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DIFFUSION COEFFICIENTS OF MIXED MICELLES OF TRITON X-100 AND SPHINGOMYELIN AND OF SONICATED SPHINGOMYELIN LIPOSOMES, MEASURED BY AUTOCORRELATION SPECTROSCOPY OF RAYLEIGH SCATTERED LIGHT

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SUMMARY

Autocorrelation spectroscopy of Rayleigh scattered light has been used to measure the diffusion coefficients of mixed micelles of Triton X-100 with sphingomyelin. Sphingomyelin is a water-insoluble lipid. It forms a solution of mixed micelles only for molar fractions of Triton X-100 above 0.32. Over the range of Triton X-100 molar fractions from 0.32–1.0 the diffusion coefficient increases from $2.6 \cdot 10^{-7}$–$4.5 \cdot 10^{-7}$ cm^2/s, indicating that the increased concentration ratio of the detergent systematically reduces the size of the mixed micelles.

Diffusion coefficients of sonicated liposomes of sphingomyelin were also measured. For specimens of unhydrogenated and partially hydrogenated sphingomyelin, respective diffusion coefficients of $8.8 \cdot 10^{-8}$ and $3.1 \cdot 10^{-8}$ cm^2/s were obtained. The corresponding Stokes radii for the two specimens are 250 and 700 Å, respectively.

INTRODUCTION

Detailed knowledge of particle size is crucial in many biological studies, if the results of those studies are to be interpreted meaningfully. In this work, autocorrelation spectroscopy of Rayleigh scattered light has been used to provide this type of information. In our studies of enzymatic activity on an insoluble lipid substrate, sphingomyelin [1, 2], two techniques were used to produce small substrate particles in order to promote measurable enzymatic rates: (a) In the work of Yedgar et al. [1], a detergent, Triton X-100, was used to produce mixed micelles of the detergent and substrate, (b) Gatt et al. [2] used ultrasonic irradiation of the pure substrate to produce liposomes. In each case it was important to know the resultant particle size.

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The interest in mixed micelles of a lipid and a detergent, and in sonicated liposomes, is of course not confined to enzymatic studies. The former are of great importance in studies of the solubilization and reconstitution of biological membranes by detergents [3, 4], and the latter are one of the favorite models for biological membranes [5]. Therefore it is also of great general interest to investigate the properties of the particles which are produced by these two methods. In this work we present measurements of the diffusion coefficients of mixed micelles of Triton X-100 sphingomyelin over a wide range of composition ratios, and of sonicated liposomes of sphingomyelin. The diffusion coefficients are used to calculate Stokes radii for these two types of particles. In the accompanying paper, these diffusion coefficients are applied, in conjunction with other measured parameters, to determining molecular weights and deducing structural models for the mixed micelles.

The measurement technique, analysis of the autocorrelation function of the scattered light, provides a highly accurate determination of the diffusion coefficients. The advantages which this and other equivalent light scattering methods enjoy over other techniques for determining diffusion coefficients have been pointed out by several workers [6–10]. The light scattering methods are inherently more accurate, do not require the establishment of a macroscopic concentration gradient, and are quicker, generally requiring seconds or minutes instead of hours or days. Indeed, as we report below, attempts to corroborate the light scattering results using an analytical centrifuge simply confirmed the greater accuracy, speed and convenience of the light scattering method. As an indicator of particle size, the advantages of this method over electron microscopy are clear. The light scattering measurements are non-destructive, and indeed can be carried out with the particles under their most favorable conditions, whereas the preparation of the specimens for electron microscopy can introduce undesirable changes.

EXPERIMENTAL DETAILS

(*A*) *Measurement of diffusion coefficients*

When a solution of monodisperse isotropic macromolecules is irradiated with a beam of monochromatic light, then the spectrum of the light scattered at an angle θ (in the medium) from the forward direction will have a Lorentzian profile centred at the excitation frequency, with half width of $K^2D/2\pi$ Hz [11, 12]. Here D is the diffusion coefficient of the particle in the solution, and K is the magnitude of the wavevector difference between the incident and the scattered light:

$$K = \frac{4\pi n}{\lambda} \sin \frac{\theta}{2} \tag{1}$$

with n the index of refraction of the solution, and λ the vacuum wavelength of the light.

