Colloid Polym Sci (2004) 282: 446–453
DOI 10.1007/s00396-003-0967-1

ORIGINAL CONTRIBUTION

C. Buron
C. Filiatre
F. Membrey
A. Foissy
J.F. Argillier

Interactions between gelatin and sodium dodecyl sulphate: binding isotherm and solution properties

Received: 18 March 2003
Accepted: 1 July 2003
Published online: 11 September 2003

C. Buron · C. Filiatre · F. Membrey
A. Foissy (✉)
LCMI, U.F.R. Sciences et Techniques,
16 Route de Gray, 25030 Besançon cedex,
France
E-mail: alain.foissy@univ-fcomte.fr
Tel.: +33-381-666297
Fax: +33-381-666530

J.F. Argillier
Institut Français du pétrole,
1–5 Avenue Bois Préau,
Rueil-Malmaison, France

Abstract The interaction between sodium dodecyl sulphate (SDS) and gelatin was studied at pH 4.5 and 6.5 where the gelatin is positively charged (i.e.p. 8). At pH 4.5 a SDS/gelatin concentration range was found where gelatin precipitates. At pH 6.5 the SDS-gelatin complex remains soluble although three SDS concentration domains were distinguished where the SDS-gelatin complex had very different affinities for the solvent. Below C_1 the complex was highly surface active but other measurements (viscosity, potentiometry, protons uptake) did not reveal any particular consequence of binding. Between C_1 and C_2 the molecular size decreased (viscosity lowering) upon charge neutralization and collapse about small SDS aggregates (17 SDS molecules per gelatin molecule). Above C_2 a cooperative binding mechanism lead to the formation of SDS aggregates; the complex stretched out and turned strongly hydrophilic (the viscosity increases, low surface activity). At saturation one gelatin molecule bound about 200 SDS molecules. Above the overlap concentration (about 3 wt%) SDS aggregates formed between several gelatin molecules, the viscosity increased continuously with SDS concentration and the binding ratio was lower than in dilute gelatin solutions. A very good correspondence was found between the different analytical data including turbidity, viscosity, surface tension, protons uptake and direct potentiometric SDS binding measurements.

Keywords Surfactant-gelatin interaction · Surfactant-protein complexation · Sodium dodecyl sulfate · Surface tension · Viscosity

Introduction

Gelatin is a major compound in the formulation of a wide variety of products covering pharmacy, cosmetics, food, photography and many more. It is used as an emulsifier, peptizer, thickener, and binder, and great interest is taken in the interaction of the protein with other ingredients, mostly cationic and ionic surfactants that are used cooperatively in formulated compounds. Our interest was in the use of gelatin for the production of microcapsules in o/w emulsions using a coacervation process, which consists principally of the precipitation of the protein by addition of an oppositely-charged surfactant [1, 2, 3]. For similar purposes several studies address the interaction between anionic surfactants and gelatin in acidic media and around its isoelectric pH [4, 5, 6]. Emphasizing the solubilization-precipitation transition, studies have explored the dependence on pH and surfactant/gelatin ratio, establishing a general concept of making microcapsules by forming an insoluble protein-surfactant layer around the oil droplets [6]. Thinking about new encapsulation processes, the layer by layer

construction of shells about nanoparticles [7, 8], we found it necessary to establish more clearly the surfactant/protein ratio range where the complex adsorbs well onto the substrates because of its amphiphilic properties, but does not precipitate. It also appeared crucial to measure precisely the equilibrium between bound and free surfactant concentrations since the latter part may unfavorably interact with further introduced molecules. In this work we have chosen sodium dodecyl sulfate as a surfactant since it is a widely used and may be considered as a generic surfactant. The gelatin molecule has a high isoelectric point (pH 8) which allows us to investigate a larger range of pH where it carries a positive charge.

A number of investigations have dealt with the SDS-gelatin system, many results of which will be discussed in this work. It has been known for a while that SDS binds with gelatin very strongly below but also above the isoelectric pH [9, 10]. The binding is easily demonstrated by the modifications induced in surface tension [10, 11, 12, 13, 14, 19], viscosity [15, 16], turbidity [17], conductivity [18, 19, 20] and pH [18, 19] when SDS is introduced in the gelatin solution. In many cases, due to the particular choice of the experimental conditions, the complex precipitates over a range of SDS/gelatin ratios and resolubilizes at higher SDS concentrations [4, 6, 11]. Studies have also investigated more deeply the structure of the SDS-gelatin complex showing that conformational transitions were associated with the aggregation of bound surfactant molecules in a large range between the critical aggregation concentration on the gelatin backbone (CAC) and the formation of free micelles in the bulk [15, 19, 20, 21, 23]. Experimental data show that the complex properties, in particular the solubility and the amphiphilic character, vary considerably in this range and depend very much on the type of gelatin and other parameters (gelatin concentration, pH).

