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# Physico-chemical studies of micelle formation on sepia cartilage collagen solutions in acetate buffer and its interaction with ionic and nonionic micelles

## Hydrodynamic and thermodynamic studies

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Sepia cartilage collagen (pepsin-extracted) in acetate buffer (pH = 2.98) forms micelles at a particular concentration below which they do not normally form. The critical micelle concentration (cmc) of the collagen was determined in buffer as well as in SDS, cetyltrimethylammonium bromide (CTAB) and Tween-80 micellar environments at different temperatures. Mutual interaction of collagen micelles with the ionic and nonionic micelles through the formation of the mixed micelle concept has also been found. The cmc of collagen decreased in the presence of SDS and Tween-80 micelles whereas it increased in the presence of CTAB micelles. This clearly suggests that the micelle formation of collagen is facilitated by the presence of SDS and Tween-80 and hindered by CTAB micelles. The various thermodynamic parameters were estimated from viscosity measurements and the transfer of collagen into the micelles of various surfactants and the reverse phenomenon was analyzed. This analysis has also been modelled conceptually as a different phase and the results have supported the above phenomenon. Our thermodynamic results are also able to predict the exact denaturation temperature as well as the structural order of water in the collagen in various environments. The hydrated volumes, $V_h$, of collagen in the above environments and intrinsic viscosity were also calculated. The low intrinsic viscosity, $[\eta]$, of collagen in an SDS environment compared to buffer and other surfactant environments suggested more workable systems in cosmetic and dermatological skin care preparations. The one and two-hydrogen-bonded models of this collagen in various environments have been analyzed. The calculated thermodynamic parameters varied with the concentration of collagen. The change of thermodynamic parameters from coil-coil to random-coil conformation upon denaturation of collagen were calculated from the amount of proline and hydroxyproline residues and compared with viscometric results. Thermodynamic results suggest that the stability of the collagen in the additive environments is in the following order: SDS > Tween-80 > buffer > CTAB.

It is well known that the structure of water is important for interactions between proteins and membrane transport phenomena. These interactions are difficult to study due to the fact that the exact shapes, sizes and structure of the proteins are not clearly known. A favourable exception is collagen, consisting of triple-stranded helices which can be regarded as rigid rods. According to X-ray diffraction patterns the rods are packed in a complex lattice [1]. The important features are that the rods are aligned along the fiber axis and that the lattice expands [2] commensurate with the water content. The studies from various sources have indicated that the structure of water absorbed in collagen is different from that of water in bulk. Broad-line NMR measurements [3–6] have shown two absorption lines instead of one. This may be the reason that the water molecules in collagen do not rotating isotropically. Moreover, their rotational relaxation times are considerably longer than in bulk. The increase of dielectric constant as well as the heat capacity of water in collagen [7, 8] indicated that the relaxation spectrum is unusually broad, unlike that of bulk water.

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*Abbreviations.* cmc, critical micelle concentration; CTAB, cetyltrimethylammonium bromide; Tween-80, polyoxyethylene sorbitan monooleate; SDS, sodium dodecyl sulfate.

Regarding details of hydration of proteins the reader is referred to an excellent article by Kuntz and Kauzmann [9]. Several models have been proposed in order to explain the structure of water in collagen. On the basis of NMR results, Chapman et al. [10] proposed that parts of the water molecules are bound at sites, but that they exchange rapidly with freely rotating water molecules. A similar two-state model was also proposed by Migchelsen and Berendsen [6]. In that model the bound molecules form hydrogen-bonded bridges between collagen C=O and N-H groups in the systematic manner suggested by Ramachandran and Chandrasekharan [11]. Suzuki and Fraser [12] presented polarized infrared absorption spectra of water absorbed in collagen in order to interpret the consistency of anisotropy with bound water having an orientation as expected by Ramachandran and Chandrasekharan [11]. According to Andronikashvill et al. [13] the amount of water incapable of freezing has been denoted as 'nonrotational bound' water, whereas the amount that is freezable has been designated as 'free'. Hence, the support for the existence of two-state models was considered.

The soluble collagen has long been used in cosmetic and dermatological skin care preparations. Collagen is attached to the upper layers of skin where it is highly beneficial for the improvement of hydration conditions. It has been observed that a smoothening of the surface structure of the skin develops with an increase in elasticity. Sodium dodecyl sulfate

(SDS) forms micelles in aqueous solution and is widely used as a solubilizer of usually insoluble biochemical materials [14], as a dissociating agent for protein–nucleic-acid complexes [15] or as a solute in electrophoresis [16]. Tween-80 and cetyltrimethylammonium bromide (CTAB) are nonionic and cationic surfactants respectively which form micelles in aqueous solution and their properties, characterization and applications have been described in our recent publications [17–20]. The interaction of protein with surfactants is thus an important consideration of the protein physico-chemists [21].

It has been proposed that steric restictions to rotation around the polypeptide backbone arising from the stereochemistry of the pyrrolidine ring act to stabilize the triple-helical structure by reducing the total configurational entropy change in the helix-coil thermal transition [22]. If it is assumed that the denaturation of collagen in both the macroscopic and molecular state is a first-order phase transition of the melting type with the free energy of unfolding $\Delta G_u = 0$, then the following relationship is predicted [23]:

$$T_d = \frac{\Delta H_r}{\Delta S_r} \quad (1)$$

where $\Delta H_r$ and $\Delta S_r$ are the enthalpy and entropy per residue respectively and $T_d$ is the thermal denaturation temperature. Since there is no accompanying enthalpy change when pyrrolidine residues are introduced into the collagen chain, it follows that any increase in heat stability should be due to a fall in entropy with an increase in the content of imino acids.

It has been shown by calorimetric measurements that the enthalpy and temperature of thermal transition depend on the pyrrolidine content [24, 25]. However, the stability of collagen structures, the transfer phenomena and other thermodynamic properties are not yet clearly understood.

Collagen fibrils reacting with surface-active substances reveal a surprising parallel between the degree of aggressiveness of the detergent and its binding intensity. Therefore, our research has been oriented towards obtaining collagen-surfactant complexes.

We have investigated [26–28] the micelle formation on various synthetic tanning materials (syntans) and its interaction with various additives. Hydrodynamic and thermodynamic studies have also been undertaken on the above systems, but the investigation of micelle formation on collagen solutions are not reported in the literature. In our recent (unpublished studies), we have shown that rat tail tendon collagen forms true solutions or is present as micelles in citrate buffer (pH 3.7) as well as in SDS environments. We have also studied the hydration and thermodynamic properties of this collagen in the above-mentioned environments (A. B. Mandal, F. Chandrasekaran, P. K., Sehgal & M. Chvapil, unpublished results). The reason for studying collagen in solution is that traditionally biologists have been concerned with the chemistry and function of cells on a molecular level and because important physiological reactions usually occur in solution. In our present studies, we report first that the pepsin-extracted sepia cartilage collagen solutions in acetate buffer (pH 2.98) form micelles. We also describe the effects of various ionic and nonionic surfactants on the micelle formation of this collagen at various temperatures. Some fundamental hydrodynamic, thermodynamic and transport parameters of the collagen in buffer and surfactant-additive environments, which have been derived from viscometric and spectrophotometric results, are presented in this report.

## MATERIALS AND METHODS

### *Extraction and purification of sepia cartilage collagen*

Extraction of the collagen was carried out according to the method of Kimura et al. [30]. Sepia cartilage was carefully removed and cut into small pieces. The tissue (200 g wet weight) was first washed with cold distilled water and then with 0.5 M sodium acetate. After the final washing, the tissue was suspended in 1.5 l 0.5 M acetic acid mixed with 100 mg pepsin (2900 units/mg) and incubated at 4°C for 48 h with stirring. The digest was then centrifuged at $10000 \times g$ for 1 h. The supernatant was precipitated with 5% NaCl, the precipitate dissolved in 0.5 M acetic acid and then dialyzed several times against 0.02 M disodium hydrogen phosphate. The dialyzate was centrifuged, the residue was dissolved in 0.5 M acetic acid and dialyzed sequentially against 0.5 M, 0.2 M and 0.01 M acetic acid. The dialyzate thus obtained was lyophilized and used in the present study. The purity of collagen was estimated to be 96–98%.

### *Reagents and solutions*

The grade, purity and characteristics of surfactants used in the present study are described in our recent publications [17–19, 31]. Tween-80 (polyoxyethylene sorbitan monooleate), a nonionic surfactant, was pure grade material of BDH with the head group containing on average 20 oxyethylene units. The critical micelle concentration of Tween-80 in aqueous solution at 25°C was checked up by surface tension and found to be 0.01 mM which agreed closely with the literature value [32]. All the other reagents are of analytical grade. Double distilled conductivity water (specific conductance 1.5–2 μS $cm^{-1}$ at 25°C) was used throughout the experiment.

### *Spectrophotometric measurements*

Spectral measurements were made on a Beckman (model 26) apparatus with automatic device using a silica cell of 1-cm path length. Prior to the measurements all the solutions were thermosttated for a considerable length of time. The temperature fluctuation was in the range of ±0.05–0.1°C.

