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Research paper

Self-assembly of micelles in organic solutions of lecithin and bile salt: Mesoscale computer simulation

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ABSTRACT

We propose a coarse-grained model for studying the effects of adding bile salt to lecithin organosols by means of computer simulation. This model allows us to reveal the mechanisms of experimentally observed increasing of viscosity upon increasing the bile salt concentration. We show that increasing the bile salt to lecithin molar ratio induces the growth of elongated micelles of ellipsoidal and cylindrical shape due to incorporation of disklike bile salt molecules. These wormlike micelles can entangle into transient network displaying perceptible viscoelastic properties.

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1. Introduction

There are two main classes of biological surfactants: phospholipids and bile salts. Phospholipids are a major component of cell membranes and bile acids play an important role in several physiological processes, such as removal of excess cholesterol, fat emulsification and solubilization of lipids in the bowel [1–7]. A typical representative of phospholipids is phosphatidylcholine, a zwitterionic surfactant, with polar groups and two hydrocarbon chains (see Fig. 1a). The polar part carries both negative and positive charges localized on the choline and phosphate groups, respectively. Phospholipids generally self-assemble in organic solvent into inverted spherical or short cylindrical micelles. Bile acids and their salts belong to the class of steroids and are often mentioned as facial amphiphiles [1–7]. Their molecules are amphiphilic due to the presence of three fused cyclohexane rings and cyclopentane ring (forming a flat surface) and the polar groups in the α-position. Bile salt molecules have the Janus-type structure, i.e., their structure can be roughly represented as a flat hydrophobic surface where hydrophilic groups are attached from one side (see Fig. 1b). Such a structure distinguishes bile salts from other surfactants. Bile salts are insoluble in pure organic solvents, and in aqueous solution they form small micelles with low aggregation number [1–7].

Due to the good biocompatibility, lecithin vesicles prepared in an aqueous solution, as well as inverted micelles formed in various organic solvents can be used to deliver a wide class of drugs [7]. Vesicles in pharmacology are used to improve the solubility of hydrophobic drug molecules, while the inverted micelles serve for uniform admission and release of drugs within a human body due to partial crystallization of a micelles surface in an aqueous environment. Lecithin-based organogels are used for transdermal drug delivery as topical medication [5–7,8,9].

Self-assembly of biological surfactant has potentially wide range of practical applications. The lecithin-based organogels attract much interest due to the good biocompatibility and easy transformation between organosol and organogel states [10]. The addition of water is one approach to grow lecithin inverted micelles. In lecithin-oil-water systems aggregation morphology depends on the water-to-lipid ratio, chemical structure of organic solvent [11,12] and temperature in the system [10]. Bile salts [11] and small polar molecules [13] also can act as gelation agents [3]. In the last time a large amount of work has been done using molecular dynamics computer simulations for studying of AOT-water reverse micelles in organic solvents [14–18] and phosphatidylcholine reverse micelles in organic solvents [19,20]. These studies cover the micelles morphology [14–17], dynamic properties [18] and effect of hydration [17,19,20]. Our article is focused on studying morphology of lecithin micelles in organic solvents where bile salt plays the key role of gelation agent. A trace amount of water in organic solutions of lecithin cannot be removed by

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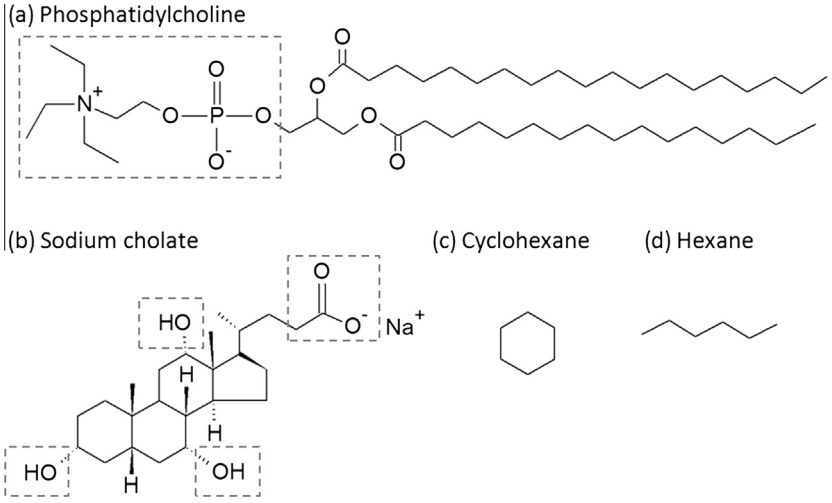


