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Physicochemical Characterization and Self-Assembly Studies on Cationic Surfactants Bearing mPEG Tail

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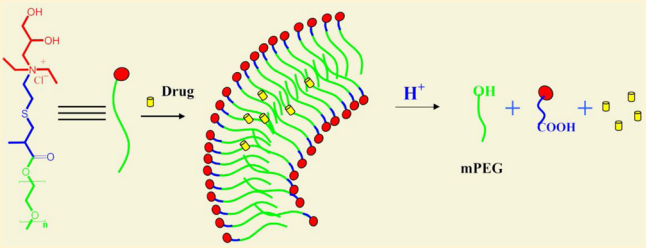
Supporting Information

ABSTRACT: Poly(ethylene glycol), PEG, is normally coupled to hydrophobic molecules to produce nonionic surfactants. However, there is no report so far on cationic surfactants in which PEG chain acts as a hydrophobic tail. In this work, two novel cationic amphiphiles containing a poly(ethylene glycol) monomethyl ether (mPEG) tail of different lengths linked to a cationic headgroup were synthesized to investigate their surface activity and self-assembling properties. The amphiphiles were shown to be surface-active with low critical micelle concentration (cmc). It has been found that although mPEG chain is hydrophilic as compared to hydrocarbon chain of equivalent length, the cmc values are lower than that of cetyltrimethylammonium chloride, a commercial cationic surfactant. The cationic surfactants have been shown to have antimicrobial activity. The fluorescence probe studies and the thermodynamic data have shown that the self-assembly is due to strong van der Waals interaction between mPEG chains as well as hydrophobic effect. The single-tailed cationic surfactants spontaneously self-assembled to form small unilamellar vesicles with hydrodynamic diameter in the range of 20−50 nm. The vesicles were characterized by fluorescence probe technique, dynamic light scattering, transmission electron microscopy, and confocal fluorescence microscopy. We have also studied encapsulation of model drugs by the vesicles and pH-triggered release kinetics.



INTRODUCTION

Surfactant molecules typically consist of a long nonpolar hydrocarbon tail and a polar hydrophilic headgroup.[1] Because of the difference in interaction of the two segments of the same molecule with water, surfactant molecules self-assembled in aqueous solution above a certain concentration, called critical micelle concentration (cmc), to give micelles or other nanosize structures. Micellar properties including shape and size of the aggregates formed depend upon the molecular architecture of the surfactant as well as on its concentration, environment, additives, and temperature.[1] Surfactants containing single, double, saturated, and unsaturated hydrocarbon tail as well as fluorocarbon tail have been reported.[1,2] However, to the best of our knowledge, there is no report, except a recent report from this laboratory,[3] on the surface activity and self-assembly formation by molecules containing poly(ethylene oxide), $(-O-CH_2CH_2-)_n$, chain acting like a hydrocarbon tail. It is expected that replacement of a $-CH_2-$ by oxygen (−O−) along the hydrocarbon, $-(CH_2)_n-$, chain would increase its interaction with water and thus will disfavor aggregate formation. Consequently, PEGs are coupled to hydrophobic molecules to produce nonionic surfactants.[4−7] Indeed, Tween-20, Triton-X-100, etc., are well-known nonionic surfactants in which the PEG chain acts as a polar headgroup. The micelle-forming ability of methoxy-PEG (mPEG) monoacrylate bearing 25−45 $-O-CH_2CH_2-$ units was first shown by Ito et al.[8] Subsequently, Mandal and co-workers[9−11] using a variety of techniques determined the cmc (0.12 mM), aggregation number (20), and hydrodynamic radius (22.4 Å) of the micelles formed by the macromonomer containing 9 $-O-CH_2CH_2-$ units. In these works, however, it was concluded that the mPEG chain acts as a hydrophilic group.

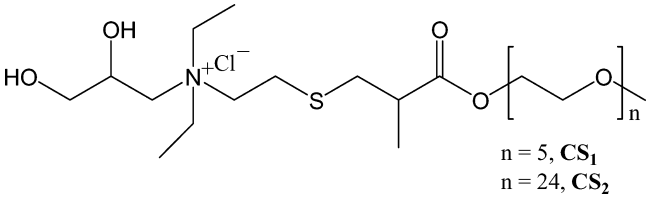
Because PEGs are known to be biocompatible, PEG-based polymers have been widely used in applications such as detergents[12] and personal care products,[13] especially drug delivery system[14−17] by virtue of their nanometer-scale size, increasing solubility in water, viscosity, flexible physical properties, and biocompatibility. So far, many micelle-forming copolymers of PEG with different hydrophobic blocks, such as poly (L-amino acids),[18,19] diacyl lipids,[20−22] etc., have been used to prepare drug loaded micelles by direct entrapment of drug into the micelle core and without covalent attachment of drug molecules to core-forming blocks. Morikawa and co-worker have reported the incorporation of carboxylic acid moieties into PEG-based nonionic surfactant showing a pH-controlled micellar system.[23] Clusters et al. have reported that modification of nonionic pluronic surfactants, PEG-poly-(propylene glycol)-PEG, with carboxylic acids at the terminal exhibits pH-dependent phase separation and micellar behavior for the purpose of the removal of metal ion and organic from

