Amphiphilic Properties of a Polymerized Glycolipid Surfactant

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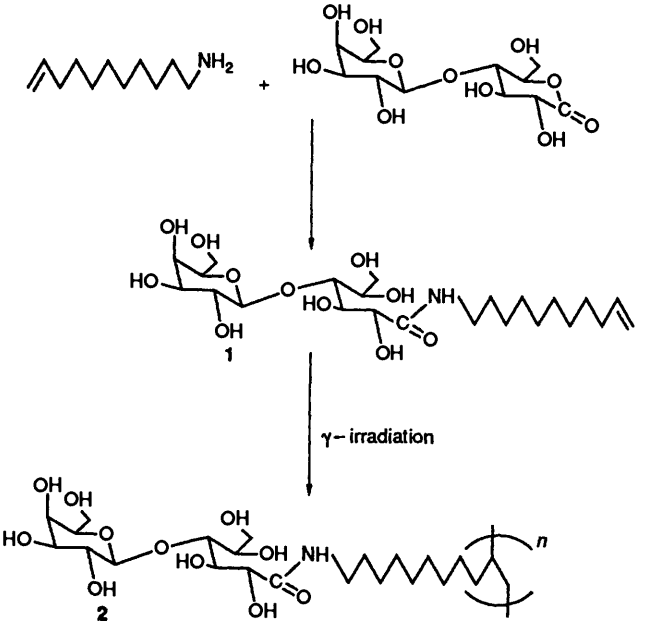
N-(Undec-10-enyl)lactobionamide was synthesized and polymerized in aqueous solution by γ-irradiation of micellar solutions, leading to the polymer. The critical micellar concentration (cmc) of monomer compound was determined by surface tension measurements (3.2×10^{-3} mol dm^{-3}); we could not detect any cmc with the polymer. The structure of the monomer and of the polymer were characterized using quasi-elastic light scattering (QELS), transmission electron microscopy (TEM) and freeze–fracture electron microscopy (FFEM). The data obtained for the polymer are in agreement with it consisting of spherical aggregates. We have investigated the micellar properties of the monomer as well as those of its corresponding polymer through the study of the solubilization of highly hydrophobic compounds and of the base-catalysed hydrolysis of hydrophobic esters. Comparative studies showed better efficiency of the polymer compared with the monomer analogue.

The membrane recognition mechanisms as well as the potentialities offered by natural or synthetic macromolecular systems as drug carriers have been widely investigated in recent years. Among the various transport systems proposed – monoclonal antibodies, glycoproteins, neoglycoproteins, microspheres, nanoparticles, synthetic macromolecular carriers or liposomes[1–8] – particular attention has been paid to polymerizable analogues of some cell-membrane components, *i.e.* phospholipids or glycolipids bearing on their hydrophobic moiety a polymerizable unit (acryloyl, vinyl, butadienyl, diacetylenyl,[9] *etc.*). Indeed, the presence of such a polymerizable group allows the preparation of ill-defined polymerized species which may show micellar properties even at very low concentration. Although inadequate, the term 'polymerized micelle' is often used to describe these macromolecular species. Owing to their ability to trap drugs in the 'micelles' they are useful not only as carriers but also as a means of targeting cells, since they can be selectively recognized by membrane lectins when the hydrophilic moiety of the surfactant is constituted by the appropriate sugar.

Larrabee[10] and Holladay[11,12] have shown that polymerized ionic micelles are able efficiently to solubilize drugs, steroids or various peptides. Similarly, micellar aggregates obtained from *N*-(*p*-vinylbenzyl)glucosamide were shown to solubilize organic compounds such as methyl orange as well as to bind concanavaline A.[13,14] In this work, we report preliminary results dealing with the potentiality of micellar aggregates obtained by polymerization of *N*-(undec-10-enyl)lactobionamide **1** as a drug carrier, and for cell targeting.

Results and Discussion

The method described by Williams *et al.*[15,16] for the synthesis of model glycolipids has several advantages: (i) the starting material, the glyconolactone, is readily obtained by oxidation of a reducing sugar; (ii) reaction with an aliphatic amine affords a condensation product in good yield without protection of the carbohydrate moiety. This process was used to synthesize compound **1** (according to Scheme 1).



