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Self-Assembly of Unconventional Low-Molecular-Mass Amphiphiles Containing a PEG Chain

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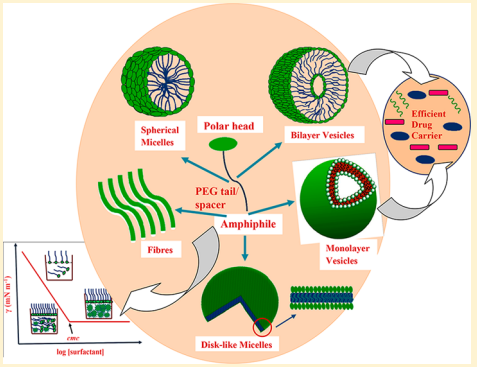
ABSTRACT: The design and synthesis of biocompatible surfactants are important for a wide range of applications in cosmetics, personal care products, and nanomedicine. This feature article summarizes our studies over the past 8 years on the design, synthesis, surface activity, and self-assembly of a series of unconventional low-molecular-mass amphiphiles containing a poly(ethylene glycol) (PEG) tail or spacer and different ionic or zwitterionic headgroups, including carboxylate, sulfonate, and quaternary ammonium salts. Despite having a so-called polar PEG chain as a tail or spacer, these ionic amphiphiles are found to have a tendency to adsorb at the air/water interface and self-assemble in pH 7.0 buffers at 298 K in the same way that conventional hydrocarbon tail surfactants do. However, they are observed to be relatively less surface-active compared to hydrocarbon tail surfactants. Although these amphiphilic molecules have less surface activity, they do self-assemble in aqueous buffer at 298 K, producing a range of microstructures, including spherical micelles, disclike micelles, and vesicles. In fact, our group is the first to report the self-assembly of PEG-tailed ionic amphiphiles in water at room temperature. Some of these molecules are also found to gel various organic liquids on heat−cool treatment or by ultrasound irradiation. We think that the present article will arouse general interest among researchers working toward the development of new biocompatible amphiphiles and soft materials.



INTRODUCTION

Surfactants find widespread applications in industrial, household, and pharmaceutical formulations. Consequently, a large variety of surfactants have been developed.[1,2] Synthetic surfactant molecules are amphiphilic in nature and are composed of a water-compatible polar head and a hydrophobic tail that is compatible with an oil phase. The hydrophilic headgroup can be either uncharged (neutral or zwitterionic) or charged (positive or negative). The hydrophobic tail is usually a long, fully saturated, or partially unsaturated hydrocarbon chain. However, surfactants having other hydrophobic groups, such as aromatic moieties,[3] steroids,[4] and vitamin E,[5] are well known in the literature. Surfactants containing more hydrophobic fluorocarbon tails have also been reported.[6] Surfactants such as phospholipids have double hydrocarbon tails.[7] Phospholipids having both positively and negatively charged centers at the headgroup are charge-neutral and hence biocompatible. Synthetic nonionic surfactants, on the other hand, usually consist of a nonionic sugar[8] or poly(ethylene glycol) (PEG)[9−11] head and a hydrocarbon tail. In fact, Triton X-100 and Tween-20 are well-known nonionic surfactants in which the PEG chain acts as a polar headgroup. Also, there are many reports on copolymers of PEG with different hydrophobic blocks, such as poly(propelyne oxide) and poly(1,2-butylene oxide), that have been found to behave like low-molecular-mass amphiphiles in water.[12−15] Mandal and co-workers using a variety of techniques exhibited aggregate formation in water by the methoxypoly(ethylene glycol) monomethacrylate (mPEG) macromonomer containing nine $[-O-CH_2-CH_2-]$ units and concluded that the mPEG chain acts as a hydrophilic headgroup.[16]

The self-assembly behavior depends on the molecular architecture of the amphiphile as well as on the concentration, temperature, nature of solvent, and presence of additives. Thus, under different conditions, amphiphilic molecules can form a wide variety of microstructures, including spherical, rodlike, and disclike micelles; reverse micelles; bilayer vesicles; and flat bilayers in solution above the cmc. For example, phospholipids with double hydrocarbon tails are known to form liposomes (vesicles) in water above a very low cmc value.[17] Synthetic double-tailed surfactants have also been shown to form vesicles in aqueous solution.[18] Although vesicular aggregates are usually produced by double-tailed surfactants, there are many reports in the literature on single-tailed surfactants, such as *N*-acyl amino acid derivatives,[19,20] and catanionic mixtures[21] that form vesicles in water. The nature of aggregates formed by the amphiphiles depends on the packing parameter, P ($P = v_h/(A_{min}l_c)$, where A_{min} is the cross-sectional area of the headgroup at the air/water interface, l_c is the extended hydrocarbon chain length, and v_h is the volume of the hydrocarbon tail(s)).[22] It has been shown that cylinder-shaped amphiphiles ($1/2 \leq P \leq 1$) produce vesicles with an aqueous core surrounded by one or more bilayer membranes.[22,23] Similarly, cone-shaped amphiphiles with $P \leq 1/3$ form spherical micelles that have a fluid hydrocarbon core. The molecules that form disclike micelles or flat bilayer structures have $P = 1$. On the other hand, truncated cone-shaped amphiphilic molecules have

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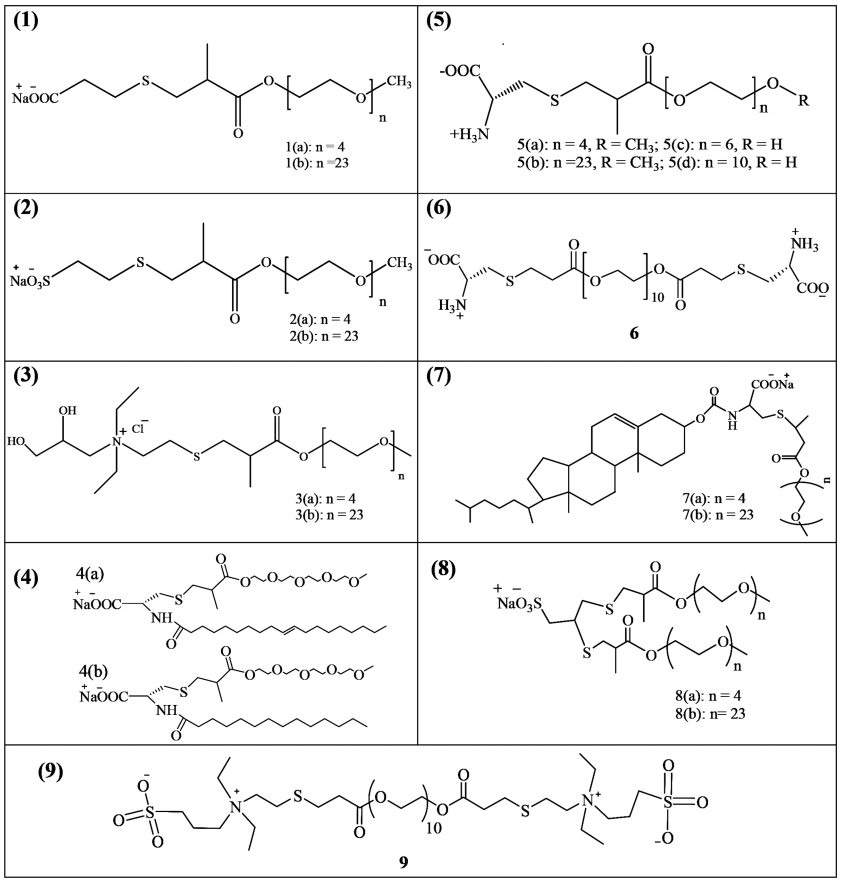
Chart 1. Chemical Structures of PEG-Based Low-Molecular-Mass Amphiphiles



$P > 1$ and form reverse micelles in nonaqueous solvents. Because of the unique structure and properties of each of the self-assemblies, they can be used for various applications, such as solubilization, encapsulation, delivery, and so forth. Therefore, understanding the solution properties and self-assembly behavior of amphiphilic molecules is important to the development of new and practical applications.

