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ORIGINAL CONTRIBUTION

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Aggregation numbers of SDS micelles formed on EHEC. A steady state fluorescence quenching study

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Abstract The present investigation proves that in the interaction between an uncharged polymer and a negatively charged amphiphilic ion (surfactant) clusters are actually formed and it provides data for the cluster concentration and the cluster size and their variation with composition. The polymer bound cluster size increases after a certain critical surfactant concentration and passes through a maximum. This maximum cluster size decreases with decreasing polymer concentration and attains a limiting value at infinite dilution. For the highest polymer concentration the cluster size is close to the size of normal surfactant micelles. The cluster concentration was determined by a fluorescence quenching technique and the amount surfactant adsorbed to the polymer by dialysis equilibrium measurements. Combining these independent sets of data permits the cluster aggregation number to be unambiguously determined. Solubilization experiments indicate the possibility to regulate the amount solubilized by varying the polymer concentration. The molecular properties of the system are sensitively monitored by the variation in two vibronic peaks in the pyrene fluorescence emission spectrum which defines a "hydrophobic index." Very good agreement is found between all three experimental methods. Finally, the model suggested is analyzed in terms of coil size and cluster-cluster distance. Depending upon the degree of adsorption saturation and the density of polymer segments in solution the interaction may switch from being intramolecular to becoming intermolecular.

Key words Aggregation numbers – ethyl hydroxyethyl cellulose – sodium dodecyl sulphate – interaction – fluorescence quenching – dye solubilization

Introduction

Recent investigations [1–4] on systems containing an uncharged polymer and a negatively charged amphiphilic ion (surfactant ion) in water solution have revealed the possibility of strong interaction, especially in certain concentration intervals containing the CMC point. It has also been found that for many polysaccharides, and in particular the cellulose derivatives, the situation is complex due to the mixed hydrophobic/hydrophilic structure. The hydrodynamic properties of such systems often change dramatically with polymer concentration. Thus the coil volume of a chain polymer has been found to change by a factor of four over fairly limited concentration changes of surfactant [1]. The hydrodynamic effect is enhanced in a nonlinear way by an increase in polymer concentration [1, 5]. Thermodynamic properties are also markedly affected as can

be seen from equilibrium dialysis experiments [1, 6]. These effects observed seem to depend critically on the chemical nature of the polymer. In particular, the balance between hydrophobic and hydrophilic properties are of importance. The most conspicuous effects occur in a concentration interval of the amphiphile from zero up to say twice the CMC concentration. The presence of small amounts of electrolytes will also change the system properties remarkably [2, 7]. The reason for these strong interactions seem to relate to a preferential cooperative adsorption (clustering) of the amphiphile to hydrophobic sites on the polymer chain. The main indication for this is the abrupt onset of redistribution (adsorption of amphiphile) at a well defined amphiphile concentration, as seen from dialysis data [1].

For the specific system ethyl hydroxyethyl cellulose (EHEC)/sodium dodecyl sulphate (SDS)/water there is also a very pronounced difference between a solution dilute with respect to polymer and a solution less dilute. In dilute polymer solutions the clustering adsorption is assumed to be entirely an intramolecular phenomenon that leads to coil shrinkage (decrease in hydrodynamic volume) when the surfactant is added. The shrinkage is interpreted to be due to multiple mixing of hydrophobic substituents (side chains) of the polymer in the same surfactant cluster. At higher polymer concentrations the surfactant clusters (micelles) are probably shared intermolecularly and some sort of three-dimensional network forms which explains the considerable viscosity increase observed [1]. Even "normal" polymer-polymer interactions will of course also contribute to the overall effect.

So far, mainly indirect arguments for this model have been available, but recent NMR studies [8, 9] in our laboratory support the cluster model just presented. With all this accumulated general knowledge it was judged important to try to apply some independent method by which the size of the clusters could be determined as well as the linear cluster density along the polymer chain. It is well known that fluorescence spectroscopy and fluorescence quenching is an appropriate technique for this [10, 11] since it allows a determination of the molar concentration of clusters. The data treatment that ultimately gives the average number, N, of molecules in the cluster must then resort to some determination or model calculation concerning the amount of redistribution of the amphiphile to the polymer. In our case dialysis equilibrium data are available [1, 6] that allow an unambiguous calculation of N from the spectroscopic data.

In this paper results will be presented for the cluster aggregation number in the system EHEC/SDS/water at 20 °C as a function of surfactant concentration up to well above the CMC point and for polymer concentrations up to slightly above the critical overlap concentration. The results are obtained from a combination of spectroscopic and quenching data with those of dialysis equilibrium measurements.

Experimental

Materials

Ethylhydroxyethylcellulose, (EHEC; fraction CST-103, MSeo = 0.7, DSethyl = 1.5) with a weight average molecular weight (Mw) of approx. 480 000 as determined from classical light scattering, was obtained from Berol Kemi AB, Stenungsund, Sweden. The cloud point (CP) of EHEC/CST-103 was observed in the interval 28–37 °C depending on the polymer concentration [4]. A typical EHEC structure is shown in Fig. 1. The standardized procedure adopted to make an aqueous EHEC stock solution is described elsewhere [1]. All EHEC/SDS solutions in this study were prepared by weighing the required

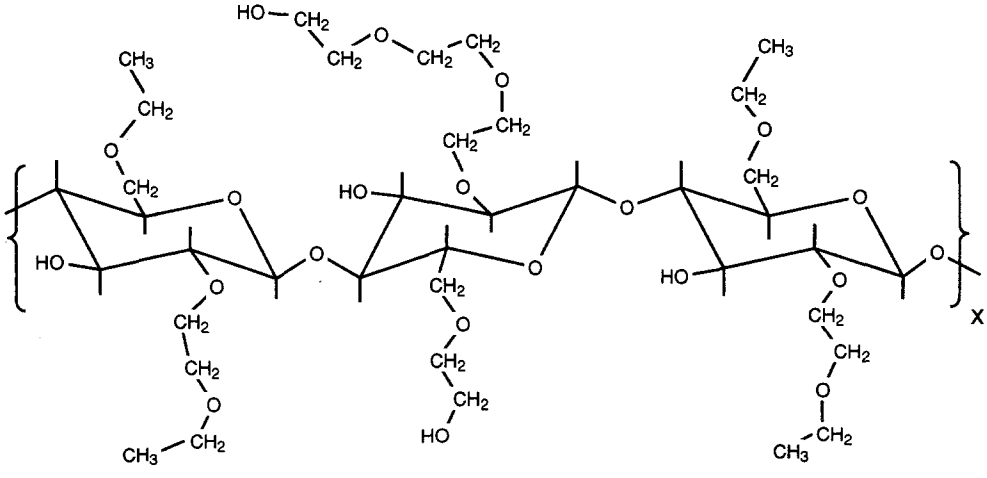


Fig. 1 Possible structure segment in ethyl(hydroxyethyl)cellulose (EHEC) with a $MS_{eo} = 2$, $DS_{ethyl} = 1.3$ and DP(degree of polymerization) = $3x$

amounts of the EHEC stock solution into appropriately diluted SDS stock solution at least 12 h before adding quencher (Benzophenone) or dye (Oil Orange SS) to let the time-dependent effects previously reported settle [4]. EHEC concentrations are expressed in percent by weight (% (w/w). SDS concentrations are calculated as moles per 1000 grams of solvent (molal) but since all solutions are dilute they will be given on the molar scale.