Scheme 1

Polymerization was performed according to the method of Larrabee[10] by 100 kGy γ-irradiation of 0.1 mol dm^{-3} aq. **1** in a 1.5 kGy h^{-1} ^{60}Co γ-ray source. The progress of the polymerization as a function of the γ-ray dose was monitored by ^{1}H NMR spectroscopy. Evidence for the structure of the poly-[*N*-(undec-10-enyl)lactobionamide] **2** was provided by comparison of ^{1}H NMR spectra of the monomer and the polymer. The only major change which accompanied polymerization was the disappearance of the vinyl proton signal and the broadening of the remaining peaks (Fig. 1)

Critical micellar concentrations (cmc) of surfactants were obtained by two different methods. The dependence of the surface tension (mN m^{-1}) *versus* concentration is shown for both the monomer **1** and the polymer **2** in Fig. 2. Only the

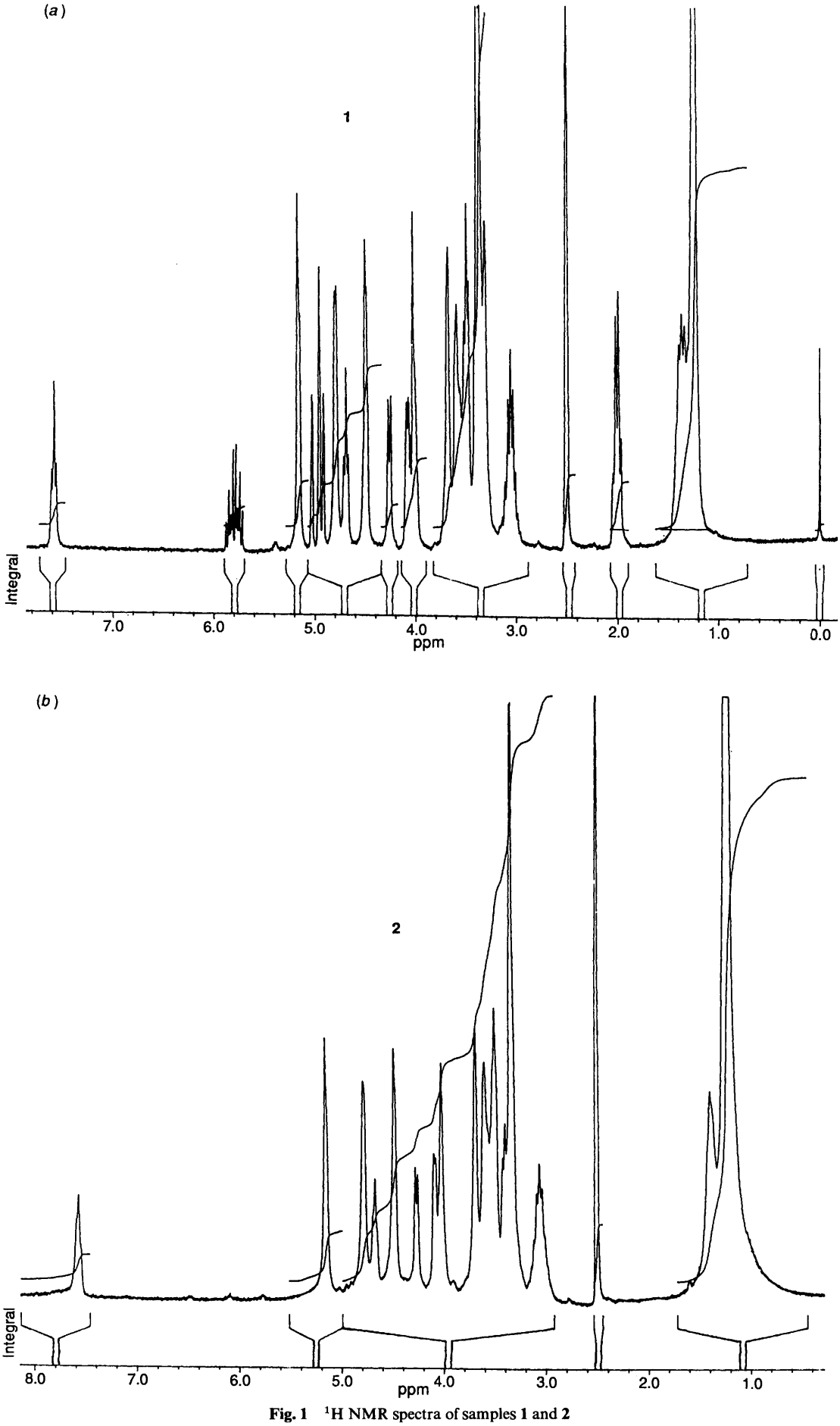


Fig. 1 ^{1}H NMR spectra of samples **1** and **2**

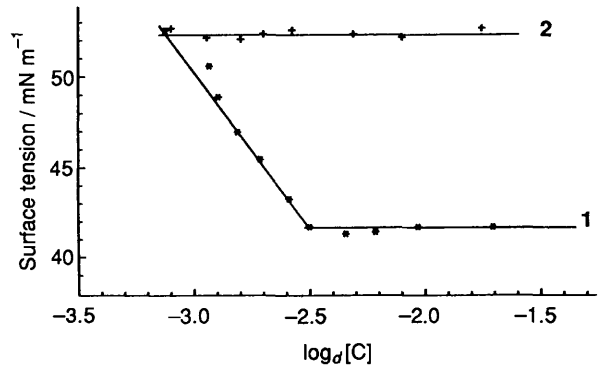


Fig. 2 Concentration dependence of the surface tension of compounds **1** and **2** in solution at 25 °C

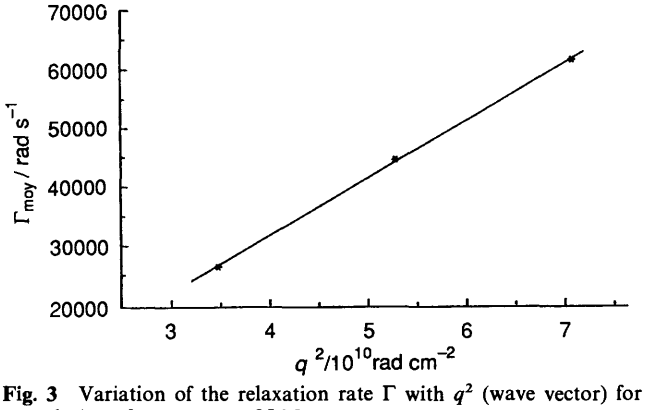


Fig. 3 Variation of the relaxation rate Γ with q^2 (wave vector) for aq. solution of monomer at 25 °C

monomer **1** exhibits a break point, characteristic of micellar behaviour: its cmc was found to be 3.2×10^{-3} mol dm^{-3}. On the other hand, as the concentration of polymer **2** was increased from very low values to 0.1 mol dm^{-3}, no significant variation of the superficial tension was detected.

Furthermore, similar results were obtained when pinacyanol chloride was used as a probe according to the procedure of Menger and Portnoy:[17] no cmc could be measured with the polymeric species.

Nevertheless, it would be difficult, from these data, to predict if polymerized micelles or other non-micellar structures are obtained. Indeed, the question is whether the lifetime of a micelle is sufficiently long compared with the lifetime of the growth macroradical chain in the micelle so that polymerization can take place before the micelle dissociates. The current information about lifetimes of micelles and growth of macroradical chains does not allow us to answer this question clearly. Sherrington[18] has reported that polymerization of the micellar structure can only occur if the propagation constant and the rate of dissociation of a monomer are roughly of the same magnitude range; according to this author, those conditions are difficult to obtain. However, these results concern only acrylic ionic surfactants, the polymerization being induced by light irradiation.

On the other hand, Nagai[19] claimed that the polymerization of a styryl non-ionic surfactant by azo initiation led to polymerized micelles, although propagation constants of styryl monomers are much lower than those of acrylic ones.[20] So, it seems difficult to be able to predict the type of topochemical polymerization which occurs with our vinylic non-ionic surfactant under γ-irradiation. Only quasi-elastic light scattering (QELS) and transmission electron microscopy (TEM) should provide an answer.

