Ultramicroscopy of association colloids formed from block copolymers

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Ultramicroscopy studies have been made of micelle formation by two poly(styrene)-poly(isoprene) block copolymers in organic solvents (N,N-dimethylacetamide and n-decane respectively) and a poly(L-glutamic acid)-poly(L-leucine) block copolymer in an aqueous solution of 0.2M NaCl at pH = 4.0. The technique provides a method of determining the number-average translational diffusion coefficient) $\bar{D}_n$, of association colloids and leads, via the Stokes–Einstein relation, to a measure of the number-average of the reciprocal hydrodynamic radius $(\overline{R_D^{-1}})_n$ for spherical particles. Particles having a radius less than approximately 30 nm were too small to be detected by the technique.

The ultramicroscopy results were compared with data obtained by laser light scattering photon correlation spectroscopy which provides a measure of the *z*-average translational diffusion coefficient. $\bar{D}_z$. An additional comparison was made by carrying out measurements on two well-characterized poly(styrene/divinyl benzene) latices.

INTRODUCTION

Under the most favourable conditions the resolving power of an optical microscope fitted with an oil-immersion objective is limited to one-third the wavelength of the light source. Thus for the middle part of the visible spectrum the least distance between two points which can still be perceived separately by this technique is 0.2 μm. Generally association colloids have dimensions much smaller than this and so cannot be studied by conventional optical microscopy.

However, using an arrangement termed an ultramicroscope, which was devised in 1903 by Siedentopf and Zsigmondy[1], it is possible to follow the movements of particles much smaller than the resolving power even though their actual geometrical size cannot be determined in the experiment. A strong beam of light is directed horizontally into the liquid containing the suspended particles and the vertically mounted microscope is focused on a region just below the surface. The observer sees bright spots of scattered light moving in a dark field, use being made of the fact that the diffraction image round a particle is considerably larger in size than the particle itself. For metallic particles which show strong scattering it is possible to observe particles down to 0.005 μm. For non-metallic particles on the other hand it is usually only possible to reach 0.02 μm which unfortunately rules out the study of micelles formed by synthetic surfactants and by many biochemical amphiphiles. Nevertheless the technique is particularly valuable for observing the random paths (Brownian motion) of organic particles 0.02 μm to 1 μm in diameter caused by uneven forces exerted on them by the molecules of the fluid medium. With present day light microscopes, ultramicroscopy studies may be carried out in a very convenient manner simply by replacing the condenser with one of special design, such as paraboloid or cardiod condenser. These condensers ensure that only scattered light enters the objective lens.

Recently we found that micelles formed by a number of block copolymers scattered sufficient light to be observable by ultramicroscopy. In this communication we report results for two polystyrene-polyisoprene block copolymers and for a poly(L-glutamic acid)-poly(L-leucine) block copolymer. The first polystyrene-polyisoprene copolymer, designated (1)/SI, was studied in N,N-dimethylacetamide which is a selectively bad solvent for polyisoprene. The micelles in this type of system are believed to consist of a compact swollen core of polyisoprene blocks surrounded by a fringe of polystyrene blocks[2]. In contrast the second polystyrene-polyisoprene copolymer, (2)/SI, was studied in n-decane which is a selectively bad solvent for polystyrene, and so in this case polystyrene blocks can be expected to be in the core and polyisoprene blocks to form the outer fringe. The poly(L-glutamic acid)-poly(L-leucine) copolymer, designated (1)/G–L, was studied in an aqueous solution of 0.2M NaCl brought to a pH = 4.0 with HCl. The polypeptide is amphiphilic consisting of one ionic block, poly(L-glutamic acid), and one hydrophobic block, poly(L-leucine). Like many other amphiphiles[3] this polypeptide, under suitable conditions in an aqueous medium, forms micelles consisting of a hydrophobic core surrounded by ionic groups.

Translational diffusion coefficients were determined for each system by ultramicroscopy and compared with values from laser light scattering photon correlation spectroscopy, p.c.s. A further check on the reliability of the ultramicroscopy procedure was provided by measurements made on two poly(styrene/divinyl benzene) lattices.