Analytical grade sodium dodecyl sulphate (SDS) was obtained from Merck, the radioactive SDS was obtained from Amersham, the quencher Benzophenone (+99%) from Aldrich and the dye Oil Orange SS (*o*-Toluenazo-*β*-Naphtol) from Tokyo Kasei Inc. and they were all used as supplied. The probe pyrene (+98%) was obtained from Janssen Chimica and twice purified by recrystallisation from absolute ethanol. All solutions were prepared with MilliQ water (Millipore) as solvent.

Instrumentation

The UV absorption was recorded on a Beckman DU-68 Spectrophotometer using ordinary quarts cuvettes with 10 mm path length. A Hitachi F-4000 Fluorescence Spectrophotometer was used to obtain the steady-state fluorescence spectra. The sonications were performed on a Engisonic instrument (typenr. B12).

Dye solubilization measurements

Solubilization of Oil Orange SS was studied by determination of the absorbance spectrophotometrically at 495 nm. EHEC/SDS solutions were prepared as described above. An excess of dye was then added and the solutions equilibrated for 3 days at room temperature on a rotating table (Infors AG CH-4103 Bottmingen). The excess of dye was then separated off by centrifugation and the absorbance of the supernatant determined, dilutions being performed for supernatants of absorbance greater than 1.3. All absorbances were recalculated to undiluted solutions.

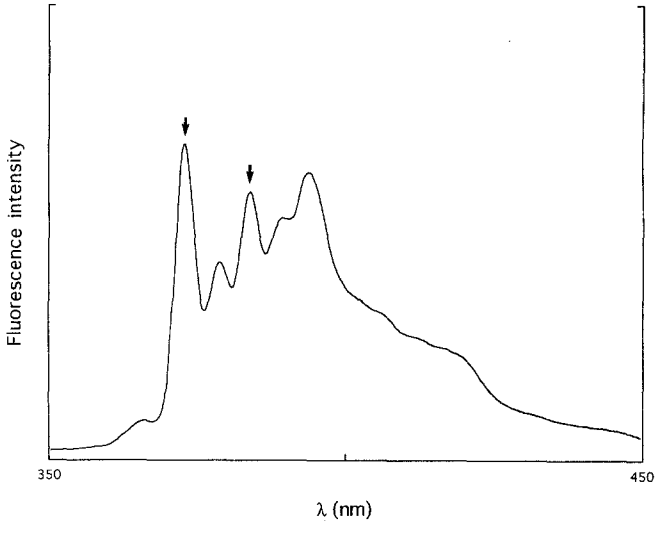
Fluorescence measurements

Emission spectra ($\lambda = 350$–450 nm) were recorded at room temperature for the probe (pyrene). Wavelength 334 nm was selected for excitation and the bandwidths set to 3 nm for excitation and 1.5 nm for emission. In the determination of mean aggregation numbers, N, ratios of fluorescence intensities in the presence (I) and absence (I^0) of quencher (benzophenone) were calculated as an average between the intensity ratios at the first vibronic peak ($\lambda = 373$ nm) and at the third vibronic peak ($\lambda = 384$ nm) in the pyrene emission spectra (see Fig. 2). EHEC/SDS solutions for spectroscopic analysis contained a pyrene concentration less than 10^{-6} M (filtered saturated water solution) and the benzophenone concentration was varied from 0 to 0.2 mM. Solutions for quenching experiments were prepared as follows. An SDS stock solution was prepared by dissolving SDS powder in pyrene saturated water. After appropriate dilution of the SDS stock with the pyrene saturated water, EHEC stock was added as the last component to get the desired overall composition. A portion of the EHEC/SDS solution containing pyrene was saturated with benzophenone by sonication for 20–30 min. After centrifugation and separation of the supernatant, aliquots of the benzophenone saturated solution were added to the EHEC/SDS/pyrene solutions to cover the desired benzophenone concentration range. The benzophenone concentrations were determined spectrophotometrically at $\lambda = 255$ nm, dilutions being performed for solutions of absorbance greater than 1.3.

Molar micelle concentrations [Micelles] were determined from plots of the logarithmic intensity ratio $\ln (I^0/I)$ versus the quencher concentration $[Q]$ according to the method of Turro and Yekta [10]. Average aggregation numbers N were then calculated by dividing the amount of amphiphile redistributed – as determined in dialysis equilibrium experiments [1, 6] – by the micelle concentration from the quenching measurements (see Theory).

The "hydrophobic index," I_1/I_3, was taken as the ratio between the intensities at the first ($\lambda = 373$ nm) and the

Fig. 2 Emission spectra for pyrene in water. Peaks 1 ($\lambda = 373$ nm) and 3 ($\lambda = 384$ nm) used in the determination of the hydrophobic index and (I^0/I) ratios are marked with arrows



third (λ = 384 nm) vibronic peaks in the pyrene emission spectra [12] (see Fig. 2 and Theory section).

Dialysis

Equilibrium dialysis experiments were carried out between EHEC SDS-solutions, the SDS solution containing a small amount radioactive SDS. Approximately 25 000 dpm/mL of activity was employed in the experiments. The experiments were performed in a dialysis cell made up of two compartments divided by a membrane (Spectrapor, mw cut-off 12–14000). The cells were rinsed throughly with deionized water before use. After the water had been removed the EHEC solution and the SDS solution were filled into their respective cell compartments. In all cases each experiment was run in triplicate in three individual cells to ensure the reliability of the results. Preliminary results indicated equilibrium was established after 48 h, but 7 days were allowed for equilibration as a precaution. The concentrations of DS-ions in the EHEC-SDS and the SDS solutions respectively were determined by scintillation counting. Experiments indicated the presence of EHEC did not cause an extinction of the scintillation.

Theory

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. Fluorescence probe techniques are among the most reliable ones for the determination of N since they are quite insensitive to intermicellar interactions [11]. This method is also quite useful in monitoring polymer-surfactant interactions [13–26]. The steady-state fluorescence method proposed by Turro and Yekta [10] has been applied in this study with pyrene as the luminescent probe and benzophenone as quencher, Q [16, 27]. Although this method has been subject to some debate [28, 29] it has been found to give reliable determinations of N when the experimental conditions are chosen with care. This means that a highly efficient probe/quencher pair should be employed, there should be a negligibly small fraction of probe and quencher in the aqueous phase, as well as low probe concentration, and the micelles (clusters) should be relatively small, approximately $N < 100$. Under these circumstances the measured ratio of luminescence probe intensities I^0/I, in the absence I^0, and presence I of quencher is related to the quencher concentration according to the expression

$$I/I^0 = \exp(-[Q]/[\text{Micelles}]) . \quad (1)$$

Normally, $[Q]$ is varied and the average molar concentration of micelles, [Micelles], can be obtained by regression calculation.