The method used in this work to study the spectrum of the scattered light is (single clipped) autocorrelation spectroscopy, in which the time-correlation in the scattered light is monitored. The theoretical and practical considerations for the application of this technique to this type of problem have been well documented [8, 9, 13–16]. This method is quite equivalent to the more established homodyne spectroscopy, [6, 7, 10, 17–19] in which the self-beating spectrum of the scattered light is measured. The autocorrelation function measured in the present work is simply the Fourier

transform of the homodyne spectrum.

The intensity autocorrelation (or "second order" autocorrelation) of a light source is given by

$$G_2(\tau) = \{I(t)I(t+\tau)\} \tag{2}$$

where $I(t)$ is the light intensity at time t, and where $\{\}$ indicates an ensemble average. The scattering is a stationary process, and it is of course an average over time which is actually measured.

The digital autocorrelator used in this work was a Malvern Autocorrelator (Precision Devices, Great Malvern, England). The autocorrelator samples $G_2(\tau)$ at 24 discrete values of autocorrelation time $\tau = mT_s$, where $m = 1,2, \ldots 24$, and T_s is a suitably selected sampling time (of order 10 μs in most of this work). A number of salient details of the operation of the digital autocorrelator are given in an Appendix.

The function $G_2(\tau)$ contains the square-modulus of the first-order autocorrelation function. Now the first-order autocorrelation function of light scattered from particles undergoing Brownian motion takes the simple form [11]

$$A_1(\tau) = A_1(0) \exp(-K^2 D\tau) \tag{3}$$

with $A_1(0)$ a normalizing constant. (The Lorentzian profile of width $K^2D/2\pi$ Hz mentioned above will be recognized as the Fourier transform of this function.) Therefore, the square modulus of this function is of the form

$$A_2(\tau) = A_2(0) \exp(-2K^2 D\tau) \tag{4}$$

This function occurs in $G_2(\tau)$ as

$$G_2(\tau) = A_2(\tau) + C \tag{5}$$

where C is a constant which depends in part on such purely instrumental factors as the coherence area of the source, i.e., on the light collection geometry [15]. Its magnitude, equal to $G_2(\infty)$, is contained in the accumulated autocorrelation data, so that $A_2(\tau)$ may be extracted from the measurement of $G_2(\tau)$. The determination of the diffusion coefficient then consists of fitting the function $A_2(\tau)$ to an exponential form and extracting D.

(*B*) *Light scattering apparatus*

The scattering cell was of $1 \cdot 2$ cm rectangular cross section. The focussed beam of a 50 mW He–Ne laser was introduced at normal incidence into the narrower side of the cell, and the light scattered through the adjacent window at some selected angle was collected. This angle was accurately defined by restricting apertures, one a vertical slit close to the cell, and the other a pin-hole about 20 cm removed, both mounted on an accurate goniometer. The scattering angle in the medium, θ, was calculated from Snell's law, using a value of the refractive index of the solution measured with a Bausch and Lomb refractometer. The light admitted by the apertures was incident upon an ITT FW-130 photomultiplier, and the train of photon pulses was analyzed by the autocorrelator.

(*C*) *Instrument calibration*

The intrinsic accuracy of the autocorrelation measurements was verified by

measuring the diffusion coefficient of a highly dilute suspension of 1090 Å polystyrene spheres (Dow Diagnostic Products, Indianapolis, U.S.A.). This was the smallest size available. It is comparable to the size of the sonicated liposomes, but almost an order of magnitude larger than that of the micelles studied. For the monodisperse scattering system provided by the polystyrene spheres (the standard deviation in particle diameter is given as 27 Å) the autocorrelation function $A_2(\tau)$ should have the form given in Eqn 4. Such a function is best handled by considering its logarithm

$$\ln A_2(\tau) = \ln A_2(0) - 2K^2 D\tau \tag{6}$$

which defines a straight line with respect to τ. Indeed, the logarithms for a number of measurements lay on a straight line with negligible deviation. Herein lies an important advantage of the autocorrelation method over the homodyne method. In the latter, a Lorentzian spectrum is expected for a monodisperse scatterer. Polydispersity is difficult to diagnose, except in severe cases, since the small systematic departures from a Lorentzian shape are not recognizable. On the other hand, the logarithm of the autocorrelation function is extremely sensitive to polydispersity, showing it as a readily observable departure of the function from lioearity [20].

