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Studies on surfactant–biopolymer interaction. I. Microcalorimetric investigation on the interaction of cetyltrimethylammonium bromide (CTAB) and sodium dodecylsulfate (SDS) with gelatin (Gn), lysozyme (Lz) and deoxyribonucleic acid (DNA)

A. Chatterjee[a], S.P. Moulik[a,*], P.R. Majhi[b], S.K. Sanyal[c]

[a]*Department of Chemistry, Centre for Surface Science, Jadavpur University, Calcutta-700032, India*
[b]*Martin-Luther-University Halle-Wittenberg, Institute of Physical Chemistry, Muehlpforte 1, D-06108 Halle/Saale, Germany*
[c]*Department of Chemical Engineering, Jadavpur University, Calcutta-700032, India*

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Abstract

The interaction of the surfactants cetyltrimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) with the biopolymers gelatin (Gn), lysozyme (Lz) and deoxyribonucleic acid (DNA) was studied by isothermal titration microcalorimetry at varied biopolymer concentration, pH and temperature. The nature of interaction of the surfactants with the biopolymers was assessed from the observed enthalpy–[surfactant] profiles. The biopolymer-induced aggregation of the surfactants was observed. The enthalpies of aggregation of amphiphiles, binding of aggregates with macromolecules, organisational change of bound aggregates, and threshold concentrations for micelle formation of surfactants in the presence of biopolymers were estimated. The results collected on the three biopolymers were analysed and compared. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Sodium dodecyl sulfate; Cetyltrimethyl ammonium bromide; Gelatin; Lysozyme; DNA; Microcalorimetry

1. Introduction

The interaction of polymers with surfactants in aqueous solution is a topic of interest for widespread applications as well as for fundamental understanding [1–15]. Most studies are focused on systems consisting of water-soluble polymers and ionic surfactants. Like synthetic polymers, biopolymers such as various proteins, DNA, etc., undergo both specific and non-specific interactions with surfactants [16–21]. Surfactants are used to extract proteins from cell membranes. Their interaction with proteins has direct relevance to the formulation and preparation of skin and body care products, and cosmetics [7,10,22–25]. These interactions are comparable with lipid–protein interactions in the membrane of living cells (where lipid

*Corresponding author. Tel.: +91-33-483-8411; fax: +91-33-473-4266.
E-mail address: cssju@yahoo.co.uk (S.P. Moulik).

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and protein remain in the form of complexes) [17,18,26,27], and can account for the transportation of lipids in the body fluids [18,28].

Quantitative understanding of the interaction (binding) process between surfactant and polymer can be achieved by way of thermodynamic studies. Like all other binding equilibria, the reversibility of the surfactant–polymer interaction process is the most important thermodynamic requirement as binding of ligands to macromolecules may in certain cases be partially irreversible [18,29]. The reversibility–irreversibility of the process is important also for clinical reasons for drugs essentially bind with proteins and DNA, and their release afterwards primarily depends on the strength of binding and the reversibility of the interaction process.

It is known that surfactants can interact with polymers in the form of smaller aggregates, and the threshold of this aggregate formation is called the critical aggregation concentration (CAC), which is essentially lower than the critical micellar concentration (CMC). In the presence of sufficient amount of surfactant, the CAC is followed by normal micelle formation. The formation of CAC and CMC can be studied by various techniques, like tensiometry, fluorimetry, conductometry, microcalorimetry, etc., of which microcalorimetry is advantageous for it can directly provide the enthalpy of the studied process. In the isothermal titration mode it can monitor stepwise interaction extents of the substrate with the polymer. The enthalpies of CAC formation and amphiphile binding can quantitatively differentiate the activity of one polymer from the other.

On a general basis, the driving forces for amphiphile association with polymer are electrostatic, dipolar and hydrophobic in nature [1,2]. Neutral polymers are known to interact with both anionic and cationic surfactants but their modes of binding are different [1,2]. The anionic surfactants are found to exhibit stronger interaction than cationics of similar chain length [1,2]. Polyelectrolytes can interact with oppositely charged surfactants (even in dilute solution) by electrostatic attraction. Ionic surfactants and polyelectrolytes of similar charge can also interact if the latter is markedly hydrophobic [1,2].

In recent years, although isothermal titration microcalorimetry has been proven to be a potential method for detailed understanding of the interaction of surfactants with polymers and the related thermodynamics of the process [8–12,30], its use in the area of biopolymer–surfactant interaction has been, on the other hand, very modest [31]. In the present work, the interaction of two oppositely charged surfactants, sodium dodecyl sulfate (SDS) and cetyltrimethyl ammonium bromide (CTAB) with the biopolymers, gelatin (Gn), lysozyme (Lz) and deoxyribonucleic acid (DNA) have been studied under varied ionic strength, pH and temperature by the isothermal titration microcalorimetric method. The nature of binding and the thermodynamics of the involved processes have been explored. It may be noted that Gn is a completely denatured protein, whereas Lz is compact and DNA has some degree of flexibility. The rigidity–flexibility of the molecules is supposed to offer varied influence on the interaction of the two surfactants (SDS and CTAB) used in the study.

2. Experimental

2.1. Materials

Gelatin (mol wt. 38 000), lysozyme (mol wt. 14 300), calfthymas DNA (mol wt. of the order of 10^7) and the surfactant SDS (99% pure) were products of Sigma Chemicals, USA. The surfactant CTAB was 99% pure product of Fluka, USA. They were used as received. The DNA sample contained 18% (w/w) water, which was determined by heating the sample to constant weight at 100 °C. Its sodium ion content was 7% (w/w) that corresponded to one sodium per nucleotide. The absorbance ratio at 260 and 230 nm was 2.1. Thus, the sample was free of protein contamination. The molar base composition (average of six reports) [32] of the calfthymus DNA is 1.0, 0.78, 0.75 and 1.01 as adenine, guanine, cytosine and thymine, respectively. For the preparation of phosphate buffer (in the range of pH 4–8), disodium hydrogenphosphate and sodium dihydrogenphosphate

(99.5% pure products of S.D. Fine Chemicals Ltd, India) were used. The pH of the buffer solution after preparation was tested with a digital pH meter of Global Electronics, India. In the preparation of DNA solution ionic strength was maintained at 0.58 using NaCl. Whenever required, its concentration has been expressed in terms of phosphate group based on spectrophotometric assay at 260 nm with molar extinction coefficient of 6600 l mol^{-1} cm^{-1}. Doubly distilled water of specific conductance 2–4 μS cm^{-1} was used for solution preparation.

