 

# The Interactions of a Homologous Series of Cationic Surfactants with Bovine Serum Albumin (BSA) Studied Using Surfactant Membrane Selective Electrodes

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The binding of a homologous series of *n*-alkyltrimethylammonium bromides to bovine serum albumin were measured in 2.7 mM phosphate buffer, pH 7.0, and at various temperatures using surfactant membrane selective electrodes as a fast and accurate method. The obtained binding isotherms have been analyzed and interpreted using the binding capacity concept, Hill equation and Wyman binding potential. The system behaved as a system with two sets of binding sites in all studied situations. The results represent the essential role of hydrophobic interactions in the first binding set. At high cationic surfactant concentrations, the occupation of the second binding set occurred, with an accompanying unfolding and exposure of numerous hydrophobic binding sites. However, this unfolding process occurred for the cationic surfactant at much higher concentrations than was required for sodium dodecyl sulfate. The Gibbs free energy change calculated on the basis of the Wyman binding potential concept decreases in the initial stages of binding and passes through a minimum, followed by occupation of the second binding set. The affinity of binding increased with increasing temperature, indicating an endothermic and essentially entropy driven process.

Studies on the structure of protein molecules in aqueous solution are formidable not only because of the complexity of the structure but also because of the absence of any really direct and general experimental method. Although many native protein structures are known at present at the level of atomic resolution, we do not have a clear concept of the physical principles of organization of these structures and the forces stabilizing them in space. The only approach to this problem is by studying the process of destruction (denaturation) of the native structure. The denaturation of proteins may be done by denaturating agents such as urea, guanidine hydrochloride, or ionic surfactants. It is important to note that the mechanism of denaturation of proteins by ionic surfactants involves the binding of the surfactant ions to sites on the protein molecules, which occurs at 1–3 mmol $dm^{-3}$ surfactant concentration.[1] This concentration range is much lower than those required for other commonly used denaturants such as urea (6–8 mol $dm^{-3}$) or guanidine hydrochloride (4–6 mol $dm^{-3}$),[2] for which the denaturation process depends primarily on the effect of these compounds on the water structure and weakening of the hydrophobic interactions in the tertiary structure of the proteins. There is evidence that the initial interaction between surfactants and proteins is predominantly ionic; the surfactant ions bind to groups of opposing charge on the protein. These initial interactions cause the protein to unfold, which results in the exposure of more binding sites. As the surfactant concentration is increased, binding becomes cooperative and, ultimately, saturation occurs.[3] Thus, for an evaluation of the mechanism of surfactant denaturation, a very accurate measurement of the binding data is essential. The common experimental method which has usually been used for obtaining the binding data of ionic surfactants to proteins is equilibrium dialysis, which is a very tedious and time consuming method.[4–6] Furthermore, at higher surfactant concentrations, the Donnan effect may be attributed to our measuring. The influence of this effect on the distribution of the surfactant ions in equilibrium dialysis is magnified when the concentration of the surfactant ion is increased relative to that of non-reactive electrolytes.

Recently, surfactant membrane selective electrodes have been successfully used for investigating physico-chemical parameters of ionic surfactants in aqueous solutions containing various additives.[7–9] These electrodes have also been successfully used for binding studies of *n*-hexadecylpyridinium bromide with bovine serum albumin (BSA).[10] The potentiometric method has several advantages, including simplicity, cheapnes, a wide dynamic range, and fast response time and provides more accurate and precise data compared with the equilibrium dialysis technique.

