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Aggregation Properties of Sodium Hyaluronate with Alkanediyl-α,ω-bis(dimethylalkylammonium Bromide) Surfactants in Aqueous Sodium Chloride Solution

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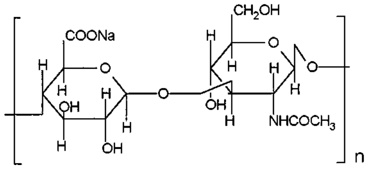
Aggregation properties of sodium hyaluronate (NaHA) with alkanediyl-α,ω-bis(dimethylalkylammonium bromide) surfactants (referred to as dimeric surfactants) in aqueous sodium chloride solutions have been studied as a function of surfactant chemical structure. Surface tension measurements indicate the unusual parabolic dependence of surface tension vs log surfactant concentration with a surface tension minimum at concentration c_{min}. The increase of surface tension above c_{min} may be related to the formation of clusters consisting of NaHA chain and dimeric surfactants at the air–water interface and in the bulk. From light scattering measurements, molecular weight, hydrodynamic radius, and second virial coefficient have been calculated. The simple calculation of the ratio of positive charge of dimeric surfactant unit per one negatively charged hyaluronate disaccharidic unit in NaHA–surfactant complex reveals that there is a slight excess of positive surfactant charges per one negatively charged disaccharidic unit in the region around c_{min} and the NaHA–surfactant complex is not far from electroneutrality. The nonlinear behavior of viscosity vs surfactant concentration in the NaHA–dimeric surfactant system depends on surfactant chemical structure. The behavior is concerned with the size increase due to complex growth and with the size shrinkage above c_{min}. A model describing the behavior of NaHA–surfactant complex in the bulk and at the interface is suggested. © 2000 Academic Press

Key Words: **sodium hyaluronate; dimeric surfactant; polymer–surfactant complex; surface tension; viscosity; light scattering.**

INTRODUCTION

Sodium hyaluronate (NaHA) is a polysaccharide with a polyanionic character (see formula).



Aqueous solutions of NaHA show not only viscous but also viscoelastic properties (1, 2). These rheological properties are due to the stiffness of the long chain and follow from the hyaluronate chain solvation and from its polyelectrolyte character (3). Broad applications for high viscosity NaHA solutions have been found in ophthalmology and eye surgery (4).

Alkanediyl-α,ω-bis(dimethylalkylammonium bromide) surfactants (denoted as dimeric surfactants [C_mH_{2m+1}–$(CH_3)_2N^+$–$(CH_2)_s$–$(CH_3)_2N^+$–C_mH_{2m+1}] $2Br^-$, s is the number of carbon atoms in the interconnecting chain referred to as spacer, m is the number of carbon atoms in the alkyl chain) attract research interest because of their potent bactericidal activity and peculiar solution properties (5–13). Measurements of surface tension and the electron micrographs indicate that the physical properties (critical micelle concentration, area per surfactant) of these systems strongly depend on spacer length. It was found that the surface area per head group appeared to go through a maximum for $s = 10$–12 (9). This state is concerned with the maximal micelle curvature and the spherical shape of micelles. The more detailed explanation of dependence of micelle shape on spacer length is given in Ref. (12). According to this, the distance distribution in micelles is given by the thermodynamic distance d_{T} between dimer head groups and by the distance d_{s} between two heads in one dimeric surfactant. It was found that in the region of spacer values $s = 4$–12, d_{T} is not expected to differ much from its value for conventional single-chain surfactants and the shape of micelles was found to be spherical or spheroidal. As s is increased, d_{s} approaches d_{T}, which is valid for the region $s = 6$–7. This region is characterized by a fully stretched spacer with the spacer length approximately identical to d_{s}. It follows from the experiments at the air–water interface (9) that at $s > 12$ the spacer becomes too hydrophobic to remain in the water phase, adopting the folded form directed into the air side of the interface. This results in a decrease in d_{s} value. On the other side, d_{s} decreases with decreasing s and for dimeric surfactant with $s = 2$ the spacer is short enough to allow for micelle growth and the formation of long threadlike micelles. These conclusions are related to the solution properties of dimeric surfactant with $s = 2$, where shear-thickening at a surfactant

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concentration of 1.4% and viscoelasticity above 2% were observed (12).

