Fluorescence probe studies upon microenvironment characteristics and aggregation properties of gemini sugar surfactants in an aquatic environment

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ABSTRACT

The micellar aggregation behavior and micropolarity characteristics of a series of N,N′-bisalkyl-N,N′-bis[(3-D-aldonylamido)propyl]ethylenediamines referred to as gemini bis(C_nX) surfactants (where C_n = n-butyl, n-octyl, n-decyl and n-dodecyl; X = gluconyl and lactobionyl, denoted as GA and LA, respectively) has been investigated by steady-state fluorescence, SSF (by emission spectra of pyrene) and time-resolved fluorescence quenching, TRFQ (using pyrene as the fluorescence probe and cetylpyridinium ion as the fluorescence quencher). Remarkably low magnitude *cmc* values were found for the studied aldonamide-type gemini surfactants, in a range from 4.5×10^{-6} M (bis(C_{12}GA)) to 2.3×10^{-3} M (bis(C_4LA)). The variation of *cmc* for bis(C_nLA) (n = 4, 8, 10 and 12) follows a linear semi-logarithmic pattern. The obtained $I_1/I_{3,c > cmc}$ ratios from 1.41 to 1.5 indicate a considerable high polarity sensed by pyrene in micelles of bis(C_nX), dependent upon both the kind of aldonyl entity, and the length of hydrophobic tail. According to TRFQ, all studied systems display micellar growth (demonstrated by values of an average aggregation number of surfactant chains per micelle) with increasing surfactant concentration typically from 100 up to 1000 times more than the *cmc*. The aggregation numbers, N_{agg} (N_{tail}) and the "polydispersity index", σ/N_ω (less than ~0.3), show that micelles of bis(C_nLA)'s of up to ca. 500*cmc*, are nearly spherical and almost monodisperse. Their spheroidal micelle growth and enhanced micellar polydispersity is observed as the surfactant concentration increases. Such a behavior was found to be strictly related to geometric aspects of a given surfactant dimeric structure.

1. Introduction

Rearrangement through self-aggregation of saccharide-derived surfactants – in which bioinspired structural motifs are embedded in their molecular structure – has been a hot topic during the past decade because these surfactants can be utilized to fabricate various solubilizing media and new forms of cosmetics, synthetic templates and drug delivery systems as well as nanostructured functional materials and membrane builders [1–3]. The incorporation of carbohydrate-based species into the polar building blocks of amphiphilic molecules leads to the creation of new interesting physico-chemical and biological functionality [3–7]. Such structures can generate a broad spectrum of aggregate morphologies resembling biological systems, e.g., micelles, vesicles, bilayers, sponge phases, lyotropic mesophases or fibers and gels [3,8,9]. Among a variety of sugar-based surfactant architectures [1,2], gemini or dimeric derivatives, consisting of two hydrophobic chains and two carbohydrate-based polar headgroups linked by a rigid or flexible spacer, seem to be the most interesting in view of their tun-

able molecular geometry and aggregate morphology, reinforced by many hydroxyl groups that readily enter into hydrogen bonding [6–13]. These structures reveal mostly temperature independent and significantly improved surface properties (markedly low surface tension and *cmc* values), reduced environmental impact, a broad spectrum of aggregates upon hydration [14,15] and usual progression of lyotropic phases in the concentrated regime [6]. Furthermore, some of their representatives display quite dramatic myelins (worm-like channels) [6,15]. Amine-type gemini surfactants reveal an extraordinary pH-induced vesicle-to-cylindrical or wormlike micelle transition [14].

Continuing our work on designing and characterizing new surface active saccharide derived systems and the characteristic features embedded in their structure [7,11,16–18] we present here the fundamental description of an aggregation behavior of N,N′-bisalkyl-N,N′-bis[(3-D-aldonylamido)propyl]ethylenediamines with varying both the hydrophobic tail, and the aldonamide type (structures and abbreviations are placed in Chart 1).

The main purpose of the present study was to elaborate the characteristics of micelles formed by bis(C_nX)'s, whose X = GA and LA (mainly *cmc* and microenvironment polarity) and the aggregation behavior of studied dimeric compounds in dilute and concentrated aqueous surfactant solutions (i.e., up to their solubility limit), by

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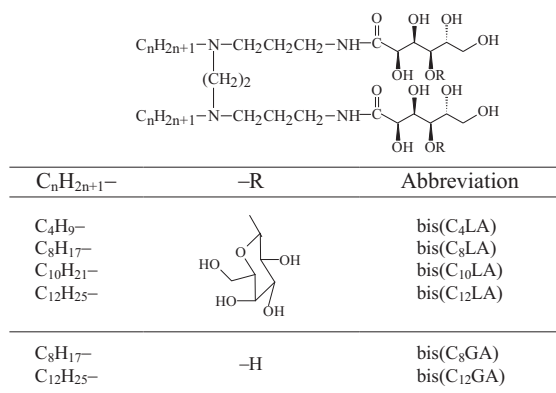*E-mail address:* kazimiera.wilk@pwr.wroc.pl (K.A. Wilk).

