EXPERIMENT 6: DETERMINATION OF THE MEAN AGGREGATION NUMBER OF A MICELLAR SYSTEM

Introduction

In this experiment, the mean aggregation number of sodium dodecyl sulfate within micelles in aqueous solution will be determined by a fluorescence probe technique.

Background

It is well known that hydrocarbons have very low solubilities in water under ordinary conditions. The fundamental reason for this situation is that hydrocarbons lack the ability to disrupt and participate in the hydrogen bonding that characterizes liquid water. This incompatibility of hydrocarbons (hydrophobic) and water (hydrophilic) can be overcome if a molecule possesses both hydrophobic and hydrophilic properties. Examples of such amphiphilic molecules are long-chain carboxylic acids or the subject of the present experiment, sodium dodecyl sulfate (SDS), $NaOSO_3C_{12}H_{25}$. Such molecules function as detergents or surfactants (*surface-active agents*) because they dramatically change many bulk properties of water.

When sufficient SDS is dissolved in water, several bulk solution properties significantly change, particularly the surface tension, which decreases, and the solubility of hydrocarbons, which greatly increases. However, the changes in these properties do not occur until a certain bulk SDS concentration is reached. This concentration is called the *critical micelle concentration* (CMC). Several experiments, including light scattering and NMR studies, show that above the CMC these monomers undergo self-organization to form structures called *micelles*. The CMC is a not a precisely defined concentration, and thus its experimental value depends somewhat on the method used to determine it.

A micelle is an organized cluster, or aggregate, consisting of a number of monomers (~ 60–120 for SDS, depending on the circumstances). Micelles are roughly spherical in shape. Technically, a micellar solution is a colloidal dispersion of organized surfactant molecules. One of the key aspects of micelle structure is that its interior consists of an associated arrangement of the hydrocarbon chain structure of the surfactant molecule (an "oil drop"). The exterior coat of the micelle structure, then, consists of the polar, ionic moieties of the amphiphilic monomers (the OSO_3^- groups, for SDS). It is the hydrocarbon-like interior of the micelle that gives it its many diverse and interesting properties.

We will assume for convenience that a micelle is constructed of definite number of surfactant molecules, called the *aggregation number N*. In actuality, however, micelles are characterized by a distribution of amphiphile aggregates, and so we will regard N as the mean aggregation number of the micellar system. The value of N depends not only on the nature of the surfactant, but also on the temperature, and perhaps surprisingly, on the concentration of electrolytes in the aqueous solution.

Theory

Consider an aqueous solution of a surfactant that has a bulk concentration $[S]_o$, which is above the CMC. If we make the simple assumption that the surfactant molecules are

present either as monomeric units or as micelles that contain N monomers, there will be a concentration of such micelles, $[M]$, which can be expressed as

$$[M] = ([S]_o - \text{CMC}) / N , \qquad (1)$$

where CMC is the concentration of free monomers in solution. In reality, a micellar solution is not a static system containing only two solutes, monomer and micelle. Micelles constantly undergo assembly and dissociation, and at a given instant in time micelles are characterized by a distribution of aggregates containing different numbers of monomer units. Thus, in Eq. (1), N represents the *mean* aggregation number, and $[M]$ accordingly represents an average micelle concentration. Because the numerator in Eq. (1) can be directly determined (the CMC can be obtained experimentally), we could find the value of N if we knew the average micelle concentration in the system.

In this experiment, we will obtain this information indirectly through a fluorimetric technique.[1] This method relies on several simple but important assumptions:

(1) A luminescent probe molecule is added to the micellar system. This probe is exclusively associated with a micelle rather than being dispersed in the aqueous medium. The luminescence intensity of the system is then proportional to the fraction of "tagged" micelles (not all micelles have a probe in them).

(2) There are many more micelles present than probe molecules. Thus, only a fraction of the micelles present contains the probe molecules; a micelle is either empty or associated with a single probe molecule.

Suppose a luminescence quencher is added to this system. The quencher essentially removes the photoexcitation energy from the probe, so that the probe does not luminesce after it absorbs light. We make the further assumptions: (3) The quencher is associated with micelles *only*; it is not solvated in the aqueous medium. (4) These solubilized quenchers occupy micelles randomly, irrespective of whether they are vacant or occupied by a luminescent probe molecule. (5) If a probe shares a micelle with one or more quenchers, the probe will not luminesce.