Received: August 8, 2012
Revised: November 19, 2012
Published: November 26, 2012

the wastewater.[24] Although aggregation of PEGs in the presence of salt and at higher temperatures has been reported in the literature,[25] there is no report so far on the room temperature surface activity and self-assembly formation of low-molecular-weight ionic amphiphiles formed by coupling mPEG with a small anionic or cationic headgroup by stable chemical bond. However, recently, we have synthesized two mPEG-based carboxylate surfactants of different chain lengths and have demonstrated their surface activity and self-assembly behavior in salt-free aqueous medium at room temperature.[3]

Nanometer-sized amphiphilic self-assembly is of significant interest for the controlled release of functional ingredients, because the association and collapse of aggregation formed by the amphiphiles are drastically controlled by various stimuli in aqueous media.[26−31] Effective pharmaceutical application requires adjustment of surface properties of nanoparticle to make them compatible with the biological environment. Cationic amphiphiles have great potential as antibacterial agents and gene delivery vehicles for cancer treatment in clinic.[1] The interaction between DNA and cationic surfactants is currently the focus of great interest in the scientific community due to their applications in biomedical sciences. The interaction between DNA−cationic surfactant system and some applications like DNA purification and extraction using quaternary ammonium surfactants, counting small quantities of DNA, and the precipitation have been well documented in recent literature.[32] It is believed that the compaction of DNA, together with the reduction of its charge, facilitates the uptake of nucleic acids through the cellular membrane. Because the strong binding of cationic surfactants to DNA causes these two effects, the complexation with cationic lipids can be used as a strategy for delivery of DNA to cells. However, common cationic surfactants containing hydrocarbon tail(s) precipitate DNA from solution. Rosa et al. have shown the precipitation maps of DNA and cationic surfactants of various chain lengths.[33] Thus, cationic amphiphiles containing hydrocarbon chain(s) are considered less biocompatible than PEG chain. With this in mind, we have synthesized two cationic amphiphiles **CS$_1$** and **CS$_2$** (see Chart 1 for chemical structures)

Chart 1. Chemical Structure of CS$_1$ and CS$_2$ Surfactants



containing an mPEG tail of different lengths (MW 232 and 1032). The low-molecular-weight PEGs (M_n < 1500 Da) are considered hydrophilic,[34] which means the head as well as the tail of our surfactants are hydrophilic. Therefore, on the basis of the current knowledge about solution behavior of PEG, the cationic amphiphiles **CS$_1$** and **CS$_2$** are not expected to be surface-active and self-assemble in water. To examine this, we have studied their surface activity and aggregation behavior in phosphate buffer as well as in salt-free water at room temperature using various techniques, including surface tension, conductivity, fluorescence, light scattering, and microscopy. We have also tested antibacterial activities of the cationic amphiphiles. It will be shown that these cationic amphiphiles form vesicles above a critical concentration. Also, entrapment and release of model hydrophobic drugs by the vesicles will be demonstrated.

RESULTS AND DISCUSSION

Interfacial Properties. The surface activity of **CS$_1$** and **CS$_2$** was studied in phosphate buffer (20 mM, pH 7). The plots of surface tension (γ mN m^{-1}) versus [surfactant] are shown in Figure 1. The surface activity as measured by the pC$_{20}$ (negative

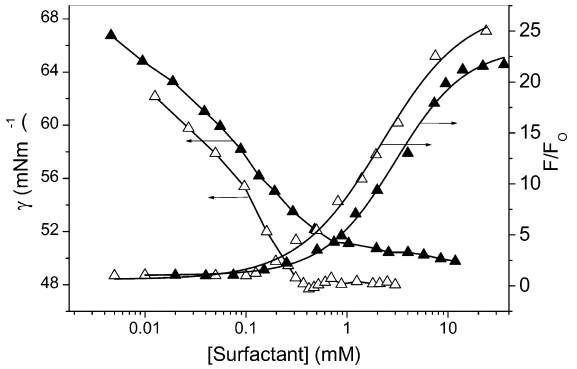


Figure 1. Plots of γ and F/F_o of AN versus [surfactant] in phosphate buffer (20 mM, pH 7) at 298 K; **CS$_1$** (▲), **CS$_2$** (△).

logarithm of surfactant concentration at which surface tension of water is reduced by 20 units) values (ca. 3) suggests spontaneous adsorption of the surfactants at the air/water interface and indicates hydrophobic nature of the mPEG tail. Although the lowest value of γ obtained is ca. 50 mN m^{-1}, these cationic surfactants are more surface-active than the commercial cationic surfactants dodecyltrimethylammonium chloride (DTAC) or cetyltrimethylammonium chloride (CTAC) with hydrocarbon tail. The concentration corresponding to the point of minimum surface tension (γ_{min}) in the surface tension plots was taken as cmc. The cmc values of **CS$_1$** (0.60 mM) and **CS$_2$** (0.41 mM) thus obtained are less than those of DTAC (15 mM) or CTAC (1.4 mM)[35] surfactants and are indicative of spontaneous aggregate formation. The smaller values of the minimum cross-sectional area (A_{min}) per headgroup of **CS$_1$** (81 Å^2) and **CS$_2$** (90 Å^2) at the air/water interface calculated using Gibbs adsorption equations[1] suggest formation of large aggregates in which the mPEG chains are tightly packed.