The principal objective in this work was to relate the composition of the complex (SDS/gelatin ratio) and its properties to the total SDS concentration in the system. The investigation was conducted at two gelatin concentrations, below and above the overlap concentration at 40°C, and two pH (4.5 and 6.5). The uptake of SDS by gelatin was measured directly using a prototype electrode. The complex formation and its properties were investigated with a combination of techniques including the measurements of surface tension, viscosity, turbidity and pH variation upon addition of SDS into the gelatin solution. All data will be shown to correlate nicely, revealing three critical SDS concentrations where the complex properties change drastically. A range of experimental conditions were found where the SDS-gelatin complex remained soluble, although it was strongly amphiphilic, even hydrophobic.

Experimental

Materials and solutions preparation

Acid-processed gelatin (type A, 300 bloom strength, moisture content 10.6%) with an isoelectric pH of about 8 was obtained from Sigma Aldrich (Saint Quentin Fallavier, France). The average molecular weight was 50,000. All runs in the study were carried out either at pH 4.5 or 6.5. Gelatin solutions were prepared by soaking the gelatin flakes in milli-Q water ($1–2\ 10^{-6}$ S.cm^{-1}) for one hour and by heating at 40°C under mild stirring. At this temperature gelatin is denatured and may be seen as a random coil.

The anionic surfactant sodium dodecyl sulfate (SDS), molecular weight 288, was purchased from Aldrich and was used without additional purification. The solutions of SDS were prepared in milli-Q water at the same pH as the gelatin solution before mixing. The pH adjustment was made using the required aliquot of a HCl solution.

Solutions of gelatin + SDS were prepared by mixing the two separate solutions at the same pH at 40°C. The pH drift upon mixing was corrected by addition of either HCl or NaOH. All measurements were made at 40°C.

Experimental procedures

The turbidity of the gelatin-SDS mixture was measured with a Turbisan MA 2000 at 40°C. The solutions were prepared 15 min before the measurements by mixing an equal volume of the gelatin solutions (1 and 7 wt %) and a SDS solution at variable concentration and similar pH. Measurements were performed at pH 4.5 and 6.5.

The viscosity was measured with a low shear 30 Contraves apparatus using a small volume couette device. The shear rate covered the range 0.01–100 s^{-1} which is well within the Newtonian (linear) regime for all the solutions. Viscometric measurements were performed at 40°C.

The binding of SDS with gelatin was measured directly using a potentiometric procedure based on a specific electrode developed by Moküs and Letellier [24]. The potential of the electrode was measured relative to the saturated calomel electrode which was separated from the gelatin solution by a salt bridge (2 M NH_4Cl in Agar gel). The solution was stirred magnetically and heated at 36°C to prevent the deterioration of the Agar gel at 40°C. Measurements were recorded when the output had stabilized, about 10min after mixing. The concentration of free SDS was evaluated by comparison between the output and a calibration curve obtained separately with solutions at concentrations below the CMC. It was verified that gelatin had no specific effect on the electrode potential. The number of SDS molecules bound with gelatin was calculated from the difference between the total content in SDS and the concentration of free molecules as measured potentiometrically. No background electrolyte was added to perform the potentiometric measurements. The binding of SDS was measured at pH 6.5 where there is complete solubility of the complex in 1 and 7% solutions. The gelatin and SDS solutions were prepared separately at pH 6.5, but the pH increased when mixing both solutions. Before performing the measurement the pH was reset at pH 6.5 by adding the required amount of an HCl solution. Both the change of pH and the amount of acid necessary to return the mixture to pH 6.5 will be discussed later.

Results

Rheological properties of pure gelatin solutions

The viscosity of the gelatin solutions at pH 4.5 and pH 6.5 was measured at different concentrations. From

these data the intrinsic viscosities [η] were calculated using the double extrapolation technique described by Huggins and Kraemer [25, 26]; they read 38 $cm^3.g^{-1}$ and 63 $cm^3.g^{-1}$ at pH 6.5 and 4.5 respectively. At pH 6.5 the overlap concentration (C*) was determined at the concentration 0.031 $g.cm^{-3}$ (3.1%) by the double logarithmic plot of the zero shear viscosity versus the coil overlap parameter $c[\eta]$ (Fig. 1). Further experiments were preformed at two gelatin concentrations, below (1 wt %) and above (7 wt %) the overlap concentration.

Solubility of SDS-gelatin solutions

The solubility of SDS-gelatin solutions was determined by turbidity measurements. Fig. 2 shows that the SDS-gelatin complex is completely soluble at pH 6.5 in the whole range of SDS to gelatin ratios and at the two gelatin concentrations 1 and 7%. On the other hand the dilute and concentrated gelatin solutions precipitate at pH 4.5 in the same range of SDS/gelatin composition, in other words between the ratios 0.3 and 1–1.6 mmol SDS/g gelatin.

