Hydrophobically Modified Pullulans: Characterization and Physicochemical Properties

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Pullulan was hydrophobically modified by reaction with dodecanoic acid, modulating the polymer-to-acid ratio. The resulting products, termed PULAU$_N$, have been characterized, and their physicochemical properties in aqueous solution, at 25 °C, are reported. The hydrophobic substitution was determined by Fourier transform infrared, FTIR, and NMR analyses. NMR also quantifies the substitution degree, *N*. Viscosity, surface tension, quasi-elastic light scattering, QELS, and gel permeation chromatography, GPC, methods were used to characterize the solution properties of PULAU$_N$. Depending on the polymer concentration and *N*, PULAU$_N$ form micelle-like aggregates. The critical micelle concentration, cmc, decreases with increasing *N*. Addition of sodium dodecyl sulfate, SDS, results in the formation of mixed micelles. In the investigated concentration range, no polymer−surfactant-based gels are formed by mixing PULAU$_N$ and SDS. According to NMR self-diffusion and QELS findings, the number-average aggregation numbers of PULAU$_N$ micelles, $\langle Z \rangle$, depend on the concentration and substitution degree. Large $\langle Z \rangle$ values are observed in the more substituted PULAU$_{5.0}$. A very slow kinetics of adsorption at the air−solution interface (some days long) is observed for PULAU$_{5.0}$. The effect, observed at concentrations below the cmc, is modulated by the concentration of hydrophobically modified polymer. The kinetics of adsorption is related to the combination of different effects. A thermodynamic model was developed to account for the above behavior. The process was rationalized by taking into account terms due to the surface spreading, self-diffusion, and elastic properties of the PULAU$_{5.0}$ chains.

Introduction

Much interest is actually devoted to investigate mixtures containing polymers and surfactants.[1,2] Studies on polymer−surfactant systems, PSS, try to understand the physicochemical aspects of the macrocolloids formed by mixing intrinsic and association colloids. The associative and dissociative interaction mechanisms,[3] the surfactant binding onto the macromolecule-(s),[4] and the structural modeling of polymer−surfactant, PS, aggregates,[5] in particular, are the subjects of significant interest.

Polymer−surfactant mixtures have technological applications as viscosity modulators and solvents and/or adsorbents in food sciences. Other uses of them are in paint industries, cosmetics, and biomedical applications.[6,7] Hence, their solvent capacity, rheological properties,[8] and surface activity[9,10] are systematically investigated.

Polymers link amphiphilic molecules by different interaction mechanisms.[11−13] In some instances, the polymers act as binding sites for surfactant molecules. Hydrophobically modified polymers, HMPs, in particular, undergo inter- and/or intra-supramolecular association and may form interconnected networks by changing the concentration of polymer and/or surfactant. Micelles, gels, and organized solutions will be formed.[14,15] The polymer concentration, added surfactant, temperature, pH, and ionic strength modulate the association mechanisms, giving rise to a wide class of soft materials, interesting for technological applications.[16,17]

Interest is currently focused on HMPs obtained from natural sources, to have biocompatible and low cost materials. Polysaccharides, in particular, have physicochemical properties strongly related to the nature and number of functional groups on the polymer chain.[18] Their solubility in water and interactions with surfactants change significantly, depending on the polymer architecture, functionalization degree, and nature of the functional groups.[19,20] Gels, solutions, precipitates, and organized phases are formed by mixing HMPs with other polymers, surfactants, or lipids.[21−25]

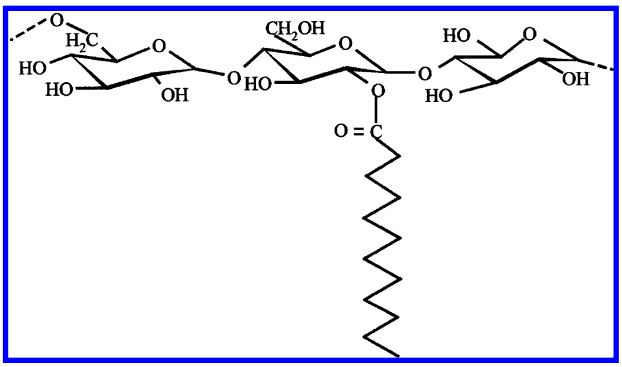

Figure 1. Schematic picture of pullulan hydrophobically modified by dodecanoic acid. The final products contain, on average, 2.5 or 5.0 alkyl groups per 100 sugar units. Ester linkage by dodecanoic acid is not specific to a given position.

In this contribution, we report on hydrophobically modified pullulans obtained by reaction with dodecanoic acid. Some derivatives were prepared and investigated, and the synthetic procedures are reported. The products, termed PULAU$_N$ (where *N* indicates the effective percent substitution degree with respect to the glucose units, PU, the polysaccharide, and LAU, the acyl group), differ in hydrophobic character (Figure 1).

We present and discuss some physicochemical properties of PULAU$_N$ in aqueous solution, at 25 °C. For this purpose, viscometric, NMR, quasi-elastic light scattering, QELS, Fourier transform infrared, FTIR, and surface tension experiments were

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performed. Additional studies investigate whether $PULAU_N$ interact with surfactants. The critical micelle concentration, cmc, in mixed SDS−$PULAU_N$ systems has been determined. In addition, the partial phase diagram of the water−$PULAU_{2.5}$−SDS system, at 25 °C, has been drawn.