Fig. 1. Chemical structure of phosphatidylcholine (a), sodium cholate (b) and solvents: cyclohexane (c) and hexane (d). Dashed lines show hydrophilic parts of phosphatidylcholine and sodium cholate.

drying and may serve as relevant control factor in morphological transitions [10]. However, small amount of water (mole ratio about 1:1) does not have a significant effect on the self-assembly of lecithin in oil, the shape of lecithin micelles is nearly spherical and the viscosity does not increase [11]. Thus, we do not take into account trace amounts of water in our coarse-grained model, focusing only on the bile salt effect on the lecithin-oil mixture.

In the experimental work [11], the zero-shear viscosity of the lecithin/bile salt mixture in cyclohexane was studied as a function of the molar ratio of bile salt to lecithin (B_0). At low B_0 the solution has low viscosity while at B_0 around 0.4 the viscosity increases significantly. And finally, when B_0 is larger than 0.5, the phase separation in two coexisting liquid phases takes place. The impact of trace amount of water on the described effect was excluded. Only the addition of sufficient amount of bile salt can cause the growing of micelles. A qualitative explanation of observed phenomena was given in Ref. [11] on the basis of some simple geometrical model of wormlike micelles. However, the question about molecular mechanisms of formation of wormlike micelles still remains open.

In order to answer this question, we have studied the morphology of the organic solutions (with hexane and cyclohexane used as solvents) of phosphatidylcholine and sodium cholate by means of mesoscopic computer simulations based on the dissipative particle dynamics method [21,22] and on a coarse-grained model specially developed for this system. In contrast to the atomistic simulations [14–20], our coarse-grained model does not include specific interactions like hydrogen bonding, but only purely hydrophobic-hydrophilic interactions. As an alternative, a specific geometry of molecules in coarse-grained representation is the key part of our model. For computer simulation of large micellar systems one certainly needs appropriate mesoscale coarse-graining scheme [23–30]. Mesoscale simulations save CPU time due to accelerate equilibration on large scales, and coarse-grained configurations can be used for reverse mapping in order to the atomistic length scales where specific interactions can play a key role [23–30]. Our goal was to build the adequate coarse-grained mesoscopic model for lecithin-bile-oil mixture which can reproduce the experimental results [11] at least qualitatively and give explanation of system self-assembly mechanisms. Thus, this work is a theoretical extension of the experimental study [11].

2. Model and simulation technique

The dissipative particle dynamics (DPD) is the mesoscopic computer simulation method for studying structural and rheological properties of polymer melts and solutions [21,22]. The DPD method utilizes coarse-grained models and "soft" potentials of intermolecular interactions, and that allows selecting a sufficiently large time step in a difference scheme of Newton's equations and enables studying molecular systems on larger spatial (about 10–1000 nm) and time (about 0.001–1 s) scales as compared to the atomistic molecular dynamics. This method correctly represents the hydrodynamic behavior of a system. DPD method is based on solving Newton's equations for a model of molecular system constructed of spherical particles with diameter $\sigma = 1$ and mass $m_i = 1$ [22]:

$$\frac{d\mathbf{r}_i}{dt} = \boldsymbol{v}_i; \quad m_i \frac{d\mathbf{v}_i}{dt} = \mathbf{f}_i,$$

where $\mathbf{f_i}$, $\mathbf{r_i}$, m_i, $\mathbf{v_i}$ are the net force, coordinate, mass, and velocity of i-th particle, respectively. Each particle is associated with a group of small molecules, some fragments of macromolecules, or with a statistical segment (Kuhn segment). Multicomponent systems can consist of particles of several different types. The particle diameter is a unit of length scale and corresponds to an average volume V_{ref} of characteristic system fragments. The net force applied to each particle consists of five terms:

$$\mathbf{f}_i = \sum_{i \neq j} \left(\mathbf{F}_{ij}^b + \mathbf{F}_{ij}^a + \mathbf{F}_{ij}^c + \mathbf{F}_{ij}^d + \mathbf{F}_{ij}^r \right),$$

where the summation is performed over all particles within the cut-off radius r_c. The spring force $\mathbf{F}_{ij}^b$ takes into account the covalent bonds between coarse-grained particles in molecules. Its amplitude could be written as:

