Microviscosity in Clusters of Ethyl Hydroxyethyl Cellulose and Sodium Dodecyl Sulfate Formed in Dilute Aqueous Solutions As Determined with Fluorescence Probe Techniques

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ABSTRACT: The microviscosity has been measured in dilute aqueous solutions of ethyl hydroxyethyl cellulose (EHEC) and sodium dodecyl sulfate (SDS) by utilizing three steady-state fluorescence probe techniques: intramolecular excimer formation by 1,3-di(1-pyrenyl)propane (P3P), fluorescence depolarization of perylene, and intramolecular rotational relaxation about bonds with (*p*-(dimethylamino)-benzylidene)malononitrile (BMN). Results obtained by the three techniques are compared. They all detect qualitatively the same behavior with a well-developed maximum in microviscosity and rigidity of the EHEC/SDS clusters formed at a surfactant concentration close to or slightly higher than the critical surfactant concentration where adsorption to the polymer starts. The EHEC/SDS clusters have, independent of composition, higher microviscosities than ordinary SDS micelles. The microviscosity is also compared with other EHEC/SDS/water system features such as the bulk viscosity, the actual adsorption isotherm, the average aggregation numbers, and the micropolarity as sensed by pyrene of the EHEC/SDS clusters formed. The maximum in microviscosity corresponds to a rather low degree of SDS adsorption to EHEC (≈0.5 mmol of SDS per gram of EHEC) and a low aggregation number (≈10) where the polymer content of each polymer-bound surfactant cluster is high. It coincides (according to the surfactant concentration) with a maximum in bulk viscosity for polymer concentrations higher than the critical overlap concentration (c^*). It is suggested that the maximum in bulk viscosity is due to a three-dimensional network of polymer and cluster tie points while the maximum in microviscosity is related to a high content of hydrophobic polymer segments which stabilizes the surfactant clusters.

Introduction

Cellulose derivatives are today used in a number of technical applications such as foods, building materials, cosmetics, and pharmaceutical products.[1] This category of substances plays an important role as components in many pharmaceutical formulations—for instance, to regulate the rheology of the system and to modulate the release rate of active substance. The interaction between polymers and surfactants in aqueous solutions is of interest from both theoretical and applied aspects, and the topic has recently been reviewed.[2,3] A number of experimental methods have been utilized to explore structures formed in polymer/surfactant systems, and fluorescence probe techniques have proved[4] to be powerful tools for obtaining microstructural information. The specific system ethyl hydroxyethyl cellulose (EHEC)/sodium dodecyl sulfate (SDS)/water has been studied in this laboratory in the dilute regime, i.e. for polymer concentrations up to slightly above the critical overlap concentration of the polymer (c^*) and for surfactant concentrations up to 3−4 times the normal critical micelle concentration (cmc). A fairly detailed description of the system has emerged[5−12] involving a cooperative adsorption of micelle-like SDS clusters onto EHEC as a basic step in the proposed mechanism.

Two types of viscosities describe and control a heterogeneous pharmaceutical system,[13,14] i.e. the bulk viscosity, which is the macroscopic resistance of a solution to flow, and the microviscosity, which governs the motion of a specific probe molecule. Since many active substances are quite small and hydrophobic, their diffusion and release rate from formulations are affected more by the microviscosity rather than by the bulk viscosity.

Many water-soluble polymers exhibit, at elevated concentrations, a pronounced maximum in bulk viscosity (or even gel formation) upon addition of surfactant.[2,3] This enhanced viscosity most likely derives from some sort of three-dimensional polymer network where mixed micelles of surfactant and polymer (clusters) act as connecting tie points. In a recent paper, Piculell et al.[15] suggested a mechanism for the formation and breakdown of a polymer/surfactant network based upon measurements of bulk viscosity and NMR self-diffusion in semidilute ($c_p > c^*$) solutions of EHEC and SDS. They concluded that a breakdown of the network occurs when the surfactant dominates in the clusters. In this study, we will use a different approach and report on the microviscosity in the EHEC/SDS/water system in dilute ($c_p \approx c^*$) solutions at 20 °C. It aims at deepening the basic understanding and description of the interaction mechanism on a molecular level for the control and application of this specific system and similar systems, especially in the field of pharmaceutical applications. We have measured the microviscosity of the system by three steady-state fluorescence probe techniques which represent three principal categories of microviscometric fluorescence probe mechanisms: first, the kinetics of intramolecular excimer formation of 1,3-di(1-pyrenyl)-propane (P3P), second, depolarization of perylene fluorescence, and third, the intramolecular rotational relaxation about bonds of (*p*-(dimethylamino)benzylidene)-malononitrile (BMN). These techniques have previously been used in the study of microviscosity in aqueous solutions of surfactant micelles[16−18] and amphiphilic self-aggregating polymers,[19−24] while the application on aqueous polymer/surfactant systems has been more rare.[4,25−29] The microviscosity is discussed in relation to other important features of the EHEC/SDS/water system such as bulk viscosity, the actual adsorption isotherm, average aggregation numbers, and micropo-

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larity as sensed by pyrene of the EHEC/SDS clusters formed.

Materials

Ethyl hydroxyethyl cellulose (EHEC; fraction CST-103, $MS_{eo} = 0.7$, $DS_{ethyl} = 1.5$) with a weight-average molecular weight (M_w) of approx. 1.9×10^5, a polydispersity index (M_w/M_n) of 2.1 as determined from size exclusion chromatography experiments (with LALLS and RI detection),[30] and an intrinsic viscosity ($[\eta]$) of 455 mL/g as determined from viscometry[30] was obtained from Akzo Nobel AB (Stenungsund, Sweden). The cloud point (CP) of EHEC/CST-103 was observed in the interval 28−37 °C depending on the polymer concentration,[8] and the surface tension (against air) was equal to 37 mN/m for a 0.2% (w/w) aqueous solution of CST-103 as determined by the pendant drop technique.[11,31]

Analytical grade (99+%) sodium dodecyl sulfate (SDS) was obtained from Merck (Darmstadt, Germany), the radioactive SDS (^{35}S) was bought from Amersham (Buckinghamshire, England), 1,3-di(1-pyrenyl)propane (P3P) and (*p*-(dimethylamino)benzylidene)malononitrile (BMN) were obtained from Molecular Probes (Eugene, OR), and perylene (99.5+%), tris(2,2′-bipyridyl)ruthenium(II) chloride ($Ru(bpy)_3^{2+}$), and 9-methylanthracene (9-MA) (98%) were purchased from Aldrich-Chemie (Steinham, Germany). All of these reagents were used as supplied. Pyrene (98+%) from Acros Chimica, (Geel, Belgium) was twice recrystallized from absolute ethanol. Spectra/Por tube dialysis membrane, with a molecular weight cutoff at 12000−14000, was obtained from Spectrum Medical Ind. (Los Angeles, CA). All solutions were prepared using Milli Q water.

