Micellization and Single-Particle Encapsulation with Dimethylammoniopropyl Sulfobetaines

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Supporting Information

ABSTRACT: Sulfobetaines (SBs) are a class of zwitterionic surfactants with a reputation for enhancing colloidal stability at high salt concentrations. Here, we present a systematic study on the self-assembly of SB amphiphiles (sultaines or hydroxysultaines) in aqueous solutions, as a function of chain length and composition, ionic strength, and in the presence of alkanethiol-coated Au nanoparticles (GNPs). The diameters of the micelles assembled from SB and amidosulfobetaine (ASB) generally increase monotonically with chain length, although ASB micelles are smaller relative to alkyl SB micelles with similarly sized tailgroups, and oleyl sulfobetaine (OSB) micelles are slightly larger. SB amphiphiles can stabilize alkanethiol-coated GNP in physiologically relevant buffers at concentrations well below their CMC, with size increases corresponding to single-particle encapsulation. SB-encapsulated GNP were prepared by three different methods with SB:GNP weight ratios of 10:1, followed by dispersion in water or 1 M NaCl. The low hydrodynamic size of the SB micelles and SB-coated NPs is within the range needed for efficient renal clearance.

INTRODUCTION

Amphiphilic molecules are well known to self-assemble into aggregate species above a critical concentration. Micelles are the simplest and most common type of self-organized assemblies, and are traditionally depicted as cores of close-packed hydrophobic tails surrounded by hydrophilic headgroups, resulting in maximum system entropy. Such micelles are ubiquitous in soaps and detergents, and have also drawn attention as nanosized capsules for dyes, drugs, and inorganic nanoparticles (NPs), and as catalyst supports in aqueous solution, wherein the biphasic interface is confined to volumes of a few nanometers.

Low-molecular-weight (LMW) amphiphiles can be classified as having headgroups that are charged (anionic or cationic), uncharged (nonionic), or amphipathic (zwitterionic). Zwitterionic surfactants are electroneutral yet easily dispersed in high ionic strength solutions, and their reputation for biocompatibility is of great interest to the pharmaceutical and cosmetic industries; for example, phosphatidylcholines are often used in emollients to retain body moisture or treat skin diseases. In materials science, zwitterionic surfactants can serve as novel supercapacitors.

Zwitterionic coatings are also well known for their ability to minimize nonspecific protein interactions across a broad pH range. This is especially important in drug delivery and nanomedicine, as the adsorption of complement proteins and other opsonins can lead to rapid clearance by circulating macrophages. Poly(ethylene glycol) (PEG) has long been used to minimize protein fouling, however, the PEG layer increases the hydrodynamic size ($d_h$) by at least several nanometers, and can prohibit renal elimination of nanosized carriers. There are also several studies suggesting that PEG may be immunogenic, possibly compromising the efficacy of PEGylated therapeutics in multidose regimens for follow-up treatments of cancer and other chronic diseases.

Previous studies have shown that renal clearance is size-dependent for NPs between 5.5 and 13 nm. Particles with $d_h$ values above this threshold have little chance of elimination through the renal pathway, whereas particles below 5.5 nm are efficiently cleared by glomerular filtration. Renal elimination is a key process for preventing the toxic accumulation of theranostic agents or NPs in the reticuloendothelial system. Micellar structures assembled from LMW zwitterions are likely to fit within this size window, although particle analysis supporting this assumption has yet to be established.