If one then, by some other experiment, can determine the molar amount of amphiphile (surfactant) that is redistributed to the micellar form, the average aggregation number N, can be calculated simply by dividing the redistributed amount by the micellar concentration. In the presence of a polymer the onset of cluster formation normally begins well below the normal CMC of the surfactant. In this case, one can assume that, at least up to concentrations somewhat below the normal CMC, all redistribution of surfactant derives from adsorption to the polymer. Hence, N will unambiguously give the aggregation number for clusters bound to the polymer. (For a more precise evaluation see section Results and discussion.) For surfactant concentrations close to and above CMC there will also be normal micelles present and the total micelle molarity [Micelles] then consists of two parts. If $[M]_p$ denotes the molarity of micelles (clusters) bound to polymer and $[M]_n$ denotes normal micelles, we have

$$[\text{Micelles}] = [M]_p + [M]_n . \quad (2)$$

Furthermore, if we let $[S]_{tot}$ denote the total molar concentration of surfactant and $[S]_{free}$ the molar concentration of free surfactant, i.e., *not* bound in any type of cluster or micelle, we have in general

$$\langle N \rangle = ([S]_{tot} - [S]_{free})/[\text{Micelles}] , \quad (3)$$

where $\langle N \rangle$ denotes some average aggregation number for all types of clusters present. In the special case that $[S]_{free} \leq$ CMC, the average number $\langle N \rangle$ as calculated from (3) will be identical to the aggregation number for clusters bound to the polymer, N_p. However, in the general case when the surfactant concentration is so high that also normal micelles are formed, $\langle N \rangle$ will be some average number of N_p and N_n, where N_n denotes the aggregation number for normal micelles.

In an equilibrium dialysis experiment one obtains the quantities $[S]_{tot}$ and $[S]_{eq}$, where $[S]_{eq}$ is the molar concentration of surfactant in equilibrium with polymer (possibly also including normal micelles). In the general case when $[S]_{eq} >$ CMC, it is then assumed that N_n is given by

$$N_n = ([S]_{eq} - \text{CMC})/[M]_n , \quad (4)$$

and for N_p one then gets

$$N_p = ([S]_{tot} - [S]_{eq}/[M]_p \quad (5)$$

$$[M]_p = [\text{Micelles}] - [M]_n = [\text{Micelles}] - ([S]_{eq} - \text{CMC})/N_n . \quad (6)$$

Under the fairly realistic assumption that N_n varies with $[S]_{eq}$ in a similar way as it varies with the total surfactant concentration in a solution free of polymer, N_p calculated according to (5) will unambiguously give the average aggregation number for clusters bound to polymers. The size of normal micelles was determined in separate experiments and agreed with literature data [10, 21, 30–32]. It should be observed that $[S]_{eq}$ denotes the equilibrium value determined in a dialysis experiment *corrected for the Donnan effect*.

Often one has for the calculation of N_p assumed the existence of a critical adsorption concentration, denoted CAC, for the interaction of surfactant with a polymer [15, 16, 25] such that all surfactant in excess of CAC is adsorbed until saturation of the polymer. Since our dialysis data as well as for instance the solubilization results to be discussed later do not support this assumption, such a procedure for the calculation of N_p has not been adopted here. This point is further discussed under the section Results and discussion.

It cannot be excluded, especially for higher polymer concentrations, that in the vicinity of CMC there will be competitive equilibria between cluster formation on the polymer chain and formation of normal micelles. In this case the equations given here must be modified. Furthermore, at higher concentrations the large number of highly charged clusters per volume unit may lead to coulombic interaction. Although the equations given in this section will be used throughout this study, for the data treatment the problems just mentioned will be discussed in connection with the results.

Results and discussion

Fluorescence quenching experiments have been performed on the system EHEC/SDS/water according to the method by Turro and Yekta [10] and using pyrene as a probe and benzophenone as quencher. The measurements have been performed for selected values of polymer (EHEC) concentration in the range 0–0.2% by weight and for surfactant (SDS) contents from 0 up to 22 mM, the maximum amount depending upon polymer concentration. An example of the pyrene emission spectrum obtained with the excitation wavelength set to 334 nm is shown in Fig. 2. From such spectra the intensity maxima I_1 and I_3 are determined. The average ratio of fluorescence intensities (I^0/I) in the presence (I) and absence (I^0) of quencher were calculated as the arithmetic average of I_1^0/I_1 and I_3^0/I_3. In Fig. 3 the logarithm of the ratio I^0/I is plotted for one high and one low polymer concentration versus quencher concentration. For the low polymer concentration there is a linear regime with a slight downward curvature at elevated quencher concentrations as observed also by others using the same probe/quencher pair [16]. From such plots the initial slope has been determined and its inverse has been put equal to [Micelles] (see Theory section). The values are given in Table 1.

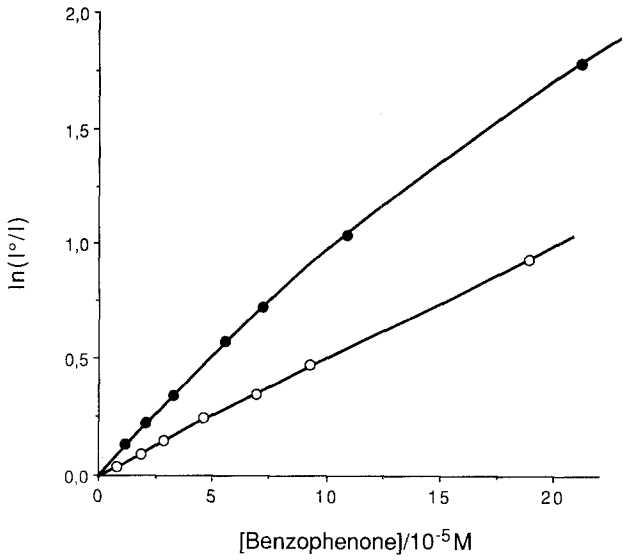


Fig. 3 Plot of $\ln(I^0/I)$ for pyrene (conc. $< 10^{-6}$ M) as a function of benzophenone concentration in aqueous EHEC/SDS-solutions at 20 °C for determination of cluster concentration according to Eq. (1). ○ 0.20% EHEC + 11.5 mm SDS, ● 0.03% EHEC + 11.5 mm SDS

From studies of the dialysis equilibrium in the same system [1, 6] data are available for $[S]_{tot}$ and $[S]_{eq}$ as defined in the Theory section. In this discussion of experimental results we will more specifically write SDS instead of the more general designation S. From such dialysis data the values of $[SDS]_{tot}$ and $[SDS]_{eq}$ in Table 1 have been calculated according to the model described in a previous paper [1], Fluorescence quenching measurements on the binary system SDS/water give values of N_n, as defined in the Theory section.

Combining the values of [Micelles], $[SDS]_{tot}$, $[SDS]_{eq}$, and N_n the average cluster aggregation number of polymer bound SDS clusters, N_p, has been calculated according to Eqs. (5) and (6). The results are found in Table 1. To illustrate the effects observed, the values of N_p have been plotted versus $[SDS]_{tot}$ and $[SDS]_{eq}$, see Figs. 4 and 5, respectively. The dialysis results are plotted in Fig. 6. It is obvious that dialysis equilibrium depends markedly both qualitatively and quantitatively on the polymer concentration. To further illustrate the dialysis equilibrium features the amount of surfactant redistributed to the polymer, y, expressed as millimoles per gram, is plotted versus $[SDS]_{eq}$ in Fig. 7 for one polymer

Table 1 Summary of experimental data and calculated values, especially average aggregation numbers and cluster concentrations in the EHEC/SDS/water system; n = number of glucose units per polymer bound SDS cluster and m = number of polymer bound SDS monomers per glucose unit.

