EPR insights into aqueous solutions of gelatin and sodium dodecyl sulfate †

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The characteristics of the EPR spectra of the spin-probe 16-doxyl-stearic acid ‡ methyl ester (16-DSE) solubilised in micelles of the anionic surfactant sodium dodecyl sulfate (SDS) have been examined as functions of SDS and gelatin concentrations. For simple SDS solutions, the rotational correlation time increases slightly with surfactant concentration whilst the polarity decreases slightly. In contrast, however, in the presence of gelatin these properties vary markedly as a function of the stoichiometric ratio of the concentration of surfactant to gelatin; the correlation time decreasing and the hyperfine coupling constant increasing with increasing surfactant concentration. In the presence of gelatin therefore, 16-DSE reports a very different micellar environment compared with the simple SDS case. Furthermore, this environment differs significantly from that observed in solutions of synthetic, non-ionic homopolymers and SDS. These features arise due to the varied characteristics of the amino acids present in the protein.

Introduction

The interaction between certain polymer and surfactant pairs in aqueous solution has been studied extensively[1,2] because of the useful properties that the polymer (rheological control, stability enhancement) and surfactant (surface tension lowering, wetting) impart to the system. Generally, interactions between non-ionic polymers and anionic surfactants,[3,4] polyelectrolytes and oppositely charged surfactants,[5,6] or hydrophobically modified polymers and anionic surfactants[6,7] are significant. Any interaction between non-ionic polymers and non-ionic surfactants is considerably weaker and largely due to excluded volume effects.

The interaction starts at a critical aggregation concentration denoted cac or cmc(1) and this concentration is substantially lower than the critical micelle concentration cmc, the concentration at which micelles would form in the absence of the polymer. The polymer must, therefore, stabilise the formation of the micelle.

NMR studies of the interaction between synthetic homopolymers such as poly(ethylene oxide) (PEO) or poly(vinylpyrrolidinone) (PVP) and anionic surfactants, such as sodium dodecyl sulfate (SDS) have shown that only those surfactant carbon atoms closest to the headgroup interact with the polymer segments. Therefore, the polymer does not penetrate the micellar core.[4,8] Neutron scattering and fluorescence studies have shown that these 'adsorbed' micelles are comparable in size to the micelles that would be formed in the absence of any polymer.[9,10]

The stabilising effect arises through the polymer segments adsorbing into the micelle palisade layer, thereby shielding part of the hydrophobic core of the micelle from the aqueous phase. There are thermodynamic penalties to this arrangement. Sections of the polymer coil are effectively constrained at the interface, which results in a loss of both translational and configurational entropy. Furthermore, there are steric interactions between the micelle headgroups and polymer segments.

A thermodynamic modelling of polymer–surfactant systems has recently been presented.[11,12] The adsorbed micelles are charged and therefore intramicellar repulsion will be present between the micelles adsorbed on the same polymer molecule. This causes an expansion of the polymer molecule. Concomitantly, the intramicellar repulsion necessitates that the solution surfactant concentration required to place a second or any subsequent micelle onto the same polymer molecule is slightly higher than for the previous micelle. This has two effects: the micelle occupancy on the polymer molecules is smooth (no polymer molecule will have significantly more micelles than the next) and second, the solution surfactant unimer (a single surfactant molecule) concentration increases. The polymer is 'saturated' when the energy penalty due to this intramicellar repulsion is too great to force a further micelle onto the polymer; micelles then form in solution. This concentration is denoted cmc(2) and occurs when the unimer concentration exceeds the cmc of the surfactant under the prevailing conditions of ionic strength and pH.[13]

In this study, we are concerned with the proteinaceous material gelatin, which is significantly different from the more frequently studied synthetic homopolymers where all the segments are identical. Gelatin comprises both ionic and non-ionic amino acids and the non-ionic amino acids are both hydrophilic and hydrophobic.[14] The principal constituents are glycine (32–35%), proline (11–13%), alanine (10–11%), hydroxyproline (9–19%), glutamic acid (7–8%), aspartic acid (4–5%) and arginine (5%).