Surface tension measurements

The surface tension of the SDS-gelatin solutions was measured at the gelatin concentration 1% at pH 6.5 and temperature 40°C. It displays (Figure 3) the frequently reported difference between the pure surfactant solution and that containing an interacting polymer [27]. The surface tension curve presents three singular points C_1 (5 10^{-4} M), C_2 (5–7 10^{-3} M) and CMC* (4 10^{-2} M), corresponding to SDS concentrations where the surface tension curve levels off (C_1, start of the plateau), where it crosses the pure SDS solution curve and starts to decrease more strongly (C_2) before it eventually reaches a second plateau at CMC*. Points like C_1 are known as the Critical Aggregation Concentrations (CAC) referring to the onset of the formation of aggregates about the polymer backbone. Between C_2 and CMC* the surface tension decreases significantly down to the value that is reached with pure SDS solutions. We note the very significant decrease of γ at low SDS concentration (below C_1) due to the presence of the gelatin. We also note that above the concentration CMC* no difference is observed any longer between pure SDS and mixed SDS + gelatin solutions. At this latter stage the air-solution interface is only occupied by SDS molecules. The complex behaviour of the γ versus SDS concentration profile results obviously from interactions between the surfactant and the protein that will be discussed below. Note that in the absence of SDS the surface tension of the gelatin solution is about 60 $mN.m^{-1}$.

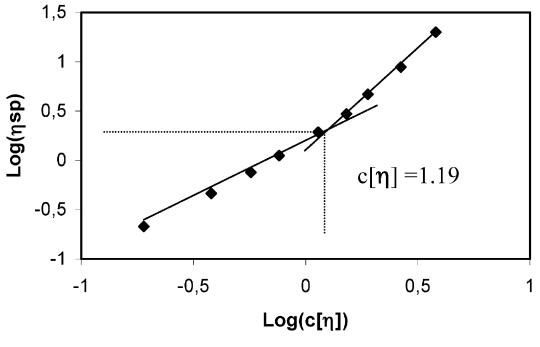

Fig. 1 Double logarithmic plot of zero shear specific viscosity vs coil overlap parameter ($c[\eta]$)

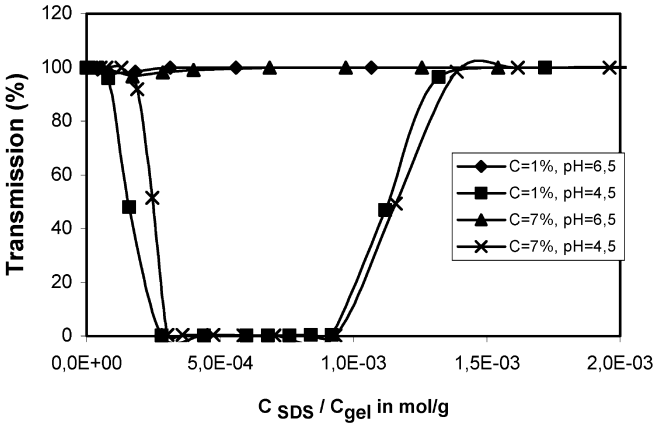

Fig. 2 Light transmission through gelatin solutions at pH 4.5 and 6.5; gelatin concentrations were 1 and 7% as shown in the figure

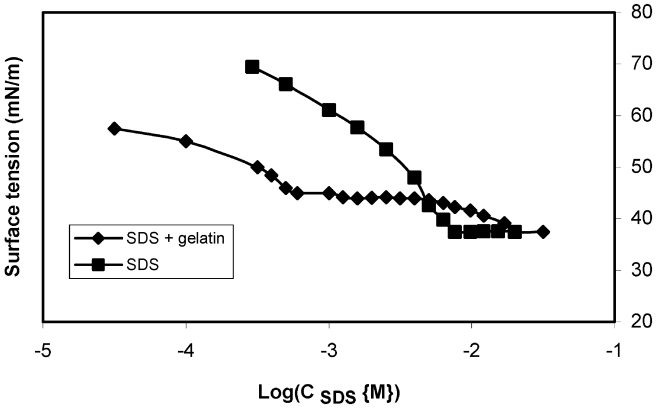

Fig. 3 Surface tension of gelatin + sodium dodecyl sulphate solutions (SDS) vs log(SDS)

Viscosity of SDS-gelatin solutions

Viscosity measurements were only performed at pH 6.5 where the SDS-gelatine complex is soluble. Viscosity data as a function of SDS concentration (Figures 4, 5) reveal also singular points and different shapes

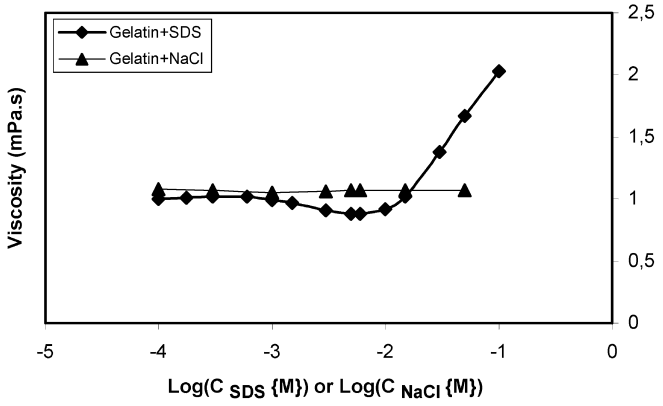

Fig. 4 Viscosity of gelatin solutions vs SDS or NaCl concentrations, for pH 6.5. (♦) Gelatin 1% + SDS, (▲) Gelatin 1% + NaCl