Here, we provide a comprehensive study on the micelle-forming properties of single-chain sulfobetaines (SBs) as a function of molecular structure and at different ionic strengths. SBs are zwitterionic surfactants with 1-(N,N)-dimethylammonium-3-sulfopropyl or -2-hydroxy-3-sulfopropyl headgroups that are widely used in the food, detergent, and petroleum industries. We focus primarily on commercially available sultaines or hydroxysultaines, some having already established
Figure 1. DLS characterization for SBs as a function of concentration in H₂O and 1 M NaCl: (a) C12SB; (b) CAHS; (c) C14SB; (d) C16SB; (e) C18SB; (f) oleyl sulfobetaine (OSB); (g) C14-amidosulfobetaine (ASB); (h) C16-ASB. All measurements performed in triplicate at room temperature (rt); samples were prepared at least 1 h in advance prior to analysis. Apparent CMC values for each SB are based on thresholds for signal detection by DLS (circled).
a reputation for low toxicity.11,28 We include a novel sultaine prepared from oleylamine for comparison against fully saturated SBs, and use dynamic light scattering (DLS) to characterize concentration-dependent micellization down to 50 μM. We also study the self-assembly behavior of SB amphiphiles in the presence of alkanethiol-coated gold nanoparticles (GNPs) as a model for nanoscale encapsulation, using several different methods of preparation. We find that SB-coated GNPs can form stable aqueous dispersions at concentrations far below the CMC of the supporting surfactant.

## RESULTS AND DISCUSSION

### Analysis by DLS

DLS was used as the primary tool for characterizing SB self-assembly behavior, as quantified by hydrodynamic size (d_h), critical micelle concentration (CMC), and aggregation number (g). DLS studies were performed on SBs as a function of concentration in both deionized water and 1 M NaCl (Figure 1). Number-based distributions were used to obtain mode peak values and CMC thresholds; although these tend to be less sensitive than intensity-based distributions (see below), they are less affected by adventitious particles that can generate artifacts or improperly skew the size distribution.26

Most SBs exhibit sharp transitions in micellization at characteristic concentrations, with aggregate sizes of 4–7 nm that remain constant at higher solute levels. In the case of C16SB in water, nanosized micelles are formed even in supersaturated solutions (>1.3 mM). The threshold values by DLS correlate well with the CMC values for lower MW surfactants (C12SB, C14SB),26 although they are higher than those based on surface tension or isothermal calorimetry.27,30 We also observed SB micellization to occur at lower concentrations in 1 M NaCl versus pure water, again in accord with an earlier work.26 Although DLS is not a standard tool for measuring CMCs in absolute terms,26 the instrument used in this study can detect signals from organic aggregates (1–100 kDa) at a limit close to 1 μg/mL.25 This gives us confidence to use DLS signal thresholds to define apparent CMCs for practical purposes, namely, comparing micelle formation in water versus 1 M NaCl (Table 1).

The maximum d_h of micelles assembled from alkyl SBs (C12–C18) increases monotonically with tailgroup length, in proportion to their extended conformations, as measured using geometry-optimized models (Figures 2 and S2). The same trend is observed for the amidopropyl SB derivatives CAHS, C14-ASB, and C16-ASB, although their micelles are smaller relative to those formed from alkyl SBs with similar tailgroup sizes. Interestingly, OSB produces nearly the same size micelles in 1 M NaCl as those of C18SB, despite having a shorter overall length (ca. 10%) due to its C9–C10 cis double bond. It is also worth noting that for all micelle-forming SBs in this study, ionic strength has no significant impact on hydrodynamic size, at least within the resolution of DLS. This suggests that hydration of the SB headgroups does not contribute meaningfully toward d_h.

All micelle-forming SBs have a lower apparent CMC in 1 M NaCl relative to that in pure water, indicating greater micellar stability at high ionic strength.26 We note that neither OSB nor C18SB formed nanosized micelles in pure water at rt, although much larger aggregates could be observed by eye and removed with a membrane filter. In contrast, all SB surfactant solutions in 1 M NaCl were optically clear, with no visible evidence of aggregates at the highest concentrations evaluated.

