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Diffusion Coefficient Analysis by Dynamic Light Scattering Enables Determination of Critical Micelle Concentration

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The critical micelle concentration is an important property of supramolecular detergents. Two dynamic light scattering approaches have been developed for critical micelle concentration analysis, i.e., concentration-dependent light scattering intensity analysis and diffusion coefficient analysis. Their utility as complementary tools for a reproducible determination of critical micelle concentration remains to be clarified. Herein, we address the question which of the two approaches is more suitable for obtaining reproducible critical micelle concentration values. We systematically compare both approaches in context with common detergent classes and benchmark utility by means of literature values. Our results show that the diffusion coefficient analysis delivers reproducible critical micelle concentration values in aqueous solutions. Our findings outline a roadmap to guide the critical micelle concentration analysis of detergents by dynamic light scattering in the future.

Introduction

The critical micelle concentration (cmc) is an important parameter that describes the lowest concentration at which detergents form micelles.[1] Knowledge on the cmc is crucial for detergent formulations that harness the solubilizing properties of micelles, for example, in domestic cleaning,[2] membrane protein purification[3–5] or drug delivery.[6–8] Cmc values are typically determined by tensiometry,[9,10] conductivity[1,11] or dye solubilization measurements.[9,12] Further methods include isothermal titration calorimetry,[13] spectrofluorimetry[14] and small angle X-ray scattering.[15] A common feature of these methods is that changes in physical properties, such as surface tension, conductivity, absorbance, fluorescence or X-ray scattering are monitored against the detergent concentration. The formation of micelles can significantly affect these physical properties, thus enabling the determination of cmc values.

The above-mentioned methods have in common that the presence of micelles is detected indirectly, which can lead to a couple of limitations. For example, conductivity measurements are only applicable to ionic detergents. Furthermore, cmc analysis can be difficult for detergents with low aggregation numbers where changes in conductivity can be negligible.[1] In the cases of dye-based solubilization methods, cmc values can be biased, because hydrophobic guests can affect aggregation properties.[9,12]

Dynamic light scattering (DLS) increasingly attracts attention as a method for cmc analysis that circumvents above-mentioned challenges.[16–21] DLS is a non-invasive technique that analyzes micellar assemblies directly by investigating time-dependent fluctuations of light scattering.[17] Commercial DLS instruments offer two possibilities to determine cmc values, i.e., (i) detergent concentration-dependent analysis of light scattering intensities[16,20] and (ii) detergent concentration-dependent analysis of diffusion coefficients of most abundant particle populations in solution.[21] Switching from solutions containing monomeric detergents to mixtures of monomers and micelles can lead to abrupt changes in observable light scattering intensities and/or diffusion coefficients of most abundant particle populations. The concentration that marks the transition between both regions is usually extrapolated and taken as cmc (Figure 1).[20,21]

Procedures for the determination of cmc values by DLS-based light scattering intensity analysis are well described in the literature.[17,18,20] However, procedures describing cmc deter-

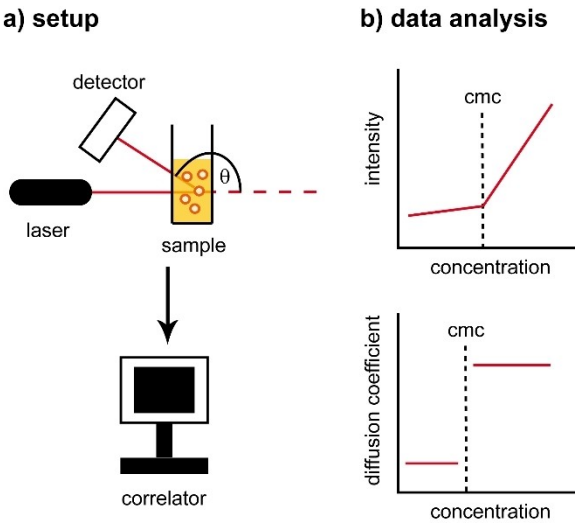

Figure 1. DLS enables cmc determination. a) Schematic of a DLS setup. b) Schematic highlighting two ways of analyzing cmc values, i.e., DLS-based light scattering intensity analysis or DLS-based diffusion coefficient analysis.

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mination via DLS-based diffusion coefficient analyses in detail are missing. Furthermore, it is not clear which of the two methods is more suitable for delivering reproducible cmc values. To increase user friendliness and scientific value of both DLS-based methodologies, herein, we systematically compare the concentration-dependent analysis of light scattering intensities and diffusion coefficients regarding their capabilities to deliver reproducible cmc values for different detergent classes.