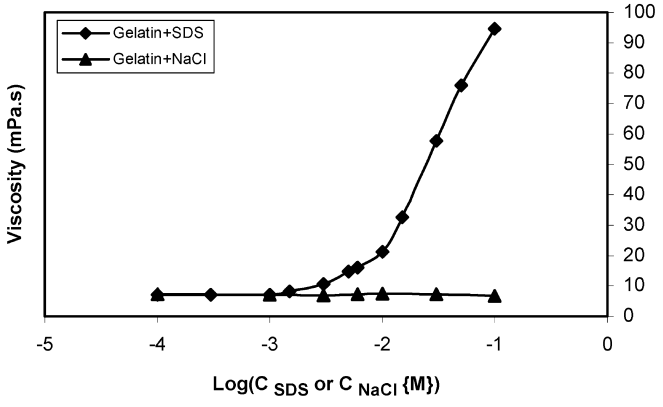

Fig. 5 Viscosity of gelatin solutions vs SDS or NaCl concentrations, for pH 6.5. (♦) Gelatin 7% + SDS, (▲) Gelatin 7% + NaCl

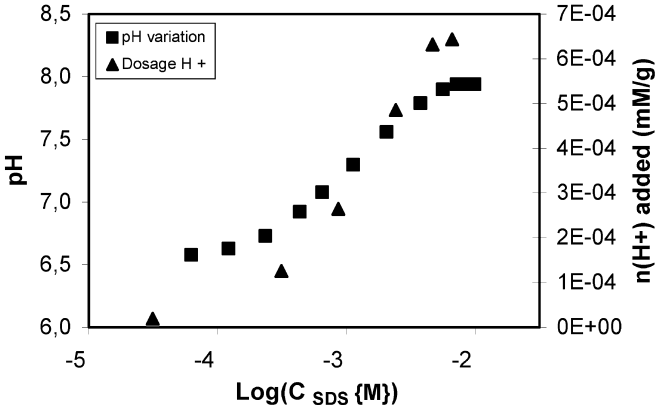

Fig. 6 Variation of pH and proton uptake upon addition of SDS in gelatin solutions

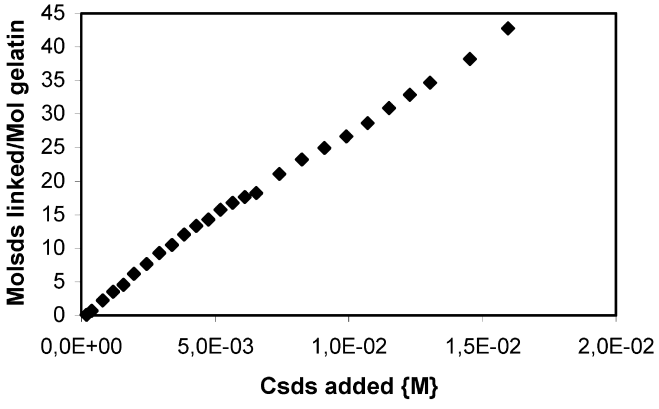

Fig. 7 Uptake isotherm of SDS by gelatin. pH 6.5, temperature is 20°C

depending on the gelatin concentration. At higher concentration (7%) the viscosity starts to rise at the SDS concentration 2 10^{-3} M and the increase is almost linear with the SDS concentration above 2 10^{-2} M. Comparatively, the addition of salt (NaCl) does not induce any viscosity change which shows that the effect is due specifically to the gelatin-SDS interaction and not to any aggregation or conformational change of the protein induced by the increase of ionic strength. Note that a pure SDS solution does not exhibit any significant increase of viscosity at a concentration below about 10^{-1} M. The dilute gelatin solution (1%) behaves differently since the viscosity decreases first in the concentration range C_1-C_2 and then increases. The increase is very steep and linear above the SDS concentration 2 10^{-2} M.

pH variations

Addition of SDS in the gelatin solutions produces an increase of pH that reveals an increase of the ionization of amino groups. The change of pH was monitored and further we have measured the amount of acid that was needed to return the solution to the initial pH. These measurements presented in Figure 6 for 1% gelatin concentration show two break-points at C_1 (start of the pH increase) and between C_2 and CMC* at 2 10^{-2} M where the protein uptake of protons reaches saturation at 0.65 mmol.g^{-1}.

Binding isotherm

The SDS-gelatin binding isotherm at low gelatin concentration clearly shows two different parts (Figure 7). At lower SDS concentration the binding varies almost linearly up to a small shoulder at the total (added) concentration C_2. The Scatchard analysis [27] of this first part gives a saturation of the gelatin molecule of about 0.4 mmol per gram. The second part of the curve is also linear but it does not show any saturation plateau up to the limit of the accessible concentration range. At the

gelatin concentration 7 wt% the uptake of SDS is significantly lower (in mole SDS per g of gelatin) than at concentration 1% and there is only one continuous adsorption curve.