EHEC %	$[SDS]_{tot}$ mM	$[SDS]_{eq}$ mM	N_p	$[Micelles]_p$ mM	$[Micelles]_{tot}$ mM	m	n	β
0.20	2.50	2.20	3.4	0.088	0.088	0.04	89	277
	3.50	2.55	8.2	0.115	0.115	0.1	68	223
	5.00	2.95	16.6	0.124	0.124	0.3	63	189
	7.00	3.42	21.5	0.167	0.167	0.5	45	196
	9.00	3.82	30.9	0.167	0.167	0.7	45	185
	10.5	4.00	32.6	0.199	0.199	0.8	39	180
	11.5	4.10	37.6	0.197	0.197	0.9	40	178
	13.0	4.28	43.8	0.199	0.199	1.1	39	176
	15.0	4.40	54.3	0.195	0.195	1.4	40	171
	17.0	5.00	58.0	0.207	0.207	1.5	38	177
	20.0	8.15	53.5	0.221	0.239	1.5	35	197
	22.0	13.6	50.8	0.165	0.265	1.1	48	191
0.05	2.50	2.40	0.9	0.114	0.114	0.05	17	604
	5.00	4.25	6.8	0.110	0.110	0.4	18	227
	7.00	5.40	15.7	0.101	0.101	0.8	19	201
	9.00	6.15	29.4	0.097	0.097	1.5	20	179
	11.0	7.15	37.3	0.103	0.106	2.0	19	185
	13.0	9.70	40.5	0.082	0.124	1.7	24	170
	15.0	12.2	34.6	0.081	0.161	1.4	24	166
	17.0	14.5	35.0	0.072	0.184	1.1	27	163
0.03	5.00	4.50	4.9	0.102	0.102	0.4	12	170
	8.00	6.10	22.0	0.086	0.086	1.6	14	122
	10.0	7.80	33.4	0.066	0.078	1.9	18	136
	11.5	8.65	33.2	0.071	0.097	2.0	16	144
	12.0	10.0	31.4	0.062	0.110	1.7	19	136
	13.0	11.0	30.6	0.065	0.127	1.7	18	135
	15.0	12.2	31.8	0.088	0.168	2.4	13	137

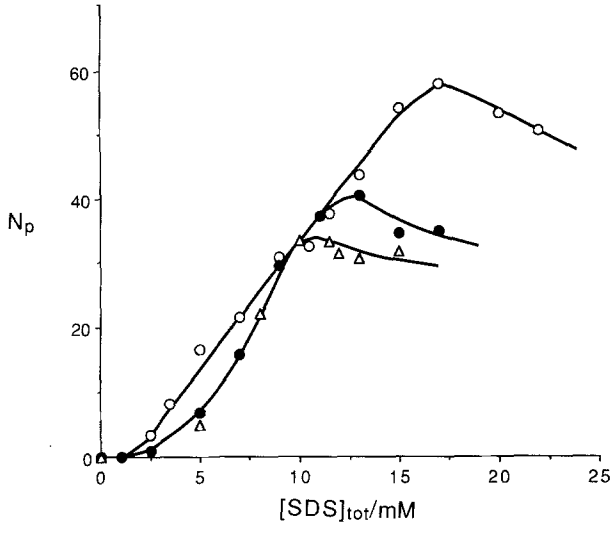


Fig. 4 Average aggregation number of polymer bound SDS clusters, N_p, as a function of $[SDS]_{tot}$ at 20 °C. ○ 0.20% EHEC, ● 0.05% EHEC, △ 0.03% EHEC

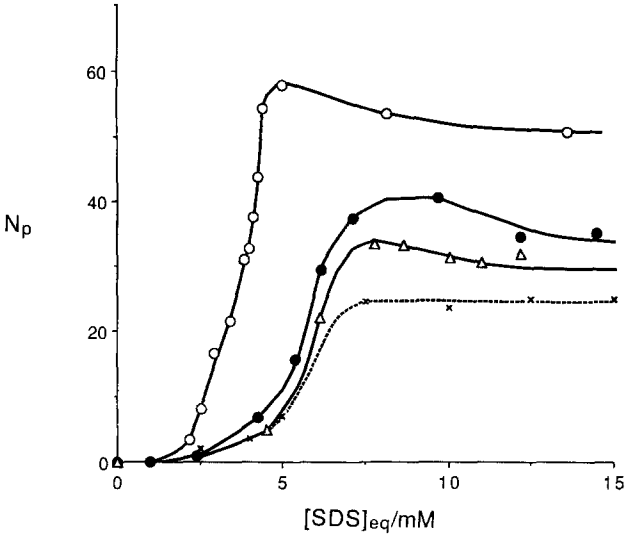


Fig. 5 Average aggregation number of polymer bound SDS clusters, N_p, as a function of $[SDS]_{eq}$ at 20 °C. ○ 0.20% EHEC, ● 0.05% EHEC, △ 0.03% EHEC, × 0% EHEC (extrapolated values)

concentration. The specific features of the dialysis equilibrium and the temperature dependence, will be discussed in more detail elsewhere [5, 6].

Figure 6, which is only based on dialysis equilibrium data, shows some fundamental features of the system worth noting before going further in the analysis. In the first place there is an onset of redistribution for $[SDS]_{eq} = [SDS]_{tot} \approx 2$ mM (previously denoted the "foot point").

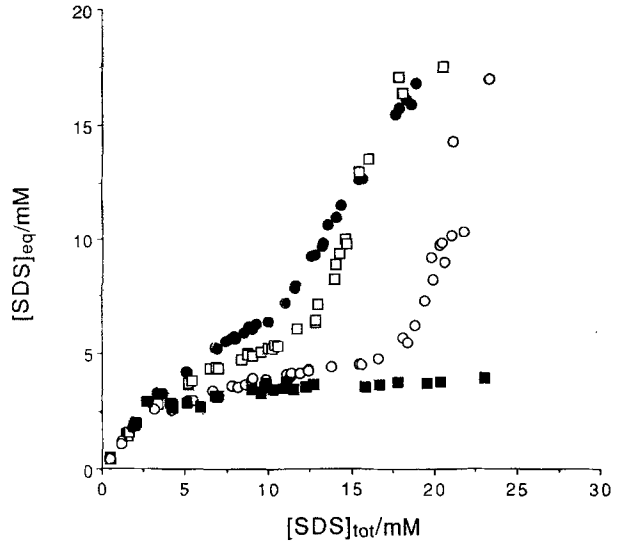


Fig. 6 Equilibrium dialysis data at 20 °C in the EHEC/SDS/water system plotted as $[SDS]_{eq}$ versus $[SDS]_{tot}$. ■ 0.50% EHEC, ○ 0.20% EHEC, □ 0.10% EHEC, ● 0.05% EHEC. Dialysis data for 0.03% EHEC, used in the calculation of N_p, was extrapolated from the primary data of the higher EHEC concentrations presented in this figure

