transition at 52.4 °C is hardly visible because the corresponding (MLV) subsystem is destroyed. We note that we did not observe the transition at 52.4 °C in the dilute DODAB solution with the concentration 0.7 mM/L, which means that the MLV

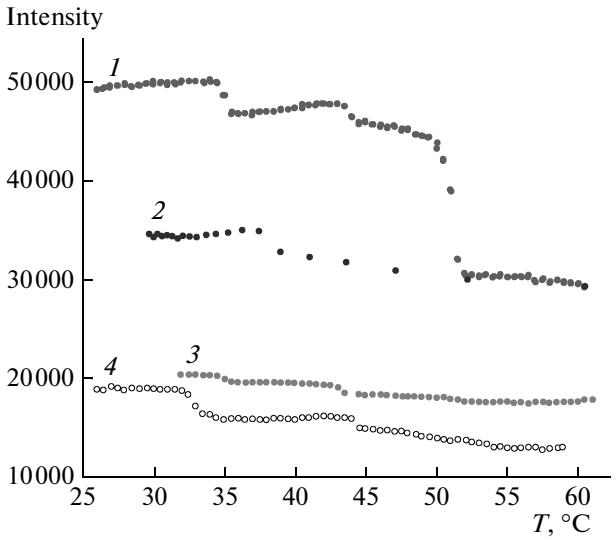

Fig. 8. Light scattering intensity as a function of temperature. Points *1* and *2* correspond to heating and cooling of the solution 7 mM/L, *3* — heating of the same solution after sonication, *4* — heating of dilute solution $c = 0.7$ mM/L

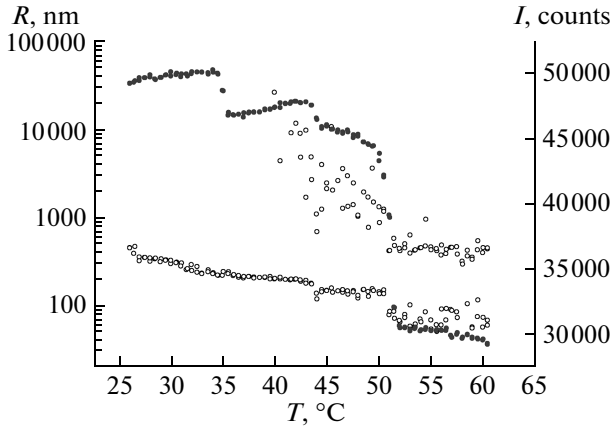

Fig. 9. Light scattering intensity and size of vesicles as a function of temperature. Open circles present the hydrodynamic radius of particle, obtained at heating

subsystem does not appear in the low-concentration solution (see curve *4*).

Dynamic light scattering of the solution was performed on a photon correlation spectrometer Photocor-Complex [14] at a scattering angle 90 degrees. The measurement results are presented in Fig. 9. Typically, the size distribution has two maximums, corresponding to small and large vesicles. The size values at the maximums decrease as the temperature increases. We believe that small vesicles are unilamellar. Large vesicles can be both ULV and MLV, they remain in the solution after sonication, when the MLV subsystem practically disappears. The scattered light intensity strongly depends on the scattering angle, which proves that the observed slow mode in the dynamic measurements is indeed the diffusion of large vesicles.

We note that the evolution of the solution occurring in calorimetric measurements was not observed in our light scattering study. We suppose that this evolution is a result of the change of ion concentration in solvent, which takes place in calorimetric cell.

4. CONCLUSION

We have for the first time used the adiabatic scanning calorimetry to study the phase behavior of a DODAB aqueous solution prepared without sonication. Measurements revealed four phase transitions at heating with the temperatures 35.2, 39.6, 44.6, and 52.4 °C and one phase transition at cooling at the temperature 40.4 °C. In general, such behavior is analogous to the behavior of the well-studied DPPC solution. The first three transitions at heating occur in ULVs. The first and third transitions correspond to the subgel–gel and gel(ripple phase)–liquid transitions; the enthalpy jumps at these transitions are 33 ± 1.5 and 49 ± 1.5 kJ/mol. The second phase transition at the temperature 39.6 °C was not observed in fresh solution, but it appears later. The enthalpy jump at this transition exceeds 7.4 kJ/mol. The ratio of this jump to the enthalpy jump at the gel–liquid transition is approximately equal to 0.15, which is close to the enthalpy jump ratio at gel–ripple phase and gel–liquid transitions for the DPPC solution, equal to 0.14 [9]. This phase transition occurs in ULVs.