Discussion

All of the experiments above demonstrate successive steps in the binding mechanism of SDS with gelatin. In 1% gelatin solutions at pH 6.5 the transitions between the different processes take place at the SDS concentration C_1 ($5\ 10^{-4}$ M, in other words $0.5\ 10^{-4}$ mol SDS/g gelatin), C_2 ($7\ 10^{-3}$ M) and CMC* ($4\ 10^{-2}$ M). Table 1 summarizes the singular SDS concentrations where a change of behaviour has been observed in the present investigation.

The different steps in the binding of surfactant molecules with polymers have often been discussed in the literature by considering data obtained via a number of analytical procedures. In the particular case of SDS and gelatin, two SDS concentration breaks have been generally reported in view of the measurements of surface tension [11], surface tension and fluorescence [20], viscosity [15], conductivity and pH [18, 19, 20], and solubility [17, 22]. Similar results were also obtained in a large number of investigations addressing all kinds of ionized and neutral surfactants mixed with polymers and polyelectrolytes. Two general references are [28, 29].

The strong decrease of surface tension at low surfactant concentration (below C_1) in the presence of gelatin, in comparison with pure SDS solutions, has been attributed to the formation of an amphiphilic complex at the interface. In that range of SDS concentrations it was claimed that no binding of the surfactant with gelatin takes place in the bulk [29] but gelatin may act as an efficient co-surfactant as do other polyions by screening the electrostatic and repulsive interactions between SDS molecules at the interface [30]. Indeed, besides surface tension measurements none of our technical investigations reveals any binding between SDS and gelatin below C_1. However, in our opinion it is very likely, as was also suggested in earlier studies [11, 31], that some interaction takes place in the form of a discrete electrostatic binding involving an exchange of the polyion counterion with an SDS molecule. Such a binding was detected recently below the CAC by Tomasic et al [32] for the system carrageenan-dodecylammonium chloride.

At concentration C_1 the binding of SDS with gelatin is clearly revealed by the effects on the viscosity and on the pH of the solution. The change of pH indicates that the interaction involves coulombic forces between the anionic groups of the surfactant and the positive sites along the gelatin backbone. The positive charges are protonated amino and guanidino groups in the arginine and lysine amino acids [15, 20]. The neutralization of the positive groups by the surfactant allows the protonation of neighbouring NH_2, which is monitored by the increase of pH. The phenomenon is well described in the literature concerning the ionization of polyelectrolytes. A similar pH rise was studied by Tavernier [18] when mixing the solutions of gelatin (from 0.1 to 5 wt %) and a sulfonic surfactant (N-methyl N-oleolyltaurine).They showed that the uptake of protons accompanied the binding of surfactant molecules up to a ratio of surfactant/gelatin (about 1 mmole/g) that was independent of the gelatin concentration, the pH and the ionic strength. In our case the maximum of additional protons uptake is 0.07 mmol/g (a few percent of the bound SDS) and it is reached at the SDS concentration $2\ 10^{-2}$ M which is between C_2 and CMC*. The quantitative difference between the results by Tavernier and this work is attributed to the fact that they worked at a lower pH (pH 5) and they used a material with an isoelectric pH much lower than ours (4.85 instead of 8).

Also Sovilj et al [19] studied the binding of SDS using the variations of conductivity and pH upon addition of SDS. They also found that the increase of pH upon addition of surfactant reached a maximum at an SDS concentration slightly below CMC*, although the binding continued to increase as shown by the variation of conductivity. The change of pH upon SDS addition makes it very clear that the binding of the anionic surfactant involves the positive groups of the protein but, since the binding continues to increase although the ΔpH remains constant at higher SDS concentrations close to CMC*, there is obviously another binding force that

Table 1 Summary of correlated SDS concentration ranges where the solution properties change abruptly

SDS concentration (M)	$C_1 = 5\times10^{-4}$	$C_2 = 5\times10^{-3}$	2×10^{-2}	CMC* = 4×10^{-2}
Log (SDS)	-3.3	-2.3	-1.7	-1.4
Solubility (1% and 7%, pH 6.5)	soluble over the whole range			
Surface tension (1% in gelatin)	1^{st}plateau	steep decrease		2^{nd}plateau
1% viscosity	decrease	increase		
7% viscosity	increase over the whole range			
ΔpH 1%	increase		stable	
Uptake isotherm 1%	increase	plateau	increase	
Uptake isotherm 7%	continuous increase			

is attributed to the hydrophobic attraction between hydrocarbon tails of surfactant molecules. Although the interaction of SDS with gelatin is essentially electrostatic at low binding ratio, an appreciable and complementary hydrophobic interaction has been reported, after NMR studies, between SDS and alkyl groups of non polar amino acid residues [33].