Experimental Section

Detergent Solutions

To evaluate DLS-based light scattering intensity and diffusion coefficient analysis for reproducible cmc determination, detergent dilutions were prepared in deionized water or salt-containing aqueous solutions with concentrations ranging from 10^{-8} to 20 mg/mL. Detergent stock solutions with a final concentration of 20 mg/mL were prepared by dissolving 120 mg detergent in 6 mL solvent. Stock solutions were filtered through a syringe filter (0.2 μm, RC) and diluted according to previously established mixing tables (Tables S1–S4).[20]

DDM (99%, Glycon), SDS, (95%, Sigma-Aldrich), SDS (99%, Thermo Fischer), DTAB (99%, Thermo Fischer), LDAO (Apollo Scientific), Fos-Choline-12 (99%, PharmaBlock) were used as supplied. Rhamnolipids (85–90%, AGAU Technologies) were enriched to purity grade 5 as described before.[22] The detergent [G1] OGD was synthesized as described before.[5] For the preparation of salt and buffer solutions, NaCl (99.5%, Carl Roth), Tris (99.9%, Carl Roth), Glycine (99%, Carl Roth), EDTA (99%, Carl Roth), and Imidazol (99%, Fisher Scientific) were used as supplied. Deionized water was obtained from a deionization system (PURELAB flex, LC134). Samples in deionized water were prepared and stored at 22.5 °C for 20 h before measurements. Samples in salt-containing solutions were prepared at 22.5 °C and measured on the same day. Stock solutions of NaCl and Tris buffer were filtered twice (0.2 μm, regenerated cellulose) before being used for the preparation of detergent concentration series.

DLS Measurements

DLS measurements were performed in Quartz Suprasil cuvettes (width×length: 2 mm×10 mm) using a Zetasizer Nano ZS ZEN3600 (Malvern, UK) equipped with a red laser (632.8 nm). The instrumental parameters were set as follows: measurement type (size), material (polystyrene latex), dispersant (water for aqueous solutions/complex solvents for salt and buffer solutions), sample viscosity parameters (use dispersant viscosity as sample viscosity), temperature (22.5 °C), equilibration time (120 s), cell type (quartz cuvettes), measurement angle (173° backscatter), measurement duration (manual), number of runs (11), run duration (10 s), number of measurements (3), delay between the measurements (0 s), data processing (general purpose, normal resolution). Data analysis was performed using the Zetasizer Software (version 7.12).

DLS-based Light Scattering Intensity Analysis

To evaluate DLS-based light scattering intensity analysis for cmc determination, the derived count rate, herein expressed as kilo counts per seconds (kcps), was averaged over three technical replicates and plotted against the detergent concentration. The resulting plot revealed two regions, i.e., lower kcps values at lower detergent concentrations and linearly increasing kcps values at higher detergent concentrations. Both regions were fitted to linear functions and the intersection of both fits was taken as cmc (Figure 2).[16,20] Data were plotted using Origin (OriginPro 2023) and