In this paper we report the interactions of a homologous series of *n*-alkyltrimethylammonium bromides with BSA using surfactant membrane selective electrodes. Serum albumin is the most abundant protein in plasma with a typical concentration of 5 g/100 mL, which contributes 80% to the colloid osmotic blood pressure.[11] It has now been determined that serum

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albumin is chiefly responsible for the maintenance of blood pH.[12]

Based largely on hydrodynamic experiments[13–15] and low-angle X-ray scattering,[16] serum albumin was postulated to be an oblate ellipsoid with dimensions of 140 × 40 Å. However, studies using $^1$H NMR indicated that an oblate elipsoid structure for albumin was unlikely. Rather, a heart-shaped structure was proposed.[17]

The BSA molecule is made up of three homologous domains (I, II, III) which are divided into nine loops (L1–L9) by 17 disulphide bonds. The loops in each domain are made up of a sequence of large–small–large loops forming a triplet. Each domain in turn is the product of two subdomains (IA, IB, etc.).

The value of 4.7 has been reported as the isoelectric pH of BSA.[18] The BSA molecule is not uniformly charged within the primary structure. A net charge of −10, −8, and 0 have been calculated for domains I, II, and III of BSA at neutral pH, respectively.[19] Various researchers has studied the structure and properties of serum albumin and its interactions with surfactants in order to understand its functionality.[20–24] However, there is no comprehensive study on the interactions of a homologous series of cationic surfactants with BSA. This led us to the study of the interactions between a homologous series of *n*-alkyltrimethylammonium bromides and BSA. A thermodynamic analysis of binding data would provide a better understanding of the mechanism involved.

## Experimental

**Materials.** BSA type F (fatty acid free), obtained from Sigma Chemical Co., was used as received. Calculations were made assuming a molecular weight of 66000 D for BSA.[25] The BSA concentration was determined with the use of $\varepsilon_{280} = 4.4 \times 10^4$ $M^{-1}\,cm^{-1}$.[26] All of the cationic surfactants, 98% purity provided by Alderich Chemical Co., were purified by repeated crystallization from an acetone/water mixture. Tetrahydrofuran (THF) obtained from Merck was purified by distillation. All solutions were prepared with double distilled water. All of the measurements were carried out in 2.7 mM sodium phosphate buffer solution, pH 7.0 and $I = 0.0046$.

**Methods. Conditioning and Casting of Membrane:** 0.5 gram of carboxylated PVC dissolved in purified THF (20 mL) was added dropwise to 100 mL of $4 \times 10^{-3}$ mol dm$^{-3}$ surfactant in distilled water. The solution was allowed to stand for 4 h at room temperature. The precipitate was filtered off and dried between two sheets of filter paper. After that, the product was stored under vacuum at 30 °C over night. Freshly distilled THF (25 mL) was added to the 40% (0.12 g) conditioned PVC and 60% (0.18 g) of plasticizers (Elvaloy 742) in a 100 mL beaker. The solution was filtered through a 10 μL Teflon millipore filter in to a 5.5 cm glass dish (preveiously cleaned with THF). The dish was then left to stand in a dust free environment for two to three days to allow the solvent to evaporate at room temperature. The membrane was approximately 0.1 mm thick. A scalpel was used to cut around the membrane and water was added to assist in the removal of the membrane from the dish. The membrane was dried between two sheets of filter paper and a cork bore was used to cut a disk for the electrode tip. The THF was used to attach the membrane to the tip of the electrode.

**Electrode Body:** A silver rod was sealed to the PVC electrode body and then coated with AgBr in 0.1 mol dm$^{-3}$ NaBr solution in a 100 mL beaker. The silver rod (as an anode) and platinum (as a cathode) were connected in series with a 4 V battery and immersed in salt solution for 15 min. Finally, the coated rod was washed with distilled water.

**Emf Measurements:** The surfactant ion-selective electrode (ISE) was used for measurement of the free concentration of surfactant ions, $[S]_f$, in equilibrium with BSA–surfactant complexes at various conditions. The potentials were measured relative to a commercial sodium ion-selective electrode (Corning 476210). In all experiments, the temperature was controlled to within ±0.1 °C by circulating thermostated water through the jacketed glass cell, and the sample solution was continuously stirred using a magnetic stirrer.