The magnitude of K was calculated from Eqn (1) using the known values of n, λ, and θ. The diffusion coefficient deduced from the slope of the straight line was $3.90 \cdot 10^{-8}$ cm^2/s, consistent to ± 1 % over several measurements. This measured value may by checked against the value calculated from the Stokes law

$$D = kT/6\pi\eta r \tag{7}$$

where k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the solute, and r is the particle radius. The calculated value of D is $3.87 \cdot 10^{-8}$ cm^2/s, which is in excellent agreement with the measured value.

(D) Preparation of micelles

Spinal cord sphingomyelin was prepared according to the technique described by Gatt et al [2]. Since hydrogenation of the sphingomyelin with Tritium gas was required in the enzymatic studies cited [1], to monitor the enzymatic activity, and since hydrogenation can influence the aggregation properties of the sphingomyelin, equivalent hydrogenation was carried out on these specimens. Details of the chemical analysis will be given in the accompanying publication. The Triton X-100 was obtained from British Drug Houses.

The sphingomyelin and the Triton X-100, each in chloroform–methanol 2 : 1 solutions, were combined in the desired ratio. The solvent was evaporated under nitrogen. Sodium acetate buffer, pH 5.0, 0.1 M, which had been filtered through a 0.01 μm cellulose acetate filter (Millipore Sartorius membrane filter), was then added. The solution was warmed to the cloud point and mixed by a Vortex cyclometer, and then cooled. The resultant clear solution was left in a closed vacuum for several hours to remove air bubbles. No further filtration could be carried out because the micelles are prone to adsorption on the filter.

(E) Preparation of sonicated liposomes

Liposomes of unhydrogenated and partially hydrogenated sphingomyelin were prepared in sodium acetate buffer, pH 5.0, 0.1 M, by ultrasonic irradiation in a

Braun-Sonic 300 W sonicator. The total duration of the sonication was 15 min for the unhydrogenated sphingomyelin, and 30 min for the hydrogenated sphingomyelin. The sonication was intermittent, to prevent excessive heating of the samples.

After sonication the liposome suspension was centrifuged for 15 min at 30 000 $\times g$. The supernatant was subjected to gel filtration through a Sepharose 4B column (Huang [21]), eluted with the above buffer. Successive fractions were tested for organic phosphorus content according to Bartlett [22]. The elution pattern consisted of two distinct peaks for both species of liposomes. Fractions at the second of these peaks (the smaller and more homogeneous particles) were chosen for study.

RESULTS AND DISCUSSION

(*A*) *Mixed micelles*

Since Triton X-100 and sphingomyelin do not form a homogeneous system of mixed micelles at arbitrarily high or low ratios of Triton to sphingomyelin, ultracentrifuge sedimentation measurements were carried out to determine the range of compositions that could usefully be investigated by light scattering. Because the details of the sedimentation measurements are given in the accompanying paper, only the main results are given here. It was found that for Triton molar fractions (X_{Tr}) below 0.32, a highly heterogeneous mixture of particles was produced; the ultracentrifuge Schlieren traces showed numerous sedimentation peaks with widely differing sedimentation coefficients. For X_{Tr} between 0.32 and 0.79 the Schlieren traces showed a single, narrow, symmetrical peak. For values of X_{Tr} above 0.79 this peak persisted, but an additional peak of lower sedimentation coefficient also appeared. For X_{Tr} equal to unity (pure Triton) a single peak was again obtained.

The solutions for X_{Tr} below 0.32, the region of extreme heterogeneity, were not investigated by light scattering. For X_{Tr} between 0.32 and 0.79, the region of the single Schlieren peak, it was tentatively concluded that a homogeneous system of mixed micelles was formed, keeping in mind that sedimentation velocity measurements do not provide complete assurance in this regard (Van Holde [23]). 8 Triton molar fractions in this range were chosen for study. The sedimentation results for X_{Tr} greater than 0.79, where a new peak appeared, suggested that around 0.79 is the maximum value of X_{Tr} for the formation of mixed micelles, and that above this molar fraction the excess Triton X-100 forms a second phase of pure Triton micelles. On this assumption, it was concluded that mixtures of such similar particles would be amenable to investigation by light scattering, and measurements were carried out for two Triton molar fractions in this range. Finally, for completeness and for comparison with other work, the case of pure Triton X-100 was included.