EXPERIMENTAL

Materials

The two polystyrene–polyisoprene copolymers were prepared by sequential anionic polymerization using benzene as solvent and sec-butyl lithium as primary initiator[4]. Each

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copolymer consisted of just two blocks – one polystyrene and the other polyisoprene. A small sample of homopolystyrene was isolated from each reaction system after the first stage of polymerization to facilitate characterization of the polystyrene blocks. Molecular characteristics of the copolymers determined from measurements in tetrahydrofuran (which is a solvent for both polymer components) were as follows:

Copolymer (1)/S-I	Total copolymer	Polystyrene block
$\bar{M}_w$ (g.p.c. and 'universal' calibration)/g mol^{-1}	170 000	85 000
$\bar{M}_w/\bar{M}_n$ (g.p.c.)	1.06	1.05
Copolymer (2)/S-I	**Total copolymer**	**Polystyrene block**
$\bar{M}_w$ (light scattering)/g mol^{-1}	54 000	12 700
$\bar{M}_w/\bar{M}_n$ (g.p.c.)	1.06	1.06

The weight % polystyrene in the copolymers as determined by ultraviolet spectroscopy of the copolymers in chloroform solution were 52 ± 3(1)/S-I) and 24 ± 3 ((2)/S-I) respectively. Before the investigations were carried out the solutions of copolymer (1)/S-I in DMA were filtered through 1.0 μm Millipore filters at 80°C and the solutions of copolymer (2)/S-I in n-decane were filtered through 0.25 μm Millipore filters at 55°C.

Poly(L-glutamic acid)-poly(L-leucine) block copolymer

Poly(γ-benzyl-L-glutamate), PBLG, was first synthesized by polymerizing γ-benzyl-N-carboxy-L glutamate anhydride in benzene at 20°C with n-hexylamine added as initiator[5,6]. The PBLG was fractionated using a procedure described previously[7] and then one of the fractions ($\bar{M}_w$ = 3.7 × 10^4 g mol^{-1}, and $\bar{M}_w/\bar{M}_n$ = 1.07) was used to initiate the polymerization of N-carboxy-L-leucine anhydride in benzene. The poly(γ-benzyl-L-glutamate)-poly(L-leucine) block copolymer obtained was fractionated by a successive precipitation method using chloroform and methanol as the solvent/non-solvent system. Finally one of these fractions was debenzylated using HBr in trifluoracetic acid solution to give the poly(L-glutamic acid)-poly(L-leucine) block copolymer sample used in the study. From proton nuclear magnetic resonance studies it was found that the number fraction of L-leucine residues in the copolymer was 0.29. Since for the fully debenzylated poly(L-glutamic acid) block, $\bar{M}_n$ = 2.0 × 10^4 g mol^{-1}, the copolypeptide was calculated to have $\bar{M}_n$ = 2.7 × 10^4 g mol^{-1} and the poly(L-leucine)block, $\bar{M}_n$ = 0.7 × 10^4 g mol^{-1}. Before use the solutions of the copolymer in the chosen solvent were centrifuged for half-hour at 10 000 rev/min; tests showed that very little of the polymer was lost from solution by this procedure.

Poly(styrene/divinyl benzene) latices

These were prepared by emulsion polymerization under conditions which led to particles which were spherical in shape and almost monodisperse in size[8]. Characterization by electron microscopy gave for the particles of latex 1, $\bar{M}_w$ = 5.3 × 10^9 g mol^{-1} and $\bar{M}_w/\bar{M}_n$ = 1.10 and for latex 2, $\bar{M}_w$ = 3.5 × 10^8 g mol^{-1} and $\bar{M}_w/\bar{M}_n$ = 1.09. The latices were filtered before use.

Ultramicroscopy

Successive displacements of colloidal particles in time intervals t (either 4 or 8 sec) were observed using an Olympus BHB optical microscope fitted with a condenser of the cardiod type. The microscope was positioned on a rigid surface in a thermostatted room. The sample was contained in a cell formed by the walls of a Teflon spacer which separated a microscope cover slip and slide. When sealing the cell care was taken to ensure (i) no air-bubbles were trapped in the solution and (ii) the cell was vapour tight to prevent solvent evaporation: when these points were not met drifts occurred making meaningful measurements impossible. The thickness of the cell (usually ≈0.1 mm) was chosen so that whilst good contrast was maintained between particles and background there was still sufficient space for free translational motion. The displacements were manually recorded with the aid of a drawing attachment which superimposed onto the microscope field the image of a marker point and recording paper. Time intervals were indicated by an electronic audio-timer.

In each experiment the mean-square value for N recorded displacements was determined.

$$\overline{d^2} = \left(\sum_{i=1}^{N} d_i^2 \right) / N$$

(where N was about 1000) and then the actual mean-square planar displacement, $\overline{l^2}$, of the colloid particles was calculated using a value for the magnification factor which had been established with the aid of a graticule.