Apart from heuristic motivations, interest in the above compounds is oriented toward biochemical applications.[26,27] Polysaccharides similar to $PULAU_N$ cover cell membranes and are, presumably, responsible for their sensitivity toward antibiotics.[28] Hence, $PULAU_N$ can coat vesicles similar to the cells to be destroyed and may be used in the drug selective release. Another possibility is using $PULAU_N$ in DNA transfection technologies.[29] Pullulan derivatives are also used in the preparation of foamlike solids for the implantation of biocompatible tissues.[30]

Experimental Section

Materials. F20 pullulan, from Hayashibara Co. Ltd, is a microbial polysaccharide obtained from *Aureobasidium pullulans*. It has a weight-average molar mass, M_w, of 1.76×10^5 D, a number-average molar mass, M_z, of $\approx 1.96 \times 10^5$ D, and an intrinsic viscosity, $[\eta]$, of 0.78 dL g^{-1}. The amount of hydration water of F20 is 7−8 wt %, and dehydration was performed, to increase the reaction yield. Finely monodisperse pullulans (Aldrich) were used in gel permeation chromatography, GPC, calibration.

Dodecanoic acid, $C_{11}COOH$ (LAUOH), of nominal 99.0% purity (Aldrich) was used as received. Its purity was controlled by the melting point, T_F (44 °C). Sodium dodecyl sulfate, SDS (Aldrich, 98.0%), was purified by standard procedures[31] and vacuum-dried.

4(*N*,*N*-dimethyl-amino)pyridine, DMAP, dicyclohexyl carbodiimide, DCC, and dodecanoyl chloride, LAUCl, were analytical purity products (Aldrich). All reagents and solvents were used as received, unless otherwise stated.

Water was doubly distilled in an all-glass apparatus, deionized, and degassed. Its ionic conductivity, at 25 °C, is ≈ 1.0 μS cm^{-1}. D_2O (99.8% isotopic enrichment) was from Merck.

Synthetic Procedures. A 5.0 wt % pullulan solution in anhydrous dimethyl sulfoxide, DMSO, was kept under argon, at 25 °C. Then DMAP was added, in a mole ratio of 1−10 with respect to the amount of dodecanoic acid to be added. A DMSO solution containing $C_{11}COOH$ and DCC (in a 1/1 ratio) was prepared separately in a vial, by adding the minimum amount of solvent. The solution was stirred up, to dissolve DCC, and introduced dropwise into the pullulan-containing vessel, under stirring. The reaction was carried out at room temperature for 24 h. The product was isolated by precipitation with cold ethanol, redissolved in a minimum amount of water, and exhaustively dialyzed against distilled water at 4 °C. The derivatized polymers were isolated by lyophilization.

Methods. *Surface Tension.* Surface tension was measured by a K10 T unit (Kruss GmbH, Hamburg, Germany), equipped with a platinum−rhodium Du Noüy ring, flamed at red color before use and washed with 0.1 molal HCl and doubly distilled water. The temperature is kept at 25 ± 0.1 °C. Surface tension, σ, data are obtained by five independent determinations on thermally equilibrated mixtures. The data accuracy is to ±0.2 mN m^{-1}. The adsorption kinetics was followed over ~100 h.

Critical micellar concentrations were determined by the inflection point of σ (mN m^{-1}) versus the solute mole fraction, X_2, according to

$$\partial\sigma = -\Gamma_2 \partial\mu_2 \approx -\Gamma_2[RT\partial \ln \gamma_2 X_2] \qquad (1)$$

where Γ_2 is the surface excess concentration of the solute and μ_2 is its chemical potential. The activity coefficients in eq 1 were set equal to 1.

TABLE 1: The Average Number of Alkyl Chains per Molecule, $\langle n \rangle$, the Intrinsic Viscosity, $[\eta]$, the Limiting Hydrodynamic Radius, R_H, and the cmc of $PULAU_{2.5}$ and $PULAU_{5.0}$, at 25 °C

substance	$\langle n \rangle$	$[\eta]$ (dL g^{-1})	R_H (nm)	cmc (wt %)
$PULAU_{2.5}$	50 ± 5	0.730	20 ± 1	2.3 ± 0.05
$PULAU_{5.0}$	90 ± 5	0.630	19 ± 1	1.8 ± 0.05

FTIR. Measurements were run with a Shimadzu 8300 FTIR spectrometer (Shimadzu, Tokyo, Japan) equipped with an attenuated total reflection, ATR, Golden Gate accessory (Specac Inc., U.S.A.).

Optical Polarizing Microscopy. Transmission optical microscopy, in white and polarized light, was performed by a CETI apparatus, mod. Topic (CETI, Antwerp, Belgium), equipped with removable optical polarizers and filters. The samples were homogenized by gentle stirring between the glass slides before investigation. Measurements were performed at room temperature. The assignment of optical anisotropic textures was made according to Rosevear's classification.[32]

Gel Permeation Chromatography. GPC was performed by a Lab-Flow 4000 high-pressure liquid chromatography, HPLC, pump (LabService Analytica, Bologna, Italy) equipped with a Varian RI-4 refractive index detector (Varian Associates, Palo Alto, CA) and TSK-Gel GMPW columns (TosoHaas, Montgomeryville, PA). The eluent was 0.1 M NaCl at 1.0 mL/min. The hydrophobic derivatives were analyzed using Polymer Laboratories PL gel MIXED B columns and dimethylformamide, DMF, as eluent at 0.8 mL/min.