$$F_{ij}^b = -K_b(r_{ij} - r_0),$$

where the bond stiffness parameter K_b is equal to $4k_bT/\sigma^2$ (T – the absolute temperature, k_B – the Boltzmann constant, in the DPD method $k_BT = 1$) and r_0 is the unperturbed bond length. If the beads i and j are not connected, then $F_{ij}^b = 0$. The elastic force of bond angle deformation F_{ij}^a is used to take into account the intramolecular stiffness. The amplitude of F_{ij}^a is determined as follows:

$$F_{ij}^{a} = -K_a\left(\varphi_{ijk} - \varphi_0\right),$$

where the bond angle stiffness parameter K_a is $5k_bT/(\sigma \cdot rad)$, φ_{ijk} – the angle between particles i, j and k, φ_0 – the unperturbed angle value. The conservative force is the soft core repulsion between the particles i and j:

$$\mathbf{F}_{ij}^{c} = \begin{cases} a_{ij}(1 - r_{ij})\hat{\mathbf{r}}_{ij}, & r_{ij} < 1 \\ 0, & r_{ij} > 1 \end{cases}$$

$$\hat{\mathbf{r}}_{ij} = \mathbf{r}_{ij/r_{ij}},$$

where a_{ij} is the maximum repulsion force between beads i and j. The remaining two forces are dissipative force (F_{ij}^{d}) which takes into account the friction of an effective medium, and random force (F_{ij}^{r}) which describes the thermal motion of the system. The system density was chosen to be equal to $\rho = 3\sigma^3$ for correct description of the water isothermal compressibility [22]. For this density value the parameter a_{ij} can be expressed in terms of the Flory-Huggins parameter as $a_{ij} = 25 + 3.27\chi_{ij}$ [22]. The Flory-Huggins parameter describing the volume interaction between species α and β depends on the Hildebrand solubility parameter δ [31,32]:

$$\chi_{\alpha\beta}\frac{V_{ref}\left(\delta_\alpha - \delta_\beta\right)^2}{RT} - \chi_s$$

where R is the gas constant, χ_s is the entropic contribution to the mixing free energy; usually $\chi_s \sim 0$ and can be omitted. According to the Hildebrand theory, mutual solubility of non-electrolytes increases with decreasing difference between their solubility parameters [33–35]. The Hildebrand solubility parameter is a square root of a cohesive energy density:

$$\delta = \sqrt{\frac{\Delta H_v - RT}{V_m}},$$

where V_m is the system volume and ΔH_v is the heat of vaporization.

Computer simulations have been performed in canonical (NVT) ensemble for systems consisting of the following molecules: (1) lecithin (phosphatidylcholine), (2) bile salt (sodium cholate), and (3) solvent (cyclohexane or hexane). We have developed a coarse-grained model shown in Fig. 2. When choosing the fragments of molecules and assigning them to different beads (types of particles), it was important to take into account the proportion of sizes of lecithin and cholic acid molecules, as well as structural features of the bile salt molecule, namely its geometrical peculiarities (planar Janus-type structure). Five different coarse-graining schemes have been tested before we have found an appropriate model. The selected coarse-grained representation for bile salt looks like a 'backless stool', whose three legs (composed of hydrophilic groups $O^{(2)}$ and $O^{(3)}$) are connected to hydrophobic skeleton and fixed by introducing the potential on the valence angles φ and choosing $\varphi_0 = 90^\circ$ and $K_a = 150$. Both lecithin charged groups, choline and phosphate, were combined into a single coarse-grained polar particle P to maintain the correct balance between the size of polar and nonpolar groups. The symbols for coarse-grained particles and corresponding fragments are marked with different color in Fig. 2, and this color scheme will be used in the snapshots of the system below.

The Askadskii semi-empirical method was utilized to calculate the solubility parameters and volumes of molecular fragments, which correspond to the coarse-grained particles P, $O^{(1)}$, $O^{(2)}$, $O^{(3)}$, C, W [36]. The obtained values of solubility parameters have been checked by means of the atomistic molecular dynamics with PCFF force field [37] which allows to calculate the cohesive energy ΔH_v - RT. Almost similar values for δ_i have been obtained for all particles except P and $O^{(3)}$. For these coarse-grained particles the Askadskii method gives wrong values, because it does not take into account the contribution of electrostatic interactions to the total energy of a system. As a result, we have used the values δ_i obtained from the atomistic molecular dynamics. The characteristics of molecular fragments are presented in Table 1.

