

Effects of Fluorinated and Hydrogenated Surfactants on Human Serum Albumin at Different pHs

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Complexation between human serum albumin (HSA) and two different surfactants, one fully fluorinated (sodium perfluorooctanoate, SPFO) and one fully hydrogenated (sodium caprylate, SO), was studied using ζ -potential measurements and difference spectroscopy. The study was carried out at three different pHs, 3.2, 6.7, and 10.0. The spectroscopy study was performed at pHs 6.7 and 10.0, given that at pH 3.2 high turbidity was observed in the wide range of surfactant concentrations. The results were interpreted in terms of the electrostatic and hydrophobic contributions to the stability of the different phases formed in the water–surfactant–HSA system. Solutions and precipitates were observed in the concentration range investigated in more detail. Using Pace methods, the thermodynamic values of the surfactant-induced conformational changes in HSA were determined for sodium perfluorooctanoate in the concentration range 2–12 mmol dm⁻³ at pH 6.7 and 5–22 mmol dm⁻³ at pH 10.0. Electrophoretic measurements were used to characterize surfactant adsorption by determining the number of molecules adsorbed on the surface of HSA and the Gibbs energy of adsorption. Finally, the interactions between human serum albumin and other anionic surfactants studied by other authors were compared with those observed in the present work.

Introduction

When preparing human serum albumin (HSA) for clinical use, it is important to study its behavior at different temperatures. Albumin is heated to 60 °C for about 10 h to inactivate the hepatitis virus. To retain protein structure in this treatment, sodium octanoate or sodium octanoate plus acetyltryptophan may be used as a stabilizer¹.

Much experimental work has been devoted to studying the interactions between surfactants and proteins in recent years, essentially in regard to the effects of sodium *n*-dodecyl sulfate (SDS) on enzymes and its denaturing effect.^{2,3} Different models have been proposed for the structures of these complexes, and it is well-established that the interaction between anionic surfactants and proteins involves initial binding of the surfactant molecules to cationic amino acid residues on the surface of the protein by ionic interactions followed by more extensive hydrophobic binding (surfactant alkyl chains bind hydrophobic regions of the enzyme close to the cationic sites) as the critical micelle concentration (cmc) of the surfactant is approached.⁴

The driving force of the energetics of protein conformations appears to be the hydrophobic effect as Creighton has pointed out⁵ or the “degree of attraction for water” as consider by Hvidt and Westh.⁶ Nonpolar groups are rather nonattractive, whereas polar or charged groups are attractive for the dipolar water molecules. So, an understanding of the stability of protein conformations is associated with the concept of hydrophobic effects or hydrophobicity. Moore et al.⁷ have examined the role of the surfactant polar head structure in protein–surfactant complexation.

The amino acid sequence of HSA is known.⁸ The number of the ionizable groups can be counted: a total of 116 acidic groups

(98 carboxyl and 18 phenolic OH) and 100 basic groups (60 amino, 16 imidazolyl, and 24 guanidyl). The absolute molecular weight of HSA is 66 436, as determined from the numbers and molar masses of the constituent amino acid residues.

The present paper analyzes the hydrophobic effects in surfactant–protein interactions, examining the role of the different hydrophobicities of the surfactant tails. The effects of fully fluorinated and fully hydrogenated surfactant concentration on human serum albumin was studied in different pHs (3.2, 6.7, and 10.0) by electrophoretic mobilities and spectroscopic measurements. The electrophoretic mobility of sodium caprylate/HSA and sodium perfluorooctanoate/HSA was measured, providing information on the adsorbed layer, the ζ -potential of the complex, and the adsorption energies. UV–vis data were used to follow the conformational changes in the HSA structure.

Materials and Methods

Materials. Human serum albumin (product no. A-1887) was purchased from Sigma. The surfactants sodium perfluorooctanoate (product no. 16988) and sodium caprylate (product no. 10241) were obtained from Lancaster MTM Research Chemical, Ltd. Three buffers were used: (1) 50×10^{-3} mol dm⁻³ glycine plus hydrochloric acid, pH 3.2; (2) NaH₂PO₄ (50×10^{-3} mol dm⁻³) plus Na₂HPO₄, pH 6.7; and (3) 50×10^{-3} mol dm⁻³ glycine plus sodium hydroxide, pH 10.0. Sodium azide (0.02% w/v) from Merck (no. 6688) was added to the three buffers, contributing 0.0031 mol dm⁻³ to the ionic strength. All other materials were of analytical grade, and solutions were made using doubly distilled and degassed water. The critical micellar concentration (cmc), in water at 25 °C, was 31.3 mmol kg⁻¹ for sodium perfluorooctanoate (González-Pérez et al., 2003)⁹ and 0.382 mol kg⁻¹ for sodium caprylate (González-Pérez et al., 2003).¹⁰