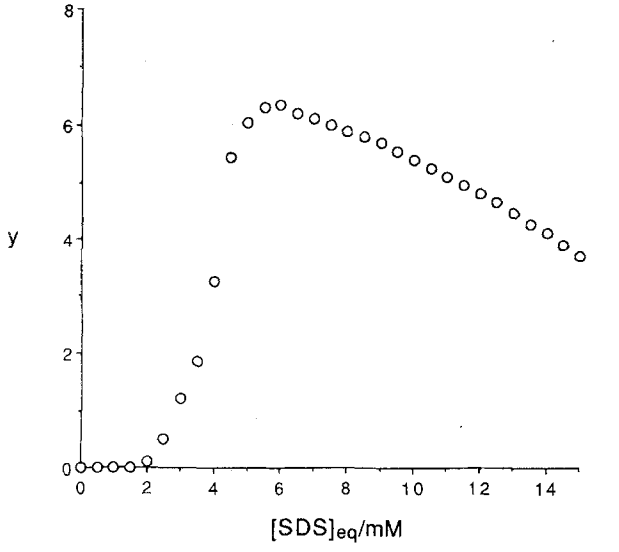


Fig. 7 Equilibrium dialysis data at 20 °C presented as a plot of y (mmoles of SDS bound per gram of EHEC) as a function of $[SDS]_{eq}$ for aqueous EHEC/SDS solutions. The EHEC concentration is 0.20%

This feature is independent of polymer concentration and marks the onset of redistribution of surfactant to the polymer. It reveals itself as a slower increase of $[SDS]_{eq}$ with increasing $[SDS]_{tot}$ above the foot point in Fig. 6 or as the onset of increase of y in Fig. 7. As the total amount of SDS present in the system increases further, one reaches a second "point of change" after which $[SDS]_{eq}$ begins to change more quickly again with $[SDS]_{tot}$ although still in a linear way. This second point of change occurs for $[SDS]_{eq} \approx$ CMC when the polymer concentration is low but the value of $[SDS]_{eq}$ for this change decreases as the polymer concentration is increased. The slope of the curve $[SDS]_{eq}$ vs. $[SDS]_{tot}$ becomes almost 1, i.e., similar to the slope before the "foot point," for the lowest polymer concentration, but has a higher value for the higher polymer concentrations. Evidently, normal micelles begin to form at this point. Whether or not the adsorption to the polymer still continues cannot be judged unequivocally from these curves. For the present investigation it should be noted, however, that *up to this point* the equilibrium dialysis provides very reliable data for the calculation of N_p.

The values of N_p, calculated as described above, show the same "foot point" as the dialysis equilibrium results after which there comes a considerable and almost linear increase in N_p with $[SDS]_{eq}$. It is found that $N_p < N_n$ for all compositions within the range investigated. It is also found that the maximum value of N_p attained decreases with decreasing polymer concentration and levels off without a maximum at about $N_p = 25$ (extrapolated value) in a solution infinitely dilute with respect to polymer, see Fig. 5. This should be compared with $N_n \approx 60$–70 for normal SDS micelles [10, 21, 30–32].

It is worth noting, by comparing Figs. 6 and 7, that the second point of change in the dialysis data corresponds to the maximum in N_p.

When N_p is plotted versus $[SDS]_{eq}$ (see Fig. 5) there are also indications of a "break point" halfway between the foot point and maximum. This break point comes out more clearly for the higher polymer concentrations. It seems to mark a composition where the rate of increase of the number of clusters per volume unit, i.e. [Micelles], begins to level out whereas the cluster size increases abruptly, see Fig. 8.

With reference to some remarks in the Theory section concerning the correction for normal micelles it is evident from the previous discussion that up to the "second point of change" the results given for N_p are entirely unequivocal. However, above this point it is unclear to what extent normal micelles begin to form and if and how the adsorption to the polymer continues. As mentioned earlier, this will affect the calculation of N_p. However, we will not carry this discussion any further here but intend to come back to this important matter in the future.

The hydrophobic index, I_1/I_3, as defined in the Theory section can be used to further clarify the solution structure at the molecular level. The ratio I_1/I_3 of the first ($\lambda = 373$ nm) and the third ($\lambda = 384$) vibronic peak in the

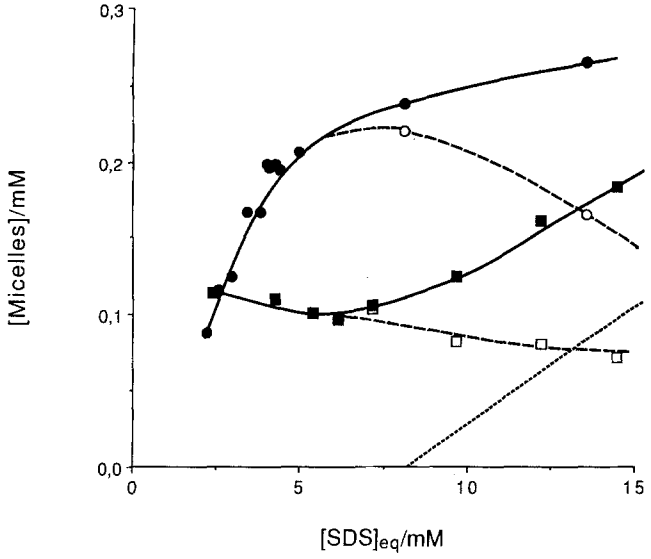


Fig. 8 Total and polymer bound cluster concentrations ($[Micelles]_{tot}$ and $[Micelles]_p$ respectively) as a function of $[SDS]_{eq}$ for aqueous EHEC/SDS solutions at 20 °C. $[Micelles]_{tot}$: ● 0.20% EHEC, ■ 0.05% EHEC. $[Micelles]_p$: ○ 0.20% EHEC, □ 0.05% EHEC. The dotted line ((- - - -) refers to an "ideal" reference situation in the binary SDS/water system assuming a fixed aggregation number 65 and CMC = 8.2 mM

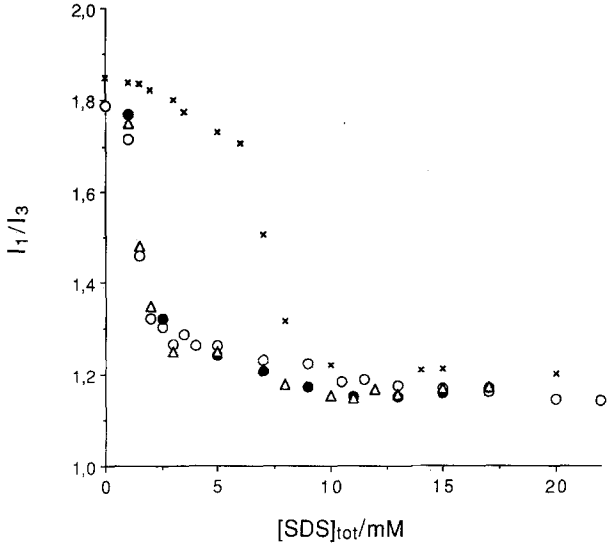


Fig. 9 Hydrophobic index (I_1/I_3) for pyrene (conc. $< 10^{-6}$ M) luminiscence in aqueous EHEC/SDS-solutions as a function of $[SDS]_{tot}$ at 20 °C. ○ 0.20% EHEC, ● 0.05% EHEC, △ 0.03 EHEC, × 0% EHEC

pyrene emission spectrum, see Figs. 2 and 9, is known to be a monitor of the pyrene microenvironment polarity [12, 33, 34]. This ratio is commonly used as a sensitive indicator of micelle or cluster formation both in the absence and presence of polymer [16, 23, 25, 26, 33].