The simulation box of the size $L_x \times L_y \times L_z = 60 \times 60 \times 60\sigma^3$ with periodic boundary conditions containing 648,000 ($\sim 10^6$) particles was used, temperature was set to 300 K.

3. Results and discussion

The computer simulation of lecithin and bile salt solutions was performed for two organic solvents – hexane and cyclohexane. The effective length of micelles and concentration of surfactant are the main factors that influence the viscosity of surfactant solutions: $\eta_0 \sim l^3\varphi_s^{15/4}$, where l is the effective micelles length, φ_s is the volume fraction of surfactant [38]. The growth of elongated structures leads to their entanglement and formation of a dynamic network that is accompanied by viscosity increase. The presence of small ellipsoidal micelles as well as branched cylinders leads to the viscosity reduction. The volume fraction of lecithin was fixed $\varphi_{lec} = 0.05$, and the amount of bile acid salt was varied, while the

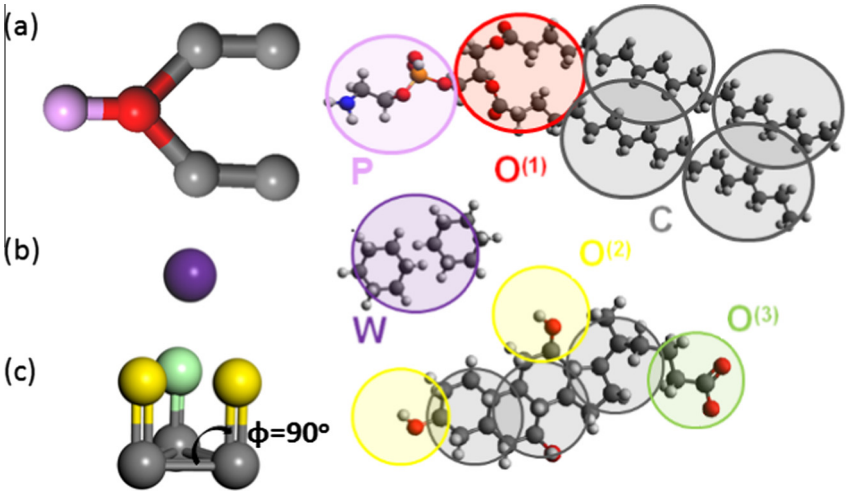
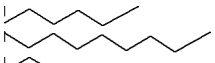
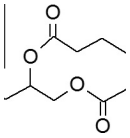
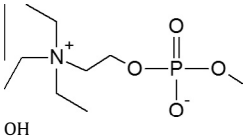


Fig. 2. The coarse-grained model of the three-component system: (a) lecithin; (b) organic solvent (cyclohexane); and (c) bile salt.

Table 1
Subsystems symbols, chemical structure of molecular fragments and their Hildebrand solubility parameter δ.

Subsystem	Structure	$\delta = \left(\frac{J}{cm^3}\right)^{\frac{1}{2}}$
W		15.5
C		16.3
W′		18.4
O(1)		21.2
P		39.4
O(2)	OH	41.0
O(3)		41.0

total volume fraction of all components, including the solvent was $\varphi_{total} = 1$. The bile salt to lecithin molar ratio $B_0 \in [0.133, 0.6]$ characterizes the composition of the system. The main parameters calculated during the simulation were the effective length of micelles, the percentage of large clusters and their volume fraction. At every set of input parameters, we have started from a random initial configuration. To control the system equilibrium process we monitored the system energy and the micelles size distribution. Typically, these observables did not vary with time after about 0.5 million simulation steps, and this was for us the criteria that the system has reached the equilibrium. The total simulation time was about 1.5–2 million simulation steps to accumulate statistic at equilibrium.

Fig. 3 shows the changes in the morphology of lecithin micelles in hexane solution upon increasing the molar ratio of bile salt to lecithin (B_0). Visual analysis shows that there is a trend towards reducing the micelles number and increasing their length due to aggregation of small micelles. As will be shown below, in our model bile salt molecules induce the transition from spherical to cylindrical micelles. Actually, there are two underlying mechanisms of such transition - the elongation (first to prolate ellipsoids and then to small cylinders) of existing spherical micelles of lecithin and their aggregation. Both mechanisms can be jointly explained by the free energy penalty associated to the two ends as being the driving force for micellar elongation [3].