On addition of SDS, gelatin-bound micelles are formed around 1 mM.[11,15] This interaction will contain both charge and hydrophobic character. ^{13}C NMR studies at ambient pH have shown that the anionic surfactant interacts strongly with the cationic and non-ionic residues of gelatin, but not with the anionic residues.[14] The onset of this interaction is accompanied by substantial increases in viscosity.[11,15] Intriguingly, a local maximum in the viscosity occurs at a surfactant concentration

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‡ 2-(14-Carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxy free radical.

corresponding to 1 micelle per gelatin molecule.[13,15,16] The viscosity at this maximum is some ten or so times greater than the SDS-free value. A series of anionic alkyl sulfates ranging from octyl to tetradecyl have been studied[13] and with the exception of the octyl, all showed a maximum occurring at this stoichiometry. This is not observed in non-ionic polymer–anionic surfactant interactions. The basis of this manifestation is still unclear, but undoubtedly related to a connivance of the mechanisms of charge and hydrophobic interactions.

The aim of this work, therefore, is to contrast the polyampholytic interactions occurring between SDS and gelatin with the corresponding interaction occurring between the equivalent segments of a non-ionic synthetic homopolymer (PEO) and SDS.

Experimental

Sample preparation

Two samples of lime processed gelatin have been used: (*a*) a photographic grade alkali-processed polydisperse gelatin (Kodak Ltd., Harrow, UK), referred to as standard gelatin (nominal molecular weight 107 000 with $M_w/M_n \approx 3.0$), with a bimodal size distribution, and (*b*) a fractionated sample, kindly supplied by Dr T. H. Whitesides, Eastman Kodak Co., derived from the standard gelatin by a fractional precipitation procedure from methanol with sodium nitrate in which the higher molecular weight fraction has been removed. Both gelatin samples have isoelectric points (iep) of 4.9–5.0. In the absence of acid, base or surfactant these gelatins form solutions with a pH of 5.8, at which they are slightly negatively charged. In the presence of SDS, solutions have pH values in the range 5.5–6.5. The two gelatin samples have very different molecular weight distributions. Other work[17] has shown that molecular weight is not an important factor in the microenvironment as reported by the spin-probe.

The spin probe, 16-doxyl-stearic acid‡ methyl ester (Fluka), and sodium dodecyl sulfate (99%, Aldrich) were used as received.

In order to minimise sample-to-sample variations, all samples were prepared from a stock gelatin solution which was prepared by warming the required amount of gelatin and distilled water to 45 °C. The solution was maintained at that temperature for 1–2 h. Due to its insolubility in water, the spin probe (concentration $<5 \times 10^{-4}$ M) was first dissolved in a surfactant solution, $c >$ cmc. To aliquots of stock gelatin and stock spin probe–surfactant solution were added varying amounts of surfactant solution (without spin probe) and distilled water. All samples were equilibrated at 45 °C for at least 1 h before being flame sealed in capillary tubes made of soda glass (Samco). The capillary tubes were placed in standard EPR tubes for the measurement. Throughout this paper, the surfactant concentration is expressed in mM units whilst the gelatin is given as percentage (w/w) in H_2O.

Electron paramagnetic resonance

EPR spectra were recorded at 45 °C on a JEOL JES-RE-2X EPR spectrometer equipped with a variable temperature accessory controlled by a gas stream. 100 kHz field modulation of amplitude 1 G and 10 mW microwave power were used. The sweep-width of the magnetic field was set at 50 G, with a scan time of 60 s using a time constant of 0.1 s. This modulation amplitude broadens the Gaussian component of the EPR lines by 0.12 G, leaving the Lorentzian component unchanged.[18] The effect of this Gaussian broadening, as well as that due to unresolved hyperfine structure, was corrected as described below. Each EPR spectrum is an average of five scans. Further measurements were performed using a Bruker ESP 300E, employing a 100 kHz field modulation of amplitude 1 G and 1 mW microwave power. The sweep-width of the magnetic field was 50 G, with a scan time of 83.9 s, using a time constant of 10.2 ms. On this machine, each EPR spectrum was averaged for at least three scans.