In Fig. 1a are shown, as examples, two separate measurements of the autocorrelation function $A_2(\tau)$, for a solution of mixed micelles with $X_{Tr} = 0.76$, at 0.028 g/ml total solute concentration. The scattering angle in the medium was 115 °. The two functions apparently have a common shape, except that one (●) is upshifted with respect to the other (○). The lower function is considered first. Its logarithm is shown in Fig. 1b (○). The linearity of the logarithm indicates that the function $A_2(\tau)$ is well described by a simple exponential, in confirmation of the results of the sedimentation velocity measurements, which indicated a monodisperse system of particles.

The upshifting of the second function in Fig. 1a was caused by gross scatterers

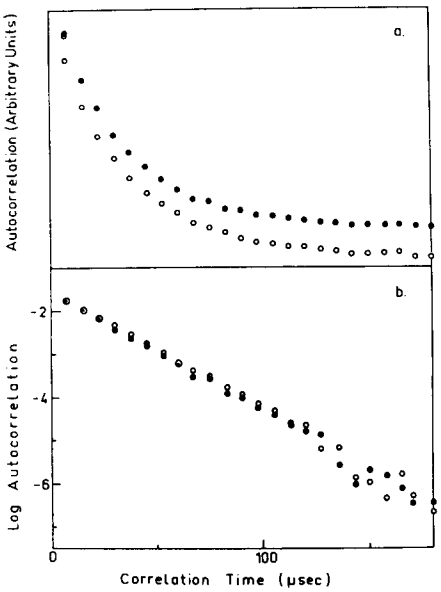

Fig. 1. (a) 2 measurements of the autocorrelation function $A_2(\tau)$ for mixed micelles. The Triton X-100 molar fraction is 0.76, and the total solute concentration is 0.028 g/ml. Scattering angle in the medium is 115°. ○, an undisturbed measurement; ●, function upshifted due to intermittent stray light. (b) Logarithm of the undisturbed autocorrelation function (○), and of the upshifted function after correction for the stray light (●).

(dust or residual bubbles) which, despite efforts to eliminate such sources of spurious scattering, were nonetheless present in sufficient numbers as to occasionally traverse the laser beam. Because these particles introduced a large momentary increase in the intensity of the scattered light, their presence was always detectable. It was found that, over the several minutes of photon counting that were required in order to accumulate statistically reliable data, the occurence of such an event was highly likely. The presence of this scattered component in one measurement, and its complete absence in the other, shows that its occurrence was indeed a matter of chance. In the Appendix it is shown that this phenomenon has the effect of merely adding a constant (with magnitude depending on the severity of the spurious scattering) to the function $A_2(\tau)$.

Since all measurements that were not troubled by this effect indicated that $A_2(\tau)$ was always well described by a simple exponential, correction for the spurious constant when it did occur was a trivial matter. The correction was made iteratively: an approximate correction was made, and then successively refined until a simple exponential fit the function over its entire range. The logarithm of the shifted function, with the appropriate correction made, is shown in Fig. 1b (●). The logarithm of this corrrected function is indeed coincident with the unshifted function, to within the accuracy of the data, confirming that the required correction is indeed only a constant.

The slope of the logarithm of $A_2(\tau)$ is $-2K^2D$, and with K known to high accuracy, D is readily extracted by means of a simple fit to a straight line. In this fitting, all points do not have equal weight [15]. The uncertainty in the function $A_2(\tau)$ decreases only slightly in absolute terms from the first to the 24th point. This is be-

