

Micelle Characterization by Dynamic Light Scattering: Bringing Viscosity into the Equation

Relevant for: Dynamic Light Scattering (DLS), Litesizer™ 500, Lovis 2000 ME, DMA™ 5000 M, cosmetics, surfactants, micelles, particle size, density, dynamic viscosity

Dynamic viscosity is an integral part of the Stokes-Einstein equation, which enables the calculation of particle size from DLS measurements. While performing calculations using the dynamic viscosity of the solvent might be an acceptable strategy for some samples, it introduces a sizeable bias in DLS results when particles strongly affect the suspension's viscosity. Micelles are composed of amphiphilic surfactant molecules which spontaneously assemble into spherical structures in solution. Here we investigated particle size in a micellar solution submitted to variations in ionic strength, pH and surfactant concentration with the Litesizer™ 500, while viscosity was measured using a Lovis 2000 ME viscometer coupled to a DMA™ 5000 M densitometer. Especially for undiluted sample we observed that the particle size calculated using the measured dynamic viscosity differed significantly from that calculated using solvent viscosity. Diluting the sample in order to diminish the particles' influence on overall viscosity is frequently used as an alternative to measuring dynamic viscosity. However this strategy was not applicable here, as we observed that dilution had a dramatic impact on the size of micelles. Together these data stress the importance of measuring the dynamic viscosity of samples to obtain accurate particle size results with DLS.

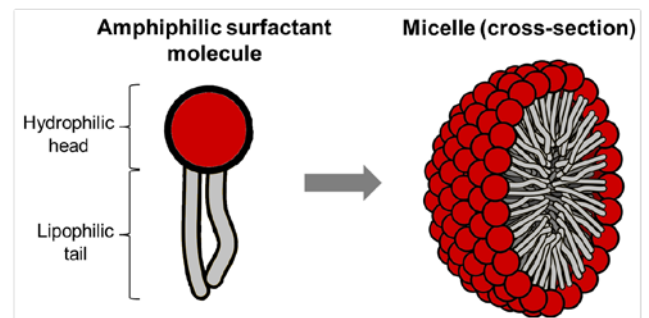


Figure 1: Schematic structure of an amphiphilic surfactant molecule and of a micelle. Assembly of surfactant molecules into micelles occurs spontaneously when surfactant concentration is above the so-called critical micelle concentration (CMC).

1 Introduction

Micelles are spherical aggregates of surfactants with surface-active and amphiphilic properties. If the concentration of surfactant is below the critical micelle concentration (CMC), surfactant molecules are free to move in solution and position themselves on surfaces. If the surfactant concentration is high enough to reach the CMC, surfactants spontaneously start to form micelles in solution (Figure 1).

When surfactant concentration increases, the number of micelles increases as well (1). Micelle properties are also influenced by pH, temperature and the ionic strength of the solvent (2).

Disodium cocoamphoacetate is a mild amphiphilic surfactant which is derived from coconut oil fatty acids of different chain lengths. It is frequently used in skin and hair conditioning products because it conditions as well as cleanses (3)(4). Moreover, it increases the foaming capacity of cosmetic formulations when used as a co-surfactant. Unlike other surfactants like sodium lauryl sulfate, which is very widely used in cosmetics, disodium cocoamphoacetate has a relatively low detergent capacity and is thus mild to the skin and non-irritating.

In the present application report the influence of pH, surfactant concentration and ionic strength on the particle size of micelles was investigated by dynamic light scattering (DLS) using the Litesizer™ 500 particle analyzer. A disodium cocoamphoacetate-based commercial micellar solution was used for this study.