Theoretical considerations

16-Doxyl-stearic acid methyl ester (16-DSE) was chosen as the spin-probe (*a*) due to its insolubility in water (no EPR signal could be detected from 16-DSE containing surfactant solutions below 8 mM—the critical micelle concentration), (*b*) because of its structural similarity to SDS and (*c*) because it, or the parent 16-doxyl-stearic acid, have been used previously to study PEO–SDS complexes[19,20] and hydrophobically modified PEO–SDS complexes.[21,22] It is tacitly assumed here that the ester locates in a broadly similar position to the acid.

Rotational correlation times

The rotational correlation time, τ_c, and micropolarity can be determined from an analysis of the EPR spectra. For very fast motion of aminoxyl§ radicals, *i.e.* $\tau_c < 10^{-11}$ s, the EPR spectrum of the radical is insensitive to the rate of molecular motion and consists of three lines of equal intensities. For fast motion, $10^{-11} < \tau_c < 10^{-9}$ s, the effective rotational correlation time to a good approximation can be calculated from eqn. (1),

$$\tau_c^{\text{uncorrected}} = 6.6 \times 10^{-10}\, \Delta H_0 \left[\sqrt{\left(\frac{V_0}{V_{-1}}\right)} + \sqrt{\left(\frac{V_0}{V_{+1}}\right)} - 2 \right] \quad (1)$$

where ΔH_0 represents the overall line-width of the central line and $V_{-1,0,+1}$ represent the peak-to-peak intensity of the high-, middle- and low-field lines respectively.

Alternatively, the approximation given in eqn. (2) can also be used.

$$\tau_b^{\text{uncorrected}} = 6.6 \times 10^{-10}\, \Delta H_0 \left[\sqrt{\left(\frac{V_0}{V_{+1}}\right)} - \sqrt{\left(\frac{V_0}{V_{-1}}\right)} \right] \quad (2)$$

The superscript 'uncorrected' in eqns. (1) and (2) refers to the fact that the lines are inhomogeneously broadened by unresolved hyperfine structure and modulation broadening. Therefore, the three lines in a spectrum are neither Lorentzian nor have the same shape. To correct these errors, Bales[23] adds corrections to the previous equations consisting of a Voigt approximation, such that the lines are a sum of Lorentzians with a Gaussian profile. The shape of the Voigt approximation curve depends only on the Voigt parameter χ which is the ratio of the Gaussian and Lorentzian line-widths. To correct rotational correlation times calculated from eqns. (1) and (2), the value of the Voigt parameter for the central line is required. Thus, eqns. (3)–(6) follow where χ is the Voigt parameter of the

$$\tau_b = S(\chi) Q_0 \tau_b^{\text{uncorrected}} \quad (3)$$

$$\tau_c = S(\chi) Q_0 \tau_c^{\text{uncorrected}} \quad (4)$$

$$\text{where } Q_0 = \frac{[-1 + \sqrt{(1 + 4\chi^2)}]}{2\chi^2} \quad (5)$$

$$\text{and } S(\chi) = \frac{(1 + 1.78\chi + 1.85\chi^2)}{(1 + 2.08\chi)} \quad (6)$$

central line. For isotropic motion, these two estimates of the correlation time are equal. For all the solutions presented in this paper, this was the case. However, to facilitate the comparison with the PEO–SDS data, it is the uncorrected data [eqn. (1)] that are presented in Fig. 3.

Polarity determination

Hyperfine coupling results from the magnetic interactions

§ Formerly referred to as nitroxide.

