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Water Loading Driven Size, Shape, and Composition of Cetyltrimethylammonium/Hexanol/Pentane Reverse Micelles

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Abstract

Cetyltrimethylammonium bromide (CTAB)/hexanol reverse micelles have found a variety of applications that demand control over physical parameters. Water content or loading is among the most basic tunable components and is the major driver of the physical properties of these systems. This study uses small-angle scattering with contrast variation to characterize these systems as a function of water loading. The scattering data were analyzed with a variety of approaches, resulting in converging physical specifications. Equations that describe basic physical parameters were determined that allow for characterization and manipulation of the CTAB/hexanol reverse micelle surfactant system. The shape of the reverse micelles was revealed to be slightly ellipsoidal and varies slightly through the water loading range. The surfactant shell is shown to contain a higher fraction of hexanol upon addition of water. Analysis reveals that the size, shape, and surfactant/cosurfactant composition are directly tunable by variation of the water content and that these properties are consequences of the balance of forces present in the reverse micelles.

Graphical Abstract

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Keywords

Reverse micelle; Small-angle neutron scattering; Small-angle X-ray scattering; CTAB/hexanol; Cetyltrimethylammonium bromide

INTRODUCTION

Reverse micelles (RMs) are assemblies of nanoscale water pools solubilized by surfactants in a hydrophobic solvent (Figure 1) and have found great utility in a number of applications such as nanoparticle [1, 2] and quantum dot templating [3], as chemical and enzymatic nanoreactor vessels [4, 5] and in drug delivery [6]. Protein encapsulation within RMs have also found use in protein studies including purification [7–9], as nanoscale reaction vessels for biocatalysis [7, 10], as a confinement based stabilizer of protein structure [11–13], and in a variety of protein NMR applications [14]. Perhaps the most well understood RM systems are those comprised of bis(2-ethylhexyl)sulfosuccinate (AOT), with a variety of methods being used to characterize the physical parameters of these systems [15–23]. Despite a large avenue of research of RM encapsulated proteins within AOT RMs, it has become apparent through protein NMR studies that nearly all proteins are unfolded when encapsulated within the core of AOT reverse micelles [24]. As such, AOT is generally impractical for exploring proteins in their native state. In distinct contrast, cetyltrimethylammonium bromide (CTAB) RMs are much more amenable to protein studies and conditions can often be found that result in stable protein encapsulation [14]. Unfortunately, CTAB RMs are far less understood than their AOT counterparts.

A variety of experimental studies [25–29] sometimes combined with simulation [30–33] have characterized the size of CTAB water cores under a variety of compounding conditions, i.e. using varying cosurfactant alcohols, alkane solvents, the molar ratio of water to surfactants or water loading (W_0), and surfactant concentrations. These studies often used measurements of RM dimensions to understand the composition of these systems, in particular the alcohol content in the RM shell. Less well characterized are other shape parameters including ellipticity and shell thickness. Recent studies have illuminated the power of small-angle X-ray (SAXS) and neutron (SANS) scattering methods to elucidate these structural features [33, 34]. Shape parameters are important in the utility of RM systems particularly in the context of quantum dot synthesis [3], nanoparticle templating [2], and NMR-based protein studies [14]. The present study seeks to understand the fundamental properties of CTAB/hexanol RMs such as size, shape, and composition as a function of

water loading, with a particular focus on the ellipsoidal shape and the partitioning of the cosurfactant alcohol, for which little information exists. The formulation used here, CTAB/hexanol/pentane, is commonly used in protein NMR studies, but the methods and insights presented here are applicable to a range of formulations of CTAB RMs.

METHODS

Reverse Micelle Sample Preparation.

All reverse micelle samples were made with 37.5 mM cetyltrimethylammonium bromide (CTAB, 99%, Sigma-Aldrich, St. Louis, MO), 450 mM hexanol (Sigma-Aldrich, 99%) as a cosurfactant, with pentane (99%, Thermo Fisher Scientific, Waltham, MA) as the solvent. These concentrations were chosen because at 75 mM CTAB and above, interparticle interactions among RMs are observed [33] and 450 mM hexanol accommodates the entire range of water loadings as determined by visual sample clarity. RM assembly was achieved by direct injection of varying water volumes into the CTAB/hexanol/pentane solution and mixing [35]. Samples comprising of the following water loading (W_0) values were used in this study: 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5, and 35. Contrast variation by SANS was achieved using 99.9% deuterium oxide (D_2O ; Sigma-Aldrich) for characterization of the aqueous core of the RM and fully deuterated CTAB (D-CTAB; Cambridge Isotope Laboratories, Cambridge, MA; D42, 98%) for characterization of the RM shell. All SANS samples contained a single deuterated component. The cetyltrimethylammonium (CTA) counterion, bromine (Br^-), provides ample scattering length density (Table S1) for SAXS characterization of the bromine distribution within the RM aqueous core [33].

Small-Angle X-ray Scattering (SAXS).

Data were collected with a Rigaku (Rigaku America, Woodland, TX) PSAXS system served by a MicroMax-007 HF microfocus rotating anode generator ($Cu_{\alpha}=1.54 \text{ \AA}$), Osmic mirror optics, a three-pinhole enclosed preflight path, and a gas-filled multiwire detector. Samples were loaded into a quartz capillary within an evacuated sample chamber maintained at 25°C during one-minute exposures. Calibration of beam center and sample-to-detector distance were performed using silver behenate. Data were converted to absolute units (cm^{-1}) using of a known polymer standard [36]. The data were then radially-averaged using SAXSGui v2.05.02 (Rigaku America & JJ X-Ray Systems ApS, Lyngby, Denmark) to produce one dimensional intensity profiles, $I(q)$ versus q ($q=4\pi\sin\theta/\lambda$, where 2θ is the scattering angle, in units of $\text{\AA}^{-1}$). In the configuration used, an accessible q of $0.0055 \text{ \AA}^{-1} < q < 0.25 \text{ \AA}^{-1}$ was recorded. The profiles were further corrected by subtraction of solvent-alone scattering to yield the final net scattering profile.