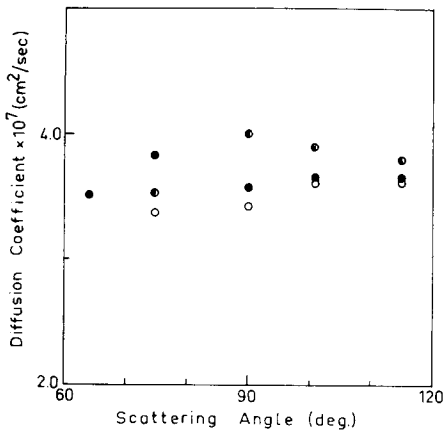

Fig. 2. Measured diffusion coefficients for 3 concentrations of mixed micelles with Triton X-100 molar fraction 0.79, plotted with respect to scattering angle in the medium. ○, 0.008 g/ml total solute concentration; ◐, 0.016 g/ml; ●, 0.032 g/ml.

cause the uncertainty in $A_2(\tau)$ is in fact the statistical uncertainty in $G_2(\tau)$. Since the constant C in $G_2(\tau)$, cf. Eqn 5, was always larger than $A_2(\tau)$, this constant largely determined the magnitude of the uncertainty. In relative terms, this error grows rapidly with higher correlation times. In addition to this, any slight inaccuracy in the correction applied to the function because of the intermittent stray light will, for the same reasons, have a small relative importance at small times, but potentially greater significance at higher times. Therefore, the value of D taken from the best fit is weighted in favour of the points at lower times.

Diffusion coefficients were measured for each micelle composition at a number of concentrations, and for each concentration at a number of scattering angles. In Fig. 2, values of D are shown for three concentrations of micelles with $X_{Tr} = 0.79$, plotted with respect to scattering angle in the medium, θ. There is a 4 % average deviation in the points about their overall mean of $3.67 \cdot 10^{-7}$ cm^2/s. Any dependence of D on angle or concentration must therefore be small, so that no unambiguous conclusions can be drawn from these results alone regarding such dependence. However, when the results for all the micellar compositions at all concentrations and angles are considered together, the following picture emerges: (1) There is no angular dependence, with the unimportant exceptions noted below. (2) There is a clear concentration dependence. With dilution from high concentrations, a small increase in the average value of D is observed. The magnitude of this dependence is quite consistent; D varies for nearly all the micellar compositions by about $0.01 \cdot 10^{-7}$ cm^2/s for each mg/ml of solute. With continued dilution, however, the average value of D again falls.

The results of Fig. 2 illustrate this concentration dependence. At the highest concentration of 0.032 g/ml, the average value of D is $3.64 \cdot 10^{-7}$ cm^2/s. At 0.016 g/ml it has risen to $3.81 \cdot 10^{-7}$ cm^2/s, and at 0.008 g/ml again fallen to $3.51 \cdot 10^{-7}$ cm^2/s. The average deviation for the points at each concentration about their respective mean is 3 %.

The concentration dependence at high concentrations can be ascribed to interactions between the micelles (Tanford [24]). The drop in D at low concentrations is

ascribable to a homogeneous impurity of particles significantly larger than the micelles (but to be distinguished from the gross scatterers that manifested themselves intermittently). Such an impurity, if present in the buffer in more or less fixed concentration, will produce such a lowering of the value of D at very low solute concentrations. In confirmation of this, measurements at increasingly higher dilutions (not shown), though less and less accurate, showed ever smaller values of D and, moreover, a dependence on angle. The impurity particles were apparently comparable in size to a wavelength of light, and their stronger forward scattering weighted the value of D to smaller values at low angles.

It should be noted that no correlation was found between the value of D and the degree of upshifting of the function (cf. Fig. 1). Thus the various data points in Fig. 2 represent measurements in which no upshifting, moderate, and even severe upshifting of $A_2(\tau)$ occurred. This is further confirmation of the validity of the type of correction made.

For each of the micellar compositions studied, the reliable data at higher concentrations (typically up to 0.03 g/ml) were used to extrapolate to the value D_0, the diffusion coefficient at zero solute concentration. (Although the extrapolation should strictly be to zero micelle concentration, the critical micellar concentration for the Triton–sphingomyelin system should be low enough that extrapolation to zero solute concentration introduces negligible error.) The values of D_0 are shown plotted with respect to X_{Tr} in Fig. 3. The dependence of D_0 on X_{Tr} is given with good accuracy by a linear relationship:

$$D_0 = [2.57\, X_{Tr} + 1.89] \cdot 10^{-7}\ \mathrm{cm^2/s}$$

with correlation coefficient of 0.96. The standard deviation in the slope is 9 %, and in the intercept 2 %. There is no physical significance necessarily attached to this linear fit, since there is no a priori reason for a strictly linear relationship between D_0 and X_{Tr}.