From Fig. 9, where intensity ratios I_1/I_3 are given for water solutions containing either only the surfactant, SDS, or both surfactant and polymer, EHEC, the sensitivity of the technique is evident. For solutions containing only SDS, the ratio I_1/I_3 decreases sharply in the interval 6–8 mM SDS (inflexion point at about 7 mM SDS), indicating the formation of normal micelles into which pyrene is preferentially distributed with a high distribution coefficient. It is also evident that the presence of polymer (EHEC) even in minute amounts (0.03% by weight) dramatically shifts the point of decrease of the ratio I_1/I_3 to a narrow region at 1–2 mM SDS (inflexion point at about 1.5 mM SDS).

Furthermore, this decrease appears to be fairly independent of the polymer concentration. As indicated earlier, the surfactant concentration at such a point is often termed *critical adsorption concentration*, denoted CAC. For the system at hand the CAC value correlates well with previously reported results from viscometry and equilibrium dialysis [1], as well as with the solubilization data given in Fig. 10.

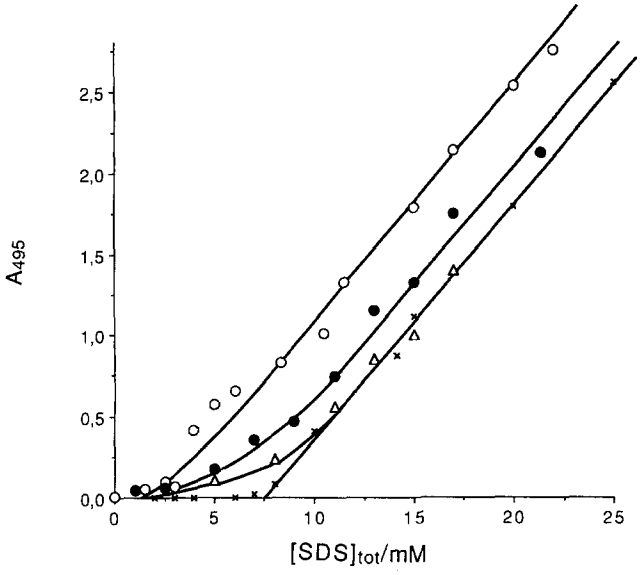


Fig. 10 Absorbance at 495 nm of solubilized Oil Orange SS, A_{495}, as a function of $[SDS]_{tot}$ in aqueous EHEC/SDS-solutions at 20 °C. ○ 0.20% EHEC, ● 0.05% EHEC, △ 0.03 EHEC, × 0% EHEC

By several techniques a fairly sharp CAC point can thus be experimentally defined in the sense that it indicates *the onset of redistribution*. It is sometimes assumed that all surfactant in excess of CAC is adsorbed until saturation on the polymer [3, 14–16, 21, 22, 24, 25, 29, 35]. Our equilibrium dialysis data as well as the solubilization results

contradict this assumption. In the system EHEC/SDS/water it is correct only for sufficiently high polymer concentrations, cf. Fig. 6. For this reason the CAC value has not been used in the calculations of N_p, but rather the dialysis equilibrium data, as already explained.

Since the normal CMC for SDS at 25 °C, as determined by many classical techniques, is often found in an interval 8.0–8.3 mM [4, 36], it should be noted that the response of I_1/I_3 to the molecular changes occurs at somewhat lower surfactant concentrations than indicated by such techniques. The same observation holds for the "foot point," normally at 2 mM in our system. The fact that the onset of interaction between EHEC and SDS is sampled at slightly lower surfactant concentrations by the hydrophobic index as compared to other methods (viscosity, equilibrium dialysis, electrical conductivity, etc.) has been observed by others [33, 37] for similar binary surfactant/water systems. The most likely explanation of this is the great sensitivity of the fluorescence technique and the low concentration and the high preferential distribution coefficient of the probe used. Thus the hydrophobic index is much more sensitive to the onset of the cooperative redistribution process than to its quantitative magnitude. In Fig. 9 the slightly lower I_1/I_3 ratio (indicating a less polar microenvironment of pyrene) in the EHEC/water solution compared to pure water should be observed.

In contrast to this, the dye solubilization measurements should present an adequate indication of the "volume" of the cluster formation together with the relative solubility in the clusters. If this is true the absorbance at the selected wavelength 495 nm, A_{495}, should correlate with the product $\langle N \rangle$[Micelles]. This product, which according to (3) is equal to $[S]_{tot} - [S]_{free}$, should be approximately proportional to the total volume fraction of clusters or micelles. On the other hand A_{495} is a measure of the total amount of dye solubilized under saturation equilibrium conditions. Hence the number β, defined by

$$\beta = A_{495}/\{\langle N \rangle [\text{Micelles}]\} = A_{495}/\{[S]_{tot} - [S]_{free}\}\ , \quad (7)$$

should be a quantitative measure of the solubilization efficiency. If the various types of clusters present in the system were identical in this respect, β should be a constant irrespective of the SDS content above the foot point. A similar relation to (7) has been discussed earlier [38, 39].

In Fig. 10 the primary results from dye solubilization experiments are presented. In the binary SDS/water system a regression calculation on the solubilization data gives a CMC value 7.5 mM, in fair agreement with the hydrophobicity index results. In the presence of polymer there appears to be some solubilization even for $[SDS]_{tot}$ smaller than 2 mM. There is apparently also a difference between low and high polymer concentrations, the solubilization being more efficient at the highest polymer concentration investigated, 0.2%, than at the lower ones. This is probably an effect of the degree of homogeneity of the polymer segment distribution in solution, since the critical overlap concentration, c^*, for the EHEC fraction used is in the vicinity of 0.2% [1]. For the polymer concentration 0.2% the A_{495} vs. $[SDS]_{tot}$ curve in Fig. 10 shows a breakpoint at the "foot point" value $[SDS]_{tot} = 2$ mM. For the lower polymer concentrations the plots A_{495} vs. $[SDS]_{tot}$ curve more smoothly from the origin upwards to an approximately linear part without any sharp making of the foot point.

A calculation of β according to (7) for the data in Fig. 10 supports the view that the presence of polymer enhances the dye solubilization (see Table 1). In the first place, β is found to be approximately constant over an extended interval in $[SDS]_{tot}$ from the foot point and upwards. Secondly, for the 0.2% polymer containing solutions an average value $\beta \approx 183\ M^{-1}$ is found, as compared to $\beta \approx 138\ M^{-1}$ for the polymer free SDS/water solution. The higher β value indicates a better solubilization capacity. From Table 1 it is seen that at 0.03% EHEC and for the "linear part," one has $\beta \approx 138\ M^{-1}$ which equals the value for the SDS/water system.