In addition to particle sizes and CMC values, DLS can measure molecular aggregation numbers (g) based on the molecular weight of the micellar materials (Table 1 and Figure S3). Aggregation numbers can be useful for formulating stoichiometries between excipient (host material) and prospective encapsulants. As expected, the number of SB molecules per micelle increases with alkyl chain length; however, the trend does not support the simple assumption of isotropic micelles, for which g should increase roughly as a function of n_c^2, where n_c is the number of tailgroup carbon

### Table 1. Physicochemical Characteristics of SB Micelles at Room Temperature

| compound   | solubility limit | apparent CMC in H_2O | CMC in 1 M NaCl | dn/dc (mL/g) | aggregation number, g |
|------------|------------------|-----------------------|-----------------|--------------|----------------------|
| C12SB      | 1.0 M (H_2O)     | 3 mM (3 mM)           | 1.7 mM (1.7 mM) | 0.151        | 49 (55)              |
| C14SB      | 138 mM (H_2O)    | 0.4 mM (0.32 mM)      | 0.2 mM          | 0.147        | 81 (83)              |
| C16SB      | 1.28 mM (H_2O)   | 80 μM (29 μM)         | 60 μM           | 0.133        | 150 (155)            |
| C18SB      | 70 μM (H_2O)     | n/a (1.5 μM)          | 50 μM           | 0.131        | 245                  |
| OSB (C18)  | 70 μM (H_2O)     | n/a                   | 80 μM           | 0.152        | 137                  |
| CAHS (C12) | >100 mM (H_2O)   | 2 mM (50 μM)          | 1.2 mM          | 0.143        | 102                  |
| C14-ASB    | 115 mM (H_2O)    | 200 μM (19 μM)        | 100 μM          | 0.139        | 121 (108)            |
| C16-ASB    | >100 mM (H_2O)   | 100 μM (9.6 μM)       | 50 μM           | 0.139        | 172 (168)            |

Values obtained using serial dilutions in 1 M NaCl. Values obtained using serial dilutions in 1 M NaCl. 

Literature values in parentheses. Solubility limits in 1 M NaCl not quantified if >100 mM. On the basis of threshold for micelle detection by DLS.

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atoms. Instead, empirical g values for C12SB through C18SB increase with \(n_C\) such that the molecular volumes remain approximately constant, implying a greater packing density. One possible explanation is a transition to anisotropic micelles at higher \(n_C\) values, but additional studies would be needed to elucidate the structures of these self-assembled NPs.

**Micellar Encapsulation of Hydrophobic Nanoparticles.** Given their widespread use as excipients in consumer products, single-chain SBs could be useful for stabilizing hydrophobic NPs intended for biomedical applications, while maintaining their hydrodynamic sizes within the threshold for renal clearance. To test this possibility, we conducted studies with the C16SB surfactant using 3 nm GNP coated with dodecanethiol (C12-SH) as the encapsulant. Micellar encapsulation is actually a hybrid phenomenon driven by the thermodynamics of both self-assembly and surface adsorption, the latter having additional parameters based on the radius of curvature and interaction potentials between surface, surfactant, and medium. The encapsulation of C12-SH-stabilized GNPs by single-chain surfactants is expected to be highly favorable, as the interdigitation of alkyl surfactant chains into the alkanethiol monolayer supports a stable interface. However, the solubilization of hydrophobic NPs can create kinetic barriers that hinder their efficient encapsulation by micellar surfactants; thus, practical mechanisms for NP encapsulation need to be addressed.

Three methods for encapsulating GNPs by SB micelles in water and 1 M NaCl were investigated: (a) the addition of citrate-stabilized GNPs to preformed SB micelles containing C12-SH; (b) the gradual removal of co-solvent from microemulsions of C12-SH coated GNPs mixed with SB micelles; and (c) the solid-state mixing of SB surfactants and C12-SH coated GNPs using mechanochemical (grinding) methods (Figure 3). Surfactants are often employed as grinding aids for reducing particle size during ball-milling processes (comminution), but to our knowledge grinding is not widely used for micelle formation and encapsulation. However, solid-state mixing in the absence of solvent has been applied for the supramolecular encapsulation of fullerenes and other polyaromatic hydrocarbons. We thus compared mechanochemical encapsulation against solution-based methods.