The following is another important methodological requirement. The micelle is continually exchanging monomers with the solvent (at a rate of roughly several thousand times per second), whereby it undergoes a complete reorganization tens of times per second. Therefore a probe being used to determine its mean aggregation number (a static concept) must "take a snapshot" of the micelle on a time scale of much less than ~ 1 – 10 ms. Luminescent probes easily satisfy this criterion since their intrinsic lifetimes are usually less than 1 μs.

The luminescence intensity of the system is proportional to the number of micelles that are occupied by a probe molecule with no quencher. Thus, for a particular (bulk) quencher concentration, the ratio of the luminescence intensity, I, to that with no quencher present, I^o, (the probe concentration being constant) is equal to the fraction of probe-containing micelles that do *not* contain a quencher molecule.

We now consider the statistics of the situation. If q quenchers are placed randomly in m micelles. the distribution of these quenchers in the micelles is governed by Poisson[2] statistics (if q and m are large). Such a distribution means that the probability of finding n quenchers in a randomly selected micelle is given by

$$P_n = \langle q \rangle^n \exp(-\langle q \rangle) / n! \ , \tag{2}$$

where $\langle q \rangle$ is the overall probability that a micelle contains at least one quencher, *i.e.* $\langle q \rangle = q/m$. Macroscopically, $\langle q \rangle = [Q] / [M]$, where $[Q]$ is the bulk quencher concentration and $[M]$ is the (mean) micelle concentration. Of particular interest to us is the probability that a micelle contains *no* quencher, because if such a quencher contained a probe, it would produce luminescence. From Eq. (2), we have for $n = 0$,

$$P_0 = \exp(-\langle q \rangle) = \exp(-[Q] / [M]) \ . \tag{3}$$

(Remember 0! = 1.) Finally, we relate the measured quantity I/I^o to the fraction of quencher-occupied micelles:

$$I/I^o = \exp(-[Q] / [M]) \ . \tag{4}$$

Substituting the expression for $[M]$ from Eq. (4) into Eq. (1) and rearranging, we have

$$ln(I^o/I) = [Q]\, N / ([S]_o - \mathrm{CMC})) \ . \tag{5}$$

We can use Eq. (5) to determine both N and the CMC, depending on the dependent variable used, $[Q]$ or $[S]_o$. In either case, the concentration of the luminescent probe is held constant throughout the experiment. If the surfactant concentration is fixed (*i.e.* constant $[S]_o$) and $[Q]$ is varied, we can use a linear least squares fit to obtain N. Alternatively, if $[Q]$ is constant and $[S]_o$ is varied, we may find *both* N and the CMC from an appropriate linear fit using Eq. (5). In the former experiment, the emission intensity decreases with increasing $[Q]$, as might be expected. However, when $[S]_o$ is increased at fixed $[Q]$, the luminescence intensity should *increase* because the number of micelles increases, thereby decreasing the probability that a given micelle will be occupied by both a probe molecule and a quencher. In this case, it is assumed that the smallest $[S]_o$ is larger than the CMC, *i.e.* we begin with a micellar system.

Procedure

Fluorescence emission intensities will be measured with a Perkin-Elmer LS-5B luminescence spectrophotometer (fluorimeter). Use extreme care in handling the fluorescence cuvettes. Do not put fingerprints in ANY of the four optical faces. Detailed instructions for the operation of the instrument will be given in the laboratory.

In this experiment, the probe molecule is the chloride salt of the divalent second-row transition metal ruthenium, which is coordinated with three molecules of the organic ligand bipyridine (bipy). This compound, $Ru(bipy)_3^{2+}$, is a very well characterized system.[3] It absorbs blue light ($\lambda_{max} \sim 450$ nm) and emits red light ($\lambda_{max} \sim 625$ nm). The lifetime of the luminescent state ($\sim 0.5\ \mu s$) is much shorter than the average lifetime of the "intact" micelle.