In polymer solutions free of surfactant almost no trace of solubilization was detected ($A_{495} = 0.009$ for 0.2% EHEC in water). Thus, it must be concluded that the polymer itself does not adsorb or redistribute the dye. For this to occur an amphiphile must be present. However, even minute amounts (much below the foot point at 2 mM) tend to give some redistribution although the main effect sets in at and above 2 mM SDS.

It is possible to calculate a solubilization ratio, δ, by a combination of known cluster concentrations (see Table 1), dye solubilization data (see Fig. 10) and the molar extinction coefficient of the dye ($\varepsilon = 1.99 \times 10^4\ M^{-1}\ cm^{-1}$ [38, 39]). This ratio describes the number of SDS/EHEC clusters per dye molecule. It has been proposed that δ for many surfactants should be one micelle/cluster per dye molecule [38] although this conclusion has been debated [40]. In the SDS/EHEC system we find that δ is a function of both polymer and surfactant concentration. For the highest EHEC concentration (0.20%) δ goes from a high value (low solubilization capacity) at the footpoint towards a limiting value of approximately two clusters per dye molecule (which equals δ in pure SDS/water solutions according to our results) when $[SDS]_{tot} > 17$ mM. The lower polymer concentrations shows similar behavior but with higher values of δ up to SDS concentrations where formation of normal non-polymerbound micelles starts.

The conclusion is that dye solubilization is a sensitive means of detection of cooperative amphiphile-polymer interaction. The method gives results analogous to equi-

librium dialysis data. However, the quantitative interpretation in terms of the amount of amphiphile being redistributed rests on the validity of relations like (7). Since this relation, at least in the system studied here, seems to be valid with good precision, it appears as if dye solubilization could be a convenient and rapid method for quantitative measurements of formation of hydrophobic clusters in water solution.

From an applied point of view the observations just made indicate a principle for increasing the solubilization capacity of a polymer/surfactant/water system by increasing the polymer concentration above c^*.

In a comparison between the hydrophobic index results and the solubilization data discussed above it must not be forgotten that there are chemical differences between the substances used and that this certainly will effect the observations.

As already mentioned the results obtained in this study seem to indicate the onset of micelle formation at or slightly above 7 mM SDS as compared to 8.0–8.3 mM normally given in the literature as the CMC for SDS in water. Earlier investigation made by us utilizing other techniques like surface tension and electrolytic conductivity tend to give values of CMC in the vicinity of 8 mM [4]. Since the onset of cooperative cluster formation is also connected with a (rapidly) growing value of the aggregation number as the SDS content is increased, it can be expected that different techniques can be sensitive to different averages of N or that N must attain a certain minimum value characteristic of the method in question before cooperativity can be detected. Since fluorescence spectroscopy is very sensitive it may not be surprising that it detects even the first stages of cooperative cluster formation leading to an "observed" CMC value somewhat lower than the "established" one 8.0–8.3 mM [4, 36]. This effect has been reported by others [33, 37].

Model of interaction between EHEC and SDS

Complexes between surfactants and nonionic water soluble polymers were identified more than 20 years ago and have since then been the subject of continued investigations [3, 41]. In this particular field the PEO/SDS/water system is probably the best known [3, 13, 15, 21, 35, 41–44] and it is believed that the following interaction model is valid. When PEO and SDS are present in the same water solution SDS adsorbs as small micelle like clusters (20 Å in radius [44], $N = 35$ [15]) along the PEO chain. The adsorption starts abrubtly when a critical SDS concentration (CAC) is exceeded and the size of these polymer bound clusters seems to be constant up to the saturation of the PEO chain. The spacing between these clusters is controlled by a sensitive balance between hydrophobic attractive and coulombic repulsive forces. After the saturation of the polymer and when the free nonpolymer-bound SDS concentration exceeds CMC, even normal micelles form.

Although there are similarities, the mechanism of interaction between EHEC and SDS is more complex than in the case of PEO and SDS and the observed effects differ in many respects between the two systems [1–4]. The origin of this different behavior resides in important differences between the two polymers. At first, EHEC is a substitution polymer with a cellulose backbone and two different types of substituents (ethyl- and hydroxyethylgroups) (see Fig. 1). Not all cellulose hydroxyl groups have substituents attached, nor can they be expected to the uniformly distributed along the chain. If also the possibility to form oligo(ethylene)chains is taken into account it is easy to imagine EHEC as a polymer with preferred binding sites for SDS in contrast to the homogenous PEO. Another difference is the high hydrophobicity of EHEC compared to the hydrophilic PEO. The cloud point (CP) of this particular EHEC-fraction (CST-103) is 28–35 °C [4] and the CP of PEO is approx. 90–100 °C [45]. This high hydrophobicity of EHEC increases the tendency of self-aggregation and phase separation. Very important for the specific properties of EHEC is also the uneven type and degree of substitution along the chain together with a high polydispersity (as indicated from SEC experiments [46]). The last difference is the chain stiffness. Cellulose derivatives are rather stiff and rigid [47, 48] whereas PEO is a much more flexible polymer. All this taken together is likely to explain the observed differences in the interaction mechanism with SDS between these two polymers.

When SDS is added to an aqueous EHEC solution, SDS clusters start to form abruptly above a certain concentration ("footpoint," CAC) and adsorb to the EHEC chain. This footpoint seems to be insensitive to polymer concentration. In contrast to the PEO/SDS-system these clusters appear to increase in size (up to a certain limiting plateu value) both with increasing polymer and surfactant concentration. The maximum in N_p (see especially Fig. 5) seems to mark the onset of formation of normal micelles. In particular the maximum in N_p is related to the maximum in the dialysis equilibrium as is also the decreasing part above the maximum. Hence the exact value of N_p depends closely on the precise interpretation of the dialysis data and in this region close to and after the maximum the competition with normal micelle formation may be sufficiently developed to require a more elaborate theory before precise conclusions can be drawn.

To illustrate the sensitivity of N_p to different ways of utilizing the basic data some results for 0.05% EHEC are shown in Fig. 11. If the true dialysis equilibrium data are