Einstein[9] showed that the diffusion coefficient for the translation of particles through the suspending liquid is given by

$$D = \frac{\overline{l^2}}{4t} \tag{1}$$

He also proved that according to the molecular kinetic theory

$$D = \frac{RT}{fN_A} \tag{2}$$

where f is the friction coefficient of the particles, R is the gas constant and N_A is Avogadro's Number. If the particles are spheres of radius R_D then according to Stokes' law

$$f = 6\pi\eta R_D \tag{3}$$

where η is the viscosity of the solvent. Combining (1), (2) and (3) yields[9]

$$R_D = \frac{2RTt}{3\pi\eta\overline{l^2}N_A} \tag{4}$$

Equation (4) combined with optical microscopy measurements of $\overline{l^2}$ for monodisperse gamboge and mastic particles of known radii was one of the methods used by Perrin[10] to obtain one of the first reasonably accurate estimates of Avogadro's Number.

For a polydisperse system the ultramicroscopy technique yields a number-average diffusion coefficient.

$$\bar{D}_n = \frac{\Sigma N_i D_i}{\Sigma N_i} \tag{5}$$

Table 1 Translational diffusion coefficients and hydrodynamic radii for poly(styrene/divinyl benzene) latices (20°C)

	Ultramicroscopy		PCS		
	$(\bar{D}_0)_n/10^{-8}$ cm^2 s^{-1}	$(\overline{R_D^{-1}})_n^{-1}$/nm	$(\bar{D}_0)_z/10^{-8}$ cm^2 s^{-1}	$(\bar{D}_0)_n/10^{-8}$ cm^2 s^{-1}	$(\overline{R_D^{-1}})_n^{-1}$/nm
Latex 1	1.72 ± 0.07	124 ± 5	1.64 ± 0.03	1.76 ± 0.03	121 ± 2
Latex 2	4.2 ± 0.2	51 ± 2	4.2 ± 0.1	4.5 ± 0.1	47 ± 1

Table 2 Ultramicroscopy and photon correlation spectroscopy results for micelles formed from copolymer (1)/G-L in aqueous solution at 24°C and for copolymer (1)/S-I in N,N-dimethylacetamide at 25°C

	Ultramicroscopy		PCS	
	$(\bar{D}_0)_n/$ 10^{-8} cm^2 s^{-1}	$(\overline{R_D^{-1}})_n^{-1}/$ nm	$(\bar{D}_0)_z/$ 10^{-8} cm^2 s^{-1}	$(\overline{R_D^{-1}})_z^{-1}/$ nm
(1)/G-L	2.4 ± 0.1	–	2.30 ± 0.05	–
(1)/S-I	1.11 ± 0.04	185	0.81 ± 0.02	253

where N_i is the number of particles having diffusion coefficient D_i and radius R_{Di}. Hence in the case of spherical particles it leads to a determination of the number-average reciprocal hydrodynamic radius

$$(\overline{R_D^{-1}})_n = \frac{\Sigma N_i R_{Di}^{-1}}{\Sigma N_i} \tag{6}$$

Laser light scattering photon correlation spectroscopy

A 500 mw argon-ion laser (Coherent Radiation Model 529) was used as the light source (λ_0 = 488 nm) and scattered radiation was detected by a Mullard 56 AVP photomultiplier. The photocurrent was analysed by homodyne spectroscopy using a Malven Instruments Model 4300 digital correlator. The mean decay constant $\bar{\Gamma}$, which was determined for different angles in the range 15°–110°, was related to the average translational diffusion coefficient $(\bar{D})_{\text{pcs}}$ for the system by[11]

$$\bar{\Gamma} = 2(\bar{D})_{\text{pcs}} K^2 \tag{7}$$

The scattering vector

$$K = 4\pi \sin(\theta/2)/\lambda \tag{8}$$

where λ is the wavelength of the light source in the medium and θ is the scattering angle. In using equation (7) it is assumed that contributions to the linewidth from internal modes of motion and rotational motion are negligible. The average translational diffusion coefficient[12–14]

$$(\bar{D})_{\text{pcs}} = \frac{\Sigma D_i w_i M_i P_i(\theta)}{\Sigma w_i M_i P_i(\theta)} \tag{9}$$

where w_i is the weight of particles having molecular weight M_i, diffusion coefficient D_i and particle scattering factor $P_i(\theta)$.

