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Can molecules with an anionic head and a poly(ethylene glycol) methyl ether tail self-assemble in water? A surface tension, fluorescence probe, light scattering, and transmission electron microscopic investigation†

Joykrishna Dey* and Saurabh Shrivastava

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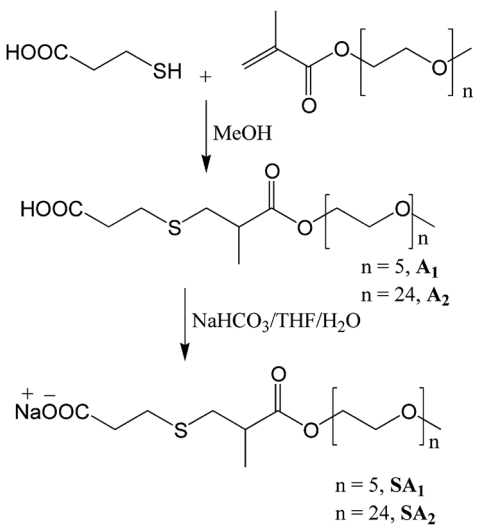
In this work, we report for the first time the synthesis and characterization of two unusual carboxylate surfactants with a poly (ethylene glycol) monomethyl ether, mPEG, tail of different chain lengths. The molecules were observed to be surface-active, and were found to self-assemble in water above a relatively low critical aggregation concentration to form disk-like micelles.

Surfactants have widespread applications in industry in household and pharmaceutical formulations. Therefore, a variety of surfactants that differ in the headgroup structure or in the hydrophobic tail have been developed.[1] Synthetic surfactant molecules typically consist of a long nonpolar hydrocarbon tail and a polar hydrophilic head. The headgroup is either uncharged or charged (positive or negative). On the other hand, surfactants with more hydrophobic fluorocarbon tails have also been reported.[2] When added to water such molecules adsorb with their head in water and tail in the air, forming a monolayer at the air/water interface. This causes reduction of the surface tension of water. Also due to the difference in interaction of the two segments of the same molecule with water, surfactant molecules self-assemble in aqueous solution above a certain concentration, called the critical aggregation concentration (CAC), to give micelles or other nano-size structures. In order to avoid the unfavorable interaction with the water molecules, the hydrocarbon tails interact with each other forming a micellar core and the polar headgroups remain on the surface of the aggregates facing the bulk water. Micellar properties including the shape and size of the aggregates thus formed depend upon the molecular architecture of the surfactant molecule.

In spite of many reports on synthetic amphiphilic molecules with different hydrophobic tails, there is no report so far on the formation of self-assembled structures by molecules containing a short poly (oxyethylene), $(–O–CH_2CH_2)_n$, chain linked to an ionic headgroup. The low-molecular-weight PEGs (M_n < 1500 Da) are considered hydrophilic.[3] It is expected that replacement of a $–CH_2–$ by oxygen (–O–) along the hydrocarbon, $–(CH_2)_n–$, chain would increase its polarity and hence increase its interaction with water. This is expected to disfavor the formation of any self-assembled structure. Consequently, PEGs have been covalently coupled to hydrophobic molecules to produce nonionic surfactants.[4] In fact, Tween-20, Triton-X-100, *etc.* are well-known nonionic surfactants in which the PEG chain acts as a polar headgroup. Many micelle-forming copolymers of PEG with different hydrophobic blocks, such as poly(L-amino acids), diacyllipids, poly(propylene oxide) or poly(1,2-butylene oxide) *etc.* have been used to prepare drug loaded micelles.[5] Morikawa and co-workers have reported the incorporation of carboxylic acid moieties into a PEG-based nonionic surfactant showing a pH-controlled micellar system.[6] However, to the best of our knowledge, there is no report on the surface activity and self-assembly of low-molecular-weight ionic amphiphiles formed by coupling of mPEG and a small anionic or cationic headgroup by a stable chemical bond.