Fig. 3 shows the variation of Γ *versus* q^2, measured by QELS, for the non-polymerized surfactant **1**. The slope of the plot gives the mutual diffusion coefficient D, calculated by linear regression procedure to be $D = (9.7 \pm 0.2) \times 10^{-11}$ m^2 s^{-1}, with a correlation coefficient $r = 0.9996$, corresponding to an apparent R_H-value of 25 Å. The mean aggregation number (N) of monomer **1** can be approximated from the relation

$$N = V_H/V_a \quad (1)$$

where V_H is the hydrated micelle volume and V_a the amphiphilic molecule volume, respectively. The value of N was found to be in the range 60–80, corresponding to a total molecular weight of ~30 000.

Since a very pronounced increase of the micellar size with time[21] was observed in the case of a similar glycolipid surfactant, we decided to duplicate these measurements after several weeks with the same solutions kept at room temperature. The hydrodynamic radius was not affected (R_H = 27 Å), indicating that no micellar growth had occurred.

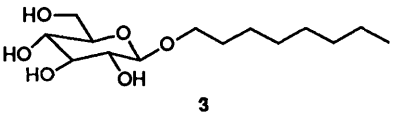
The experimental parameter (the mutual diffusion coefficient) is dependent on the size of the aggregates and on the interactions between aggregates. As the interactions (repulsion ones) affect the mutual diffusion coefficient by increasing its value as the concentration is increased, the experimental D-value is greater than the mutual diffusion coefficient measured, for example, at the cmc where no interactions occur in the medium. Consequently, the apparent R_H-value obtained from D by the Stokes–Einstein equation is underestimated.

These results indicate that the micellar size is small with respect to the wavelength of the light. The good correlation coefficient suggests that these species are monodisperse.

When the same measurements were performed on the polymer solution, it was not possible to obtain an exponential decrease of the autocorrelation function and the scattered intensity was always very weak. As the apparent radius of the polymeric aggregates is an important parameter, we decided to apply TEM analysis to the polymeric solution.

Fig. 4 shows typical TEM photographs of sample **2** in aq. solution. We can observe the formation of spherical aggregates disposed into clusters. The radius determined, 180 Å, is much larger than that of monomeric micelles. Such a size can explain the difficulties encountered when attempting to detect these species by QELS. The above results suggest that the relaxation time of micelles should be slightly lower than the lifetimes of the growth macroradical chains. Furthermore, we checked the size and the shape of the polymeric surfactant aggregates by means of freeze-fracture electron microscopy (FFEM). The micrographs are reported in Fig. 5. The poly-[*N*-(undec-10-enyl)lactobionamide] **2** gels were shown to consist of monodisperse and spherical (or globular) particles of 180 Å radius, in agreement with TEM results.

In order to substantiate observations related to polymerized species, we have undertaken a comparative study of the micellar properties (additive solubilization, micellar catalysis) of both the monomeric and polymeric surfactants. Solubilizing power with respect to a highly hydrophobic substrate in aq. solutions, and catalytic activity using base-catalysed hydrolysis of an ester as a probe, were determined. The influence of these surfactants on the water solubility of sparingly soluble 3-acetylphenanthrene (0.023 mmol dm^{-3}) is represented in Fig. 6. For the sake of comparison we achieved the same studies with a non-ionic detergent, the octyl β-D-glucopyranoside **3**, widely used for the solubilization and purification of hydrophobic membrane proteins.[22]