Experimental Section

Preparation of Solutions. The standard procedure for preparing EHEC stock solutions was described in a previous paper.[7] After preparation, the EHEC stock solution was rinsed from low molecular weight material and salts using a dialysis tube membrane. Dialysis was performed against Milli Q water (Millipore) during 1 week, after which the stock solution was filtered through 0.8 μm filters (Millex-AA, Millipore, SA (Molsheim, France)) for removal of undissolved polymer, microgels, and dust particles. The conductivity of the dialyzed EHEC stock solution was as low as 4−6 μS, which corresponds to a concentration of sodium chloride of approximately 0.03 mM. Finally, the concentration was determined by drying samples to constant weight at 105 °C. All EHEC/SDS solutions in this work were prepared by weighing the desired amounts of EHEC stock solution into appropriately diluted SDS solutions at least 24 h before the samples were used for experiments in order to let the previously reported time-dependent effects settle.[8] EHEC concentrations are given in percent by weight and are 0.05, 0.20, or 0.30% (w/w). SDS concentrations are calculated as moles per kilogram of solvent but since all the solutions used in this study are dilute, SDS concentrations are given as molarities. For experiments with BMN, P3P, and perylene, these probes were dissolved in acetone, pro analysi (Merck (Darmstadt, Germany)). Aliquots of the probe−acetone solutions were added to the EHEC/SDS/water solutions to achieve final approximate probe concentrations of 1×10^{-5}, 1×10^{-6}, and 1×10^{-6} M for BMN, P3P, and perylene, respectively. For BMN, absolute measurements of the fluorescence intensity were performed and care was therefore taken to ensure a constant probe concentration throughout all experiments. In order to avoid formation of P3P microcrystals, a problem also described for the closely related probe dipyme,[20] the P3P aliquots were added to a 100 mM SDS stock solution and then further diluted. The final EHEC/SDS/water/P3P solutions were kept for equilibration on a rotating table in the dark for at least 3 days. For quenching experiments with $Ru(bpy)_3^{2+}$−9-MA as the probe−quencher pair, the EHEC/SDS/water solutions contained approximately 1×10^{-6} M $Ru(bpy)_3^{2+}$ and $(0.1-5) \times 10^{-4}$ M 9-MA. The quencher was dissolved in acetone and incorporated into a small amount (5 mL) of each EHEC/SDS/water−probe solution by addition of aliquots. These solutions were then mixed with the corresponding quencher-free solutions to achieve appropriate concentrations with respect to 9-MA.

Fluorescence Measurements. All fluorescence measurements were recorded on a SPEX Fluorolog 2 Model FL1T2 steady-state spectrofluorometer in the "s" mode with 0.5 mm excitation and emission slits. The cell holder was thermostated to 20 °C using a circulating water bath. P3P was excited at $\lambda = 348$ nm and emission spectra were recorded between 350 and 500 nm. The I_M/I_E ratio was taken as the monomer ($\lambda \approx 377$ nm) to excimer ($\lambda \approx 485$ nm) intensity peak height ratio. Fluorescence depolarization experiments were performed with perylene as the probe, excitation recorded at $\lambda = 412$ nm and emission recorded at $\lambda = 443$ nm, using manually operated prism polarizers. BMN was excited at $\lambda = 444$ nm and narrow wavelength range emission spectra were run to find the emission peak at about $\lambda = 490$ nm.

For hydrophobic index (I_1/I_3) measurements, the excitation of pyrene was performed at $\lambda = 334$ nm and emission spectra were recorded. The I_1/I_3 ratio was taken as the first ($\lambda = 377$ nm) and third ($\lambda = 388$ nm) intensity peak height ratio in the fine vibrational spectrum of pyrene since this ratio is a sensitive indicator of the pyrene microenvironment polarity.[32]

Quenching experiments were performed with $Ru(bpy)_3^{2+}$ as the probe and 9-MA as the quencher. Excitation of $Ru(bpy)_3^{2+}$ was performed at $\lambda = 450$ nm and the emission was recorded at $\lambda = 625$ nm. In these experiments, the emission slit was set to 1.25 mm. In order to determine average aggregation numbers of the polymer-bound surfactant clusters (N_p), the steady-state fluorescence quenching method originally proposed by Turro and Yekta[33] on anionic micelles (SDS) (and later successfully employed on uncharged polymer/SDS/water systems[9,10,34−37]) was combined with equilibrium dialysis according to a methodology described[9] and validated[10] recently. The steady-state fluorescence quenching method works well for determination of average aggregation numbers if a highly efficient probe−quencher pair is used, a very low fraction of both probe and quencher is in the aqueous phase, and the cluster sizes determined do not exceed about 100.[38,39] N_p was finally calculated for this system (EHEC/SDS/water) by dividing the amount of EHEC-bound SDS, as determined by equilibrium dialysis, by the micellar concentration of the EHEC-bound SDS clusters, as determined by the fluorescence quenching experiments.