Polymer–surfactant interactions are a subject of intensive research. Research work may be divided into two main groups—studies of polymer–surfactant in the bulk and at the interface. Works discussing the bulk interactions focus on neutral, uncharged polymer–surfactant interactions (14–17) and on charged polymer–conventional ionic surfactant interactions as well. Interactions between positively charged polymer and anionic surfactant (18–21) as well as between polyanion and cationic surfactant (22, 23) were studied. It was found that ionic polymer–ionic surfactant systems are sensitive to precipitation depending on the concentration of added electrolyte (18, 19, 22). The process is reversible and can be recovered from the precipitation state to the single phase state by changing the electrolyte concentration. Some studies focus on interactions between anionic surfactant and polyelectrolytes with variable hydrophobicity (24, 25) and between anionic surfactants and hydrophobically modified anionic polyelectrolytes as well (26). Other works are devoted to polypeptide–anionic surfactant interactions (27, 28) and protein–anionic surfactant interactions (29).

Works concerned with polymer–surfactant interactions at the interfaces are focused on adsorption properties between nonionic polymer–anionic surfactant (30) and nonionic polymer–cationic surfactant (31). Surface viscoelasticity was observed in the system of ionic polyelectrolyte–oppositely charged surfactant (32). Other works concern the adsorption of single-chain surfactants on natural polysaccharides (33–35) and the adsorption of gelatin with different kinds of cationic and anionic surfactants at the solid–water interface (36). Another important area of research is protein–surfactant interactions at the interfaces (37–38).

In the last paper (39), we dealt with rheological properties in NaHA–NaCl–alkanediyl-α,ω-bis(dimethylalkylammonium bromide) surfactant systems depending on the number of carbon atoms in the spacer. The aim of this paper is to extend the investigation to aggregation properties determined by surface tension and static and dynamic light scattering as a function of the number of carbon atoms in the spacer.

MATERIALS AND METHODS

Bisquarternary ammonium salts were prepared by the reaction of tertiary diamines with 1-bromoalkanes as described (40). Surfactants were purified by manifold crystallization from a mixture of acetone–methanol. Thin-layer chromatography and elementary analysis confirmed sample identity.

Microbial NaHA was obtained from Contipro (Ústí n. Orlicí, Czech Republic).

NaCl–NaHA stock solution was prepared by stirring it and letting it stand overnight. NaCl–NaHA–surfactant solution was prepared by surfactant dissolution in the stock solution. This solution was found to be single phase for the following spacer number values $s = 6, 8, 10, 12$. For $s = 2$, precipitation occurred at all investigated surfactant and salt concentrations.

A KRUSS K12 tensiometer and a computer-controlled Lauda CD 15 capillary viscometer were used for surface tension and viscosity measurements. Experiments were carried out at 25°C.

The Otsuka Electronics dynamic light scattering photometer DLS-700 with an argon ion laser (wavelength, 488 nm) was used for static and dynamic light scattering measurements. The region of scattering angles reached from 20° to 150°. For calculation of the molecular weight of NaHA–surfactant aggregates, the value of the refractive index increment $dn/dc = 0.175$ at 25°C was used (2). Hydrodynamic diameter d was calculated by using the Einstein–Stokes formula, $d = kT/3\pi\eta_0 D$, where k is the Boltzmann constant, T is the temperature, η_0 is the solvent viscosity, and D is the diffusion coefficient. The cell housing was filled with di-*n*-butyl phthalate and kept at 25°C. Solvent and solutions were filtered three times through Millipore membrane filters.