Fluorescence Probe Studies. The self-assembly formation by **CS$_1$** and **CS$_2$** in phosphate buffer was also indicated by the enhancement of fluorescence intensity as well as by the blue shift of the emission maximum of the hydrophobic fluorescent probe, AN (Figure S1, Supporting Information). The blue shift accompanied by a large increase in intensity of the fluorescence spectrum of AN in the presence of **CS$_1$** and **CS$_2$** at concentrations above their cmc values must be due to its incorporation into the nonpolar environment.[36] This means that the cationic surfactants self-assemble to form hydrophobic domains and is consistent with the results of surface tension studies. The plots of relative intensity (F/F_o, where F and F_o are the fluorescence intensities at λ = 460 nm in the presence and absence of surfactant, respectively) as a function of [surfactant] for both **CS$_1$** and **CS$_2$** are depicted in Figure 1. On the other hand, the variation of spectral shift ($\Delta\lambda = \lambda_{max}$(water) − λ_{max}(surf)) with [surfactant] is shown in Figure S2 of the Supporting Information. The cmc values (0.25 mM for **CS$_1$** and 0.15 mM for **CS$_2$**) as obtained from the fluorescence titration

dx.doi.org/10.1021/la303210f | Langmuir 2012, 28, 17247−17255

curves are slightly less than those obtained from surface tension measurements. However, the cmc value obtained from the surface tension plot is more accurate because, unlike fluorescence titration curves, it exhibits a sharp break.

The polarity of the microenvironment of the self-assembled structures formed by $\mathbf{CS_1}$ and $\mathbf{CS_2}$ was studied by using pyrene as an external 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 pyrene fluorescence spectrum (Figure S3, Supporting Information) is known to change with the polarity of the solvent.[37] The I_1/I_3 value was observed to be highest in water (1.70) that decreased with the increase in surfactant concentration. The variation of I_1/I_3 with [surfactant] is shown in Figure S2. The large decrease in I_1/I_3 value clearly suggests formation of aggregates, and the pyrene probe is solubilized within its microenvironment, which is relatively less polar as compared to bulk water. The low polarity ($I_1/I_3 \approx 1.22$) of the microenvironment of pyrene molecule is consistent with the large $\Delta\lambda$ value of the AN probe (see Figure S2) and can be attributed to tight packing of the mPEG chains forming the hydrophobic domain of the aggregates. This means that the mPEG chain acts like a hydrocarbon tail of conventional surfactants. Mandal and co-workers have also shown partial hydrophobic character of the mPEG chain by NMR measurements of the mixed micelles of mPEG monoacrylate macromonomer and SDS surfactant.[38] The hydrophobic character of the mPEG chain is further supported by the thermodynamic data as discussed below.

For block copolymers containing PEG chain, it has been reported that PEG chain undergoes dehydration in the presence of salt as well as with the increase of temperature leading to aggregation in water.[39] Therefore, fluorescence measurements using AN probe were also performed in water in the absence of any salt. The spectral shift as well as intensity rise confirm self-assembly formation in water (see Figure S4, Supporting Information). The increase of $\Delta\lambda$ and F/F_o values with surfactant concentration is shown in Figure 2. However,

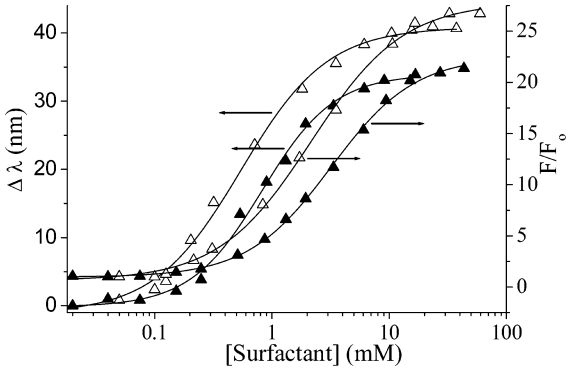


Figure 2. Plots of spectral shift ($\Delta\lambda$) and F/F_o of AN as a function of [surfactant] in water at 298 K; $\mathbf{CS_1}$ (▲), $\mathbf{CS_2}$ (△).

the cmc values (0.3 mM for $\mathbf{CS_1}$ and 0.2 mM for $\mathbf{CS_2}$) as obtained from the inflection points are slightly higher than that in phosphate buffer. This is due to increased ionic strength of the buffer solution that reduces the cmc value of ionic surfactants. Thus, the hydrophobicity of the mPEG chain cannot be attributed to dehydration in the presence of buffer salt as observed with block copolymers containing PEG chain. The hydrophobicity of mPEG chain cannot be attributed to temperature-induced dehydration either, as the present study was carried out at room temperature. In support of this conclusion, we have measured fluorescence spectra of AN probe in 2 mM surfactant solutions at different temperatures. For both surfactant solutions, a decrease in F/F_o (where F_o is the maximum intensity at 430 nm at room temperature) was observed with the increase of temperature (Figure 3). The

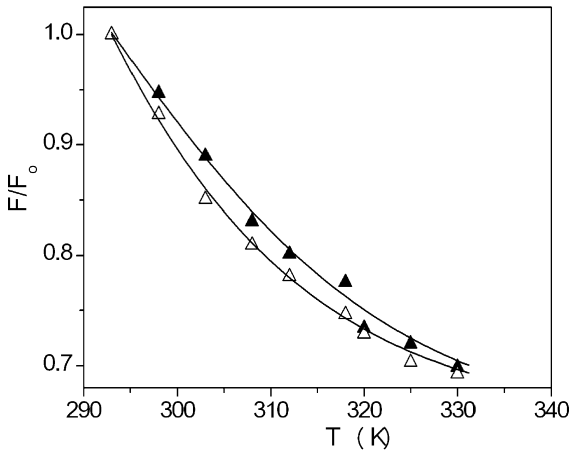


Figure 3. Plot of relative fluorescence intensity (F/F_o) of AN versus temperature (T) in 2 mM $\mathbf{CS_1}$ in (△) pure water and (▲) phosphate buffer (20 mM, pH 7).

control experiment in water, however, did not show a significant change in the fluorescence intensity with the increase of temperature. This suggests that the decrease in fluorescence intensity is either due to increase in polarity of the microenvironment or due to partial disruption of the aggregates, causing release of the probe molecule in bulk water. If the aggregation was caused by dehydration of the mPEG chain, then one would expect an increase in fluorescence intensity of the AN probe upon increase of temperature due to decrease in polarity of the microenvironment.