Equilibrium Dialysis. The equilibrium dialysis experiments were performed at 20 °C using the same type of dialysis cells as used previously.[7,9,10,12,34] The cell consists of two cell compartments, each of 2 mL volume, separated by a membrane (Spectra/Por with a M_w cutoff of 12000−14000) according to the same principle as developed by Fischman and Eirich.[40] The polymer solution was placed in one of the cell compartments and the SDS solution in the other. The SDS solutions contained a small amount of ^{35}S, enough to achieve an activity of approximately 25 000 cpm/mL. The cells were placed in an air thermostat at 20 °C for 1 week before the ^{35}S content on each side was determined by scintillation counting. Preliminary results indicated that equilibrium was reached after 48 h although 7 days was used as a precaution. The equilibrium dialysis method gives both quantitative and qualitative information about the binding of SDS to EHEC from which the SDS concentration in equilibrium with EHEC ($[SDS]_{eq}$), the amount of SDS bound to EHEC (y), and the total SDS concentration ($[SDS]_{tot}$) can be calculated. Since the system studied is salt-free, a correction for the Donnan effect has been made as described previously.[7] The mass balance equation then becomes

$$[SDS]_{tot} = [SDS]_{eq} + c_p y \qquad (1)$$

where c_p is the polymer concentration in grams per liter. This methodology has been utilized in several recent investigations of interactions in aqueous mixtures of nonionic cellulose derivatives and surfactants.[7,9,10,12,34]

Bulk Viscosity Measurements. Bulk viscosity of the EHEC/SDS/water solutions was measured using ordinary Ostwald capillary viscometers with approximate flow times of 100 s for pure water at 20 °C, which were immersed in a

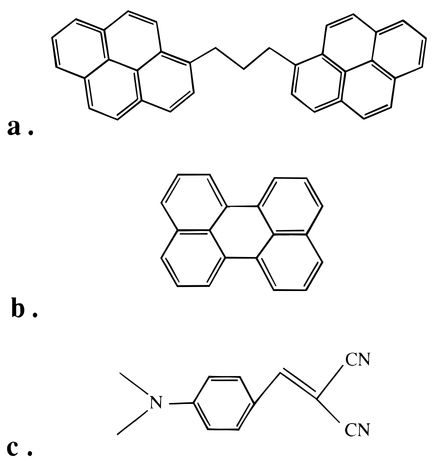

Figure 1. Structures of the probes used in this study: (a) P3P (1,3-di(1-pyrenyl)propane); (b) perylene; (c) BMN (*p*-(dimethylamino)benzylidene)malononitrile)

water thermostat. The reduced specific viscosity, η_{sp}/c ($\eta_{sp} = (\eta - \eta_0)/\eta_0$), was calculated, treating the system as a quasibinary one, from viscometric data, where c is the polymer concentration and η and η_0 are the viscosities of solution (EHEC/SDS/water) and solvent (SDS/water), respectively.

Results and Discussion

Methodological Aspects and Qualitative Observations. The microviscosity or rigidity of polymer-bound SDS clusters formed on the nonionic cellulose ether EHEC was determined by utilizing three different steady-state fluorescence probe techniques. These probes represent three principal categories of microviscometric fluorescence probe mechanisms which all are based on an analysis of some form of probe molecule motion within the microenvironment of the polymer-bound surfactant clusters: intramolecular excimer formation with P3P, fluorescence depolarization with perylene, and intramolecular rotational relaxation about bonds with BMN. Figure 1 shows schematic chemical structures of P3P, perylene, and BMN. Fluorescence probe techniques have previously been used to investigate the microviscosity of various systems such as aqueous micellar solutions[16−18] and amphiphilic self-aggregating polymers.[19−24] Due to the complexities involved in extracting microviscosity information from fluorescence probe techniques[16,17,41,42] (such as the location in and perturbation of a cluster or micelle, the use of an isotropic solvent reference system with known viscosities as a calibration curve, etc.), all values presented herein are used and should be read only as qualitative observations to express relative changes within the microenvironment of the probes solubilized in the EHEC/SDS clusters and not to present absolute values of microviscosity.

The extent of intramolecular excimer formation and emission of P3P is dependent on the local friction of the probe imposed by its microenvironment. Hence, the monomer to excimer intensity ratio, I_M/I_E, provides a qualitative index of the microviscosity as sensed by the probe. Pyrene is an example of an intermolecular excimer forming probe. However, the use of pyrene in microviscosity studies of micelles is limited due to the need of a rather high probe concentration, resulting in distortion of the micellar structures, and furthermore its analysis must take the probe distribution into account.[16] The method of intramolecular excimer formation can be applied with a very low probe concentration and does not have the drawbacks mentioned above.

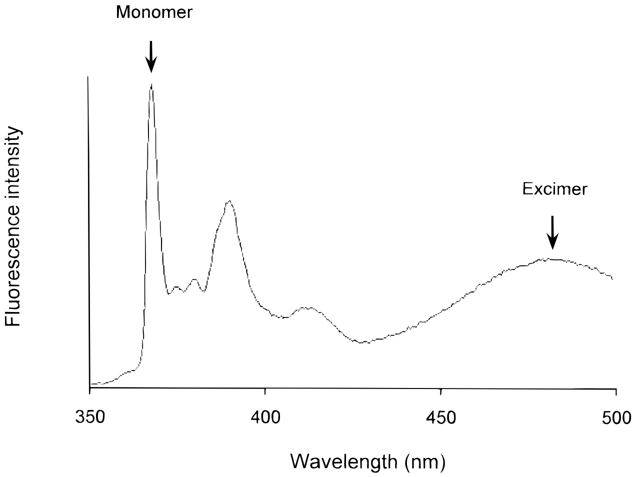

Figure 2. Emission spectra of P3P in a 0.05% EHEC/50 mM SDS/water solution at 20 °C. The monomer intensity peak, I_M, at $\lambda = 377$ nm and excimer intensity peak, I_E, at $\lambda = 487$ nm are marked with arrows in the figure.

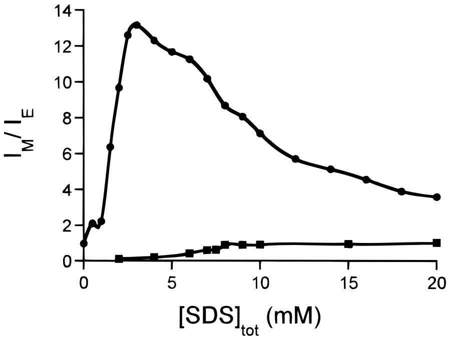

Figure 3. Monomer to excimer intensity ratio, I_M/I_E, of P3P as a function of the total SDS concentration, $[SDS]_{tot}$, at 20 °C: (●) 0.20% aqueous solutions of EHEC; (■) the binary system SDS−water.