Generally phospholipids tend to form inverted spherical micelles in organic solvents [10]. Visual analysis of molecular structure shows that lecithin molecules self-assemble in pure hexane solution into small micelles of approximately spherical shape (ellipsoidal shape with not very high asymmetry axes). The structure of the micelles in the lecithin/bile salts mixture in the organic solvent depends mainly on their molar ratio. Fig. 4a shows a particular micelle at the molar ratio $B_0 = 0.53$. Upon incorporation into the structure of inverted micelles of lecithin, bile salt molecules increase the volume of the core occupied by the hydrophilic groups of lecithin, while their contribution to increasing of the surface area occupied by the hydrophobic particles is less significant because the volume of the surface layer of a lecithin micelle is larger than the volume of its core. As a result, the volume of the micelle core grows faster than the volume of the surface layer, so that the surface layer looses its ability to cover the core of spherical shape. The need for increasing the effective surface of micelles causes the formation of highly elongated structures reminiscent of curved "flattened" cylinders (having elliptic cross-section). The non-polar beads of bile salt and lecithin form the surface contact-

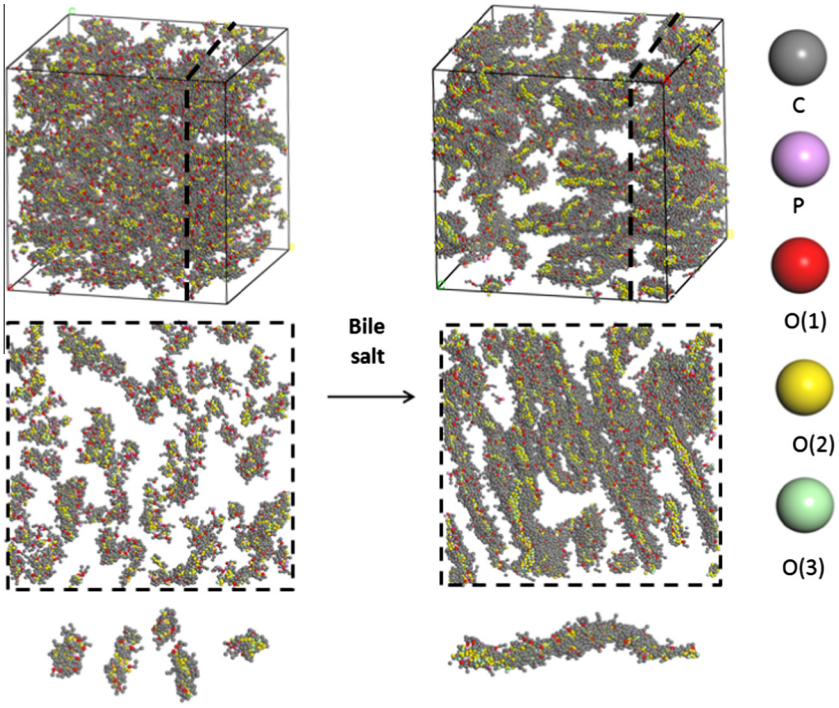


Fig. 3. Morphological changes in the lecithin-cyclohexane solution upon increasing the concentration of bile salt. The molar ratio of bile salt to lecithin is $B_0 = 0.13$ (on the left) and $B_0 = 0.53$ (on the right). The solvent particles are not shown. The bottom part of the figure demonstrates a section of the modeling cell by the plane shown as dotted line in the upper part of the figure.