Chart 1. N,N$'$-bisalkyl-N,N$'$-bis[3-D-aldonylamidopropyl]ethylenediamines bis(C$_n$X) where X = GA or LA.

means of emission fluorescence spectra and time-resolved fluorescence quenching method; as the latter has been recognized as one of the most convenient tools for the estimation of micelle aggregation numbers [19–27]. This contribution extends our previous studies on synthesis, surface activity and environmental impact of the mentioned aldonamides [10,11,25] by investigations of their concentration-induced micellar growth, mainly driven by the surfactant geometric size and the hydrophobic interactions. Points imparting the additional attraction and novelty to the objects are the effect of changes in molecular structure and in the environment at the interface around the gemini (dimeric) polyol-type compounds on their self-aggregation behavior.

2. Experimental

2.1. Materials

All aldonamide-type gemini surfactants studied, bis(C$_n$X) (n = 8, 10, 12; X = LA and GA), were synthesized according to the synthetic methodologies described in our previous papers [10,11]. All reagents and solvents were the highest grade available commercially, dried, or freshly distilled as required. Pyrene was purchased from Molecular Probes, Inc. (Eubene, OR 97402) and cetylpyridinium chloride from Aldrich Chemical Co. (Milwaukee, WI). Water used for all experiments was doubly distilled and purified by means of a Millipore (Bedford, MA) Milli-Q purification system.

2.2. Methods

The values of *cmc*'s related to presently synthesized N,N$'$-bisbutyl-N,N$'$-bis[(3-D-aldonylamido)propyl]ethylenediamine (bis(C$_4$LA)) and N,N$'$-bisdecyl-N,N$'$-bis[(3-D-aldonylamido)propyl] ethylenediamine (bis(C$_{10}$LA)) were measured by means of a Krüss K12 automatic tensiometer (Hamburg, Germany) with a du Nuoy Pt-Ir-ring as in the case of other bis(C$_n$X)'s [11] and found to be 2.0×10^{-3} M and 4.4×10^{-5} M, respectively.
Fluorescence measurements were recorded on an IBH 5000U spectrometer, used as a single-photon sensitive spectrofluorimeter. The steady-state fluorescence spectra were recorded by means of an additional Model 5000U-06 steady-state accessory with a xenon lamp, operated at an excitation wavelength of 335 nm. Time-resolved fluorescence quenching data were collected with the time-correlated single-photon counting technique on the IBH 5000U spectrometer using a hydrogen filled lamp as the excitation source. Sample preparation and fluorescence decay recording were as described in detail by others [21,23,26]. Pyrene was excited at 335 nm and its emission recorded at 381 nm. Measurements were carried out in a thermostated l cm^3 cuvette holder at 25 ± 0.1 °C.

The critical micelle concentration (*cmc*) values of the investigated surfactants were determined from the measurements of the pyrene polarity index, I_1/I_3, i.e., the intensities ratio of the first and third vibronic peaks in the fluorescence emission spectra of pyrene [27,28]. All *cmc* values were estimated from the plots of I_1/I_3 ratio vs. surfactant concentration. Additionally, the value of I_1/I_3 at a concentration well above the *cmc* provided information on the micropolarity sensed by pyrene in its micellar microenvironment [27,29,30].

The time-resolved fluorescence decay measurements were performed using pyrene as the fluorescence probe (*P*) and cetylpyridinium ion as the fluorescence quencher (*Q*). In all experiments the probe concentration, [*P*], was kept at low level, such that [*P*]/[*MI*] < 0.05, where *MI* denotes micelles concentration in M. The molar concentration ratio [*Q*]/[*MI*] was adjusted as to be close to 1. The surfactant concentration was selected in such a way as to receive the most accurate results, i.e., for bis(C$_n$X) (n = 8, 12; X = LA and GA) having extremely low *cmc*'s the measurements were performed in the range of 100–1000*cmc*. The decay curves were recorded in the absence and in the presence of quencher. Since the molecules of the probe and quencher remain in a micelle for a time long compared to the probe fluorescence lifetime, the deconvolution of fluorescence decay curves was possible. Quantitative analyses of fluorescence decay curves have been performed by means of the IBH Micellar Quenching Model (Fit 11 module in the IBH Das 6 Analysis packet) in one step by fitting the Infelta–Tachiya equation [31,32].

3. Results and discussion

3.1. Micelle formation and microenvironment characteristics at diluted surfactant concentration: steady-state fluorescence (SSF)

In the case of N,N$'$-bisalkyl-N,N$'$-bis[(3-D-aldonylamido)propyl] ethylenediamines, bis(C$_n$X) (n = 8, 12; X = LA and GA), we have observed on the basis of surface tension measurements [11] that the decisive driving force for micelle formation are hydrophobic interactions between C$_n$'s and dominating hydrogen bond interactions between aldonamide-type headgroups. Further details related to the structural organization of gemini bis(C$_n$X)'s aggregates have been elaborated here by means of the SSF technique using the measured values of the fluorescence probe properties in such organized systems. In order to acquire more complex characteristics of micelle formation and microenvironment polarity of bis(C$_n$X), additional analogs, i.e., n-hexyl and n-decyl derivatives, have been included. Pyrene was used as the probe because its intensities of the first (I_1) and the third (I_3) vibronic peaks are highly sensitive to the polar nature of the surrounding medium [27,29–31].