At surfactant concentrations below the critical micelle concentration (cmc), the surfactant was dissociated completely. Therefore, the logarithm of the concentration of surfactant against the emf gives a Nernstian slope. Obviously, the potential of the electrode should be measured relative to the reference electrode. The cell configuration used was:

$$\text{Ag} \,\big|\, \text{AgBr} \,\big|\, \begin{matrix}\text{internal}\\ \text{solution}\end{matrix} \,\big|\, \begin{matrix}\text{PVC}\\ \text{membrane}\end{matrix} \,\big|\, \begin{matrix}\text{test}\\ \text{solution}\end{matrix} \,\big|\, \begin{matrix}\text{reference}\\ \text{electrode}\end{matrix}$$

The following equations can be written for different electrode potentials, according to the Nernst's equation:

$$E_{sur^+} = E^\circ_{sur^+} + \frac{RT}{F} \ln a_{sur^+} \tag{1}$$

$$E_{Na^+} = E^\circ_{Na^+} + \frac{RT}{F} \ln a_{Na^+} \tag{2}$$

where $T$, $R$, $F$, $a_{sur^+}$, and $a_{Na^+}$ are absolute temperature, gas constant, Faraday's constant, activity of surfactant ion, and activity of sodium ion, respectively. $E_{sur^+}$, $E_{Na^+}$, $E^\circ_{sur^+}$, and $E^\circ_{Na^+}$ indicate the sodium, surfactant, and corresponding standard electrode potentials, respectively.

The potential of each electrode depends upon the logarithm of the activity of the surfactant and sodium ions. In this way, cells without a liquid junction were constructed which to respond to two ionic species concentrations, namely the surfactant monomer ion, $[S]_f$, and co-ion (where $Na^+$), $[C]_s$, which comes from the backing electrolyte.

The electrochemical cell can be considered to be between the surfactant electrode and the sodium electrode as a reference electrode:

$$E_{cell} = E_{sur^+} - E_{Na^+} \tag{3}$$

where $E_{cell}$ is the potential of the electrochemical cell between surfactant and sodium electrodes.

The activity coefficient for an ionic species measures the deviation from ideal behavior resulting mainly from interionic interactions of an electrostatic nature. At low ionic strength, the mean activity coefficient of different ions, irrespective of charge and shape, leads to unity.[27] Based on the above discussion, it is reasonable to assume that:

$$\gamma_{Na^+} = \gamma_{sur^+} \approx 1 \tag{4}$$

and with this assumption the monomer concentration of the surfactant ion can be determined below and above the cmc using the following equations:

$$E_{cell} = E^\circ_{sur^+/Na^+} + 2.303 \frac{RT}{F} \log \frac{[S]_f \gamma_{sur^+}}{[C]_s \gamma_{Na^+}}. \tag{5}$$

At a constant sodium ion concentration, which applies to this

experiment, this assumption leads immediately to:

$$E_{\text{cell}} = E^{\circ\prime}{}_{\text{sur}^+/\text{Na}^+} + 2.303\frac{RT}{F}\log[S]_{\text{f}} \quad (6)$$

where

$$E^{\circ\prime}{}_{\text{sur}^+/\text{Na}^+} = E^{\circ}{}_{\text{sur}^+/\text{Na}^+} - 2.303\frac{RT}{F}\log[C]_{\text{s}}. \quad (7)$$

Since the surfactant is dissociated completely into ions below the cmc, the plot of $E_{\text{cell}}$ against $\log[S]_{\text{f}}$. obeys Nernstian behavior. Hence, the slope of the line is refered to $(+2.303RT/F)$ and the intercept, $E^{\circ\prime}{}_{\text{sur}^+/\text{Na}^+}$. This calibration line can be used for the determination of monomer surfactant ion concentration above the cmc by adjusting data on the calibration line. A least mean squares method was used for determination of the slope, $2.303RT/F$, and intercept, $E^{\circ\prime}{}_{\text{sur}^+/\text{Na}^+}$, for each set of data.