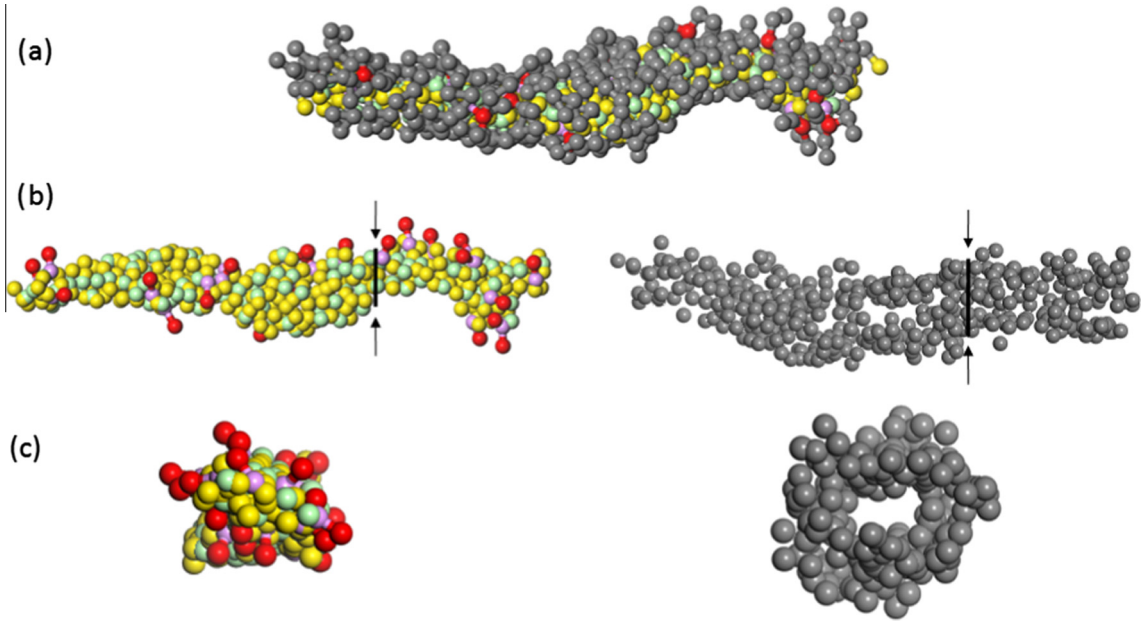


Fig. 4. The typical morphology of an elongated micelle formed by bile salt and lecithin in organic solvents: (a) all particles; (b) only polar groups (on the left) and only non-polar groups (on the right); and (c) cross-section of the micelle by a plane indicated by the arrows. The color scheme corresponds to Figs. 2 and 3.

ing with the solvent, while the polar groups are located in the insoluble core of the micelles (Fig. 4b and c).

Simple geometric considerations (quite similar to those presented in Ref. [11]) help to understand the observed processes. Due to special Janus-type structure the bile salt molecules contribute more significantly to increasing the volume of the surface layer of a spherical reverse lecithin micelle in comparison to the contribution to the volume of its core. Hydrophilic and hydrophobic groups in lecithin and bile salt molecules are joined together, and the maximum block length is equal to two (for lecithin molecular model). From molecular geometry of proposed model (see Fig. 2) it can be seen that the lecithin tends to form spherical micelles, while disklike micelles are the most favorable morphology for bile salt (due to the flat geometry of molecules). The volume ratio of the hydrophilic and hydrophobic particles in the case of pure lecithin solution is equal to two, and it depends on the molar ratio of bile salt to lecithin, in particular, it is equal to $7/5 = 1.4$ for $B_0 = 1$. What geometric objects do we get if all hydrophilic particles are completely covered by all hydrophobic particles? Let us consider the ideal case: there are only three types of possible objects – spheres, cylinders or disks. Hydrophilic core (interior) of these objects should be compact, so the sphere can have radius at most two (if two hydrophilic groups of lecithin molecules are pulled in a string), and the disk should have a thickness of the inner part equal to two. The radius of the cylinder also should be almost two (remember that the unit of length is the diameter of a bead). The energetically not favorable contribution from the edges of a cylinder and a disk will not be taken into account, therefore the length of a cylinder can be arbitrarily large, as well as the diameter of a disk. The large number of small objects (disks or cylinders) will be preferable in comparison to a single large object due to the entropic contribution to free energy. The core of any shape should be covered by a hydrophobic surface layer with a linear size about one. For spheres, the ratio of the surface layer volume V_{surf} to the volume of the core V_{core} is equal to 2.4 (almost the same as the volume ratio of hydrophobic to hydrophilic particles for pure lecithin). For cylinders, $V_{surf}/V_{core} = 5/4 = 1.25$, and it corresponds approximately to the volume ratio of hydrophilic and hydrophobic particles in the lecithin/bile salts mixture with the molar ratio $B_0 = 1$. This simple geometrical consideration confirms that our coarse-grained model (Fig. 2) reasonably takes into account the most crucial features of the real system and therefore is suitable for studying the growth of wormlike micelles.