RESULTS AND DISCUSSION

Surface Tension

In Fig. 1a, the dependence of surface tension on log concentration c for dimeric surfactant (denoted as 12-s-12, $s = 8, 10, 12$) in 0.40 M NaCl solution is shown. The relations are straight lines

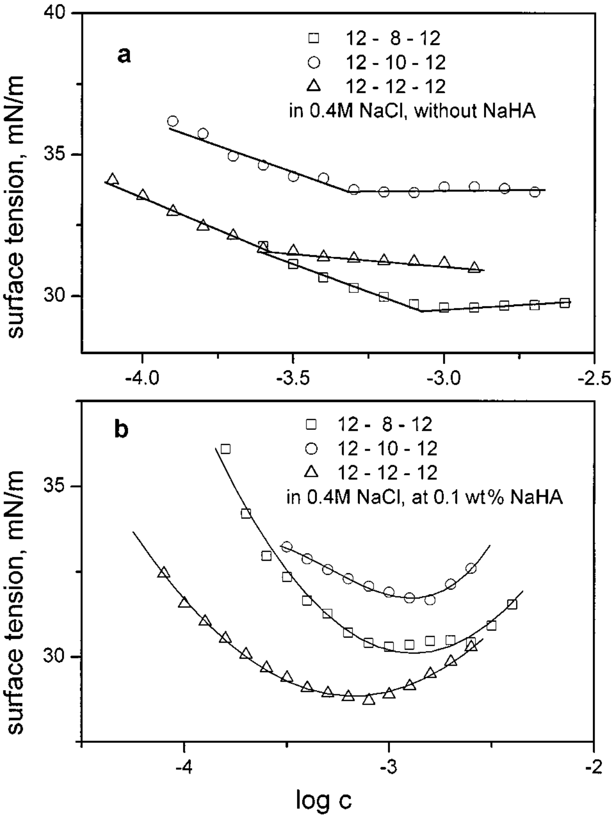

FIG. 1. Surface tension of NaCl–12-s-12 dimeric surfactant system in aqueous 0.40 M NaCl solution vs log c at different surfactant structures (variable parameter s). (a) Without NaHA. (b) With 0.1 wt% NaHA.

with the sharp intercept assigned to the critical micelle concentration (cmc). When NaHA is added (Fig. 1b), a broad minimum instead of a line intercept is observed. The minimum of surface tension vs log *c* may indicate that the process of micellization of surfactant during the presence of NaHA in solution should not be considered a sharp phase transition. The interaction between NaHA chain and surfactant occurs in the broad region of surfactant concentration. The absence of the cmc has been observed in the amphiphilic polyelectrolyte–dodecyltrimethylammonium chloride (DTAC) system (25). The fit of the measured data was carried out using parabolic dependence (solid curves in Fig. 1b). The nonlinear increase in surface tension above the concentration of minimum surface tension seems to be unusual. The amount of surface active monomers decreasing the surface tension at the air–water interface should be diminished despite the increasing surfactant concentration. This observation is discussed later in the light scattering section.

The plot of surface tension vs log *c* in NaHA–NaCl–12-*s*-12 solution at a concentration of NaCl $c_s = 0.25$ M is shown in Fig. 2a. The broad minimum of surface tension is observed in these solutions as well as in Fig. 1b. The concentration at minimum surface tension $c_{\min}$ is plotted as a function of spacer carbon number *s* in Fig. 2b. The cmc of the NaCl–12-*s*-12 dimeric surfactant system without NaHA, obtained from Fig. 1a, is also included in Fig. 2b. It follows that, whereas linear dependence of log cmc vs *s* in the absence of NaHA is observed, the quantity log $c_{\min}$ is approximately constant at low values of *s* and decreases with increasing *s* up to another constant level. This sigmoidal change of log $c_{\min}$ may indicate structural changes in NaHA–NaCl–12-*s*-12 in the intermediate region of *s*, as observed in the case of 0.25 M NaCl. As for the system with 0.40 M NaCl, since there are not enough data points, the dependence beyond the measured region is marked by a dashed curve. However, we assume that sigmoidal dependence appears even in the system with 0.40 M NaCl and is shifted to higher *s* values as the NaCl concentration is increased (Fig. 2b). The point of inflection at the sigmoidal curve representing the transition state is shifted from $s = 9$ at 0.25 M NaCl to $s = 11$ at 0.40 M NaCl.