Determination of ζ -Potential. Zeta potential (ζ -potential) measurements of the protein–surfactant complex were recorded using a Malvern Instruments, Ltd. Zetamaster 5002 by taking the average of five independent measurements at the stationary level. The measurement

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was taken 1 day after sample preparation. The cell used was a 5 mm \times 2 mm rectangular quartz capillary. The temperature of the experiments was 298.15 ± 0.01 K controlled by proportional temperature controller HETO. ζ -potential was calculated from the electrophoretic mobilities, μ_E , using the Henry equation¹¹

$$\zeta = \frac{3\mu_E\eta}{2\epsilon_0\epsilon_r f(\kappa a)} \quad (1)$$

where ϵ_0 is the permittivity of vacuum, ϵ_r and η are the relative permittivity and viscosity of water, respectively, a is the particle radii, and κ the Debye length. The function $f(\kappa a)$, which depends on particle shape and, in this system, on the protein-surfactant complex, was determined by

$$f(\kappa a) = \frac{2}{3} - \frac{9}{2\kappa a} + \frac{75}{2\kappa^2 a^2} - \frac{330}{\kappa^3 a^3} \quad (2)$$

valid for $\kappa a > 1$.

All measurements were taken using solutions containing a protein concentration of 1.25 g L^{-1} .

Difference Spectroscopy. Difference spectra were measured using a Beckman spectrophotometer (model DU 640), with 6 microcuvettes, which operates in the UV-vis region, from 190 to 1100 nm of the electromagnetic spectrum wavelength. Absorbance was measured at 25 °C using a Beckman (DU Series) temperature controller, following the Peltier methods for temperature control.

All measurements were made using HSA solutions with a fixed concentration of 1.25 g dm^{-3} in carefully matched quartz cuvettes (50 μL capacity) in the wavelength range 220–500 nm. For absorbance difference spectra, the cells were filled with protein and surfactant solutions, using the protein solutions as reference. Stock solutions of defined protein concentrations were prepared by dissolving a certain amount of freeze-dried protein in an appropriate buffer volume. All cuvettes were filled and placed in the same orientation for all tests. Measurements were made after HSA and surfactant had been incubated for over 30 min time, during which the difference spectra did not change.

All absorbance measurements reported refer to surfactant concentrations below the critical micelle concentration (cmc) of surfactants in the different media used in this work.

Results and Discussion

The interaction between protein (HSA) and anionic surfactants was studied by monitoring the change in calculated ζ -potential and spectroscopic measurements upon addition of surfactants, sodium perfluorooctanoate (SPFO), and sodium caprylate (SO), with the same HSA concentration. This study was carried out at pH's 3.2, 6.7, and 10.0 and a constant temperature of 25 °C.

Electrophoretic Mobility. The binding of the two anionic surfactants to human serum albumin at pH's 6.7 and 10.0 was expected to produce an increase in the net charge on the protein-surfactant complex. This is illustrated in Figures 1 and 2, which show a plot of the ζ -potential of the protein-surfactant complex as a function of surfactant concentration for native HSA solutions at 25 °C, for both surfactants and different pH's. Pure HSA molecules, in the absence of added surfactant, showed ζ -potential values of -9.6 mV and -28.3 mV for pH 6.7 and pH 10.0, respectively. Surfactant (SPFO, SO) addition increased the effective net charge on the protein, suggesting the formation of HSA-surfactant complexes. However, in acid medium (pH 3.2), the pattern of HSA binding was different, as seen in Figures

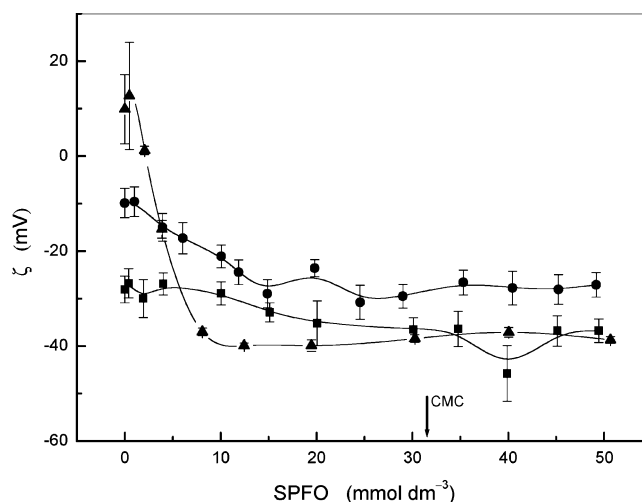


Figure 1. Influence of sodium perfluorooctanoate (SPFO) concentration on the ζ -potential of human serum albumin ($0.5 \times 10^{-3} \text{ kg dm}^{-3}$) in aqueous solutions, at 25 °C: (Δ) pH 3.2, (\bullet) pH 6.7, (\blacksquare) pH 10.0. The arrow indicates the critical micelle concentration.