The steady-state fluorescence anisotropy (r) value of DPH probe is often used as an index of membrane rigidity of liposomes or vesicles.[40] The r-values of the DPH probe in the presence of 2 mM $\mathbf{CS_1}$ (0.177) and $\mathbf{CS_2}$ (0.165) surfactants are much larger than those of spherical micelles of conventional cationic surfactants DTAC ($r \approx 0.050$) and CTAC ($r \approx 0.060$) and are indicative of more ordered microenvironment around DPH probe. Further, the large r-value is consistent with the small values of polarity index (I_1/I_3) and A_{min} as discussed above and suggests the existence of bilayer aggregates, such as vesicles in which the mPEG chains are tightly packed in the bilayer. The critical concentration (cmc) above which the vesicles are formed will henceforth be called the critical vesicle concentration (cvc). The vesicles formed in solution are equilibrium structures and are stable over time. The stability was confirmed by the absence of any significant change in fluorescence intensity of AN probe (see Figure S5, Supporting Information) even 3 days after the sample preparation.

Thermodynamics of Self-Assembly Formation. The thermodynamic parameters of aggregation of both $\mathbf{CS_1}$ and $\mathbf{CS_2}$ in salt-free water were obtained from the following equations:[41,42]

$$\Delta G^{\circ} = (1 + \beta)RT \ln \frac{\text{cmc}}{C_r} \quad (1)$$

dx.doi.org/10.1021/la303210f | Langmuir 2012, 28, 17247−17255

Table 1. Critical Vesicle Concentration (cvc), Degree of Counterion Binding (β), and Standard Gibbs Free Energy Change (ΔG^o), Enthalpy Change (ΔH^o), and Entropy Change (ΔS^o) of Aggregation of CS_1 and CS_2 Surfactants in Salt-Free Water at Different Temperatures

T (±0.1 K)	cvc (±0.1 mM)	β (±0.03)	ΔG^o (kJ mol^{-1})	ΔH^o (kJ mol^{-1})	ΔS^o (J mol^{-1}K^{-1})
CS_1					
293	1.17	0.79	−29.50 ± 0.61	−20.90 ± 0.51	29.0 ± 2.7
298	1.28	0.66	−27.50 ± 0.59	−20.10 ± 0.51	25.0 ± 2.6
303	1.39	0.61	−26.70 ± 0.58	−20.10 ± 0.53	22.0 ± 2.6
308	1.50	0.52	−25.40 ± 0.56	−19.80 ± 0.53	18.0 ± 2.5
313	1.63	0.41	−23.70 ± 0.55	−18.90 ± 0.53	15.0 ± 2.4
CS_2					
293	0.41	0.82	−35.00 ± 1.20	−22.90 ± 0.54	41.0 ± 4.4
298	0.47	0.67	−32.00 ± 1.00	−21.70 ± 0.54	34.0 ± 3.7
303	0.53	0.56	−29.50 ± 0.93	−20.90 ± 0.54	30.0 ± 3.6
308	0.59	0.52	−28.70 ± 0.87	−21.10 ± 0.55	26.0 ± 3.2
313	0.64	0.44	−27.80 ± 0.83	−20.70 ± 0.55	23.0 ± 3.2

$$\Delta H^\circ = -(1+\beta)RT^2\left[\frac{\mathrm{d}\ln(\mathrm{cmc})}{\mathrm{d}T}\right] \quad (2)$$