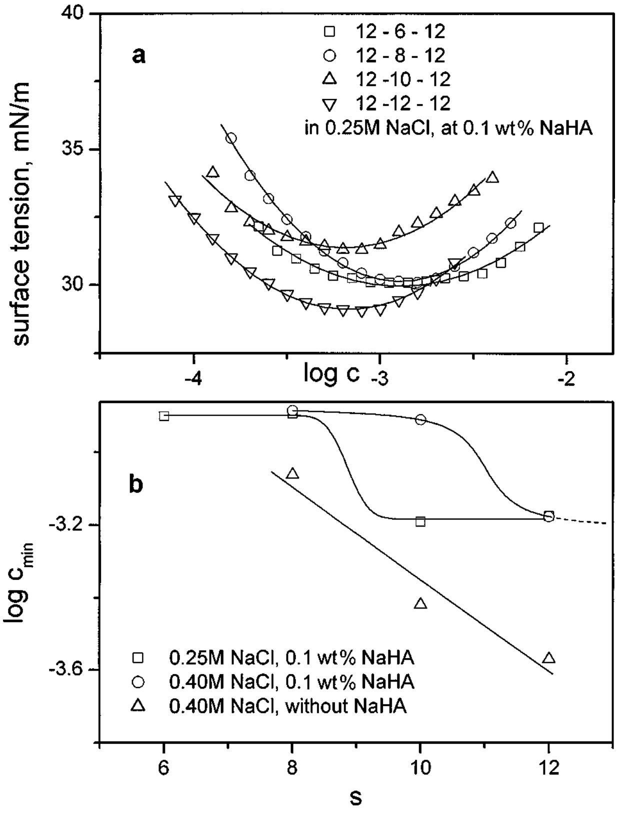

FIG. 2. (a) Surface tension of 0.1 wt% NaHA–NaCl–12-*s*-12 dimeric surfactant system in aqueous 0.25 M NaCl solution vs log *c* at different surfactant structures (variable parameter *s*). (b) Plot of log $c_{\min}$ obtained from Figs. 1b and 2a vs parameter *s*. The cmc of 0.40 M NaCl–12-*s*-12 dimeric surfactant system without NaHA is included as symbol △.

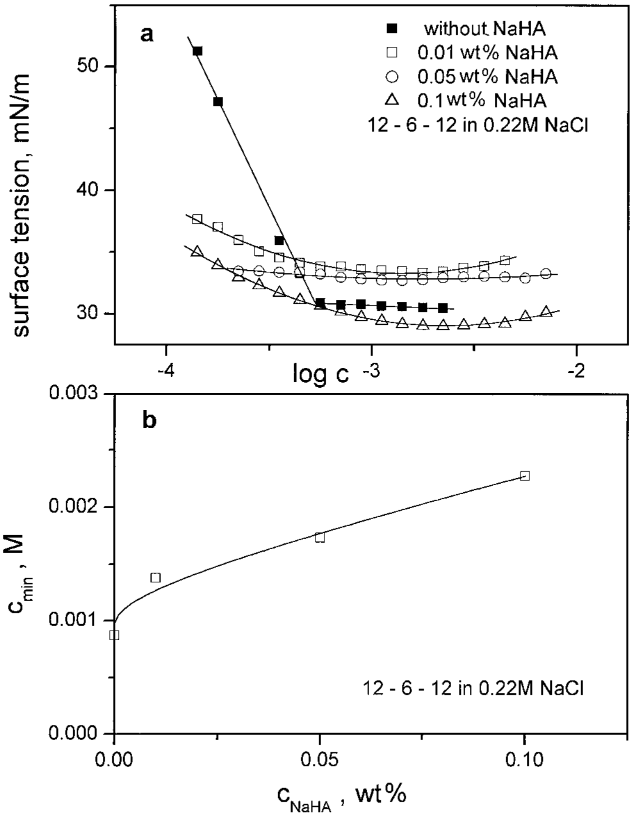

FIG. 3. (a) Surface tension of NaHA–NaCl–12-*s*-12 dimeric surfactant system in aqueous 0.22 M NaCl solution vs log *c* at different NaHA concentration c_{NaHA}. (b) Plot of surfactant concentration at minimum viscosity $c_{\min}$ obtained from Fig. 3a vs c_{NaHA}.