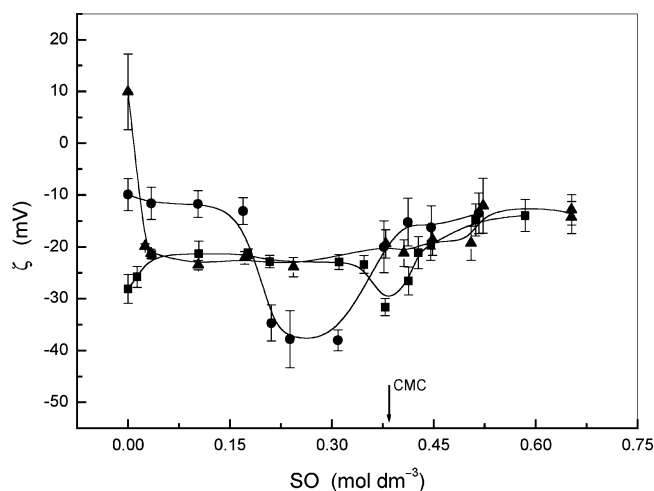


Figure 2. Influence of sodium caprylate (SO) concentration on the ζ -potential of human serum albumin in aqueous solutions (protein, $0.5 \times 10^{-3} \text{ kg dm}^{-3}$), at 25 °C: (Δ) pH 3.2, (\bullet) pH 6.7, (\blacksquare) pH 10.0. The arrow indicates the critical micelle concentration.

1 and 2. The net charge of the protein-surfactant complex changed from positive ($\zeta = +9.9 \text{ mV}$) in the native protein in the absence of surfactant to negative upon surfactant addition. This is consistent with previously reported isoelectric points of 4.2,¹² 4.9,¹³ and 4.7–4.9¹⁴ for HSA. At pH's 6.7 and 10.0, ζ -potential values were negative, and the plots of ζ -potential versus surfactant concentration were different for SPFO and SO. The adsorption of surfactants onto proteins did not change the sign of the ζ -potential, and all negative values became more negative as the surfactant concentration increased, reaching the cmc. This negative ζ -potential value suggests that the hydrophobic interaction was predominant. In the interaction with the fluorinated surfactant, SPFO, the ζ -potential decreased with surfactant concentration at low concentrations and finally remained constant, an effect observed by Kayes.¹⁵ However, in the interaction with the hydrogenated surfactant, SO, after an initial decrease in ζ -potential, the system reached a plateau and then a minimum for all pH's, especially clear at 6.7 and 10.0, when the cmc is approached and finally the ζ -potential increased. Since the driving force for binding is mainly a hydrophobic interaction, as described in previous papers, this

minimum may be regarded as the concentration¹⁶ where SO molecules begin to form hemimicelles on the surface of the HSA.

These two different behaviors, for SPFO and SO in the interaction with HSA, had previously been observed in the interaction with the same surfactants and other proteins (lysozyme, catalase, and hemoglobin).¹⁷ In the interaction with SPFO, the protein surface seems to be full, and surfactant aggregation is reached. It is well-known that anionic surfactants induce denaturation in many globular proteins. Shirahama et al.¹⁸ observed that the free electrophoretic mobilities of the saturated polypeptide–sodium dodecyl sulfate complexes were virtually constant. Probably, there is a conformational change in the protein due to the interaction with fluorinated surfactant. When the conformational change is reached, the ζ -potential remains constant. This effect was observed in spectrometric experiments. The structure of the surfactant–protein complex can be described by a string of constant-sized micelles distributed randomly along the hydrophobic patches of the denatured random coil.¹⁹

In the interaction with SO, however, there was a difference; the initial change was not as notable as that observed in the interaction with SPFO, except in the case of pH 3.2. Probably, close to the surfactant cmc there is competition between the SO–protein interaction and SO micellization, or that interaction of SO micelles with surface protein cannot exist. This behavior was observed at pH's 6.7 and 10.0, when the protein charge is negative upon addition of the hydrogenated surfactant.

At pH 3.2, the protein changes phase to form precipitates. This can be explained by taking into account previous works,^{20,21,22} where the acid-induced structural changes of HSA have been studied. In agreement with these works, at pH values below 4, albumin undergoes expansion to the E form (less compact and increasingly asymmetric molecule) as a result of an abrupt opening of the protein molecule. This involves a breakup of salt bridges and of attractive hydrophobic interactions, and the protein undergoes a decrease in helical content from 55% to 45% attributed to helix \rightarrow β -sheet and helix \rightarrow coil transition. Consequently, the protein is more accessible to surfactant interaction, and the complexes HSA–SPFO and HSA–SO formed are not stable and precipitate under these conditions.