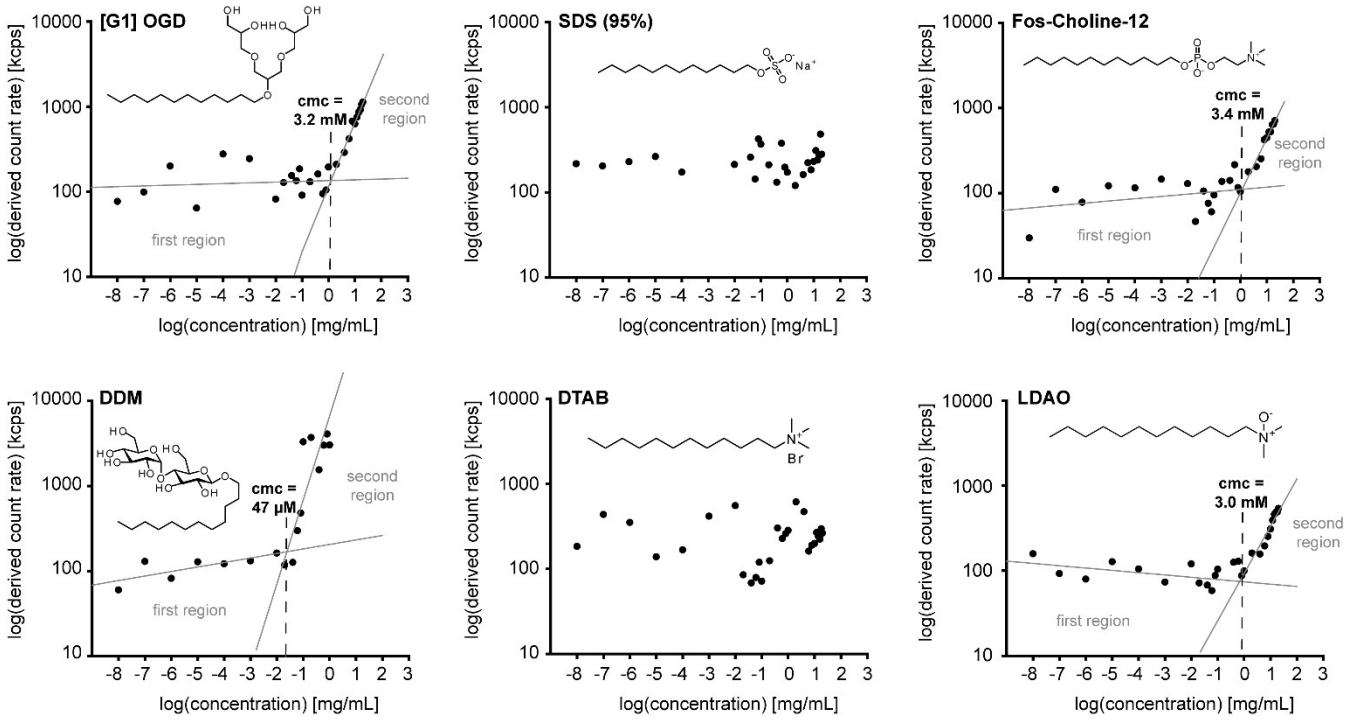

Figure 2. DLS-based light scattering intensity analysis for cmc determination in deionized water. Double-logarithmic charts showing derived count rates over detergent concentrations for different detergents. Lower count rates below cmc and linearly increasing count rates above cmc were fitted to linear trend lines and labelled with "first region" and "second region," respectively. Intersections were highlighted with dashed lines and taken as cmc.

exported as PDF files. Figures for the manuscript were made with Adobe Illustrator CS2, and exported as PNG files.

DLS-based Diffusion Coefficient Analysis

To evaluate the capabilities of the DLS-based diffusion coefficient analysis for cmc determination, the diffusion coefficient of the most abundant particle population was extracted from the recorded DLS data. The data output of the Zetasizer software displays the relative intensity of scattered light against detected diffusion coefficients of particle size distributions in solution (Figure 3). The difficulty of identifying the most abundant particle size distribution from multimodal size distributions relates to the fact that, according to the Rayleigh approximation, the intensity of scattered light scales exponentially with the hydrodynamic radius $(R_H)^6$ of particles.[23,24] The intensity of scattered light obtained from larger particles is therefore naturally higher compared to smaller particles, thus rendering an identification of most abundant particles from diffusion distribution profiles by intensity difficult.[23] To identify most abundant particle distributions, we analyzed the data output "particle size distribution by volume," which reflects more likely the relative abundance of particle distribution in solution without overestimating the presence of bigger particles as it would be the case in "diffusion coefficient by intensity" plots.[25] To identify diffusion coefficients of the most abundant particle distributions in solution, we identified the R_H value of the most abundant particle population in the data output "particle size distribution by volume," and identified the related diffusion coefficient (Figure S1).

To determine cmc values of detergents, the diffusion coefficients of the most abundant particle populations were plotted against detergent concentrations (Figure 3). The resulting plots showed two regions. At lower detergent concentrations, lower diffusion coefficients were obtained which we assigned as background signal. At higher detergent concentrations, higher diffusion coefficients were obtained which we assigned as micelles. The average of the highest detergent concentration with a lower diffusion coefficient and the lowest detergent concentration with a higher diffusion coefficient was taken as cmc (Figure 3). Data were plotted using Origin (OriginPro 2023) and exported as PDF files. Figures for the manuscript were made with Adobe Illustrator CS2, and exported as PNG files.

DOSY-NMR Reference Measurements

To evaluate whether changes in diffusion coefficients detected by DLS below the cmc of detergents are caused by the detection limit of the DLS instrument or a change in supramolecular properties of detergents, we performed diffusion ordered spectroscopy (DOSY)-NMR measurements. Detergent solutions were prepared in deuterated water (D_2O, 99.9 %) as described above. Samples were divided. One half was investigated with DLS as described above; the other half was investigated with ^{1}H DOSY-NMR using a 600 MHz spectrometer AVANCE III HD equipped with a helium cooled cryoprobe (Bruker BioSpin GmbH). Data were plotted using Origin (OriginPro 2023) and exported as PDF files. Figures for the manuscript were made with Adobe Illustrator CS2, and exported as PNG files.