Herein, we report for the first time, the synthesis and micelle-forming properties of two carboxylate molecules bearing an mPEG tail of different chain lengths. In contrast to standard low-molecular-weight surfactants, the head as well as the tail of our molecules are hydrophilic. In this work, we have done chemical coupling of poly (ethylene glycol) methyl ether methacrylate (M_w 300 and 1100) to



Scheme 1 Chemical structures of the mPEG-derived anionic surfactants.

Department of Chemistry, Indian Institute of Technology Kharagpur, Kharagpur, 721 302, India. E-mail: joydey@chem.iitkgp.ernet.in; Fax: + 91-3222-255303

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3-mercaptopropionic acid to obtain corresponding carboxylic acid derivatives, A_1 and A_2, respectively (see Scheme 1 for chemical structures). The details of materials used and synthetic procedures are given in the ESI†. Carboxylic acid-containing amphiphiles are significantly important for the controlled release of functional ingredients because the association and collapse of micelles or aggregates formed by the amphiphiles can be controlled by various stimulants.[7] Thus the interfacial as well as self-assembly behavior of the sodium salts (SA_1 and SA_2) of the corresponding carboxylic acids was investigated. We used a combination of surface tension, fluorescence, dynamic light scattering (DLS), and transmission electron microscopic (TEM) measurements to elucidate the behavior of the molecules in water. A good agreement between the results obtained using different techniques was observed.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker SEM 200 instrument using TMS (tetramethylsilane) as the internal standard. A Perkin Elmer model 883 IR spectrometer was used for recording the FT-IR spectra. The pH measurements were done with a digital pH meter Model pH 5652 (EC India Ltd., Kolkata) using a glass electrode. The FT-IR spectrum was recorded using a Perkin Elmer model 883 IR spectrometer. For solid samples, KBr pellet was used as the solvent.

The surface tension (γ) of the surfactant solutions was measured by the Du Nüoy ring detachment method with a surface tensiometer (Model 3S, GBX, France) at 298 ± 0.1 K. Surfactant solutions of different concentrations were made in Milli-Q water (pH 6.6). The temperature of the solution was controlled by a Thermo-Neslab RTE-7 circulating water bath with a temperature accuracy of ±0.1 °C.

Steady-state fluorescence measurements of 1-anilino naphthalene (AN), pyrene (Py), and 1,6-diphenyl-1,3,5-hexatriene (DPH) were performed on a Perkin Elmer LS-55 spectrophotometer equipped with an automated polarization accessory, using a quartz cell of 10 mm path-length. The excitation wavelengths were 340 and 350 nm for AN and DPH, respectively. The excitation slit width (band pass) was set at 2.5 nm for excitation and 2.5–10 nm for the emission depending upon the sample concentration. Emission spectra of Py were measured with a SPEX Fluorolog-3 (Horiba, FL3-11) spectrophotometer at an excitation wavelength of 335 nm using excitation and emission slit widths of 2 nm and 5 nm, respectively. In all experiments, background spectra, either of water alone or of water containing surfactant were subtracted from the corresponding sample spectrum. The temperature of the samples was controlled using the water jacketed magnetically stirred cell holder in the spectrometer connected to a Thermo Neslab RTE-7 circulating water bath.

DLS measurements were carried out using a Zetasizer Nano (Malvern Instrument Lab, Malvern, UK) optical system equipped with a He–Ne laser operated at 4 mW at $\lambda_0 = 633$ nm, and a digital correlator. Surfactant solutions prepared in Milli-Q water were filtered through a Millipore Millex syringe filter (0.45 μm) directly into the scattering cell.

TEM measurements were performed with a JEOL-JEM 2100 (Japan) electron microscope operating at an accelerating voltage of 200 kV at room temperature. The specimen was prepared by immersing a 400 mesh size carbon-coated copper grid into the surfactant solution (2 mM for SA_1 and 5 mM for SA_2) for 30 s followed by blotting the excess liquid, stained with 1% aqueous phosphotungstic acid (pH 7.0) and air dried. The specimens were kept in desiccators for further drying until before measurement.