Both the determination of values of critical micelle concentration, and the microenvironment polarity estimation were carried out by means of SSF. The fluorescence spectra of pyrene, in aqueous solution of bis(C$_n$X), were recorded at different surfactant concentrations. As surfactant concentration increases the fluorescence intensity increases, and the structure of spectrum changes (see Fig. 1). The ratio I_1/I_3 of the intensities of the bands that appear at 372 nm (I_1) and 390 nm (I_3) were measured. Fig. 2 presents variations of the studied surfactants concentration vs. the observed I_1/I_3 ratios in the fluorescence emission spectra of pyrene. The plots show a clear break that defines the *cmc*, whose values for bis(C$_n$X)'s are listed in Table 1. The *cmc*'s obtained by surface tension measurements (values in parentheses) are also included for comparison, indicating the reliability of the SSF approach. As it can be noticed, the bis(C$_n$X)'s can form micelles at an exceptionally low concentration (for comparison see data for single-head single-tail surfactants presented in Table 1), ranging from 4.5×10^{-6} M (bis(C$_{12}$GA)) to
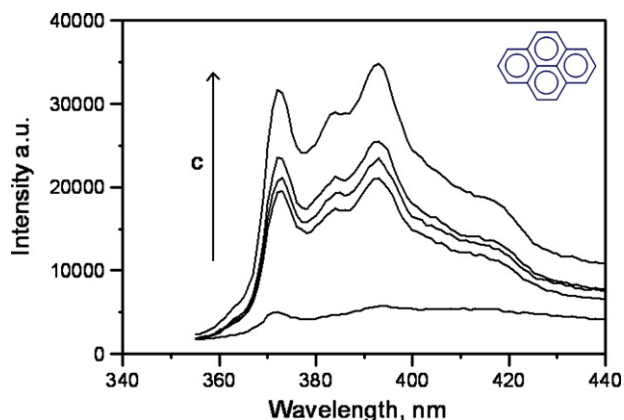
Fig. 1. Fluorescence spectra of pyren (5×10^{-7} M) in aqueous solution of bis(C_8LA).

Fig. 2. Variation of pyrene polarity ratio I_1/I_3 with the surfactant concentration for (a) bis(C_nLA) where $n = 4$, 8, 10 and 12 and (b) bis(C_nGA) where $n = 8$ and 12.

2.3×10^{-3} M (bis(C_4LA)). The variation of *cmc* for bis(C_nLA) ($n = 4$, 8, 10 and 12) follows a linear semi-logarithmic pattern (regression coefficient is ca. 0.998). Such a phenomenon is similar to one previously observed in the case of surface tension measurements also for other gemini sugar surfactants [14,15], and many various series of ionic dimeric surfactants [29] having a hydrophobic tail not exceeding C_{16} as well as single-head single-tail polyoxyethylene [33] and saccharide-derived surfactants [18].

The polarity of the micellar interior was expressed as the local polarity of the solubilized site of pyrene. It has been suggested [29] that the first few methylene groups in the hydrocarbon tail lying near to the polar headgroup may be in contact with water. Thus, water molecules may somehow penetrate into the hydrophobic core of micelles. The estimated $I_1/I_{3,c > cmc}$ ratios from the SSF measurements for bis(C_nX)'s ranging from 1.41 to 1.50 (placed in Table 1; for comparison: water $I_1/I_3 = 1.87$, dioxane $I_1/I_3 = 1.5$, 1,2-propandiol $I_1/I_3 = 1.45$, methanol $I_1/I_3 = 1.35$, ethanol $I_1/I_3 = 1.18$, n-pentanol $I_1/I_3 = 1.02$, n-hexane $I_1/I_3 = 0.58$ [34]) indicate a considerably high polarity sensed by pyrene in the studied systems. It means that the probe is solubilized in the palisade layer of the micelle as previously been achieved by other workers [21,35]. Furthermore, it can be noticed that the nature of X has strong influence on the polarity of the pyrene micellar binding sites. It has to be emphasized here that the obtained values of $I_1/I_{3,c > cmc}$ are comparable to those of cationic ammonium dimerics [29] but considerably higher than that in the case of gemini 1,2-bis(N-hexaethyleneglycol dodecylamide) [30] or single-head single head glucopyranoside-type surfactant [31]. Additionally, from the $I_1/I_{3,c > cmc}$ values there
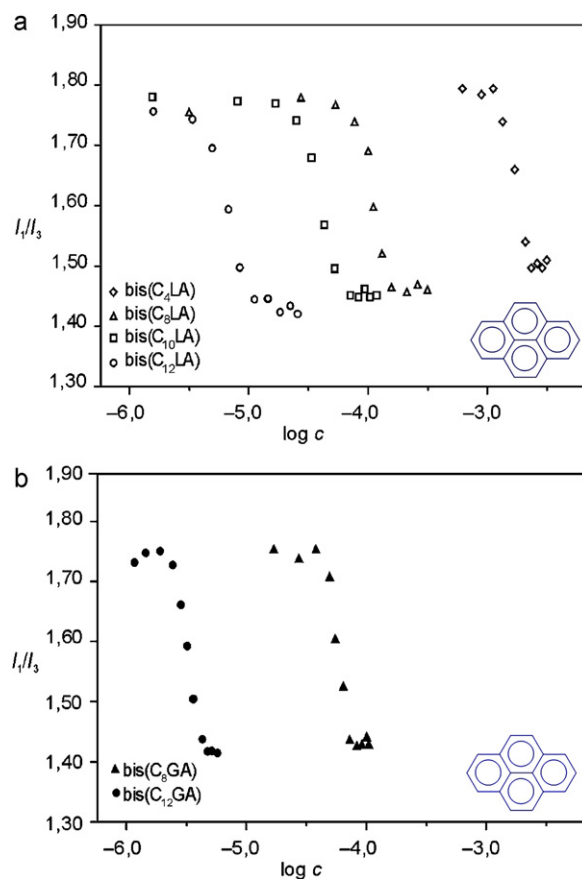
were estimated values of the apparent dielectric constant (D) of the medium (i.e., the pyrene environment inside the micelle) [27,32] (the applied relation of $I_1/I_{3,c > cmc}$ vs. D is given in the footnote of Table 1 along with the apparent values of D). Both magnitudes, i.e., $I_1/I_{3,c > cmc}$ and D, indicate that forming of more water imbued structure occurs in the aqueous micelles of bis(C_nX)'s whose aldonylamido-type headgroups are appreciably hydrated due to the presence of numerous hydroxyl groups. Our recent contribution [11] has shown that bis(C_nX) ($n = 8$, 12; X = LA, GA) form small, spherical aggregates just upon micellization as evident from steady-state fluorescence quenching (SSFQ).