NMR Spectroscopy. The samples, prepared with D_2O on a mole fraction basis, were flame-sealed into 5 mm NMR glass tubes and equilibrated at room temperature for some days. A Bruker AVANCE AQS600 unit was used (Bruker Bio-Spin Co., Millerica, MA). The experimental conditions, acquisition times, and number of transients were previously checked on HM polysaccharide gels.[33] ^1H NMR spectra of $PULAU_{2.5}$ and $PULAU_{5.0}$ in perdeuterated DMF are reported below.

The experimental conditions for DOESY are the following. A diffusion time between 400 and 1000 ms and bipolar rectangular gradient pulses between 1.0 and 2.3 ms long were used. The eddy current delay was set constant to 25 ms, when the gradient pulse recovery time was 0.1 ms.[34] The resulting accuracy on self-diffusion coefficients is 1−2%, when the temperature, T, is to ±0.1 °C. The polymer hydrodynamic radii, R_H, calculated from the limiting self-diffusion values, $D°$, by using the Stokes−Einstein equation are reported in Table 1. They are in good agreement with those obtained by QELS measurements.

Viscosity. Ubbelohde viscometers, model 52503, (Schott-Geräte GmbH, Germany) were used. The flow times, t, were 2−300 s, to minimize spurious kinetic effects. The viscometers are placed in a water bath (Heto, Allerød, Denmark) and thermostated to within ±0.01 °C. Each flow time is the mean value of five measurements; the resulting accuracy is to ±0.2 s.

Density measurements were performed by a DMA 60 vibrating densimeter (Anton Paar, Austria), working at 25.000 ± 0.003 °C with a resolution of $\pm 5.0 \times 10^{-6}$ g cm^{-3} on ρ values (g cm^{-3}). More details on the densimeter setup and measuring procedures are given elsewhere.[35]

Laser Light Scattering. The vertically polarized laser light passes through a pinhole, is focalized into the cell (thermostated at 25 ± 0.05 °C), passes through the sample, and reaches a 2030 AT photomultiplier, located on a rotating arm (Brookhaven Instruments, Redditch, Worchestershire, U.K.).

The position of the diffusion angle is selected by rotating a 200 MS goniometer (Brookhaven Instruments, Redditch, Worchestershire, U.K.). Measurements were performed at 45, 90, 120, 135, and 150°. The signal is collected by a digital correlator. Before entering the cell, the solutions are filtered by 0.22 μm membranes, to avoid dust. Details on QELS unit setting are given elsewhere.[36,37]

The normalized autocorrelation function of the scattered electric field, $g_1(\tau)$, was expanded in cumulants as

$$\ln|g_1(\tau)| = -\Psi_1\tau + \left(\frac{\Psi_2}{2}\right)\tau^2 \quad (2)$$

and the apparent self-diffusion coefficient is obtained by

$$D_{app} = \left(\frac{\Psi_1}{q^2}\right) \quad (3)$$

The R_H values are obtained by D_{app} values through the Stokes−Einstein relation.

Results

To rationalize the behavior of PULAU$_N$, the hydrophobic substitution degree, N, the intrinsic viscosity, [η], the hydrodynamic radii, R_H, the critical micella concentration, cmc, and the partial phase diagram of the water−SDS−PULAU$_{2.5}$ system, at 25 °C, were investigated. Data relative to the thermodynamics of association with SDS and to the kinetics of adsorption at interfaces are reported in the Discussion section.

Hydrophobic Substitution Degree. Different products were prepared. The experimental conditions were such to give 6.0 and 9.0% nominal hydrophobic substitution degrees, N (average number of dodecanoyl groups per 100 glucose units). The success of the derivatization reaction was assessed by the presence of the ester stretching peak of C═O in the FTIR spectra, at ≈1730 cm^{-1}. ^{1}H NMR quantitative analysis (Figure 2) indicates effective N values of 2.5 (40 ± 4% of the nominal one) in PULAU$_{2.5}$ and 5.0 (yield ≈55 ± 3%) in PULAU$_{5.0}$.

GPC. PULAU$_N$ were analyzed using DMF as eluent, to assess the molecular weight with respect to pullulan. By using DMF as solvent, intra- or intermolecular association of the hydrophobic derivatives, taking place in aqueous media and giving rise to significant changes in the hydrodynamic volumes, is prevented and the real molecular weight can be measured.

According to GPC, no polymer degradation takes place during the derivatization, since the molecular weights of PULAU$_N$ are equivalent, within the limits set up by the experimental data accuracy, to that of the native product. Thus, changes in the macromolecule size observed with the subsequent techniques will only be ascribed to polymer association in water-based media.