$$\Delta S^\circ = \frac{\Delta G^\circ - \Delta H^\circ}{T} \quad (3)$$

where ΔG^o, ΔH^o, and ΔS^o are the change of standard Gibb's free energy, enthalpy, and entropy of aggregation of the surfactant, respectively, β (=1 − α) is the degree of counterion binding, and C_r (=1 mol L^{-1}) is the reference concentration. The cvc values at various temperatures were determined from conductivity (κ) measurements. The plots of κ versus [CS] at various temperatures (20−40 °C) are shown in Figure S6 of the Supporting Information. The plots exhibit two straight lines intersecting at the corresponding cvc value. Values of α at different temperatures were calculated as the ratio of the slope of the line above the cvc to the slope of the line below the cvc following Evan's method.[43] The thermodynamic parameters thus calculated from the cvc and β values using eqs 1−3 have been listed in Table 1. It is interesting to note that β values of CS_1 and CS_2 are closely equal despite the large difference in mPEG chain lengths. This is a striking difference between the present surfactants and the conventional cationic surfactants (e.g., CPC and DPC or CTAC and DTAC) bearing a hydrocarbon tail. It is well-known that cmc as well as the degree of counterion dissociation (α) of conventional surfactants decrease (i.e., β value increases) as the hydrocarbon tail length increases.[1] The data in Table 1 suggest that the degree of counterion binding, β, decreases continuously with the increase of temperature. Both ΔG^o and ΔH^o are negative at all temperatures and change very little over the temperature range. The large negative value of ΔG^o for both surfactants clearly indicates spontaneous formation of large aggregates, such as vesicles. The thermodynamic data are thus consistent with the results of surface tension and fluorescence probe studies. The negative values of ΔH^o show that the process of aggregation of the surfactants is exothermic. This means that the major attractive force for aggregation of surfactant molecules is van der Waals interaction. On the other hand, the entropy change, ΔS^o, is positive and has a decreasing tendency with the increase in temperature, indicating formation of more disordered aggregates. Similar results were also reported by others for dodecyltrimethylammonium bromide (DTAB) surfactant. In the case of DTAB, magnitudes of ΔH^o (−1.77 kJ mol^{-1}) and ΔG^o (−18.4 kJ mol^{-1})[44] are smaller than those of **CS_1** and **CS_2**. However, in the case of **CS_1** and **CS_2**, the contribution of the entropic term to the ΔG^o value is much less in comparison to the enthalpic term, which suggests that perhaps van der Waals interaction is more important than hydrophobic effect in the aggregate formation. The magnitudes of ΔH^o and ΔG^o for **CS_1** (or **CS_2**) clearly suggest that vesicle formation is energetically as well as entropically more favorable in the case of **CS_1** and **CS_2** than in the DTAB surfactant.

It is well-known that the behavior of PEG in solution is very complex. The PEG (or mPEG) chain as characterized by short carbon units separated by oxygen atom has good solubility in water as well as in nonhydroxylic organic solvents, such as chloroform. Although a lot of work was carried out, both experimentally[45−48] and theoretically,[49,50] the solution behavior of PEG is still not completely understood. From these studies, however, one can conclude that the hydrophilic−lipophilic balance (HLB) in these polyethers where conformation of molecule plays a very important role[49] determines their solution behavior. In the present work, the mPEG chain has been modified by linking it with a cationic headgroup through a short (5C) hydrocarbon chain. This perhaps has caused a change in conformation of the mPEG chain and hence HLB, making them behave like conventional ionic surfactants bearing hydrocarbon tail. However, unlike conventional surfactants, the aggregation is not due to the hydrophobic effect as indicated by the small positive entropy change (see Table 1). Therefore, the only driving force for spontaneous micelle formation could be van der Waals interactions. Because unsubstituted mPEG molecules are not known to aggregate in water, at this moment, it is believed that the strong van der Waals interactions among the mPEG chains in the case of **CS_1** and **CS_2** are associated with the conformational change caused by the ionic headgroup.

Size and Shape of the Aggregates. The size of the surfactant self-assemblies can be expressed in terms of either average aggregation number (N_{agg}) or by mean hydrodynamic radius (R_h). The N_{agg} value can be determined by various methods,[51,52] of which the fluorescence quenching method first developed by Tachiya[53] has been employed by many researchers.[9,54−59] However, this is an indirect method that relies on some assumptions.[55] Thus, the N_{agg} value obtained from this measurement is often misleading. On the other hand, the accuracy of the light scattering method is limited by the polydispersity of surfactant aggregates in solution. Because **CS_1** and **CS_2** surfactants form vesicles, the N_{agg} value is expected to be much larger than the micellar aggregates of conventional

dx.doi.org/10.1021/la303210f | Langmuir 2012, 28, 17247−17255

cationic surfactants, such as CTAB (N_{agg} = 95)[52] or CPC (N_{agg} = 52).[52] The formation of large aggregates is also indicated by the small A_{min} and large β values obtained from surface tension and conductivity measurements, respectively. The hydrodynamic size of the vesicles was therefore measured directly by use of dynamic light scattering (DLS) technique. The corresponding size distributions are shown in Figure 4.

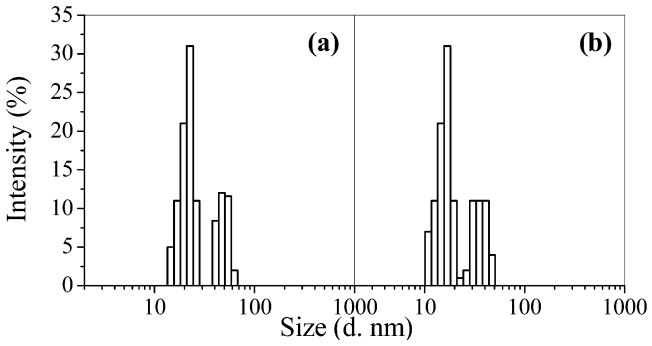


Figure 4. Size distributions of SUVs in 2 mM solution of (a) $\mathbf{CS_1}$ and (b) $\mathbf{CS_2}$ in phosphate buffer (20 mM, pH 7).

Although size distributions appear to be bimodal in nature, their combined width is still narrow (10−70 nm) and therefore can be taken as monomodal distributions. As seen, the mean hydrodynamic diameters of the aggregates formed by $\mathbf{CS_1}$ (~30 nm) and $\mathbf{CS_2}$ (~25 nm) in aqueous buffer are large in comparison to normal spherical micelles of DTAC (3.68 nm)[60] surfactant. The N_{agg} value of the large aggregates can be obtained from the measured A_{min} and R_h values using the equation:[61]

$$N_{agg} = 8\pi(R_h)^2/A_{min} \quad (4)$$

The N_{agg} values of the surfactants $\mathbf{CS_1}$ (7980) and $\mathbf{CS_2}$ (4362) thus obtained are very high and rule out the existence of small micelles. Similar large values of N_{agg} were also reported for other bilayer-forming amphiphiles by other researchers.[61,62] The high value of N_{agg} is consistent with the vesicular aggregates and can be ascribed to SUVs, which have narrow size distributions. The mean hydrodynamic diameters of the vesicles formed by $\mathbf{CS_1}$ and $\mathbf{CS_2}$ in aqueous buffer are closely similar to those observed in respective transmission electron micrographs (TEM) as shown below.