2.2. *Method* (*microcalorimetry*)

The microcalorimetric measurements were taken with an OMEGA Isothermal Titration Calorimeter of Microcal Inc., Northampton, USA. In an experiment, 1.325 ml of aqueous buffered biopolymer solution of desired concentration was taken in the calorimeter cell. The reference cell contained the same concentration of the biopolymer solution. Under computer controlled condition, a preset (programmed) number of 5–20-μl aliquots of temperature equilibrated concentrated surfactant solution (~20 times its CMC) were injected into the cell at 4-min intervals. The heat flow in or out of the cell per injection and the corresponding enthalpy change per mole of the added surfactant were obtained using a Microcal Origin software. Control run using water instead of biopolymer solution to follow the corresponding dilution of the surfactant was made following the same procedure as above. The heats of dilution of the biopolymers with water were also measured following the same procedure and were observed to be very small compared to the heats of dilution of the surfactants in the presence of biopolymers and were thus neglected. All measurements were duplicated to check the reproducibility. The temperature variation was made by circulating water from a thermostated bath (NESLAB, RTE100, USA) around the adiabatic jacket of the calorimeter cells. The above procedure was basically similar to that followed earlier in the study of micellisation of surfactants [33–37] and their interaction with polymers [8–12,30].

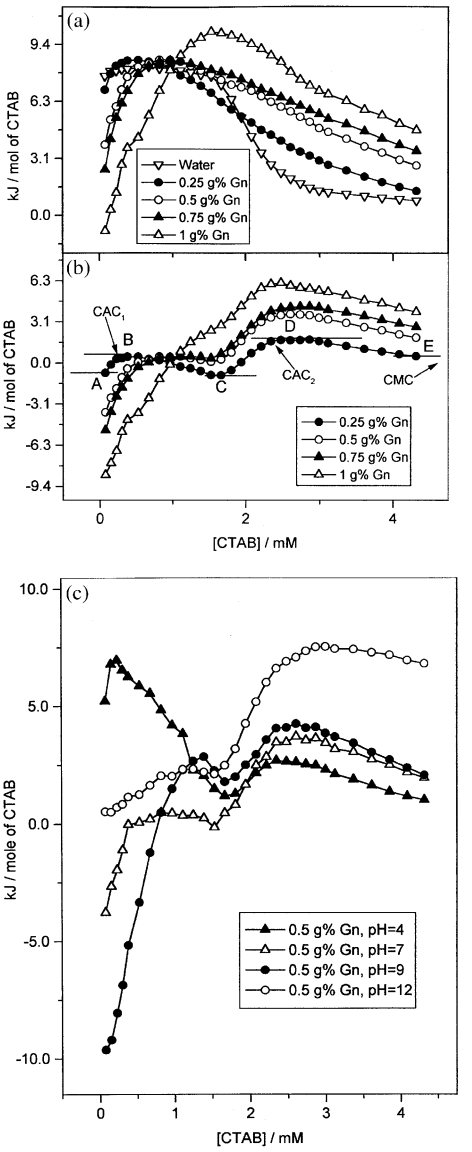

Fig. 1. (a,b) Enthalpy of dilution of CTAB vs. [CTAB] profiles in aqueous Gn solution at 303 K and at pH 7. [CTAB] in the syringe = 20 mM; volume of water or water–Gn solution in the cell = 1.325 ml; total 325-μl CTAB solution added in 30 installments. (a) Plots of actual dilution enthalpy values. (b) Plots of dilution enthalpy values subtracting the contributions of the corresponding controls. (c) Enthalpy of dilution of CTAB vs. [CTAB] profiles in 0.5 g% Gn at 303 K and at different pH estimated relative to the corresponding enthalpy of dilution of pure CTAB. Measurement protocols are the same as in (a,b).

Table 1
The interaction of CTAB with Gn under different conditions

[Gn] (g%)	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^b_{CAC_1}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(*a*) *The effect of concentration of Gn at pH 7 and temperature 303 K*					
0.25	0.375 (2.47)	4.60	1.23 (2.70)	−1.41	−1.76
0.50	0.523 (2.47)	–	4.26 (3.63)	−0.39	–
0.75	0.67 (2.47)	–	5.63 (3.97)	−0.16	–
1.0	1.25 (2.47)	–	10.1 (3.24)	1.28	–
PH	CAC_1 (CAC_2) (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^b_{CAC_1}$ (kJ mol^{-1})		
(*b*) *The effect of pH with 0.5 g% of Gn at 303 K*					
4	0.230 (2.34)	1.74 (1.52)	−5.77		
7	0.523 (2.47)	4.26 (3.63)	−0.39		
9	1.25 (2.47)	12.5 (2.46)	−1.61		
12	1.10 (2.86)	1.80 (5.43)	−0.21		