Viscometric Findings. The intrinsic viscosity, [η], was extrapolated from η_{sp}/C versus C plots (in g per 100 mL of water) for pullulan, PULAU$_{2.5}$, and PULAU$_{5.0}$, respectively. Data were interpreted according to

$$[\eta] = KM^{\alpha} \quad (4)$$

where α and K are the same as pullulan ($K = 2.21 \times 10^{-2}$ mL g^{-1} and $\alpha = 0.66$, at 25 °C).[38] This approximation is fulfilled when effective N values are moderate.[39] The hydrodynamic volumes are 0.78, 0.73, and 0.63 dL g^{-1} for pullulan, PULAU$_{2.5}$, and PULAU$_{5.0}$, respectively. Accordingly, PULAU$_N$ retain a more compact conformation than the starting polymer even at high dilution, to reduce contact of alkyl groups with water. The effect is marked for the more substituted PULAU$_{5.0}$ (Table 1).

Hydrodynamic Radii. Light scattering gives the dependence of hydrodynamic radii, R_H, on the concentration of PULAU$_N$ (Figure 4). In binary systems, R_H values remain nearly constant up to the cmc and increase thereafter. Within the limits set up by data accuracy, the R_H values of PULAU$_{2.5}$ and PULAU$_{5.0}$ in molecular form are nearly constant (20 ± 2 nm) and close to that of pullulan.[40]

Micelle Formation. Association begins at concentrations above 2.3 wt % for PULAU$_{2.5}$ and 1.8 wt % for PULAU$_{5.0}$. Changes in the foam lifetime, viscosity, and apparent turbidity are concomitant to micelle formation. The apparent hydrodynamic radii of micelles are ≈40 nm for PULAU$_{2.5}$ and ≈80 for PULAU$_{5.0}$. We did not extend the investigation to high polymer content because of the significant solution viscosity.

Added SDS leads to the formation of mixed micelles. The dependence of cmc values on the PULAU$_N$ mole fraction (Figure 5) indicates nonideality of mixing in the formation of micellar aggregates.[41]

Phase Diagram. The partial phase diagram of the system water−SDS−PULAU$_{2.5}$, at 25 °C, is reported in Figure 6. Above the cmc (at fixed PULAU$_{2.5}$ wt %), the solution viscosity decreases upon addition of SDS. Thus, no micelle-mediated interconnections between polymer chains occur, in disagreement with the behavior observed in the system water−SDS−hydrophobically modified cellulose, HM-EHEC.[42] A gel phase is formed at high PULAU$_{2.5}$ wt %, but no gels are formed in dilute concentration regimes. Two phase regions are observed, one between the solution phase and the gel phase and the other between the solution and the liquid crystalline phase. The kinetics of phase separation in the gel−solution systems is long, presumably because of the high viscosity of the PULAU$_{2.5}$-rich phase.

The hexagonal liquid crystalline phase, observed by optical polarizing microscopy at high SDS content, dissolves tiny amounts of PULAU$_{2.5}$. No cholesteric order can be inferred from optical anisotropic textures.

Discussion

Micelle Formation. PULAU$_N$ are not typical association colloids, and questions can be raised as to whether aggregates formed by them can be considered true micelles. The original definition of micelles, that is, a reversible association−dissociation of many molecules in a poor solvent containing the molecular species, fits with the present systems.[43] It must also be considered that mixtures containing high amounts of SDS form micelles in the presence of PULAU$_N$, and it is difficult to reconcile the existence of micelles for certain mole fractions only. Let us assume, in the following, that the macromolecule participates in micellar association if micelles nucleate onto its alkyl chains. In such limits, current definitions of cmc can be used.

cmc values in mixtures containing PULAU$_{5.0}$, or PULAU$_{2.5}$, and SDS were determined to quantify the role of hydrophobic modification in mixed micelle formation. Data were transformed in mole fraction units and reported as a function of the percent of PULAU$_{5.0}$, $X_{PU,5.0}$, or PULAU$_{2.5}$, $X_{PU,2.5}$. Because of the very different molar masses with respect to SDS, the mole fractions