### *Viscosity measurements*

The viscosity of different concentrations of collagen solution was measured by an Ostwald viscometer at various temperatures using sodium acetate/acetic acid buffer (pH 2.98) as well as with 0.08 M SDS, 8 mM CTAB or 0.8 mM Tween-80 in the buffer (pH of the solution = 2.98). The flow times of the buffer, surfactant additives in the buffer and water were taken separately at various temperatures in order to estimate the absolute viscosity values of collagen solutions. The viscometer was calibrated using 10% and 20% sucrose solution in water, the results are in good agreement with the values available in the literature. The viscometer gives a flow time of 240 s for water at 25°C. The temperature of measurement was accurate within ±0.1°C. Uncertainties in temperature and density measurement and the flow detection imparted a maximum error of ±0.7% to the measured viscosity. Details of viscosity measurements are provided in our recent publications [31, 33].

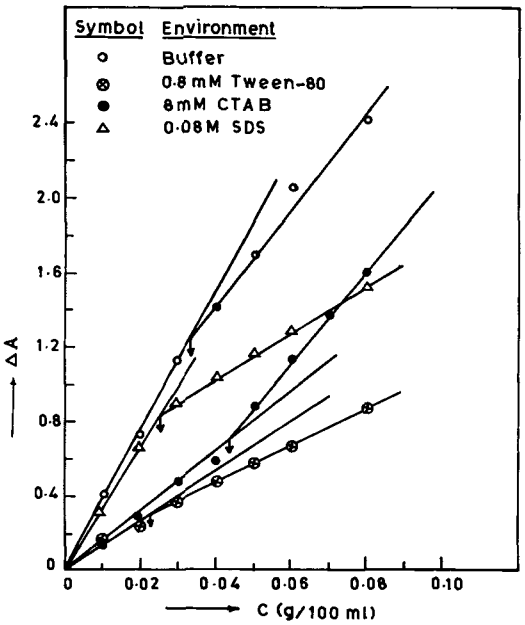


Fig. 1. *Difference absorbance at 230 nm (28°C, pH 2.98) plotted against sepia cartilage collagen concentration in sodium acetate/acetic acid buffer, 0.08 M SDS, 0.8 mM Tween-80 and 8 mM CTAB*

### *Determination of critical micelle concentration of collagen*

Spectrophotometric determination of the critical micelle concentration (cmc) of collagen was performed by taking its ultraviolet spectrum in the presence and absence of surfactant additives and plotting the absorbance at $\lambda_{max}$ (230 nm) of the main band against concentration of collagen. The full spectra were taken to investigate fully the effects of the additives on the nature of the native spectra in micellar and non-micellar states of the collagens. The full spectrum of sepia cartilage (pepsin-extracted) collagen solutions in acetate buffer as well as in the environment of surfactant additives is given in our recent publication [20]. Since no dye was used to determine the cmc of the collagen, the possibility of contamination was ruled out. The plot of differential absorbance vs concentration of collagen is shown in Fig. 1.

In the determination of the cmc of the collagen in the absence and presence of surfactant additives, a series of solutions were made at a fixed concentration of the additive (0.08 M SDS, 8 mM CTAB and 0.8 mM Tween-80 in the present case) and varying concentrations of the collagens. The relative viscosity of each solution was plotted as a function of concentration of collagen. The abrupt changes in the value of the initial slopes at a particular concentration were considered as the cmc points below which they do not form micelles. The experiment was performed at various temperatures and at acidic pH (pH = 2.98).

## RESULTS AND DISCUSSION

### *Viscosity and micelle formation of collagen*

For a spherical shape of the particles, it is possible to calculate the hydrated specific volume, $V_h$, of collagen solutions using the following Einstein [34] equation:

$$\eta/\eta_0 = \eta_{rel} = 1 + a\,V_h \cdot c \qquad (2)$$

where $\eta_{rel}$ = relative viscosity of the solution; $\eta$ and $\eta_0$ are the viscosities of the solution and the solvent respectively; $c$

Table 1. *The hydrated specific volume, $V_h$ and critical micelle concentration (cmc) of sepia cartilage collagen solutions in acetate buffer and in 0.08 M SDS, 0.8 mM Tween-80, 8 mM CTAB*
The pH of the solution = 2.98. The cmc values obtained by the spectrophotometric method are shown in the parenthesis

| Temp. | $V_h$ for spherical shape model ($a$ = 2.5) | | | | $V_h$ for elongated rod shape model ($a \gg 2.5$) | | | | cmc in | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | buffer | SDS | Tween-80 | CTAB | buffer | SDS | Tween-80 | CTAB | buffer | SDS | Tween-80 | CTAB |
| °C | dl/g | | | | ml/g | | | | g/dl | | | |
| 20 | 1.43 | 0.57 | 1.25 | 1.43 | 1.73 | 3.46 | 1.58 | 2.16 | 0.025 (0.025) | 0.020 (0.020) | 0.015 (0.025) | 0.039 (0.04) |
| 25 | 1.72 | 0.50 | 1.11 | 1.67 | 1.68 | 1.83 | 1.14 | 1.81 | 0.030 | 0.025 | 0.017 | 0.050 |
| 30 | 1.78 | 0.80 | 2.00 | 1.82 | 1.53 | 1.01 | 1.43 | 1.37 | 0.035 (0.032) | 0.030 (0.028) | 0.025 (0.025) | 0.060 (0.05) |
| 35 | 1.32 | 0.53 | 1.43 | 1.33 | 1.69 | 1.47 | 1.12 | 1.41 | 0.060 | 0.070 | 0.048 | — |
| 40 | 1.25 | 0.89 | 1.67 | 1.05 | 1.98 | 2.65 | 1.38 | 1.25 | — | — | — | — |
| 45 | 1.00 | 0.57 | 1.11 | 0.80 | 1.85 | 1.29 | 2.21 | 1.15 | — | — | — | — |
| 50 | 0.87 | 0.47 | 1.25 | 0.74 | 1.69 | 1.26 | 1.06 | 1.10 | — | — | — | — |

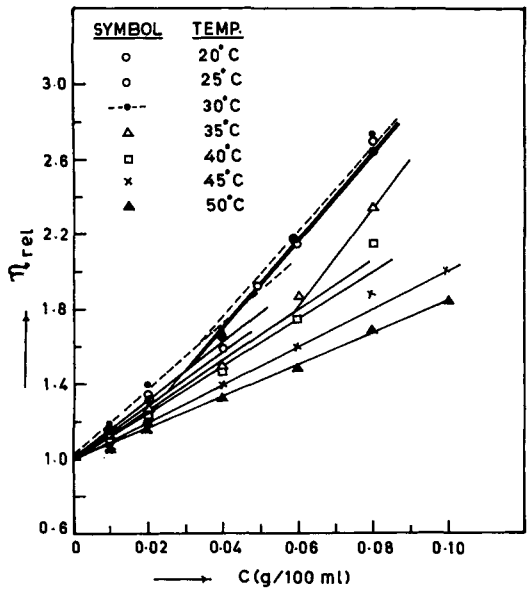


Fig. 2. *Plot of* $\eta/\eta_0$ *vs* c *using Einstein equation on sepia cartilage collagen in sodium acetate/acetic acid buffer (pH = 2.98) at various temperatures*

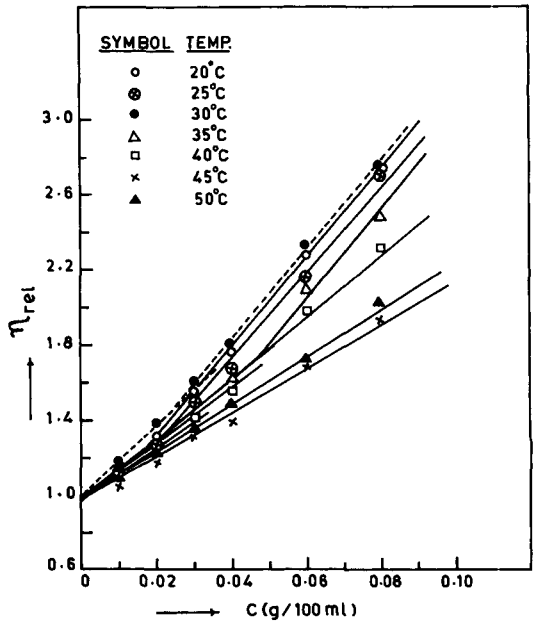


Fig. 4. *Plot of* $\eta/\eta_0$ *vs* c *using Einstein equation on collagen in 0.8 mM Tween-80 (pH 2.98) at various temperatures*

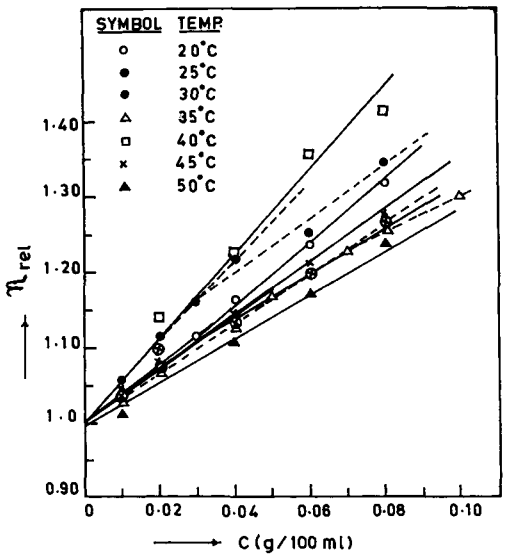


Fig. 3. *Plot of* $\eta/\eta_0$ *vs* c *using Einstein equation on collagen in 0.08 M SDS (pH 2.98) at various temperatures*