3. Results

3.1. Gelatin–CTAB and gelatin–SDS systems

In Fig. 1, the enthalpy of dilution of CTAB in the presence of Gn expressed per mole of injectant vs. the [CTAB] is plotted in 'a', and the said dilution enthalpies relative to that of the controls (pure CTAB) are presented in 'b'. The results are shown for four concentrations of gelatin, 0.25, 0.5, 0.75 and 1.0 g% (w/v). Each curve in 'b' can be divided into several sections. For curve 1 (0.25 g% Gn), these sections are AB, BC, CD and DE (which essentially should converge at zero enthalpy). This state is yet to be reached in other curves, only a tendency is there. Like most other surfactant–polymer systems, the initial rise from A→B is due to the interaction of the surfactant monomers with Gn culminating into aggregate formation with the critical aggregation concentration (CAC) [8–12,30] at B. The segment BC of the curve denotes the enthalpy change due to the association of the small amphiphile aggregates with the protein. The section CD represents the enthalpy change associated with Gn induced aggregate modification (structural and otherwise, their size may increase) yielding an altered kind of aggregate ending at D, called the second CAC, i.e. CAC_2 (the modified critical aggregation concentration) [30]. The section DE virtually represents the enthalpy of formation of free micelle in biopolymer solution. The point E (nearing zero enthalpy) is close to the self-aggregated (i.e. CMC) state of CTAB in solution [9–12,30], which at the zero enthalpy state (obtained by extrapolation) is 4.6 mM. The other curves (2–4) at different [Gn] are, on the whole, comparable with curve 1. The CAC_1 and CAC_2 values virtually remain unaltered. Only the zero enthalpy states are yet to be reached in them because of higher [Gn]. The values of CAC_1, CAC_2, CMC and the apparent enthalpies of different stages expressed per mole of surfactant are presented in Table 1a.

The profiles of the enthalpy of dilution of CTAB in the presence of Gn (0.5%) vs. [CTAB], estimated relative to the corresponding enthalpy of dilution of pure CTAB, at pH values 4, 7, 9 and 12 are presented in Fig. 1c. The CAC_1 and CAC_2 values are found to depend on pH; the first two at pH 4 and 7 show comparable CAC_1 (although with large displacement of the profiles). At pH 9 and 12 the CAC_1 values are higher but close. The increase in CAC_2, on the other hand, is mild. The apparent enthalpies of different associated processes (as discussed in connection with Fig. 1) are presented in Table 1b. All the curves in Fig. 1c tend to reach the zero enthalpy or the level of formation of free micelle, and the extent of the

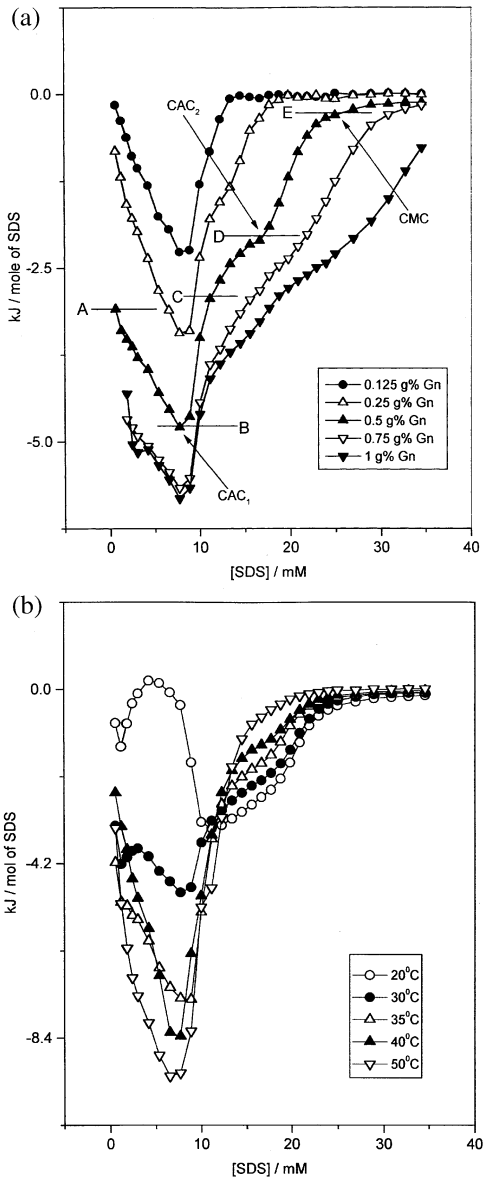

Fig. 2. (a) Enthalpy of dilution of SDS vs. [SDS] profiles in aqueous Gn solution at pH 7 and at 303 K estimated relative to the corresponding enthalpy of the dilution of pure SDS. [SDS] in the syringe = 160.1 mM; volume of water or water–Gn solution in the cell = 1.325 ml; total 325-μl SDS solution added in 30 installments. (b) Enthalpy of dilution of SDS vs. [SDS] profiles in 0.5 g% Gn solution at pH 9 and at different temperatures estimated relative to the corresponding enthalpy of dilution of pure SDS. Measurement protocols are the same as in (a).

difference increases with pH. At higher pH (> isoelectric pH of 4.8), the interaction of cationic surfactant CTAB with Gn is significant. Thus, electrostatic factor has a sizeable contribution in the interaction process.

The enthalpies of dilution of SDS in the presence of Gn at 0.125, 0.25, 0.5, 0.75 and 1.0 g% of Gn at 303 K relative to the enthalpy of dilution of SDS in water are presented in Fig. 2. Well-defined CAC_1 and ill defined CAC_2 points (indistinct CAC_2 at 0.125 g% Gn) are observed along with CMC points in the curves. A representative curve at 0.5 g% Gn is marked (as in Fig. 1), for getting the different interactional enthalpies which are recorded in Table 2a. Here the delayed reaching of the CMC points with increasing [Gn] is distinct; it is yet to be reached at [Gn] = 1.0 g%. For [Gn] = 0.5 g% at pH 9, the effect of temperature on the enthalpy profile is striking and it is illustrated in Fig. 2b. The CAC_1 formation is endothermic at 293 K but are more and more exothermic at the other temperatures, 303, 308, 313 and 323 K. The tendency of CAC_2 formation becomes less prominent. The CAC_1, CAC_2 and the dissected apparent enthalpy values are also shown in Table 2b. The pH of the medium has a minor say on the interaction of SDS with Gn in the range of 4–9 studied at 303 K (curves not shown). At pH 12, although the pattern remains the same, the heat change is greater at [SDS] < 10 mmol dm^{-3}. The CAC_1, CAC_2 and the apparent enthalpy values of the involved stages are presented in Table 2c. The enthalpy profiles of Gn–CTAB and Gn–SDS are different. Both CTAB and SDS can appreciably interact with Gn depending on environmental conditions in which electrostatic and hydrophobic processes contribute.