To visualize the morphology of the aggregates, TEM of the surfactant solutions was measured. The TEM images in Figure 5a clearly reveal the existence of small unilamellar vesicles (SUVs) in dilute solution (<2 mM) of both surfactants. However, the vesicles have distorted spherical shapes. The size of the SUVs lies in the range from 20 to 50 nm. Because TEM measurements by the staining method have been criticized in the literature, we have taken confocal fluorescence microscopic images of the 2 mM $\mathbf{CS_1}$ and $\mathbf{CS_2}$ solutions containing carboxyfluorescein (FC). The optical micrographs, OM (Figure 5b), also reveal monodisperse aggregates of diameter ca. 35 nm, which have aqueous core confirming vesicles. However, an attempt to show the bilayer in the OM by use of a hydrophobic fluorescent probe (coumarin 153) failed because the thickness of the bilayer is expected to be ca. 3 nm, which is beyond the limit of resolution (~100 nm). The results of both types of microscopic measurements are similar and are also consistent with the fluorescence probe studies.

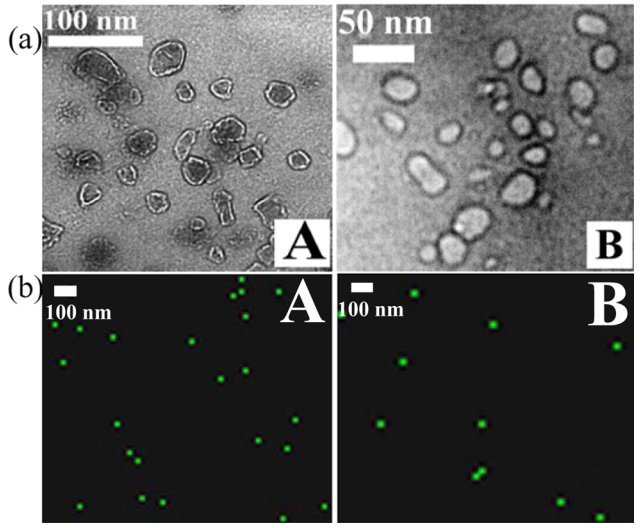


Figure 5. (a) Negatively stained (with 1% w/v uranyl acetate) TEM micrographs and (b) confocal fluorescence microscopic images of 2 mM solution of (A) $\mathbf{CS_1}$ and (B) $\mathbf{CS_2}$ in phosphate buffer (20 mM, pH 7).

The SUVs, as expected, are positively charged, which is indicated by the ζ-potential values measured in 2 mM $\mathbf{CS_1}$ (+ 28 mV) and $\mathbf{CS_2}$ (+ 34 mV) solution. It should be noted that size distributions (not shown here) of the SUVs formed by both surfactants remained almost unchanged when measured after 72 h. This suggests that the vesicles are stable equilibrium structures.

Drug Encapsulation and Release Studies. As the mPEG tail of the cationic surfactants contains a hydrolyzable ester linkage, hydrophobic drug molecules solubilized within the bilayer membrane can be released in an acidic pH. To examine this, we monitored the release of DPH probe upon acidification (pH ≈ 3) by measuring its fluorescence spectra at different time intervals. At low pH, the ester linkage is slowly hydrolyzed, thus disrupting the vesicle membrane, which releases the DPH molecules. Because DPH is poorly soluble in water and hence weakly fluorescent, the relative emission intensity F/F_o (where F_o is the initial intensity at λ = 450 nm at room temperature) decreased with time following a first-order rate ($k \approx 1.2 \times 10^{-2}$ min^{-1} for $\mathbf{CS_1}$ and 9.8×10^{-3} min^{-1} for $\mathbf{CS_2}$) as shown by the plots in Figure 6. The rate constants are almost equal, indicating that the ester linkages of both amphiphiles in the vesicle bilayer are equally accessible to water. The DPH release

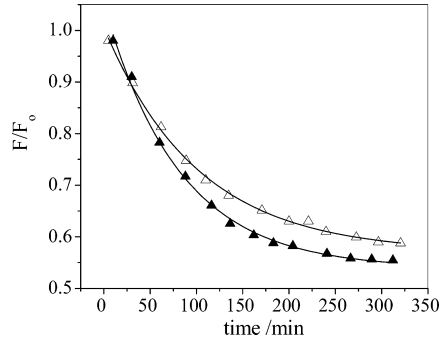


Figure 6. Hydrolysis-triggered release of DPH probe in pH 3: (▲) $\mathbf{CS_1}$ and (△) $\mathbf{CS_2}$.

dx.doi.org/10.1021/la303210f | Langmuir 2012, 28, 17247−17255

rate is expected to be slower at 3 < pH < 8 because the rate of ester hydrolysis becomes slower around neutral pH.