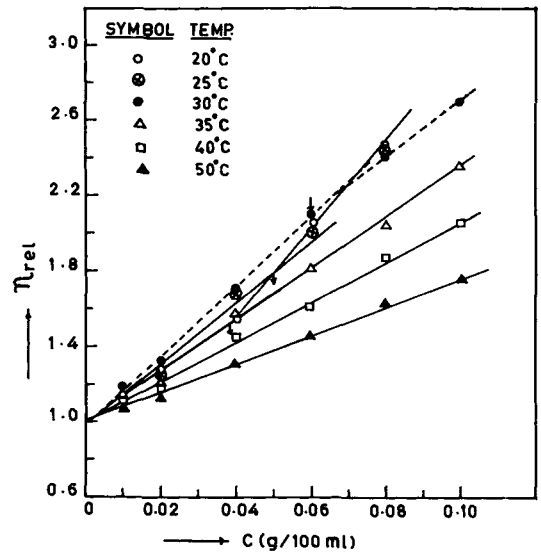


Fig. 5. *Plot of* $\eta/\eta_0$ *vs* c *using Einstein equation on collagen in 8 mM CTAB (pH 2.98) at various temperatures*

is the concentration of the collagen solution. For spherical particles $a = 2.5$ and for non-spherical particles $a > 2.5$. The plot of $\eta/\eta_0$ vs concentration of collagen in solutions in the absence and presence of surfactant environments at various temperatures is shown in Figs 2–5. The $V_h$ values for elongated rod-shaped particles of collagen (Table 1) were computed after obtaining $v$ values from the following Simha-Einstein [35] equation:

$$[\eta] = v\left(\bar{v}_{\text{collagen}} + \frac{\delta}{\varrho_{H_2O}}\right) \qquad (3)$$

where $\bar{v}_{\text{collagen}}$ = partial specific volume of collagen, $\delta$ = g water bound /g collagen, $\varphi_{H_2O}$ = density of water and $v$ is the asymmetry or shape factor. $v = 2.5$ for spherical shape of the particles and $v > 2.5$ for non-spherical shape. The values of $\delta$, $\bar{v}$ and $v$ are given in Table 2.

Eqn (2) is generally valid for electrolyte solutions; in the present case of sepia cartilage collagen, although these are not electrolyte solutions, Eqn (2) is still valid up to the critical micelle concentration of collagen. Beyond this Eqn (2) is not applicable due either to the miceller aggregation of collagen solutions (Figs 1–5; hydrophobic interaction is responsible for micelle formation) or to the prominent interaction between the segments in collagen solutions. Similar results are also found with rat tail tendon collagen solutions (our unpublished results). We have also recently found [36] that Eqn (2) is valid for collagen solutions up to 0.035 g/dl for warm water fish

Table 2. *The intrinsic viscosity, [η], assymmetry factor (shape factor), ν, and some physico-chemical parameters of sepia cartilage collagen solutions in acetate buffer and in 0.08 M SDS, 0.8 mM Tween-80, 8 mM CTAB*
pH of the solution = 2.98

| Temp. | [η] in | | | | ν in | | | | $\bar{v}$ | δ |
|---|---|---|---|---|---|---|---|---|---|---|
| | buffer | SDS | Tween-80 | CTAB | buffer | SDS | Tween-80 | CTAB | | |
| °C | dl/g | | | | | | | | ml/g | g/g |
| 20 | 8.50 | 1.50 | 7.20 | 6.00 | 701.96 | 123.87 | 594.60 | 495.51 | 0.71 | 0.50 |
| 25 | 9.00 | 2.40 | 8.55 | 8.10 | 768.33 | 204.87 | 729.90 | 691.47 | 0.70 | 0.47 |
| 30 | 9.90 | 6.75 | 11.85 | 11.25 | 874.59 | 596.31 | 1046.88 | 993.87 | 0.68 | 0.45 |
| 35 | 6.45 | 3.00 | 10.50 | 7.80 | 585.00 | 272.10 | 952.32 | 707.43 | 0.67 | 0.43 |
| 40 | 5.10 | 2.70 | 9.75 | 6.75 | 475.17 | 251.55 | 908.40 | 628.89 | 0.65 | 0.42 |
| 45 | 4.20 | 3.45 | 3.9 | 5.40 | 406.20 | 333.69 | 377.22 | 522.27 | 0.63 | 0.40 |
| 50 | 3.90 | 2.85 | 6.00 | 5.10 | 384.27 | 280.83 | 591.20 | 502.50 | 0.60 | 0.41 |

(bigeye tuna, carp and cat) and 0.045 g/dl for cold-water fish (halibut). The cmc obtained by relative viscosity and spectrophotometric methods agree well, as shown in Table 1.

The intrinsic viscosity [η] has been determined using the following equation:

$$\eta_{sp}/c = [\eta] + [\eta]^2 K \cdot c \qquad (4)$$

where $\eta_{sp} = [(\eta/\eta_0) - 1]$ = specific viscosity of the solution; $K$ is the Huggins' constant. The results of [η] obtained from the intercept of the plot of $\eta_{sp}/c$ vs $c$ are presented in Table 2.

*Hydrated volume and interaction of surfactant with collagen*

It is generally accepted [31] that the hydration decreases with the increase in temperature in the case of nonionic surfactants and some proteins. We have also observed the same phenomenon [37] in collagens of shark skin, bovine skin, desamido and type-III from human placenta (our unpublished results). But in case of rat tail tendon collagen [29] in citrate buffer (pH 3.7), the hydration volume increases with the increase in temperature up to 26 °C (from 5 °C to 26 °C) beyond which it was found to decrease with the increase in temperature, as with bigeye tuna, carp, cat fish collagens [36] etc. In our present case of sepia cartilage collagen, the hydration volume decreases with the increase in temperature up to 30 °C (from 20 °C to 30 °C) in each surfactant environment except Tween-80; beyond 30 °C the opposite trend was observed (see Table 1, for elongated rod-shaped (model). It can also be seen in Table 1 that the cmc of sepia cartilage collagen increases with the increase in temperature irrespectively of the environment. In other words, the formation of the aggregate of collagen is hindered by an increase in temperature. At constant temperature, the cmc of sepia cartilage collagen in SDS and Tween-80 environments is always smaller than that in the buffer which clearly suggests that micelle formation of the collagen is facilitated in the presence of SDS and Tween-80, probably owing to the formation of mixed micelles, whereas in CTAB the micelle formation of the above collagen was hindered. The surfactants form micelles in aqueous solution. The concentration of surfactant in collagen was kept 10 times higher than the respective cmc so that their interaction with collagen is more prominent. Now, the question can be raised, whether collagen will be incorporated into the micellar core of the surfactant or the surfactant will be incorporated into the micelle of collagen. The reverse situation is not possible in SDS and Tween-80 at temperatures below the denaturation temperature of collagen but is possible above it. The reverse situation is possible in CTAB even below the denaturation temperature of collagen. We will discuss this later in this paper.

The helix-coil phase transition during thermal denaturation in acetate buffer (pH 2.98) was followed by specific viscosity measurements. The plots of $\eta_{sp}/c$ vs temperatures in buffer as well as surfactant additive environments are shown in Figs 6–9. The denaturation temperature of collagen was determined as the mid-point temperature of the $\eta_{sp}/c$ vs temperature plot. We conjecture that the appearance of the second maxima may be due to the secondary denaturation of collagen but we do not have direct evidence for this at present.

The question of stability of collagen in cosmetic or dermatological emulsions must be answered and as a general rule, we can say that the stability of collagen is higher in an emulsion system than alone.

Nicoli and Benedek [38] found that the heat denaturation of lysozyme increases the hydrodynamic radius from 1.85 nm to 2.18 nm and not to the 4.9 nm calculated for a random coil. Our results for sepia cartilage collagen suggest that hydrodynamic volume (rather hydrodynamic radius) increases with the increase in temperature below the denaturation temperature of collagen.

## THERMODYNAMIC PARAMETERS

*Enthalpy, entropy and free energy*

The enthalpy of activation, $\Delta H^*$, was calculated using the following Eyring equation [39]:

$$\log \eta = \left[\log\left(\frac{hN}{V} - \frac{\Delta S^*}{2.303R}\right)\right] + \frac{\Delta H^*}{2.303R}\left(\frac{1}{T}\right) \qquad (5)$$

where $\eta$ = absolute viscosity of collagen solutions; $h$ = Plank's constant; $N$ = Avogadro's number; $V$ = molar volume of the solvent medium; $R$ = universal gas constant and $T$ = temperature on the absolute scale.