3.2. Lysozyme–CTAB and lysozyme–SDS systems

The enthalpy of dilution of CTAB vs. [CTAB] plots at four different [Lz] = 0.2, 0.35, 0.5 and 1.0 g% (w/v) obtained by the isothermal titration microcalorimetric procedure relative to the dilution of CTAB in water are presented in Fig. 3. The CAC_1 values remain unaltered whereas the CAC_2 values increase mildly with [Lz]. The apparent enthalpies of different interaction stages are pre-

Table 2
The interaction of SDS with Gn under different conditions

[Gn] (g%)	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^b_{CAC_1}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(*a*) *The effect of concentration of Gn at pH 7 and temperature 303 K*					
0.125	7.68 (–)	13.3	−2.13 (–)	–	2.23
0.25	7.68 (13.3)	18.7	−2.63 (0.46)	1.65	1.33
0.50	7.68 (17.1)	23.9	−1.71 (0.674)	2.12	1.89
0.75	7.68 (22.9)	34.5	−0.996 (2.11)	1.79	1.64
1.0	7.68 (22.9)	–	−0.78 (1.67)	1.73	–
Temp (K)	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^b_{CAC_1}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(*b*) *The effect of temperature with 0.5 g% of Gn at pH 9*					
293	4.18 (18.2)	24.9	1.58 (1.07)	−3.55	2.14
303	7.68 (18.2)	23.9	−1.06 (1.25)	1.85	1.69
308	8.25 (17.7)	22.9	−3.29 (0.96)	4.91	1.39
313	7.10 (17.7)	22.9	−5.84 (1.02)	6.11	1.09
323	7.10 (–)	21.9	−5.94 (–)	–	9.26
pH	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^b_{CAC_1}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(*c*) *The effect of pH with 0.5 g% of Gn at 303 K*					
4	7.68 (17.7)	23.4	−1.08 (0.694)	2.12	1.89
7	7.68 (17.1)	23.9	−1.71 (0.674)	2.12	1.89
9	7.68 (18.2)	23.9	−1.06 (1.25)	1.85	1.69
12	7.68 (15.5)	–	−5.80 (1.37)	2.55	–

sented in Table 3a. The pH has marked effect on the interaction process (results not illustrated). While the CAC_1 points are fixed and well defined, the CAC_2 points are less prominent and they follow the order with pH as $CAC_2^7 > CAC_2^9 > CAC_2^{12}$ (the superscript indicates pH). In Table 3b, CAC_1, CAC_2 and the enthalpy values at different pH are presented. The effect of temperature on the system Lz (0.35%)–CTAB at pH 9 has shown increase in CAC_1 with temperature; the CAC_2 has become prominent virtually with no shift up to 313 K. At 323 K, it has shifted to a higher concentration. At 313 and 323 K, the enthalpy values are yet to reach the level zero (i.e. the interaction of the aggregate to Lz is incomplete and the formation of free micelles in solution is yet to start). Again, the results are not illustrated to save space. The apparent enthalpy values of the interaction processes along with the CAC_1 and CAC_2 values are contained in Table 3c.

The system Lz–SDS has shown striking difference in the pattern compared to Lz–CTAB as well as Gn–CTAB and Gn–SDS. The stepwise enthalpy values realised with the addition of SDS at four different concentrations of Lz = 0.25, 0.5, 0.75 and 1.0 g% (w/v) treated in the same manner as in the case of Gn–SDS are presented against [SDS] in Fig. 4. In each curve in the figure, there appear two maxima corresponding to CAC_1 and CAC_2. There are two minima as well, the first corresponds to the end of binding of the smaller SDS aggregates with Lz, and the second designates the end of structural and organisational changes of the higher (secondary) aggregates (corresponding to CAC_2) upon increased addition of SDS. The enthalpy corresponding to the process is designated as $\Delta H^{org}_{CAC_2}$. Thereafter, normal micelle formation occurs in solution as in the other cases previously described. This state is yet to be reached for [Lz] = 1.0 g%. Both CAC_1 and CAC_2 have increased

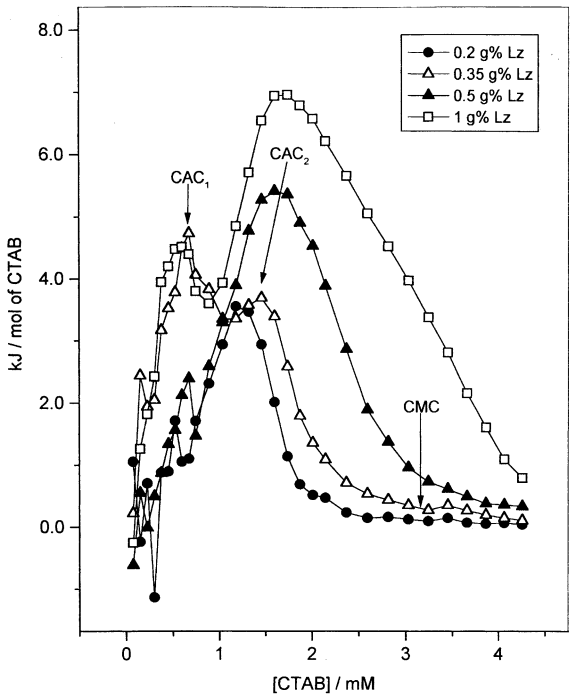

Fig. 3. Enthalpy of dilution of CTAB vs. [CTAB] profiles at pH 7 in aqueous Lz solution at 303 K estimated relative to the corresponding enthalpy of dilution of pure CTAB. [CTAB] in the syringe = 20 mM; volume of water or water–Lz solution in the cell = 1.325 ml; total 320-μl CTAB solution added in 30 installments.

with [Lz]. These results indicate that Lz–SDS interaction is a complex process; we have herein presented only a simplified analysis. The apparent enthalpy corresponding to each process is designated as for the other systems discussed above. The results extracted from the measurements are summarised in Table 4a. The measurements at pH 4, 7, 9, 10.5 and 12 at 303 K have shown variations in the nature of the course (results not exemplified). The CAC_1, CAC_2, and CMC have finally decreased with pH after a small increase at pH 7. These results are presented in Table 4b. At pH = 12, Lz is isoelectric and its interaction with SDS is electrostatically weak which is not so at lower pH. The distinction in behaviour between lower pH and pH = 12 is thus obvious. The thermodynamic features with CTAB at pH 12 are also conspicuous. The effect of temperature on the binding process at pH 9 has been also studied. The values of CAC_1 have remained unaltered whereas the CAC_2 value at 323 K has increased while those at 303 and 313 K have remained unaltered. The CAC values and the enthalpies of interaction are presented in Table 4c.