Small-Angle Neutron Scattering (SANS).

SANS data were collected at the NG-B 30m instrument at the NIST Center for Neutron Research (NCNR, Gaithersburg, MD) at 25°C with collection times of five minutes for each sample. Scattered neutrons were detected with a $64 \text{ cm} \times 64 \text{ cm}$ two-dimensional position-sensitive proportional counter with a $5.1 \text{ mm} \times 5.1 \text{ mm}$ resolution. Data reduction was performed using the NCNR Igor Promacro (Wavemetrics, Inc., Lake Oswego, OR) NCNR package [37]. Raw counts were normalized to a common monitor count and corrected for

empty cell counts, background counts, and non-uniform detector response. Data were placed on an absolute scale using a polymer standard and normalized to the incident beam intensity. The data were then radially-averaged to produce one-dimensional $I(q)$ versus q profiles. The scattered intensities from the samples were further corrected for buffer scattering and incoherent scattering from hydrogen in the samples. Data from sample-to-detector distances of 1 m (q -range 0.03–0.48 Å⁻¹) and 4 m (q -range 0.001–0.12 Å⁻¹) at a wavelength of 6 Å (10% λ/λ (FWHM)) were merged. We observed good agreement between R_g and $I(0)$ determined from inverse Fourier analysis using GNOM [38] and those determined by classical Guinier analysis [39] using the program PRIMUS [40].

Shape fitting of SANS data.

SANS data corresponding to RM samples with D₂O as the only deuterated component and RM samples with D-CTAB as the only deuterated component were fit in SasView [41]. Initial model parameters were generated by individual fitting of the SANS D₂O scattering to ellipsoidal models and the SANS D-CTAB scattering to hollow ellipsoidal models [42]. SLD values from Table S1 were used. The background, scale, equatorial radius of ellipsoid (R), and ellipsoidal semi-axis ratios (s) were extracted from this analysis. Shell thickness (t) was included and allowed to float in the SANS D-CTAB fitting. After initial values were generated, global fitting was performed by simultaneous fitting of s to the D₂O and D-CTAB data. Polydispersity fitting used a Gaussian distribution of R_g in all models. Fits to spherical and hollow-sphere models were performed, with and without polydispersity of the radius, and confirmed that ellipsoidal and hollow ellipsoid models are the best models to describe these data.

Fitting of SAXS data.

SAXS scattering arises primarily from the Br⁻ counter-ion of CTAB. Based on the concentration of the different components in the micelle and the scattering length densities in table S1, 95% or more of SAXS scattering arises from Br⁻. SAXS scattering could not be robustly fit using any of the shell or core models provided by SasView [41], perhaps because the average Br⁻ density varies continuously through the aqueous region of the RM, while the low density of scattering centers is not well modeled by a continuous geometric form factor. We therefore fit the SAXS data directly by generating ‘atomic’ models consisting of point scattering centers spaced by 3 Å arranged in either a solid or hollow ellipsoidal array. The ellipsoidal array was characterized by a primary axis of length R_A , secondary axes lengths $R_B = R_C$, where the axial ratio $s = R_A/R_B = R_A/R_C$ as for the SANS data fitting. Axial ratio values of greater than or less than one correspond to prolate or oblate ellipsoids, respectively. The hollow ellipsoid array was also characterized by a shell thickness T . The scattering intensity at the scattering angle $q = 2 \sin \theta/\lambda$ from the N atoms in the model is given by the Debye formula [43].

$$I(q) = \sum_i^N \sum_j^N \sin(2\pi q d_{ij}) / (2\pi q d_{ij}) \quad (1)$$

where d_{ij} is the distance between atoms i and j . The parameters R_A , s (and T for the hollow model) that minimized the sum of the square of the residuals between the experimental and calculated intensities $I(q)$ were obtained by a grid search over s values between 1.0 and 2.0 in increments of 0.1, T values from 10 Å to 30 Å in 1 Å increments and R_A values from 10 Å to 30 Å in 1 Å increments. These limits were found to be large enough to include the best-fit models for water loadings between 15 to 35.

RESULTS

Guinier and pair distance distribution analysis

As described previously [33], the Br^- counterion in the CTAB/hexanol RM system provides a convenient route to characterization by SAXS due to its comparatively high scattering-length density (SLD) (Table S1). Br^- is more concentrated towards the inner surface of the surfactant layer as found previously by Poisson-Boltzmann electrostatic modeling and molecular dynamics simulations, though Br^- ions are found throughout the water core (Figure 1) [33]. The distribution is termed a ‘pseudo-shell’ in this study. Selective deuteration of the CTAB component or the water core of the RMs provides selective contrast variation for SANS characterization of the CTAB surfactant phase and water phase, respectively (Table S1) [33, 44]. This allows for characterization of three distinct physical components within the reverse micelle: the RM core that is largely composed of water, the pseudo-shell in the outer region of the aqueous phase that is formed by the Br^- counter-ions, and the surfactant shell that is formed by CTAB and hexanol. Though the neutron SLD of bromine is slightly higher than water, its overall concentration in the core of RMs is low compared to water (~3.5%); we found it to be dispensable in fitting SANS data. The water loading range in this study was chosen to reflect small, roughly spherical RMs that are useful in protein NMR and other applications. In order to accommodate this range of water loading values, ($W_0 = 15$ to 35), 450 mM hexanol was used for all samples. Water loading values of less than 15 and greater than 35 were unstable under these conditions, as assessed by visual clarity of the sample. We have previously established that the onset of inter-micellar interactions can be detected in the small-angle scattering (SAS) data for surfactant concentrations of 75 mM or higher for $W_0 = 20$ RM systems [33]. Therefore, here we used a concentration of 37.5 mM surfactant for all water loadings, well below the threshold for inter-micelle interactions. High-quality SAS profiles were collected for the water core by using D_2O for SANS (Figure 2a) by utilizing the high SLD Br^- for SAXS (Figure 2b) and for the surfactant shell by using deuterated CTAB (D-CTAB) for SANS (Figure 2c). These data were converted to $\ln(I(q))$ vs. q^2 for Guinier analysis via linear fitting of the low q^2 range [39]. All data series were found to be linear in the low q range via Guinier analysis indicating that significant inter-micelle interactions are not present. Guinier analysis also gave initial estimates of R_g and $I(0)$ (Table S2).