The plot of $\log \eta$ vs $1/T$ for obtaining the transition temperature is shown in Figs 10–13. The $\Delta H^*$ values of collagen before and after denaturation were calculated from the slope; results are given in Tables 3–5 together with the Gibb's free energy of activation, $\Delta G^*$ obtained using Eqn (6):

$$\Delta G^* = RT \ln\left(\frac{\eta V}{hN}\right) \qquad (6)$$

where the symbols have their usual significance.

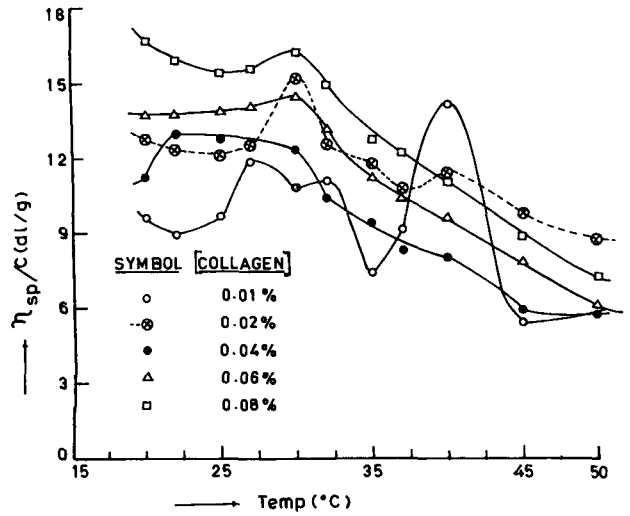


Fig. 6. *Plot of* $\eta_{sp}/c$ *vs temperature for sepia cartilage collagen in sodium acetate/acetic acid buffer (pH = 2.98) as the solvent medium at various concentrations of collagen*

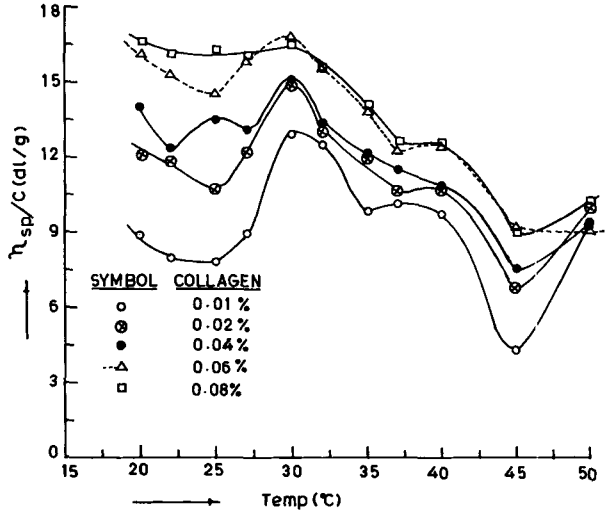


Fig. 8. *Plot of* $\eta_{sp}/c$ *vs temperature for collagen in 0.8 mM Tween-80 (pH 2.98) at various concentrations of collagen*

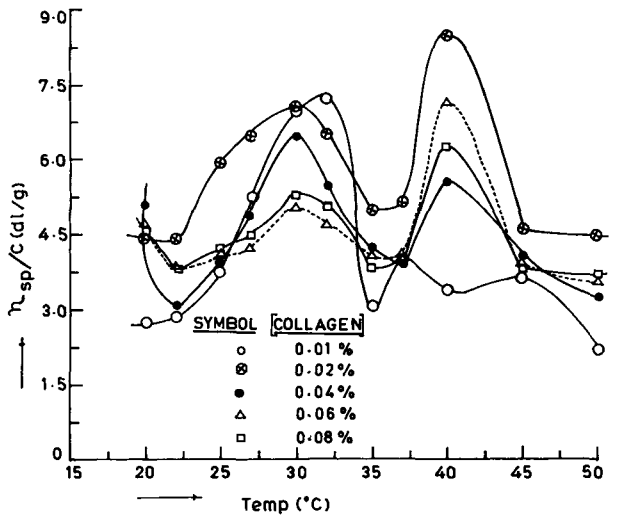


Fig. 7. *Plot of* $\eta_{sp}/c$ *vs temperature for collagen in 0.08 M SDS (pH 2.98) at various concentrations of collagen*

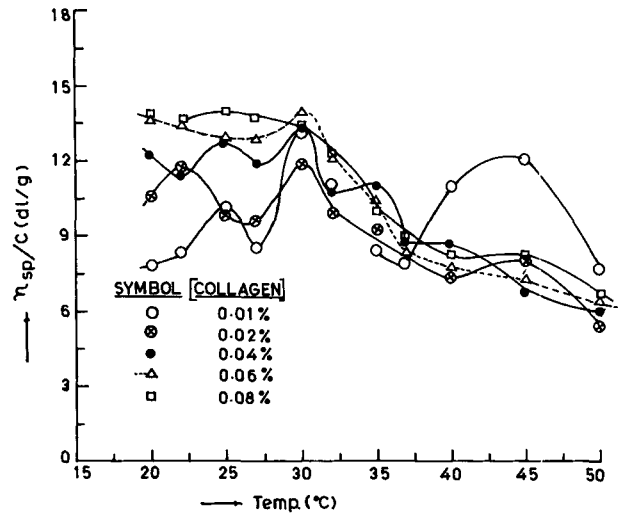


Fig. 9. *Plot of* $\eta_{sp}/c$ *vs temperature for collagen in 8 mM CTAB (pH 2.98) at various concentrations of collagen*

After conjunction of $\Delta G^*$ and $\Delta H^*$ in the following Gibb's equation:

$$\Delta G^* = \Delta H^* - T \cdot \Delta S^* \tag{7}$$

the entropy of activation, $\Delta S^*$, was calculated at the corresponding temperatures. The $\Delta G^*$ and $\Delta S^*$ values for collagen in buffer as well as in environments containing surfactant additives are also given in Tables 3–5. In this regard, it may be noted that it is possible to calculate the value of $\Delta S^*$ from the intercept of the plot of log $\eta$ vs $1/T$ utilizing Eqn (5); however, the intercept is not very sharp and hence we have avoided such calculations. Details of calculations of thermodynamic parameters from viscosity measurements can be found in our recent publications [31, 33].

In Tables 3–5, it can be seen that over the entire range of temperature investigated, the $\Delta G^*$ values decreased with the increase in temperature in various environments irrespective of the source of collagen, e.g. rat tail tendon [29], shark skin, bovine skin, desamido, type III (human placenta) [37], bigeye tuna, carp, cat, halibut [36]. It is also important to note that no appreciable change of $\Delta G^*$ was observed above the denaturation temperature of collagen, but at that temperature and a little below, there is a remarkable change in $\Delta G^*$: the transfer of $\Delta G^*$ from the lower temperature to a higher one is more negative in the denatured state of the collagen and is accompanied by a greater irreversibility of the process. This denaturation was further substantiated by the consideration of the entropy of activation, $\Delta S^*$. Generally for collagens $\Delta S^*$ increases with the increase of temperature, but at the denaturation temperature an anomalous increase of $\Delta S^*$ starts. The values of $\Delta G^*$, $\Delta S^*$ and $\Delta H^*$ for water at 25°C are 9.15 kJ $mol^{-1}$, 17.05 J $K^{-1}$ $mol^{-1}$ and 15.01 kJ $mol^{-1}$ respectively [31]. The increase of all these thermodynamic activation parameters of collagen in buffer as well as in the presence of surfactant additives in the present study have supported the idea that the structure of water in the collagen is ordered.

In Tables 3–5, it can also be seen that all the thermodynamic parameters ($\Delta G^*$, $\Delta H^*$ and $\Delta S^*$) increase with increase in concentration of sepia cartilage collagen in

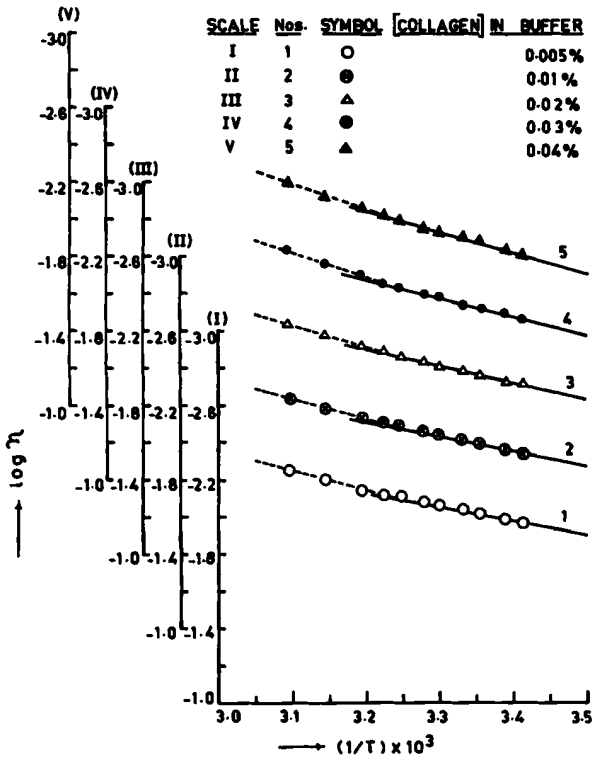


Fig. 10. *The plot of log η vs 1/T using Eyring equation on sepia cartilage collagen at various concentrations in sodium acetate/acetic acid buffer (pH 2.98)*