As viscosity is an integral part of the Stokes-Einstein equation (see Equation 1), it is essential to know the viscosity of the solution in order to calculate particle size accurately.

$$R_H = \frac{k_B T}{6\pi \eta D}$$

D Translational diffusion coefficient [m²/s]

k_B Boltzmann constant [m²kg/Ks²]

T Temperature [K]

η Viscosity [Pa.s]

R_H hydrodynamic radius [m]

Equation 1: The Stokes-Einstein equation

To this end, we coupled DLS measurements with dynamic viscosity measurements of the micellar solution using the Lovis 2000 ME, a rolling ball viscometer which is perfectly suited for viscosity measurements of dilute solutions. The Lovis 2000 ME measures the runtime of a ball in a sample-filled capillary. The density value of the sample is used to calculate the dynamic viscosity from the runtimes determined by the Lovis 2000 ME. In order to determine both parameters within a single run, the Lovis 2000 ME was combined with a DMA™ 5000 M density meter.

This setup enabled us to compare particle size values calculated either using the viscosity of the sample's diluent (water) or using the dynamic viscosity values obtained from the Lovis 2000 ME measurements.

2 Experimental Setup

2.1 Sample Preparation

A commercial micellar solution containing disodium cocoamphoacetate was purchased from a local drugstore.

To investigate the effect of ionic strength, the solution was diluted 1:2 with different concentrations of NaCl (10, 30, 50, 150, 300 or 600 mM in deionized water).

To investigate the influence of pH the solution was first diluted 1:2 with filtered deionized water. The pH was then adjusted using 1 M HCl or 1 M NaOH solutions.

To investigate the effect of surfactant concentration and viscosity, the micellar solution was either measured pure ("undiluted") or diluted with filtered deionized water to 1:2, 1:10 and 1:100 dilutions.

2.2 Viscosity Measurements

The dynamic and kinematic viscosities of the four sample dilutions (undiluted, 1:2, 1:10 and 1:100) were determined using a DMA™ 5000 M density meter coupled with a Lovis 2000 ME viscometer. Triplicate measurements were performed for every sample dilution. The method settings for the Lovis 2000 ME are given in Table 1.

Table 1: Lovis 2000 ME viscometer settings

Parameter	Setting
Temperature	25 °C
Angle	50°
Measuring mode	Repeated mode
Measurement cycles	n = 3
Maximum allowed Variation Coefficient [%]	0.1

DMA™ 5000 M measurements were performed at 25 °C and thermal equilibrium in triplicates.

2.3 Dynamic Light Scattering Measurements

A Litesizer™ 500 particle analyzer was used for all dynamic light scattering (DLS) measurements. Measurement series were performed in disposable cuvettes at 25 °C. The measuring angle, number of runs, focus position and optical filter settings were automatically set by the instrument. Every sample was equilibrated before the measurement to avoid temperature gradients.

3 Results and Discussion

3.1 Viscosity Measurement Results

The measured density and dynamic viscosity values are displayed in Table 2. As expected, both the density and viscosity of the micellar solution decrease when the sample is diluted in deionized water. To verify the measurement quality of the Lovis 2000 ME, the Variation Coefficient as well as the Forward/Backward deviations were recorded and are displayed in Table 3.

Table 2: Density and dynamic viscosity measurements of 4 dilutions from a micellar solution, at 25 °C (mean ± SD, 3 measurements)

Sample dilution (in Δ H ₂ O)	Density [g/cm ³]	Dynamic viscosity [mPa.s]
Undiluted	1.01239 ± 0.00001	1.240 ± 0.001
1:2	1.00469 ± 0.00001	1.084 ± 0.003
1:10	0.99934 ± 0.00001	0.954 ± 0.001
1:100	0.99729 ± 0.00001	0.905 ± 0.001

Table 3: Variation coefficients and forward/backwards deviations of 4 dilutions from a micellar solution (n = 3 measurements)

Sample dilution (in Δ H ₂ O)	Variation Coefficient [#] [%]	Forward/Backward Deviation [‡] [%]
Undiluted	0.01	0.05
1:2	0.01	0.07
1:10	0.02	0.05
1:100	0.02	0.06

[#] The variation coefficient gives the deviation [%] between the runtimes of the sample's measurement cycles on the Lovis 2000 ME.

[‡] The Forward/Backward deviation describes the deviation [%] between the forward and the backward runtime of the Lovis 2000 ME.