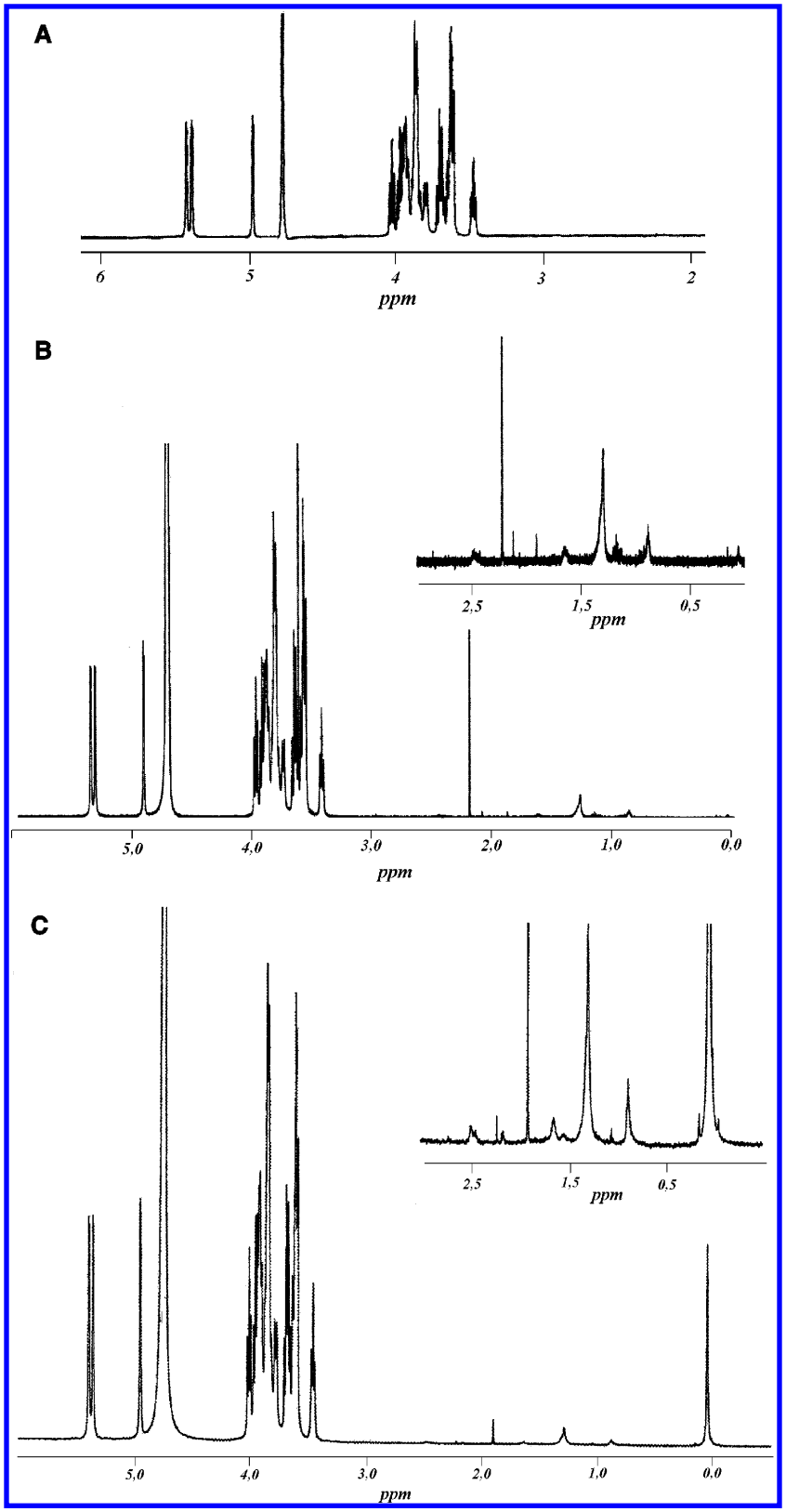

Figure 2. ^{1}H NMR spectra of (A) pullulan, (B) PULAU$_{2.5}$, and (C) PULAU$_{5.0}$, in perdeuterated DMSO at 300 K. Concentrations are 0.30 wt % in all cases.

of HMPs, $X_{PU,N}$, are expressed in terms of the mole fraction of alkyl chains, $\langle n \rangle$, according to

$$X_{PU,N} = \left\{ \frac{\langle n \rangle M_{\langle n \rangle,PU,N}}{\langle n \rangle M_{\langle n \rangle,PU,N} + M_{SDS} + M_{wa}} \right\} \qquad (5)$$

where $M_{\langle n \rangle,PU,N}$, M_{SDS}, and M_{wa} are the mole numbers of PULAU$_N$, surfactant, and water, respectively.

The average number of alkyl chains per macromolecule, $\langle n \rangle$, inferred from NMR, is 50 ± 5 in PULAU$_{2.5}$ and 90 ± 5 in PULAU$_{5.0}$. Hence, the cmc values of PULAU$_{5.0}$ ($X_{PU,5.0} = 1.6 \times 10^{-4}$) and PULAU$_{2.5}$ ($X_{PU,2.5} = 1.9 \times 10^{-4}$) are comparable

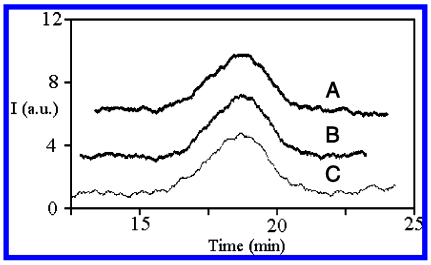

Figure 3. GPC of (A) pullulan, (B) PULAU$_{2.5}$, and (C) PULAU$_{5.0}$, in DMF, at 25 °C. The signal intensity is in arbitrary units.

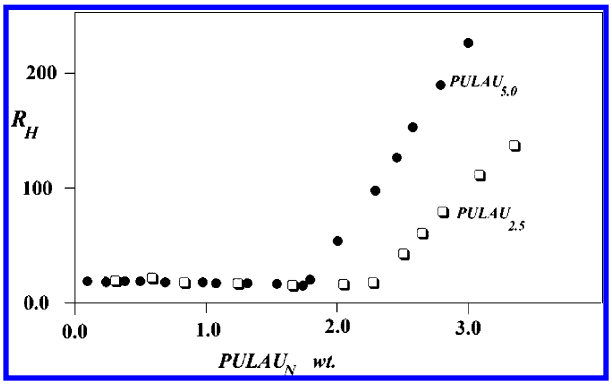

Figure 4. Plot of the apparent hydrodynamic radius of PULAU$_{5.0}$ (●) and PULAU$_{2.5}$ (□), R_H (nm), versus the solute wt % in water. Data were obtained from QELS, at 25.0 °C.

with that of SDS ($X_{SDS} = \sim 1.4 \times 10^{-4}$). The cmc values for both mixed systems are reported in parts A and B of Figure 5, respectively.