The transition at the temperature 52.4 °C occurs in another statistical subsystem of the solution, and we believe that this is the MLVs. The enthalpy jump at this transition is equal to 97 ± 4.4 kJ/mol, and the analysis of our observations suggests that it is most probably a subgel–liquid phase transition. Both the main transition in ULVs and the subgel–liquid transition in MLVs are first-order phase transitions; the first of them can be overcooled by 4.2 °C. Most probably, the subgel–gel transition in the ULV phase is also a first-order phase transition, because fitting of the heat capacity for a critical behavior provides the exponent value larger than unity. Since the gel–ripple phase transition in ULVs is weak, it is impossible to determine its order from our data.

Only one phase transition was observed at cooling of the solution from 60 °C. This transition at 40.4 °C is a liquid–gel transition in ULVs. The absolute value of the enthalpy jump at this transition is equal to the enthalpy jump at the gel–liquid phase transition at heating if the ULV concentration remains constant. Transitions to the subgel phase in unilamellar and multilamellar vesicles at cooling have a large relaxation time, which does not allow observing them by adiabatic calorimetry. These transitions can be observed at heating from 3 °C, when the relaxation time is small. Slow evolution of the solution prepared without intense mixing occurs during the measurements process. It consists in a change of concentrations of unilamellar and multilamellar vesicles. The fraction of the unilamellar phase in the DODAB solution with concentration 7.24 mM/L increased from 60 to 94 % during the 42 days of measurements.

In general, the phase state of DODAB solution reveals a strong hysteresis and depends not only on the concentration and temperature but also on the sample preparation protocol and the history of temperature variation. It follows that the phase behavior depends on the temperature range and the temperature change rate at the measurement process. At cooling, the gel–subgel transition in ULVs and the liquid–subgel transition in MLVs allow large overcooling and have slow kinetics. To observe the complete phase behavior of the solution with four phase transitions, it was necessary to use runs with cooling to the temperature 3 °C and aging of the solution at low temperatures for hours. For runs in the temperature range 20–60 °C, only the transition between gel and the liquid phase in ULVs can typically be observed.

The use of ultrasonic mixing or extrusion allows obtaining a reproducible uniform state of lipid solution, but in general this state is far from equilibrium and relaxes very slowly. This fact and finite-size effects make the study of near-transitional behavior in sonicated systems senseless.

We are indebted to T. Narayanan from ESRF for supplying us with unpublished data on the DODAB bilayer structure and the useful discussions. We thank E. Kats and V. Lebedev for the helpful discussions. This work was supported in part by the RFBR (Grants №10-08-01057-a (V. V.) and 09-02-01346-a (A. M.)).

REFERENCES

1. J. F. Nagle and S. Tristram-Nagle, Biochim. Biophys. Acta **1469**, 159 (2000).
2. P. Saveyn, P. Van der Meeren, M. Zackrisson et al., Soft Matter **5**, 1735 (2009).
3. C. R. Benatti, M. J. Tiera, E. Feitosa et al., Thermochem. Acta **328**, 137 (1999).
4. J. Cocquyt, U. Olsson, G. Olofsson et al., Colloid Polym. Sci. **283**, 1376 (2005).
5. R. O. Brito and E. F. Marques, Chem. Phys. Lipids **137**, 18 (2005).
6. L. Coppola, M. Youssry, I. Nicotera et al., J. Colloid Interf. Sci. **338**, 550 (2009).
7. E. Feitosa, P. C. A. Barreleiro, and G. Olofsson, Chem. Phys. Lipids **105**, 201 (2000).
8. E. Feitosa, F. R. Alves, E. M. S. Castanheira et al., Colloid Polym. Sci. **287**, 591 (2009).
9. M. Kodama, H. Hashigami, and S. Seki, Biochim. Biophys. Acta **814**, 300 (1985).
10. E. E. Entov, V. A. Levchenko, and V. P. Voronov, Int. J. Thermophys. **14**, 221 (1993).
11. V. P. Voronov, Zh. Eksp. Teor. Fiz. **118**, 163 (2000).
12. H. Aoki, T. Koto, and M. Kodama, J. of Thermal Analysis and Calorimetry **64**, 299 (2001).
13. *Handbook of Optical Materials*, ed. by M. J. Weber, CRC Press LLC (2003).
14. http://www.photocor.com.