An important fact about this probe is that $Ru(bipy)_3Cl_2$ is soluble in water. This property would seem to disqualify it as a probe, since we have stipulated that the probe and quencher must be solubilized in or associated with a micelle, rather than also being present as water-solvated species. Detailed studies have shown that in the presence of submicellar concentrations of SDS (*i.e.* less than the CMC), $Ru(bipy)_3^{2+}$ forms an insoluble compound with the surfactant, $Ru(bipy)_3(OSO_3C_{12}H_{25})_2$.[4] However, at higher SDS concentrations, where micelle formation occurs , this compound is solubilized by the micelle. It is believed that the $Ru(bipy)_3^{2+}$ cation is not in the hydrocarbon-like interior of the micelle but is instead closely associated the negatively charged outer mantle of the micelle. Furthermore, it is presumed that the quencher, which is solubilized in the hydrocarbon-like interior of the micelle, will quench the $Ru(bipy)_3^{2+}$ luminescence if they happen to share the same micelle.

The quencher in this experiment is 9-methyl anthracene, which has a very low solubility in pure water but can be solubilized in SDS micelles. The absorption spectrum of SDS-solubilized 9-methylanthracene is consistent with the interpretation that the solute is in a hydrocarbon-like medium.

Part 1. Fixed Surfactant Concentration. The probe concentration in each solution to be studied should not exceed 7.2×10^{-5} M in $Ru(bipy)_3Cl_2$. An aqueous stock solution of $\sim 7 \times 10^{-3}$ M will be available in the lab. Prepare two 50 ml quantities of a solution that is 0.045 M in SDS. The amounts of SDS added to each volumetric flask should be identical, within ~ 1 mg. Dissolve all the solute in an amount of water that does not fill the neck of the flask. Heating speeds up the process. Fill one of the volumetric flasks to the mask after the solution cools to room temperature. Make up 5 ml of a 0.15 M solution of 9-methylanthracene in absolute ethanol. Inject 200 μl of this stock solution into the other SDS solution and fill to the mark. The ethanol acts as a coupling agent that facilitates the solubility of the hydrocarbon in the micelle solution. The minute amount of ethanol is presumed to have a negligible effect on the micellar system. If the solution becomes cloudy, heat it slightly. Sonicating the solution is also helpful.

Make up a series of solutions from the solutions of SDS with quencher (*A*) and the SDS without quencher (*B*). Prepare four solutions by pipetting 2, 4, 6, and 8 ml of *A* into 10-ml volumetric flasks and filling to the mark with *B*. Into two other 10-ml volumetric flasks separately add solutions *A* and *B*. In all, there will be six 10-ml samples ranging from 0 to 100 % of the quencher concentration., each having the same SDS concentration. Finally, to each solution add 100 μl of the $Ru(bipy)_3^{2+}$ stock solution. It is important to keep these deliveries as uniform as possible so that the resulting probe concentrations are equal. [The concentrations of $Ru(bipy)_3^{2+}$ can be checked by measuring the absorbance at 450 nm on the Cary 219 UV/VIS spectrophotometer, available in the laboratory.] Each of the six samples should be clear, pale yellow-orange in appearance. Label each solution.

For each solution, measure the luminescence intensity in the fluorimeter. Set the excitation and wavelengths to 450 and 625 nm, respectively. It is not necessary to scan the luminescence spectra. Begin with the sample having no quencher. Also record the temperature of the micellar system.

Part 2. Fixed Quencher Concentration. Hold the $Ru(bipy)_3^{2+}$ concentration at ~ 7×10^{-3} M, as in part 1. Keep the quencher concentration at ~ 1×10^{-4} M while varying the surfactant concentration between ~ 0.01 and 0.05 M. Prepare two 50-ml solutions of SDS in purified water, one 0.01 M (*A*) and the other 0.05 M (*B*). Make up 5 ml of a 0.050 M solution of 9-methylanthracene in absolute ethanol. Inject 100 μl of the 9-methylanthracene stock solution into the SDS solutions, heating or sonicating if necessary. Next prepare five solutions of *A* and *B* by adding 1, 2, 4, 6, and 8 ml of *A* to 10-ml volumetric flasks. Fill to the mark with solution *B*. Finally, add to each 10-ml mixture 100 μl of a 7×10^{-3} M aqueous solution of $Ru(bipy)_3^{2+}$ (the same stock solution used in part 1). Mix the solutions well and label them.