The adsorption of SA_1 or SA_2 at the air/water interface was studied by surface tension (γ, mN m^{-1}) measurements at different concentrations (C). The variation of γ upon addition of SA_1 and SA_2 in water has been shown by the plots of γ *versus* log C in Fig. 1. The γ-value decreases nonlinearly with log C and shows a characteristic break and remains constant thereafter. This shows spontaneous adsorption of both the molecules at the air/water interface in spite of having a hydrophilic tail. In fact, the surface activity of aqueous PEG400 at around 30 °C has been reported earlier by others.[8] It is observed that for both surfactants, the γ value does not decrease much in comparison to conventional anionic surfactants, sodium dodecyl sulfate (SDS) and sodium lauryl sarcosinate (SLS). Although the γ_{min} values of SA_1 (52.2 mN m^{-1}) and SA_2 (51.1 mN m^{-1}) are much higher than those of SDS (32.5 mN m^{-1})[9] and SLS (41 mN m^{-1}),[11] the surface activity as measured by pC_{20} (negative logarithm of surfactant concentration required to reduce the surface tension by 20 units) value (~3) is almost equal to that of SDS (2.6)[9] or SLS (3.3).[10] The values of the cross-sectional area per headgroup (A_{min}) of SA_1 (135 Å^2) and SA_2 (151 Å^2) at the air/water interface calculated using Gibbs adsorption equations[1a] suggest formation of aggregates in which the carboxylate headgroups are less tightly packed. This might be due to the methyl substituent at the 6-position of the hydrocarbon bridge connecting the headgroup with the mPEG tail.

The concentration corresponding to the breakpoint of the γ *vs.* log C plot can be taken as the CAC of the anionic surfactant. The CAC values thus obtained are 2.25 mM and 0.25 mM for SA_1 and SA_2, respectively. In spite of having a hydrophilic tail the CAC values of SA_1 and SA_2 surfactants are much lower than those of SDS (7.92 mM)[9] and SLS (15 mM)[10] which have a hydrocarbon tail and is indicative of spontaneous aggregate formation at concentrations greater than the CAC value. In fact, for other PEG based surfactants, equally low CAC values have been reported in the literature.[11] The decrease of CAC value with mPEG chain length shows the effect of increase of hydrophobicity of the PEG chain. Zaslavsky *et al.* reported a comparative study showing the hydrophobic character of PEG of various molecular weights.[3] However, they reported that hydrophobicity does not vary with the molecular weight, which explains a small decrease in CAC value with chain length of the mPEG tail.

Three different fluorescent molecules AN, Py, and DPH were used as extrinsic probes for studying the self-assembly behavior of the

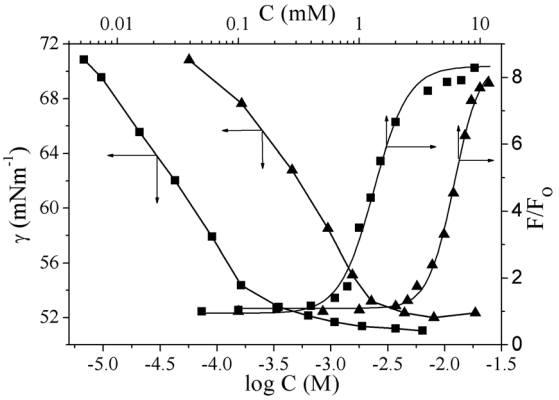

Fig. 1 Plots of surface tension (γ) and relative fluorescence intensity (F/F_o) of AN *versus* log C in water at 298 K: (▲) SA_1, (■) SA_2.

anionic surfactants. Since these probe molecules are hydrophobic and are poorly soluble in water, they have been used as probe molecules to study the change of the microenvironment of its solubilization site.[12] These probe molecules bind preferentially to the hydrophobic domains of aggregates resulting in enhancement of fluorescence intensity, shift of the emission maximum or change in fluorescence anisotropy. Therefore, the fluorescence characteristics of these probes when bound to the hydrophobic domains can shed light on the aggregate structure.