The values of D_0 for X_{Tr} equal to 0.86 and 0.93 (open circles in Fig. 3), for

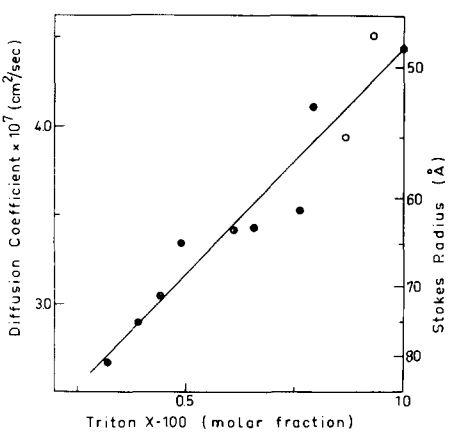

Fig. 3. The extrapolated diffusion coefficient D_0 as a function of Triton X-100 molar fraction. At molar fractions indicated by ○, an additional phase coexists with the mixed micelles. The Stokes radius is indicated by the reciprocal scale to the right.

which a new phase was evident in the sedimentation measurements, are consistent with the rest of the data. This indicates that the new phase is similar in size to the mixed micelles at high X_{Tr}. If the new phase indeed consists of micelles of pure Triton X-100, then the average value of D_0 for the two species should, as observed, smoothly bridge the transition from $X_{Tr} = 0.79$ to pure Triton, as the concentration of the pure Triton micelles is increased.

The scatter in the points is greater at higher values of X_{Tr}. Anticipating conclusions given below, the higher values of D_0 in this region correspond to smaller particles. Their scattering intensity, for a given concentration of solute, was almost an order of magnitude smaller than from the larger micelles at lower X_{Tr}. Not only were the measurements intrinsically less accurate, but they also had to be carried out at higher concentrations, so that larger extrapolations were required.

For comparison, measurements of D were made with a Beckman model E analytical centrifuge run at 6000 rev./min. Micelles with X_{Tr} equal to 0.36, 0.79 and 1.0 were run at a number of concentrations, and the results were extrapolated to determine D_0. This extrapolation was unreliable indeed, since the data showed no better consistency than about 25 %, far short of the good consistency of the light scattering data. The results obtained were in rough agreement with the light scattering data.

The radii of the micelles can be calculated from D_0 using Eqn 8. This calculation requires the assumption that the micelles are spherical, and that there is no slip between the solvent and the micellar surface (Tanford [24]). For pure Triton X-100 there is evidence that the assumption of spherical shape may be valid [25]. Its validity for the case of the mixed micelles will be discussed in the accompanying work. The Stokes radius according to Eqn 8 is indicated in Fig. 3 by the reciprocal scale of the right-hand ordinate.

To examine the influence of hydrogenation on the micellar size, specimens were prepared at several representative Triton molar fractions using unhydrogenated sphingomyelin. The values of D_0 which were found were in all cases consistent with those for the hydrogenated sphingomyelin.

(*B*) *Liposomes*

The scattering intensity per unit concentration of solute was high for the liposomes. Therefore measurements could be made at very low concentrations, so that the concentration dependence of the diffusion coefficient was not a consideration. Good quality exponentials were obtained for $A_2(\tau)$, indicating that the gel-filtration fractions were monodisperse.

For the partially hydrogenated sphingomyelin, two fractions at the second elution peak were examined. Concentrations in both were less than 10^{-4} g/ml. The respective diffusion coefficients were 3.07 and $3.18 \cdot 10^{-8}$ cm^2/s. The accuracy, based on many measurements, was 3 %. The corresponding Stokes radii are 705 Å and 680 Å, both $\pm$20 Å. The large diameters are consistent with the high scattering intensities obtained. The small difference between the radii is in the proper sense: decreasing radius for successive gel-filtration fractions. The accuracy of the results is well in excess of the calibration accuracy of a gel-filtration column.