3.2 Effect of Ionic Strength on Micelle Particle Size

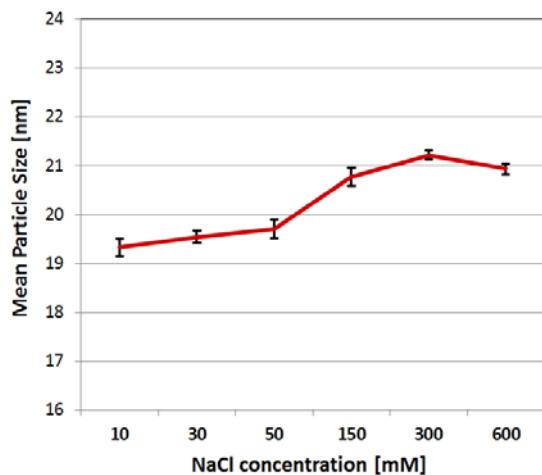


Figure 2: Mean micelle particle size at different ionic strengths. Results are expressed as mean ± SD from 6 consecutive measurements. The given concentrations refer to NaCl solutions which were diluted 1:2 with the sample (the NaCl concentration of which is unknown).

Changing the ionic strength of the solvent only had a moderate impact on micelle particle size, as shown in Figure 2. Although significant, the increase in mean particle size of the sample remained limited to approximately 10 % when the ionic strength was multiplied by a factor of 60. A likely explanation for this limited effect is that the micelles under investigation are amphoteric, which means that they carry both negatively and positively charged functional groups

resulting in a neutral net charge. These micelles are thus not stabilized by ionic effects, so increasing the ionic strength of the solvent does not impact their size.

3.3 Effect of pH on Micelle Particle Size

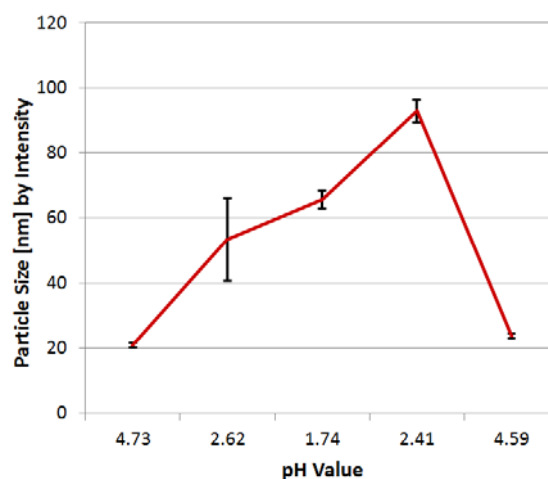


Figure 3: Variation of micelle particle size (main peak by intensity) during a pH ramp. The x-axis shows the pH values in chronological order. Results are expressed as mean ± SD from 6 consecutive measurements.

In contrast to ionic strength, pH had a strong impact on micelle formation and size, as shown in Figure 3 and Figure 4. When the pH of the stock solution was decreased from 4.7 to 1.74, the micelles changed their structure and the particle size increased (from about 20 nm to approximately 90 nm). This was likely due to the fact that when protons are added to the micelles, the surfactant molecules are not ionic anymore. This results in a disruption of micelle assembly, leading to an aggregation of the particles. Furthermore, the initial monomodal distribution of micelle size (Figure 4, top panel) was lost during the acidification process, as the size distribution for pH = 2.6 (Figure 4, middle panel) showed at least 3 discrete peaks.

This phenomenon proved reversible, as the micelle particle size was restored to its original value of about 20 nm when the pH of the solution was readjusted to 4.6. This indicated that the surfactant molecules were deprotonated again and that the micelles reassembled into their original structures.