For all data sets, increasing water loading values resulted in higher effective initial scattering intensities ($I(0)$). This is expected, as the resultant intensity should vary with concentration and volume of the scattering object:

$$I(q) \propto \frac{N}{V} V_{particle}^2 (\Delta\rho)^2 FF(q) S(q) \quad (2)$$

Where $\frac{N}{V}$ is the number of reverse micelles per unit volume (concentration), $V_{particle}$ is the volume of the individual particle, ρ the contrast, $FF(q)$, which is the form factor or scattering component from a rotationally averaged single particle, and $S(q)$ is the interparticle structure factor which we found to have no impact on the scattering as assessed by Guinier analysis.

The shape distribution (pair distribution) function provides a description of the population of interatomic vectors between all pairs of atoms within the scattering volume. The function shows the probability of finding two scattering atoms separated by a distance r . Values determined from Guinier analysis were compared to values extracted from fitted pair distribution functions $P(r)$ (Table S2). As suggested by the Guinier analysis, no indications of aggregation or interparticle interactions were observed in the $P(r)$ functions [45, 46]. To facilitate comparison among samples, all $P(r)$ functions were normalized to integrals of 1.0. All three of the data sets (SANS core, SAXS and SANS shell) showed a decrease in the maximum of $P(r)$ as water loading increased (Figure 3). The distribution of interatomic vectors broadened with an increasing maximum dimension observed for higher W_0 samples, indicating the transition from smaller to longer interatomic distances. R_g values were extracted from the $P(r)$ functions and for each component characterized (D₂O core, Br⁻ shell, and D-CTAB shell) a linear increase is observed as a function of increasing W_0 . Lines were fit to R_g values for each data set and correlation coefficients (R^2) greater than 0.996 were obtained for all three (Figure 4). The slopes of each fitted line were: $0.88 \pm 0.1 \text{ \AA}/W_0$ for SANS for the D₂O core, $1.03 \pm 0.03 \text{ \AA}/W_0$ for the SAXS Br⁻ pseudo-shell and $1.29 \pm 0.02 \text{ \AA}/W_0$ SANS D-CTAB shell. The R_g values determined from SAXS scattering of the Br⁻ pseudoshell demonstrates a linear trend with a slope between the slopes of the water core and the CTAB shell (Figure 4). This is consistent with a shell that lies near, but not beyond, the surface of the water core as observed previously [33].

Ellipsoidal shape fitting of SAS data

Guinier and $P(r)$ analyses provide the radius of gyration (R_g) (the global average value of center-to-mass distances) in a model-free manner. However, the entire $I(q)$ vs q scattering profile provides a more rigorous test for shape and model form factors [42, 47, 48]. Our previous molecular dynamics studies have suggested that CTAB reverse micelles have an ellipsoidal shape [33]. Using SAS calibrated MD simulations, a CTAB RM at $W_0 = 20$ was found to have a prolate ellipsoidal semi-axis ratio (s) which fluctuated between 1.1 and 1.8, averaging about 1.4 through the course of a 12 ns molecular dynamics simulation. Therefore for the present study, we used a solid ellipsoidal form factor to model the D₂O SANS data and a hollow ellipsoidal shell form factor to model the D-CTAB SANS data.

Fitting was performed in SasView and included global fitting of the axial ratio between D₂O data and D-CTAB data for each water loading value. Quality of fits were assessed by

reduced χ^2 and fit residuals. Polydispersity was tested by fitting the SANS data to ellipsoidal form factors (D₂O sample) or hollow-ellipsoidal form factors (D-CTAB sample). A Gaussian distribution function was applied to the radius of the ellipsoid or hollow-ellipsoid with the best-fit standard deviation of the Gaussian function (σ) reported. Polydispersity of the ellipsoidal radius was also included and improved fitting to the data, while polydispersity in the axial ratio did not improve fits. SAXS data were excluded from this analysis due to its complex distribution and lack of a simple form factor that may describe the complex Br⁻ ion distribution. Polydispersity in R_g significantly improved fits to the D₂O and D-CTAB data and resulted in high-quality fits through the scattering curves (Figure 5). Polydispersity fitting in the core models ranged from nearly no polydispersity for $W_0 = 15, 17.5$, and 20 to ranging from $\sigma = 3.8 \pm 0.3 \text{ \AA}$ to $6.8 \pm 0.0 \text{ \AA}$ for W_0 from 22.5 to 35.0 (Table S3). Polydispersity in the inner radius of the hollow-ellipsoid for D-CTAB data fitting ranged from $\sigma = 2.7 \pm 0.5 \text{ \AA}$ for $W_0 = 15$ to $8.0 \pm 0.2 \text{ \AA}$ for $W_0 = 35$.