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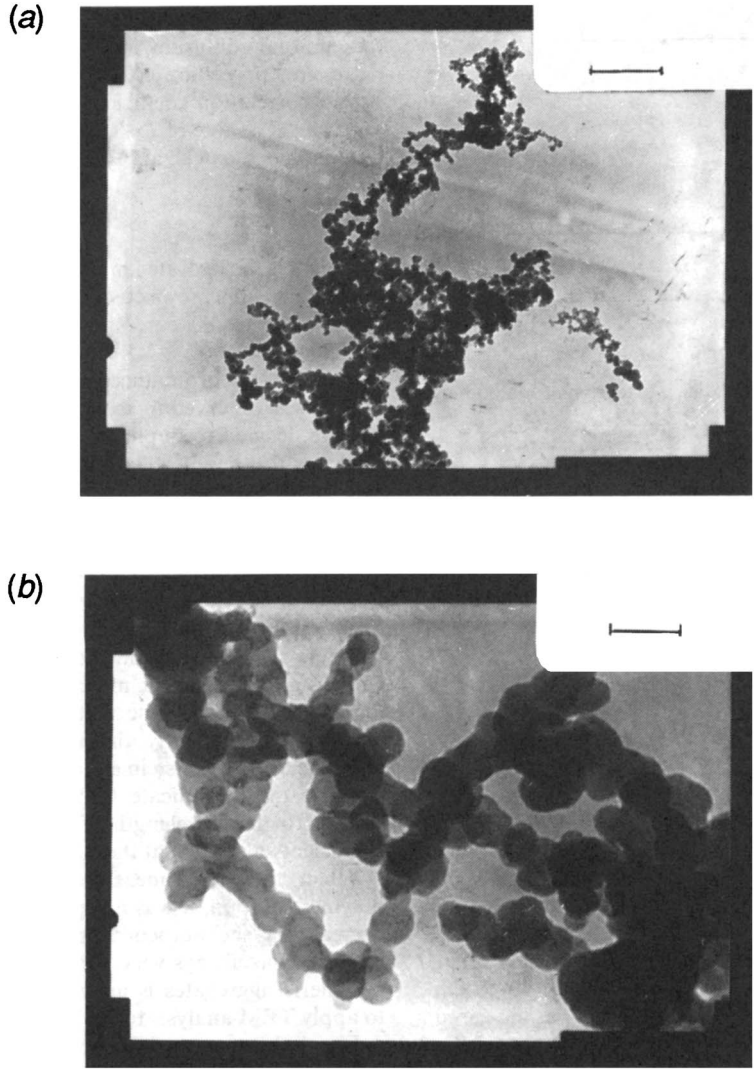

Fig. 4 TEM photographs of an aq. solution of compound **2** (3×10^{-3} mol dm^{-3}). (*a*) 20 000 magnification (scale 500 nm); (*b*) 140 000 magnification (scale 70 nm).

Data presented in Fig. 6 call for several comments. (i) While the solubilizing power of the monomer appears at concentrations superior to cmc, the polymerized analogue increases the solubility of 3-acetylphenanthrene at very low concentrations (< 1 mmol dm^{-3}); (ii) with the polymeric surfactant, a break appears in the plot *S versus* [surfactant] at 20 mmol dm^{-3} (the concentration of the polymer is expressed in terms of lactobionamide units). The break could result from a polymerized micellar aggregation; this behaviour has already been observed with ionic polymerized micelles;[23] (iii) in both cases and for high surfactant concentrations, the solubility exhibits linear behaviour. Moreover, whatever the concentration, the polymer **2** is still more efficient than the monomer **1**. For a 100 mmol dm^{-3} concentration, the monomer **1** produces a 260-fold increase in the solubility compared with that observed in pure water, while this enhancement reaches 490 for the polymer **2**. Finally, we note that, within the same concentration range, the solubilizing power of glycoside **3** appears at concentration above its cmc-value (25 mmol dm^{-3} [24]), and remains inferior to those of compounds **1** and **2**. Hence it would appear that polymerization favours interactions between the solute and the micellar aggregate. This property suggests that the polymer is more prone to promote catalytic activity than is the monomeric analogue.

In order to elucidate the contribution of polymerization to the catalytic effect observed with non-polymeric micelles, we studied the base-catalysed hydrolysis of 4-nitrophenyl hexanoate. As with solubilization, we have included octyl β-D-glucopyranoside **3** in our studies. In our comparative study rate constants for compounds **1**, **2** and **3** at different concentrations are reported in Fig. 7.

Data obtained with compound **1** give the normal plot with a catalytic effect appearing only for concentration above the cmc and reaching a plateau for a concentration roughly equal to 10 mmol dm^{-3}. For this concentration, we expect the substrate to be fully adsorbed within the micelles.