For applications in biological and medicinal fields, the amphiphilic molecules must be biocompatible as well as biodegradable. Among single hydrocarbon chain surfactants, amino acid-derived small molecular amphiphiles have proven promising not only because of cheap, raw materials and ease of synthesis but also because of biocompatibility. The self-assembly of amino acid-based surfactants has been reviewed in the literature.[24−27] On the other hand, nonionic surfactants consisting of PEG as the head and poly(lactic acid),[28] proteins,[29,30] poly(L-amino acid)s,[12,31] diacyl lipids[32−34] as the tail have recently been developed. This is because PEG has low toxicity and reactivity, a flexible structure, and good water solubility. Also, the stealth effect of the so-called hydrophilic PEG chain makes them useful in developing functional materials and drug delivery systems (DDS). Therefore, PEGylation has recently been applied to oligonuleotides,[35−38] biodegradable hydrogelators,[39,40] and dendrimers.[41−44] They have been conjugated to many pharmaceuticals to overcome the limitations of low solubility, a short circulation lifetime, and immunogenicity.[45] Thus, PEG-based polymers have been widely used in detergents, personal care products, and drug delivery.

One of the interesting properties of PEG is its thermal response in aqueous solution. It is known that PEGs exhibit both lower critical solution temperature (LCST) and upper critical solution temperature (UCST) phenomena in water leading to phase separation as a result of self-assembly due to temperature elevation.[46] Such a phenomenon is not observed with conventional ionic surfactants. The aggregation of PEGs in the presence of a salt has also been reported in the literature.[47] The formation of aggregates or clusters of PEG molecules in water, though controversial, is well known.[48] The self-assembly of methoxy-PEG monomethacrylate (mPEG) bearing 25 to 45 [−O−CH_2−CH_2−] units was first shown by Ito and co-workers.[49] Zhou and Brown reported that the PEG molecules aggregate in methanol (MeOH) at room temperature.[50] While there are reports suggesting that aggregation is an inherent property of PEG molecules in water,[51−53] some authors argued that the aggregation of PEG molecules is a result of impurities.[54−56] On the basis of the results of light-scattering measurements, others have eliminated the possibility of aggregate formation by PEGs in water, MeOH, or acetonitrile (ACN) at room temperature.[57,58]

However, amphiphiles containing PEG chains have become a topic of interest due to their biocompatibility and anomalous behavior in water.[59,60] Consequently, for the development of a more effective material and drug design, an in-depth understanding of the physicochemical properties of the compounds containing PEG chains is required. Our interest in the PEG-chain-containing amphiphiles is motivated by the huge biological and industrial importance of PEG molecules in a wide range of applications, involving protein crystallization,[61−63] the modification of surfaces and membranes for biocompatibility,[64,65] and the control of particle aggregation in solution.[66] Although a large volume of experimental work on PEG/water

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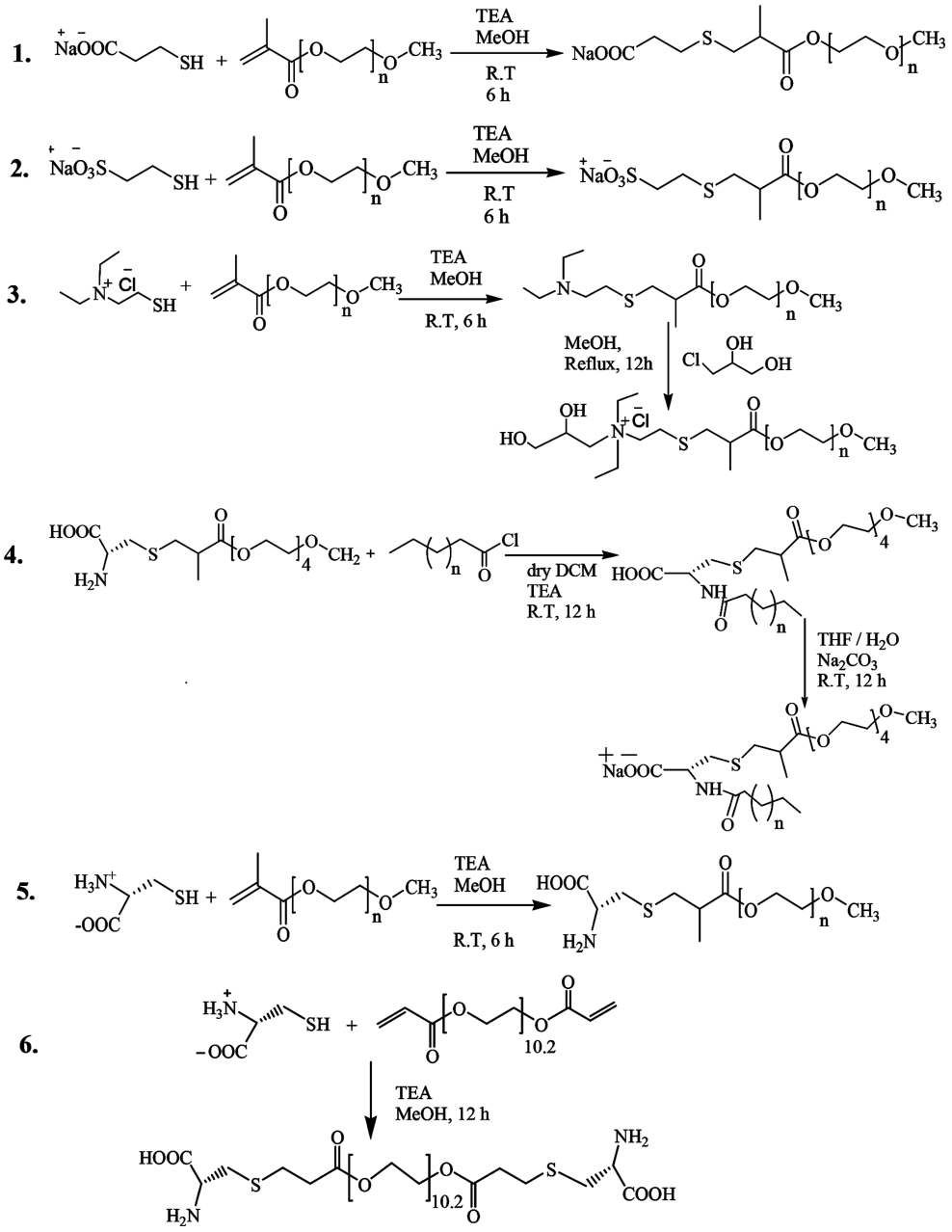
Scheme 1. Synthesis Schemes for Representative Amphiphiles



systems has been carried out to understand this interesting system, there are no reports on the self-assembly of ionic compounds containing the mPEG tail, except those published earlier by our group.[67−74] In these works, our major goal was to find out whether PEG-chain-containing ionic compounds can self-assemble in water at room temperature. If so, what kind of microstructure they will form, and will the molecules behave in the same way as conventional amphiphiles with hydrocarbon tails? This feature article summarizes recent studies on the structure and self-assembly properties of a number of unconventional amphiphilic molecules bearing mPEG as tail or PEG as a spacer. (See Chart 1 for structures.)

SYNTHESIS OF PEG-TAILED AMPHIPHILES

The detailed design, synthesis, and chemical identification of these molecules can be found in our earlier publications.[67−74] Herein, we include only a general scheme for the synthesis of representative molecules (Scheme 1). Briefly, poly(ethylene glycol)methyl ether methacrylate or poly(ethylene glycol) diacrylate was reacted with 3-mercapto-propionic acid, sodium 2-mercaptoethanesulfonate (mesna), *N*,*N*-diethylaminoethanethiol, or L-cysteine in nonaqueous media containing triethylamine (TEA) to produce the desired precursor molecule. The sodium salt was obtained from the corresponding carboxylic acid derivative upon reaction with sodium bicarbonate. Similarly, a quaternary ammonium salt was prepared by refluxing the corresponding amine with (±)-3-chloro-1,2-propanediol.