High quality fits of the SANS D₂O experimental data were obtained (Figure 5). The R_g values determined from shape fitting D₂O SANS data to a prolate ellipsoid generally agreed well with those determined from $P(r)$ analysis (Figure 6) and Guinier analysis (Tables S2 and S3), although they are somewhat lower at high water loading. This lowering is due to the inclusion of polydispersity, in which the higher R_g end of the Gaussian distribution contributes more to the average R_g value than the lower end, requiring a lower average R_g value to fit the data. Best-fit axial ratios of the long axis to the short axes of the prolate spheroids varied between $s = 1.75 \pm 0.07$ and $s = 1.09 \pm 0.10$ from lowest to highest water loading values. Through the water loading series, this averages to a very similar value as the axial ratio of $s = 1.4$ found via SAS benchmarked MD simulations of the $W_0 = 20$ RM system [33].

The ellipsoidal shell model fitting of the D-CTAB SANS data (Figure 5) resulted in R_g values that correlated well to the Guinier analysis (Table S3) and ($P(r)$) analysis (Figure 7), although they are also slightly lower due to the down-averaging effect of polydispersity. The hollow ellipsoid form factor used to fit the D-CTAB SANS data has the shell thickness as a floating parameter. The resulting values ranged between $20.0 \pm 0.4 \text{ \AA}$ and $22.8 \pm 0.6 \text{ \AA}$ (Figure 7), a range consistent with the end-to-end length of CTAB observed in our previous simulations [33]. There is a trend towards slight thinning of the surfactant layer at higher water loading with a low correlation ($R^2 = 0.60$, Figure 7).

The bromine counter ions were previously determined via MD and electrostatic calculations to have a complex distribution within the RMs [33]. The shape of this distribution can be described as a ‘pseudo-shell’, where the Br⁻ ions are distributed to a higher degree away from the core of the RMs, towards the inner surface of the surfactant layer. This tendency to form a shell-like distribution within the RM water core is best explained by repulsion among the Br⁻ counter-ions. However, the center of the core of the RMs still retains some Br⁻ ions at local concentration of about 650 mM, as compared to the approximately 10-fold higher concentration observed near the inner surface of the RM [33]. This is a relatively complex model as it has characteristics of a shell, but does not have a well-defined empty core. Due to the Br⁻ distribution having both shell- and core-like characteristics, we fit the scattering data to both models for comparison (Figures S1 and S2). Fitting routines were similar for

each model and gave an axial ratio between 1.4 ± 0.1 to 1.7 ± 0.1 across the W_0 series for the solid ellipsoid model and 1.4 ± 0.1 for all W_0 values with the hollow ellipsoidal model. Both shell model fitting and core model fitting resulted in R_g values that correspond well with R_g values determined from $P(r)$ and Guinier analysis (Figure 8 and Table S4). The shell fitting model gave estimates for the thickness of a bromine shell as well as the radius of the hollow core of this shell, with the thickness of the shell going up as water loading is increased and the radius of the hollow core going down (Figure 8). The increasing shell thickness is expected as the radius of the water core is increased upon adding water to the system as repulsive forces among the Br⁻ ions force them to occupy more space. However, the decreasing hollow core diameter is not well explained by the electrostatic repulsion model and may be an artifact of fitting a shell model to a complex distribution of Br⁻ ions. While fitting the Br⁻ scattering to these models seems to result in an incomplete description of the counter-ion distribution and precludes using it for compositional analysis, it does provide additional validation of R_g values from $P(r)$ and shape fitting the SANS data.

Compositional analysis

Based on the core and shell ellipsoid geometries determined from D₂O and D-CTAB data, we derived estimates of the ‘structure’ and composition of the micelles as follows. For the ellipsoid, the axial ratio is defined as $s = R_A/R_B = R_A/R_C$ where R_A , R_B and R_C are the ellipsoid semi-axes lengths. Note that $s > 1$ and $s < 1$ correspond to prolate and oblate ellipsoids, respectively. For s we use a mid-range value of 1.4, which is the average value seen in our previous molecular dynamics simulation at $W_0 = 20$ [33], the value from the fitting of the SAXS data, and about the average seen in our global fit of SANS data; although as we show, the results are very insensitive to the exact value. Since the volume of this ellipsoid is given by $V_e = \frac{4\pi}{3}(R_A R_B R_C)$, it is convenient to define the geometric average radius $R' = (R_A R_B R_C)^{1/3}$, which is just the radius of a sphere of the same volume, so that

$$V_e = \frac{4\pi}{3R'^3} \quad (3)$$

with $R_A = s^{2/3}R'$ and $R_B = R_C = s^{-1/3}R'$. The radius of gyration of an ellipsoid is given by

$$R_g = \sqrt{\frac{3}{5}} \sqrt{(R_A^2 + R_B^2 + R_C^2)/3} \quad (4)$$

which for $s = 1$ gives the usual factor of $\sqrt{3/5}$ relating R_g to the radius of a sphere. Using the relations above, we can obtain an exact expression for the volume of the aqueous phase from the measured R_g (core) and the estimated ellipticity, s :

$$V_{eq} = \frac{4\pi}{3}R'^3 = \frac{4\pi}{3}(R_g^3 s^{1/3} / \sqrt{(s^2 + 2)/5})^3 \quad (5)$$

For a sphere the factor $f = s^{1/3}/\sqrt{(s^2 + 2)/5}$ is just $1/\sqrt{3/5} = 1.2910$. For the plausible value $s = 1.4$ this factor is 1.26, which differs by less than 3% from the sphere value. Thus estimates of the aqueous core volume from R_g will be largely insensitive to the deviations from ideal spherical geometry. From the volume of the aqueous phase and the molar volume of water, $29.9 \text{ \AA}^3$, an estimate of the number of water molecules in the aqueous core, N_w , is obtained (Table 1, columns 4 and 5). The number of CTAB molecules, N_c , is obtained by dividing the latter by the water loading (Table 1, column 6). The surface area of an ellipsoid is given by [49]:

$$A_e \approx 4\pi \left((R_A^P R_B^P + R_B^P R_C^P + R_C^P R_A^P)/3 \right)^{1/p} \quad (6)$$