3.3. DNA–CTAB and DNA–SDS systems

The effect of pH at 4, 6 and 8 on the interaction of CTAB with DNA (0.02 g%) at $\mu = 0.58$ and at 303 K has been examined (Fig. 5a,b). Here, the enthalpy values without and with subtraction of the heats of dilution of SDS are presented to exhibit what difference the latter can make towards the understanding of the process. Two maxima appear in 'b' whereas there is only one maximum in 'a'. It is observed (Table 5a) that both the CAC_1 and CAC_2 have remained unchanged with a rise in height in the case of CAC_2 at pH 8 (Fig. 5b). The effect of the thermal condition of the solution at 0.02 g% [DNA] and pH 6 has been also investigated (results not displayed). The trends at temperatures 303 and 333 K are parallel but that at 318 K has shown shifted CAC_1 and reverse heat change thereafter without an indication for CAC_2. The CAC and enthalpy values obtained at different temperatures are presented in Table 5b.

It may be added that microcalorimetric study of the DNA–SDS system has not exhibited any visible interaction in terms of enthalpy change for the process.

3.4. Comparison of results

It was observed that for Gn–CTAB, CAC_1 increased with increasing [Gn] but CAC_2 remained more or less invariant. On the other hand, for the Gn–SDS system, CAC_1 was independent of [Gn] whereas CAC_2 increased with increasing [Gn]. For the Lz–CTAB system, the CAC_1 increased up to 0.5 g% of Gn and subsequently decreased; the CAC_2 showed a mild increase. Both CAC_1 and CAC_2 of SDS increased by interacting with [Lz]. The effect of pH on the CAC was system-specific. For Gn–CTAB, CAC_1 appreciably increased with pH up to 9, then it mildly declined. The CAC_2

Table 3
The interaction of CTAB with Lz under different conditions

[Lz] (g%)	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^b_{CAC_1}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(*a*) *The effect of concentration of Lz at pH 7 and temperature 303 K*					
0.20	0.52 (1.17)	2.59	1.95 (2.50)	−0.66	−3.48
0.35	0.67 (1.46)	3.24	2.80 (0.335)	−1.38	−3.42
0.50	0.67 (1.60)	3.86	3.0 (3.94)	−0.92	−5.05
1.0	0.56 (1.60)	–	3.26 (3.36)	−0.91	–
pH	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^b_{CAC_1}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(*b*) *The effect of pH with 0.35 g% of Lz at 303 K*					
4	0.600 (–)	2.14	1.92 (–)	–	−7.73
7	0.670 (1.46)	3.24	2.80 (0.335)	−1.38	−3.42
9	0.375 (1.80)	4.26	1.80 (9.04)	0.46	−4.65
12	0.375 (2.59)	4.26	21.2 (0.48)	−13.7	−4.91
Temp (K)	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^b_{CAC_1}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(*c*) *The effect of temperature with 0.35 g% of Lz at pH 9*					
293	0.300 (1.87)	3.66	1.11 (7.64)	−0.83	−2.67
303	0.375 (1.87)	4.26	1.80 (9.04)	0.46	−4.65
313	0.743 (1.73)	–	3.79 (15.9)	1.10	–
323	0.743 (2.14)	–	5.06 (16.6)	2.18	–

witnessed a mild increase although. The CAC_1 of SDS in the presence of Gn remained invariant with pH; the CAC_2 also showed a similar behaviour with a decrease at pH 12. The CAC_1 for the Lz–CTAB system had a mild increase with pH between 4 and 7. Thereafter, CAC_1 decreased to a constant value at pH 9 and 12. The CAC_2 increased with pH in the range of 7–12 and it was not recognisable at pH 4. For the Lz–SDS system, CAC_1 increased from pH 4 to 9 and then decreased. On the other hand, CAC_2 had shown a mild decrease up to pH 10.5 and a sharp decrease at pH 12. The CAC_1 and CAC_2 both remained invariant on pH for the interaction of CTAB with DNA. The CAC_1 of SDS passed through a maximum at 308 K but its CAC_2 showed a mild decline with temperature by interacting with Gn. For Lz–CTAB, CAC_1 increased with temperature while CAC_2 remained unchanged up to 313 K, and then had shown an increase at 323 K. The CAC_1 of SDS, on the other hand, did not change with temperature in the presence of Lz but the CAC_2 had increased at 323 K. The CAC_1 of CTAB passed through a maximum at 318 K in the presence of DNA. The CAC_2 remained constant at 303 and 333 K, and the result at 318 K was inconclusive.