To confirm our conclusions by more quantitative arguments, we have performed the cluster analysis for specified particles to study the number of beads per micelle. First, we have fixed the cutoff radius r_c – the maximal distance between adjacent particles belonging to the same cluster (see Fig. 5). Then, we have selected an arbitrary seed particle i of the specified type and calculated the distances to the neighboring beads. All beads j (of the same type with i), for which $r_{ij} < r_c$, were defined as belonging to one cluster and used for searching for other beads in this cluster. Finally, we have searched for a new seed particle of specified type which has not yet been included in any cluster and repeated the search for other beads in this cluster. The procedure was completed when all beads have been checked. All calculations were performed for $O^{(2)}$ beads, and the cutoff radius was set to be equal to $r_c = 1\sigma$. The beads $O^{(2)}$ correspond to hydrophilic part of the bile

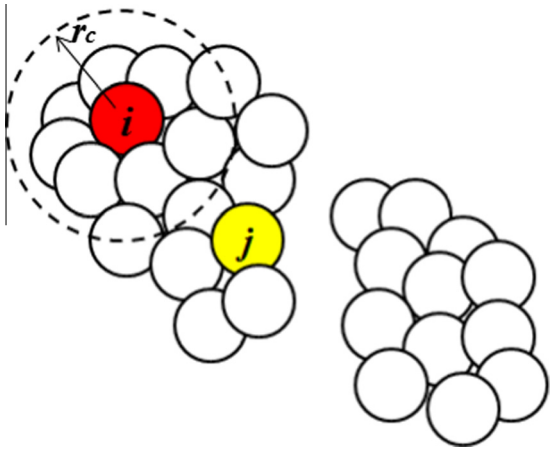


Fig. 5. Scheme of cluster analysis.

salt and are located in the micelle core, and this reduces the probability of the merging of two closely located micelles in a single cluster [39]. We have tested several other values of the cutoff radius $r_c < 1.4\sigma$, but we have not observed any significant difference in the system behavior.

In Fig. 6 we show the volume fraction of clusters Φ as a function of their size, i.e., of the number of particles per cluster. One can observe clearly the increasing of the volume fraction of large micelles upon increasing the bile salt concentration. At low $B_0 = 0.26$ the system consists mainly of relatively small clusters of the size less than 200 particles. At $B_0 = 0.53$ the main contribution comes from the clusters consisting of more than 600 particles while the number of small micelles decreases. The elongated wormlike micelles with large number of particles grow up by the aggregation of small micelles. This is confirmed by the data shown in Figs. 3 and 6.

Fig. 7 presents the growth of the average cluster length and the percentage of long micelles with increasing the bile salt concentration in organic solvents, hexane and cyclohexane. At the molar ratio $B_0 < 0.3$ one can observe the small clusters with the average length about $0.05L$. The average cluster size grows dramatically, reaching values about $1.2L$ at $B_0 \sim 0.5$. The micelles length in this case is larger than the simulation box size, but smaller than $\sqrt{2}L$ (the length of the diagonal of the box). At critical value of the bile salt concentration $B_0 \sim 0.53$ one observes the macrophase separation, i.e., the lecithin/bile salt micelles aggregate and form a single micelle (the shaded region in Fig. 7). This may correspond to the destruction of dynamic network of wormlike micelles and considerably decreasing of the viscosity [12]. Such behavior is in qualitative agreement with the experimental data [11] (and even in quantitative agreement, as regards the values of the bile salt to lecithin molar ratio).

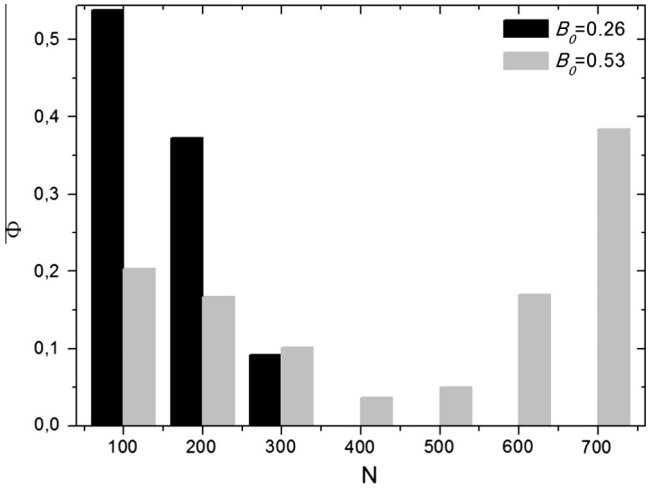


Fig. 6. The volume fraction of clusters Φ versus the number of particles per cluster for lecithin/bile salt mixture in hexane; the molar ratio is $B_0 = 0.26$ (black bars) and $B_0 = 0.53$ (grey bars).