Using $s = 1.4$, equation 6 gives a surface area of $A_{aq} = 1.019(4\pi R'^2)$, just 1.019 times the area of a spherical core of the same volume. (Table 1, column 7). In other words, the uncertainty due to the asymmetry of the micelle is about 2%. Previous molecular dynamics simulations indicated that the mean interfacial areas occupied by CTAB and hexanol molecules are $\sim 33 \text{ \AA}^2$ and $\sim 20 \text{ \AA}^2$, respectively. From the former and N_c we obtain the interfacial area covered by CTAB (A_c) and the fraction of total area it covers, f_a (Table 1, columns 8 and 9). Finally, from the hexanol molar interfacial area we estimate the number of hexanol molecules at the interface needed to provide complete coverage of the aqueous phase (Table 1, column 10). The numbers in Table 1 should be considered only as reasonable estimates due to the various model assumptions and estimates involved: idealized prolate ellipsoidal geometry, estimation of the values for axial ratio, the interfacial areas occupied by CTAB and hexanol, and the assumption of total coverage of the interface by the two surfactants. The correction factors for ellipticity with $s = 1.4$ are 1.027 and 1.019 for the aqueous core radius and area terms, respectively. These are both close to unity and do not vary much for s values in the range 1.1–1.8 so the shape term is not a major source of uncertainty. Thus, the trends in size and composition with water loading seen in Table 1 are robust.

Physical trends include the linear relationship of radius of aqueous phase with water loading, and the approximately linear decrease in fraction of interface covered by CTAB with increasing water loading. Clearly, as the water loading increases, the aqueous phase becomes larger, and the surfactant phase becomes more enriched in hexanol relative to CTAB (Table 1, column 11). This enrichment in hexanol accounts for the slight trend towards a thinner CTAB layer at higher water loadings inferred from perdeuterated CTAB SANS scattering data based on fitting to a hollow ellipsoid model (Figure 7). As the percentage of the shorter hexanol molecules in the surfactant layer increases, the CTAB molecules have more freedom to adopt kinked conformations in their aliphatic tail, and thus are slightly shorter on average.

The analysis of the empty micelle scattering data can be summarized in terms of two empirical, linear ‘equations of state’, derived from fitting the number of components as a function of water loading:

$$N_c = 10W_0 + 8 \quad (7)$$

$$N_h = 27W_0 - 252 \quad (8)$$

where the water loading, W_0 is the independent variable used to compound the micelles, N_c and N_h are the average number of CTAB and hexanol molecules in the resulting reverse micelle. The observed increase in molar ratio of hexanol to CTAB with increasing water loading in Table 1 is reflected in the very different intercepts for these two equations. From the first equation of state we can derive empirical equations for the number of water molecules and the size of the aqueous phase:

$$N_w = 10W_0^2 + 8W_0 \quad (9)$$

$$R_s = \left(V_m \frac{3N_w}{4\pi} \right)^{1/3} \quad (10)$$

where V_m is the molar volume of water, $29.6 \text{ \AA}^3$. These compositional equations and equations of state are plotted in Figure 9.

DISCUSSION

A comprehensive experimental characterization of the size, shape and compositional parameters of CTAB/hexanol reverse micelles over water loadings ranging from $W_0 = 15$ to 35 is presented here. Though the size of CTAB/alcohol RMs have been characterized previously through a variety of techniques [30, 31, 50–53], a higher resolution view and modeling accuracy is provided here using small-angle scattering (SAS) with contrast variation. Multiple characterizations for each W_0 value were performed by utilizing the SLDs of deuterium in SANS, for the D_2O core and the D-CTAB shell, and of the Br^- counter ion in SAXS. Various data analysis strategies were performed on each data set including Guinier and $P(r)$ analysis for determination and confirmation of R_g and $I(0)$ values, and shape form factor fitting for the determination of R_g , asphericity, and shell thickness of these RM systems. From these analyses, equations of state as a function of water loading were determined for numbers of water, CTAB, and hexanol molecules. These equations provide a robust parameterization for composition and size of CTAB/hexanol RMs in pentane and illuminate important physical properties of these systems.

We previously characterized CTAB/hexanol RMs at $W_0 = 20$ using a combined SAS and molecular dynamics (MD) approach [33]. This study used an initial estimate of the R_g of the Br^- shell within the RM as a starting point for construction of a series of MD simulations

with varying N_c . The results presented here demonstrate that both an ellipsoidal core and an ellipsoidal shell model describe the Br⁻ ion distribution reasonably. However, neither model fully describes the Br⁻ ion distribution, further demonstrating the complexity of this component. $P(r)$ functions through the previous MD trajectories were compared against the $P(r)$ determined from SAXS and SANS scattering data with the best fit obtained from the RM composed of $N_c = 140$ –166. This corresponds to approximately 20–30% fewer CTAB molecules than the $W_0 = 20$ RMs characterized in the current study and is reflected in smaller values of V_{aq} , N_w , R_s , and N_h [33]. There are key differences that may account for this discrepancy. To accommodate the widest range of W_0 values in this study (15 to 35), the hexanol concentration was 450 mM, as compared to 300 mM used previously for the singular W_0 value of 20, which may affect the size of the CTAB/hexanol RMs. However, this is counter to previous determinations of the effect of alcohol cosurfactant on CTAB RMs that demonstrate an increase in alcohol concentration is expected to *decrease* the RM radius [27, 54]. This discrepancy may be due to the previous study employing 4–6° C for the SAS experimental conditions, while the experiments in the current study were performed at 25° C. This suggests that temperature plays a role in the size of CTAB RMs and a lower temperature induces a smaller size.