The influence of NaHA concentration in NaHA–NaCl–12-6-12 solution is presented in Fig. 3. The decrease in surface tension at the highest NaHA concentration in the system is observed below the level of surface tension of surfactant without NaHA (Fig. 3a). The surface activity at the interface has been reported for some mixed systems of nonionic polymer (ethylhydroxyethylcellulose) and anionic surfactant sodium dodecyl

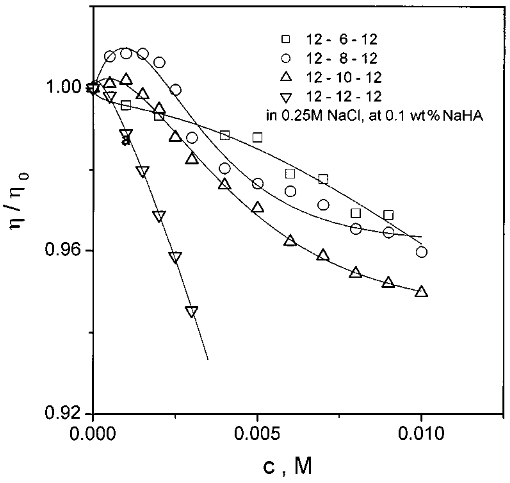

FIG. 4. Relative viscosity η/η_0 of 0.1 wt% NaHA–NaCl–12-*s*-12 dimeric surfactant system in aqueous 0.25 M NaCl solution vs surfactant concentration *c* at different surfactant structures (variable parameter *s*). η_0 means viscosity of NaHA in 0.25 M NaCl solution at zero surfactant concentration.

sulfate (SDS) at very low polymer concentrations (30). Similar to our observations, surface tension decreases, with increasing polymer concentration, below the level of surface tension of aqueous SDS solution (30). $c_{\min}$ also depends on the NaHA concentration c_{NaHA} (Fig. 3b). $c_{\min}$ is approximately linearly increased with NaHA concentration, with deviation from the linear dependence at low c_{NaHA}.

Viscosity

The observations obtained from viscosity measurements as a function of surfactant concentration (Fig. 4) correspond to the assumption of NaHA–surfactant interactions at low surfactant concentrations where the viscosity increase was found. η_0 and η are viscosities of 0.1 wt% NaHA in 0.25 M NaCl solution at zero and finite surfactant concentrations, respectively. The most pronounced effect of viscosity enhancement was registered at $s = 8$. A very similar viscosity dependence on surfactant concentration was observed in the amphiphilic copolymer–DTAC system (24). Nonlinear dependence of viscosity in systems of ethyl hydroxyethyl cellulose–SDS (17) and adsorbed cationic surfactant hexadecyltrimethylammonium bromide on cellulose (33) and carboxymethylcellulose (34) has been observed as a function of surfactant concentration.

The explanation of the nonlinear viscosity dependence results from several approaches. The common explanation is based on the occupation of charged polymer sites by surfactant monomers resulting in the viscosity increase. It is supposed that the viscosity maximum corresponds with $c_{\min}$. The process of micellization is believed to act against the occupation of charged polymer sites by surfactant monomers. One of the possible explanations of the observed viscosity changes is that polymer chains wrap around micelles as is stated in a case of amphiphilic copolymer–DTAC (24) resulting in the viscosity decrease. The viscosity maximum in our case is only a few percentages higher than unity (within 1%—Fig. 4). We assume that there is probably no formation of a network among NaHA coils at the point of maximum viscosity.

Dynamic and Static Light Scattering

For the purpose of light scattering experiments performed on hyaluronate–dimeric surfactant complex, we chose dimeric surfactant with the spacer length $s = 8$ carbon atoms. All investigated surfactants ($s = 6$, 8, 10, 12) form spherical or spheroidal micelles in aqueous solution (12). According to viscocity experiments (Fig. 4), the most pronounced viscosity enhancement was found for the complex NaHA–12-8-12 surfactant.