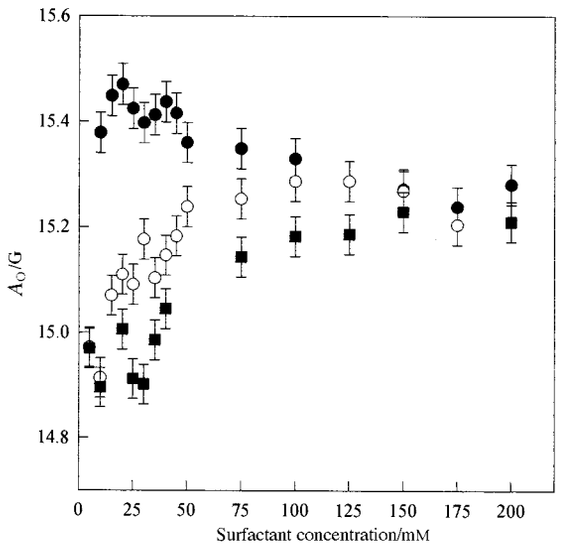
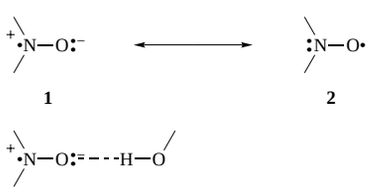


Fig. 1 Hyperfine coupling constant for 16-DSE solubilised in SDS micelles as a function of SDS concentration: (●) no gelatin; (○) 1.67 wt% fractionated gelatin; and (■) 5 wt% fractionated gelatin

between the electron and nuclear spins of atomic neighbours. In the case of aminoxyl radicals, hyperfine coupling to the ^{14}N yields three possible spin states ($m = -1, 0, +1$), and thus three lines are observed in the spectrum. The hyperfine coupling constant is determined as half the separation of the two outermost lines. The hyperfine coupling constant varies with the local polarity in the vicinity of the aminoxyl group. This variation has been interpreted[21] to be due to a shift of the equilibrium illustrated in Scheme 1.



Scheme 1

It is well-known that polar solvents or those that can provide a hydrogen bond will stabilise form **1** thereby increasing A_o. In the case of micellar environments, the aminoxyl group can be engineered to remain in the micelle such that A_o reveals information regarding the polarity of the location of the spin probe.

Results

To facilitate comparisons between the gelatin–SDS and synthetic, non-ionic polymer–SDS systems, the results will be presented first and discussed in a later section.

Hyperfine coupling constant

Fig. 1 shows the behaviour of the hyperfine coupling constant of the spin-probe 16-DSE solubilised in SDS solutions, as a function of SDS concentration containing 0, 1.67 and 5 wt% gelatin. In the absence of gelatin, the hyperfine coupling constant *decreases* slightly with increasing SDS concentration, in good agreement with other work.[24] In the two gelatin containing solutions, the hyperfine coupling constant *increases* significantly with increasing SDS concentration. Up to 150 mM SDS, the hyperfine coupling constant is lowest (*i.e.* the least polar) at any given SDS concentration for the system containing most gelatin and highest for the gelatin-free system. The micellar environment is therefore very different in the presence of gelatin. The hyperfine coupling constant is similar for all three systems at high SDS concentrations.