Figures 1 and 2 show an initial abrupt change, at low surfactant concentration for pH's 3.2 and 6.7, which corresponds with the initial interaction assumed to be an electrostatic interaction between the surfactant and the specific binding sites of the HSA in the different medium, i.e., an interaction between anionic surfactant and the cationic parts of HSA, more evident in the acid medium where the protein charge is positive. At pH 10, this change in ζ -potential at low concentrations is smaller than in the other media, probably because of the effective charge of the protein. In this medium, pH 10.0, the charge is more negative, and then, the initial electrostatic interaction with the surfactant is less effective. There are probably fewer charge interactions, since at this pH, the protein and the surfactants have the same charge sign. This behavior is consistent with that reported in previous results.^{15,23–25}

If we consider the slope of the ζ -potential–log(surfactant concentration) as adsorption due to the hydrophobic effect taking place (i.e., the whole of the concentration under study, where the protein and the surfactant have the same charge sign), the following equation can be used to calculate the number of

adsorption sites per unit area, N_1 :^{15,26}

$$\frac{d\zeta}{d \log c} = \frac{4.606k_B T}{ze} \left[\frac{\sinh(ze\zeta_1/2k_B T) - \sinh(ze\zeta_2/2k_B T)}{\cosh(ze\zeta_2/2k_B T)} \right] \times \left[\frac{\sqrt{8n_0\epsilon k_B T} [\sinh(ze\zeta_1/2k_B T) - \sinh(ze\zeta_2/2k_B T)]}{zeN_1} - 1 \right] \quad (3)$$

where ζ_1 and ζ_2 are the potentials on the line, c is the concentration, n_0 is the ionic concentration, ϵ is the relative permittivity of the medium, k_B is the Boltzmann constant, z is the ion charge, and T is the absolute temperature.

The adsorption constant κ_2 can be calculated from the equation

$$\frac{1}{c} = \kappa_2 \left[\frac{zeN_1}{\sqrt{8n_0\epsilon k_B T} [\sinh(ze\zeta_1/2k_B T) - \sinh(ze\zeta_2/2k_B T)]} - 1 \right] \quad (4)$$

Here, c is chosen as the concentration at the potential midpoint between ζ_1 and ζ_2 . The standard free energy of adsorption, $\Delta G_{\text{ads}}^\circ$, can be obtained from the equation

$$\kappa_2 = \exp(-\Delta G_{\text{ads}}^\circ/k_B T) \quad (5)$$

The standard free energies of adsorption, evaluated from eq 3, are plotted in Figure 3 for the interaction between HSA and the surfactants SO and SPFO at pH 10.0. Similar behavior was observed for the other pH's. The shape of these curves shows that $\Delta G_{\text{ads}}^\circ$ is large and negative at low surfactant concentrations where binding to the high-energy sites occurs and linearly decreases as saturation is approached. The values of the free energies of adsorption in the saturation can be assumed constant, -12 and -18 kJ mol⁻¹ for SO and SPFO, respectively. Similar behavior was found for the systems propranolol hydrochloride/human serum albumin,²⁷ sodium *n*-dodecyl sulfate/lysozyme²⁸ and nafcillin/human serum albumin.²⁹ As observed in Figure 3, there is a difference in the interaction between the protein and the two types of surfactants. The Gibbs energy of interaction is larger for SPFO than for SO. This suggests that the adsorption process is energetically more favorable for SPFO. The number of adsorption sites is shown in Table 1. This number decreases as the pH changes from acidic to basic. When the two surfactants are compared, no significant differences are observed. Probably, at pH 3.2 the HSA molecule is more extended, and the protein charge is positive (the first electrostatic interaction affects positively), and the binding sites increase in this medium.

It is well-known that anionic surfactants, such as SDS, induce denaturation in many globular proteins. Shirahama et al.¹⁸ observed that the free electrophoretic mobilities of the saturated polypeptide–SDS complexes were practically constant.