Between C_1 and C_2 the viscosity decrease in 1 wt.% gelatin solutions results from the reduction of size induced by charge neutralization and by the hydrophobic attraction between aliphatic tails of the bound SDS molecules. The formation of aggregates (micelles) on the polymer chain, starting at the CAC (C_1 in this work) has been largely demonstrated in the literature. In the case of SDS and gelatin direct evidence was given in several studies by using environmentally sensitive fluorescent probes that have a strong affinity for hydrophobic domains [15, 20, 23, 29]. Between concentrations C_1 and C_2 the binding isotherm (Figure 7) obeys the Langmuir equation, as found formerly by Vinestky et al [5]. The equilibrium concentration of free (unbound) SDS molecules increases in parallel with the progressive uptake by the protein, which is different from regular micellization in the bulk where the unimers' concentration remains approximately constant when the total surfactant concentration increases. Between bulk micellization and aggregation about the protein the difference is that, in the former case, the chemical potential of the surfactant molecule remains the same within micelles; when the total concentration of surfactant increases the number of micelles increases but not the micelle size. The equilibrium resembles that between two different phases. In the second case the chemical potential of the surfactant in the SDS-protein complex depends on the complex stoichiometry. Whitesides et al [20] have described an electrostatic model to explain the progressive binding of SDS on the gelatin backbone as a function of the free SDS concentration. They explained that when SDS molecules bind with gelatin the change that they induce in the effective charge of the complex lowers its affinity for additional uptake. At the onset of the negative charge excess of the complex, that is revealed by the increase of viscosity (at concentration C_2, Fig. 4) and the break in the binding isotherm (Figure 7), the SDS uptake is 0.34 mmol/g (about 17 SDS molecules per gelatin molecule) which amounts to about one quarter of the aggregation number in the bulk. Stabilization of smaller than regular micelles is due to the strong electrostatic screening and possibly to the contribution of hydrophobic residues of the gelatin molecule [15, 20, 23]. Another study by Magdassi et al [4, 6] with a gelatin molecule that ressembles ours (Mw = 50,000; isolectric pH 8) gave a binding ratio at 17 SDS /gelatin molecules at pH 6.1, which is quite similar to our result. In the later work we note interestingly that the protein has no net charge and precipitates at this ratio. The lack of solubility has certainly the same physical origin as the decrease of viscosity in our study. The comparison will be further discussed below.

Arora et al [34] studied the binding of SDS and sodium octyl sulphate with various gelatin derivatives (methyl ester, acetylated, formilated gelatins) using equilibrium dialysis. They found that the uptake isotherms always divided in two sections as we have found, each fitting correctly the Langmuir plot but with a second part (higher surfactant concentration) that did not show any saturation. At low concentration the rather linear increase of binding with surfactant concentration was attributed to the electrostatic linkage of the negative surfactant with the positive residues and the unlimited binding at higher concentration to the aggregative hydrophobic forces between hydrocarbon tails of additional surfactant molecules and those of already bound ones. Using the Scatchard procedure they calculated binding of 33 SDS molecules per molecule of gelatin (about one half of a bulk micelle) at a point equivalent to our C_2 concentration but which may not be quantitatively compared to our measurement (17 per mole of gelatin) since the authors do not indicate the origin and the molecular weight of their sample.

Although it is not explicitly discussed in other studies, the singular point at concentration C_2 in 1 wt % solutions (SDS concentration 5×10^{-3} M, binding ratio SDS/gelatin = 0.5 mmol/g from Figure 7) is remarkable since designates the concentration at the start of three different physical phenomena, i) the viscosity starts to increase, ii) the surface tension decreases significantly and iii) the binding isotherm reveals a clear break (see Table 1). Above C_2 the increase of viscosity shows that the polymer size increases, which is due to the influence of repulsive interactions between the negative charges carried by the excess of bound SDS molecules. Correspondingly the complex turns strongly hydrophilic; it is therefore displaced from the interface by free SDS molecules, which explains the steep decrease of the surface tension. Actually between C_2 and CMC* the surface tension in gelatin solutions is higher than in pure SDS solutions (Figure 3). The reason is that, at the same total concentration, the large fraction of bound SDS molecules leaves a lower concentration of surface active molecules than in the gelatin-free solution. With the reasonable assumption that the CMC is determined by the concentration (activity) of free surfactant molecules, we may calculate at CMC* that the bound SDS molecules is the difference between CMC* and CMC, which is about 4 mmol/g or 200 molecules per gelatin molecule. The physical phenomena that are observed at C_2 in this work correspond very well to the resolubilization of the complex reported by other investigators [4, 5, 6, 17]. That the complex remains soluble at pH 6.5 in our case is attributed to the ratio of ionized carboxylic groups.

Using fluorescence quenching Whitesides [20] determined that near CMC* the aggregation number of SDS bound with isolated protein molecules (low gelatin concentration range) was very similar to that of free micelles in the bulk (between 52 and 61, depending on the pH and the ionic strength of the solution). Combining the fluorescence results and the binding isotherm obtained from dialysis experiments they estimated that a maximum of 5 micelles was bound with a gelatin molecule of molar mass about 60,000 in the range pH 5 to 6. From the difference between CMC and CMC* we have found a maximum binding ratio about 4 mmol/g at pH 6.5, which is above that found by Sovilj [19] (1.6 mmol/g) using ΔpH and conductivity measurements, but which agrees quite well with the former study since it makes 200 SDS molecules (about 4 micelles) per gelatin molecule assuming an average Mw = 50,000 for our sample (see Figure 8).

All comments above addressed the dilute solution of gelatin at pH 6.5. We shall now extend the discussion to the results obtained at lower pH and in more concentrated solutions.