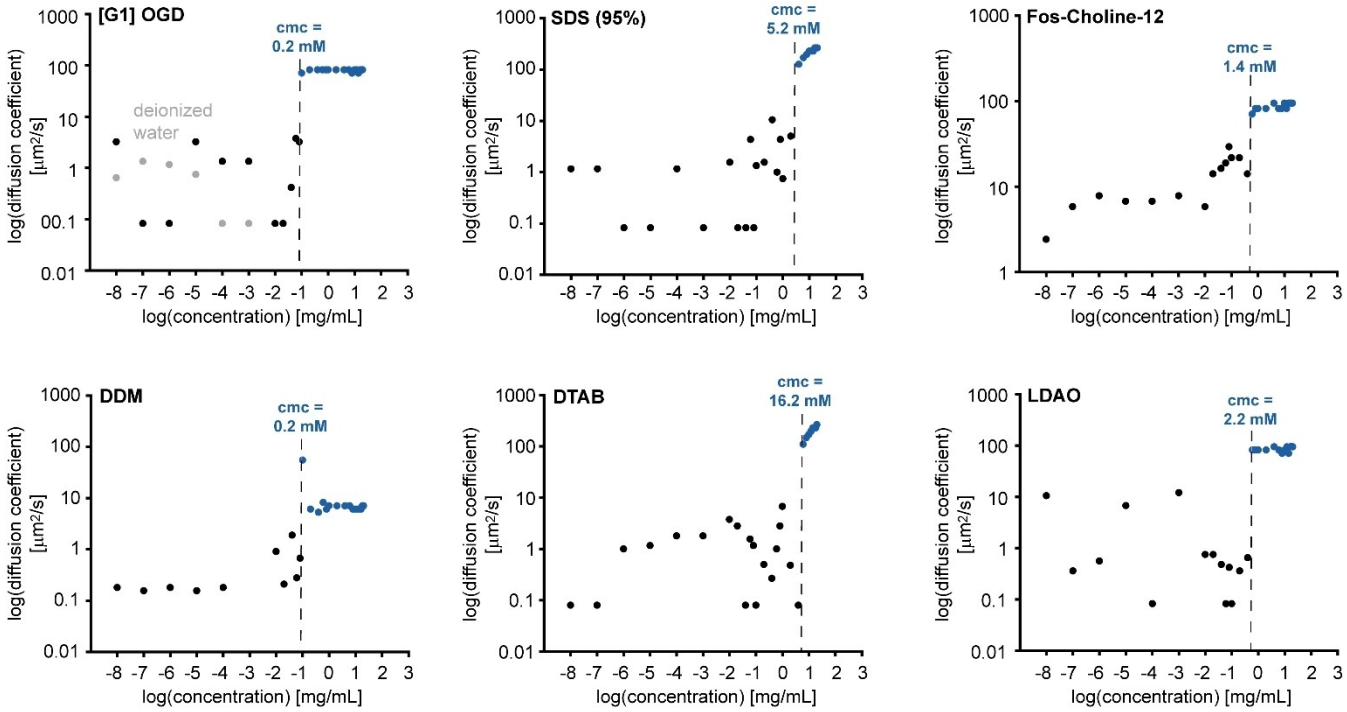

Figure 3. Concentration-dependent diffusion coefficient analysis of non-ionic [G1] OGD, non-ionic DDM, anionic SDS, cationic DTAB, zwitter-ionic Fos-Choline-12 and zwitter-ionic LDAO in deionized water. Double logarithmic charts showing the diffusion coefficients obtained from the particle size analysis of aqueous detergent solutions by DLS. Data points assigned as background signal are shown in black. Data points assigned as detergent aggregates are shown in blue. As shown exemplarily for [G1] OGD, diffusion coefficients obtained from six measurements with pure deionized water are shown in grey. Smaller diffusion coefficients were obtained below cmc and assigned as background signal. Larger diffusion coefficients were obtained above cmc and assigned as micelles.

Results and Discussion

DLS-based Light Scattering Intensity Analysis Determines Relative Critical Micelle Concentration Values

DLS-based light scattering intensity analysis is more frequently communicated for cmc determination of aqueous detergent solutions than DLS-based diffusion coefficient analysis.[16–21] Accuracy of the DLS-based light scattering intensity approach and applicability to common detergent classes, i.e., non-ionic, cationic, and anionic detergents, have not yet been systematically investigated.[17,18,21]

To investigate utilities and limitations of this approach, we recapitulated the general analysis procedure. The principle of cmc determination by DLS light scattering intensity analysis is based on the observation that mixtures of detergent monomers and micelles can scatter more light compared to dispersions of detergent monomers.[17] To monitor changes in light scattering, a series of aqueous solutions with different detergent concentrations was analyzed by DLS. The logarithm of the obtained sample scattering intensity, herein expressed as derived count rate in kilocounts per second (kcps), was plotted against the logarithm of the detergent concentration. As exemplified for the non-ionic detergent [G1] OGD, the resulting plot can show two regions (Figure 2a). At lower detergent concentrations, the scattering intensity is lower, because no micelles are present in solution (Figure 2a, first region). At higher detergent concentrations, the scattering intensity increases linearly with increasing detergent concentration due to the presence of micelles (Figure 2a, second region). Both regions were fitted to linear functions and the intersection was taken as cmc (Figure 2a).