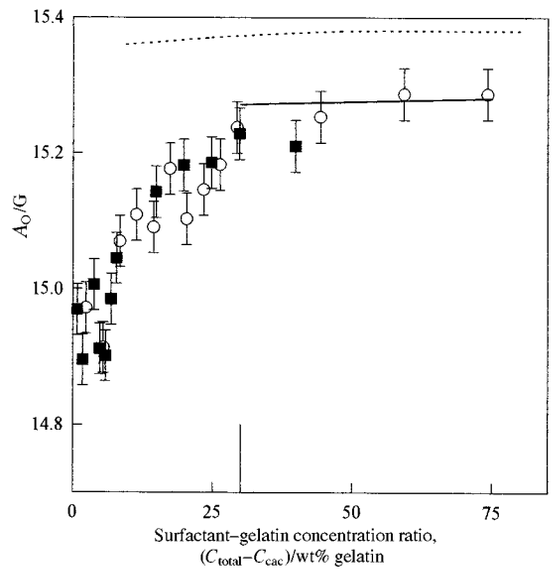


Fig. 2 Hyperfine coupling constant for 16-DSE solubilised in SDS micelles as a function of normalised SDS–gelatin concentration ratio: (solid line) no gelatin (○) 1.67 wt% fractionated gelatin; and (■) 5 wt% fractionated gelatin. The dashed line corresponds to the PEO–SDS systems at 25 °C.

Fig. 2 shows the same data as Fig. 1 normalised by dividing by the gelatin concentration. Since all the gelatin data overlay, the important factor in these systems would appear to be the composition of the gelatin–SDS micelle complex. In order to facilitate a comparison between the gelatin–SDS (45 °C) and literature PEO–SDS data (25 °C) (broken line), a further correction has been applied to remove the cac dependence. Thus, the abscissa is presented as $[(C_{total} - C_{cac})/C_{polymer}]$ (NB for gelatin–SDS, the cac = 1 mM whereas PEO–SDS, the cac = 4 mM). Data for simple SDS (45 °C) (solid line) are also included.

Approaching a surfactant–polymer concentration ratio of 30 (≈150 mM for 5 wt% gelatin) indicated by the vertical line in Fig. 2, the distinction between gelatin-bound SDS micelles and gelatin-free micelles is minimal. This concentration is significant as it corresponds to the saturation of the gelatin, $c >$ cmc(2), and above this concentration, there are also SDS micelles present in solution. Our main interest is therefore the region up to this saturation concentration.

Rotational correlation time

Fig. 3 shows the calculated rotational correlation times for 16-DSE in the SDS micelles as a function of gelatin concentration for three concentrations of SDS, 10, 20 and 55 mM. In this case, the gelatin concentration has been varied yet the data can be normalised by the approach taken in Fig. 2. This strongly reinforces the hypothesis that it is the composition of the gelatin–SDS micelle complex that is the important feature in these systems. Due to the nature of this normalisation, the SDS-only data cannot be plotted,but τ_c increases smoothly with increasing SDS concentration from $\tau_c = 1.7 \times 10^{-10}$ s at 30 mM SDS passing through $\tau_c = 2.7 \times 10^{-10}$ s at 150 mM SDS.

Discussion

Deuterium electron spin echo modulation ESEM has been used[19,20] to probe the depth of penetration of a series of aminoxyl labelled doxyl-stearic acid spin-probes into the micelle. A maximum in the penetration depth was observed when the spin-probe was located at the 12-position. The depth for the 16-position labelled probe was estimated to be approximately 0.5 nm; just inside the hydrophobic core. Furthermore, according to studies involving the quenching of pyrene fluorescence by copper ions,[25] these types of probe are readily accessible to the copper ions present in solution. The nature of the quenching

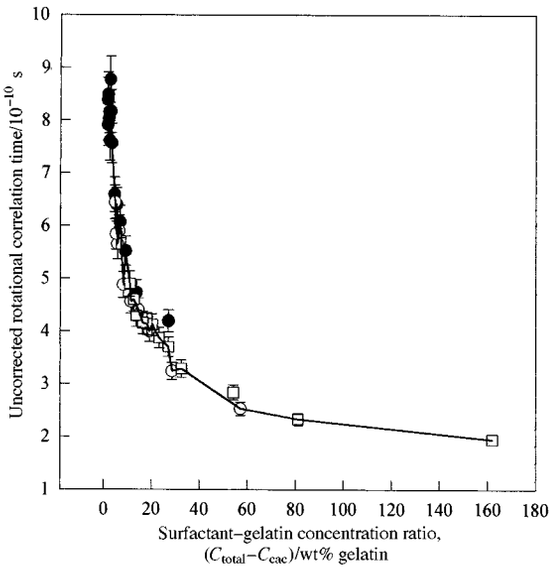


Fig. 3 Rotational correlation time for 16-DSE solubilised in SDS micelles as a function of normalised SDS–standard gelatin concentration ratio (gelatin concentration varied): (●) 10 mM SDS; (○) 20 mM SDS; and (□) 55 mM SDS