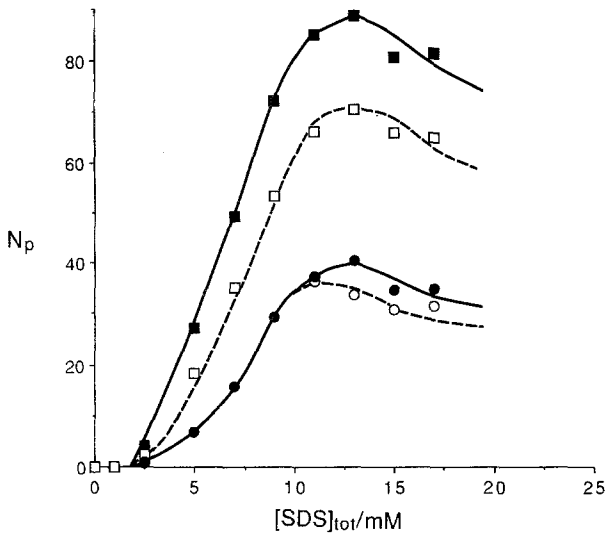


Fig. 11 Results from model calculations of N_p with different assumptions as described in the main text. The resulting values of N_p are plotted as a function of $[SDS]_{tot}$ for aqueous 0.05% EHEC and SDS solutions assuming a CAC = 2 mM (■) or using dialysis data from 0.20% EHEC (□) and comparing with results calculated from 0.05% EHEC dialysis data assuming CMC = 8.2 mM (○) or CMC = 7.0 mM (●)

used the lower curves are obtained. As seen, they are not very sensitive to the exact choice of CMC (7 or 8.2 mM, cf, the previous discussion). On the other hand, if the CAC value of 2 mM is used to sharply mark the onset of cooperative clustering, quite large values of N_p are obtained. Finally, if the dialysis equilibrium for 0.2% EHEC is used the results will fall somewhere in between. In all cases the shape of the dependence of N_p on $[SDS]_{tot}$ is preserved and the position of the maximum remains unchanged. With reference to the previous discussion, the results with CMC = 7 mM and true dialysis data for 0.05% EHEC appear to be the most reliable ones for this particular case.

From the experimental data for the polymer bound cluster concentration, $[M]_p$, and an average molecular weight for a glucose unit including substituents it is possible to calculate the average number of monomer units, n, in the polymer chain per polymer bound SDS cluster (see Table 1). This number is an indicator of the average distance between clusters along the chain. The results from such calculations show a clear difference between high and low polymer concentrations. This is consistent with the anticipated model that at increased polymer concentrations the tendency by different polymer molecules to share a cluster should increase and n should be large. Even the cluster size will become larger (see below).

It is also possible to calculate the average number of polymer bound SDS monomers m per polymer glucose (monomer) unit from $[M]_p$. N_p, and the average molecular weight of the polymer glucose unit. This number indicates the amount of SDS that is bound to the polymer and can be compared with the dialysis data, see Table 1 and Figs. 6 and 7. By comparing m with the parameter y (mmoles SDS bound per gram of EHEC) in Fig. 7 it is seen that m and y follow almost the same shape of dependence of $[SDS]_{eq}$ with a maximum in SDS adsorption to the polymer slightly below CMC ($[SDS]_{eq}$ < CMC). The numerical values of m varies between 0 and 2, i.e., up to approximately two SDS monomers per polymer glucose unit can be adsorbed, the exact value depending on both the polymer and $[SDS]_{eq}$ concentrations.

Both at low and high polymer concentrations the cluster size N_p tends to level off at increased concentrations of surfactant. The reason for this could be somewhat different in dilute and more concentrated polymer solutions. In a dilute polymer solution, with little interaction between different chains, an equilibrium is likely to build up between polymer hydrophobic sites and free surfactant. Approaching CMC, a situation is reached where also competition with the formation of normal micelles will contribute. In a concentrated polymer solution, on the other hand, and especially above c^*, several polymer molecules are likely to share one cluster and the higher concentration of hydrophobic substituents tends to increase the cluster size, N_p. The dye solubilization correlates with the cluster size in the sense that a certain cluster size must be reached before a proportionality is reached between the amount of dye solubilized and the increase in amount of SDS bound in a cluster. This is evident from Fig. 10 since only at sufficiently high SDS concentrations, where N_p is large, do the curves become linear and parallel.

In a concentrated polymer solution the clusters tend to bind the polymer molecules together until the polymer species become more or less "saturated" with clusters. When more SDS is added normal micelles begin to form. A particular polymer molecule may then solubilize its hydrophobic sites just as well in a normal "free" micelle and the networking tendency will decrease. This competition will favor the normal micelles as their relative number increases, which will be the case when the total SDS concentration increases, and the network will disintegrate with a decrease in viscosity as a consequence. This is probably one reason for the decrease of adsorption, cluster concentration, and cluster size shown in Figs. 5, 7 and 8 after the maximum level.

In conclusion, let us make a few important quantitative observations borne out by the experimental results. The fluorescence quenching studies directly give the cluster concentration in the system, see Fig. 8. The main basic result is that in a dilute polymer solution (c < 0.05% EHEC) the cluster concentration is fairly constant up to

the point where normal micelles begin to form. (To be precise, there is a small decrease in cluster concentration over the interval 2 mM < $[SDS]_{eq}$ < 6 or 8 mM). On the contrary, in a more concentrated polymer solution, the cluster concentration increases strongly in a similar interval, cf. Fig. 8. After the CMC point the increase in cluster (micelle) concentration, for the dilute polymer system, gradually approaches the increase characteristic of the binary SDS/water system. In the more concentrated polymer system the situation is quite different, however, and above $[SDS]_{eq}$ = 4 mM, which seems to mark the onset of formation of normal micelles, the rate of increase of cluster concentration is less steep. Nor does the average cluster size increase. From this, one may possibly conclude that the concentrated polymer solution provides an environment which is capable of solubilizing molecularly dispersed surfactant even above a critical micelle concentration.

The observation of an almost constant cluster concentration in dilute polymer solution leads to the important result that the number of "sites" on which clusters form is constant (or almost constant). All these sites appear to become active after the foot point and the aggregation number increases gradually from very small values up to 30–40 at saturation. A quantitative theory must take this into account.

If the previous hydrodynamic data [1] are combined with the cluster concentrations given here (Table 1 and Fig. 8), and if it is assumed that at low polymer concentrations all clusters must reside inside the coil region, an "effective" cluster concentration inside the coil of the order of 1 mM is obtained. This would correspond to the concentration of normal micelles in the SDS/water system when $[SDS]_{tot} \approx 100$ mM. The distance between clusters inside the coil is estimated from our data to be of the order of 100 Å, which is large enough for the Debye–Hückel theory to hold. In these dilute polymer solutions the distance between coils (assuming close packing) is of the order of 1500 Å and the coil diameter is about 500 Å.

Calculations based on our data show that in a more concentrated polymer solution (0.2% EHEC) the distance between coils will be comparable to the coil diameter. As long as the polymer sites are not saturated the intermolecular interaction can then be expected to be strong and networks will form resulting in high bulk viscosities. As more clusters form (see Fig. 8) they also quickly become saturated (N_p approaches its maximum) and a dramatic drop in viscosity could be expected. This viscosity profile as well as the strong interaction region (high values of the Huggins constant) were borne out by our previous hydrodynamic results [1].

It may be concluded from the discussion above that the results presented in this paper give substantial and quantitative support for the interaction model suggested earlier [1, 4]. According to this model there is a marked difference between the macroscopic consequences of the amphiphile-polymer interaction in a dilute polymer solution and a polymer solution sufficiently concentrated to have a homogeneous segment distribution. Thus the interaction goes from a more or less exclusive intramolecular form to one which is strongly intermolecular and even leads to network formation (high viscosity). At a sufficiently high amphiphile concentration the networking tendency is decreased, for reasons just explained.

The cluster formation also promotes solubilization in a way that provides interesting means for "storing" hydrophobic components by a viscosity regulating mechanism in aqueous systems. This is presently being further explored.

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