Having recapitulated the general analysis procedure, we investigated the impact of fitting bias on obtainable cmc. Light scattering intensities varied for each concentration between repeats, which is what we expected from dynamic, self-assembling systems, such as micellar assemblies. Additionally, it can be difficult to define which data point lies below and which lies above the cmc due to variations in the count rate values around the cmc. Consequently, data sets obtained from one concentration series could be fitted to multiple linear equations with similar R^2 values. For example, in the cases of [G1] OGD and DDM, we obtained cmc values between 2.84–3.38 mM and 35–47 μM from three fits of comparable quality, respectively (Figures S2 and S3). This led us to average cmc values with standard deviations of 3.09 ± 0.22 mM for [G1] OGD and 42 ± 5 μM for DDM (Figures S2 and S3). Average cmc values obtained for [G1] OGD and DDM from a second independent repeat were 3.11 ± 0.34 mM and 38 ± 9 μM and of similar quality compared to our first measurement. This underlined that DLS-based intensity analysis can deliver stable cmc values for individual detergents under the experimental conditions employed.

To evaluate whether DLS-based light scattering intensity analysis can reproduce literature cmc values, we extended our set of detergents by zwitter-ionic lauryldimethylamine oxide (LDAO) and Fos-Choline-12. For detergents with cmc values in the millimolar range, such as [G1] OGD, LDAO, and Fos-Choline-12, we obtained cmc values that were consistently 2–3.8 times higher compared to cmc values reported in the literature (Table 1) (Figure 2). Interestingly, the cmc value obtained for [G1] OGD was 3.8 times higher than the literature value, which was determined also determined by DLS-based light scattering intensity analysis. This indicates that the fitting bias can reduce the reproducibility of cmc data between different laboratories. Conversely, in case of a detergent with a cmc in the lower micromolar range, such as DDM, DLS-based light scattering intensity analysis underestimated the literature cmc value by factor 4.3 (Table 1). This is very likely a result of the double-logarithmic nature of the derived count rate versus detergent concentration plots. Attempts to extrapolate cmc values by linear regression are prone to over- or underestimate actual cmc values under the conditions employed. However, cmc values obtained by DLS-based light scattering intensity analysis were in the same order of magnitude as literature cmc values, suggesting that this method enables a relative comparison of cmc values.

The last question to be addressed in this evaluation was whether DLS-based light scattering intensity analysis is applicable to all detergent classes. In addition to non-ionic and zwitter-ionic detergents, we included anionic sodium dodecyl sulfate (SDS) and cationic dodecyl trimethylammonium bromide (DTAB). To our surprise, in the cases of SDS and DTAB, we did not obtain a noticeable change in light scattering intensity (Figure 2c and d). The morphology of all detergent micelles investigated here is similar, but the aggregation numbers of SDS and DTAB are smaller compared to DDM, LDAO, and Fos-Choline-12 (Table S5). Although the expected cmc values of

Table 1. DLS-based light scattering intensity and diffusion coefficient analysis of cmc values in aqueous solutions. Summary of utilized detergents, detergent classes, literature values, and cmc values determined with DLS-based light scattering intensity and diffusion coefficient analysis. Cmc values derived from scattering intensity and diffusion coefficients are averages of two independent repeats. The literature value for DDM was taken from the product description of DDM (CAS: 69227–93-6) distributed by Merck (Germany).

detergent	detergent class	cmc (literature)	cmc (diffusion coefficient)	cmc (intensity)
[G1] OGD	non-ionic	0.5–0.8 mM[3,26]	0.2 mM	3.10 mM
DDM	non-ionic	0.1–0.6 mM	0.3 mM	0.04 mM
SDS 95%	anionic	3.7–4.6 mM[27]	5.2 mM	-
DTAB	cationic	15 mM[28]	14.6 mM	-
LDAO	zwitter-ionic	1–2 mM[29]	1.7 mM	3.0 mM
Fos-Choline-12	zwitter-ionic	1.5 mM[30]	1.4 mM	3.4 mM

SDS and DTAB laid within our tested concentration ranges, it is interesting to see that the presence of micelles formed by SDS and DTAB did not lead to a detectable increase in light scattering intensity (Figure 2c and d). To investigate if light scattering was impaired by electrostatic repulsion between ionic micelles, we plotted the derived count rates from SDS and DTAB solutions in aqueous NaCl (Figure S4). Again, we observed no increase in light scattering intensity above the cmc, which indicates that light scattering is not impaired by electrostatic repulsion. Similar absence of a detectable increase in light scattering intensity was observed from non-ionic C8E4 before,[20] which suggests that this observation is linked to the light scattering behavior of micellar assemblies rather than the ionic character of detergent headgroups. Since C8E4, SDS, and DTAB have consistently lower aggregation numbers compared to DDM, LDAO, and Fos-Choline-12, we expect that the micelles are too small to cause a noticeable increase in light scattering intensity (Table S5).