was diffusion controlled at a rate approaching that of copper ions in bulk water. All of the data in liquid systems are consistent with a model of the spin-probe executing rapid motion near the micelle surface. Hence, the data presented here are concerned with structural perturbations occuring within this region. Other spin-probes would report on different regions of the micelle or the continuous phase.[24]

Hyperfine coupling constant

The interpretation of the hyperfine coupling constant is non-trivial as several factors contribute to its behaviour including (*a*) the charged nature of the headgroup and amino acid residues as well as the presence of any bound counter-ions and (*b*) replacement of some water molecules previously in contact with the extremities of the micellar core[14] by the adsorbed polymer. It has been proposed that the most important of these for synthetic polymers is the charged nature of the headgroup and associated counter-ions.

The hyperfine coupling constant of 16-DSE has been measured for a series of relevant surfactants in solution: SDS, $A_o = 15.30 \pm 0.05$ G; $C_{12}E_8$, $A_o = 14.50 \pm 0.05$ G; whereas for hydrophobically modified PEO, $C_{12}EO_{200}C_{12}$, $A_o = 14.85 \pm 0.05$ G. The interpretation of these values is informative as an insight into the gelatin–SDS system. The charges in the SDS headgroup result in the higher polarity for the spin-probe in micellar SDS. The smaller aggregation number ($N_{agg} = 15$)[26] of the polymeric surfactant $C_{12}EO_{200}C_{12}$, compared to $C_{12}E_8$ ($N_{agg} = 55$–62)[27] results in a smaller micelle and thus, large surface area to volume ratio. Proportionally, the spin-probe spends more time at the surface of the micelle and thus in contact with the aqueous phase. A_o therefore is higher. These simple comparisons show, in particular, that charge has the largest effect on A_o.

Consider the interaction between PEO and SDS. In the regions between the interdigitated charged headgroups, there are water molecules in contact with the extremity of the hydrophobic core. On adsorption of the homopolymer, the polymer segments will displace some of the water molecules rendering the micellar surface less polar. Thus A_o decreases. However, A_o for 16-DSE in PEO–SDS is largely insensitive to PEO concentration;[19] $A_o = 15.41 \pm 0.05$ G, 100 mM SDS, 0 wt% PEO and $A_o = 15.36 \pm 0.01$ G, 100 mM SDS, 10 wt% PEO. From these numbers, it may be assumed that this effect is relatively weak. The same cannot be said for the gelatin–SDS system. However, it should be noted that the PEO–SDS study[19] was performed in D_2O and whilst this may affect the value of A_o, any trend with increasing surfactant concentration should be comparable.

On addition of SDS to both 1 wt% and 2.5 wt% hydrophobically modified PEO, $C_{12}EO_{200}C_{12}$, solutions, A_o increases sharply ($A_o = 15.05 \pm 0.05$ G at 40 mM SDS) before subsequently decreasing much more slowly to $A_o = 14.90 \pm 0.05$ G at 180 mM SDS. When the data are corrected for the relative concentrations of polymer and surfactant, the aggregates formed over a range of SDS concentrations were found to be rather similar.[21]

Consider now the gelatin–SDS system. At very low SDS concentrations, the hyperfine coupling constant is close to the value of the C_{12} aggregates found in solutions of $C_{12}EO_{200}C_{12}$. The hyperfine coupling constant increases with increasing SDS concentration, ultimately to a value identical to pure SDS micelles.