At the concentration 7% and pH 6.5 the interaction between gelatin and SDS presents two principal differences in comparison with the dilute solution: there is a continuous increase of viscosity starting at the SDS concentration 10^{-3} M, and the binding isotherm shows a continuous increase with no intermediate break. Also we note that the viscosity is an order of magnitude higher and that the SDS uptake is significantly lower than in the dilute gelatin solution (Figure 7). We have shown in the first section that the polymer coils overlap in gelatin solutions at 7%. The consequence is that, instead of an intramolecular shrinking of discrete protein molecules induced by small SDS aggregate(s), the surfactant micelles cooperatively bridge the gelatin molecules. Similar observations were made by Tavernier [18] and Greener [15]. In the latter study a correlation was found between the size of the alkyl group and the onset of thickening, demonstrating that the phenomenon is related to the tendency of surfactant molecules to aggregate. By analyzing the dependence of the zero shear viscosity on the gelatin and the SDS concentrations, the authors deduced that each SDS micelle is bound with a maximum of three gelatin molecules. This ratio is qualitatively in line with the lower binding presented in Figure 8 for gelatin solutions at 7%.

At pH 4.5 the protein molecule is quite below its isoelectric point. It carries a high density of positive groups that constitute adsorption sites for SDS molecules. It is therefore understandable that the SDS-gelatin complex precipitates when its charge is completely neutralized and moreover when the hydrocarbon tails of SDS enhance the hydrophobic character. Logically precipitation (charge neutralization) and redissolution of the complex at pH 4.5 takes place at exactly the same

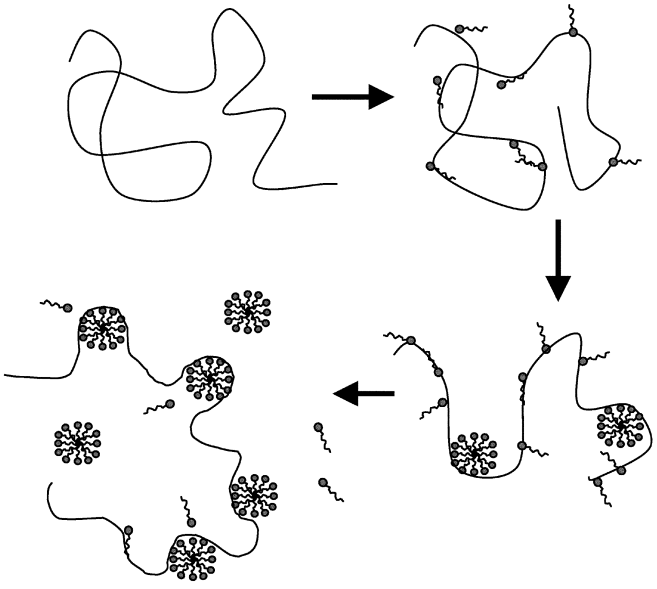

Fig. 8 Sketch of the molecular complex between SDS and gelatin

SDS/gelatin ratios in the 1 and 7% gelatin solutions (the two curves merge in Figure 2). Redissolution occurs at higher SDS ratio when the complex turns negative. The correspondance between precipitation and charge neutralization has been studied by Arora et al [35] in the system gelatin-triethanolamine lauryl sulphate at pH 2, 3 and 4; by Chen and Dickinson [17, 22] for the complex formed with sodium lauryl ether sulphate and gelatin, by Vinetsky et al [6] and Knox and Parshall [11] for the complex SDS-gelatin. Our data reveal clearly that a break in physical properties (increase of viscosity, resolubilization, decrease of viscosity) takes place at the concentration where an excess of charge of the complex results from the cooperative binding of SDS molecules via the hydrophobic attraction between hydrocarbon tails. At pH 6.5 the SDS-gelatin complex remains soluble at charge neutralization in our investigation (about concentration C_2) because the carboxylic groups ionization makes it sufficiently hydrophilic.

Conclusions

We have shown that the binding of SDS with gelatin is of a different kind below (1 wt%) and above (7 wt%) the critical concentration C*. In the dilute regime SDS interacts with a single gelatin molecule producing conformational changes that are revealed by the variation of the viscosity of the solution. Three particular SDS concentrations were identified that correspond to different stages in the binding ratio and in the behaviour of the complex molecule. Below the concentration C_1, the surface tension is the only property that reveals some

interaction between SDS and gelatin. Between concentrations C_1 and C_2 the viscosity decreases, which is caused by the contraction of the gelatin molecule due to the electrostatic screening of the positive charges and to the intramolecular association between the hydrocarbon tails of SDS. Above concentration C_2 the cooperative binding of SDS molecules leads to the formation of large micelles, probably similar to those found normally in the bulk. At this stage the molecule extends, becoming more hydrophilic, correspondingly the viscosity increases and the complex is displaced from the solution interface by free SDS molecules. At still higher SDS concentrations free (unbound) micelles form in the solution and the surface tension reaches that given by pure SDS solutions.