The CMC of both CTAB and SDS increased with the biopolymer concentration; the exact points, on the other hand, remained unreached at relatively higher [biopolymer]. The CTAB–Gn, SDS–Gn (at 1.0 g%), and SDS–Lz (at 1.0 g%) can be cited as examples. At constant [Gn] and temperature, pH had no effect on the CMC of SDS. It had an increasing effect on the CMC of CTAB with Lz. An increase followed by a decrease of CMC of SDS with Lz was observed. The CMC of CTAB mildly increased in the presence of DNA at pH 8. In the temperature range of 293–323 K, the CMC of SDS mildly decreased in the presence of Gn. With increase in temperature, the CMC of CTAB increased by interacting with Lz. Such

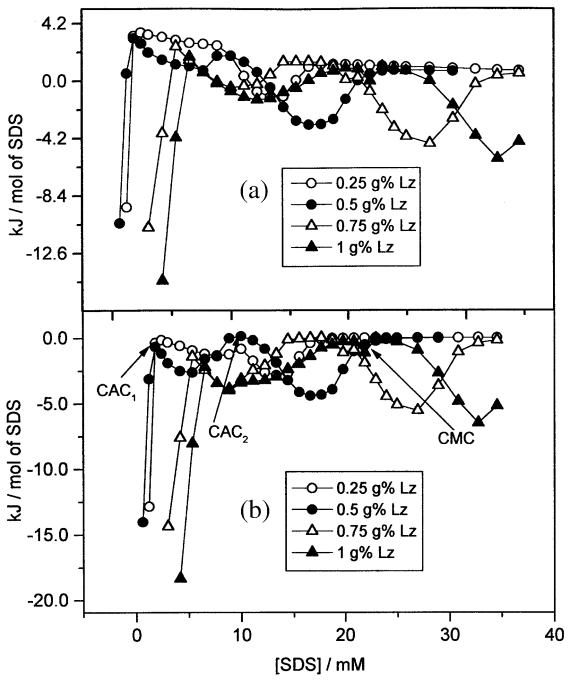

Fig. 4. Enthalpy of dilution of SDS vs. [SDS] profiles at pH 7 in aqueous Lz solution at 303 K. Measurement protocols are the same as in Fig. 2a. Illustration protocols are the same as in Fig. 1a,b.

interaction of SDS with Lz resulted a maximum in CMC at 313 K. The CMC of CTAB upon interaction with DNA produced a minimum at 318 K.

3.5. Discussion

Since the nature of biopolymer-bound aggregates (with reference to CAC_1 and CAC_2) is not known, detailed thermodynamic analysis and correct evaluation of the enthalpies of the complex binding and other related processes are not possible at this stage. Only the direction of the net heat released (exothermic or endothermic) and its apparent magnitude is reported. However, since the surfactant–biopolymer interaction normally ends up with high equilibrium constant [38], the reported binding enthalpies directly obtained from microcalorimetry are, therefore, not far from the true values.

The results collected on the CTAB–Gn, SDS–Gn, CTAB–Lz and SDS–Lz systems at pH lower and higher than the isoelectric pH reveal that while hydrophobic interaction is the major factor at lower pH for CTAB and at higher pH for SDS, electrostatic interaction is the primary factor at higher pH for CTAB and lower pH for SDS. Such interactions may lead to configurational change of the otherwise compact Lz molecule, a separate study of which would be worthwhile.

The enthalpy profiles in calorimetric runs presented in Figs. 1–5 are comprised of differential values. The integral enthalpy (ΔH_i) can be obtained by summing up all the differential enthalpies in a run and dividing it by the total number of injections. These results for the studied systems are presented in Table 6. The concentration dependent ΔH_i values of the system Gn–CTAB fitted to a polynomial of degree three (corr. coeff. = 1) gave an intercept of -1.385 kJ mol^{-1}, which is the [Gn] independent ΔH_i for the binding of CTAB with Gn. Such result for Gn–SDS is 0.078 kJ mol^{-1}. Similar treatment of results on Lz–surfactant (CTAB and SDS) systems also yielded [biopolymer] independent ΔH_i values; these are 3.29 and 1.38 kJ mol^{-1} for CTAB and SDS interaction with Lz, respectively. The ΔH_i values obtained at different pH and temperature are presented in Table 6. For Gn–CTAB, the ΔH_i decreased with increasing pH and at higher pH it substantially increased (from a curve fitting procedure, the minimum was found to be at pH 8.68). The ΔH_i for the Gn–SDS system remained almost independent of pH.

The thermodynamic studies of binding of surfactants with biopolymers using microcalorimetric method are rare except the recent work of Spink and Chaires [31] on the CTAB–DNA system by the ITC method. Gani et al. [38] have studied the interaction of CTAB with Gn, collagen, bovine serum albumin (BSA) and DNA, as well as BSA–DNA, DNA–Gn and DNA–collagen mixtures by equilibrium dialysis method. References of other works on similar systems are cited therein [38]. They have reported the enthalpy of binding of CTAB with DNA (expressed in a non-conventional unit of kJ kg^{-1} of DNA) at 296 and 306 K and at pH 5.0 to be -253 and $+131$, respectively.

Table 4
The interaction of SDS with Lz under different conditions

[Lz] (g%)	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^b_{CAC_1}$ (kJ mol^{-1})	$\Delta H^{org}_{CAC_2}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(*a*) *The effect of concentration of Lz at pH 7 and temperature 303 K*						
0.25	2.40 (9.97)	17.7	12.7 (0.562)	−1.22	−2.02	2.84
0.50	4.18 (13.3)	26.9	13.4 (2.76)	−1.94	−4.56	4.37
0.75	5.36 (14.4)	32.7	13.0 (3.90)	−2.47	−5.58	5.37
1.0	6.53 (19.8)	–	11.6 (3.97)	−1.78	−6.45	–
pH	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^b_{CAC_1}$ (kJ mol^{-1})	$\Delta H^{org}_{CAC_2}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(*b*) *The effect of pH with 0.5 g% of Lz at 303 K*						
4	3.0 (12.2)	24.9	14.5 (2.68)	−2.15	−4.58	4.50
7	4.18 (13.3)	26.9	13.4 (2.76)	−1.94	−4.56	4.37
9	4.18 (11.1)	22.9	13.3 (2.72)	−1.395	−3.40	3.04
10.5	1.81 (11.1)	22.9	15.2 (3.59)	−1.58	−4.14	4.34
12	1.21 (7.68)	18.7	1.59 (2.68)	−7.48	–	7.08
Temp (K)	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^b_{CAC_1}$ (kJ mol^{-1})	$\Delta H^{org}_{CAC_2}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(*c*) *The effect of temperature with 0.5 g% of Lz at pH 9*						
303	4.18 (11.1)	22.9	13.3 (2.72)	−1.40	−3.40	3.04
313	4.18 (11.1)	30.8	2.68 (6.82)	−1.41	−2.91	1.79
323	4.18 (14.4)	22.9	−7.20 (8.91)	−5.65	−0.43	0.74