Spectroscopic Measurements. To evaluate the effect of the two surfactants on HSA, difference spectra were collected at 280 nm for native versus surfactant–protein complex. Figures 4 and 5 show the absorbance change for the 280 nm difference spectral band as a function of surfactant concentration at 25 °C. The data show that, for pH's 6.7 and 10.0, in the case of the interaction between SPFO and protein, there is a transition region over which the absorbance changes steeply with surfactant concentration, moreso in the neutral medium. At pH 6.7, the transition is in the concentration range 2–12 mmol dm⁻³, while at pH 10.0, the range is 5–22 mmol dm⁻³. However, for SO–protein interactions, no transition region is observed in this

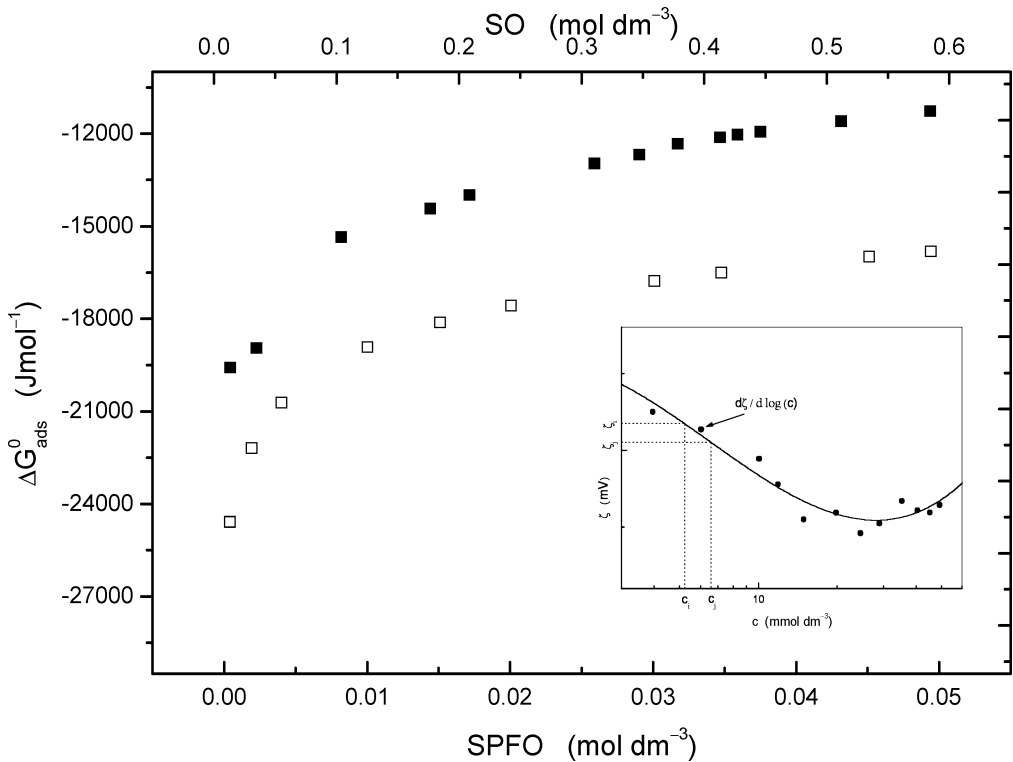


Figure 3. Gibbs energy of adsorption on human serum albumin ($0.5 \times 10^{-3} \text{ kg dm}^{-3}$) as a function of the concentration of sodium perfluorooctanoate (SPFO) (□) and sodium caprylate (SO) (■) in aqueous solutions, at 25 °C and pH 10.0. Inset illustrate the method: First, experimental points are fitting to the best polynomial equations, where ζ_i and ζ_j are ζ -potential values of this fitting, choice around a experimental point. The derivative permits calculation of κ_2 and then $\Delta G_{\text{ads}}^\circ$ through eqs 3, 4, and 5.

Table 1. Number of Adsorption Sites, $N_1 \text{ (m}^{-2}\text{)}$, at 25 °C and Different Media

	pH 3.2	pH 6.7	pH 10.0
SO	1.1×10^{15}	9.9×10^{14}	4.8×10^{14}
SPFO	1.0×10^{15}	1.7×10^{14}	1.2×10^{14}

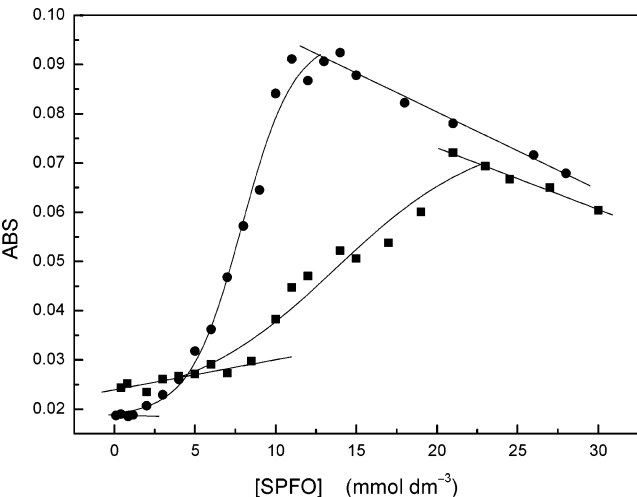


Figure 4. Effect of sodium perfluorooctanoate on human serum albumin stability. Difference spectral band (280 nm) of human serum albumin ($0.5 \times 10^{-3} \text{ kg dm}^{-3}$) in different buffered media: pH's 6.7 (●) and 10.0 (■).