Antimicrobial Activity. Because cationic surfactants are known to have bactericidal activity, both CS_1 and CS_2 were tested for gram-positive as well as gram-negative bacterium. Against gram-positive *B. subtilis* bacteria, the values of minimum inhibitory concentration (MIC) of CS_1 (10 μg/mL or ~17 μM) and CS_2 (40 μg/mL or ~30 μM) surfactants are observed to be lower than that of commercial cationic surfactant cetyltrimethylammonium bromide, CTAB (44 μg/mL).[63] On the other hand, the MIC values against gram-negative *E. coli* bacteria were 40 μg/mL (~68 μM) and 70 μg/mL (~54 μM), respectively, for CS_1 and CS_2. It is important to note that MIC values of CS_1 and CS_2 are much less than their respective cvc values in buffer medium. Similar results have also been reported for hydantoin drug for which the MIC value is close to its cmc value.[64] The MIC values of CS_1 are comparable to benzalkonium chloride with MIC value of 14 μg/mL for gram-positive *B. subtilis* bacteria and 28 μg/mL for gram-negative *E. coli* bacteria.[65] Also, the MIC values of CS_1 and CS_2 are much lower than those of bis-quaternary ammonium salts (cationic gemini surfactants) (MIC > 512 μg/mL).[66,67] Thus, CS_1 and CS_2 have reasonably good antibacterial activity against both gram-positive and gram-negative bacteria.

CONCLUSIONS

Two novel cationic surfactants CS_1 and CS_2 of different mPEG chain lengths were synthesized and characterized. The surface activity of the cationic surfactants is as good as commercial surfactants of comparable hydrocarbon tail length. Both surfactants are characterized by low cmc values and large aggregation numbers. In dilute solutions of concentration greater than cmc, these surfactants have been shown to spontaneously form small unilamellar vesicles (SUVs) with diameters in the range of 20−70 nm. The negative values of ΔH^o and positive values of ΔS^o clearly show that the vesicle formation is due to both energetic and entropic effects. However, the large value of ΔH^o suggests that the spontaneous aggregation of the surfactants is mainly due to van der Waals interaction between mPEG chains and the mPEG chain acts like hydrocarbon tail. This is perhaps due to the conformational change of the PEG chain caused by the ionic headgroup. However, a more detailed study on the effects of (i) the nature of ionic headgroup and (ii) linker chain length on the conformational change of PEG chains of structurally similar amphiphiles is required for proper understanding. A detailed experimental as well as theoretical investigation on structurally similar molecules with different ionic head groups is currently underway in this laboratory, and this issue will be taken up in a separate publication. To our knowledge, this is the first report on the surface activity and self-assembly formation by mPEG-based cationic surfactants at submillimolar concentration at room temperature. We have demonstrated encapsulation of DPH, a hydrophobic model drug, and hydrolysis-triggered release behavior of the SUVs formed by the surfactants. The surfactants can thus be used for pH-triggered gene delivery. Further, in comparison to traditional cationic surfactants, both CS_1 and CS_2 show reasonably good antimicrobial activity against gram-negative as well as gram-positive bacteria at a concentration 10 times less than their respective cvc values. We believe that this Article will spark new research in this field.

EXPERIMENTAL SECTION

Materials. Poly(ethylene glycol)methyl ether methacrylate (MW 300 and 1100), *N,N*-diethylaminoethanethiol, and (±)-3-chloro-1,2-propanediol were purchased from Aldrich and were used without further purification. The fluorescence probes 1-anilino-naphthalene (AN), 1,6-diphenyl-1,3,5-hexatriene (DPH) pyrene, coumarin 153, and carboxyfluorescein (CF) were obtained from Aldrich and were purified by repeated recrystallization from an ethanol−acetone mixture. Analytical grade sodium dihydrogen phosphate and disodium monohydrogen phosphate were procured locally and were used directly from the bottle. Milli-Q water (18 MΩ) water was used for the preparation of aqueous solutions.

The cationic amphiphiles were synthesized in the laboratory by the Michael addition reaction of *N,N*-diethylaminoethanethiol with poly(ethylene glycol)methyl ether methacrylate according to the reported method[68] followed by quaternization by refluxing with (±)-3-chloro-1,2-propanediol. Details of the synthesis and chemical identifications of the intermediates and products, including ^{1}H and ^{13}C NMR spectra (Figures S7−14), are included in the Supporting Information.

Methods and Instrumentation. *General Instruments.* The ^{1}H NMR and ^{13}C NMR spectra were recorded on an AVANCE DAX-400 (Bruker, Sweden) 400 MHz NMR spectrometer in $CDCl_3$ solvent using TMS (tetramethylsilane) as internal standard. The FT-IR spectra were measured with a Perkin-Elmer (model 883 IR) spectrometer. For solid samples, KBr pellet was used as solvent. The pH measurements were done with a digital pH meter model 5652 (EC India Ltd., Calcutta) using a glass electrode. The elemental analysis was carried out with a MICRO CHNS analyzer (S. no.: 15082029).

Surface Tension Measurements. The surface tension (γ) of the surfactant solutions was measured by Du Nüoy ring detachment method with a surface tensiometer (model 3S, GBX, France) at 298 ± 0.1 K. Ethanol−HCl solution was often used for cleaning the platinum ring, and it was burnt in oxidizing flame by use of a Bunsen burner. The instrument was calibrated and checked by measuring the surface tension of distilled water before each experiment. Surfactant solutions of different concentrations were made in phosphate buffer (20 mM, pH 7) using Mili-Q water. For each concentration, three measurements for γ were performed, and their mean was taken as the value of the equilibrium surface tension. The temperature of the solution was controlled by a Thermo-Neslab RTE-7 circulating water bath with a temperature accuracy of ±0.1 °C. The solution was equilibrated for 5 min before a measurement was taken.