## Results and Discussion

Figure 1 shows the plot of emf versus the logarithm of total surfactant concentration, $\log[S]_{\text{t}}$, in the absence of BSA at specified experimental conditions. It is obvious that at low concentrations of surfactant (below the cmc), the emf is directly proportional to $\log[S]_{\text{t}}$ with a Nernstian slope (57–60 mV). However, at higher concentrations, the resulting plots show a distinct break at the cmc. An increase in surfactant concentration causes a decrease in $[S]_{\text{f}}$ in the concentration range above the cmc. The decrease in monomer surfactant concentration above the cmc was confirmed by Hall's theory in 1981.[28] However, many workers have assumed that the monomer concentration is constant above the cmc.[29,30] The cmc values obtained for dodecyltrimethylammonium bromide (DOTAB), tetradecyltrimethylammonium bromide (TTAB), and hexadecyltrimethylammonium bromide (HTAB) are $1.45 \times 10^{-2}$, $3.6 \times 10^{-3}$, and $9.2 \times 10^{-4}$, respectively, which are in good agreement with the literature values.[31]

Figures 2 and 3 show the emf as a function of $\log[S]_{\text{t}}$ in the presence of BSA. The curves in this figures can be divided in to three distinct regions as follows:

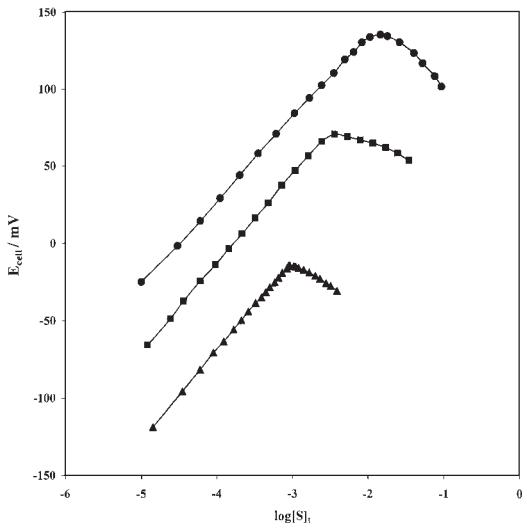


Fig. 1. Electromotive force of surfactant ions against its logarithm of total concentration in the absent of BSA at 2.7 mM phosphate buffer, pH 7, and 27 °C: DTAB(●), TTAB(■), and HTAB(▲).

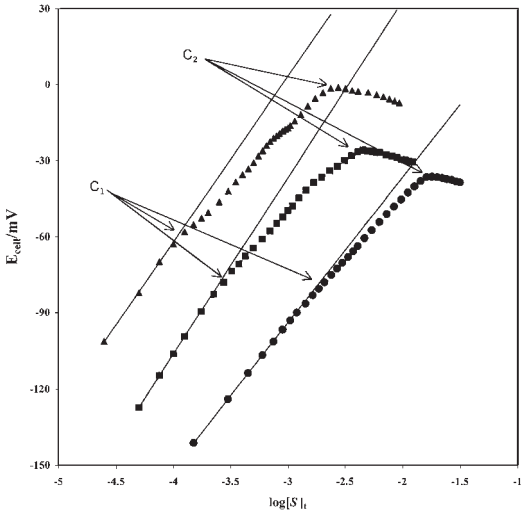


Fig. 2. Electromotive force of surfactant ions against its logarithm of total concentration in the present of (0.1% w/v) BSA at 2.7 mM phosphate buffer, pH 7, and 27 °C: DTAB(●), TTAB(■), and HTAB(▲).