It has been shown that P3P,[43] like the closely related 1,1-dipyrenyl methyl ether (dipyme),[44] is a molecule capable of forming an intramolecular excimer. In Figure 2 is shown a typical example of the fluorescence emission spectrum for P3P in a mixture of EHEC and SDS in water. Figure 3 presents this monomer to excimer intensity ratio, I_M/I_E, for P3P in aqueous 0.20% EHEC solutions as a function of the SDS concentration, and as a reference, I_M/I_E values for the binary SDS/water system are also given. At the critical SDS concentration where interaction between EHEC and SDS starts, here referred to as c_1 ($\approx$1 mM), the monomer to excimer intensity ratio increases at first sharply with the total SDS concentration up to a maximum followed by an asymptotic decrease toward the region of, but still significantly higher than, the I_M/I_E ratio found for normal SDS micelles. It is known[7,9,10,12] from previous investigations utilizing other techniques such as equilibrium dialysis and dye solubilization that c_1 for this specific EHEC fraction and SDS falls in the region of 1.5 mM SDS (the exact value depending on the technique used). The sharp increase in microviscosity is found between 1 and 3 mM $[SDS]_{tot}$ and correlates thus with our previous findings. At the maximum the microviscosity index is approximately 13 times higher as compared to the index found for both the binary 0.20% EHEC and the micellar SDS solutions, giving evidence for a high ordering of the solution structure and a high rigidity of the EHEC/SDS aggregates initially formed in the solution. However, it is evident

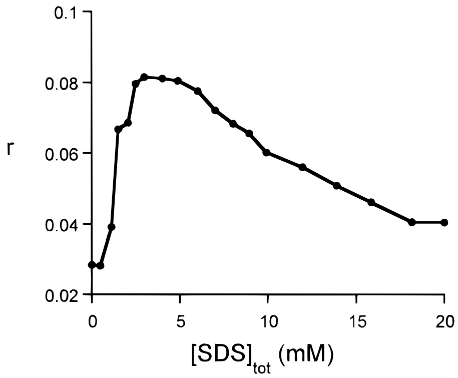

Figure 4. Emission anisotropy, *r*, of perylene as a function of the total SDS concentration, [SDS]$_{tot}$, in 0.20% aqueous solutions of EHEC at 20 °C.

that, independent of composition and within the concentration range studied, the polymer-bound surfactant clusters give microviscosities which are much higher than those of normal free SDS micelles. A similar observation has also been made by others[26,28,29] for related aqueous polymer/surfactant systems. The cmc for the binary SDS/water system is detected as a small but significant increase in the I_M/I_E ratio at about 8 mM [SDS]$_{tot}$ (which is in good agreement with the cmc found for SDS in the literature[45]). At SDS concentrations higher than cmc (up to 80 mM; results not shown in Figure 3), there is a slight increase in the I_M/I_E ratio for the SDS/water system, which might be due to an increased packing in the pallisade region of the micelles, assuming a growth in aggregation number at increased surfactant concentration. Parthasarathy and Labes[46] also observed an increase in microviscosity with micellar size and concentration for the closely related surfactant sodium decyl sulfate (SDecS), contrary to Komaromy-Hiller and Wandruszka,[47] who observed a decrease in anisotropy and microviscosity for Triton X-114 at surfactant concentrations above the cmc. These two surfactants give most likely a different structure of the micelle interior which is monitored by the probes. As the micelles grow, the bulky and rigid surfactant Triton X-114 probably gives a more ordered micelle interior and an increased volume compared to the more flexible SDecS, resulting in an increased freedom for rotation for the probe, which gives a lower microviscosity. An increase in microviscosity with aggregation number has also been reported for some alkyltrimethylammonium bromide surfactants.[48]

The traditional method for studying microviscosity has been fluorescence depolarization,[49] which is a measure of the rotational diffusion of the fluorophore during its excited lifetime. In a steady-state determination of depolarization, the fluorescence intensities are measured through a polarizer oriented parallel ($I_{||}$) and perpendicular ($I_{\perp}$) to the plane of polarization of the excitation beam. From these intensities, the steady-state emission anisotropy (*r*) is given by

$$r = (I_{||} - I_{\perp})/(I_{||} + 2I_{\perp}) \tag{2}$$

Perylene and diphenylhexatriene (DPH) are two probes which have been used to determine microviscosities in the study of surfactant,[47,49] polymer/surfactant,[26−28] and amphiphilic self-aggregating polymer aggregates[50] utilizing fluorescence depolarization measurements. Figure 4 shows the emission anisotropy (*r*) of perylene in 0.20% EHEC/water solutions as a function of the SDS concentration. As the SDS concentration is increased in the aqueous EHEC solution, the anisotropy increases at first rapidly from 0.028 in the pure EHEC/water solution up to 0.082 at approximately 3.5 mM SDS after which *r* declines asymptotically to 0.040 at 20 mM SDS. Measurements of binary micellar SDS/water solutions give an emission anisotropy on the order of 0.01. Qualitatively perylene detects the same behavior in the EHEC/SDS/water system as P3P with a sharp increase in microviscosity up to a maximum at about 3−4 mM SDS at which there exists a strong ordering in the solution and a high microviscosity and rigidity of the polymer-bound surfactant clusters formed followed by a decreasing microviscosity at higher SDS contents. The anisotropy measurements also show that perylene has a lowered rotational mobility, independent of composition, in all aqueous EHEC/SDS solutions compared to both the binary EHEC/water and the micellar SDS/water solutions. However, the anisotropy, *r*, is necessarily not directly proportional to the microviscosity since it depends also on the lifetime of the probe according to the Perrin equation[51]

$$r_0/r = 1 + kT\tau/\eta V_0 \tag{3}$$

where r_0 is the limiting value of emission anisotropy obtained in the absence of rotational freedom, τ is the average lifetime of the fluorophore excited state, T is the absolute temperature, k is the Boltzmann constant, V_0 is the effective molecular volume of the probe, and η is the microviscosity of the probe. As pointed out by Schinitzky and Inbar,[52] the lifetime of the probe increases with increasing microviscosity at constant temperature, resulting in a nonlinear relationship between *r* and the microviscosity. In the EHEC/SDS/water system, this effect should be most pronounced close to the onset of interaction (c_1) where the microviscosity and the lifetime of the probe reach their maximum. This effect might partly explain the small qualitative difference between the dependencies of *r* and I_M/I_E on [SDS]$_{tot}$.