Table 1

Critical micelle concentration, *cmc*, pyrene polarity index, I_1/I_3 and apparent dielectric constant of the medium, D for the N,N´-bisalkyl-N,N´-bis[(3-D-aldonylamido)propyl]ethylene diamines (bis(C_nX) where $n = 4$, 8, 10, 12; X = GA and LA) obtained by steady-state fluorescence[a] method at 25 °C.

Surfactant (abbreviation)	*cmc* [mol × dm⁻³]	$(I_1/I_3)_{c > cmc}$	App.D[b]
bis(C_4LA)	2.3×10^{-3} (2.0×10^{-3})[c]	1.50	39.5
bis(C_8LA)	1.5×10^{-4} (1.3×10^{-4})[d]	1.46	36.3
bis(C_{10}LA)	4.7×10^{-5} (4.4×10^{-5})[c]	1.45	35.5
bis(C_{12}LA)	9.7×10^{-6} (8.9×10^{-6})[d]	1.43	33.9
bis(C_8GA)	7.2×10^{-5} (6.7×10^{-5})[d]	1.43	33.9
bis(C_{12}GA)	4.5×10^{-6} (3.8×10^{-6})[d]	1.41	32.3
N-decyl-N-methylgluconamide	$-(1.29 \times 10^{-3})$[e]	–	–
N-dodecyl-N-methylgluconamide	$-(1.46 \times 10^{-4})$[e]	–	–
N-decyl-N-methyllactobionamide	$-(2.31 \times 10^{-3})$[e]	–	–
N-dodecyl-N-methyllactobionamide	$-(2.49 \times 10^{-4})$[e]	–	–

[a] SSF: probe = pyrene; $\lambda_{exc} = 335$ nm; $\lambda_{em,1} = 372$ nm; $\lambda_{em,2} = 381$ nm.
[b] Calculated [31] from the relation: $(I_1/I_3)_{c > cmc} = 1.00461 + 0.01253D$.
[c] Determined from surface tension measurements in the present work.
[d] Determined from surface tension in [11].
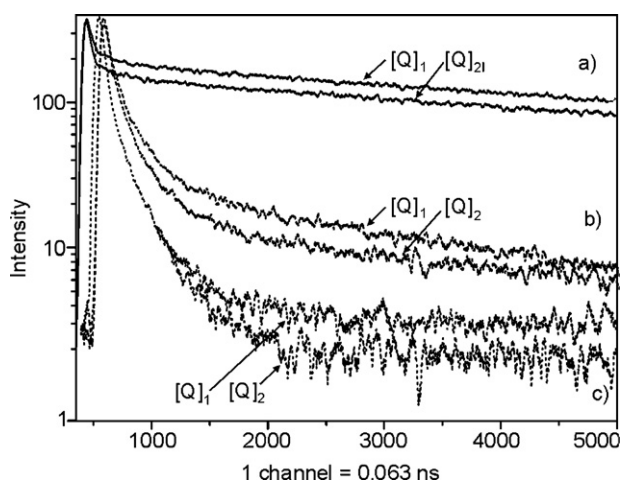[e] Determined from surface tension in [18].