A variety of experimental studies have characterized the size of CTAB RMs and along with the current study provide insight into the impact of RM composition on size. Combined chemical-trapping and time-resolved fluorescence quenching experiments found that the radius of the water pool as 29.1 ± 1.5 Å for a $W_0 = 22.8$ in CTAB/isooctane/hexanol RMs [25], which compares well with the 26.4 ± 0.2 Å for $W_0 = 22.5$ in the present study despite the differences in solvent (isooctane) and concentration of hexanol used (~900 mM). Lang et al. determined N_c and R_s on CTAB/isooctane/chloroform reverse micelles using a time-resolved fluorescence quenching approach [26]. This study characterized RMs from $W_0 = 10$ to $W_0 = 30$ and for 75 mM CTAB determined that the R_s ranged from 23.3 Å to 65.2 Å over this range in a linear manner. This is significantly larger than the R_s range determined here for CTAB/hexanol/pentane RMs and is reflected in an elevated N_c compared to the current study, from 153 to 1225 over the W_0 range of 10 to 30. The difference in cosurfactant and the very different solvent (chloroform) expectedly impact the size parameters of CTAB RMs and illustrates the large impact that these components may have on W_0 -driven size dependencies.

A more directly comparable measurement is in the work of Palazzo et al. where the effect of increasing W_0 on the hydrodynamic radius of the similar CTAB/pentanol/hexane were determined using pulsed-field gradient spin-echo NMR [27]. W_0 values of between 7 and 20 were measured while keeping the CTAB concentration at 60 mM and pentanol concentration at about 500 mM. The resulting dependence of hydrodynamic radius on W_0 was linear and ranged from ~30 Å for $W_0 = 7$ RMs to ~55 Å for $W_0 = 20$ RMs, correlates very well to the ~55 Å radius of R_s plus shell thickness determined by the current study. Besides minor differences in the concentrations of CTAB and alcohol, the major parameter that is different in the study by Palazzo et al. is the use of pentanol as opposed to hexanol used here, however there is little or no expected difference between pentanol and hexanol on CTAB RM radius [30].

The current study provides additional insight for the role of cosurfactant. The overall concentration of hexanol was kept consistent through the W_0 series. Incorporation of a higher ratio of hexanol to CTAB in the RM shell is observed upon increasing W_0 . The effect of cosurfactant on the size of CTAB RMs has been thoroughly investigated by several studies [27, 28, 54, 55], which determined that increasing the amount of alcohol results in a smaller hydrodynamic radius. This contrasts to the current results that demonstrate an increase in the radius (via increasing W_0) drives the incorporation of more alcohol at the interface. The partitioning behavior of the cosurfactant alcohols may explain these differences. Palazzo et al. also characterized the shell thickness as a function of alcohol content and found that in conditions most similar to those used here, the shell thickness was 22 ± 3 Å [27], comparable to the 20.0 ± 0.4 to 22.8 ± 0.6 Å found in the current study. There was also a trend observed in of thinning of the surfactant layer as a function of alcohol concentration, with the surfactant shell thickness decreasing to less than 10 Å thick, presumably with an associated increase in alcohol content in the shell. This study was performed under a very high (20-fold) ratio of alcohol to CTAB. This thinning is reflected to a much smaller degree in the result presented here, where the shell thickness decreases (and fraction of hexanol in the shell increases) as a function of water loading and is found to be correlated to dilution of CTAB at the interface upon increasing water content. Similarly, an earlier study found a higher fraction of hexanol in the shell, determined for CTAB RMs in dodecane and at $W_0 = 20.3$ with a core radius determined to be 32 Å [56], similar to our results for $W_0 = 20$. Despite the highly similar result for radius, Atik et al. found that in order to form stable RMs, 2.2 hexanol molecules per CTAB molecule reside in the shell as compared our result of $1.33 N_H/N_C$. These studies and the present illustrate that despite similar size parameters in these systems, the alcohol content in the shell may vary greatly. The results presented here lead to the conclusion that the ratio of cosurfactant hexanol to primary surfactant CTAB is determined by the balance of forces in the surfactant layer itself and it is partially independent of the bulk hexanol concentration.

CTAB RMs have recently been discovered to have an ellipsoidal shape as opposed to the previously assumed spherical shape [31]. The major strength of utilizing SAS to characterize CTAB RMs as employed here is the ability to experimentally characterize the ellipticity of these systems. Few studies have been able to measure ellipticity and three rely on MD simulation for determination of this parameter [31–33]. One recent study characterized ellipticity of CTAB/butanol/isooctane RMs and the impact of a hydrotrope on the size and shape parameters using SAXS [34]. Radius of the core of these RMs were measured over the range of $W_0 = 12$ to 30 and were determined to be 27 Å to 55 Å, with the larger size than the present study again likely attributable to differing compositions. Interestingly, this study found that these reverse micelles were ellipsoidal with a range of ratios of ~5 to ~8.4 in the $W_0 = 12$ to 30 with a much higher ellipsoidal ratio upon addition of hydrotrope to the RM core. These particles are somewhat larger and much more ellipsoidal than those measured in the current study. A major difference in these RM systems is that the previous study used a nearly 10-fold greater CTAB concentration (352 mM), which is well above the threshold of interparticle interactions that we previously observed at about 75 mM. The shape model in this previous study accounted for interparticle interactions, but these interactions may lead to a highly heterogeneous RM morphology that is not easily described by a single shape form-

factor. In fact, the previous study found evidence of larger ellipsoidal and smaller spherical RM populations at $W_0 = 20$ and 30, characterized by a broad tail region in the scattering curves, which is not found for our systems (Figure 2).