SINGLE PEG-TAILED ANIONIC AMPHIPHILES

Low-molecular-weight PEGs are highly soluble in water because of the strong hydrogen-bonding interaction of water molecules with the electron-rich oxygen atoms in the polyether chain. However, PEGs are also known to be soluble in organic solvents, including tetrahydrofuran (THF), chloroform (CF), dimethyl

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Table 1. Surface Activity (pC_{20}), cmc, Micropolarity (I_1/I_3), Microviscosity (η_m), Hydrodynamic Diameter of Micelles (d_h(nm)), and Thermodynamic Parameters ($\Delta_{mic}G^o$, $\Delta_{mic}H^o$, $T\Delta_{mic}S^o$) of the Micellization Process of 1(a,b), 2(a,b), 3(a,b), 4(a,b), 5(a−d), and 6 in Phosphate Buffer (20 mM, pH 7.0) at 298 K

amphiphile	cmc (mM)	pC_{20}	I_1/I_3	η_m (mPa s)	d_h (nm)	$\Delta_{mic}G^o$ (kJ mol^{-1})	$\Delta_{mic}H^o$ (kJ mol^{-1})	$T\Delta_{mic}S^o$ (kJ mol^{-1})
1a	2.25	∼3.00	1.47	70.9[a]	40			
1b	0.25	∼3.00	1.38	69.0[a]	50			
2a	2.0	2.30	1.63	46.0	150	−14.66	0.27	14.93
2b	0.9	2.40	1.61	22.0	5	−16.99	0.43	17.43
3a	0.25	∼3.00	1.22	67.9[a]	50	−27.50	−20.10	7.45
3b	0.15	∼3.00	1.22	50.6[a]	40	−32.00	−21.70	10.13
4a	0.1	5.25	1.02	49.6	50	−48.25	−1.13	47.08
				22.3	6	−38.98	0.42	39.34
4b	0.15	4.84	1.02	55.8	300	−44.74	−0.61	44.40
				25.8	11	−37.56	1.78	39.04
5a	1.0	2.28	1.54	65.0	50−250	−16.81	0.42	17.23
5b	0.2	2.36	1.46	79.0	50−250	−21.81	2.20	24.01
6	0.6	2.30	1.38	70.1	250	−18.26	0.19	18.45

[a]Calculated from the corresponding r value from the respective reference and assuming τ_f equal to 5.4 and 4.6 ns, respectively, for shorter- and longer-chain amphiphiles.[71]

sulfoxide (DMSO), and MeOH. It is interesting that the solubility of PEG in these solvents decreases in the order THF > CF > DMSO > MeOH > water.[75] This suggests that in comparison to THF, CF, DMSO, and MeOH, water is a bad solvent for PEG and is itself amphiphilic in nature. The hydrophobic ethylene (−CH_2−CH_2−) units and the hydrophilic oxygens (−O−) in the PEG chain are responsible for its amphoteric character.[76] In fact, PEGs are known to reduce the surface tension (γ) of water. For example, the γ value of water in the presence of 0.4% w/w (= 13 mM) PEG_{300} reaches a limiting value of ca. 50 mN m^{-1}.[77] The surface activity of an amphiphilic molecule is usually measured by the pC_{20} parameter, which is defined as the negative logarithm of the concentration of amphiphile required to reduce the γ of water by 20 units at room temperature. The amphiphiles that have $pC_{20} \geq 3$ are usually considered to be surfactants.[78] Therefore, simple PEGs cannot be called surfactants. Although the surface activity depends on the chain length, nature of the headgroup, environment, and temperature, the conventional hydrocarbon chain surfactants have $pC_{20} \geq 3$.[79]

The surface activity of all of the PEG-chain-containing amphiphiles was measured in phosphate buffer at 298 K, and the relevant data are listed in Table 1. The results can be compared with those of sodium dodecyl sulfate (SDS) and sodium lauryl sarcosinate (SLS) surfactants that are widely used in industry. However, SLS and SDS are not good examples for this comparison because they are structurally different from those of PEG-tailed ones. Therefore, here we examine the possibility of using the PEG-tailed surfactants in place of SDS and SLS. It is observed that both amphiphiles **1a** and **1b** with the carboxylate (−COO^-) headgroup have reasonably good surface activity as indicated by the pC_{20} values.[67] Interestingly, the pC_{20} values of **1a** and **1b** are comparable and the cmc values are less than that of SLS.[80] On the other hand, the data in Table 1 show that amphiphiles **2a** and **2b** having the sulfonate (−SO_3^-) headgroup[68] have cmc values that are almost equal to that of the corresponding hydrocarbon chain surfactants, sodium dodecyl sulfonate ($C_{12}H_{25}SO_3Na$) and SDS.[79] The pC_{20} values of **2a** and **2b**, within the experimental error range, are equal to that of $C_{12}H_{25}SO_3Na$ (2.3).[79] However, it should be noted that not only **2a** and **2b** but also $C_{12}H_{25}SO_3Na$ have cmc values greater than that of SDS. In fact, $C_{12}H_{25}SO_3Na$ is less surface-active than SDS.[79] It is observed that amphiphiles **2a** and **2b** are less surface-active than amphiphiles **1a** and **1b**, which can be attributed to the difference in ionization behavior of the −COO^- and −SO_3^- headgroups. As the −COO^- group is partially hydrolyzed to produce carboxylic acid (−COOH), which facilitates the formation of an acid−soap dimer, the amphiphile becomes more surface-active. As with hydrocarbon tail surfactants, the cmc value is observed to decrease with the increase in the mPEG chain length, showing the effect of increased hydrophobicity of the mPEG tail. This is, however, in contrast to the report of Zaslavsky et al., which suggests that the hydrophobic character of the PEGs is independent of the chain length.[81] Therefore, the small difference in cmc values must be associated with the conformational change in the mPEG tail with the chain length. In fact, molecular dynamics (MD) simulations and spectroscopic data have shown that the longer PEG chain has random coil structure, in contrast to the helical structure of the shorter PEG chain.[82] In other words, longer PEG chains are more flexible and the shorter PEG chains have stiff rodlike structure in an aqueous environment.

Privat and co-workers reported that PEG chains can form helices due to different types of interactions of water molecules with the hydrophobic and hydrophilic parts of the chain.[83] The results of MD simulation and spectroscopic data suggest that the most likely conformation of the PEG chain is helical with −O− atoms stuck inside the helix, and thus PEG−water interactions are strong inside the helix.[84] The structural model proposed for the PEG/water system consists of a PEG chain surrounded by an extended region with a hydration sheath.[85,86] A coil of water molecules hydrogen bonded to two sites of ether oxygen around the PEG chain is found. Thus, the PEG molecule forms a loose coil in water. It has been suggested that water spirals are formed in such a way that more water molecules accumulate in the cavities surrounding the oxygen atoms of the PEG chain.[87] Thus, the reduction of the surface tension of water and the clustering of PEG molecules above ∼13 mM and at 303 K can be attributed to the helicity of the chain.[87]

The self-assembly properties of the amphiphiles were investigated by different techniques, including fluorescence, dynamic light scattering (DLS), transmission electron microscopy (TEM), and isothermal titration calorimetry (ITC). The fluorescence studies were performed using hydrophobic, environmentally