Viscosity-sensitive fluorescence emission of malononitriles through intramolecular rotational relaxation about bonds represents an alternative method to measure microviscosity. As the microviscosity of a medium increases, the rotational relaxation of the malononitrile dye bonds is increasingly hindered and the fluorescence emission intensity increases. It has been shown[53] that, for a series of malononitrile derivatives, the fluorescence quantum yield is solvent viscosity dependent. This method has been applied in the study of microviscosity of phamaceutical systems[13] as well as monitoring polymerization rates[54] and for the study of phospholipid bilayers and surfactant micelles.[55] In a recent report, LaPorte et al.[13] showed that the malononitrile derivative BMN was able to to differentiate between the bulk viscosity and the microviscosity in aqueous solutions of methyl cellulose (MC) in the concentration range from 1 to 2.25% (w/w). Figure 5 presents the fluorescence emission intensity of BMN in aqueous 0.20% EHEC solutions as a function of the SDS concentration. Also BMN monitors qualitatively the same trends in the EHEC/SDS/water system as seen with P3P and perylene, here with a sharp increase in fluorescence emission intensity and microviscosity up to a maximum followed by a declining region as the SDS concentration is raised from 0 to 20 mM. The polymer-bound surfactant clusters give a higher microviscosity than ordinary SDS micelles. The fluorescence emission intensity of BMN is raised for the binary SDS/water system in the region

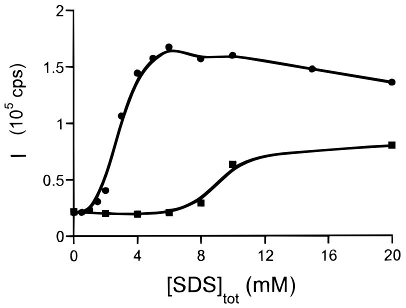

Figure 5. Fluorescence intensity, *I*, of BMN as a function of the total SDS concentration, $[SDS]_{tot}$, at 20 °C: (●) 0.20% aqueous solutions of EHEC; (■) the binary system SDS–water.

of cmc, which correlates well with the P3P measurements shown below. However, there also exist some differences between BMN and P3P/perylene in behavior: At first, the increase in microviscosity is not as abrupt for BMN as was found for P3P/perylene, and in the case of BMN, the increase stretches between 1 and 6 mM SDS in comparison with 1 and 3 mM SDS for P3P and perylene. Second, the maximum in microviscosity for the EHEC/SDS/water system is seen at 6 mM SDS for BMN while it was found at 3 mM SDS for P3P and perylene. It is also found that the increase in emission intensity for BMN at the cmc for the SDS/water system comes at a slightly higher SDS concentration than the increase in I_M/I_E for P3P. Third, the microviscosity of a 0.20% EHEC solution is significantly lower than that of a binary micellar SDS/water solution while the reversed relationship and equality between the two solutions was seen with perylene and P3P, respectively. These three differences in behavior between the probes can possibly be explained by considering their chemical structure, as is schematically shown in Figure 1, hydrophobicity, water solubility, and distribution coefficient between surfactant clusters (micelles) and water. BMN is a single aromatic ring structure which has some water solubility.[13] P3P has two aromatic rings and perylene has four aromatic rings, and they both have a very low water solubility. The order of their water solubility is BMN $>>$ P3P $>$ perylene. Their hydrophobicity and distribution coefficient to surfactant clusters (micelles) are most likely in the opposite order, BMN $<<$ P3P $<$ perylene. Thus the differences between the probes can possibly be rationalized in the following way: BMN has the highest water solubility and the lowest distribution coefficient to surfactant clusters and micelles of the three probes. As the surfactant clusters are formed on the polymer chain, BMN distributes less effectively and quantitatively than P3P and perylene, and hence it is not surprising that the increase in microviscosity stretches more and that the maximum in microviscosity is detected at a slightly higher SDS concentration for BMN than for P3P and perylene. In comparison of the microviscosity of a 0.20% EHEC and a micellar SDS solution as measured by these probes, it is found that the hydrophobic probe perylene samples a higher microviscosity in the polymer solution while BMN, which has the lowest hydrophobicity, samples a higher microviscosity in the micellar SDS solution. Perylene interacts probably intensively with the hydrophobic polymer, thus hindering the rotational movements of the probe. BMN, on the other hand, interacts less intensively with the polymer and senses a higher rigidity in the normal SDS micelles. Another factor which also might affect the microviscosity results is the precise probe location[17] in a micelle or polymer-bound surfactant cluster. This information is not easily obtained, but it seems reasonable to assume that BMN is located closer to the aqueous cluster (micellar) surface where exposure to water is more pronounced compared to perylene and P3P, which both are solubilized deeper inside the hydrophobic interior.

All three fluorescence probe techniques applied on the EHEC/SDS/water system give qualitatively the same picture concerning the microviscosity behavior of the polymer-bound surfactant clusters formed in the solutions. All techniques show a sharp increase and a well-developed maximum in microviscosity of the clusters formed at SDS concentrations slightly higher than c_1. The maximum in microviscosity gives evidence of very rigid and viscous structures formed in these polymer–surfactant solutions. At surfactant concentrations higher than that corresponding to the maximum, the measurements show asymptotically decreasing values of microviscosity with increasing SDS concentration. However, the polymer–surfactant clusters formed, independent of composition, have microviscosities which are higher than those of ordinary SDS micelles but since the measured microviscosity most likely is an average of all clusters or micelles present in the solution, it becomes more statistically weighted toward normal micelles as the SDS concentration is raised.

Microviscosity in Relation to Some Basic EHEC/SDS/Water System Features. As concluded above, all three fluorescence probe techniques utilized in this study detect qualitatively the same behavior of the microviscosity in aqueous EHEC/SDS solutions, but since P3P yields a sensitive measure of the system's rigidity, gives highly reproducible results, and is easy to use when handled with care (see Experimental Section), we have chosen this probe when comparing the microviscosity with other characteristics on the system.