For NP encapsulation in monophasic solution (aqueous assembly), a 2.4 wt % solution of C16SB was prepared in water or 1 M NaCl, followed by addition of C12-SH (6.5 mol %) with agitation by vortex action to form mixed micelles. DLS was conducted on serial dilutions of the C16SB/C12-SH mixtures, indicating that the corresponding micelles had the same \(d_h\) (5 nm) as those produced from C16SB alone, but with a higher CMC (0.2 mM) and a sharper transition (Figure 4a). Similar observations were made for the C16SB/C12-SH mixtures prepared in 1 M NaCl (Figure 4b). Citrate-stabilized 3 nm GNPs were then added to a 0.04 wt % solution of C16SB/C12-SH, with vortex mixing for 2 min. On the basis of simple weight ratios, roughly 10% of these mixed micelles contain GNPs, which is the same estimate based on an initial micelle-to-GNP ratio of 10:1. DLS analysis revealed an increase in mean \(d_h\) from 5.0 to 6.2 nm (Figures 4a and S4a). The stability of the GNPs encapsulated by mixed micelles in 1 M NaCl was also monitored for up to 1 week, with only modest changes in \(d_h\) and optical density (Figure S4b,c), whereas the citrate-stabilized GNPs began to aggregate within minutes after dispersion in 1 M NaCl without C12-SH, and with or without C16SB (Figure S4d).

The effective CMC of the mixed-surfactant encapsulation system is considerably lower than that of the free surfactant. For example, GNP encapsulated by C16SB could be detected after dilution to 50 \(\mu\)M surfactant (19.6 \(\mu\)g/mL), well below its CMC value of 80 \(\mu\)M, with further dilutions in concentration still possible. The enhanced stability can be attributed to cooperative adsorption of SB surfactants and C12-SH onto the 3 nm GNPs. The lower effective CMC may also be helpful in addressing some safety concerns over the use of LMW surfactants for drug delivery, namely, their premature dissolution and dispersion into off-target cells and organs.
In this regard, we note that although single-chain surfactants have a reputation for hemolytic activity, zwitterionic species appear to be less toxic than their charged counterparts, and no toxicological effects of dimethylammoniopropyl SBs have been reported thus far.

For NP encapsulation under microemulsion conditions, a 0.04 wt % solution of C16SB (in water and 1 M NaCl) was combined in a 1:1 ratio with C12-SH coated GNPs in toluene with sonication to produce a milky solution, followed by removal of the organic phase by centrifugal evaporation for several hours until a clear phase was achieved, with readjustment in volume to restore the original molarities. DLS analysis of the final dispersions indicated similar $d_h$ values (ca. 7 nm) to those of the micelle-stabilized GNPs prepared under monophasic conditions, again with a lower effective CMC relative to the free C16SB (Figure 4). The stability and similarity of the encapsulated products and the absence of hysteresis effects using either preparation method indicate thermodynamic control of encapsulation.

For NP encapsulation by solid-state mixing, C16SB was combined with C12-SH-coated GNPs in dry powder form using the same mole ratio as above, then ground by mortar and pestle for several minutes until a homogeneous mixture was achieved. Dispersions of this solid in water or 1 M NaCl produced optically clear solutions that again yielded particles with a mean size of 6.6 nm. TEM imaging with negative staining confirmed the micellar encapsulation of individual GNPs, as well as a significant population of empty micelles (Figure 5). We note that although grinding is a convenient method for micellar encapsulation, its efficiency may be lower than the other two approaches, as determined by a gradual loss in dispersion stability after 12 h.

**CONCLUSIONS**

Single-chain sulfobetaines form well-defined micelles above their CMCs at both low and high ionic strength. DLS was found to be a useful and practical surrogate for classical methods of CMC determination and yielded quantitative values for hydrodynamic size and aggregation number. The increases in micelle diameters were directly related to surfactant chain length but independent of ionic strength, although the latter had a significant impact on CMC values. Three methods of encapsulating 3 nm Au particles with dodecanethiol within SB micelles were investigated, all of which produced assemblies of 6–7 nm, confirming single-particle encapsulation. The stability of encapsulated GNPs at low concentrations indicate these assemblies to be stable below the CMCs of the supporting SB surfactant, and within the size window for rapid renal clearance.