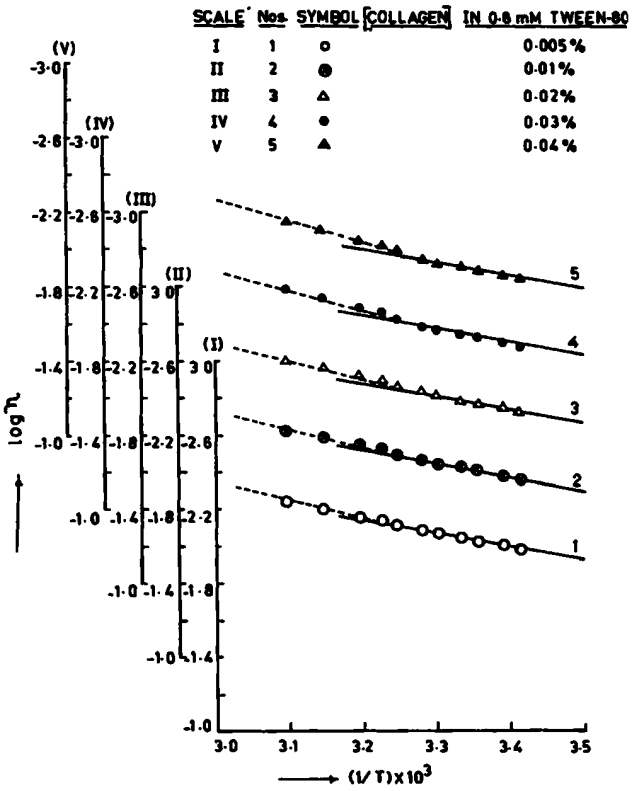


Fig. 12. *The plot of log η vs 1/T using Eyring equation on collagen at various concentrations in 0.8 mM Tween-80 (pH 2.98)*

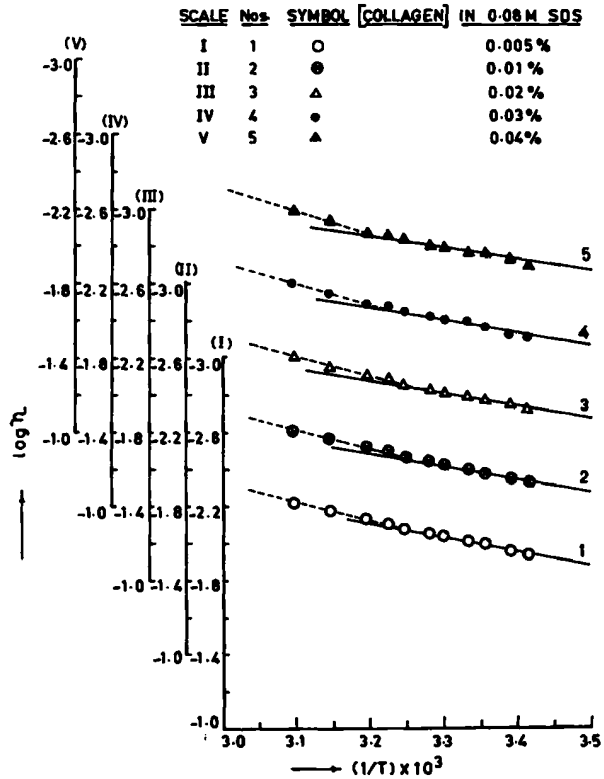


Fig. 11. *The plot of log η vs 1/T using Eyring equation on collagen at various concentrations in 0.08 M SDS (pH 2.98)*

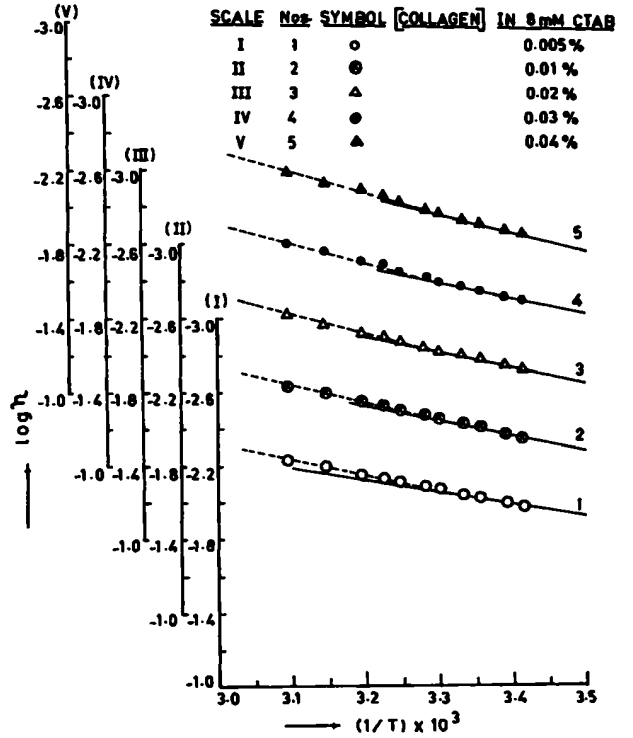


Fig. 13. *The plot of log η vs 1/T using Eyring equation on collagen at various concentrations in 8 mM CTAB (pH 2.98)*

Table 3. *The various thermodynamic activation parameters of sepia cartilage collagen at different concentrations in buffer as well as in 0.08 M SDS and the transfer of activation parameters of collagen from buffer to SDS environment*
pH of the solution = 2.98

| [Collagen] | Temp. | Buffer | | | SDS | | | Transfer of activation parameters of collagen | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $\Delta G^*$ | $\Delta H^*$ | $\Delta S^*$ | $\Delta G^*$ | $\Delta H^*$ | $\Delta S^*$ | $(\Delta G^*)_{tr.}$ | $(\Delta H^*)_{tr.}$ | $(\Delta S^*)_{tr.}$ |
| g/dl | °C | kJ mol$^{-1}$ | | J K$^{-1}$ mol$^{-1}$ | kJ mol$^{-1}$ | | J K$^{-1}$ mol$^{-1}$ | kJ mol$^{-1}$ | | J K$^{-1}$ mol$^{-1}$ |
| 0.005 | 20 | 9.51 | | 15.05 | 9.59 | | 13.94 | +0.08 | | − 1.11 |
| | 22 | 9.43 | | 15.21 | 9.54 | | 14.02 | +0.11 | | − 1.19 |
| | 25 | 9.35 | 1392 | 15.35 | 9.45 | | 14.20 | +0.10 | − 0.24 | − 1.15 |
| | 27 | 9.30 | | 15.40 | 9.41 | 13.68 | 14.24 | +0.11 | | − 1.16 |
| | 30 | 9.20 | | 15.53 | 9.36 | | 14.26 | +0.16 | | − 1.27 |
| | 32 | 9.19 | | 15.51 | 9.33 | | 14.27 | +0.14 | | − 1.24 |
| | 35 | 9.13 | | 39.42 | 9.25 | | 14.37 | +0.12 | | −25.05 |
| | 37 | 9.10 | | 39.24 | 9.19 | | 35.34 | +0.09 | | − 3.90 |
| | 40 | 9.07 | 21.27 | 38.98 | 9.11 | 20.15 | 35.28 | +0.04 | − 1.12 | − 3.70 |
| | 45 | 8.85 | | 39.05 | 9.02 | | 34.99 | +0.17 | | − 4.06 |
| | 50 | 8.71 | | 38.89 | 8.88 | | 34.90 | +0.17 | | − 3.99 |
| 0.04 | 20 | 10.33 | | 46.42 | 9.86 | | 5.82 | −0.47 | | −40.60 |
| | 22 | 10.25 | | 46.38 | 9.76 | | 6.11 | −0.49 | | −40.27 |
| | 25 | 10.12 | 23.99 | 46.34 | 9.68 | | 6.32 | −0.44 | −12.43 | −40.02 |
| | 27 | 10.07 | | 46.21 | 9.64 | 11.56 | 6.39 | −0.43 | | −39.82 |
| | 30 | 10.03 | | 45.87 | 9.61 | | 6.41 | −0.42 | | −39.46 |
| | 32 | 9.96 | | 45.82 | 9.59 | | 6.47 | −0.37 | | −39.35 |
| | 35 | 9.81 | | 55.91 | 9.48 | | 6.77 | −0.33 | | −49.14 |
| | 37 | 9.75 | | 55.73 | 9.42 | | 46.81 | −0.33 | | − 8.92 |
| | 40 | 9.62 | 27.00 | 55.64 | 9.38 | 23.97 | 46.48 | −0.24 | − 3.03 | − 9.16 |
| | 45 | 9.35 | | 55.58 | 9.25 | | 46.17 | −0.10 | | − 9.41 |
| | 50 | 9.12 | | 55.46 | 9.10 | | 45.89 | −0.02 | | − 9.57 |