The most basic and fundamental information concerning the polymer–surfactant interaction is the adsorption isotherm describing the quantitative binding of surfactant onto the polymer. Equilibrium dialysis experiments provide this kind of quantitative information about SDS binding onto EHEC, and it has previously been utilized in this laboratory for several studies of interaction between nonionic cellulose derivatives and surfactants.[7,9,10,12,34] Figure 6a shows the relation between the monomer to excimer intensity ratio, I_M/I_E, of P3P and the adsorption parameter, y (millimoles of SDS adsorbed per gram of EHEC), for EHEC/SDS/water solutions as a function of the SDS concentration in equilibrium with EHEC, $[SDS]_{eq}$. This adsorption parameter y has been calculated with a correction for the Donnan effect according to a model described in recent papers.[7,12] It is seen in Figure 6a that in the early onset of SDS adsorption to EHEC the microviscosity or rigidity of the solution increases abruptly. The maximum in microviscosity is seen to correspond to a rather low degree of adsorption, approximately $y \approx 0.5$ (which equals about 0.1 SDS monomer per polymer glucose unit). The second break point in the adsorption isotherm at $y \approx 2$, which recently has been characterized[10] as a shift from SDS adsorption to aggregates of EHEC to adsorption of SDS to single deaggregated EHEC chains, seems to coincide with the "shoulder" in microviscosity after the maximum. This fact shows that as the aggregated EHEC chains are changed into a deaggregated state, the decrease in microviscosity of the

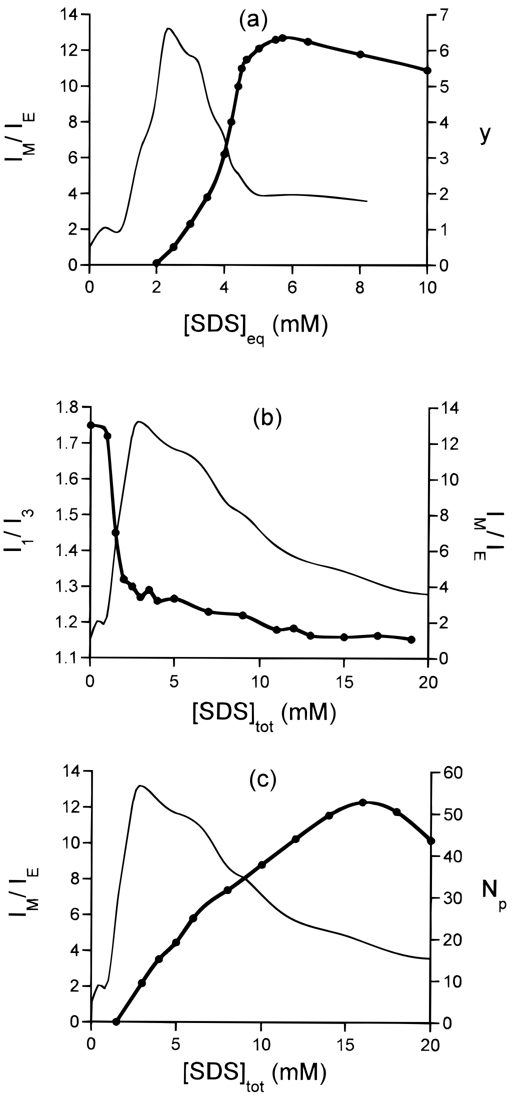

Figure 6. Thin lines in a−c represent the monomer to excimer intensity ratio of P3P, I_M/I_E, as a function of the SDS concentration in 0.20% aqueous solutions of EHEC at 20 °C. Bold lines with ● markers represent (a) the adsorption isotherm of SDS adsorbed to EHEC as determined by equilibrium dialysis (millimoles of SDS adsorbed per gram of EHEC, y, as a function of the equilibrium SDS concentration, $[SDS]_{eq}$), (b) the hydrophobic index of pyrene, I_1/I_3, as a function of the total SDS concentration, $[SDS]_{tot}$, and (c) average aggregation numbers of polymer-bound SDS clusters formed in 0.20% EHEC/water solutions as a function of the total SDS concentration, $[SDS]_{tot}$.

solution and its polymer-bound SDS clusters accelerates. The abrupt decrease in microviscosity ends as the maximum in adsorption, y_{max}, is reached and normal micelles start to form in the solution.[9,34] Evidently, the declining region in microviscosity corresponds to a rather broad range in adsorption values (y), i.e. from 0.5 to 6 mmol of SDS per gram of EHEC.

It is known that the intensity ratio I_1/I_3 of the first and third vibronic peaks in the pyrene emission spectrum can serve as a monitor of pyrene microenvironment polarity.[32] I_1/I_3 decreases as pyrene is transferred from a hydrophilic to a more hydrophobic microenvironment and it is well established that this type of change is seen when micelles or clusters are formed both in the absence[32] and in the presence[9,34,56,57] of polymer.

Figure 6b shows the relation between the intensity ratio I_1/I_3 of pyrene and the monomer to excimer intensity ratio I_M/I_E of P3P for EHEC/SDS/water solutions as a function of the SDS concentration. As the polymer−surfactant clusters are formed at and above c_1, the microviscosity increases simultaneously as the micropolarity decreases in the system. The micropolarity of the clusters is almost constant and comparable to that of ordinary SDS micelles[9,34] approximately from c_1 and upward when the surfactant concentration is raised until also normal and not polymer-bound micelles are formed in the solutions. Both these fluorescence probe techniques agree extremely well concerning the determination of c_1, which equals 1.5 mM $[SDS]_{tot}$ for this specific system. The onset of interaction between EHEC and SDS at c_1 is detected at a slightly lower surfactant concentration by the use of these fluorescence probe techniques compared to other methods such as equilibrium dialysis and viscometry (see Figures 6a and 7, respectively). This has been observed and reported earlier for related systems[9,32,34,58] and can most likely be attributed to a very high sensitivity of the fluorescence probe techniques and the low concentration and high distribution coefficient of the probes to the polymer-bound surfactant clusters. Thus fluorescence probe techniques are much more sensitive to the onset of cooperative adsorption and cluster formation than to its quantitative magnitude.