For a polydisperse system in which all the particles are small compared with the wavelength of light the $P_i(\theta)$'s = 1. Hence the z-average diffusion coefficient $\bar{D}_z$ may be obtained from the slope of the plot of $\bar{\Gamma}$ versus $2K^2$. If the system also contains particles of the order of the wavelength of light for which $P_i(\theta) \neq 1$ except at $\theta = 0$ it becomes necessary to determine the limiting slope of the plot as $K \to 0$ to obtain $\bar{D}_z$. From $\bar{D}_z$ for spherical particles the z-average reciprocal hydrodynamic radius

$$(\overline{R_D^{-1}})_z = \frac{\Sigma R_{Di}^{-1} w_i M_i}{\Sigma w_i M_i} \tag{10}$$

may be calculated using equations (1) and (4).

RESULTS AND DISCUSSION

Translational diffusion coefficients determined by ultramicroscopy, $(\bar{D}_o)_n$, and p.c.s., $(\bar{D}_o)_z$, for the colloid particles of the two latices are given in *Table 1*. In the p.c.s. measurements were made at four concentrations over the range $c = 0.5 \times 10^{-4} \to 5 \times 10^{-4}$ g cm^{-3}: there was found to be no significant dependence of the diffusion coefficient on concentration. The ultramicroscopy measurements were made at $c = 2 \times 10^{-6}$ g cm^{-3}. The z-average values from p.c.s. were converted to number-average values by using distributions of particle sizes determined from electron microscopy. Comparison of these number-average values (column 6) with those from ultramicroscopy (column 3) shows that two techniques yield results in good agreement. The hydrodynamic radii, $(\overline{R_D^{-1}})_n^{-1}$, given in *Table 1* were determined from the diffusion coefficients using equations (2) and (3).

Results for copolymer (1)/S-I in DMA at 25°C and for copolymer (1)/G-L in an aqueous solution of 0.2M NaCl (pH = 4.0) at 24°C are given in *Table 2*. For both samples no significant dependence of $\bar{D}_z$ on c was detectable, measurements being made up to $c = 5 \times 10^{-4}$ g cm^{-3}. At the temperatures chosen for the studies we believe that both copolymers are almost completely in the micelle form i.e. if we consider micellization can be treated in terms of an association[15]

$$NP_1 \rightleftharpoons P_N \tag{11}$$

where P_1 and P_N are the free-chain and micelle forms, then the position of equilibrium lies overwhelmingly to the r.h.s.

For copolymer (1)/G-L the ratio $(\bar{D}_0)_n/(\bar{D}_0)_z = 1.04$. This indicates the micelles have a fairly narrow distribution of particle sizes. Ultramicroscopy showed, however, that the micelles were not spherical since the light they scattered was observed to fluctuate in intensity. Ellipsoidally shaped micelles would seem to be the most likely explanation of this effect, although other shapes cannot be ruled out since any anisotropic particle would appear to 'twinkle' provided the rotation was not too rapid. Conventional light scattering studies on the micelles from (1)/G-L gave $\bar{M}_w = 2.0 \times 10^9$ g mol^{-1}.

For copolymer (1)/S-I ultramicroscopy revealed a distribution of micelle sizes since the points of light had different intensities. However, the micelles did not 'twinkle' which suggested that they had a spherical shape; the average hydrodynamic radii of the micelles given in *Table 2* were calculated using equations (1) and (4). The polydispersity in size is confirmed by the ratio $(\bar{D}_0)_n/(\bar{D}_0)_z = 1.37$. Additional evidence is provided by the electron micrograph given in *Figure 1* which was obtained for a specimen that had been isolated from solution by a recently developed 'spreading-drop'procedure[2].

The micelles shown in *Figure 1* were stained with osmium tetroxide which selectively reacts with olefinic bonds of the polyisoprene and serves to enhance contrast between the two types of blocks; thus polyisoprene appears black and polystyrene grey. On final evaporation of the solvent during the specimen preparation[2] the micelles collapse to form particles having a density similar to that of bulk polymer. The shapes are no longer spherical because the polymer particles tend to flow and wet the surface. Because of this distortion no attempt was made to estimate quantitatively the molecular weight characteristics from the micrographs. Nevertheless, it is clear from *Figure 1* that there is a distribution of micelle sizes. A possible source of this polydispersity could be non-attainment of thermodynamic equilibrium. Further studies will be required in order to investigate the point. However,it is worth pointing out that recent studies by us of a polystyrene-polyisoprene block copolymer of lower molecular weight, in which thermodynamic equilibrium was clearly established, formed micelles which were essentially monodisperse in size.