A single fraction of unhydrogenated sphingomyelin was examined, also taken at the second elution peak. The solute concentration was $1.5 \cdot 10^{-4}$ g/ml. A diffusion coefficient of $8.8 \cdot 10^{-8}$ cm^2/s ($\pm$4 %) was measured, corresponding to a much smaller

Stokes radius of 246 Å ($\pm$10 Å). The large influence of hydrogenation on the liposome radius, which is in striking contrast to the lack of influence in the mixed micelles, is considered in our accompanying publication.

The liposomes were also examined under an electron microscope, using negative staining with 1 % ammonium molybdate [5]. The micrographs showed qualitative agreement with the above results.

CONCLUSIONS

For the mixed micelles studied in this work, autocorrelation spectroscopy of the Rayleigh scattered light gives an accurate indication of the influence of increased Triton X-100 molar fraction on the diffusion coefficient. The fuller significance of this is considered in our accompanying work.

The measurement of diffusion coefficients as a function of concentration showed a small concentration dependence. As a rule, this dependence is measured only incidentally, to allow extrapolation to D_0. However, with the light scattering technique, where no macroscopic concentration gradients exist, this dependence should be simply related to the particle interactions and can therefore provide a convenient probe of those interactions [26]. Further work in this direction is indicated.

The results for the liposomes serve to underline further the advantages of this method, namely that it is fast, accurate, convenient, and requires small quantities of solute.

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APPENDIX

(*A*) *Operation of the digital autocorrelator*

The second-order autocorrelation of a light source is given by

$$G_2(\tau) = \{I(t)\, I(t+\tau)\},$$

where $I(t)$ is the light intensity at time t, and where $\{\}$ will here be taken to denote a time average. The autocorrelator cannot, of course, sample $I(t)$ continuously and instantaneously. Rather, it samples $N(t)$, the number of photon pulses detected in successive sampling-time intervals of duration T_s. Every sample $N(t)$ is multiplied with the succeeding 24 samples, i.e., $\tau = mT_s$, with $m = 1, 2, \ldots 24$, and successive sets of 24 products are accumulated into 24 registers (the storage capacity of the model used). This efficient handling of the data renders the autocorrelator much faster than a single channel homodyne spectrometer (Cummins and Swinney [19]).

In order to allow rapid operation in real time, however, the calculation of the autocorrelation products must be simplified. For this, the quantity $N(t)$ is replaced by its "clipped" value $N_c(t)$, as follows: $N(t)$ is compared to a present "clipping level" K, a number close to $\{N(t)\}$, the mean value of $N(t)$, so that

$N_c(t) = 0$ if $N(t) < K$

$N_c(t) = 1$ if $N(t) \geqslant K$

Thus the autocorrelator builds up the function

$$G_2^c(mT_s) = \{N_c(t)\, N(t+mT_s)\}$$

The clipping introduces slight statistical loss, but no distortion of the measured function (Jakeman [14]), so that the "single clipped" autocorrelation function $G_2^c(mT_s)$ differs from $G_2(mT_s)$ by mere normalizing factors.

(B) The influence of strong intermittent scattering

In principle, scattering from large particles complicates the autocorrelation function because such scatterers theoretically introduce two spurious components into the autocorrelation function: firstly, a component which is essentially constant in time, since the diffusion coefficients of large particles are essentially zero compared to that of a micelle, and secondly, a cross term or heterodyne component of the form $\exp(-K^2D\tau)$ (Cummins and Swinney [19]).

The measurement of the function $G_2^c(\tau)$ is based upon the fact that $I(t)$, and hence $N(t)$, is a fluctuating quantity, and that part of the fluctuations are correlated in time. The setting of the clipping level close to $\{N(t)\}$ assures optimum detection of these fluctuations above and below the mean intensity. When a large particle traverses the laser beam, however, the scattering intensity may be increased by several orders of magnitude. At the very least, the intensity will be far above the clipping level. In such situations, correlation is lost [14, 15]. This occurs because $N_c(t)$ is virtually always unity when such particles are present in the beam, so that all photon counts that are then sampled are uniformly added to all 24 channels. Because of this suppression of the time dependence of the correlation, the heterodyne component, though present in principle, never appears, and the measured function is incorrect by an added constant.

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