At higher gelatin concentration (above the molecular overlap starting at concentration 3.1%) the viscosity only increases upon addition of SDS. The thickening results from a cooperative association of several gelatin molecules via SDS micelles that form at lower concentration than the regular CMC.

Different binding processes were therefore observed depending on the uptake ratio. At lower concentration SDS binds electrostatically with discrete positive groups of the protein but does not significantly modify the conformation. Between concentrations C_1 and C_2 the binding density makes possible the aggregation of the surfactant tails but the aggregation number remains much lower than in regular micelles (17 molecules per gelatin molecule). Above C_2 additional binding of SDS takes place through hydrophobic attraction between surfactant tails, causing an excess of negative charge and the stretching of the protein. In addition to the increase of viscosity, the later binding step is associated with a strong decrease of the surface tension, due to the displacement of the SDS-gelatin complex from the interface by regular SDS molecules. At the maximum the binding ratio of SDS is 200 molecules per gelatin molecule (Mw = 50,000) which is equivalent to about 4 regular micelles per molecule.

A very good coherence was found between the results of the different techniques used in the experimental analysis. The transitions between the binding steps were easily identified at the increasing concentrations $C_1 = 5\ 10^{-4}$ M, $C_2 = 5\ 10^{-3}$ M and CMC* = $4\ 10^{-2}$ M in solutions at pH 6.5 and gelatin 1 wt %. At pH 4.5 the binding of SDS lead to an electrostatic collapse and precipitation at low concentration followed by resolubilization when the excess of negative charge was sufficient, thanks to the hydrophobic binding of additional SDS molecules. The resolubilization of the SDS-gelatin complex at pH 4.5 and the expansion of the molecule at pH 6.5 are therefore of the same physical nature: an electrostatic excess producing a more hydrophilic complex in the first case and electrostatic repulsions in the second case.

References

1. Luzzi LA, Gerraughty RJ (1964) J Pharm Sci 53:429
2. Luzzi LA, Gerraughty RJ (1967) J Pharm Sci 56:634
3. Okada J, Kusai A, Ueda S (1985) J Microencapsulation 2:163
4. Magdassi S, Vinetsky Y (1995) J Microencapsulation 12:537
5. Vinetsky Y, Magdassi S (1997) J Colloid Interface Sci 189:83
6. Vinetsky Y, Magdassi S (1997) Colloids Surf, A 122:227
7. Moya S, Dahne L, Voigt A, Leporatti S, Donath E, Möhwald H (2001) Colloids Surf, A 183:27
8. Decher G, Hong JD (1991) Ber Bunsenges Phys Chem 95:1430
9. Pankurst KGA, Smith RCM (1944) Trans Faraday Soc 40:465
10. Knox WJ, Wright JF (1965) J Colloid Interface Sci 20:177
11. Knox WJ, Parshall TO(1970) J Colloid Interface Sci 33:16
12. Knox WJ, Parshall TO (1971) J Colloid Interface Sci 40:290
13. Wustnek R, Hernel H, Kretzschmar G (1983) J Colloid Inetrface Sci 93:419
14. Asnacios A, Langevin D, Argillier JF (1996) Macromolecules 29:7412
15. Greener J, Contestable BA, Bale MD (1987) Macromolecules 20:2490
16. Howe, Wilkins AG, Goodwin JW (1992) J Photogr Sci 40:234
17. Chen J, Dickinson E (1995) Colloids Surf, A 100:255
18. Tavernier BH (1983) J Colloid Interface Sci 93:419
19. Sovilj V (1998) Colloid Polym Sci 276:328
20. Whitesides TH, Miller DD (1994) Langmuir 10:2899
21. Griffiths PC, Roe JA, Bales BL, Pitt AR, Howe AM (2000) Langmuir 16:8248
22. Chen J, Dickinson E (1995) Colloids Surf, A 100:267
23. Henriquez M, Abuin E, Lissi E (1993) Colloid Polym Sci 271:960
24. Mokus M, Kragh-Hansen U, Letellier P, Le Maire M, Moller JV (1998) Anal Biochem 264:34
25. Huggins ML (1942) J Am Chem Soc 64:2716
26. Kraemer EO (1938) Ind Eng Chem Res 30:1200
27. Scatchard G (1949) Ann New York Acad Sci 51:660
28. Kwak JCT (1998) Polymer-surfactant systems. Surfactant Science Series, Marcel Dekker, New York
29. Griffiths PC, Cheung AYF (2002) Mater Sci Technol 18:591
30. Godard ED (1993) Interactions of surfactants with polymers and proteins. CRC Press, Boca Raton, FL
31. Isemura T, Tokiwa F, Ikeda S (1955) Bull Chem Soc Japan 28:555
32. Tomasic V, Tomasic A, Filipovic-Vincekovic N (2002) J Colloid Interface Sci 256:462
33. Miller DD, Lenhart W, Antalek BJ, Williams AJ, Hewitt JM (1994) Langmuir 10:68
34. Arora JPS, Soam D, Singh SP, Kumar R (1984) Tenside Detergents 21:87
35. Arora JPS, Pal C, Dutt D (1991) Tenside Surf Det 28:215