Molecular weight M_{ps} of NaHA–surfactant aggregates determined by static light scattering indicates the increase with surfactant concentration (Table 1). Decrease of the second virial coefficient A_2 as well as increase of molecular weight M_{ps} of hyaluronate–surfactant aggregates indicates that the NaHA–surfactant complex grows continually with increasing surfactant concentration, and the negative charge of NaHA is electrostatically neutralized by complex formation with cationic surfactant. On the contrary, the nonlinear change of the coil size is observed, according to dynamic light scattering results. The hydrodynamic diameter d_{90} is calculated from the diffusion coefficient D_{90} at the angle of 90° by using the Stokes–Einstein formula (Table 1). The angle-independent smaller size of NaHA–surfactant complex has been found at low and high surfactant concentrations far from $c_{\min}$. Similarly, viscosity shows an increase up to $c_{\min}$ and a subsequent decrease at high surfactant concentration (Fig. 4). Viscosity nonlinear behavior is related to the size increase due to the complex growth and to the size shrinkage above $c_{\min}$, as seen in Fig. 5a.

The hyaluronate coil shrinkage is governed by the electrolyte concentration (22, 23) by shielding of repulsive interactions of negatively charged sites on NaHA chains. The role of positive Na^+ ions on NaHA coil size is effectively replaced by the positively charged free dimeric micelles that are possibly present in the solution above surfactant cmc. Free micelles act as positive polyions and may be responsible for the rather steep viscosity decrease (Fig. 4). However, the molecular weight of the NaHA–dimeric surfactant complex was found to be high in the region

TABLE 1
Dynamic and Static Light Scattering Results in System of 0.1 wt% NaHA, 0.25 M NaCl, and 12-8-12 Dimeric Surfactant

c (10^{-3} M)	D_{90} (10^{-9} cm^2/s)	d_{90} (nm)	M_{ps} (10^6 g/M)	A_2 (10^3 (cm^3/g)^2)
0.50	298	17	2.18	3.04
0.83 ($c_{\min}$)	87	57	3.30	1.77
2.00	97	51	3.17	1.64
8.00	285	18	7.96	0.80

Note. D_{90}, diffusion coefficient at 90°; d_{90}, hydrodynamic diameter at 90°; M_{ps}, molecular weight; A_2, second virial coefficient; c, surfactant concentration.

far above $c_{\min}$ concentration (Table 1). This discrepancy of viscosity and molecular weight that results at high concentrations above $c_{\min}$ is only apparent because the hyaluronate coil size is dominantly governed by the electrostatic interactions with electrolyte ions or free micelles that are inside of the NaHA coil, as seen in the suggested model in Fig. 5a. The hyaluronate molecular weight obtained from static light scattering measurements is the weight-averaged molecular weight of single hyaluronate chain with adsorbed surfactants that was found to increase with increasing surfactant concentration.

The increase in molecular weight of NaHA–surfactant aggregate at high surfactant concentration implies the assumption that dimeric surfactant is incorporated into NaHA coils. It is possible to calculate the number n_{ps} of dimeric surfactant units in NaHA aggregate as

$$n_{\mathrm{ps}} = (M_{\mathrm{ps}} - M_{\mathrm{p}})/M_{\mathrm{s0}}, \tag{1}$$

Where M_{ps} is the molecular weight of the NaHA–surfactant complex, M_{p} is the molecular weight of NaHA without surfactant addition, and M_{s0} is the molecular weight of surfactant dimer. When considering the number of disaccharidic units n_{p} (see the formula of NaHA)

$$n_{\mathrm{p}} = M_{\mathrm{p}}/M_{\mathrm{p0}}, \tag{2}$$

where M_{p0} is the molecular weight of one disaccharidic unit (390.3 g/M), it is possible to calculate the ratio ρ—number of positive charge of dimeric surfactant unit per one negatively charged hyaluronate disaccharidic unit in NaHA–surfactant complex—as follows