In contrast, with the polymer **2**, the catalytic effect appears at lower concentrations and increases up to a maximum. In all cases the maximum catalytic activity is superior to that observed for the monomer **1**. This can be seen in the k_ψ values ($k_\psi = k_2/k_{H_2O}$). When the maximum is reached, $k_\psi = 16$ with the polymer and 11 with the monomer. In addition, no time-dependent micellar properties were found for compounds **1** or **2**. Reproducible results were obtained with solutions either freshly prepared or stored several weeks at room temperature when base-catalysed hydrolysis of ester was performed.

Finally, it is interesting to note that octyl β-D-glucopyranoside **3** does not exhibit catalytic activity under the same conditions since its cmc-value (25 mmol dm^{-3}) is larger than

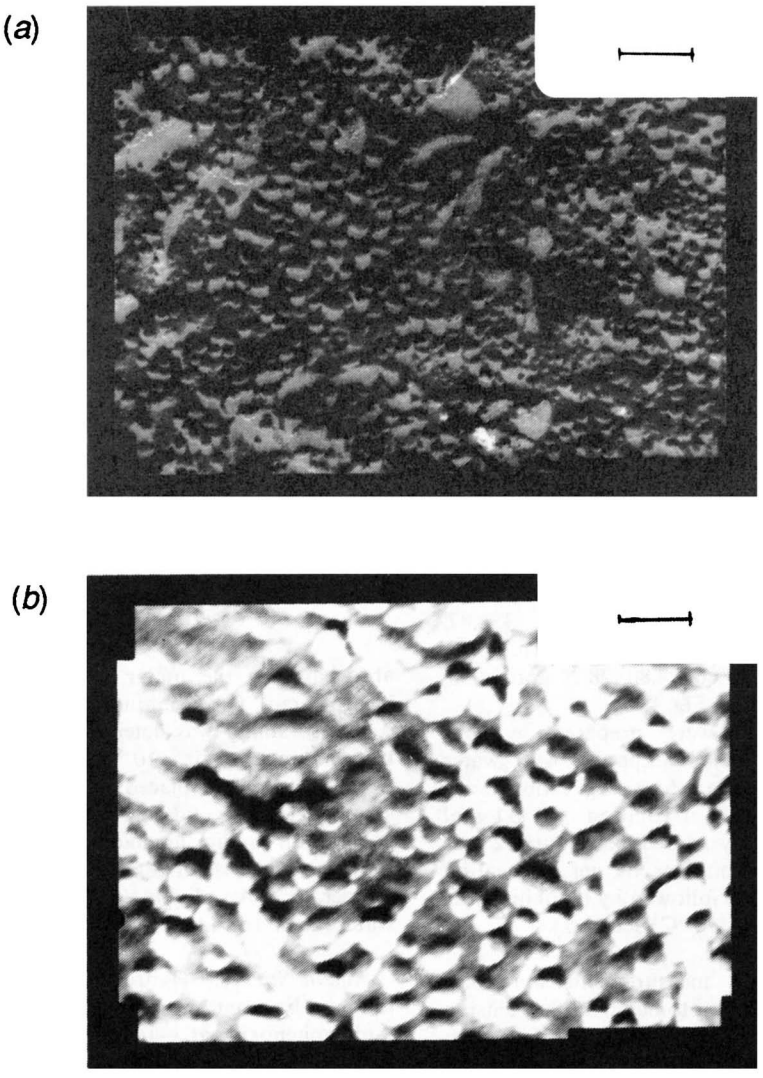

Fig. 5 Freeze-fracture electron micrographs of an aq. gel of polymer **2** (3×10^{-3} mol dm^{-3}). (*a*) 100 000 magnification (scale 100 nm); (*b*) 200 000 magnification (scale 50 nm).