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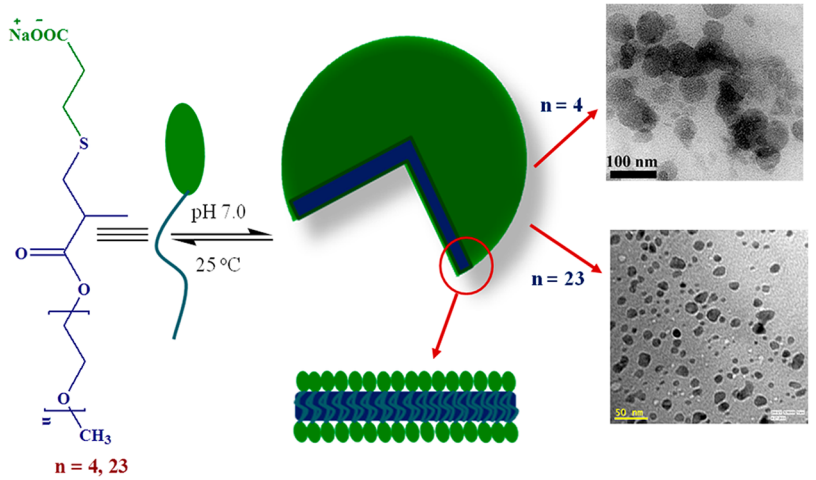

Figure 1. Formation of disclike micelles by amphiphiles **1a** and **1b** in water at 298 K.

sensitive fluorescent probes *N*-phenyl-1-naphthyl amine (NPN), pyrene, and 1,6-diphenyl-1,3,5-hexatriene (DPH), which are poorly soluble in water. The emission intensity of NPN is enhanced, accompanied by a large blue shift of the emission maximum in going from water to a nonpolar solvent.[67−71,74] Similarly, the ratio of the intensities of the first (I_1) and third (I_3) vibronic bands (I_1/I_3) decreases on going from water (1.79) to a less polar solvent.[88] On the other hand, the steady-state fluorescence anisotropy (r) of DPH increases with the viscosity of the local environment.[89] The change in fluorescence properties of these probes indicated microdomain formation above a critical concentration (cmc) of the amphiphiles. The microdomains were observed to have polarity that was less than that of bulk water. With regard to the micropolarity index, I_1/I_3, of the aggregates produced by amphiphiles **1a**, **1b**, **2a**, and **2b** when compared to those of hydrocarbon chain surfactants, it is observed that for the mPEG chain-containing amphiphiles the microenvronment of the probe molecule is more polar compared to that of the anionic surfactants, SLS (1.078)[89] and SDS (1.14),[88] which are known to form micelles in water. Furthermore, the ζ-potential values of the aggregates formed by **2a** and **2b** amphiphiles were found to be negative, suggesting that the surface of the aggregates was constituted of negatively charged $-CO_2^-$ or $-SO_3^-$ headgroups. This means that the microdomains are formed by the mPEG chains. The 2-D ^{1}H NOESY NMR spectra also suggested an interaction among the mPEG chains in the aggregate. The fluorescence anisotropy measurements using DPH probe showed that unlike **2b**, the mPEG chains of **1a**, **1b**, and **2a** are tightly packed in the microdomains. The r value of the DPH probe in micelles usually falls in the range of 0.05−0.10, whereas bilayer aggregates, e.g., vesicles, usually have $r > 0.14$.[90] In the solutions of **1a**, **1b**, and **2a** amphiphiles, the r value of DPH is observed to be in the range of 0.174−0.193. Thus, the microviscosity (η_m) value calculated using r and fluorescence lifetime (τ_f) data of DPH is higher for bilayer vesicles.[91] Indeed, the η_m values (Table 1) for the aggregates formed by **1a**, **1b**, and **2a** amphiphiles are twice that of **2b** and are also observed to be higher than the micellar aggregates of SDS (16.33 mPa s), dodecyltrimethylammonium bromide (DTAB, 13.22 mPa s), and cetyltrimethylammonium bromide (CTAB, 17.77 mPa s), indicating bilayer membrane formation for **1a**, **1b**, and **2a** amphiphiles.[91] Indeed, the TEM images of **1a** and **1b** amphiphiles revealed the existence of disclike micelles (Figure 1), which, like vesicles, have a bilayer arrangement of amphiphiles, except at the edges.[67] In the bilayer membrane, the mPEG chains are more tightly packed in comparison to micelles in which the hydrocarbon chains are more fluid. This imparts rigidity to the bilayer membrane, thus restricting the free rotation of the cylinder-shaped DPH molecule and increasing the r value.[91] Although the formation of disclike micelles, often referred to as bicelles, is rarely observed in a solution of a single-component surfactant system, the existence of bicelles in mixtures of two or more amphiphiles is often reported.[92,93] This is because they have a tendency to grow to form large, flat lamellar structures and are inherently unstable.

The DLS and TEM measurements also confirmed the formation of large aggregates by the **1a** and **1b** amphiphiles, the mean hydrodynamic diameters (d_h) of which are in the range of 20−50 nm (Table 1) and are higher than those of micelles (3−5 nm) produced by hydrocarbon chain surfactants.[94,95] However, the existence of slightly larger aggregates was observed in solutions of **2a** (150 nm). Interestingly, despite having a shorter mPEG chain, amphiphile **2a** is observed to form small unilamellar vesicles (SUVs). In contrast, **2b** is found to produce small micellar aggregates having a mean d_h of about 5 nm. The TEM images of the solutions also showed the formation of small spherical micelles by the amphiphile **2b**.[68] It is interesting that although **1b** and **2b** have the same chain length they produce aggregates of different shapes. This must be associated with the nature of headgroups of the amphiphiles. In the case of **1b**, the acid−soap dimer formation increases the P value as indicated by the formation of stable and larger aggregates such as disclike micelles. The self-assembly process of **2a** and **2b** is presented in Figure 2. While the SUVs of **2a** are found to be stable at the physiological temperature as well as in the presence of a high concentration of NaCl, they transformed into small micelles in the presence of 100 mM L-lysine hydrochloride salt, but in the presence of the same concentration of choline chloride, only the size of the SUVs is reduced. Thus, the SUVs of **2a** can be used in the salt-induced release of hydrophiplic as well as hydrophobic pharmaceutical agents.

The spontaneous self-assembly process in the cases of **1a**, **1b**, **2a**, and **2b** amphiphiles is supported by the results of ITC experiments with amphiphiles **2a** and **2b** as representative examples. The thermodynamic data are collected in Table 1. The negative values of the standard Gibbs' free energy change ($\Delta_{mic}G^o$) suggest spontaneous aggregate formation by **2a** and **2b**

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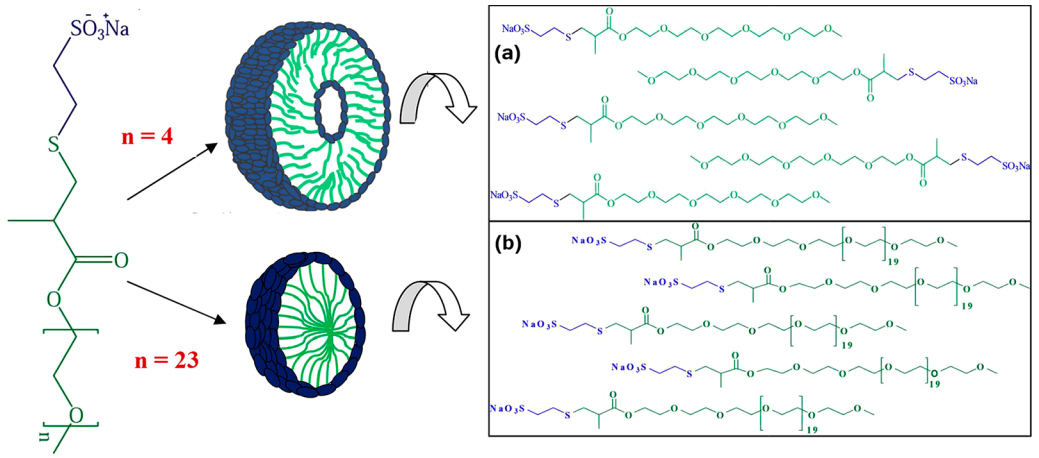

Figure 2. Schematic representations of the formation of bilayer vesicles and micelles, respectively, by amphiphiles **2a** and **2b** in aqueous solutions at 298 K and the spatial arrangement of (a) **2a** and (b) **2b** amphiphiles in the self-assembled state.