Table 4. *The various thermodynamic activation parameters of sepia cartilage collagen at different concentrations in 0.8 mM Tween-80 and the transfer of activation parameters of collagen from buffer to Tween-80 micellar environment*
pH of the solution = 2.98

| [Collagen] | Temp. | 0.8 mM Tween-80 | | | Transfer of activation parameters of collagen | | |
|---|---|---|---|---|---|---|---|
| | | $\Delta G^*$ | $\Delta H^*$ | $\Delta S^*$ | $(\Delta G^*)_{tr.}$ | $(\Delta H^*)_{tr.}$ | $(\Delta S^*)_{tr.}$ |
| g/dl | °C | kJ mol$^{-1}$ | | J K$^{-1}$ mol$^{-1}$ | kJ mol$^{-1}$ | | J K$^{-1}$ mol$^{-1}$ |
| 0.005 | 20 | 9.43 | | 18.09 | −0.08 | | + 3.04 |
| | 22 | 9.35 | | 18.22 | −0.08 | | + 3.01 |
| | 25 | 9.29 | | 18.25 | −0.06 | + 0.81 | + 2.90 |
| | 27 | 9.25 | 14.73 | 18.27 | −0.05 | | + 2.87 |
| | 30 | 9.18 | | 18.31 | −0.02 | | + 2.78 |
| | 32 | 9.14 | | 18.33 | −0.05 | | + 2.82 |
| | 35 | 9.08 | | 18.34 | −0.05 | | −21.08 |
| | 37 | 9.02 | | 32.68 | −0.08 | | − 6.56 |
| | 40 | 8.98 | | 32.49 | −0.09 | | − 6.49 |
| | 45 | 8.88 | 19.15 | 32.28 | +0.03 | − 2.12 | − 6.77 |
| | 50 | 8.78 | | 32.09 | +0.07 | | − 6.80 |
| 0.04 | 20 | 10.25 | | 10.11 | −0.08 | | −36.31 |
| | 22 | 10.16 | | 10.33 | −0.09 | | −36.05 |
| | 25 | 10.12 | | 10.35 | 0 | | −35.99 |
| | 27 | 10.07 | 13.21 | 10.47 | 0 | −10.78 | −35.74 |
| | 30 | 9.99 | | 10.62 | −0.04 | | −35.25 |
| | 32 | 9.94 | | 10.71 | −0.02 | | −35.11 |
| | 35 | 9.81 | | 41.30 | 0 | | −14.61 |
| | 37 | 9.67 | | 41.47 | −0.08 | | −14.26 |
| | 40 | 9.64 | | 41.18 | +0.02 | | −14.46 |
| | 45 | 9.45 | 22.59 | 41.14 | +0.10 | − 4.41 | −14.44 |
| | 50 | 9.34 | | 40.83 | +0.22 | | −14.63 |

Table 5. *The various thermodynamic activation parameters of sepia cartilage collagen at different concentrations in 8 mM CTAB and the transfer of activation parameters of collagen from buffer to CTAB micellar environment*
pH of the solution = 2.98

| [Collagen] | Temp. | 8 mM CTAB | | | Transfer of activation parameters of collagen | | |
|---|---|---|---|---|---|---|---|
| | | $\Delta G^*$ | $\Delta H^*$ | $\Delta S^*$ | $(\Delta G^*)_{tr.}$ | $(\Delta H^*)_{tr.}$ | $(\Delta S^*)_{tr.}$ |
| g/dl | °C | kJ mol$^{-1}$ | | J K$^{-1}$ mol$^{-1}$ | kJ mol$^{-1}$ | | J K$^{-1}$ mol$^{-1}$ |
| 0.005 | 20 | 9.45 | | 17.19 | −0.06 | | + 2.14 |
| | 22 | 9.38 | | 17.32 | −0.05 | | + 2.11 |
| | 25 | 9.32 | 14.49 | 17.36 | −0.03 | +0.57 | + 2.01 |
| | 27 | 9.26 | | 17.41 | −0.04 | | + 2.01 |
| | 30 | 9.19 | | 17.48 | −0.01 | | + 1.95 |
| | 32 | 9.17 | | 25.75 | −0.02 | | +10.24 |
| | 35 | 9.09 | | 25.74 | −0.04 | | −13.68 |
| | 37 | 9.05 | 17.02 | 25.69 | −0.05 | −4.25 | −13.55 |
| | 40 | 9.01 | | 25.58 | −0.06 | | −13.40 |
| | 45 | 8.92 | | 25.48 | +0.07 | | −13.57 |
| | 50 | 8.79 | | 25.46 | +0.08 | | −13.43 |
| 0.04 | 20 | 10.16 | | 36.56 | −0.17 | | − 9.86 |
| | 22 | 10.09 | 20.88 | 36.51 | −0.16 | −3.11 | − 9.87 |
| | 25 | 10.02 | | 36.41 | −0.10 | | − 9.93 |
| | 27 | 9.96 | | 36.34 | −0.11 | | − 9.87 |
| | 30 | 9.84 | | 36.74 | −0.19 | | − 6.13 |
| | 32 | 9.79 | | 39.66 | −0.17 | | − 6.16 |
| | 35 | 9.62 | | 39.82 | −0.19 | −5.12 | −16.09 |
| | 37 | 9.50 | 21.88 | 39.94 | −0.25 | | −15.79 |
| | 40 | 9.43 | | 39.76 | −0.20 | | −15.88 |
| | 45 | 9.34 | | 39.42 | −0.01 | | −16.16 |
| | 50 | 9.18 | | 39.32 | +0.06 | | −16.14 |

acidic solutions. This increase was also found for acidic solutions of collagen from rat tail tendon, shark skin, bovine skin and cat fish. At acidic pH, the increase of $\Delta G^*$ with the increase in concentration of collagen has also been observed in both the desamido and type-III (human placenta) collagens (our unpublished results) but the decrease of $\Delta H^*$ and $\Delta S^*$ with the increase in concentration for these collagens suggested the opposite behaviour compared to shark skin, bovine skin, rat tail tendon, cat fish and sepia cartilage collagens. However, there is a tendency of all the above thermodynamic parameters to decrease with the increase in concentration of rat tail tendon and sepia cartilage collagens in SDS environment compared to buffer environment and this is somewhat similar to the behaviour of desamido and type-III collagen in buffer only. The fact that the above thermodynamic parameters of these collagens decrease in SDS and Tween-80 environments clearly suggests a different kind of ordering and stable structure than that present in pure collagens (i.e. in buffer only). The difference in thermodynamic parameters between buffer and surfactant additive environments at various concentrations of collagen, as well as at various temperatures, is also shown in Tables 3–5. It can also be seen (Table 3) that in sepia cartilage collagen at concentrations of 0.02 g/dl and above, although the values of $\Delta G^*$ in SDS at temperatures below the denaturation state are always less than that in buffer, at temperatures above the denaturation temperature $\Delta G^*$ tends to increase. This clearly suggests that more work has been done by the system through the more stable collagen-surfactant complexes at higher temperatures.

The enthalpy of collagen transition strongly depends on the hydroxyproline content. The dependence between the enthalpy value, calculated per 1000 residues, and the hydroxyproline content of $\eta^{Hyp}_{1000}$ can be expressed to a first approximation by the following linear empirical equation [40]:

$$\Delta^{RC}_{CC}H_{1000}\,(25\,^{\circ}C) = (2400 + 35.8\,\eta^{Hyp}_{1000})\,\text{kJ mol}^{-1} \quad (8)$$

where $\Delta^{RC}_{CC}H$ is the change in enthalpy of collagen from coiled-coil to random-coil conformation. The first term of this equation corresponds to transition enthalpy of a collagen-like structure that does not contain hydroxyproline, for example (Pro-Pro-Gly)$_n$ polymer.

As for the entropy of collagen transition, it is evident that it should depend on at least three variables: the content of the amino acids, that of the hydroxyproline and that of the proline residues. Earlier it was assumed by Harrington [41] that the entropy contribution of residues containing pyrrolidine rings at collagen melting is zero, while the average contribution of all other residues is 17 J K$^{-1}$ mol$^{-1}$. However, it is now evident that the entropy contribution of hydroxyproline is far from zero. As for the proline residues, it is also very unlikely that they contribute nothing to the entropy of collagen chains in the random-coil state [42]. At the same time, the assumption that the entropy contribution of amino acids is 17 J K$^{-1}$ mol$^{-1}$ is also inconsistent with the entropy values obtained calorimetrically for collagens if the entropy dependence on the imino acid content is taken into consideration. The origin of the observed effect is not quite clear. It might be the result of some flexibility of imino acid residues in the polypeptide chain when it is in a random-coil conformation. But it might also be an effect of ordering of water around collagen, when it is in the triple-helical conformation if this conformation corresponds to the ice-lattice structure [43, 44]. Using these values for the proline and amino acid residues, it is possible