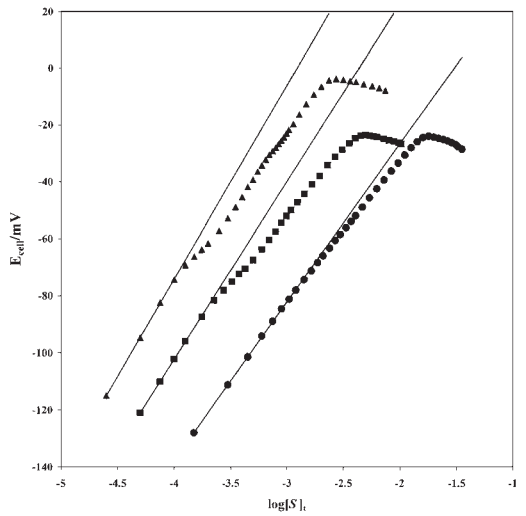


Fig. 3. Electromotive force of surfactant ions against its logarithm of total concentration in the present of (0.1% w/v) BSA at 2.7 mM phosphate buffer, pH 7, and 37 °C: DTAB(●), TTAB(■), and HTAB(▲).

I) The first region, which is at very low concentrations of surfactant, shows a Nernstian slope, which is approximately equal to the corresponding value in the absence of BSA. Hence, it can be concluded that there is no measurable interaction between BSA and surfactant at low concentrations.

II) The second region begins with a distinct break, shown as $C_1$ in the plot. This deviation from linearity is due to the interactions of the surfactant with BSA, and continues until the second distinct break, shown as $C_2$, is reached.

III) The third region is located after $C_2$, where the monomer concentration gradually decreases as the total concentration of surfactant is increased. In this region, aggregation of surfactant ions and micelle formation occurr.

By considering the fact that the emf is reduced in the presence of BSA, the amount of surfactant bound to BSA can be

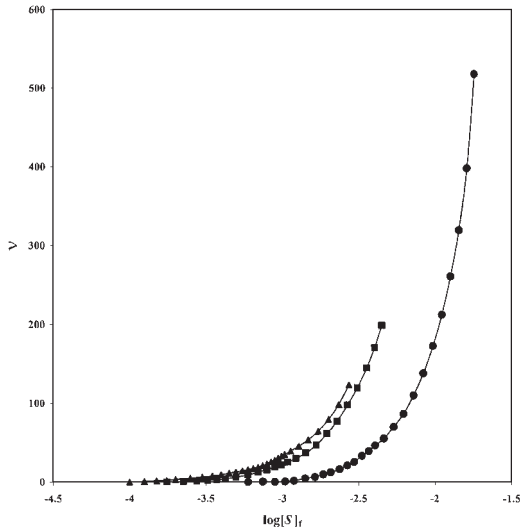


Fig. 4. Binding isotherms for DTAB(●), TTAB(■), and HTAB(▲) on interaction with BSA at pH 7 and 27 °C.

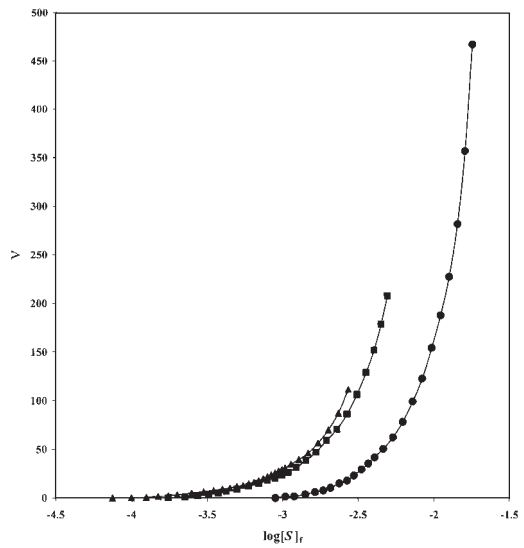


Fig. 5. Binding isotherms for DTAB(●), TTAB(■), and HTAB(▲) on interaction with BSA at pH 7 and 37 °C.

calculated.

The average number of surfactant molecules bound per BSA molecule has been calculated as:

$$\nu = \frac{[S]_t - [S]_f}{C_p} \tag{8}$$

where $C_p$ is the total concentration of BSA.