Fits including polydispersity of R_g values improved fits, particularly in smoothing peak features in the mid-q range (Figure 5). Polydispersity is modeled by applying a Gaussian (or other) function to a fitted variable, resulting in a ‘blurred’ boundary. In the current study, the only variable that benefited from being distributed via a Gaussian function is R_g in ellipsoidal and hollow-ellipsoid models. For perfectly spherical RMs, this would represent a distribution of RM sizes present in the sample. For ellipsoidal RMs, however, fluctuations in the axial ratio would result in a ‘blurred’ boundary that resembles that modeled by applying a Gaussian to a spherical shape (Figure S3). Previous MD study of CTAB/hexanol RMs with a W_0 of 20 revealed an average s of 1.4, with fluctuations of ± 0.3 . The average value is reflected with the best-fit shape parameters in the current study, and polydispersity of radius in the spherical models may reflect the fluctuations of the axial ratio over time. It is interesting to note that applying a Gaussian distribution to s in the ellipsoidal models did not result in improved fit and may reflect the difficulty in capturing minor shape fluctuations via SAS methods. Though there is likely a small degree of size and shape heterogeneity in the system used in our study [54], the conditions used in the current study precluded contamination of the major population by interparticle interactions and resulted in monodisperse size and shape with small fluctuations.

Our analysis of aqueous core shape shows that ellipticity values in the range 1.1–1.8 increase the surface area of the aqueous phase by just a few percent relative to spherical geometry. Interfacial forces at the aqueous/surfactant boundary are a major factor driving the formation and stabilization of the RM. Since fluctuations in this area of only a few percent are enough to produce the observed asymmetry, these can presumably be produced with very modest increases in energy compared to the ‘minimum interfacial energy’ state of perfect sphericity. This would explain the prevalence of modestly asymmetric RMs and their non-spherical character helps explain the ability to partially align these species in a strong magnetic field [57].

CONCLUSIONS

We have presented a robust characterization of cetyltrimethylammonium/hexanol/pentane reverse micelles across a water loading series using multiple data analyses of small-angle neutron and small-angle X-ray scattering data. These results serve as a guide for construction of reverse micelles with specific size, shape, and composition parameters. Including radius polydispersity improved form factor fitting, but contributions to apparent polydispersity may originate from fluctuations in either size or shape. The size characterizations compare favorably to previous studies with minor differences observed among systems with differing compositions, particularly in systems with highly differing alcohol compositions, further illuminating the impact of this component on the overall physical parameters. The nature of alcohol at the interface of cetyltrimethylammonium reverse micelles is known to have implications in size and shape parameters, interfacial hydration [28], and rigidity of the shell [54]. A significant conclusion from this work is that

the micelle number, the micelle size and the surfactant/cosurfactant composition all follow surprisingly simple ‘equations of state’ with respect to the controlled variable, the water loading. This simplifies the problem of compounding the reverse micelles. It is plausible that similar ‘equations of state’ should exist for other reverse micelle systems, and so these should be investigated. If they exist, they would be especially useful for systems using surfactant mixtures, where the number of variable to be considered when compounding the micelles is larger. The equations of state allowed further insight into the role of the cosurfactant in the reverse micelle shell. We observe that the shell is enriched in cosurfactant alcohol as water content is increased, as observed in composition parameters and confirmed by the observation of thinning of the reverse micelle shell. This is an interesting result in the context of previous observations that reverse micelle size is typically decreased upon introduction of more cosurfactant alcohols and suggests a propensity for highly plastic partitioning behavior by these alcohols that is driven by both the alcohol concentration in bulk and the available water core surface area. Small-angle scattering data modeling allowed a robust characterization of the shape of these reverse micelles, which are found to be slightly ellipsoidal. Interestingly, the ellipticity varies only slightly across the water loading range despite other studies reporting greatly varying ellipsoidal shapes [30–34]. This suggests that the water content alone does not drive ellipticity and that the cosurfactant and solvent have a much larger impact on this parameter.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic of the composition of the reverse micelles. The bulk solvent pentane is depicted in gray, the CTAB surfactant shell in green, hexanol cosurfactant in black, the Br⁻ counter-ion pseudo-shell in red, and the water core in blue. The schematic is intended to qualitatively illustrate the distribution of the RM components and is not drawn to scale.

Figure 2.

SAS data for 37.5 mM CTAB RMs in pentane with 450 mM hexanol and the following water loadings: 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5, 35. Radially integrated intensities (I) as a function of scattering distance (q) are plotted (See Methods). Panel (a) shows the scattering profiles of the water core of the RM using D_2O as the selective scattering agent in SANS, panel (b) shows the SAXS profiles of the Br^- pseudo-shell utilizing the X-ray SLD of the Br^- counter-ion of CTAB, and panel (c) shows the scattering profiles of the surfactant shell using D-CTAB in SANS. Arrows indicate the increase of initial intensities ($I(0)$) as a function of increasing water loading for all SAS data sets. For clarity, error bars are only included for data collected on $W_0 = 25$ in each data set and are representative of the experimental error observed for all water loadings. Error bars in the low q -range for all experiment sets are smaller than the thickness of the lines used to depict the experimental data.

Figure 3.

Pair distribution function $P(r)$ fitted from SANS data of D₂O filled RMs (panel a, blue), from SAXS (panel b, red), and from RMs composed of D-CTAB (panel c, green). Fitting was performed in GNOM for the water loading series: $W_0 = 15, 17.5, 20, 22.5, 25, 27.5, 30, 32.5$, and 35 . The integral of each $P(r)$ curve is normalized to 1.0 for comparison. Direction of arrows indicates the trend of maxima in $P(r)$ with increasing water loading.

Figure 4.