The values are lower (−84.3 and +43.7) when converted into our unit of expression (kJ mol^{-1} of CTAB). The maximum amount of binding of CTAB to DNA reported by Gani et al. [38] was at 1:1 as CTAB: nucleotide (mole/mole) at 296 K. In our experiment, the maximum [CTAB] used was 6.66 mmol dm^{-3} which is more than 1000-fold of the maximum uptake of CTAB reported by Gani et al. [38]. The maximum binding amounts of CTAB with BSA, collagen and Gn reported by Gani et al. [38] are also lower than [CTAB] used in the present study on the lower side of [biopolymer]. We can, therefore, consider that in our experiments the binding sites of Gn, Lz and DNA were totally interacted with the monomers and aggregates (micelles) of the ligands (CTAB and SDS). On the higher side of [biopolymer], binding remained incomplete in several cases. The large enthalpy change reported by Gani et al. [38] compared to us (Table 4) is, thus, surprising. Except the Gn–CTAB system, the other [biopolymer] independent integral enthalpies are positive. The interaction of SDS also ended up in positive enthalpy change. The order of their magnitudes is $(\Delta H_i)_{Gn/CTAB} < (\Delta H_i)_{Gn/SDS} < (\Delta H_i)_{DNA/CTAB} < (\Delta H_i)_{Lz/SDS} < (\Delta H_i)_{Lz/CTAB}$, which is the order of the increasing rigidity of the biopolymers (GN<DNA<Lz). Since a single low concentration (0.02%) of DNA was used, the result has been taken in the comparison. The effects of pH and temperature on the ΔH_i are too complex to correlate until results of further studies are available.

The DNA–surfactant interaction is primarily of electrostatic in nature. This is supported by the ability of CTAB to combine with *E. coli* and T4 DNA to make the helix compactly condense [31,39,40]. Isothermal titration calorimetry and light scattering measurements have been used to study the thermodynamic and the configurational aspects of the interaction. It has been reported [31] that at CTAB: DNA phosphate mole ratio (R) in the range of 0.7–1.0, the helix gets saturated with

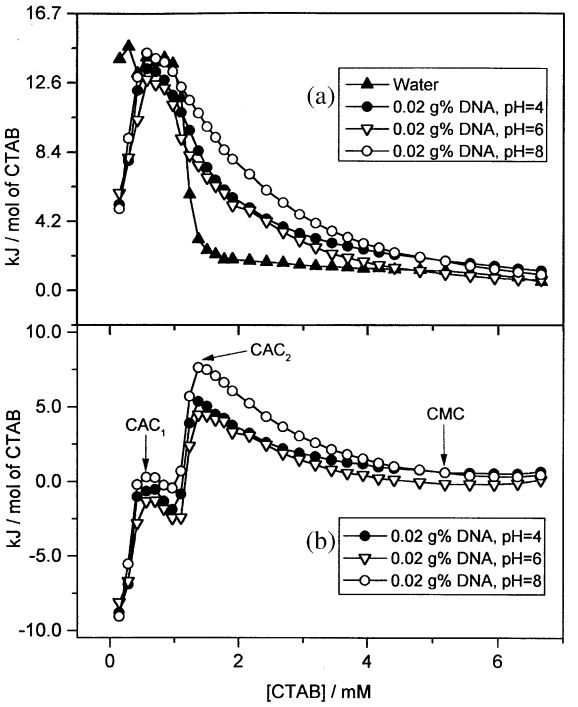

Fig. 5. Enthalpy of dilution of CTAB vs. [CTAB] profiles in 0.02 g% DNA solution at $\mu = 0.58$ and at 303 K at different pH. [CTAB] in the syringe = 30.94 mM; volume of water or water–DNA solution in the cell = 1.325 ml; total 324 μl of aliquot (CTAB solution) added in 30 installments. Illustration protocols are the same as in Fig. 1a,b.

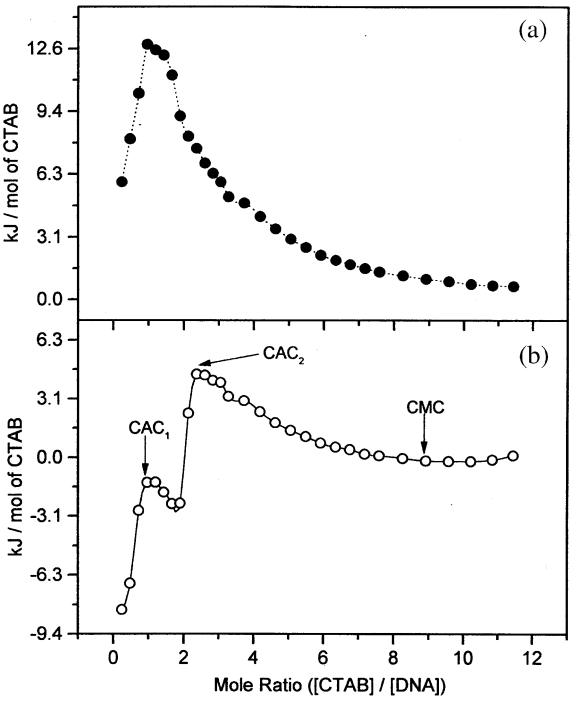

Fig. 6. Enthalpy of dilution of CTAB vs. mole ratio of CTAB and DNA in 0.02 g% DNA solution at 303 K, pH 6 and $\mu =$ 0.58. The data points are generated from those in Fig. 5a,b.