Thermodynamics. The cmc of the mixed systems, cmc*, does not linearly depend on the amount of PULAU$_N$. This behavior is more significant in PULAU$_{2.5}$. The resulting effect on cmc* values is presumably due to the combination of micelle nucleation on PULAU$_N$ and to the formation of mixed micelles.

No thermodynamic studies are reported on mixed micelles containing HMPs and surfactants; thus, we refer to models introduced by Holland and Rubingh[44,45] and developed, independently, by Rosen and co-workers.[46−50] There, the solute−solute interaction parameter, $\beta_{i,j}$, related to the solute−solute pairwise interaction in mixed micelles, is expressed as[41]

$$\beta_{i,j} = N_A\left[\frac{(W_{ii} + W_{jj} - 2W_{ij})}{RT}\right] = \frac{\ln\left[\frac{\alpha_i \mathrm{cmc}^*}{X_i \mathrm{cmc}_i}\right]}{(1 - X_i)^2} \qquad (6)$$

where W_{ii}, W_{jj}, and W_{ij} are the pairwise interactions in pure and mixed systems, α_i, the mole fraction of the ith surfactant in the mixture, cmc*, the critical concentration of the mixed system, X_i, the mole fraction of surfactant in such aggregates, and cmc$_i$, the critical concentration of the ith surfactant. Synergistic [$\beta_{i,j} < 0$] or antisynergistic effects may occur. We assume that $\beta_{i,j}$ values, determined by using eq 6, are constant in the whole mole fraction range.

As indicated in Figure 5, $\beta_{i,j}$ is positive. In other words, more surfactant than the ideal case is required to form mixed aggregates.[50] This is consistent with the hypothesis that alkyl chains in HMPs are binding sites for micelle nucleation.[51] The effect is more relevant for lower substitution degrees, as can be inferred by comparing parts A and B of Figure 5. In fact, the formation of mixed micelles requires extra work to counteract conformational constraints on the polymer chains; that is why lower hydrophobic modification results in slightly less ideality of mixing.

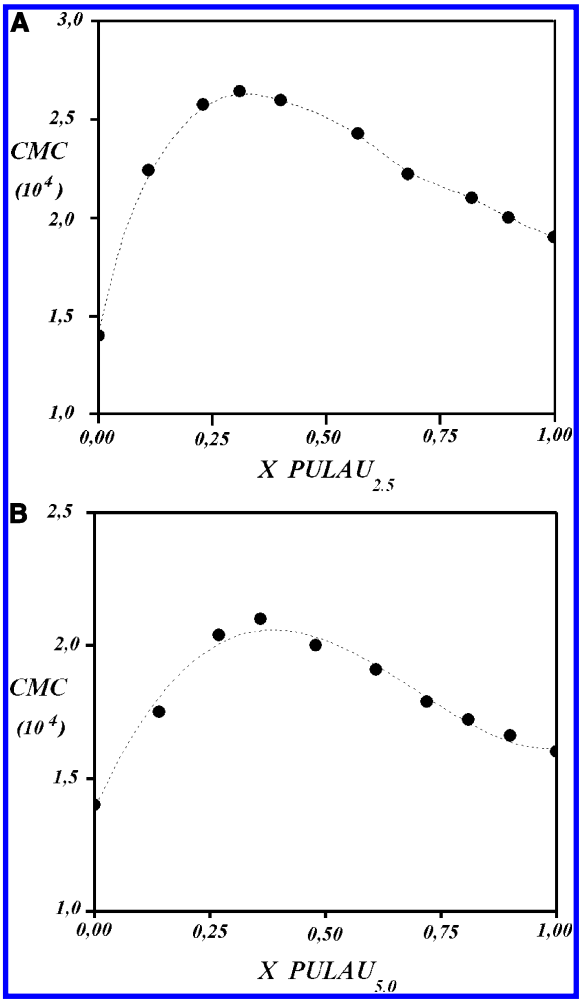

Figure 5. Dependence of the cmc* (in 10^4 mole fraction units) on the PULAU$_N$ mole fraction in the mixture for the systems (A) SDS−PULAU$_{2.5}$ and (B) SDS−PULAU$_{5.0}$, at 25.0 °C. The cmc data were obtained from surface tension and/or QELS.

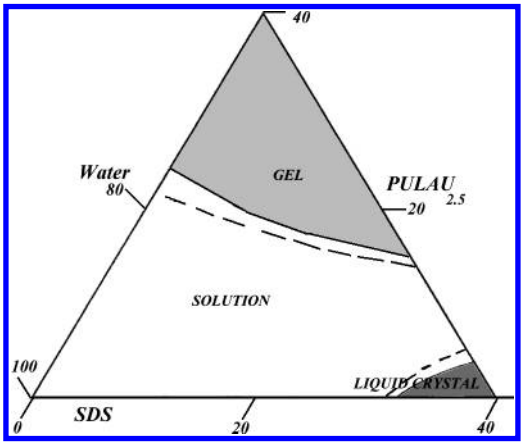

Figure 6. Partial phase diagram of the system water−SDS−PULAU$_{2.5}$, at 25 °C. Data were obtained by visual observation and optical microscopy (with or without optical polarizers). The borders of two phase regions are indicated by dashed lines.