Fig. 3. Fluorescence decay curves for bis(C_8LA): (a) $c = 50cmc$ ([Q]$_1 = 1.50 \times 10^{-4}$, [Q]$_2 = 7.50 \times 10^{-4}$), (b) $c = 350cmc$ ([Q]$_1 = 6.00 \times 10^{-4}$, [Q]$_2 = 3.00 \times 10^{-3}$) and (c) $c = 1000cmc$ ([Q]$_1 = 7.00 \times 10^{-4}$, [Q]$_2 = 2.80 \times 10^{-3}$).

3.2. Micellar growth at concentrated surfactant solution: time-resolved fluorescence quenching (TRFQ)

The TRFQ technique measures the time-resolved fluorescence intensity of a probe solubilized in micellar aggregates in absence and presence of a quencher [22–24,36,37]. Fluorescence quenching in micelles has been observed to be a first-order kinetic process [22,36]. Under the conditions that probes and quenchers do not migrate between micelles within the lifetime frame of the excited probe and assuming a Poissonian distribution of the quencher among the micelles, the Infelta–Tachiya equation [38,39] for the fluorescence intensity $F(t)$ vs. time (t) has the following form:

$$F(t) = F(0)\exp\left[-\frac{t}{\tau_0} + \langle n\rangle\left\{\exp\left(-k_Q t\right) - 1\right\}\right] \quad (1)$$

where $\langle n\rangle = [Q]/[MI]$ (where $[MI] = c - cmc$) is the average number of quenchers per micelle, $F(0)$ is the fluorescence intensity at time zero, and k_Q is the intramicellar quenching rate constant, τ_0 is the probe fluorescence lifetime, determined in a separate experiment in the absence of the quencher.

Typical families of decay curves are displayed in Fig. 3. All $F(t)$ functions have the same slope after the quencher-induced fast decay which indicates that the distributions of probe and quencher among micelles can be considered as frozen on the fluorescence time scale (about 1 μs). The values of fluorescence lifetime τ_0 were successfully determined (see Table 2).

Each decay curve for different sets of experimental parameters (i.e. type of surfactant, its concentration in aqueous solution, quencher concentration) was analyzed separately to yield the fitting k_Q parameter whose values for the studied systems are placed in Table 2. As can be seen, the values of the intramolecular quenching rate constant (k_Q) for all bis(C_nX)'s decrease with increasing surfactant concentration and the length of hydrophobic chain. On both the theoretical [22,36,40], and experimental basis [21,26,35] a reduction of this parameter has generally been attributed to an increasing size of the examined micelles.

For gemini structure of surfactants [27] in which a given spacer links two surfactant monomers, the average aggregation number of quenchers per micelle, $\langle n\rangle$, is related to the average aggregation number of surfactant chains per micelle, N_{tail}, equal to $N_R \times N_{agg}$ (where $N_R = 2$ as denoting a number of hydrophobic substituents and N_{agg} is the average aggregation number for traditional surfactants). The N_{tail} magnitude can be thus obtained from the non-exponential decay parameters by means of the following equation:
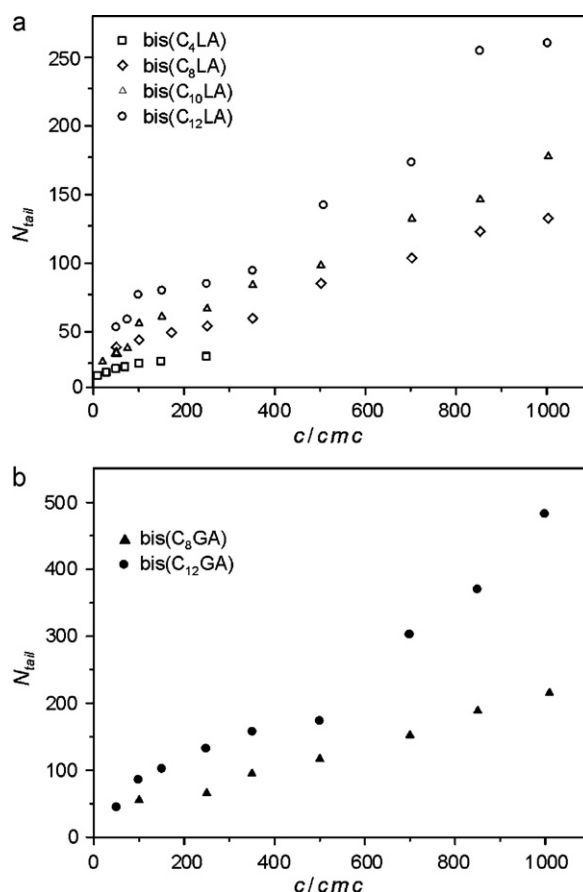

Fig. 4. Variation of the micelle aggregation numbers, N_{tail}, with normalized surfactant concentration, c/cmc, for (a) bis(C_nLA) where $n = 4, 8, 10, 12$ and (b) bis(C_nGA) where $n = 8, 12$ at 25 °C.