Extraction of R_g (Å) values as a function of W_0 from pair distribution functions for the water core of SANS of D_2O (blue points), the Br-pseudo-shell from SAXS (red points), and the surfactant shell from SANS of D-CTAB (green points). Errors from extractions are smaller than the radius of the symbols used in the plot and ranged from $\pm$ (0.04 to 0.17) Å. Exact values and error are given in Table S2. The best-fitted line to each data set is shown as black lines.

Figure 5.

Comparison of experimental D₂O and D-CTAB scattering profiles for the upper, lower and mid-range values of W_0 to best-fit ellipsoidal core/shell models with polydispersity in R_g and global fitting of s . Fitting was done with SasView and quality was assessed by reduced χ^2 and fit residuals. Polydispersity was modeled via a Gaussian distribution of R_g values. Experimental data shown as dots with $W_0 = 15$ in red, 25 in cyan, and 35 in blue. Best-fit profiles are shown as lines. (a) An ellipsoidal core model was applied to measurements via SANS performed on CTAB RMs with D₂O and all other components non-deuterated for characterization of the water core. Background, scale, radius of the sphere, ellipsoidal ratio, and the width of the Gaussian applied to the radius were allowed to float. Ellipsoidal ratio was globally fit by simultaneous fit with the corresponding D-CTAB data. Curves were normalized to 1 at the lowest measured q for convenient comparison. (b) An ellipsoidal shell model was applied to measurements via SANS performed on CTAB RMs with D-CTAB and all other components non-deuterated for characterization of the RM surfactant shell. Background, scale, inner radius of the hollow-sphere, shell thickness, and the width of the Gaussian applied to the inner radius were allowed to float. Ellipsoidal ratio was globally fit by simultaneous fit with the corresponding D₂O data. Curves were normalized to 1 at the lowest measured q for convenient comparison. Fitted parameters for all nine values of W_0 are provided in Table S3.

Figure 6.

Size and shape of water core as a function of W_0 from SANS scattering profiles of D_2O containing micelles. R_g derived by fitting the scattering curve to an ellipsoid form factor in SasView (yellow). For comparison, R_g values from pair distance distribution extraction analysis are shown (blue). The best-fitted line to the $P(r)$ derived R_g values is shown (black). The best-fit radii of equivalent volume sphere are shown as black circles. Most error bars are smaller than the radius of the symbols.

Figure 7.

Water loading dependence of the radius of gyration derived from the shape fitting protocol applied to SANS scattering profiles of perdeuterated CTAB containing RMs. R_g from best-fit hollow prolate ellipsoid (yellow). Also shown are the corresponding R_g values derived from pair distance distribution extraction analysis (green). The best-fitted line to pair distance distribution extraction extracted R_g values is shown as a solid black line. The thickness of the shell and cavity radius of the equivalent volume sphere of the hollow ellipsoid are shown as gray and black circles, respectively. The fitted the shell thickness trend is depicted as a dashed line. Error bars are smaller than the radius of the symbols.

Figure 8.

Water loading dependence of the radius of gyration derived from the shape fitting protocol applied to SAXS on CTAB RMs. The predominant scatterers are the bromine counterions from CTAB. R_g from best-fitted hollow prolate ellipsoid of axial ratio 1.4 obtained by fitting scattering curve from $q=0-0.15\text{\AA}^{-1}$ (yellow). Also shown are the corresponding R_g values derived from pair distance distribution extraction analysis (red). The best-fitted line to pair distance distribution extracted R_g values is shown as a black line. The thickness of the shell and cavity radius of the equivalent volume sphere of the hollow ellipsoid are shown as gray and black circles, respectively. Error bars for shape-fitted parameters reflect the $1\text{\AA}$ grid-search step size. Errors of R_g extracted from $P(r)$ are smaller than the radius of the symbols

Figure 9.

Reverse micelle compositions and equations of state for CTAB/hexanol reverse micelles. a) Average number of CTAB (orange) and hexanol (magenta) molecules per RM, from Table 1. Equations of state for CTAB (equation 7) and hexanol (equation 8) are plotted as black lines. b) Average number of water molecules per RM as a function of W_0 plotted as calculated in Table 1 (cyan) with the associated equation of state plotted as a black line (equation 9). The second ordinate-axis is the spherical radius of the water core as a function of water loading (gold) and the associated equation plotted as a black line (equation 10).

Table 1.Structure and composition of empty micelles^a

W_0	R_g (Å)	R_s (Å)	V_{aq} (Å ³)	N_w	N_c	A_{aq} (Å ²)	A_c (Å ²)	f_a (%)	N_h	$N_h:N_c$
15	20.2	25.8	71700	2390	159	8500	5260	62	162	1.02
17.5	22.1	28.2	93500	3120	178	10150	5880	58	214	1.20
20	24.5	30.8	122400	4080	204	12150	6730	56	271	1.33
22.5	26.4	33.4	156600	5220	232	14320	7660	54	333	1.44
25	28.1	36.0	194600	6490	260	16550	8560	52	399	1.54
27.5	30.7	38.8	245500	8180	298	19320	9820	51	475	1.60
30	32.3	40.6	280400	9350	312	21110	10280	49	541	1.74
32.5	33.9	42.6	324100	10800	332	23250	10970	47	614	1.85
35	35.7	44.4	366000	12200	349	25210	11500	46	686	1.97

^a W_0 = water loading, R_g = radius of gyration of water core, R_s = radius of equivalent sphere, V_{aq} = volume of the water core, N_w = number of water molecules per RM, N_c = number of CTAB molecules per RM, A_{aq} = surface area of the water core, A_c = interfacial area covered by CTA molecules in the inner surfactant shell surface, f_a = percentage of the interfacial surface covered by CTA, N_h = number of hexanol molecules per RM. $N_h:N_c$ is the ratio of numbers of hexanol to CTA that is contained in the RM shell.