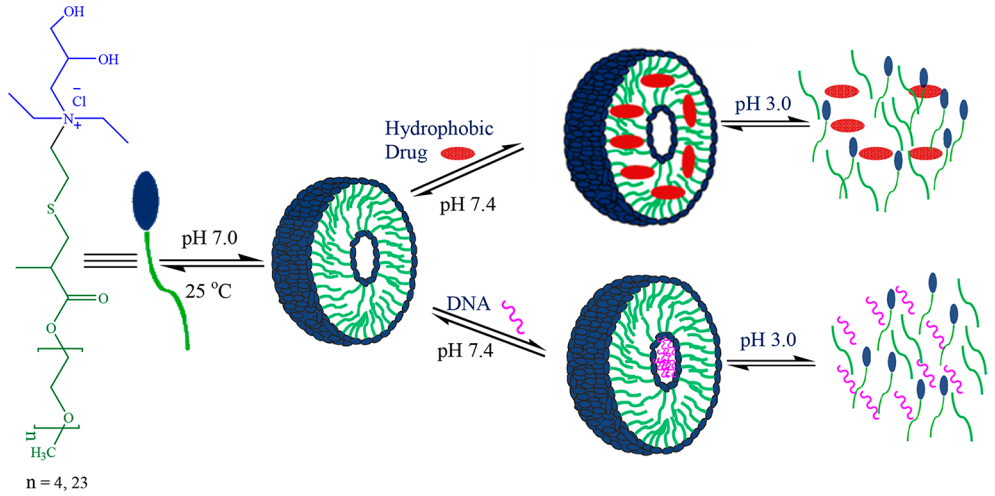

Figure 3. Schematic representation of vesicle formation by cationic amphiphiles **3a** and **3b** showing the encapsulation at pH 7.4 and release at pH 3.0 of hydrophobic model drugs and DNA.

amphiphiles at 298 K.[68] On the other hand, a small standard enthalpy change ($\Delta_{mic}H^{o}$) compared to a large, positive standard entropy change ($\Delta_{mic}S^{o}$) suggests that the self-assembly process is entropy-driven (hydrophobic effect). In other words, the hydrophobic interaction among the mPEG chains is the driving force for the self-assembly process. Thus, the self-assembly of the amphiphiles containing the mPEG chain in water is similar to that of conventional hydrocarbon tail surfactants.[79,80,89] This means that the mPEG tail behaves like hydrocarbon chains. In the cases of PEG chain-containing amphiphiles, the large entropy gain must be associated with the conformational change of the helical mPEG chain upon micellization as a consequence of breaking a large number of hydrogen bonds. Since the PEGs alone do not form micelles, it is quite obvious that the ionic headgroup is responsible for the conformational change that facilitates aggregation. However, because the $\Delta_{mic}G^{o}$ values of **2a** and **2b** amphiphiles are slightly less negative than those of hydrocarbon chain surfactants, the mPEG chain is comparatively less hydrophobic than the equivalent hydrocarbon chain. Therefore, it can be said that the water present in the hydration layer is released by the change in conformation from helix to random coil accompanied by an increase in the entropy of the system, thereby facilitating the aggregation process. Indeed, the conformation of PEG chains responds to their environment, which is an important feature of its properties, including solubility. Thus, the conclusion regarding the conformational change of the PEG chains drawn from the results of fluorescence, DLS, and ITC measurements is consistent with the literature reports.

SINGLE PEG-TAILED CATIONIC AMPHIPHILES

To firmly establish the aggregation of mPEG-tailed amphiphiles, we also examined the self-assembly properties of two cationic amphiphiles, **3a** and **3b** (Chart 1).[69] Both **3a** and **3b** are found to have surface activity similar to that of corresponding anionic amphiphiles with $-COO^{-}$ and $-SO_3^{-}$ headgroups (Table 1). Despite the similar surface activity, the cmc values of cationic amphiphiles **3a** and **3b** are observed to be less than those of **1a**, **1b**, **2a**, and **2b**. The cmc values of **3a** and **3b** are also less than those of cationic hydrocarbon chain surfactants, such as dodecyltrimethylammonium chloride (DTAC, 15 mM) and tetradecyltrimethylammonium chloride (TTAC, 1.4 mM).[96,97] A low cmc means that amphiphiles **3a** and **3b** have a higher tendency to form aggregates than DTAC and TTAC surfactants. This can be attributed to stronger hydrogen-bonding interactions between headgroups. This is indicated by the large

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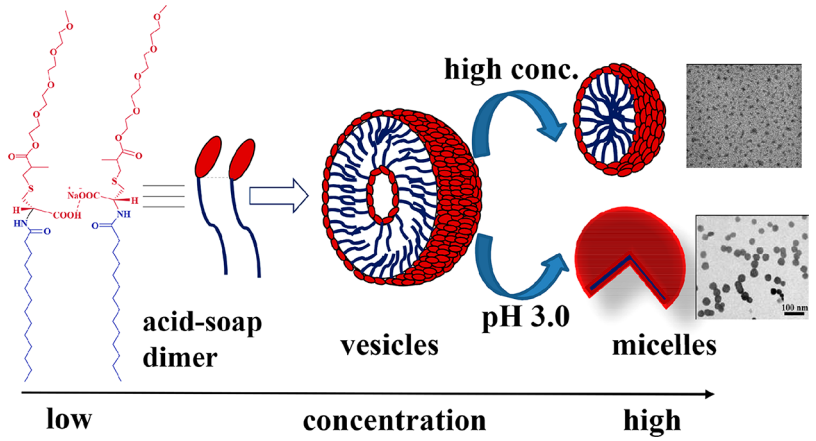

Figure 4. Schematic representation of the concentration- and pH-dependent vesicle-to-micelle transition of amphiphiles **4a** and **4b** at 298 K.

negative $\Delta_{mic}H^{o}$ values in the cases of **3a** and **3b** (Table 1).[69] In fact, in these cationic amphiphiles, the driving force for the self-assembly is the large enthalpy change compared to the entropy gain, which is in contrast to the results reported for DTAC and TTAC surfactants.[96,97] In comparison to the anionic amphiphiles, the higher degree of counterion binding to the headgroup may also reduce the electrostatic repulsion among the bulky ammonium groups facilitating the self-assembly process. Consequently, the reduction of the A_{min} value increases the magnitude of P, and thus large aggregates such as vesicles are formed. The $\Delta_{mic}G^{o}$ values of **3a** and **3b** are observed to be less than those of DTAC and TTAC.[97] Also, in contrast to the small spherical micelles of the DTAC (3.68 nm) surfactant,[98] both **3a** and **3b** produce SUVs with d_h values in the range of 20−70 nm (Table 1) in dilute solution. That the mPEG chains of **3a** and **3b** are organized to form a bilayer membrane is indicated by the low I_1/I_3 (1.22) and high r (~0.170) values (Table 1).[90,91] It is important to note that the I_1/I_3 values of the vesicles of **3a** and **3b** are not only less than those of the aggregates of **1a**, **1b**, **2a**, and **2b** amphiphiles but also less than that of DTAC (1.37) micelles.[88] The higher r value clearly suggests that the η_m and hence the rigidity of the bilayer membrane of the vesicles of **3a** and **3b** are higher than those of the hydrocarbon core of DTAB and CTAB micelles. This is attributed to the strong hydrogen-bonding attractive forces at the headgroup that bring PEG chains much closer, thus limiting the penetration of water molecules in the bilayer membrane.

The vesicles of **3a** and **3b** are observed to encapsulate water-soluble carboxyfluorescein dye within the aqueous core and hydrophobic model drug DPH in the bilayer membrane of the SUVs. We have also demonstrated the slow release of the dye molecules at acidic pH due to the hydrolysis of the ester linkage in the molecule as shown in Figure 3. Thus, they can be used for the simultaneous pH-triggered delivery of hydrophobic chemotherapeutic drugs and water-soluble proteins and DNA. Furthermore, we have shown that the amphiphiles **3a** and **3b** have good antimicrobial activity against Gram-positive and Gram-negative bacteria at a concentration almost 10 times less than their respective cmc value. The MIC values of **3a** (10−40 μg/mL) and **3b** (40−70 μg/mL) were observed to be comparable to that of benzalkonium chloride (14 μg/mL)[99] but much less than that of cationic gemini surfactants (MIC > 512 μg/mL).[100,101] As PEG chains are not known to interact with cell surfaces, we believe that the cationic headgroup plays the major role in rupturing the bacterial cell wall.