$$N_{tail} = \frac{\langle n\rangle (c - cmc)}{[Q]} \quad (2)$$

where c is the total surfactant concentration, cmc is the critical micelle concentration and $[Q]$ is the bulk molar quencher concentration. Table 2 lists the values of the average aggregation numbers, i.e., N_{agg} (N_{tail}), for the different solutions of bis(C_nX)'s, whose concentrations are far above the cmc. At the concentration 250 times higher than the cmc N_{tail} increases in the order bis(C_4LA) < bis(C_8LA) < bis(C_8GA) < bis(C_{10}LA) < bis(C_{12}LA) < bis(C_{12}GA). According to the TRFQ N_{tail} estimates, the bis(C_4LA) micelles are spherical in shape over the entire range of surfactant concentrations studied (c/cmc is varied from 50 to 250, i.e., to its solubility limit). A more noticeable but still modest increase of N_{tail} with surfactant concentration is seen for bis(C_8LA) and bis(C_{10}LA) for which the micelles remain spherical up to ca. 500cmc. This increase becomes important with bis(C_{12}LA) and bis(C_8GA) which both form spherical micelles up to approx. 350cmc, and very importantly, for bis(C_{12}GA)–up to ca. 100cmc.

The variations of N_{tail} (which is equal to $2N_{agg}$) vs. normalized concentration of bis(C_nX), c/cmc (Fig. 4) indicates that the micelles of bis(C_nX) in the aqueous phase are susceptible to an almost linear growth as the surfactant concentration increases as treated theoretically by Jódar-Reyes and Leermakers [41]. Furthermore, for most concentrations studied the resulting "quencher average" aggregation numbers were found to slightly decrease with the quencher concentration indicating a broadening of the micellar size distribution. Such a phenomenon has already been observed in the literature for various ionic or ionic micelles which

Table 2
Aggregation parameters of N,N′-bisalkyl-N,N′-bis[(3-D-aldonylamido)propyl]ethylenediamines (bis(C$_n$X) where n = 4, 8, 10, 12; X = GA and LA) obtained by time-resolved fluorescence quenching[a] at 25 °C.

Surfactant (abbreviation)	c/cmc	τ_0 [ns]	N_{tail}	N_{agg}	$k_{\text{Q}} \times 10^{-7}$ [s^{-1}]	$N_{\text{tail}}k_{\text{Q}} \times 10^{-10}$	σ/N_ω	$N_{\text{tail}}/N_{\text{theoret.}}$[b]
bis(C$_4$LA)	10	6.36	17.8	8.9	1.81	3.22	0.15	0.8
	30	6.48	20.8	10.4	1.51	3.15	0.15	1.0
	50	6.50	22.8	11.4	1.36	3.19	0.16	1.1
	70	6.39	20.8	12.0	1.33	3.19	0.17	1.1
	100	6.38	26.8	13.4	1.20	3.21	0.19	1.2
	150	6.41	28.2	14.1	1.15	3.24	0.20	1.3
	250	6.33	31.8	15.9	1.02	3.24	0.22	1.5
bis(C$_8$LA)	50	3.73	39.0	19.5	0.79	3.11	0.17	0.8
	100	3.77	44.3	22.2	0.72	3.18	0.19	0.9
	150	3.75	49.6	24.8	0.63	3.14	0.21	1.0
	250	3.86	54.2	27.1	0.59	3.19	0.23	1.1
	350	3.89	60.0	30.0	0.52	3.12	0.35	1.3
	500	3.78	85.4	42.7	0.38	3.28	0.28	1.8
	700	3.77	103.8	51.9	0.33	3.38	0.31	2.2
	850	3.79	123.2	61.6	0.26	3.27	0.34	2.6
	1000	3.72	132.8	66.4	0.27	3.59	0.36	2.8
bis(C$_{10}$LA)	100	1.72	56.0	28.0	0.57	3.17	0.18	0.9
	150	1.93	60.8	30.4	0.51	3.13	0.20	0.9
	250	1.96	66.6	33.3	0.47	3.16	0.22	1.0
	350	1.76	83.8	41.9	0.37	3.14	0.25	1.3
	500	1.77	98.1	49.0	0.33	3.25	0.28	1.5
	700	1.80	132.2	66.1	0.25	3.36	0.31	2.1
	850	1.69	146.2	73.1	0.23	3.38	0.34	2.3
	1000	1.73	177.4	88.7	0.18	3.39	0.37	2.8
bis(C$_{12}$LA)	100	0.97	76.8	38.4	0.42	3.16	0.18	0.9
	150	1.03	80.1	40.0	0.39	3.17	0.21	1.0
	250	1.05	85.0	42.5	0.38	3.20	0.24	1.0
	350	0.98	94.6	47.3	0.34	3.19	0.28	1.1
	500	0.99	141.8	70.9	0.26	3.75	0.30	1.7
	700	0.99	173.6	86.8	0.20	3.50	0.33	2.1
	850	0.96	254.8	127.4	0.14	3.57	0.36	3.1
	1000	1.01	260.1	130.0	0.13	3.61	0.40	3.9
bis(C$_8$GA)	100	4.60	54.6	27.3	0.63	3.46	0.22	1.2
	150	4.55	52.3	26.2	0.63	3.31	0.32	1.1
	250	4.76	65.2	32.6	0.52	3.44	0.36	1.4
	350	4.80	94.3	47.2	0.39	3.65	0.38	2.0
	500	4.61	116.2	58.1	0.31	5.80	0.42	2.5
	700	4.59	151.7	75.8	0.23	6.13	0.43	3.2
	850	4.63	188.2	94.1	0.19	6.46	0.42	4.0
	1000	4.50	202.2	101.1	0.13	6.71	0.42	4.3
bis(C$_{12}$GA)	100	2.65	86.2	43.1	0.39	3.36	0.17	1.0
	150	2.63	102.6	51.3	0.33	3.34	0.20	1.2
	250	2.74	129.8	64.9	0.26	3.34	0.28	1.6
	350	2.77	158.0	79.0	0.21	3.43	0.35	1.9
	500	2.66	173.0	86.5	0.22	6.72	0.39	2.1
	700	2.65	301.4	150.7	0.13	7.27	0.41	3.6
	850	2.67	370.4	185.2	0.12	8.38	0.42	4.4
	1000	2.60	482.4	241.2	0.09	9.81	0.44	5.8