For each solution, measure the luminescence intensity at 625 nm (with $\lambda_{exc} = 450$ nm). Analyze the data from the recast form of Eq. (5):

$$[\ln(I^\circ/I)]^{-1} \; \cancel{\ln(I/I^\circ)} = ([Q]\,N)^{-1}\,[S]_o - (\mathrm{CMC}/[Q]\,N) \; . \qquad (6)$$

Obtain *N* and CMC from the slope and intercept of a linear least squares fit. In your report, compare your results with those reported by Turro and Yekta.[1] Be sure to discuss possible sources of error in your determination.

Questions

While writing up your report, consider the following questions: (1) Assume that the hydrocarbon-like interior cavity of the SDS micelle has a diameter of ~ 3 nm and that it contains three solubilized benzene molecules. Calculate the "local" concentration of benzene in the micelle. (2) Assuming that at 298 K the CMC for a certain surfactant is 6×10^{-3} M and that its mean aggregation number is 70, estimate the concentration of micelles if the bulk surfactant concentration is 0.08 M. (3) A quantity (390 mg) of *n*-hexane is solubilized in 100 ml of the surfactant in question 2. Assuming that all the solubilized *n*-hexane molecules are in the micelles, what is the mean occupation number of *n*-hexane in the micelles?

References

1. N. J. Turro and A. Yekta, "Luminescent Probes for Detergent Solutions: A Siple Procedure for Determination of the Mean Aggregation Number of Micelles," J. Am. Chem. Soc. **100**, 5951 (1978). (included in this manual)

2. See, for example, J. R. Taylor, *An Introduction to Error Analysis: The Study of Uncertainties in Physical Measurements*, 2nd ed. (University Science Books, Mill Valley, CA, 1997), Chap. 11.

3. The $Ru(bipy)_3^{2+}$ ion is tris(2,2'-biphyridine)ruthenium(II) and is commonly called "ruthenium tris bipee."

4. D. Meisel, M. S. Matheson, and J. Rabani, "Photolytic and Radiolytic Studies of $Ru(bipy)_3^{2+}$ in Micellar Solutions," *J. Am. Chem. Soc.* **100**, 117 (1978).

that the $C_3H_5Cl^+$ skeleton is derived from the neutral reactant, perhaps by formal H_2^- abstraction; a complementary isotope-labeling study will clearly be useful.

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References and Notes

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(5) The arbitrary scales for Figures 1 and 2 are the same and represent relative photodissociation cross sections.
(6) J. L. Beauchamp, D. Holtz, S. D. Woodgate, and S. L. Patt, *J. Am. Chem. Soc.*, **94**, 2798 (1972).
(7) In Figure 2(B) and in the discussion of Figure 2(C), the comparison drawn between the observed product-ion spectrum and the calculated spectrum for a particular mixture of known ions is not intended to constitute a precise composition analysis of the product-ion population, but only to show that the observed results are fully consistent with a mixture of ions.

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Luminescent Probes for Detergent Solutions. A Simple Procedure for Determination of the Mean Aggregation Number of Micelles

Sir:

One of the most fundamental and important structural parameters of micellar aggregates is the aggregation number, or the average number of detergent molecules in a micelle unit.[1] The measurement and establishment of aggregation numbers is therefore of great significance. We report here a simple procedure for measuring the mean aggregation numbers of detergent solutions. The method is based on the quenching of a luminescent probe by a hydrophobic quencher.

The mean aggregation number of micelles may be derived from luminescence quenching measurements if "static" or "active" sphere[2] quenching of a micellar donor by a micelle associated quencher is dominant. Suppose a solution contains a well-defined but unknown micelle concentration [M] and a macroscopic concentration of quencher [Q]. If Q is selected so that it resides exclusively in the micellar phase, then the molecules of Q will be distributed among the available micelles in some fashion. If a luminescent molecule D, which is also completely associated with micelles, is now added to the system, D will partition itself both among micelles containg Q and among "empty" micelles. We select Poisson statistics to describe the distribution of D and Q among micelles in the tertiary system D, Q, M. If D is luminescent only when it occupies an empty micelle (i.e., D* is completely quenched when it occupies a micelle containing at least one Q), then the *measured* ratio of luminescence intensities (I/I°) in the presence of Q to that in the absence of Q is related by the very simple expression

$$(I/I^\circ) = \exp\{-[Q]/[M]\} \qquad (1)$$

The simplicity of this expression derives from the assumption that only D* in micelles containing no Q emit.[3] This assumption can be tested experimentally since, as a function of increasing [Q], the luminescence lifetime should not change even though the luminescence intensity is decreased, i.e., "static" quenching occurs. [M] can be related to the measurable