AMPHIPHILES CONTAINING BOTH PEG AND HYDROCARBON CHAINS

After we established that PEG chains also behave like hydrocarbon chains, it was thought that when both hydrocarbon and PEG chains are covalently linked to a polar $-COO^{-}$ group the resulting amphiphilic molecule might behave like a double-tailed surfactant. Therefore, to examine whether such amphiphiles act like a single-chain or double-chain surfactant, we designed and synthesized two amphiphilic molecules, **4a** and **4b**, that have the same headgroup (L-cysteine) and PEG chains but have hydrocarbon chains of different lengths. Moreover, **4a** has an unsaturated double bond in the hydrocarbon chain (Chart 1).[70]

The surface tension measurements showed that for both **4a** and **4b** the γ_{min} values (30 mN m^{-1}) are much less than those of amphiphiles **1**, **2**, and **3** and are nearly equal to those of conventional hydrocarbon chain surfactants. Furthermore, the pC_{20} values (Table 1) of **4a** and **4b** are not only greater than those of **1**, **2**, and **3** but also are greater than those of conventional anionic surfactants, such as SDS and $C_{12}H_{25}SO_4Na$,[79] suggesting higher surface activity of the amphiphiles. In fact, **4a** and **4b** are found to behave like conventional neutral surfactants in which the hydrocarbon chain acts as the tail and the PEG chain acts as a polar headgroup. However, unlike conventional neutral surfactants that form micelles, both **4a** and **4b** self-assemble in dilute aqueous solution to form unilamellar vesicles (ULVs), the bilayer membrane of which is constituted by the hydrocarbon chain. A very large negative $\Delta_{mic}G^{o}$ value (Table 1) compared to the single PEG chain amphiphiles supports the conclusion that the hydrocarbon chain acts as the tail. On the other hand, a large $T\Delta_{mic}S^{o}$ value compared to the $\Delta_{mic}H^{o}$ value suggests that the aggregation is due to the hydrophobic effect. The ULVs of **4a** and **4b** produced at pH 7 are observed to transform into small spherical micelles on increasing surfactant concentration at 298 K as shown in Figure 4, but the stability of the ULVs increased when cholesterol (Chol) was added to the solution. Chol is known to enhance the rigidity of the bilayer membrane of vesicles.[102] Interestingly, the spherical micelles are found to transform into disclike aggregates on lowering the solution pH to 3.0. This conclusion is supported by the results of fluorescence probe, DLS, and TEM measurements.[70] This is due to the protonation of the $-COO^{-}$ group at pH 3, which leads to the formation of a neutral molecule that self-assembles to form larger aggregates as the electrostatic repulsion among the headgroups is eliminated. It is observed that these amphiphiles, like the other PEG-chain-containing amphiphiles, did not

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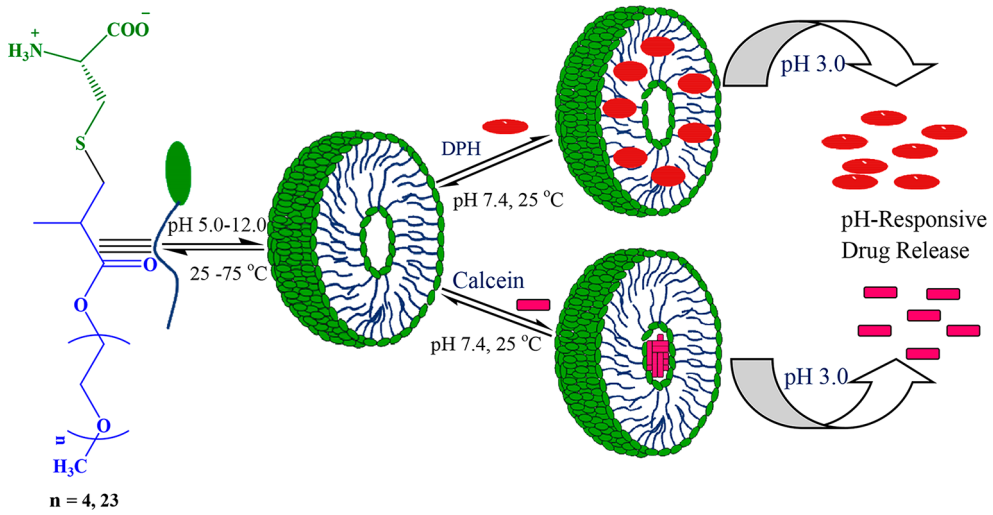

Figure 5. Schematic representation showing vesicle formation by amphiphiles **5a** and **5b** at pH 7.4, the encapsulation of model drug molecules (DPH and Cal), and drug release at pH 3.0.

exhibit any LCST phenomenon at pH 7, but aqueous solutions of both **4a** and **4b** showed the appearance of turbidity on heating to above 316 K at pH 3. At higher temperatures, the PEG chains become dehydrated, which not only leads to the growth of micelles but also facilitates the self-association of micelles, resulting in the formation of large aggregates as indicated by the appearance of turbidity in the solution. As encapsulated drug molecules diffuse out of the micelles or vesicles during any structural transition, the vesicle-to-micelle transition and LCST phenomenon at lower pH exhibited by amphiphiles **4a** and **4b** can be exploited in the development of pH-triggered DDSs for hydrophobic therapeutic agents.

SINGLE PEG-TAILED ZWITTERIONIC AMPHIPHILES

Because zwitterionic amphiphiles have the ability to form different self-assemblies at different pH values, they are attractive candidates for DDS in pharmaceutical formulations. Since their headgroup has both positive and negative charges, zwitterionic amphiphiles exhibit pH-dependent behavior and are less irritating to skin and eyes, enabling them to find practical applications in personal care, cosmetics, and household cleaning. Therefore, we conjugated L-cysteine amino acid to mPEG methyl methacrylate (MW = 300 and 1100) to produce zwitterionic amphiphiles **5a** and **5b** (Chart 1).[71] As the pI values of the amphiphiles are in the range of 6.6−7.1, both exist mostly in the zwitterionic form at pH 7. However, both amphiphiles are observed to reduce the γ of phosphate buffer (20 mM, pH 7). But despite different chain lengths, the pC_{20} values (Table 1) of **5a** and **5b** are observed to be almost equal. In other words, the surface activity of the amphiphiles is similar to that of the anionic amphiphiles containing mPEG chains.

Fluorescence studies using different probe molecules confirmed aggregate formation above the cmc in phosphate buffer at 298 K. The $\Delta_{mic}G^{o}$, $\Delta_{mic}H^{o}$, and $\Delta_{mic}S^{o}$ data (Table 1) clearly suggest entropy-driven spontaneous aggregate formation due to the well-known hydrophobic effect.[71] This means that both **5a** and **5b** behave in the same way as the other PEG-chain-containing amphiphiles. The microenvironments of the aggregates of **5a** and **5b** are found to have I_1/I_3 values similar to those of propionaldehyde and acetone solvent, respectively. The higher η_m values (Table 1), on the other hand, indicate the formation of bilayer structures in the aqueous solutions of **5a** and **5b**. The bilayer aggregate formation is again a consequence of the absence of electrostatic repulsion among headgroups, which leads to tight packing of the PEG chains in the bilayer. The DLS measurements confirmed the existence of aggregates of both **5a** and **5b** amphiphiles in two different size ranges of 20−80 and 200−500 nm. The TEM images confirmed the existence of SUVs in dilute solutions and large unilamellar vesicles (LUVs) in concentrated solutions. The vesicles are found to be most stable at neutral pH, but reduction as well as the increase in pH decreased the stability of the vesicles. However, the vesicles are observed to be quite stable under physiological conditions (pH 7.4, 310 K). The vesicles formed by the amphiphiles have been shown to encapsulate water-soluble model drug calcein within the aqueous core of the vesicles. The calcein-loaded vesicles have been shown to exhibit pH-triggered drug release in acidic pH as presented in Figure 5.