CTAB and gets condensed. There is an initial exothermic heat change at $R < 0.2$. Thereafter, the process shows endothermicity up to $R \simeq 0.8$ whose

Table 5
The interaction of CTAB with DNA under different conditions

pH	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^{b}_{CAC_1}$ (kJ mol^{-1})	$\Delta H^{org}_{CAC_2}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(a) The effect pH with 0.02 g% of DNA at 303 K						
4	0.62 (1.37)	5.18	8.23 (7.23)	−1.33	–	−4.73
6	0.62 (1.37)	5.18	6.78 (6.94)	−1.15	–	−4.63
8	0.62 (1.37)	5.56	9.31 (8.08)	0.73	–	−7.21
Temp (K)	CAC_1 (CAC_2) (mM)	CMC (mM)	ΔH_{CAC} (kJ mol^{-1}) CAC_1 (CAC_2)	$\Delta H^{b}_{CAC_1}$ (kJ mol^{-1})	$\Delta H^{org}_{CAC_2}$ (kJ mol^{-1})	ΔH_{CMC} (kJ mol^{-1})
(b) The effect of temperature with 0.02 g% of DNA at pH 6						
303	0.62 (1.51)	4.30	6.78 (6.94)	−1.15	–	−4.63
318	0.97 (–)	2.43	23.1 (–)	−4.96	–	6.02
333	0.62 (1.51)	2.94	13.6 (3.60)	−0.66	−2.35	2.21

magnitude then declines and subsequently rises in the range of $R=1.0-1.5$. The scattering intensity of the DNA–CTAB mixture also decreases at $R>0.8$. We have done ITC experiments on CTAB–DNA with R values up to 11.44 (Fig. 6). Instead of constant enthalpy change at $R\simeq 0.7-1.0$ report-

Table 6
The integral enthalpy of interaction of CTAB and SDS with the biopolymers (Gn, Lz and DNA) at different concentrations, pH and temperature

System: Gn/CTAB						
[Gn] (g%)	0	0.25	0.50	0.75	1.0	
ΔH_i (kJ mol^{-1} at 303 K)	−1.39	0.53	1.27	1.46	1.70	
pH	4	7	9	12		
ΔH_i (kJ mol^{-1} at 303 K)	3.17	1.26	0.78	4.24		
System: Gn/SDS						
[Gn]/g%	0	0.125	0.25	0.50	0.75	1.0
ΔH_i (kJ mol^{-1} at 303 K)	0.078	−0.51	−1.07	−2.20	−3.01	−3.29
pH	4	7	9	12		
ΔH_i (kJ mol^{-1} at 303 K)	−2.25	−2.20	−2.32	−2.26		
T/K	293	303	308	313	323	
ΔH_i (kJ mol^{-1})	−1.25	−2.32	−2.86	−2.56	−3.05	
System: Lz/CTAB						
[Lz]/g%	0	0.20	0.35	0.50	1.0	
ΔH_i (kJ mol^{-1} at 303 K)	3.29	0.96	2.06	2.13	4.0	
pH	4	7	9	12		
ΔH_i (kJ mol^{-1} at 303 K)	0.32	2.06	−0.46	9.27		
T/K	293	303	313	323		
ΔH_i (kJ mol^{-1})	−0.48	−0.46	−0.66	1.63		
System: Lz/SDS						
[Lz]/g%	0	0.25	0.50	0.75	1.0	
ΔH_i (kJ mol^{-1} at 303 K)	1.38	−1.42	−3.05	−4.0	−4.67	
pH	4	7	9	10.5	12	
ΔH_i (kJ mol^{-1} at 303 K)	−2.90	−3.05	−2.93	−2.63	−4.70	
T/K	303	313	323			
ΔH_i (kJ mol^{-1})	−2.93	−2.0	−1.25			
System: DNA (0.02 g%)/CTAB						
pH	4	6	8			
ΔH_i (kJ mol^{-1} at 303 K)	0.86	0.23	1.86			
T/K	303	318	333			
ΔH_i (kJ mol^{-1})	0.23	−4.41	0.15			

ed by Spink and Chaires [31], a sharp second maximum (called CAC_2) has been observed (Fig. 6b). In Fig. 6a (uncorrected for the heat of dilution of CTAB), maximisation at $R \simeq 1$ is observed which corresponds to CAC_1. The results presented by Spink and Chaires [31] were masked by the heat of dilution of CTAB and a sharp maximum called the CAC_2 was not observed. In the process of condensation of the double helix after saturation with CTAB, the whole system reorganises with reorganisation of CTAB molecules attached to the DNA chain, which have been designated as the CAC_2. We have also observed sharp decline in the intensity of the scattered light of the DNA–CTAB solution (measured by the dynamic light scattering method with a DLS-700 instrument of Otsuka Electronics, Japan) at $R > 0.7$. The solution acquired turbidity, and on standing precipitation of the formed complex from solution was observed. In high salt (NaCl) environment (1.5 mol dm^{-3}) CTAB failed to form the complex justifying further the requirement of electrostatic factors for the CTAB–DNA interaction.

The statement of Spink and Chaires [31] that in the ITC experiment, increase in enthalpy above $R \simeq 1.0$ was due to normal micelle formation of CTAB in the post-binding stage with DNA has not been corroborated by our results. We have observed micellisation process (of CTAB) to occur at $R \simeq 8.9$ at 303 K and pH 6 (Fig. 6b). Also, an initial exothermic heat change at $R < 0.2$ reported by Spink and Chaires [31] has not been observed by us. The errors in their measurements were also appreciable as shown from large fluctuations of the data points. Our results are much consistent in this respect. For deriving information on the configurational changes of the interacting systems, neutron scattering measurements would be advantageous.

4. Conclusions

1. The surfactant CTAB and SDS interact with the biopolymers Gn, Lz and DNA forming two types of induced aggregates prior to the formation of normal micelles in solution.
2. The cationic surfactant CTAB favourably interacts with the anionic DNA, while the anionic surfactant SDS does not interact. The surfactant–DNA interaction is, therefore, essentially electrostatic in nature. The surfactant (CTAB, SDS)–protein (Gn, Lz) interaction is governed by both electrostatic and hydrophobic interactions.
3. The DNA helix gets condensed upon interaction with CTAB and at [CTAB]/[DNA] ratio ≥ 1.0, the complex becomes insoluble.
4. The interaction of surfactants is complex and biopolymer-specific, and appreciably depends on pH and temperature.

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