Table 6. *Chemical and thermodynamic characteristics of helical part of sepia cartilage collagens*
$\eta^{Pro}_{1000} = 102.5$, $\eta^{Hyp}_{1000} = 70$, $\eta^{Pro+Hyp}_{1000} = 172.5 \cdot$ pH of the solution $= 2.98$. The asterisks indicate the values of $\Delta^{RC}_{CC}H$ (res), $\Delta^{RC}_{CC}S$ (res) and $\Delta^{RC}_{CC}G$ (res) which have been calculated from the amount of proline and hydroxyproline residue. Assuming $\Delta^{RC}_{CC}G$ (res) = 0 at denaturation, the denaturation temperature has been calculated and is given in the parenthesis. The other values were obtained from the transition temperature by the plot of log $\eta$ vs $1/T$

| [Collagen] | Environment | Thermodynamic characteristics at 25 °C | | | | | |
|---|---|---|---|---|---|---|---|
| | | denaturation temperature | $\Delta H_b$ | $\Delta H_a$ | $\Delta^{RC}_{CC}H$ (res) | $\Delta^{RC}_{CC}S$ (res) | $\Delta^{RC}_{CC}G$ (res) |
| g/dl | | °C | kJ mol$^{-1}$ | | | J K$^{-1}$ mol$^{-1}$ | kJ mol$^{-1}$ |
| 0.005 | Buffer | 30 (32) | 13.92 | 21.27 | 7.35 (4.91)* | 24.07 (16.42)* | 0.177 (0.01)* |
| | 0.08 M SDS | 37 (33.05) | 13.68 | 20.15 | 6.47 | 21.14 | 0.170 |
| | 0.8 mM Tween-80 | 37 (33.30) | 14.73 | 19.15 | 4.42 | 14.43 | 0.119 |
| | 8 mM CTAB | 32 (28.5) | 14.49 | 17.02 | 2.53 | 8.39 | 0.030 |
| 0.01 | Buffer | 32 (34.98) | 15.32 | 21.88 | 6.56 | 21.30 | 0.213 |
| | 0.08 M SDS | 37 (35.64) | 13.21 | 21.19 | 7.98 | 25.92 | 0.276 |
| | 0.8 mM Tween-80 | 37 (33.63) | 14.18 | 20.19 | 6.01 | 19.64 | 0.157 |
| | 8 mM CTAB | 30 (29.9) | 17.03 | 19.15 | 2.12 | 6.99 | 0.036 |
| 0.02 | Buffer | 32 (35.14) | 18.24 | 22.52 | 4.28 | 13.89 | 0.141 |
| | 0.08 M SDS | 37 (36.09) | 12.74 | 21.92 | 9.18 | 29.79 | 0.303 |
| | 0.8 mM Tween-80 | 35 (33.64) | 13.90 | 21.29 | 7.39 | 24.16 | 0.190 |
| | 8 mM CTAB | 27 (27.18) | 18.76 | 20.42 | 1.66 | 5.33 | 0.012 |
| 0.03 | Buffer | 32 (35.03) | 21.27 | 25.53 | 4.26 | 13.83 | 0.139 |
| | 0.08 M SDS | 37 (35.75) | 12.10 | 22.69 | 10.59 | 34.32 | 0.363 |
| | 0.8 mM Tween-80 | 35 (33.67) | 13.60 | 21.88 | 8.28 | 27.00 | 0.234 |
| | 8 mM CTAB | 27 (27) | 20.10 | 21.12 | 1.02 | 3.40 | 0.007 |
| 0.04 | Buffer | 33 (36) | 23.99 | 27.00 | 3.01 | 9.68 | 0.115 |
| | 0.08 M SDS | 37 (34.2) | 11.56 | 23.97 | 12.41 | 40.39 | 0.374 |
| | 0.8 mM Tween-80 | 37 (30) | 13.21 | 22.59 | 9.38 | 31.12 | 0.106 |
| | 8 mM CTAB | 27 (27) | 20.88 | 21.88 | 1.00 | 9.35 | 0.007 |

to estimate the entropy contribution of hydroxyproline from the known entropies of collagen melting. This estimate gives the value of 103 J K$^{-1}$ mol$^{-1}$. Thus, the entropy of collagen transition at 25 °C can be written by the following empirical expression:

$$\Delta^{RC}_{CC}S_{1000}\ (25°C) = [11 \times (1000 - \eta^{Pro+Hrb}_{1000}) + 5 \times \eta^{Pro}_{1000} + 103\,\eta^{Hyp}_{1000}]\ \text{J K}^{-1}\ \text{mol}^{-1} \quad (9)$$

Regarding details for estimating $\Delta^{RC}_{CC}H$ and $\Delta^{RC}_{CC}S$ values, we refer to the recent review of Privalov [40]. The $\Delta^{RC}_{CC}H$ and $\Delta^{RC}_{CC}S$ values calculated from the amount of proline and hydroxyproline residues, as well as viscometric results, are given in Table 6.

*Unfolding of the triple helix*

The plot of log $\eta$ vs $1/T$ (Eyring's equation) is similar to the Van't Hoff plot of the equilibrium constant of micro-unfolding ($R$ ln $K$ vs $1/T$). As can be seen from the value of log $\eta$, the concentration of the unfolded form in the temperature range below denaturation is quite low, but it increases with increase in temperature. On reaching the denaturation temperature range, the functional dependence of log $\eta$ vs $1/T$ breaks abruptly. Hence one can conclude that there are two qualitatively different processes of collagen structure unfolding, the nondenaturational and the denaturational. Since the slope of a curve on Eyring's plot corresponds to the enthalpy of a process, it follows from the figures presented (Figs 10–13) that the nondenaturational and the denaturational unfoldings of collagen structure are characterized by qualitatively different enthalpies. The enthalpy of the nondenaturational process ($\Delta H_b$ in the present case) is small and is practically the same (about 25 kJ mol$^{-1}$) for all the collagens reported by Privalov [40]. But in the present case of sepia cartilage collagen studied, the $\Delta H_b$ values obtained ranged over 13.9–24 kJ mol$^{-1}$ depending on the concentration of collagen. In the case of cat fish collagen [36], the above $\Delta H_b$ values ranged over 19–30 kJ mol$^{-1}$ (in the concentration range 0.01–0.06%). Comparing this with the average calorimetric enthalpy of collagen melting (5.5 kJ mol$^{-1}$), one can conclude that the nondenaturational unfolding of the collagen structure involves about 4–6 residues, i.e. one or two triplets, while the denaturational process involves an amount of triplets larger by two orders, which unfold simultaneously.

The other fact that attracts attention is the significant difference between the values of log $\eta$ for various collagens at the same temperature. Keeping in mind that $RT$ ln $(\eta V/hN)$ is the Gibb's energy of the process, it follows that the change in Gibb's energies from coiled-coil to random-coil conformation, which can be denoted as $\Delta^{RC}_{CC}G$, are different for collagens from various species and vary between environments. The values of $\Delta^{RC}_{CC}G$ obtained are presented in the last column of Table 6. The denaturation enthalpy values determined by McClain and Wiley [25] were in good agreement with the value of $\Delta H = 4.8$ kJ mol$^{-1}$ reported by Flory and Spurr [45] for rat tail tendon collagen, and the value of $\Delta H = 4.6$ kJ mol$^{-1}$ determined by Kuntzel and Doehner [46] for bovine

hide powder. Harrington and Rao [47] derived values of $\Delta H = 4.66$ kJ mol$^{-1}$ for rat skin and 4.74 kJ mol$^{-1}$ for cod skin.

Data from our present investigation indicated that the denaturation enthalpy varies with concentration as well as the nature of additive environments. Utilizing direct calorimetric measurements, Privalov and Tiktopulo [48], however, reported an increase in both $\Delta H$ and $\Delta S$ with increasing imino acid content. Our results are similar to the findings of Privalov and Tiktopulo [48].

The energy per triple helix of sepia cartilage collagen in the activated state, calculated by Eqn (6), gives values of 9.5–10.4 kJ mol$^{-1}$ in our study while the data of Privalov and Tiktopulo [48] revealed values of 18.4, 14.8, 10.8 and 9.6 kJ mol$^{-1}$ from rat, pike, cod and whiting skin respectively. The one-hydrogen-bonded model [49] may appear to be possible only if forces other than hydrogen bonding contribute to enthalpy. Cooper [50] and Privalov [51] advocated a re-evaluation of the earlier concepts of macromolecular stabilization by structural organization of the solvent around the molecule, while Segal [52] has proposed residue-specific interaction, such as prolyl-prolyl attractions. The low values of enthalpy of collagen in SDS and Tween-80 environments suggest that the two-hydrogen-bonded model may be possible in these environments, whereas the one-hydrogen-bonded model is likely to be possible in buffer and CTAB environments. We do not know the reasons for this.

*Micelle formation and energy transfer*

To decide upon the micellar state of affairs from the view point of thermodynamics, two conjectures were invoked. In the first, micelles and the medium are considered to be a single phase as are regular solutions. In the second, micelles are treated as a different phase than the solvent containing the monomeric species. Utilizing the phase equilibrium model, the standard free energy change for formation of micelles in buffer may conveniently by written as

$$\Delta G_m^\circ = RT \ln (\text{cmc})_b \cdot \gamma_b \qquad (10)$$

where $(\text{cmc})_b$ = cmc in buffer and $\gamma_b$ = activity coefficient of the free species in buffer; the other symbols have their usual significance. Neglecting activity effects [17, 26, 28, 53, 54], the cmc values obtained in this study when compared with those in pure buffer can help in evaluating the standard free energy of transfer from buffer (aqueous) to the additive environments thus

$$(\Delta G^\circ)_{tr.} = RT \ln [(\text{cmc})_{\text{additive}}/(\text{cmc})_{\text{buffer}}]. \qquad (11)$$

Such free energies of transfer are given in Table 7. It can be seen from Table 7 that the transfer energies became negative and the negative values decreased with the increase in temperature. These results clearly suggest that the transfer of collagen from buffer to SDS and Tween-80 environments at temperatures up to the denaturation temperature of collagen are possible, whereas the reverse (i.e. the transfer of SDS and Tween-80 from buffer to the collagen environments) became difficult. The reverse situation is only possible at temperature above the denaturation state of collagen where positive values of $(\Delta G^*)_{tr.}$ have been observed. The reverse situation is also possible in CTAB environment irrespective of temperature.