Kinetics of Adsorption. As indicated in Figure 7, the adsorption at the air−solution interface is completed in very long times. The related kinetic constants linearly depend on the amount of PULAU$_{5.0}$. Such behavior is unusual compared to simple surfactants.[52] It is well-known, however, that HMPs show fast,[53] slow,[54] or very slow[55] adsorption kinetics, depending on the substitution degree and polymer chain rigidity. A complete

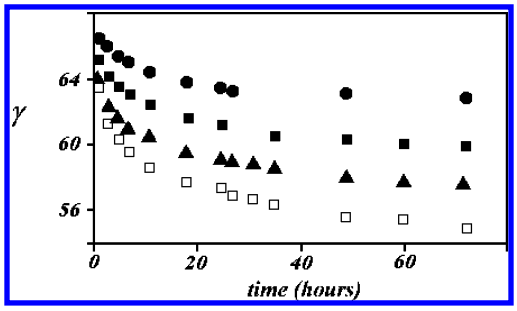

Figure 7. Dependence of surface tension, σ (mN m^{-1}), on time (h) for solutions containing 0.75 (●), 1.0 (■), 1.25 (▲), and 1.50 (□) wt % PULAU$_{5.0}$, at 25.0 °C. Data are fitted according to a first-order kinetic equation.

spreading of PULAU$_N$ chains at the air−solution interface is not reasonable because the full stretching of the sugar units between adjacent alkyl groups is not realistic.

Statistical thermodynamics approaches relate the surface coverage to bulk composition. Models reported so far deal with homopolymers partitioned between the bulk and surface phases.[56−60] We refer, conversely, to HMPs, whose alkyl groups are preferentially anchored at the interface. The alkyl chains occupy part of the surface phase, depending on the substitution degree and concentration. The equilibrium configuration is governed by the concentration, pullulan chain elasticity, and steric hindrance of sugar units located between two alkyl chains. The latter contributions avoid complete surface coverage and monolayer formation.

Polymer adsorption at interfaces can be expressed as[61,62]

$$\left(\frac{\pi\sigma^\circ}{kT}\right)=\left\{\left(\frac{n(x-1)}{2x}\right)\ln\left[1-\left(\frac{2xM\sigma^\circ}{n\sigma}\right)\right]-\ln\left[1-\left(\frac{xM\sigma^\circ}{\sigma}\right)\right]\right\} \quad (7)$$

where π is the surface pressure, σ, the total surface area, and σ°, the area occupied by an alkyl chain. In eq 7, $\sigma = N^\circ\sigma^\circ$, where N° is the number of cells in the surface phase, and n is a coordination number, M is the number of molecules linked to the surface (the number of links per polymer is surely ≥ 1), and x, the number of alkyl chains per macromolecule, is proportional to the substitution degree. Equation 7 can be rewritten as

$$\left(\frac{\pi\sigma^\circ}{kT}\right)=\left\{\left(\frac{n(x-1)}{2x}\right)\ln\left[1-\left(\frac{2xM}{nN^\circ}\right)\right]-\ln\left[1-\left(\frac{xM}{N^\circ}\right)\right]\right\} \quad (7')$$

and for high x values (where x is the number of monomers in the chain times the substitution degree, Mon·SD), it transforms into

$$\left(\frac{\pi\sigma^\circ}{kT}\right)=\left(\frac{n}{2}\right)\ln\left[\frac{nN^\circ-2M(\mathrm{SD\cdot Mon})}{nN^\circ}\right]-\ln\left[\frac{N^\circ-M(\mathrm{SD\cdot Mon})}{N^\circ}\right] \quad (8)$$

$$\exp^{(\pi\sigma^\circ/kT)}=\exp^{(\pi/\pi^\circ)}\approx\left[\frac{nN^\circ-2M(\mathrm{SD\cdot Mon})}{nN^\circ}\right]^{(n/2)}\left[\frac{N^\circ}{N^\circ-M(\mathrm{SD\cdot Mon})}\right] \quad (8')$$

In other words, spreading is controlled by the coordination number, substitution degree, and molecular weight. Rearrangement of eq 8′ gives[62]

$$\exp^{[\pi\sigma^\circ/kT]}=\left[\frac{nA-2A^\circ}{nA}\right]^{[n/2]}\left[\frac{A}{A-A^\circ}\right] \quad (9)$$

where A is the surface area and A° is the area covered by alkyl chains. A° can be related to the area covered at time t, and eq 9 gives give the spreading kinetics in terms of surface coverage as

$$\left[\frac{\partial\exp^{[\pi/\pi]}}{\partial t}\right]=\left\{\frac{\partial\left[\frac{(nA-2A^\circ)}{nA}\right]^{n/2}\left[\frac{A}{A-A^\circ}\right]}{\partial t}\right\} \quad (10)$$