The average aggregation number, N, i.e. the average number of surfactant monomers in a micelle, is a fundamental parameter for the characterization of micelles and polymer−surfactant aggregates. The steady-state fluorescence quenching method[33] was combined with results from equilibrium dialysis to determine the average aggregation number of EHEC-bound SDS clusters, N_p, according to a methodology applied and described in earlier reports.[9,10,34]

Figure 6c shows the relation between the index of microviscosity (I_M/I_E) and the average aggregation number of the polymer-bound surfactant clusters (N_p) formed in EHEC/SDS/water solutions as a function of the total SDS concentration. For this specific system, the values of N_p grow continuously from c_1 ($\approx$2 mM) toward a weakly developed maximum ($N_p \approx 53$) which is followed by a slight decrease upon raising the surfactant concentration.[9] A similar behavior with an increase in size of the polymer-bound clusters with surfactant concentration has also been found for the poly(ethylene oxide) (PEO)/SDS/water,[10,37,59] the hydroxypropyl cellulose (HPC)/SDS/water,[36] and the hydroxypropyl methyl cellulose (HPMC)/SDS/water[34] systems. As a comparison, it should be mentioned that the average aggregation number of normal SDS micelles (N_n) is reported[33,37,60] to fall between 60 and 70, which we have also experimentally verified. Evidently, the EHEC-bound SDS clusters have, for all compositions investigated, lower aggregation numbers than ordinary SDS micelles ($N_p < N_n$), which also has been reported for related aqueous polymer−surfactant systems.[9,34−37,59,61] The maximum in microviscosity is found to correspond to a very low value of N_p ($\approx$10) and in general it is seen that the regime of high microviscosity and rigidity for the polymer-bound clusters formed in these solutions correspond to aggregation numbers less than 30. These observations are consistent with a recent report by Anthony and Zana,[26] who studied the interaction between the cationic surfactant dodecyltrimethylammonium chloride (DTAC) and negatively charged polyelectrolytes (copolymers of maleic acid) in which they found the largest microviscosities for the smallest polymer-bound surfactant aggregates.

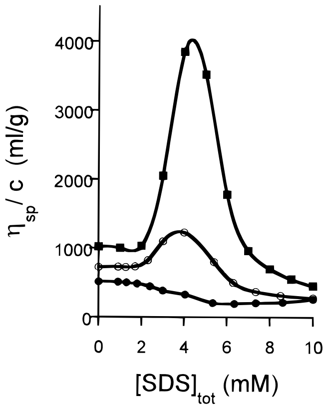

Figure 7. Bulk viscosity in the EHEC/SDS/water system presented as the reduced specific viscosity, η_{sp}/c, as a function of the total SDS concentration, $[SDS]_{tot}$, at 20 °C: (●) 0.05% EHEC; (○) 0.20% EHEC; (■) 0.30% EHEC.

It was also proposed by Anthony and Zana that the large microviscosity of polymer-bound aggregates reduces the efficiency of quenching of the probe pyrene, resulting in large values of pyrene lifetime in these aggregates. However, this is not a general behavior of binary surfactant/water solutions. For example, an opposite behavior to ours has been found for some binary aqueous micellar alkyltrimethylammonium bromides[48] where the microviscosity increases with the growth of micelles. As mentioned above, the SDS/water system gives also a slight increase in N_n and microviscosity with the surfactant concentration above cmc. When the maximum in N_p is reached for the EHEC/SDS/water system, it has been suggested[9,10] that normal micelles start to form and the average microviscosity of all clusters and micelles (polymer-bound and free) at higher surfactant concentrations is weighted toward the increasing fraction of normal micelles, resulting in asymptotically decreasing I_M/I_E values toward the level corresponding to the binary SDS/water system.

Bulk viscosity is one of the main methods used for the characterization of polymer−surfactant interactions, and it is especially useful when studying systems where strong interactions exist such as the interaction between some nonionic cellulose ethers and SDS.[7,8,34,62,63] It has been shown[7] that the hydrodynamic behavior of an aqueous bulk solution of EHEC and SDS depends critically on both polymer and surfactant concentration. Figure 7 presents the bulk viscosity behavior, here expressed as η_{sp}/c, as a function of the total SDS concentration and for three different EHEC concentrations. The polymer concentrations were selected to cover the range from well below (0.05%) to higher (0.30%) than the critical overlap concentration, c^*, which for this specific EHEC fraction (CST-103) is 0.22%[30] (defined as $1/[\eta]^{64}$ and $[\eta] = 455$ mL/g for CST103). A pronounced maximum in η_{sp}/c is developed at $[SDS]_{tot} \approx 4$ mM for the higher (0.20, 0.30%) polymer concentrations while the lowest (0.05%) passes through a small minimum as the surfactant concentration is increased. This minimum in η_{sp}/c reflects a coil shrinkage of EHEC as SDS starts to adsorb,[7] and a similar behavior has also been found in the HPMC/SDS/water system.[34] The onset of interaction (c_1) is detected to about 2 mM independent of polymer concentration with this viscometric technique. At the highest SDS concentration investigated (10 mM), it is seen that η_{sp}/c is very similar and approaches the same value for all three polymer concentrations. Figure 8 shows the corresponding measurements of the microviscosity index (I_M/I_E) for the three EHEC concentrations as a function of the total SDS concentration. The microviscosity exhibits a rather different behavior compared to the bulk viscosity although similarities between them also exist: As already shown above (in Figure 3 for 0.20% EHEC), there is a rapid increase in microviscosity from about 1 mM SDS up to a sharp and pronounced maximum at approximately 3 mM followed by an asymptotic decrease in I_M/I_E when raising the surfactant concentration further. All three polymer concentrations investigated give roughly the same qualitative behavior and even the maximum in microviscosity seems to be almost unaffected by the polymer concentration. This reflects the similarity in rigidity and ordering of the clusters formed below and above c^* and points to the important difference between the behavior of bulk and microscopic levels of a polymer−surfactant solution such as the EHEC/SDS/water system investigated. In binary EHEC/water solutions, I_M/I_E is constant at concentrations up to c^* (0.05−0.20%). The polymer concentrations above c^* (0.30%) give a significantly higher index of microviscosity, indicating an increased rigidity of these polymeric clusters probably formed intermolecularly. In a number of studies of amphiphilic self-aggregating polymers, the formation of intra- and intermolecular polymeric micelles or clusters has been reported.[19−24,65] The "shoulder" in the microviscosity curve after the maximum (which seems to be related to the aggregation state of EHEC and the SDS adsorption to EHEC (see discussion above)) seems to be shifted toward higher $[SDS]_{tot}$ and also to decrease for 0.30% EHEC, consequently leading to higher values of microviscosity for this higher polymer concentration, for a given surfactant concentration. However, it must be remembered that the adsorption differs among the three polymer concentrations compared; i.e. the adsorbed amount of SDS per gram of EHEC (y) is lower for the highest EHEC concentration at a given $[SDS]_{tot}$.