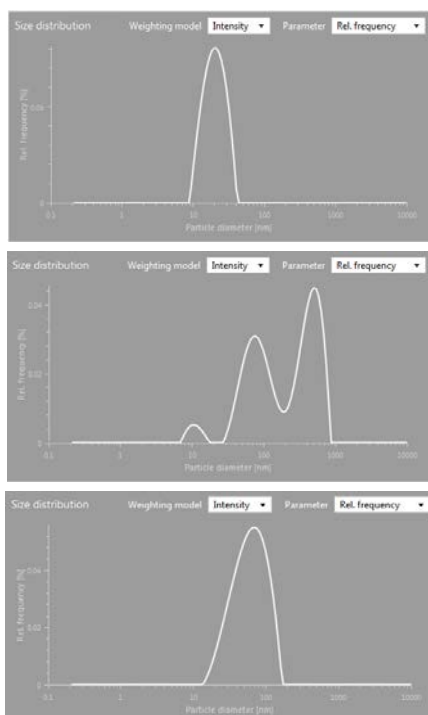


Figure 4: Particle size distribution (by intensity) of a micellar solution at pH 4.7 (top panel), pH 2.6 (middle panel) or pH 1.7 (bottom panel). One representative run out of 6 is depicted.

3.4 Effect of Viscosity and Surfactant Concentration on Micelle Particle Size Values Returned by DLS

We investigated the effect of surfactant concentration by performing serial dilutions of the micellar suspension in deionized water. DLS measurements were performed on the undiluted sample as well as on 1:2, 1:10 and 1:100 dilutions. Since dilution was likely to have a large impact on the suspension's viscosity, we measured the dynamic viscosity of every dilution with the Lovis 2000 ME (see section 3.1). Table 4 and Figure 5 show the mean particle size calculated for the different dilutions either using the viscosity of water ("uncorrected" data set) or the measured viscosity values ("viscosity-corrected" data set).

Of all physical parameters tested in this study, surfactant concentration had the most dramatic impact on micelle particle size. Indeed, particle size increased from around 10 nm in the stock solution to over 100 nm in the 1:100 dilution. Whether this is due to the enlargement of individual micelles in response to the increasing hydration or reflects micelle aggregation remains to be determined.

Table 4: Particle size (by intensity) of micelles calculated either using the viscosity of water ("Uncorrected") or the dynamic viscosity values returned by the Lovis 2000 ME ("Viscosity corrected")

Sample dilution (in Δ H ₂ O)	Uncorrected Particle Size [#] [nm] (Rel. SD in %)	Viscosity-corrected Particle Size [#] [nm] (Rel. SD in %)	Correction rate [‡]
Undiluted	14.66 ± 0.46 (3.14 %)	10.38 ± 0.24 (2.31 %)	- 41 %
1:2	20.32 ± 0.33 (1.55 %)	16.47 ± 0.26 (1.55 %)	- 23 %
1:10	37.98 ± 0.57 (1.51 %)	35.77 ± 0.87 (2.44 %)	- 6 %
1:100	120.8 ± 16.8 (13.89 %)	107.8 ± 10.7 (9.88 %)	- 11 %

[#] Results are expressed as mean ± standard deviation from 5 consecutive measurements. Relative standard deviations are given between parentheses.

[‡] Corresponds to the relative change in calculated particle size when the sample's dynamic viscosity is taken into account. Correction = (1 - (Uncorrected / Viscosity-corrected)) x 100.

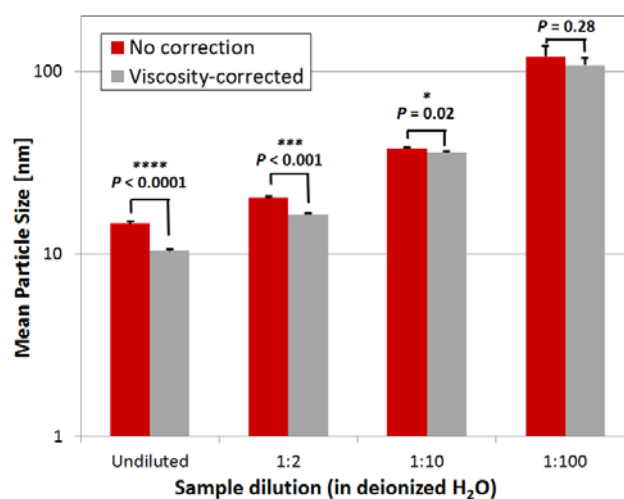


Figure 5: Effect of surfactant concentration on micelle particle size: comparison of data calculated using the viscosity of water (red bars) or the viscosity measured by the Lovis 2000 ME (gray bars). Results expressed as mean ± SD from 5 measurements. Statistical significance was evaluated by standard *t* test between uncorrected and viscosity-corrected data sets, P-values are displayed for each individual sample dilution.