Equation 10 is purely phenomenological and does not give any information on the forces controlling the adsorption nor does it explain why a given kinetic process is slow or fast. Some assumptions leading to eq 10 are misleading. In particular, the hypothesis that only alkyl chains are surface-adsorbed is questionable. If it is not true, eqs 7, 7′, 8, and 8′ should be completely rewritten.[63]

Let us express, alternatively, the kinetic process in terms of a modified Gibbs adsorption isotherm and define the derivative of surface tension, $(\partial\gamma/\partial t)$, as

$$-\left(\frac{1}{RT}\right)\left(\frac{\partial\gamma}{\partial t}\right)=\left(\frac{1}{RT}\right)\left(\frac{\partial\pi}{\partial t}\right)=\left[\frac{\partial(\Gamma_2\ln a_2)}{\partial t}\right]=\Gamma_2\left(\frac{\partial\ln a_2}{\partial t}\right)+\ln a_a\left(\frac{\partial\Gamma_2}{\partial t}\right) \quad (11)$$

where Γ_2 is the surface excess concentration of PULAU$_{5.0}$ and a_2 is its activity in the bulk.

Equation 11 gives a surface spreading term and a contribution related to self-diffusion. The former term is controlled by the maximum surface concentration and by the elasticity of chains.[64] Taking into account the elastic term, defined as $K_2\lambda_2$, one can rewrite eq 11 as

$$\left(\frac{1}{RT}\right)\left(\frac{\partial\pi}{\partial t}\right)=\Gamma_2\left(\frac{\partial\ln a_2}{\partial t}\right)+\ln a_a\left(\frac{\partial\Gamma_2}{\partial t}\right)+K_2\left(\frac{\partial\left(\frac{1}{\lambda_2}\right)}{\partial t}\right) \quad (11')$$

where λ_2 is the average length of pullulan segments between two alkyl chains and K_2 is the elastic constant of such segments.

The diffusive contribution, that is, the former term on the right-hand side of eq 11′, can be compared with experimental values from NMR and QELS. According to such values, the order of magnitude of the diffusive term is shorter than an hour and comparable in magnitude to HM-EHEC, having fast spreading kinetics.[65] Thus, purely diffusive mechanisms can be ruled out.[66]

Corrections for the diffusive contributions, integration, and rearrangement of eqs 11 and 11′ indicate that, at equilibrium,

$$\left(\frac{\ln a_2}{K_2}\right)=\left[\frac{\partial\left(\frac{1}{\lambda_2}\right)}{\partial\Gamma_2}\right]=\left(\frac{\partial\lambda_2}{\partial n_2}\right) \quad (11'')$$

where n_2 is the surface excess concentration of PULAU$_{5.0}$. In other words, the system behavior resembles an ensemble of boats, held together by springs and floating in closed space. In this case, the equilibrium is controlled by the balance of spreading and elastic terms (Figure 8).

It is noteworthy that the kinetics of adsorption of PULAU$_{5.0}$ is similar to that observed in pullulans hydrophobically modified by cholesterol.[67] This is a further indication that spreading is controlled by the elasticity of pullulan chains and not by the hydrophobic substitution degree.

Conclusions

The physicochemical characterization of pullulans hydrophobically derivatized with dodecanoic acid indicates that PULAU$_N$

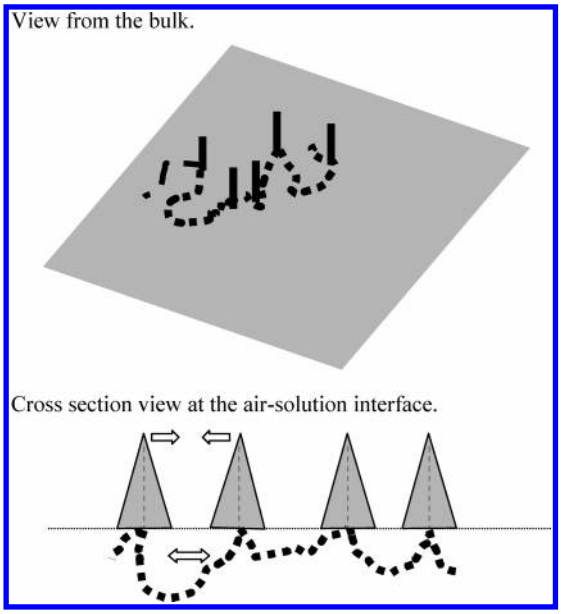

Figure 8. Schematic model of the forces controlling $PULAU_{5.0}$ adsorption, in terms of interfacial spreading and elastic constraints due to polysaccharide chains.

form micelles, alone or in mixtures with surfactants. The aggregation numbers of pure $PULAU_N$ are modulated by the substitution degree and increase in proportion with *N*. Surface tension and light scattering indicate that the formation of mixed aggregates is not ideal. Presumably, micelles rich in SDS coexist with $PULAU_N$-rich ones. No micelle-interconnected networks are formed.

Spreading kinetics at the air−solution interface is extremely slow and is presumably related to the balance of spreading and elastic terms. The above effects are also concomitant with a long foam lifetime (several hours), which can be useful in "ad hoc" applications, for instance, in the flotation of minerals.

Biomedical applications of the above substances are extremely interesting from both fundamental and applied viewpoints, in vesicle coating, for instance, and require further investigation.

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