In these solutions, gelatin as a whole is slightly negatively charged. ^{13}C NMR studies at ambient pH have shown that the cationic and non-ionic residues of gelatin interact strongly with the anionic surfactant.[14] If the interaction is predominantly between the non-ionic residues and the surfactant, one would expect the polarity at the micelle surface to decrease due to the displacement of water molecules. With increasing SDS concentration, the relative proportions of non-ionic residues and anionic surfactant in the gelatin–SDS micelle complex will change in favour of the surfactant. The complex will become more negatively charged. Hence, the hyperfine coupling constant would be expected to increase towards the pure SDS value. This scenario agrees with the experimental observations. However, a similar mechanism would be present in the PEO–SDS system yet the A_o data show little change across the same relative concentration range.[19] To account for the observed changes in magnitude of the hyperfine coupling constant in the gelatin–SDS system, we suggest there is some charge neutralisation. Furthermore, addition of a little SDS to the uncharged 'pure' C_{12} end-groups present in the $C_{12}EO_{200}C_{12}$ solutions[21,22] results in a substantial increase in A_o ($A_o = 14.88$ G no SDS to $A_o = 15.05$ G at 20 mM SDS, 2.5 wt% polymer). This is due to the increase in charge of the mixed aggregate. This magnitude of change in A_o is comparable to that observed in the gelatin–SDS case. In the $C_{12}EO_{200}C_{12}$–SDS system, a subsequent gradual decrease in A_o with increasing SDS concentration is observed due to the interaction between the SDS and the non-ionic segments of the PEO backbone ($A_o = 14.95$ G at 180 mM SDS, 2.5 wt% polymer). Hence, the displacement of water is a much weaker contribution to the changes seen in A_o than the charge effects.

The behaviour of the hyperfine coupling constant observed in the gelatin case involves a change in the charge on the gelatin–SDS micelle complex rather than the displacement of water molecules. Cationic residues of gelatin interacting with the anionic surfactant lead to some charge neutralisation. A less polar environment results and A_o decreases. At low SDS concentrations, it is probable that the charged interactions between cationic residues and the anionic micelle dominate. A_o is therefore at its lowest value. With increasing SDS concentration, the gelatin–SDS micelle complexes are diluted with non-ionic residues as well as the anionic surfactant. Thus, the charge increases[11] and hence, the polarity of the micelle. Ultimately, the micelle takes on the character of a pure SDS micelle.

Rotational correlation time

The correlation time data of Fig. 3 show that the rotation of the spin-probe is much more restricted in the gelatin-bound micelles compared with the simple SDS micelle. This motion is however, still isotropic. The gelatin residues adsorbed around the headgroups and within the outer regions of the hydrophobic core restrict the motion of the spin-probe. With increasing SDS–gelatin concentration ratio, the proportion of gelatin residues present in a single micelle decreases. The dynamics of the spin-probe tend towards that of the gelatin-free SDS

micelle. Above the saturation concentration, the distinction between those SDS micelles formed in the presence of gelatin to those in simple SDS solutions is minimal.

Conclusions

The interaction between gelatin and SDS shows many similarities with the frequently studied synthetic polymer–anionic surfactant systems. In this paper, some of its unique character is presented.

The spin-probe 16-DSE solubilised in SDS micelles shows that at low SDS concentrations, the gelatin adsorbs onto the micelle surface and greatly restricts the motion of the spin-probe. The considerably less polar environment suggests that a very high proportion of the cationic residues are present at the micelle surface. The cationic residues can be regarded as 'pinning' the gelatin to the micelle surface and are saturated at very low SDS concentrations. The strength of this binding results in a much more rigid environment for the spin-probe. τ_c is therefore high. At higher SDS concentrations, the composition of the micelle is much more rich in SDS and non-ionic residues and thus the polarity increases. The gelatin can take on a much more extended conformation and the rotation of the spin-probe becomes more fluid. τ_c decreases. Approaching the saturation level, the distinction between the gelatin-bound SDS micelle and the gelatin-free SDS micelle is negligible.

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