**EXPERIMENTAL SECTION**

**Materials.** Most sulfobetaines were purchased from Sigma-Aldrich and used as received unless otherwise stated. 3-(N,N-Dimethyloctadecylammonio)sulfopropyl betaine (C18SB) was purchased from Be Pharm; 3-(N,N-dimethylpalmitoyl-aminopropyl)ammonio)sulfopropyl betaine (amidosulfobetaine-14 or C14-ASB) and 3-(N,N-dimethylmyristoyl-aminopropyl)ammonio) sulfopropyl betaine (amidosulfobetaine-16 or C16-ASB) were purchased from G-Biosciences; 3-
purchased from Nanopartz and purified by ultracentrifugation at 10,000 rpm for 30 min to remove larger colloidal species from solution, with a final mean size of 3 nm (Figure S1a).

**Instrumentation.** Optical absorption spectra were recorded on a Cary-50 UV–Vis spectrophotometer (Varian). DLS measurements were carried out using a Zetasizer Nano ZS system (Malvern) equipped with noninvasive backscatter optics and a He–Ne laser at 633 nm. All DLS data were collected at 25 °C in triplicate, with $d_0$ values based on number-weighted size distributions (see below).

Transmission electron microscopy (TEM) images were acquired using a Tecnai T20 (FEI) operating at 200 kV with a CCD camera (Gatan US1000, 2k × 2k pixels). Negative staining was performed using 1% phosphotungstic acid (PTA). Particle size distributions based on TEM image analysis were calculated using SigmaScan Pro (SPSS Inc.).

**Synthesis of OSB.** Oleylamine was converted to OSB in two steps, using Eschweiler–Clarke methylation to form $N,N$-dimethyloleylammonio followed by ring opening of γ-sultone (Scheme 1). Reagent-grade cis-oxyamine (1.0 g, 3.74 mmol) was first heated at 50 °C for 1 h under vacuum in a 250-mL round-bottomed flask to reverse carboxylation and dispel trace CO$_2$, then cooled to rt. The carbonate-depleted oleylamine was treated with 88% formic acid (0.98 g, 18.7 mmol) and a 37% aqueous solution of formaldehyde (0.82 mL), with observation of gas evolution upon addition. The reaction mixture was heated to reflux for at least 8 h, with further gas evolution observed during the first few hours. The reaction mixture was concentrated to dryness then redisolved in toluene (1 mL), neutralized with aqueous NaOH (1 mL of a 1 M solution), then concentrated by rotary evaporation and further dried by azotropic distillation with toluene. The product could be purified by chromatography, or taken forward to the next step without further purification. $^1$H NMR (400 MHz, CDCl$_3$): δ 5.50–5.37 (m, 2H), 2.28–2.32 (m, 2H), 2.26 (s, 6H), 1.99 (q, 4H, J = 6.2 Hz), 1.47 (quint, 2H, J = 6.5 Hz), 1.20–1.38 (m, 22H), 0.86 (t, 3H, J = 6.8 Hz). $^{13}$C NMR (101 MHz, CDCl$_3$): δ 129.90, 129.79, 59.72, 45.16, 32.58, 31.89, 29.72, 29.51, 29.46, 29.30, 29.22, 27.40, 27.35, 27.18, 22.67, 14.10.

**Scheme 1. Synthesis of OSB**

Dimethyloleylamine (200 mg, 0.677 mmol) was dissolved in anhydrous ethyl acetate (EtOAc; 0.1 mL), treated dropwise with 1,3-propanesultone (124 mg, 1.01 mmol) in anhydrous EtOAc (0.2 mL), then heated to 70 °C for 6 h using an oil bath, or for 1 h using a microwave reactor during which a white precipitate formed. The reaction mixture was centrifuged to collect the white solid, which was washed with cold EtOAc and ether then dried in vacuum to yield 240 mg of OSB as a white solid (85% yield). $^1$H NMR (400 MHz, CD$_2$OD): δ 5.53 (m, 2H), 3.50 (m, 2H), 3.30 (m, 2H), 3.08 (s, 6H), 2.86 (t, 2H, J = 6.9 Hz), 2.18 (m, 2H), 2.00 (m, 4H), 1.77 (m, 2H), 1.32 (m, 26H), 4.41 (t, 3H, J = 6.6 Hz). $^{13}$C NMR (100 MHz, CD$_2$OD): δ 130.85, 130.74, 65.53, 63.77, 51.23, 33.60, 30.03, 30.76, 30.57, 30.43, 30.22, 28.14, 27.41, 23.71, 23.48, 19.89, 14.45. ESI-MS: calc for C$_{23}$H$_{47}$NO$_5$S [M]$^+$ m/z 418.33; found: m/z 418.40. This compound is stable for at least 1 year if carefully stored at −20 °C under argon to prevent air oxidation.