concentration range. It is clear that SO does not produce a well-behaved transition; however, it produces a very profound noncooperative effect on the structure of the protein. Above SO concentrations of 0.2 mol dm^{-3} at pH 6.7 and 0.3 mol dm^{-3} at pH 10.0, the sample became turbid and the absorbance increased continuously, with a new gel phase appearing above

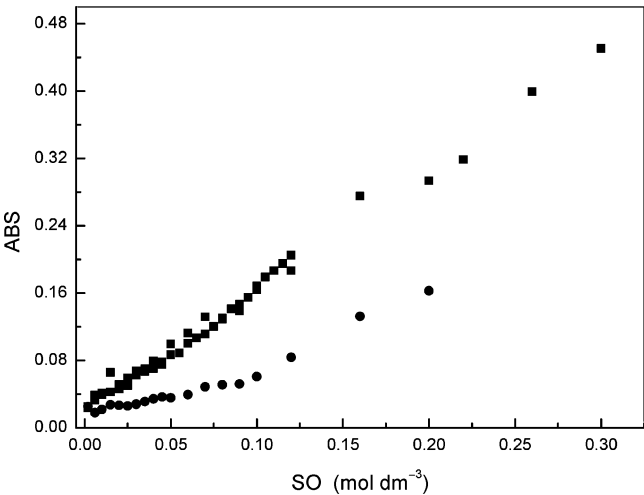


Figure 5. Effect of sodium caprylate (SO) on human serum albumin. Difference spectral band (280 nm) of human serum albumin ($0.5 \times 10^{-3} \text{ kg dm}^{-3}$) in different buffered media: (●) pH's 6.7 and (■) 10.0.

these surfactant concentrations. All transitions occurred at surfactant concentrations below the cmc.

Thermodynamically, as a first approximation, the denaturation process can be considered a transition between the two macroscopic states: the native state (N) and a denatured state with $\bar{\nu}$ bound surfactant ligands ($\text{DS}_{\bar{\nu}}$)



where $\bar{\nu}$ is the average number of surfactant molecules bound to denatured complex $\text{DS}_{\bar{\nu}}$. The two-state character of the transition is a consequence of the cooperative nature of the protein unfolding. The analysis of equilibrium unfolding transition requires extrapolation of the baseline for the native and

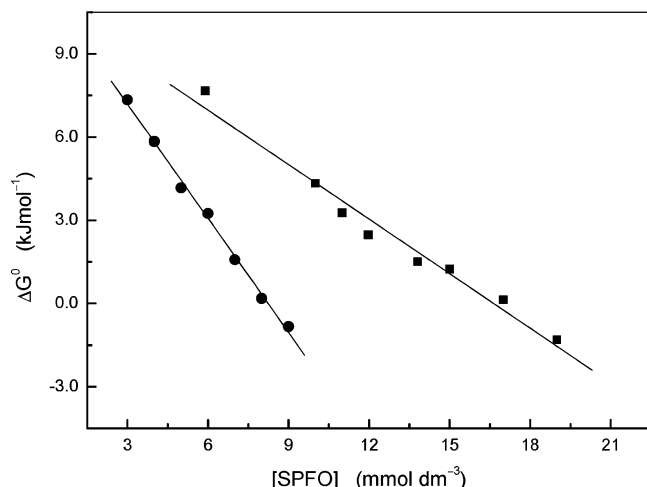


Figure 6. Standard Gibbs energy difference between the folded and unfolded conformations (ΔG°) of human serum albumin (0.5×10^{-3} kg dm $^{-3}$), in aqueous solutions of pH 6.7 (●) and pH 10.0 (■) as a function of sodium perfluorooctanoate (SPFO) concentration. The ΔG° values were calculated using eq 8. The solid line represents eq 10 with the average parameters given in Table 2.

unfolded protein into the transition region to determine the fraction of denatured molecules, F_D , as a function of the unfolding parameters³⁰

$$F_D = \frac{A_N - A_{OBS}}{A_N - A_D} \quad (7)$$

where A_{OBS} is the absorbance observed, and A_N and A_D are the absorbance for the native and denatured conformations, respectively.

The difference in the standard Gibbs energy between the folded and unfolded conformations can then be calculated as

$$\Delta G^\circ = -RT \ln \left[\frac{F_D}{1 - F_D} \right] = -RT \ln \left[\frac{A_N - A_{OBS}}{A_{OBS} - A_D} \right] \quad (8)$$

where R is the gas constant and T is the absolute temperature.