GELATION OF ORGANIC SOLVENTS

It was serendipitously found that amphiphile **5a** with a shorter PEG chain could gelate a number of organic solvents, but **5b** with a longer PEG chain showed no gelation ability.[72] Therefore, we investigated the gelation behavior of this novel family of L-cysteine-based low-molar-mass amphiphiles (**5a**, **5b**, **5c**, and **5d**) containing PEG tails in a range of organic solvents. It should be remembered that while **5a** and **5b** have methoxy ($-OCH_3$) groups, the structure of **5c** and **5d** contains a free hydroxyl ($-OH$) group at the end of the PEG chain (Chart 1). It is interesting to note that while amphiphiles **5a** and **5c** could produce organogels in nonpolar as well as in nonhydroxyl polar organic solvents above a critical gelation concentration (CGC), amphiphiles **5b** and **5d** either produced a clear solution or remained insoluble.[72] This can be attributed to the higher molecular weight and random coil structure of the latter amphiphiles which decide their solubility in a particular solvent. Although **5c** exhibited gelation in polar solvents such as nitrobenzene (NBZ), dimethylformamide (DMF), and propylene carbonate (PC), **5a** could gelate only NBZ solvent on heating−cooling (HC) treatment. All of the organogels, except that of **5a** in dioxane solvent, were transparent. Interestingly, the CF, dichloromethane (DCM), and benzene (BZ) organogels were found to form spontaneously at 298 K without the need for any HC treatment. However, the gelation time increased from 7 to 24 h in the order

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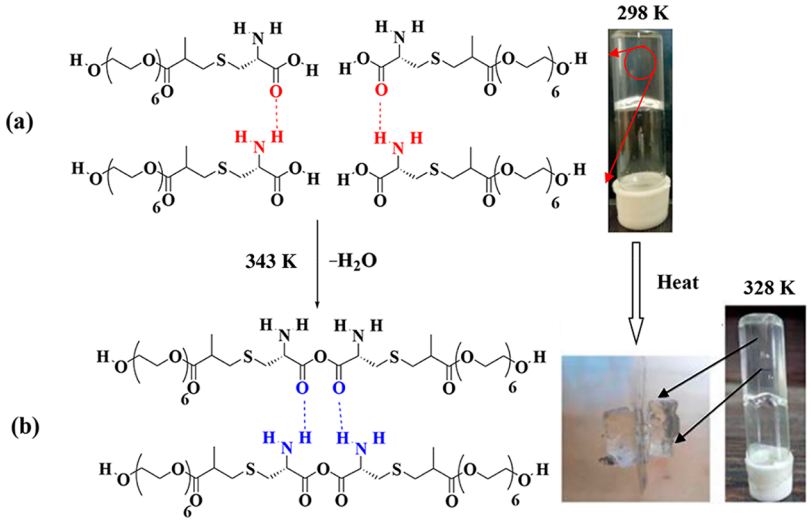

Figure 6. Photographs showing vials containing benzene gels of **5c** at 298 and 328 K and the molecular arrangement of **5c** showing the hydrogen-bonding interaction between molecules at (a) 298 and (b) 343 K.

DCM < CF < BZ. The gelation occurred almost instantaneously when the temperature was raised to 323 K. When heated, the organogels become stronger due to a stronger hydrogen-bonding interaction as shown in Figure 6a, which is confirmed by the FTIR spectrum.[72] This is supported by the fact that **5c**, which has a terminal −OH group, produces a stronger organogel than does **5a**. It is important to note that instead of a gel-to-sol transition at higher temperatures, heat-set organogels are produced in CF, DCM, and BZ solvents as a result of anhydride formation through the elimination of water molecules as shown in Figure 6b. This is supported by the ^{13}C NMR spectrum of the product obtained after evaporation of the BZ organogel at 343 K.[72] Also, we observed the solvent dependence of the morphology of the three-dimensional (3-D) network of the organogels of both **5a** and **5c**. The CD spectrum of the DCM organogel of **5a** showed the generation of supramolecular chirality during gelation. We have shown that the CF organogel could be disrupted by adding a small percentage of ethanol, which is an indirect proof of the role of the hydrogen-bonding interaction in the gelation process. This feature of the gel can be exploited to develop injectable and in-situ-forming DDSs. It is hypothesized that the presence of alcohol disrupts the interaction between gelator molecules, which maintains the formulation in a sol state, allowing the formulation to be injected at room temperature.[103] Upon parenteral injection of the formulation, ethanol is expected to gradually diffuse into the surrounding aqueous environment, and the gelator molecules will self-assemble to recreate the 3-D gel network at physiological temperature. The implant (depot) thus formed in situ will slowly degrade in vivo with time, and the payload will be released. Thus, the organogels can be used as parenteral implants in the systemic, sustained delivery of low-molecular-weight hydrophobic drugs or as a lubricant between bone junctions to cure rheumatism. However, this is just a proof of concept, and more research in this direction is currently underway in this laboratory. The biocompatibility of CF can also become an issue for such applications because CF is an FDA-approved class 2 category solvent.[104]

Because of their viscous nature and having great media to convey ultrasound (US) energy, gels are more useful materials in medical treatment than water. In the recent literature, there is a surge of interest in the use of US waves in medical diagnosis and transdermal drug delivery.[105] Interestingly, when the suspension of **5c** is treated with US, gelation occurs with a much lower CGC value. We demonstrated that US irradiation controls the gel properties at the molecular level. Our group has shown how US treatment changes the gel structure formed by the **5c** gelator in DMF and *N*-methylpyrrolidone (NMP) solvents (Figure 7).

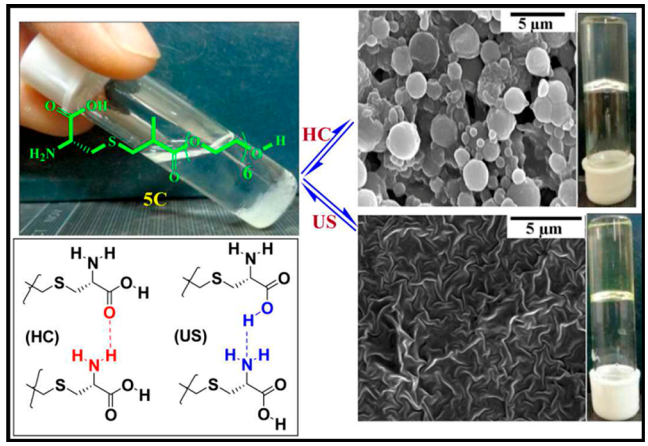

Figure 7. DMF gels of **5c** obtained by ultrasound (US) and heating–cooling (HC) treatment showing different morphology and intermolecular hydrogen-bonding interactions among gelator molecules.

For example, the gelator, **5c**, is observed to produce disc-shaped aggregates in DMF on HC treatment, but in NMP solvent, it forms long ribbons on US irradiation.[73] This is associated with a US-induced conformational change of the headgroup, which favored intermolecular hydrogen bonding to initiate 1-D growth of the self-assembly. This led to a 13-fold increase in the mechanical strength of the US-induced organogels relative to that of the organogels obtained by the HC treatment. The US-induced organogels are also found to be more thermally stable than the heat-set gels.