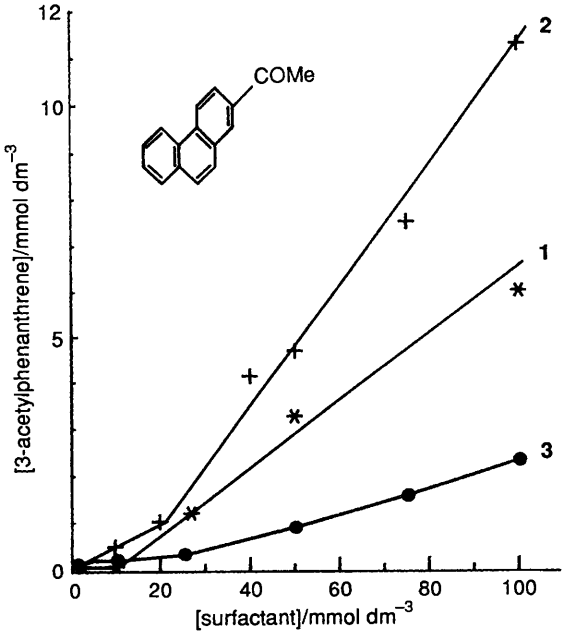

Fig. 6 Aq. solubility of 3-acetylphenanthrene in the presence of surfactants **1**, **2** and **3** at various concentrations

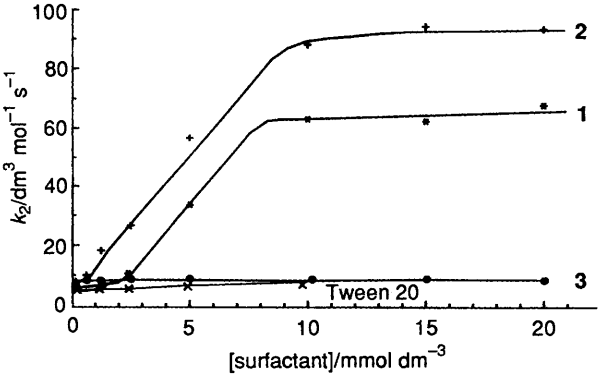

Fig. 7 Catalytic effect as a function of the concentrations of surfactants **1**, **2** and **3** in the hydrolysis of 4-nitrophenyl hexanoate at 25 °C

the concentration range used in this kinetic study (0–20 mmol dm^{-3}).

On the other hand, Tween 20 (cmc 5×10^{-5} mol dm^{-3}), a non-ionic, currently used surfactant, exhibits no catalytic activity in the same range of concentrations. It is possible that the numerous hydroxy groups present in the hydrophilic head of compound **1** or **2** play an important role in the stabilization

of the negatively charged transition state of the reaction, probably by hydrogen bonding.

In conclusion, we have shown that polymerized micelles provide a favourable microenvironment for the solubilization of hydrophobic solutes and for the catalysis of base-catalysed ester hydrolysis. Our current investigations deal with the assessment of the ability of such polymerized micelles to be recognized by appropriate membrane lectins. The latter aspect is a crucial step for the utilization of such polymeric species as drug carriers and for cell targeting. Complementary studies are in progress in our laboratory.

Experimental

Materials.—Undec-10-enylamine was prepared from undec-10-en-1-ol by treatment of the tosyl derivative with sodium azide, followed by reduction with sodium borohydride.[25] Aminolysis of lactobionolactone, derived by dehydration of lactobionic acid,[15] with undec-10-enylamine in hot methanol afforded *N*-(undec-10-enyl)lactobionamide **1** in 90% yield. Purification by recrystallization from methanol–diethyl ether gave pure monomer; m.p. 156–157 °C; $[\alpha]_D$ +32.5 (*c* 1 in water); δ_C[250 MHz; $(CD_3)_2SO$; Me_4Si] 172.06, 138.87, 114.65, 104.62, 82.96, 75.74, 75.28, 72.02, 71.48, 71.16, 70.50, 68.27, 38.34, 33.19, 29.23, 28.88, 28.86, 28.80, 28.29 and 26.38; δ_H[250 MHz; $(CD_3)_2SO$; Me_4Si] see Fig. 1.

Solutions for polymerization were prepared, without sonication, under nitrogen in sealed ampoules with water degassed and saturated with nitrogen before preparation.

After polymerization by 100 kGy irradiation of a 0.1 mol dm^{-3} aq. solution in a ^{60}Co γ-ray source, compound **2** was separated from the remaining monomer by gel permeation chromatography on Sephadex G50 followed by lyophilization; $[\alpha]_D$ +23° (*c* 0.5 in water); m.p. > 170 °C (decomp.).