Diffusion Coefficient Analysis Enables Absolute Cmc Analysis of Non-ionic and Ionic Detergents

Having evaluated the DLS-based light scattering intensity analysis, we moved on to evaluate the DLS-based diffusion coefficient analysis.[21] For this purpose, we monitored changes in diffusion coefficients of most abundant particle distributions in solution against the detergent concentration. Briefly, a series of aqueous solutions with different detergent concentrations was analyzed by DLS. The logarithm of the diffusion coefficients of most abundant particle size distributions in solution was plotted against the logarithm of the detergent concentration (Figure 3).[21] At detergent concentrations below cmc, multimodal size distribution profiles were obtained and the most abundant diffusion coefficients were small (Figure 3).

This appeared counterintuitive at first glance. Smaller diffusion coefficients indicate larger particles, which is the opposite to what we expected for detergent monomers below cmc compared to micelles. Control measurements with deionized water indicated that particle size distributions below cmc are background signals that indicate the absence of micelles (Figure 3a). This conclusion was further supported by a comparison of correlograms, which did not show significant differences between deionized water and detergent solutions below cmc (Figure S5). Accordingly, in diffusion distribution profiles by volume we observed monomodal particle distributions with larger diffusion coefficients at detergent concentrations above cmc, which we assigned to micellar aggregates (Figure 3).[31–33] The average of the highest detergent concentration with lower diffusion coefficient and the lowest detergent concentration with higher diffusion coefficient was taken as cmc (Figure 3).

To consolidate our hypothesis that smaller diffusion coefficients detected by DLS below cmc indicate the absence of micellar aggregates, we performed control measurements for [G1] OGD by ^{1}H DOSY NMR in deuterated water. In contrast to DLS, which analyzes time-dependent fluctuations of light scattering caused by particle diffusion, ^{1}H DOSY NMR analyses the diffusion of protons directly, which enables a differentiation of detergent monomers and micelles.[34,35] Our ^{1}H DOSY NMR experiments revealed higher diffusion coefficients at concentrations below cmc, which we assigned to the monomeric detergent species (Figure 4).[34,35] Diffusion coefficients of monomers could not be detected by DLS using the same samples as investigated by ^{1}H DOSY NMR (Figure 4). Our comparative analysis indicates that diffusion coefficients detected by DLS below the cmc indeed resemble the background signal of the solvent/detergent mixture, which supports the validity of the DLS-based diffusion coefficient procedure for cmc determination (Figure 4).

To clarify the utility of DLS-based diffusion coefficient analysis for cmc determination, we again analyzed fitting bias, reproducibility, and applicability to common detergent classes. First, we investigated the effect of fitting bias on cmc analysis. Our diffusion coefficient plots showed two distinguishable regions below and above cmc (Figure 3). The way we determined the cmc required no fitting and gave no room for the determination of different cmc values per analyzed concentration series.

Accuracy is determined by the distance between neighboring concentrations around cmc and needs to be adjusted to the scope of the investigation. For an unknown detergent, first a cmc range can be identified and a more precise value can be determined by decreasing the distance between neighboring concentrations within that range. Compared to the DLS-based light scattering intensity approach, DLS-based diffusion coefficient analysis delivered unambiguous cmc values, regardless

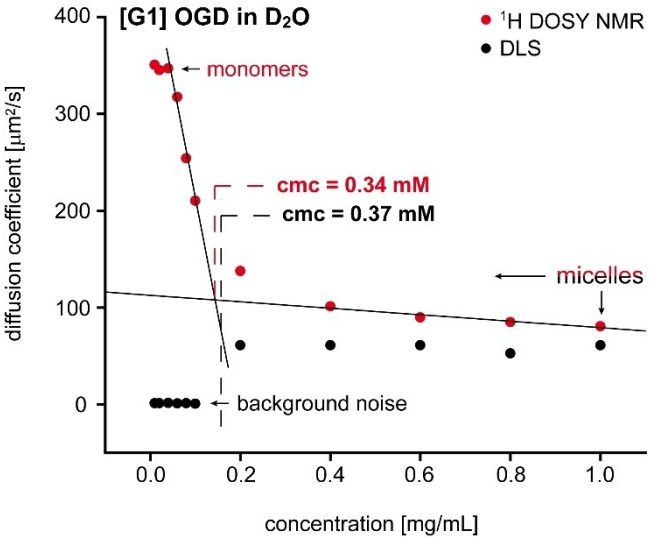

Figure 4. Comparison of DLS and ^{1}H DOSY NMR. The plot shows diffusion coefficients obtained from the same [G1] OGD samples in deuterated water by DLS and ^{1}H NMR DOSY for different concentrations. Larger diffusion coefficients were detected below cmc with ^{1}H NMR DOSY, which is in line with the presence of monomers and supports the hypothesis that smaller diffusion coefficients detected by DLS below cmc are background noise. ^{1}H DOSY NMR data were fitted to linear functions whose intersection was taken as cmc. Both methods delivered similar cmc values under the employed conditions.

of the detergent investigated under the employed experimental conditions (Figure 3).