4. Conclusions

We have investigated the effect of the bile salt concentration on the micelles formation in lecithin/bile salt organic solutions by means of mesoscopic computer simulations. The details of chemical structures of all components were taken into account by a proper coarse-grained model including both a suitable substitution of fragments of lecithin, bile salt and solvent molecules by coarse-grained beads and an appropriate choice of parameters of hydrophobic-hydrophilic interaction potential. Specific interactions have not been included in the model, so that it is the specific shape of molecules which is responsible for correct system behavior. This model allows to reproduce general features of processes in the real system and to understand the mechanism of self-assembly on the molecular level. We have shown that upon increasing the bile salt concentration, the number of large micelles and their average size grow up, and the ellipsoidal lecithin micelles are transformed into elongated structures. We have not observed any perceptible branching of these wormlike micelles. The asymmetric growth of micelles is caused by packing changes in the lecithin tails region upon addition of surfactants of specific shape. Such wormlike micelles may entangle with each other and form a dynamic network, so that the solution behaves as a gel. We have not performed analysis of entanglements and calculations of diffusion coefficient and viscosity because DPD method is not suitable for studying dynamic properties due to soft potentials and absence of real excluded volume in the system. The further increase of the bile salt concentration leads to the macrophase separation and accompanying disappearance of gel-like properties. Our conclusions have been confirmed by the images of morphological

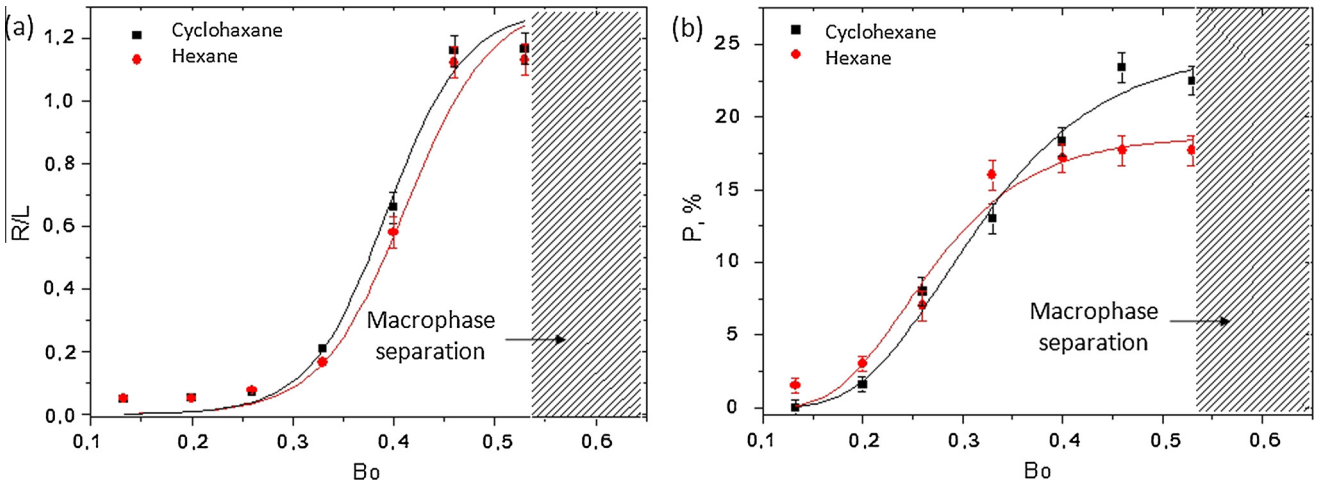


Fig. 7. (a) Normalized average cluster length R/L (R - cluster length, L - simulation cell size) and (b) percentage of big clusters ($N > 200$) with respect to lecithin – bile salt molar ratio B_0 in cyclohexane (black line) and hexane (red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

structures and by quantitative analysis of physical observables. These results of computer simulations explain rather well the experimental data [11], and even show a reasonable quantitative agreement for hexane and cyclohexane as regards the values of the bile salt to lecithin molar ratio at which the morphological transitions are observed. However, fine details in the chemical structure of a hydrocarbon solvent could have dramatic effect on aggregation and phase behavior of lecithin reverse micelles [3]. The above coarse-grained model can be modified to enable simulation of other solvents including water.

Acknowledgements

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