$$\rho = 2n_{\mathrm{ps}}/n_{\mathrm{p}}. \tag{3}$$

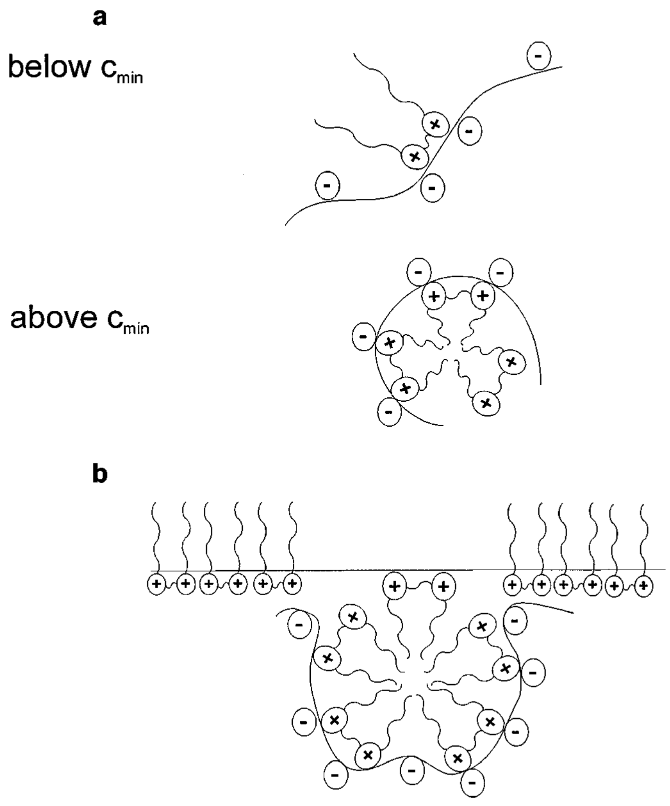

FIG. 5. Model of the NaHA–dimeric surfactant interaction: (a) in solution, (b) at interface.

TABLE 2
Aggregation Parameters of NaHA–Dimeric Surfactant Complex

c (10^{-3} M)	M_{ps} (10^6 g/M)	n_{ps}	ρ
0	1.51	0	0
0.50	2.18	959	0.50
0.83 ($c_{\min}$)	3.30	2561	1.32
2.00	3.17	2375	1.23
8.00	7.96	9229	4.77

Note. M_{ps}, molecular weight; n_{ps}, number of bound dimeric surfactant units in NaHA aggregate; ρ, number of positive charge of dimeric surfactant unit per one negatively charged hyaluronate disaccharidic unit in NaHA–surfactant complex; c, surfactant concentration.

The results are shown in Table 2. It follows that in the region around $c_{\min}$ there is a slight excess of positive surfactant charges per one negatively charged disaccharidic unit and the NaHA–surfactant complex is not far from electroneutrality. The excess adsorption of surfactant is observed at high surfactant concentrations.

At the interface, the question of unusual surface tension increase above $c_{\min}$ (Figs. 1b and 2a) may be related to the presence of surfactants adsorbed on the free NaHA chains at the air–water interface, as presented in the model in Fig. 5b. The presence of such clusters at the interface would decrease surface activity. It is important in this model that the number of clusters is proportional to surfactant concentration, which could explain the increase in surface tension above $c_{\min}$ with increasing surfactant concentration.

SUMMARY

The results of surface tension measurements in the system of NaHA–NaCl–12-s-12 dimeric surfactant show a broad region of surface tension decrease, indicating the NaHA–surfactant interaction. The increase in surface tension above $c_{\min}$ may be related to the adsorption of clusters, consisting of free NaHA chains and dimeric surfactant, to the air–water interface.

The plot of viscosity vs surfactant concentration shows a slight maximum at $c_{\min}$ and a viscosity decrease at high surfactant concentrations. Viscosity nonlinear behavior is related to the size increase due to the complex growth and to the size shrinkage following from the interaction with electrolyte ions and free micelles.

Nonlinear dependency of hydrodynamic diameter obtained from light scattering measurements coincides with viscosity measurements. On the other hand, aggregation size increases with surfactant concentration. The ratio of positive charge of dimeric surfactant unit per one negative charged hyaluronate disaccharidic unit in NaHA–surfactant complex indicates that there is a slight excess of positive surfactant charges per one negatively charged disaccharidic unit in the region around c_{min} and the NaHA–surfactant complex is not far from electroneutrality. The strong adsorption of surfactant is observed at high surfactant concentrations.

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