Figures 4 and 5 show the binding isotherms (a semi logarithmic plot of the average number of surfactant bound per BSA molecule, $\nu$, against the logarithm of the free surfactant concentration, $[S]_f$) for the interactions of DOTAB, TTAB, and HTAB as a homologous series of cationic surfactants, with BSA at the specified conditions. All the isotherms are for the free surfactant concentrations below the cmc values. All plots are concave, characteristic of cooperative binding.

The plot of binding capacity, $\theta$, versus $\log[S]_f$ can be a very useful approach for analysing the binding isotherms[32,33] $\theta$ is the

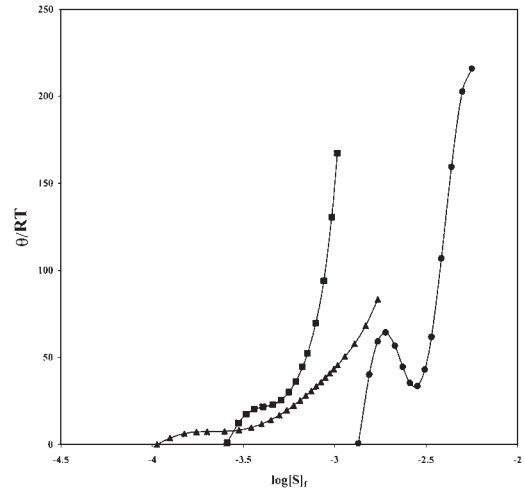


Fig. 6. The plot of $\theta/RT$ versus $\log[S]_f$ for DTAB(●), TTAB(■), and HTAB(▲) on interaction with BSA at pH 7 and 27 °C.

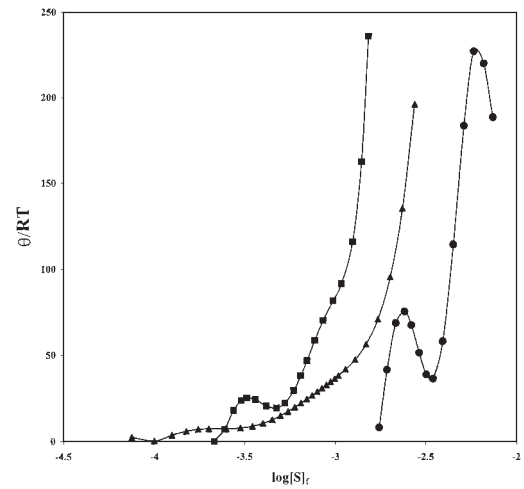


Fig. 7. The plot of $\theta/RT$ versus $\log[S]_f$ for DTAB(●), TTAB(■), and HTAB(▲) on interaction with BSA at pH 7 and 37 °C.

derivative of the binding isotherms representing the change in the number of moles of surfactant bound per mole of protein ($\nu$), which is related to $\log[S]_f$ according to the following equation:

$$\theta = \frac{\partial \nu}{\partial \mu_s} = \frac{\partial \nu}{RT\partial \ln[S]_f} = \frac{\partial \nu}{2.303RT\partial \log[S]_f}. \tag{9}$$

Where $\mu_s$ is the chemical potential of the surfactant. Other parameters have been previously defined. The value of $\theta$ at any $\nu$ can be determined by calculating the slope of the binding isotherms. Figures 6 and 7 show the plots of $\theta/RT$ versus $\log[S]_f$ for the binding of DOTAB, TTAB, and HTAB to BSA at 27 and 37 °C, respectively. The main feature of these figures is the existence of two maxima for all binding isotherms, and therefore the binding data should be fitted to the Hill equation for two sets of binding sites (Eq. 10).[34,35] However, the first maximum appears at lower concentration values of surfactant as the hydrocarbon chain of the surfactant is increased. The second maxima does not completely appear for TTAB