To investigate reproducibility, we compared our cmc values with literature. DLS-based diffusion coefficient analysis delivered cmc values that were close to literature values of detergents with cmc values in the micromolar and millimolar range, like DDM, LDAO and Fos-Choline-12 (Table 1). Since literature cmc values of [G1] OGD have been determined by DLS-based light scattering intensity analysis before,[3,26] our present data obtained from DLS-based diffusion coefficient analysis suggest that its cmc is 0.2 mM and thus lower than previously reported cmc values, i.e., 0.5–0.8 mM (Table 1).[3,26] Apart from SDS (95% purity), deviations between cmc values determined by DLS-based diffusion coefficient analysis and literature were smaller than 7%, which is a common deviation and underlines the capability of this method to deliver reproducible cmc values.[1]

To investigate the applicability to common detergent classes, we again included DDM, LDAO, Fos-Choline-12, SDS, and DTAB in our analysis. In contrast to DLS-based light scattering intensity analysis, we found that DLS-based diffusion coefficient analysis can be used to determine the cmc of non-ionic, zwitter-ionic, cationic and anionic detergents (Table 1) (Figure 3). This includes weakly scattering detergents with low aggregation numbers, like SDS and DTAB, which again emphasized the utility of the DLS-based diffusion coefficient analysis for cmc determination.

Cmc Analysis in Aqueous Salt-containing Solutions

For many applications, like protein purification,[36,37] micellar detergent solutions are not prepared in deionized but in aqueous buffers containing salts or other additives with known impact on cmc.[13b,36,38–41] Mixtures of water, detergents, and salt are more complex than binary mixtures of water and detergents. This led us to the question whether DLS-based diffusion coefficient analysis can be used to determine reproducible cmc values in salt-containing aqueous solutions.

To test, whether DLS-based diffusion coefficient analysis is applicable for cmc determination in salt-containing aqueous solutions, we applied this method to SDS in 0.015 M sodium chloride solution and DTAB in 0.01 M sodium chloride solution. In line with the literature, we observed a reduction in cmc values for anionic SDS and cationic DTAB (Figure S6) (Table 2).[38,42] We observed similar trends for anionic rhamnolipid in a more complex Tris buffer system (Figure S6) (Table 2). The observation that cmc values of ionic detergents in water are reduced in the presence of salt is generally expected. The charge of ionic head groups is better shielded by counter ions when the salt concentration is increased.[43]

Shielding charged detergent head groups with counter ions reduces electrostatic repulsion between detergent monomers, enhances their aggregation tendency and reduces their cmc.[39,43,45] Control experiments with non-ionic DDM supported this explanation. Similar cmc values were obtained in deionized water and Tris buffer under the experimental conditions employed (Table 2).[46] Taken together, our results underline the applicability of DLS-based diffusion coefficient analysis for determining cmc values of detergents in salt-containing solutions and buffer.

Practical Implications for Cmc Analysis

The question that motivated this study was, which of the two approaches, i.e., DLS-based light scattering intensity and diffusion coefficient analysis, is more suitable for obtaining reproducible cmc values. Having systematically compared both approaches, we can now say that the DLS-based diffusion coefficient analysis is more suitable for the determination of cmc values since literature values in aqueous solution could be readily reproduced. Detergent synthesis can be tedious and resource consuming.[5,47,48] Conveniently, DLS is a non-invasive technique and utilized analytes could be almost fully recovered. The preparation of a full concentration series (10^{-8} to 20 mg/mL) and subsequent DLS measurements, as described here, took us one working day per detergent. Given the concentration range around the cmc is known, the concentration

Table 2. DLS-based diffusion coefficient analysis of cmc values in aqueous salt solutions. Summary of utilized detergents, buffer conditions, literature values, and cmc values determined with DLS-based diffusion coefficient analysis.

detergent	buffer conditions	cmc literature	cmc diffusion coefficient
SDS (99%)	0.015 M NaCl	4.2 mM[38]	3.6 mM
SDS (99%)	deionized water (control)	8.2 mM[44]	8.7 mM
DTAB	0.01 M NaCl	13.5–14.1 mM[42]	10.5 mM
DTAB	deionized water (control)	15 mM[28]	14.6 mM
DDM	200 mM NaCl, 20 mM Tris, 25 mM Imidazole, pH 8	n.a.	0.3 mM
DDM	deionized water (control)	0.1–0.6 mM	0.3 mM
Rhamnolipid	200 mM NaCl, 20 mM Tris, 25 mM Imidazole, pH 8	0.007–0.4 mg/mL[22][a]	0.2 mg/mL
Rhamnolipid	deionized water (control)	n.a.	3.0 mg/mL

[a] Rhamnolipids are natural products with batch-to-batch variability in molecular composition and whose cmc values in various aqueous, salt-containing solutions were reported to be in the given concentration range. n.a.-not available.

window to be taken into consideration can be narrowed and two independent measurements become readily feasible per day. Furthermore, our DLS-based diffusion coefficient analysis experiments could reproduce trends in cmc values when shifting solvent environments from deionized water to more complex salt-containing solutions.