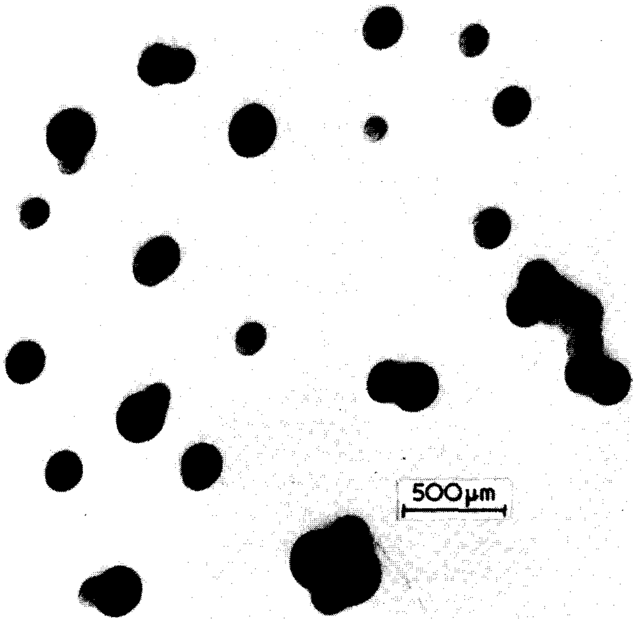

Figure 1 Electron micrograph of collapsed micelles isolated by a spreading-drop technique from micelle solutions of the polystyrene-polyisoprene copolymer in DMA. The specimen was stained in solution with OsO_4 and shadowed on the carbon substrate with C/Pt. (The clumping of the particles occurs during the drying stage[2].) The scale mark is 500 nm

In the case of copolymer (2)/S-I in decane at 23.5°C conventional light scattering studies indicated that both micelles and free-coils were present in significant amounts; studies made at 6.5°C where the micelle form was definitely favoured gave $\bar{M}_w \simeq 2.0 \times 10^6$ g mol^{-1}. Unfortunately, the micelles scattered insufficient light to be detected by ultramicroscopy. A small number of colloid particles were observed in the solution, but because of their size it was concluded these consisted of clusters of micelles. The clusters appeared to be spherical in shape but polydisperse in size. The translational diffusion coefficient of the clusters, their average hydrodynamic radius and number density in the solution are given in *Table 3*. The data show that as the copolymer concentration increases both the hydrodynamic radius and number density of the clusters increases. The clusters are probably either compact coil-like micelles formed by stringing together spherical micelles or simply globular associates of spherical micelles. The z-average translational diffusion coefficients determined at several concentrations by P.C.S. are also given in *Table 3*, but they cannot be meaningfully interpreted at this stage since they contain contributions from three types of species: free coils, micelles and clusters.

From the results reported in this paper it is clear that ultramicroscopy can play an important role in studies of non-ionic micelles. In favourable circumstances it provides a measure of the number-average diffusion coefficient of the micelles (provided $R > 30$ nm) and in general is a sensitive method for studying their colloidal stability. In work currently underway we are using ultramicroscopy to investigate a micelle/free-coil transition. The technique is proving particularly useful since it enables us to determine the number density of micelles as a function of concentration and temperature without significantly disturbing the system. The results of this study together with other applications of ultramicroscopy in this field will be reported in due course.

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Table 3 Ultramicroscopy and PCS results for copolymer (2)/S-I in n-decane at 23.5°C

Ultramicroscopy				PCS	
$c/10^{-4}$ g cm^{-3}	$\bar{D}_n/10^{-8}$ cm^2 s^{-1}	No. density/10^7 cm^3	$(\overline{R_{\bar{D}}^{1}})_n^{-1}$/nm	$c/10^{-4}$ g cm^{-3}	$\bar{D}_z/10^{-8}$ cm^2 s^{-1}
87	0.96 ± 0.04		252	41.6	8.0 ± 0.2
43	1.11 ± 0.04		218	29.9	7.3 ± 0.2
27	1.65 ± 0.07	14.3	146	24.9	7.5 ± 0.2
19	1.76 ± 0.07	12.5	137	9.9	7.1 ± 0.2
16	2.23 ± 0.09	8.4	108	8.9	7.0 ± 0.2
10	2.52 ± 0.10	5.1	96		
6	2.61 ± 0.10	1.7	92		

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