Conductivity Measurements. Electrical conductivity measurements were performed with a Thermo-Orion digital conductivity meter (model 150 A+) using a conductivity cell having a cell constant of 0.467 cm^{-1}. A measured volume of the surfactant stock solution in salt-free water of known concentration was taken in a water-jacketed beaker. The solution was gently stirred magnetically, and the temperature of the solution was controlled by a Thermo-Neslab RTE-7 circulating water bath with a temperature accuracy of ±0.1 °C. The solution was equilibrated for 5 min to get a constant conductivity value. Conductivity (κ) was measured at different dilutions of the stock solution at a fixed temperature.

Fluorescence Measurements. Steady-state fluorescence measurements 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 measurements started 3−4 h after the sample preparation. The excitation wavelengths were 335 nm for pyrene, 340 nm for AN, and 350 nm for DPH. The excitation slit width (band-pass) was set at 2.5 nm for excitation and 2.5−10 nm for the emission depending upon sample concentration. Emission spectra of pyrene 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 and 5 nm, respectively. In all experiments, background spectra, either of water (or buffer) alone or of water containing surfactant, was 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.

Dynamic Light Scattering. The dynamic light scattering (DLS) measurements were carried out using a Zetasizer Nano (Malvem Instrument Lab, Malvern, U.K.) optical system equipped with a He−Ne laser operated at 4 mW at λ_o = 632.8 nm, a digital corelator. The scattering intensity was measured at a 173° angle to the incident beam. Surfactant solutions were prepared in phosphate buffer using Milli-Q water at least 3 h before measurement. The solution was filtered through a Millipore Millex syringe filter (0.45 μm) directly into the scattering cell. Prior to measurements, the scattering cell was rinsed several times with the filtered solution. The DLS measurement started 5−10 min after the sample solution was placed in the DLS optical system to allow the sample to equilibrate at the bath temperature. For all light scattering measurements, the temperature was 303 ± 0.5 K. The apparent hydrodynamic radius (R_h) of the surfactant aggregates was obtained using the Stokes−Einstein equation, $D = k_BT/(6\pi\eta R_h)$, where k_B is the Boltzmann constant and η is the solvent viscosity at temperature T.

Zeta Potential Measurements. The surface zeta potential of the aggregates was measured using a Zetasizer Nano ZS (Malvern Instrument Laboratory, Malvern, U.K.) optical system equipped with an He−Ne laser operated at 4 mW ($\lambda_o \cong$ 632.8 nm) at 298 K.

Transmission Electron Microscopy. Transmission electron micrographs (TEM) were obtained 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 30 s followed by blotting the excess liquid, staining with 1% aqueous uranyl acetate, and air-drying. The specimens were kept in desiccators for further drying until before measurement.

Confocal Fluorescence Microscopy. All confocal fluorescence microscopy (CFM) imaging experiments were performed with a FV 1000 Olympus confocal microscope equipped with a laser scanning module (LSM) microscope and a PLAPON 60× oil immersion objectives. The numerical aperture (NA) of LSM was 1.42. For CF-labeled vesicles, we used a 488 nm laser and a 520 nm filter. For encapsulation of dyes into the vesicles, the surfactant mixture and dye solution of desired concentration in methanol were gently mixed and then dried by rotary evaporation in a round-bottom flask. The thin film of surfactant mixtures thus produced was soaked in water overnight, and then the appropriate volume of buffer was added to obtain a clear solution. The excess dye was removed by dialysis for 10−12 h in a biodialyzer using an ultrafiltration cellulose acetate membrane (pore size 1000 Da MWCO, diameter 16 mm). An aliquot of the undiluted vesicle solution was pipetted into the microscope glass slides (Riviera, 25.4 × 76.2 mm) and sealed with a coverslip and left to sit coverslip down for few minutes before analysis. All vesicles were imaged at room temperature, and image projections of dye-trapped vesicles were digitally magnified and analyzed using FV10-ASW 1.6 Viewer software.

Antibacterial Activity Measurements. The minimum inhibitory concentration (MIC) of the surfactants was determined against gram-negative bacteria, *Escherichia coli* strain, and gram-positive bacteria, *Bacillus subtilis* strain, as a test control by slight modification of the described method of the National Committee for Clinical Laboratory Standards (NCCLS).[69] MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The MICs were taken as the minimal concentration showing no growth (absence of turbidity) after 24 h of incubation at 303 K for bacteria. MICs were determined using a serial dilution method. In our experiment, we have found that the MIC values of mPEG-based cationic surfactants are significantly lower than the MIC value of commercial cationic surfactant CTAB. That indicates the effectiveness of the low concentration of $\mathbf{CS_1}$ and $\mathbf{CS_2}$ against *Escherichia coli* and *Bacillus subtilis*.

■ ASSOCIATED CONTENT

Supporting Information

Details of synthesis, chemical identification, ^{1}H and ^{13}C NMR spectra of $\mathbf{CS_1}$ and $\mathbf{CS_2}$, fluorescence spectra of AN and pyrene, and fluorometric and conductometric titration curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the Department of Science and Technology, New Delhi, for support of this work (SR/S1/PC-18/2005). S.S. acknowledges the Indian Institute of Technology Kharagpur for a partial research fellowship. We thank Dr. R. Sen of the Department of Biotechnology for assistance with the antibacterial measurements.

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