Most importantly, these data confirmed that the viscosity plays an important role for accurate particle size measurements of samples when sample viscosity significantly differs from solvent viscosity. In the present sample, for the undiluted solution, the mean particle size calculated using the sample's measured viscosity was around 4 nm lower than that calculated using the viscosity of water (see Table 4, 4th column). As the particle size calculated using the "real" sample viscosity can be mathematically considered as the exact value, we can thus conclude that the

uncorrected particle size value has an error of, or is overestimated by, 41 %. Due to the narrow standard deviation of the DLS measurements, this error proved statistically very significant ($P < 0.0001$, see Figure 5).

This error still proved very significant ($P < 0.001$) for the 1:2 dilution, with particle size still being overestimated by 23 % (3.85 nm) when calculated using the viscosity of water rather than the sample's. For the 1:10 sample dilution, and as the sample's dynamic viscosity approached that of water, the error narrowed to 6 % (2.21 nm) but was still significant ($P = 0.02$).

When the sample was diluted 1:100, micelle particle size values soared over 100 nm, but DLS measurement series also returned enlarged standard deviations (see Table 4, bottom line), likely pointing towards an instability of the micelles in the solution. Taking the sample's viscosity into account for this dilution still led to a - 11 % correction of particle size, corresponding to a difference of 13 nm. However, the error did not prove statistically significant anymore.

4 Conclusion

Dynamic viscosity is an integral part of the particle size calculation from DLS measurements. The operator who does not have a viscometer at his or her disposal is faced with two choices when dealing with a sample of unknown dynamic viscosity:

- One is to measure the concentrated sample and calculate the particle size using the dynamic viscosity of the sample's diluent, knowing that the result's accuracy will be negatively impacted.
- The other is to perform DLS on serial dilutions of the sample until the returned values (calculated using the solvent's viscosity) are stable over at least 2 dilutions. This will indicate that a point has been reached where the difference between the sample's and the solvent's viscosities has become so negligible that it does not impact particle size values anymore.

While this latter approach is suitable for particles that remain stable when diluted, it is doomed to fail in the case of particles which size and behavior vary depending on their concentration, either because they interact between themselves or with their solvent. In that case, decreasing the concentration of the particles changes their nature so that every dilution effectively becomes a distinct sample.

Micelles are such a sample. Here we demonstrate that the particle size of micelles, as determined by DLS using the Litesizer™ 500, can vary in function of the solvent's ionic strength and its pH, but that the most dramatic changes in micelle particle size are observed when the sample is simply diluted. Indeed, we observed that particle size increased 10-fold when

the stock solution was diluted 1:100 in deionized water.

Thus, the only option to obtain an accurate estimation of micelle particle size in our stock solution was to combine DLS with dynamic viscosity measurements. These were performed on a Lovis 2000 ME viscometer coupled with a DMA™ M density meter. We then compared particle size values calculated either using the viscosity of water or the measured sample viscosities, for the stock solution as well as for different dilutions.

Our data confirm that, for the undiluted or weakly diluted samples, particle size values were very largely overestimated when calculated using the viscosity of water rather than the sample's. Due to the high reproducibility of Litesizer™ measurements, this difference persisted and was still significant down to a sample dilution of 1:10.

These data highlight the fact that the measurement of concentrated samples by DLS must be coupled to that of the sample's dynamic viscosity, or risk returning largely erroneous particle size values. Using the Lovis 2000 ME viscometer coupled with the DMA™ M density meter allowed us to very significantly improve the accuracy of particle size measurements for concentrated micelle samples. This approach is of particular importance for the characterization of sensitive samples such as micelles, for which performing dilutions as a way to diminish the influence of viscosity cannot be considered because it dramatically changes the nature of the particles.

5 References

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