Table 7. *The standard free energy of transfer of sepia cartilage collagen from buffer to surfactant additive environment at different temperatures from cmc measurements*

| Temp. | $(\Delta G^\circ)_{tr.}$ for | | |
|---|---|---|---|
| | 0.08 M SDS | 0.08 mM Tween-80 | 8 mM CTAB |
| °C | kJ mol$^{-1}$ | | |
| 20 | −0.544 | −1.245 | +1.083 |
| 25 | −0.452 | −1.407 | +1.266 |
| 30 | −0.388 | −0.848 | +1.358 |
| 35 | +0.395 | −0.572 | – |

## CONCLUSION

The enthalpy and the entropy of the collagen transition from coiled-coil to the random-coil state are specific characteristics of the given collagen molecule and vary greatly from species to species and from environment to environment. Both of them correlate with the stability of collagen and both increase with the increase of stability expressed either as temperature of transition or the Gibbs energy. This last fact is in drastic conflict with the relations expected from the existing models of collagen structure. According to our results (Table 6), the value of $\Delta_{CC}^{RC}G$ for sepia cartilage collagen is positive irrespective of the presence of surfactant additive environment. It is also seen from Table 6 that the value of $\Delta_{CC}^{RC}G$ for sepia cartilage collagen in SDS and Tween-80 environments are always greater than buffer environment, whereas in CTAB environment $\Delta_{CC}^{RC}G$ value decreased significantly compared to buffer environment. It may be concluded that the presence of SDS and Tween-80 stabilized the collagen structure, whereas CTAB disorganized the collagen structure. The stability of the collagen in these environments is in the following order: SDS > Tween-80 > buffer > CTAB.

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## REFERENCES

1. Miller, A. & Parry, D. A. D. (1973) *J. Mol. Biol. 75*, 441–446.
2. Rougvie, M. A. & Bear, R. S. (1953) *J. Am. Leather Chem. Assoc. 48*, 735–739.
3. Berendsen, H. J. C. (1962) *J. Chem. Phys. 36*, 3297–3313.
4. Fung, B. M. & Trautmann, P. (1971) *Biopolymers 10*, 391–398.
5. Dehl, R. E. & Hoeve, C. A. J. (1969) *J. Chem. Phys. 50*, 3245–3247.
6. Migchelsen, C. & Berendsen, H. J. C. (1973) *J. Chem. Phys. 59*, 296–299.
7. Hoeve, C. A. J. & Lue, P. C. (1974) *Biopolymers 13*, 1661–1669.
8. Hoeve, C. A. J. & Kakivaya, S. R. (1976) *J. Phys. Chem. 80*, 745–749.
9. Kuntz, I. D. & Kauzmann, WE. (1974) *Adv. Protein Chem. 28*, 239–345.
10. Chapman, G. E., Danyluk, S. S. & McLauchlan, K. A. (1971) *Proc. R. Soc. Lond. B 178*, 465–470.
11. Ramachandran, G. N. & Chandrasekharan, R. S. (1968) *Biopolymers 6*, 1649–1655.
12. Suzuki, E. & Fraser, R. D. B. (1974) in *Peptides, polypeptides and proteins* (Blout, E. R., Bovey, F. A., Goodman, M. & Lotan, N., eds) p. 449, Wiley, New York.
13. Andronikashvili, E. L., Mrevlishvili, G. M., Japaridze, G. Sh., Sokhadze, V. M. & Kvavadze, K. A. (1976) *Biopolymers 15*, 1991–2000.
14. Guidotti, G. (1972) *Annu. Rev. Biochem. 41*, 731–752.
15. Hayashi, K. & Ohba, Y. (1974) *Proc. Natl Acad. Sci. USA 71*, 2419–2429.

16. Shapiro, A. L., Vinuela, E. & Maizel, J. V. Jr (1967) *Biochem. Biophys. Res. Commun. 28*, 815–819.
17. Mandal, A. B., Ray, S. & Moulik, S. P. (1980) *Indian J. Chem. 19A*, 620–626.
18. Mandal, A. B. & Moulik, S. P. (1982) *ACS Proceedings in solution behaviour of surfactants – theoretical and applied aspects* (Mittal, K. L. & Fendler, E. J., eds) vol. 1, pp. 521–541, Plenum Press, New York.
19. Mandal, A. B., Gupta, S. & Moulik, S. P. (1985) *Indian J. Chem. 24A*, 670–674.
20. Mandal, A. B., Ramesh, D. V., Sehgal, P. K. & Dhar, S. C. (1986) *Leather Sci. 33*, 15–20.
21. Ray, A., Reynolds, J. A., Polet, H. & Steinhardt, J. (1966) *Biochemistry 5*, 2606–2616.
22. Harrington, W. F. & Sela, M. (1958) *Biochim. Biophys. Acta 27*, 24–31.
23. Flory, P. J. & Garrett, R. R. (1958) *J. Am. Chem. Soc. 80*, 4836–4840.
24. McClain, P. E. & Wiley, E. R. (1971) *Fed. Proc. 30*, 1249–1254.
25. McClain, P. E. & Wiley, E. R. (1972) *J. Biol. Chem. 247*, 692–697.
26. Mandal, A. B., Mukherjee, D. & Ramaswamy, D. (1981) *Leather Sci. 28*, 283–288.
27. Mandal, A. B., Ramaswamy, D., Das, D. K. & Santappa, M. (1982) *Colloid Polymer Sci. 260*, 702–707.
28. Mandal, A. B., Kanthimathi, M., Govindaraju, K. & Ramaswamy, D. (1983) *J. Soc. Leather Technol. Chemists 67*, 147–158.
29. Reference deleted.
30. Kimura, S., Takema, Y. & Kubota, M. (1981) *J. Biol. Chem. 256*, 13230–13237.
31. Mandal, A. B., Ray, S., Biswas, A. M. & Moulik, S. P. (1980) *J. Phys. Chem. 84*, 856–859.
32. Becher, P. (1967) in *Nonionic surfactants* (Schick, M. J., ed.) vol. 1, Ch. 15, pp. 478–515, Marcel Dekker, New York.
33. Mandal, A. B., Ramesh, D. V., Chandrasekaran, F. & Dhar, S. C. (1985) *Leather Sci. 32*, 127–133.
34a. Einstein, A. (1906) *Ann. Phys. 19*, 289.
34b. Einstein, A. (1911) *Ann. Phys. 34*, 591–596.
35. Simha, R. (1940) *J. Phys. Chem. 44*, 25–31.
36. Rose, C., Kumar, M. & Mandal, A. B. (1987) *Biochem. J.*, in the press.
37. Reference deleted.
38. Nikoli, D. F. & Benedek, G. B. (1976) *Biopolymers 15*, 2421–2430.
39. Eyring, H. (1980) in *Rheology of polymers* (Vinogradov, G. V. & Malkin, A. Ya., eds) Ch. 2, p. 106, Mir Publishers, Moscow.
40. Privalov, P. L. (1982) *Adv. Protein Chem. 35*, 55–104.
41. Harrington, W. F. (1964) *J. Mol. Biol. 9*, 613–617.
42. Brown, F. R., Hopfinger, A. J. & Blout, E. R. (1972) *J. Mol. Biol. 63*, 101–115.
43. Berendsen, H. J. C. & Mighelsen, C. (1965) *Ann. N. Y. Acad. Sci. 125*, 365–379.
44. Lim, V. I. (1981) *FEBS Lett. 132*, 1–5.
45. Flory, P. J. & Spurr, O. K. Jr (1961) *J. Am. Chem. Soc. 83*, 1308–1318.
46. Kuntzel, A. & Doehner, K. (1939) *Angew. Chem. Int. Ed. Engl. 52*, 175–190.
47. Harrington, W. F. & Rao, N. V. (1967) in *Conformation of biopolymers* (Ramachandran, G. N., ed.) vol. 2, p. 513, Academic Press, New York.
48. Privallov, P. L. & Tiktopulo, E. I. (1970) *Biopolymers 9*, 127–131.
49. Rich, A. & Crick, F. H. C. (1961) *J. Mol. Biol. 3*, 483–490.
50. Cooper, A. (1971) *J. Mol. Biol. 55*, 123–134.
51. Privalov, P. L. (1968) *Biophysics 13*, 1117–1119.
52. Sehgal, D. M. (1969) *J. Mol. Biol. 43*, 497–501.
53. Gratzer, W. B. & Beaven, G. H. (1969) *J. Phys. Chem. 73*, 2270–2275.
54. Mandal, A. B. (1985) *J. Surface Sci. Technol. 1*, 93–98.