As reported earlier the AN probe is weakly fluorescent in water, but its fluorescence intensity increases manifold when dissolved in nonpolar solvents accompanied by a large blue shift of the emission maximum.[13] The large blue shift of the emission maximum (λ_{max}) and intensity change of AN fluorescence (not shown here) in the presence of SA_1 or SA_2 at concentrations above CAC suggest formation of aggregates in which the probe molecules are solubilized in the nonpolar environment of the aggregates. The variations of relative fluorescence intensity (F/F_o) and spectral shift, $\Delta\lambda$ ($= \lambda_{water} - \lambda_{sample}$) of AN as a function of [surfactant] have been shown in Fig. 1, and Fig. 2, respectively. The large increase of F/F_o and $\Delta\lambda$ values within a relatively narrow concentration range around CAC indicates solubilization of AN within hydrophobic domains. The CAC values obtained from the inflection points of the plots are close to the corresponding value obtained from surface tension measurements.

The hydrophobicity of the microenvironment of the aggregates was also measured by the use of Py as a fluorescent probe. The intensity ratio (I_1/I_3) of the first (I_1, 372 nm) to the third (I_3, 384 nm) vibronic peaks of the Py fluorescence spectrum is known to change with the polarity of the solvent.[14] The plots of I_1/I_3 *versus* [surfactant] have been shown in Fig. 2. The concentration at the inflection point corresponds to the CAC value and is closely equal to the value obtained from the fluorescence titration using the AN probe. The minimum value of I_1/I_3 ratio for both surfactants is much less than that in water (1.70), which indicates that the Py molecule is solubilized in a less polar environment of the aggregates. It is observed that the polarity ratio reaches minimum values of 1.47 and 1.38, for SA_1 and SA_2 surfactants, respectively. For SLS and SDS surfactants, on the other hand, the minimum I_1/I_3 ratio observed is 1.20, which is less than those of SA_1 and SA_2 surfactants. This is because the Py molecules which are normally solubilized in the micelle core/headgroup interfacial region encounter very few or no water molecules around them in the case of spherical aggregates of SDS or SLS surfactants. According to the results of surface tension studies of SA_1 and SA_2 surfactants the headgroups are less ordered at the aggregate interface and thus allow penetration of water molecules causing enhancement of micropolarity of the Py probe.

To further understand the structural change that occurs at concentrations above the CAC of the amphiphiles, we performed steady-state fluorescence anisotropy (r) measurements using DPH as a probe molecule. As reported earlier, the r-value gives an idea about the rigidity of the microenvironments of the aggregates.[15] Relatively higher r-values of SA_1 (0.186) and SA_2 (0.193) surfactants at a concentration above CAC suggest an ordered microenvironment around the DPH probe in the aggregates formed by SA_1 and SA_2 surfactants. Since surface tension data suggested less tight packing of the headgroups and DPH being a cylindrical-shaped molecule, it is solubilized within the bilayer membrane consisting of mPEG chains. The higher values of r of DPH in the presence of SA_1 and SA_2 surfactants compared to hydrocarbon surfactants, such as SDS ($\sim$0.05) and SLS ($\sim$0.06) suggest the existence of large bilayer aggregates.

It is worth mentioning here that the above conclusions drawn from the surface tension and fluorescence studies are consistent with the literature reports.[8,16] According to Privat and co-workers, PEG chains consisting of short hydrophobic ethylene groups separated by hydrophilic oxygen atoms, can form helices due to different types of interaction of water with the hydrophobic and hydrophilic parts of the chain.[8] Indeed from molecular dynamics simulation in combination with spectroscopic data it was shown that the most likely configuration of the PEG chain is helical with oxygen atoms stuck inside the helix and PEG–water interactions are strong inside the helix.[17] Thus such a helical PEG chain can be considered as an amphiphilic polymer. The helicity of the PEG chain seems to be the driving force for clustering of hydroxyl terminated PEG chains in water above 200 mM and near 30 °C.[8]

To investigate the shape of the aggregates, TEM images (Fig. 3(a)) were taken in aqueous solutions of SA_1 and SA_2 surfactants. The specimens were prepared using concentrations above their respective CAC values. The images (A) and (B) reveal large aggregates of 20–50 nm diameters. The distributions of hydrodynamic diameters of the