Table 1. Parameters Derived from Eq. 10 for the Binding of Cationic Surfactants to BSA

| | Temperature /°C | $g_1$ | $n_1$ | $K_1 \times 10^{-2}$ /$M^{-1}$ | $g_2$ | $n_2$ | $K_2 \times 10^{-2}$ /$M^{-1}$ |
|---|---|---|---|---|---|---|---|
| DOTAB | 27.0 ± 0.1 | 24.0 ± 1.2 | 6.19 ± 0.30 | 5.50 ± 0.03 | 172 ± 8 | 2.58 ± 0.12 | 2.00 ± 0.09 |
| | 37.0 ± 0.1 | 33.0 ± 1.6 | 4.30 ± 0.21 | 4.44 ± 0.22 | 140 ± 7 | 2.51 ± 0.13 | 2.05 ± 0.10 |
| TTAB | 27.0 ± 0.1 | 12.0 ± 0.6 | 5.89 ± 0.29 | 25.3 ± 1.2 | 120 ± 6 | 2.62 ± 0.13 | 8.50 ± 0.43 |
| | 37.0 ± 0.1 | 14.0 ± 0.7 | 7.37 ± 0.48 | 30.7 ± 2.0 | 90.0 ± 4.2 | 2.50 ± 0.12 | 10.3 ± 0.5 |
| HTAB | 27.0 ± 0.1 | 6.2 ± 0.3 | 5.04 ± 0.22 | 51.0 ± 2.2 | 57.3 ± 2.9 | 1.75 ± 0.09 | 9.82 ± 0.53 |
| | 37.0 ± 0.1 | 7.5 ± 0.4 | 4.64 ± 0.25 | 47.8 ± 2.5 | 40.0 ± 2.0 | 2.39 ± 0.14 | 14.2 ± 0.9 |

and HTAB, which may be due to the fact that TTAB and HTAB, with lower cmc values compared to DOTAB, form micelles before full occupation of the second binding set. The Hill equation for two sets of binding sites is:

$$\nu = \left\{\frac{g_1(K_1[S]_f)^{n_1}}{1+(K_1[S]_f)^{n_1}}\right\} + \left\{\frac{g_2(K_2[S]_f)^{n_2}}{1+(K_2[S]_f)^{n_2}}\right\} \quad (10)$$

where $g_1$, $K_1$, and $n_1$ are the number of binding sites, the binding constant, and the Hill coefficient for the first binding set, respectively, and $g_2$, $K_2$, and $n_2$ are the corresponding parameters for the second binding set. Fitting was done using a computer program for non-linear least-square fitting.[36] The results are shown in Table 1, which indicate that the values of $K_1$ are larger than $K_2$, and show that the initial interactions are stronger. The positive cooperativity in the first set ($n_1 > 1$) is due to the fact that the anti-cooperative electrostatic interaction is not the only interaction in the first binding set. This conclusion can also be reached by comparing the values of $K_1$ for DOTAB, TTAB, and HTAB with each other. The values of $K_1$ increase due to the increasing hydrocarbon chain length of the surfactants. Therefore, the hydrophobic interactions between the hydrocarbon tail of the surfactant and hydrophobic patches on the surface of the protein play an important role in the initial interactions of cationic surfactants with BSA. The large number of binding sites in the second set indicates that the binding is accompanied by unfolding and exposure of numerous hydrophobic binding sites. The concentration at which unfolding occurs is decreased by increasing the length of the hydrocarbon tail of the surfactants. This implies that the hydrophobic interactions have an essential role in the denaturation process of BSA.

Another common approach for determining the binding constant and evaluating the binding mechanism has been proposed by Wyman, who introduced the binding potential concept, $\Pi_\nu$.[37] The value of the binding potential at any specified $\nu$ is calculated from the area under the binding isotherms according to the following equation:

$$\Pi_\nu = RT\int_0^{\ln[S]_f} \nu \mathrm{d}\ln[S]_f. \quad (11)$$

This is related to an apparent overall macroscopic binding constant, $K_{app,\nu}$, as follows:[38]

$$\Pi_\nu = RT\ln(1 + K_{app,\nu}[S]_f^\nu). \quad (12)$$

Values of $K_{app,\nu}$ were determined by application of Eq. 12, and used to determine the values of the Gibbs free energy of binding per mole of surfactant, $\Delta G_\nu$:

$$\Delta G_\nu = -\frac{RT}{\nu}\ln K_{app,\nu}. \quad (13)$$

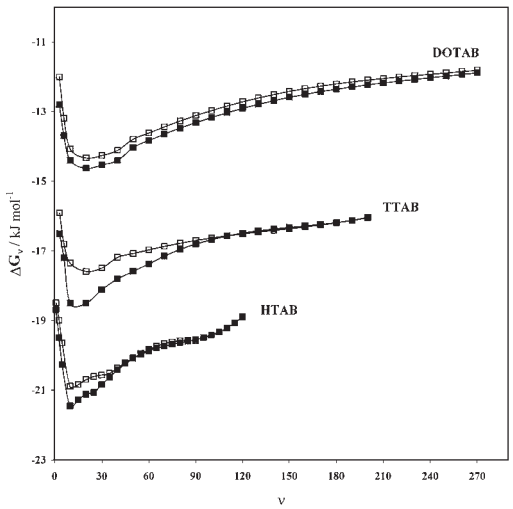


Fig. 8. Variation of Gibbs free energy of binding per mole of surfactant on interaction with BSA as a function of $\nu$: (□) 27 °C, (■) 37 °C.

Figure 8 shows the variation of $\Delta G_\nu$ versus $\nu$ for the binding of DOTAB, TTAB, and HTAB to BSA. It is obvious that the affinity of binding is increased with increasing length of the hydrocarbon tail of the surfactants. This is further evidence that the hydrophobic tail of the surfactant plays an important role in the interaction with BSA. However, all curves in Fig. 8 have a minimum and approach a limiting value with increasing $\nu$. The limiting value of $\Delta G_\nu$ (approximately −12, −16, and −18 kJ mol$^{-1}$ for DOTAB, TTAB, and HTAB, respectively) can be predominantly related to hydrophobic binding. The minima are located in the first binding set region, which has a high binding energy with the ionic sites. However, the variation of $\Delta G_\nu$ with respect to $\nu$ dose not show the trends expected from the cooperativity coefficients (Table 1). $\Delta G_\nu$ becomes less negative after $\nu > g_1$, although from Table 1 the Hill coefficients are greater than one for both binding sets of all studied surfactants. This is due to this fact that $\Delta G_\nu$ obtained from the apparent macroscopic binding constants, $K_{app,\nu}$, that contain the statistical contribution and its variation cannot show this kind of cooperativity.[39]

The values of $\Delta G_\nu$ are more negative at the higher temperatures, which represents the endothermic and essentially entropy driven process. With respect to the entropic nature of hydrophobic and enthalpic nature of electrostatic interactions, this observation represents the more predominant role of hydrophobic interactions in the overall process.

## Conclusions

Analysis of binding data for the interactions of cationic surfactants with BSA represents a system with two sets of binding sites. Both electrostatic and hydrophobic interactions are involved in the first binding set at lower concentrations of surfactants. At higher surfactant concentrations, in which the occupation of the second binding set was occurred, the cationic surfactant resembled sodium dodecyl sulfate (SDS), i.e., binding cooperatively to BSA with an accompanying unfolding and exposure of numerous hydrophobic binding sites. However, this unfolding process occurs for the cationic surfactants at much higher concentrations than is required for SDS.[40] Therefore, the cationic surfactants are not suitable as substitutes for SDS certain procedures, such as determination of molecular weight by gel electrophoresis, in which the denaturating action of the surfactant is an essential feature.

The trend of the variation of $\Delta G_v$ confirms our binding data analysis on the basis of two sets of binding sites. The limiting values of $\Delta G_v$ at high concentrations of surfactants are approximately equal to $\Delta G_{\text{micelle}}$, which indicates that the structure of the BSA–surfactant complex and the related micelle are similar.

The endothermicity of the process also indicates that the hydrophobic interactions play an important role in the BSA–surfactant binding, whereas electrostatic interactions play only a minor one.

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