Seen from the perspective of micellization theory, the DLS-based diffusion coefficient analysis comes also with a limitation. The definition for cmc considered in our work assumes that the cmc is defined as the detergent concentration above which micelles are formed. However, this could happen in two ways: (1) At lower detergent concentrations, e.g., below cmc, detergents could be present as monomers or mixtures of monomers and oligomers, while at higher concentrations, e.g., above cmc, detergent monomers co-exist with micelles.[49] (2) Alternatively, micellization could occur over a broad concentration range, hence at lower detergent concentrations, e.g., below cmc, detergents are present as monomers, while at higher concentrations, e.g., closely above cmc, detergents are present as monomer-micelle equilibrium that is shifted towards micelles when the detergent concentration is increased.[50,51] The interpretation of the sigmoidal change in diffusion coefficients detected by ^{1}H DOSY depends on the utilized micellization model. According to the first micellization model (1), the decrease in diffusion coefficients detected by ^{1}H DOSY can be interpreted as formation of oligomers and the concentration that marks the transition towards the second plateau at lower diffusion coefficients is taken as cmc (Figure 4).[52] When analyzing the ^{1}H DOSY data through the lens of the second micellization model (2), the decrease in diffusion coefficients can be interpreted as weighted average of the monomer-micelle ratio in solution.[51] Accordingly, the cmc would be taken as the concentration that marks the transition between the diffusion coefficients of detergent monomers and the decrease in diffusion coefficients at higher detergent concentrations. Since our cmc values obtained by DLS-based diffusion coefficient analysis match cmc data obtained by other techniques, we decided to apply the first micellization model (1) (Tables 1 and 2). In the case that the second micellization model is more accurate (2), the DLS based diffusion coefficient analysis would be prone to overestimate the actual cmc since monomeric detergent signals cannot be detected under the employed conditions. To enable a reinterpretation of our cmc data, we provided the DLS data files for download with the paper in a data repository–see manuscript section "Supporting Information."

However, from a practical point of view, since applications that harness solubilizing properties of detergents typically require the presence of micelles as the most abundant particle population in solution, we conclude that analyzing DLS-based diffusion coefficient data through the lens of micellization model (1) is a meaningful complementary strategy for cmc analysis. Although the detection limit of the DLS instrument is unclear, we reproduced literature cmc values for detergents with cmc values ranging from 200 μM to 14.6 mM, which indicates that cmc values within this area can be determined by DLS-based diffusion coefficient analysis (Tables 1 and 2).

Conclusions

Here we evaluated the utility of DLS-based light scattering intensity and diffusion coefficient analysis for the determination of cmc values of detergents in aqueous solution. The evaluation of the DLS-based light scattering intensity analysis led us to three conclusions. First, cmc values obtained by DLS-based light scattering intensity analysis likely over- or underestimate literature values due to the double-logarithmic nature of the analysis and fitting inaccuracy under the experimental conditions employed. Second, DLS-based light scattering intensity analysis may not reproduce absolute cmc values but can be used to compare relative changes in cmc values. Third, cmc values may only be obtained for micellar assemblies that sufficiently scatter light under the experimental conditions employed. In contrast, the DLS-based diffusion coefficient analysis can reproduce literature cmc values in deionized water, including weakly scattering detergents. DLS-based diffusion coefficient analysis can also be used for the determination of cmc values in more complex, salt-containing solutions and buffers. In contrast to ^{1}H DOSY NMR, DLS-based diffusion coefficient analysis does not exhibit the sensitivity required to differentiate diffusion coefficients of detergent monomers from micellar aggregates. Seen from a broader perspective, our systematic comparison outlined DLS-based diffusion coefficient analysis as a complementary technique for the determination of cmc values, which will facilitate the characterization of detergents in the future.

Supporting Information Summary

Supporting Information is provided with the article for download and includes additional references [53–62]. DLS data files obtained from the Zetasizer Software for all detergents are provided with the paper for download from the data repository Zenodo (https://doi.org/10.5281/zenodo.13860620).

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Zenodo at https://doi.org/10.5281/zenodo.13860620, reference number 13860619.

Keywords: Detergent · Dynamic light scattering · DOSY · Diffusion coefficient · Cmc

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