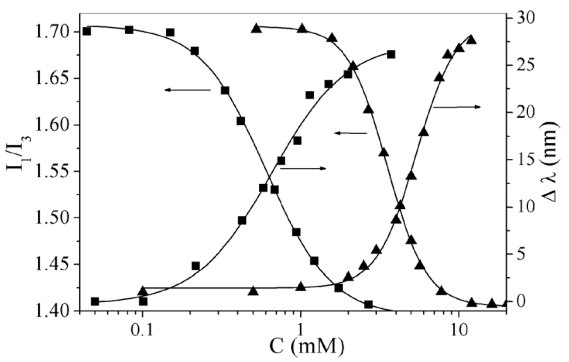

Fig. 2 Plots of shift of emission maximum, $\Delta\lambda$ of AN, and polarity ratio I_1/I_3 of Py *versus* [surfactant] in water at 298 K: (▲) SA_1, (■) SA_2.

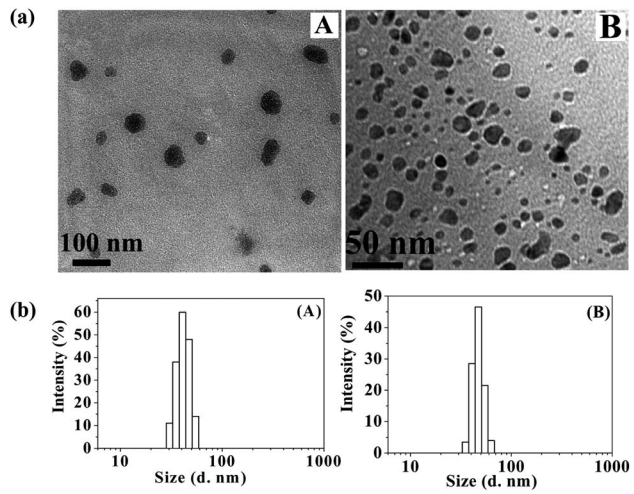

Fig. 3 (a) Negatively stained (1% sodium phosphotungstate) TEM micrographs of aqueous (A) SA_1 (5 mM), and (B) SA_2 (2 mM) solutions; (b) size distribution of the aggregates of (A) SA_1 and (B) SA_2 in water at 298 K.

aggregates were also obtained by direct measurement using the DLS technique. The corresponding size distribution has been depicted in Fig. 3(b). The results of the DLS measurement also confirm the existence of large aggregates in the size range of 40 nm to 90 nm. Since spherical micelles of surfactants having a hydrophobic chain length of 1.5 nm are expected to have diameters in the range of 3–5 nm, these large aggregates with tightly packed mPEG chains can be attributed to disk-like micelles. This is consistent with the results of fluorescence probe studies, which suggested formation of aggregates with relatively polar microenvironment of Py probe. Also in the case of disk-shaped micelles, the probe molecules are partially exposed to bulk water, thus showing slightly higher values of polarity ratio, I_1/I_3. However, the anisotropy value of DPH probe is much higher than that of spherical micelles of ionic surfactants, which is consistent with the more rigid bilayer core of the disk-like micelles.

In conclusion, we have synthesized two unusual carboxylate surfactants SA_1 and SA_2 that have a hydrophilic mPEG tail. The anionic surfactants have reasonably good surface-activity and they self-assemble in water to form large disk-like aggregates of diameter in the range of 20–80 nm. The driving force for aggregation is the helicity of the mPEG chain which is alternately hydrophobic and hydrophilic in nature. In contrast to the common notion, the mPEG chain of the surfactants has been shown to act like a hydrophobic tail. The CAC values of the surfactants are relatively lower than the corresponding hydrocarbon surfactants, SDS or SLS. The low CAC value and the mPEG chain make SA_1 and SA_2 surfactants more biocompatible than conventional fatty acid soaps. Despite large increase in the chain length of mPEG, the CAC value of the SA_2 surfactant is reduced only by 90%. The microenvironment of the bilayer disk is slightly polar but more rigid than that of spherical micelles of anionic SDS or SLS surfactants. These are the first examples of mPEG-derived anionic surfactants that form micellar aggregates in water.

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