ZWITTERIONIC BOLAAMPHIPHILES WITH PEG AS A SPACER

Bolaamphiphiles are widely employed in formulating stable nanocarrier systems. Extensive research is in progress to assess their safety profile to establish them as safe excipients for

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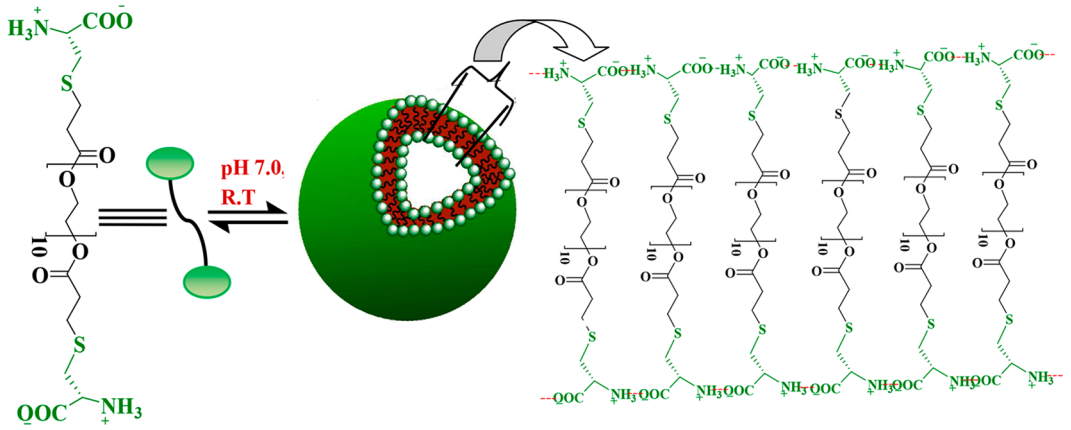

Figure 8. Schematic representation of a monolayer vesicle structure formed by amphiphile **6**. Reproduced with permission from ref 70. Copyright 2016 American Chemical Society.

pharmaceutical applications. Thus, we were interested in developing a first-in-class bolaamphiphile containing PEG backbone as the spacer. We have developed a pH-sensitive zwitterionic bolaamphiphile, poly(ethylene glycol) di(propionyl cysteine) (**6**, Chart 1), bearing PEG as a spacer and L-cysteine as the polar headgroup.[74] A zwitterionic bolaamphiphile with a PEG spacer is advantageous because the amphiphile is pH-sensitive. Furthermore, because both PEGs and L-cysteine are biocompatible and ecofriendly, the self-assembled structures of the amphiphiles in an aqueous medium can find applications in cosmetics and pharmaceuticals. Therefore, we intended to examine if this new class of amphiphiles formed any micelle in aqueous solution at room temperature. Accordingly, the surface activity and self-assembly behavior of compound **6** was investigated in pH 7.0 buffers at 298 K. Like the single-headed amphiphiles discussed above, **6** is also found to decrease the γ value of water with the increase in concentration, suggesting its amphiphilic nature and its spontaneous adsorption at the air/water interface. The pC_{20} value of **6** (Table 1) is similar to that of the single-headed amphiphiles and thus can be considered to be a reasonably good surfactant.[74]

The fluorescence probe studies indicated aggregate formation by the amphiphile, **6**, in aqueous solutions of neutral, acidic, and alkaline pH. This is further confirmed by the results of DLS, TEM, and AFM studies. The amphiphilic molecule is shown to self-organize, producing monolayer vesicles in very dilute as well as in concentrated solutions as shown in Figure 8.[74] The thermodynamic data (Table 1) suggest that the vesicle formation is spontaneous and is driven by entropy gain (hydrophobic effect). Thus, the process is similar to those of the single-headed amphiphiles, **1**−**5**. However, it should be noted that in the case of **6**, vesicle formation is less favorable than for the single-PEG-chain-containing amphiphiles described above. This is obviously due to the higher degree of hydration of the two zwitterionic headgroups. The results of the 2-D NOESY experiment (^{1}H NMR) in D_2O undoubtedly suggested the interaction among PEG spacers that constitute the monolayer. The I_1/I_3 index and η_m data (Table 1) support this conclusion. The existence of monolayer vesicles is confirmed by both TEM and AFM measurements. Although the vesicles are observed to be fairly stable with respect to the changes in amphiphile concentration and temperature, the mean d_h value of the monolayer vesicles is observed to be sensitive to the solution pH. This suggests that any pharmaceutical agent solubilized in either the aqueous core or the monolayer membrane can be released upon the change in solution pH.

CONCLUSIONS AND OVERVIEW

Our group is the first to report room-temperature self-assembly in an aqueous medium of molecules containing a PEG tail and ionic head. Over almost a decade of research, we now have a reasonably good understanding of the general physicochemical properties and solution behavior of PEG-tailed amphiphilic molecules. Despite having a so-called polar PEG tail or spacer, these molecules have reasonably good surface activity and they self-assemble to form diverse microstructures, including spherical and disc-shaped micelles, vesicles, and ribbons under different conditions at room temperature. On the basis of the literature reports and thermodynamic data, the aggregation of these amphiphiles can be attributed to the conformational change in the PEG chain from a helix in bulk water to a random coil in the aggregate as a result of the release of water molecules from the hydration layer. This is associated with the increase in the entropy of the system which facilitates the aggregation process. An interesting and important result is the formation of disclike micelles by amphiphiles **1** and **4**, which are rarely observed with single-surfactant systems. In fact, very recently, we have observed the formation of disclike micelle by the corresponding tertiary amines of **3a** and **3b** (unpublished results). We have shown that when both hydrocarbon and PEG chains are covalently linked to the anionic carboxylate headgroup the resultant molecule (**4**) behaves like a conventional hydrocarbon chain surfactant. Currently, our group has been working on the design and development of various other PEG-tail-containing amphiphiles. In fact, we have observed an enhancement of the stability of vesicles when the hydrocarbon chain is replaced by a Chol moiety to obtain amphiphiles **8a** and **8b** (unpublished results). Our group has also shown vesicle formation by double-mPEG-tailed amphiphiles (**7a** and **7b**) in water at room temperature (unpublished results). Furthermore, we have synthesized and studied the aggregation behavior of bolaamphiphile **9** containing a pH-silent sulfobetaine ($-N^+(C_2H_5)_2SO_3^-$) headgroup. Although compound **9** appears to be more hydrophiphilic, it is found to be surface-active and produces stable monolayer vesicles at room temperature (unpublished results).

This work was further extended to synthesize random copolymers having mPEG chains and pH- and/or redox-sensitive carboxylate, zwitterionic (L-cysteine), or cationic (2-dimethylaminoethyl) groups covalently linked to a hydrocarbon backbone.[106−108] In aqueous solution, these random copolymers were shown to form polymersomes in which the bilayer membrane

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consists of the mPEG chains. Also, these highly hemocompatible and cell-viable (or nontoxic) copolymers were shown to exhibit the pH- and/or redox-triggered release of encapsulated hydrophilic guest molecules. Indeed, the unusual microenvironment of these aggregates that have long-term stability during storage can physically entrap both polar and nonpolar molecules and therefore can be employed in drug-delivery applications. Since the structurally related polymers bearing PEG as side chains are found to be noncytotoxic, the low-molecular-mass amphiphiles with anionic headgroups are also expected to be at least noncytotoxic if not biocompatible. Thus, the vesicle-to-micelle transition and LCST phenomenon at lower pH exhibited by amphiphiles **4a** and **4b** can be exploited in the development of pH-triggered DDSs for hydrophobic therapeutic agents. Similarly, owing to sufficient stability under physiological conditions (pH 7.4, 310 K) the vesicles of amphiphile **6** can be used as pH-triggered DDS. On the other hand, the disclike micelles of amphiphiles **1a**, **1b**, **4a**, and **4b** can be used in the structure determination of proteins by the NMR technique.[109] Some of the low-molecular-mass amphiphiles were also found to gel a number liquids, including CF, DCM, and DMF at room temperature either by just US or HC treatment. These gels can be a more useful material in medical treatment owing to their more viscous nature and having a medium to convey US energy. We believe that in the near future these amphiphiles will have promising applications in cosmetics, personal care products, and drug delivery. However, at this stage, these studies are largely proof-of-concept in nature and need to be understood regarding their biocompatibility and interaction with cells before any formulation of pharmaceutical agents can be realized.

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Notes

The authors declare no competing financial interest.

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