Physical Measurements.—NMR measurements were performed on a Bruker AC 250 spectrophotometer. Surface tension measurements were made at 25 °C using a Tensimat–Densimat TD 2000 by application of the Wilhelmy-plate method. QELS measurements were carried out at 25 °C using an argon-ion laser operating at 5145 Å (Amtec MM 100 spectrophotometer) on a monomeric aq. solution (2.263×10^{-2} mol dm^{-3}). The scattered light was collected at a variable scattering angle. The time-averaged intensity and time-dependent correlation function of the scattered intensity were recorded on a BI-2030 autocorrelator. The autocorrelation function of the photoelectric current of the photomultiplier was analysed using the cumulant method[26,27] [equation (2)] where Γ is the

$$\Gamma = Dq^2 \qquad (2)$$

relaxation rate, $q = (4\pi n/\lambda_0) \cdot \sin(\theta/2)$ is the wave vector, n is the index of refraction of the scattering medium, λ_0 the wavelength of the incident light, and θ the scattering angle. *D*-Values can be used for the calculation of the hydrodynamic radius R_H, from the Stokes–Einstein relation [equation (3)] for

$$R_H = kT/6\pi\eta D \qquad (3)$$

spherical particles where η is the coefficient of viscosity of the solvent, k is Boltzmann's constant and R_H is only an apparent hydrodynamic radius.

TEM observations were carried out on a JEOL JEL CX 2000 transmission electron microscope. The accelerating voltage was 100 kV. We used carbon grids covered with a Formvar film (Pelanne Instruments). These grids were prepared in two ways: (i) casting a drop of a dilute solution (3×10^{-3} mol dm^{-3} in water) on the grid; (ii) plunging the grid in the same solution. After drying at room temperature under atmospheric pressure, the grids were observed by TEM with no supplementary preparations (colouration, carbon film, *etc.*).

Freeze-fracture experiments were conducted with a Reichert cryofract 180 at −150 °C under low pressure (10^{-7} mmHg) on samples prepared in two metallic cups from a polymeric aq. solution (3×10^{-3} mol dm^{-3}). Replicas were obtained by shadowing the specimen with platinum/carbon at different angles. After being washed, freeze-fracture replicas were observed by TEM.

Cmc determinations were carried out at 25 °C according to the procedure of Menger and Portnoy,[17] following the absorbance at 610 nm of a pinacyanol chloride solution (9×10^{-6} mol dm^{-3}) in the presence of different surfactant concentrations.

Solubility Studies.—3-Acetylphenanthrene was purified by crystallization and the UV spectrophotometric study was performed at 252 nm (ε 17 000 dm^3 mol^{-1} cm^{-1} in MeOH) with a Gilford 250 spectrophotometer.

The solubility (*S*) is expressed by equation (4) where *A* is the

$$S = (A/\varepsilon l) \cdot (V_T/V_A) \qquad (4)$$

absorbance, ε the molar extinction coefficient, l the optic length (1 cm), V_A the aliquot volume and $V_T = V_A + 2$ cm^3.

The solubility was determined within a surfactant concentration range of 10^{-3}–10^{-1} mol dm^{-3} in the aq. phase. In a 2 cm^3 flask were placed aq. solution (water or surfactant solution) (1 cm^3) and ketone (50 mg). The mixture was stirred gently until equilibrium had been reached (this is attained when the absorbance, *A*, remains constant) and was then kept at room temperature. An aliquot (V_A; 50–200 mm^3) was diluted with water (2 cm^3) and the adsorbance (*A*) was measured.

Kinetic Studies.—Hydrolysis of 4-nitrophenyl hexanoate was followed by monitoring of the appearance of the corresponding 4-nitrophenoxide at λ 400 nm. A stoppered 10 mm cuvette was filled with buffered solution (1 cm^3) at pH 10 (carbonate buffer); micellar solution (1 cm^3) (monomeric or polymeric) and 10^{-3} mol dm^{-3} ester solution in acetonitrile (20 mm^3). The solution was stirred and the cuvette was placed in the thermosttated cell holder (25 ± 0.1 °C) of a Gilford 250 spectrophotometer. First-order plots were linear to greater than 80% of the reaction. The observed rate constants (k_{exp}) were evaluated from the adsorbance–time data by use of a least-squares computing method. Each rate-value is the mean of at least three independent determinations differing by less than 3%.

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