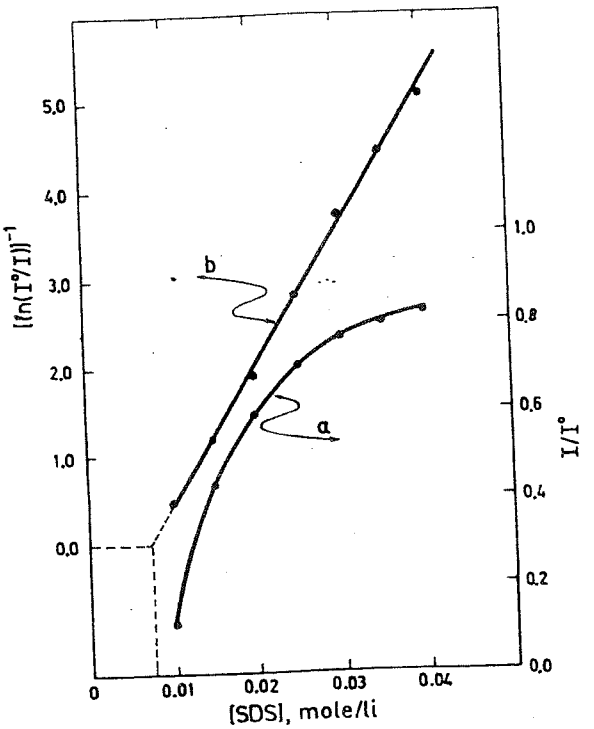

Figure 1. Detergent concentration dependence of 7.2×10^{-5} M, D = $Ru(bipy)_3^{2+}$ luminscence intensity. Q = 9-methlanthracene fixed at 1.05×10^{-4} M. Curve a: intensity normalized relative to that in the absence of Q. Curve b: analysis according to eq 3 of text. Excitation and emission wavelengths were at 450 and 630 nm, respectively (25 °C).

macroscopic concentration of detergent, [Det], and the mean aggregation number, $\bar{n}$, by the expression

$$[M] = \frac{[\text{Det}] - [\text{free monomer}]}{\bar{n}} \qquad (2)$$

where the free monomer concentration in equilibrium with the micellar aggregates is almost equal to the critical micelle concentration, cmc. The combination of expression 1 and 2 leads to

$$\ln (I^\circ/I) = \frac{[Q]\bar{n}}{[\text{Det}] - [\text{free monomer}]} \qquad (3)$$

We are in a position to evaluate both the aggregation number, $\bar{n}$, and the concentration of free monomer in equilibrium with micelles by measuring I°/I as a function of [Q] at fixed [Det] and by measuring I°/I as a function of [Det] at fixed [Q].

The experimental system of this report uses luminescent donor D = $Ru(bipy)_3^{2+}$, luminescence quencher Q = 9-methylanthracene, and detergent = sodium dodecyl sulfate (SDS). The system meets the requirement of a water-insoluble quencher and micelle-associated donor.[4] Furthermore, several evaluations of the aggregation number of SDS have been reported in the literature as a function of experimental variables.[5-8]

At $[D] \leq 7.2 \times 10^{-5}$ M, eq 1 is obeyed. Measurements of luminescence lifetime of D* yielded a constant value at 0.48 μs (±3%) *even under conditions where quenching had reduced the luminscence intensity by an order of magnitude.* Comparable quenching in a homogeneous solvent such as acetonitrile (no SDS) resulted in the expected (Stern–Volmer) decrease of both luminscence intensity and lifetime.[9]

To verify the functional form of eq 3, two sets of experiments were run, in each keeping one of the variables constant. Figure 1a shows the dependence of luminescence intensity keeping [Q] fixed at 1.05×10^{-4} M. The luminescence intensity *increases* as [SDS] is *increased.* This observation is readily explained by our model. Increased micelle concentration plays a protective role by keeping the donor and quencher molecules

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