The equilibrium constant (K) for eq 6 is

$$K = \frac{[DS_{\bar{v}}]}{[N][S]^{\bar{v}}} = \frac{K_s}{[S]^{\bar{v}}} = \frac{F_D/(1 - F_D)}{[S]^{\bar{v}}} \quad (9)$$

where K_s is the ratio of denatured to native molecules. Figure 6 shows that ΔG° and $\ln K_s$ are a linear function of $[S]$ for a temperature of 25 °C, consistent with the relationships

$$\Delta G^\circ = \Delta G_w^\circ - m[S] \quad (10)$$

$$\ln K_s = \ln K_w + \frac{m}{RT}[S] \quad (11)$$

where ΔG_w° is the Gibbs energy at zero concentration of denaturant. Thus, to obtain the values ΔG_w° , a reliable procedure for the extrapolation of ΔG° ($[S]$) to zero denaturant concentration, i.e.

$$\Delta G_w^\circ = \Delta G^\circ([S])_{\lim[S] \rightarrow 0} \quad (12)$$

is required. Numerous observations of many different proteins have shown that, in the transition range, the Gibbs energy of unfolding is, in a relatively narrow range, a linear function of denaturant concentration.³¹ These observations led to the so-called linear extrapolation method (LEM) for determination of

Table 2. Parameters Characterizing the Unfolding of HSA by SPFO at 25 °C in 50 mM Glycine Buffer (pH's 6.7 and 10.0)^a

	$[C]_{1/2}$ mmol $^{-1}$	ΔG_w° kJ mol $^{-1}$	m kJ mol $^{-1}$ M $^{-1}$	\bar{v}	$\ln K$	ΔG_{hc}° kJ mol $^{-1}$	$\Delta(\Delta_{tr}G^\circ)^Z$ kJ mol $^{-1}$
pH 6.7	7.9	11.3	1372	3.0	14.4	-35.8	-47.1
pH 10.0	12.9	11.9	730	3.5	14.4	-35.7	-47.6

^a The correlation coefficient of the plot in Figure 6 is better than 0.99.

the Gibbs energy of unfolding in the absence of denaturant (eq 10) where the so-called denaturant m -value is a measure of the dependence of ΔG° on surfactant concentration, $[S]$, and the $m[S]$ term thus represents the difference in transfer Gibbs energy between the unfolded and native states.

The most attractive feature of this analysis is that it allows one to estimate the stability of a protein over a range of conditions of interest. On this point, following Plaza del Pino and Sánchez-Ruiz,³² a difference should be noted between the *thermodynamic stability*, which is the work required to disrupt the native protein structure under particular solution conditions, and the *thermal stability*, which is understood simply as the temperature at which a protein denatures in a particular solvent and pH.

Thermodynamic stability (stabilization free energy) is directly related to the equilibrium between native and denatured states of the protein. This indicates that the higher the thermodynamic stability the lesser the probability for the native protein molecule to unfold because of thermal fluctuations and the shorter the time interval during which each protein molecule is unfolded. This, in turn, determines sensitivity of the protein to hydrolysis or inactivation due to chemical modifications or aggregation of unfolded polypeptide chains. Thus, thermodynamic stability (stabilization free energy) of a protein is a parameter which is directly related to a real lifetime of proteins in solutions under native conditions.³³

The values of ΔG_w° and m were calculated using eqs 10 and 11. These parameters are both derived from the linear plots fitted by least-squares in the transition curves. Table 2 shows the values of ΔG_w° and m for pH's 6.7 and 10 for the interaction with SPFO; values for the interaction with SO are not shown because there was no transition region, as shown in Figure 5.

It follows from eq 6 that

$$\ln K = \ln K_s - \bar{v} \ln [S] \quad (13)$$

At a surfactant concentration of 1 mol dm $^{-3}$, $\ln K = \ln K_s$, and the equilibrium constant, K , corresponds to the unfolding transition in a surfactant-saturated complex, approximating that in a highly hydrophobic environment with a corresponding Gibbs energy change ΔG_{hc}° . By using least-squares analysis of the plots of $\ln K_s$ versus $\ln [SPFO]$, the values of ΔG_{hc}° were obtained. The slopes of these plots, \bar{v} , and the values of $\Delta G_{hc}^\circ - \Delta G_w^\circ$ defined as $\Delta(\Delta_{tr}G^\circ)$ correspond to the difference in the Gibbs energies of transfer of the unfolded and native state from water to a hydrophobic environment as previously described.³⁴ All values of the denaturant unfolding parameters are shown in Table 2.