[a] TRFQ: probe = pyrene, quencher = cetylpyridinium bromide, λ_{exc} = 335 nm.
[b] Calculated according to directions given in the literature [43–46].

become more polydisperse as they grow [22,26]. The theory of a polydispersity-assisted quenching process–originally studied by Almgren and Löfroth [24] and later extended by Warr and Grieser [42] to steady-state and time-resolved experiments – concludes that in the absence of probe and quencher exchanges the measured average aggregation number referred to as the apparent average aggregation number, obtained by means of Eq. (2), has been shown to depend on the ratio $|Q|/[MI]$ in the following way:

$$N_{\text{tail}} = N_\omega - \frac{\sigma^2[Q]}{2[MI]} \tag{3}$$

where N_ω is the weight average aggregation number and σ^2 is the variance of the micellar weight distribution [42]. The "polydispersity index" σ/N_ω can be determined from a plot of N_{tail} vs. $[Q]/[MI]$ (see Fig. 5) and the numbers thus estimated are also listed in Table 2. Accordingly, the σ/N_ω parameter is less than ~0.3 for all aqueous solutions of bis(C$_4$LA), up to 500 cmc for other lactobionyl analogs, and up to ca. 150 cmc for gluconyl ones indicating that these micelles

are nearly spherical [43] and almost monodisperse as in the case of globular C$_{12}$E$_6$ micelles observed by Warr and Grieser [42]. More important decreases of N_{tail} upon $[Q]$ – as proved by polydispersity index higher than at least 0.4 – are seen for bis(C$_8$GA) and bis(C$_{12}$GA); for the latter under the entire c/cmc range studied, thus, indicating micellar polydispersity in these solutions.

Some support for the above drawn conclusions comes also from the intramicellar rate constant, k_{Q}, which is proportional to the reciprocal of the viscosity, sensed by the probe and the quencher in their motion in the micelle [35]. Its decrease achieved along with increasing surfactant concentration for all bis(C$_n$X)'s reflects a slightly enhanced packing of the studied micelles as their aggregation number increases. For spherical or quasi-spherical micelles, k_{Q} is nearly proportional to N_{tail} [40] and the relevant quantity that many authors found interesting to be compared are the values of $N_{\text{tail}}k_{\text{Q}}$. Values of this product may be treated as a size-corrected measure of the reciprocal of the microviscosity sensed by the probe and quencher in the diffuse motion which brings them together. For the studied systems, the estimated $N_{\text{tail}}k_{\text{Q}}$ magnitude (also placed
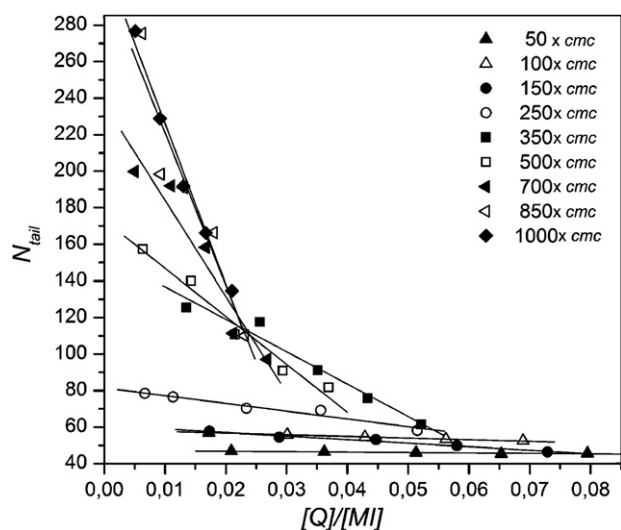
Fig. 5. Typical relationship of N_{tail} vs. $[Q]/[MI]$ for bis(C_8GA). The straight lines are weighted linear least-squares fits.

in Table 2) is being slightly increased (ca. 10%) over the range of c/cmc concentrations for bis(C_nLA)'s micelles. But for the micelle growth of bis(C_nGA)'s the $N_{tail}k_Q$ parameter is increased approximately twice for $n = 8$ and three times for $n = 12$ under the studied conditions.