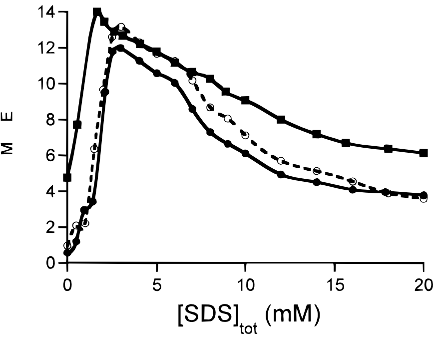

Figure 8. Monomer to excimer intensity ratio of P3P, I_M/I_E, as a function of the total SDS concentration, $[SDS]_{tot}$, at 20 °C: (●) 0.05% EHEC, (○) 0.20% EHEC, and (■) 0.30% EHEC.

The microviscosity results obtained in this study of the EHEC/SDS/water system give new information on the complex interaction pattern and support a previously proposed model.[7,9,10] As SDS is added to an aqueous EHEC solution, the interaction takes place only at surfactant concentrations larger than a specific concentration here termed c_1. At c_1 SDS is adsorbed cooperatively to the polymer as small micelle-like clusters which grow in size (or aggregation number) as the surfactant concentration is increased.[9] The small EHEC-bound clusters (with $N_p < 30$) formed at surfactant concentrations higher than, but still close to, c_1 have a very high microviscosity which is several times larger than the microviscosity of ordinary free SDS micelles, a behavior also observed by others.[26,28,29] This effect of a high ordering and rigidity of the cluster and solution structure derives most likely from a high polymer content in these small polymer-bound clusters where a single or several polymer chains (depending on the polymer concentration) may wrap around and solubilize its hydrophobic segments in a cluster. Probably the covalent bonds existing between polymer segments also contribute to a stabilization of these "mixed micelles" (clusters) between SDS and EHEC which may hinder the motional freedom of a probe and hence give rise to this high microviscosity. In the composition range investigated, the microviscosity has been shown to be almost invariable with the polymer concentration, in contrast to the bulk viscosity. In a dilute polymer solution ($c_p < c^*$), there is an intramolecular clustering process operating which leads to coil shrinkage and a decrease in bulk viscosity, while for higher polymer concentrations ($c_p \geq c^*$), the clustering adsorption tends to become intermolecular in nature where one cluster is shared by several polymer chains creating a three-dimensional network and high bulk viscosities.[7,9] Evidently, even though the macroscopic level of viscosity shows large variations with polymer concentration, the polymer-bound clusters monitored by the fluorescence probes have almost identical structure concerning micropolarity (I_1/I_3)[9] and microviscosity as the content of polymer in a solution is varied. This behavior illustrates the difference in mechanism creating the maximum in bulk viscosity and microviscosity, which occurs at almost the same surfactant concentration. The maximum in bulk viscosity derives from a three-dimensional network of polymer and cluster tie points, while the maximum in microviscosity is related to a high polymer content of the surfactant clusters; i.e. the lower the aggregation number, the higher is the polymer content and consequently the microviscosty (a relation also found for a polyelectrolyte/surfactant system[26]). As the SDS adsorption to EHEC continues, the relative fraction of polymer in each cluster decreases and consequently the microviscosity as well. The tendency to form networks is lost at higher $[\mathrm{SDS}]_{\mathrm{tot}}$ when normal free micelles are formed into which the polymer also can solubilize its hydrophobic sites or segments, resulting in a decrease in bulk viscosity. This molecular description of the formation and growth of EHEC/SDS clusters is in harmony with the recent statement by Piculell et al.,[15] who argued that a polymer/surfactant network, such as the one formed in the EHEC/SDS system, breaks down as soon as the added surfactant dominates in the clusters.

The c_1 for this specific EHEC/SDS/water system is found to fall between 1 and 2 mM SDS[7,9,10,12] where the fluorescence probe techniques utilizing P3P (microviscosity sensitive) and pyrene (micropolarity sensitive) are in high correlation and found in the lower part of the c_1 range. The effective microviscosity of ordinary SDS micelles as determined with P3P and BMN were 20 and 3 cP, respectively. In these experiments calibration curves of ethanol/glycerol mixtures of known viscosities were used,[13] although as already mentioned above, the procedure can be criticized since no continuous phase can perfectly represent an organized assembly such as a micelle or a polymer-bound cluster.[16,17,41,42] A comparison with literature values also reveals a large variation in microviscosity of SDS micelles using different fluorescence probe techniques but the major part of the reported values is about 20 cP. Our values found for the SDS micelles are in good agreement with other studies in which P3P[43,66] (19−21 cP) and for BMN, a closely related malononitrile dye were used[55] (6−9 cP). The effective microviscosity of the EHEC-bound SDS clusters is much higher than in ordinary SDS micelles—at least 10 times higher. An increased microviscosity of polymer-bound clusters compared to normal free micelles has also been observed in other polymer/surfactant systems[26,28,29] Some studies of amphiphilic self-aggregating polymers and surfactants have reported a somewhat different behavior compared to the EHEC/SDS/water system in which the microviscosity decreases when going from polymeric micelles to polymer−surfactant aggregates and eventually to ordinary micelles.[28,29] A forthcoming report will treat several different nonionic polymer/surfactant systems in order to test the generality of the observations in this paper.

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