**Quantification of Saturation Conditions in Water and 1 M NaCl.** This procedure was developed to obtain accurate concentration values of solutions saturated with surfactants having low solubility. For solubility in pure water, saturated solutions were prepared by dispersing 50 mg of surfactant in 10–100 mL (depending on initial observations), then heated to reflux with a condenser to remove all signs of turbidity or precipitation. The solutions were gradually cooled to rt and left standing for at least 2 days without evaporation, to achieve full equilibrium between solvation and precipitation. The precipitated surfactant was collected by centrifugation in a tared container and carefully separated by extracting the supernatant with a pipette, and was then passed through a 0.2 μm filter (>99% volume recovery). The supernatant was dried in a tared vial by lyophilization, then weighed with ±0.01 mg precision for estimating solution saturation. The precipitate was also carefully dried and weighed to confirm minimum attrition in mass recovery.

For solubility in 1 M NaCl, saturated solutions were prepared in the same manner as above, except that the precipitate was collected after centrifugation and washed carefully with water (3 × 1 mL) to remove residual salt. The precipitate was dried in a tared vial by lyophilization, then weighed with ±0.01 mg precision for estimating solution saturation.

**Hydrodynamic Size Analysis and Aggregation Number by DLS.** All DLS analyses were performed in triplicate at rt using number-based distributions, with hydrodynamic sizes ($d_0$) of micelles based on mode peak values. Serial dilutions were prepared down to 10 μM, which remained above the limit of detection of our instrument (see below). Aggregation numbers ($g$) were estimated by measuring the refractive index increment (dn/dc) of aqueous SB solutions using an Abbe refractometer, and incorporating these values into Debye plots to obtain the effective mass of the micellar ensemble, divided by the molecular mass of the SB unit (Table 1 and Supporting Information).

**Synthesis of Alkanethiol-Capped GNPs.** Dodecanethiol (C12-SH)-capped GNP were prepared at rt using the procedure reported by Jeff et al. with some modifications. An aqueous solution of HAuCl$_4$ (0.0458 mmol) in a 5 mL round-bottomed flask was treated with rapid magnetic stirring with 0.1 M tetraoctylammonium bromide in toluene (0.102 mmol, 1.02 mL). The initially yellow solution immediately turned into a biphasic mixture with an orange organic phase; the mixture was vigorously shaken several times until the aqueous phase was completely colorless. The biphasic
mixture was then treated with 42 mM C12-SH in toluene (0.023 mmol, 0.55 mL), followed by dropwise addition of 0.4 M NaBH4 in water (0.503 mmol, 1.25 mL) with vigorous stirring, causing the organic phase to rapidly densify to wine-red then brownish-black. The biphasic reaction mixture was allowed to stir for another 12 h prior to separation of the organic layer, which was mixed with 80 mL of absolute ethanol then cooled and stored at −20 °C overnight for precipitation. The supernatant was then carefully separated from the precipitate, which was resuspended in fresh ethanol and stored at −20 °C under an argon atmosphere. TEM and DLS analysis indicated a mean size of 3.2 nm, and mode d6, of 3.0 nm (Figure S1b).

**ASSOCIATED CONTENT**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b00288.

Characterization of citrate- and dodecanethiol-capped AuNPs; molecular models of sulfobetaines, sample data analysis for molecular aggregation numbers; characterization of SB-encapsulated GNPs (PDF)

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**Notes**

The authors declare no competing financial interest.

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