3.3. Geometric aspects of the gemini surfactant structure

The amphiphilic character of saccharide-derived surfactants is reinforced by many hydroxyl groups that readily enter into hydrogen bonding. This leads to specific self-association behavior which is found to be essentially governed by simple intermolecular and intramolecular geometric packing constraints and temperature insensitive surface properties [3,7,8,13]. The studied gemini bis(C_nLA)'s and bis(C_nGA)'s consist of two hydrophilic head groups, two hydrophobic chains, and a spacer linked at or near the head groups [10,11]. The present findings make it possible to conclude that the unique dimeric structure of the studied bisaldonamidoamines is responsible for their micellar growth as proved mainly by the apparent average aggregation numbers increase along with surfactant concentration over quenching analyses conditions. According to Israelachvili et al. [43] the values of the surfactant packing parameter, P, were calculated as the ratio between the hydrocarbon tail region volume and the product of the hydrocarbon chain length and the area per head group at the interface. The P values estimated for bis(C_8LA), bis(C_{12}LA), and bis(C_8GA), bis(C_{12}GA) as, 0.33, 0.31 and 0.37, 0.35, respectively, indicate that the dimeric aldonyl derivatives would rather tend to organize into quasi-spherical aggregates, and furthermore, at high concentration regime spherocylindrical micelles would be preferably formed. Furthermore, the aggregation number $N_{theoret}$ can be expressed – for the calculation purposes – as the ratio between the micellar core volume and the volume of the hydrocarbon chain, for which all the needed detailed equations are available from published material [44–47]. Some qualitative support for the bis(C_nX)'s aggregation behavior can be acquired also from the values of the elongation degree of aggregates, denoted as the $N_{tail}/N_{theoret}$ ratio [47] (also included in Table 2) which show that, as the micelles of bis(C_nX)'s grow, the micelle shape progressively changes and the micelle polydispersity increases. Recalling that $N_{tail}/N_{theoret} > 1$ at the highest concentrations studied, the most probable visualized picture of micelle elongation may be as it is shown in Chart 2 where N_0 denotes a spherical micelle of a given bis(C_nX), which
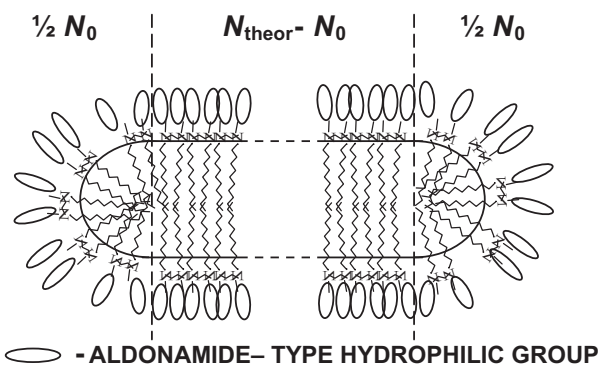

Chart 2. Visualization of elongated micelles formed by N,N′-bisalkyl-N,N′-bis[(3-D-aldonylamido)propyl]ethylenediamines.

is formed close to the cmc, and $N_{theoret}$ is the aggregation number of an elongated micelle respective to $c = 1000cmc$. Assuming, that the micellar shape is thus spherocylindrical or elongated (i.e., a cylinder with two hemispherical caps) with the radius of the hydrophobic core equal to the fully extended alkyl chains (in Å: $l_C = 1.265n_C + 1.5$) [45], the lengths of those spherocylinders are for bis(C_8LA), bis(C_{10}LA), bis(C_{12}LA) and bis(C_8GA), bis(C_{12}GA), respectively, 9.2, 10.3, 13.1 and 10.8, 17.9 nm which is in accord with length prediction [47]. The micelle structure is dependent on the configuration and bulkiness of the linkage between the hydrophilic and hydrophobic groups. More bulky linkages, such those in (N-acetyl N-dodecyl)lactosylamine described by Kjellin et al. [26], promote small spherical micelles, whereas larger oblate ellipsoidal or spherocylindrical micelles are formed when there is a lower steric hindrance, as for surfactants examined in present studies; the more bulky lactobionamide-type surfactants have lower aggregation numbers than the gluconamide ones.

4. Conclusions

The growth of micelles formed by the N,N′-bisalkyl-N,N′-bis[(3-D-aldonylamido)propyl]ethylenediamines in water at concentrated surfactant solutions can be successfully monitored by TRFQ. The variations of N_{agg} vs. normalized concentration of bis(C_nX), c/cmc can lead one to conclude that all studied gemini structures form at first micelles with aggregation numbers expected for the spherical objects and then, due to individual geometrical reasons, reveal a further concentration dependent growth up to cylindrical micelles. This tendency is more noticeably experienced in the gluconamide-type series than in the lactobionamide-type one. SSF measurements of $I_1/I_{3,c > cmc}$ values, which are indicative of the polarity of the probe environment, confirm a more hydrophilic nature of the aldonylamide-derived system studied in comparison to the classical one-chained nonionic surfactants. Thus, the hydrophilic region of aggregates formed consists of the aldonylamide-type entities and extensively entrapped water molecules.

Acknowledgment

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