The results of the human serum albumin denaturation and the values of the parameters obtained from the chemical denaturation suggest that the protein structure was affected by the fluorinated surfactant at pH's 6.7 and 10.0. However, as the alterations produced by SO are not cooperative, it is no possible to calculate a Gibbs free energy change. The values of the thermodynamic parameters of denaturation are in agreement with the same values of other unfolded proteins.

The results reveal that, in the interaction between the fluorinated surfactant and HSA, a complex is formed, and the protein undergoes a conformational change at pH's 6.7 and 10, as observed in Figure 4. However, in the interaction of HSA—hydrogenated surfactant, by electrophoretic measurements, a complex was formed, but a significant conformational change in the protein at both pH's was not observed. This difference can be attributed to the high hydrophobicity of the fluorinated surfactant compared with that of the hydrogenated one.

There were no significant differences in the thermodynamic parameters between pH's 6.7 and 10.0. The interaction was stronger (cooperative) at pH 6.7, as observed by the high values of parameter m , and the rest of the thermodynamic parameters were quite similar for both media. In principle, the value of ΔG_w° is independent of the surfactant used to induce the transition, but in this case the medium is different (different pH's), and ΔG_w° is larger at pH 10.0, probably because the protein has a different conformation at this basic medium.²¹

Hence, the interaction between fluorinated surfactant and HSA will be more favorable at pH 6.7 than at pH 10.0. m values show close dependence on the variation of the medium, suggesting that the unfolded form of the protein obtained from the chemical denaturants depends on the hydrophobicity of the surfactant and on the electrical charge of protein.

As the surface activity of fluorocarbon surfactants is stronger than that of the hydrocarbon surfactant with corresponding carbon number, the binding of fluorocarbon surfactants to protein is expected to be the same as that of hydrocarbon surfactants. However, in a previous paper,³⁵ Deep and Ahluwalia reported the importance of the hydrophobic part of the surfactant in binding bovine serum albumin (BSA). In the present experiments with HSA—surfactants, the same conclusion, i.e., the interaction of HSA—fluorinated/hydrogenated surfactant is affected by the protein charge, which depends on the medium and on the hydrophobicity of the surfactant. Farrugia and Picó³⁶ found, in the unfolding process of human serum albumin induced by guanidine chloride and urea, a similar result ($\Delta G_w^\circ = 14.2 \text{ kJ mol}^{-1}$ and 17.1 kJ mol^{-1} , respectively).

Conclusions

Electrophoretic mobilities and spectroscopic techniques have been used to characterize the interactions of two different chemical denaturant surfactants, sodium perfluorooctanoate (SPFO) and sodium caprylate (SO). The ζ -potential of HSA in its interaction with both surfactants showed similar behavior at pH 3.2. The HSA is protonated in this medium, and its interactions with surfactants are initially, at low surfactant concentration, electrostatic. There is a change from positive to negative in the surface charge; after the initial interaction, the ζ -potential is constant until the cmc is reached. At high surfactant concentration, the interaction is probably hydrophobic. In this medium, no stability was found with time. The complex precipitated one day after the preparation of the samples, probably because the protein structure was more open, leading to loss of stability of the HSA—surfactant complex.

In the other media (pH's 6.7 and 10.0), the behavior was different. The interaction of the protein with SPFO for pH's 6.7 and 10.0 do not lead to the formation of the precipitate, and the system was stable for the concentration range studied. However, a precipitate of the HAS—SO complex was found at high SO concentration, above 0.2 mol dm^{-3} and 0.3 mol dm^{-3} for pH 6.7 and pH 10.0, respectively.

At pH 6.7, the interactions observed using the ζ -potential measurements of HSA showed significant changes in ζ -potential

of HSA. The interactions were most likely hydrophobically driven when both species carried the same charge sign. In the case of interactions with SO, there was a minimum in the curve of the ζ -potential, close to the cmc. After the minimum, the ζ -potential decreases with an increase of the concentration of SO, and the formation of hemimicelles is possible. The Gibbs energy of adsorption determined by the Kayes method showed, in all cases, that the interactions between the fluorinated surfactant and HSA are more favorable than those of the hydrogenated one; this finding confirms the magnitude of the hydrophobicity of the surfactant.

The spectroscopic measurements of the interactions between HSA and the two surfactants showed two different trends. The interactions with SPFO presented a conformational change of the protein in the surfactant concentration range studied, always below the cmc. However, in the interactions with the hydrogenated surfactant, a very profound noncooperative effect on the structure of HSA is produced.

The parameters characterizing SPFO unfolding of HSA at 25 °C in buffer (pH's 6.7 and 10.0) are very close. Hence, the results suggest that the effects of the fluorinated surfactant on protein structure at these pH's are probably quite similar. The elevated value of parameter m and the Gibbs energy of unfolding at pH 6.7 is consistent with the most compact structure of HSA at pH 10.0.

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