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Laser Raman spectroscopic study of water in gelatin–surfactant solutions and gels

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Abstract A Raman spectroscopic study was carried out on water in gelatin at 4% w/v in gel (25 °C) and sol (40–60 °C) states at various concentrations (0.5, 1, 5, 10 and 15 mM) of anionic surfactant, sodium dodecyl sulfate (SDS). The in-phase collective stretching mode vibration of hydrogen-bonded -OH oscillators, centered around 3250 cm^{-1} in a tetrahedral network of water molecules, was observed to be significantly affected by temperature and the presence of SDS. According to our observation this may be due to the thinning of the hydration water around the gelatin molecules due to strong thermal agitation. The peak center of the collective bands of water decreased linearly with SDS concentration in the gel state which implied that with the increase in concentration of SDS, the -OH oscillators gradually lost their attachment to gelatin chains and were replaced by SDS molecules. Ultimately this resulted in a thinning of the hydration layer around the gelatin and the oscillation frequency of -OH oscillators moved towards 3250 cm^{-1} at 1 mM SDS concentration resulting in increased coupling of -OH oscillators to form the tetrahedral network at the critical micelle concentration (cmc) of SDS. The variation in the peak amplitudes and the systematic reversal of their trend about the cmc axis was surprising. At 40 °C the amplitude of the peak at 3250 cm^{-1} increased drastically due to a possible coil expansion by about 7–8% which accommodated more interstitial water into the pseudonetwork leading to an increase in the number of nearest neighbors and for about 6% increase in the *C* value. However, at the cmc the peak amplitude was observed to be independent of temperature. Continuous shifting of the peak center and full width at half-maxima towards lower values was observed with increasing SDS concentrations in the gel state.

Key words Raman spectroscopy – -OH stretching – Gelatin – Sodium dodecyl sulfate – Gelation

Introduction

Water is the most significant constituent of living cells and its unique physical and chemical properties have been studied most extensively over the last several decades. The unique feature of the solvation of water has been recognized and it is one of the most studied liquids. The hydrogen bonds (H bonds) which play the most crucial role in many interactions and conformational changes are the focus of attention of many scientists. Water, which is interconnected by weak H bonds forms a tetrahedral network. In the liquid state, two models of water structures have been commonly accepted:

1. Water molecules are connected to neighboring molecules and form a long non-terminating linear chain.
2. Water molecules through intermolecular H bonding form tetrahedral networks [1].

Both models attributed the water structure to the bending and stretching of intermolecular H bonds. The profile of -OH stretching mapped by Raman spectroscopy is known to be useful in providing in-depth understanding of the microscopic thermodynamical environment of macromolecular sols, gels and networks. The properties of water–soluble polymers are largely dependent on the water–polymer interactions. Similarly, the viscoelastic characteristics of polymeric networks and gels depend on the physicochemical properties of the solvent medium. The polymer–solvent interaction decides the structure of gels and networks in a given thermodynamic environment. This interaction provides a distinction between the bulk, interstitial and hydration water. The water manifests itself in deciding the conformations and macroscopic properties of polymers in solutions. The properties of polymers in aqueous solution show different trends due to the interaction with water.

Recently, much attention has been paid to probing the effect of water on polymers such as polyacrylamide, polyethylene glycol, polyacrylic acid, various native proteins and H_2O–D_2O, H_2O_2–H_2O systems by IR [2–3], NMR [4] and other techniques. However, as molecular vibrations have shorter relaxation times than the rotational arrangements of water molecules, Raman spectroscopy is the most versatile method to study the structure of water in the liquid phase.

The -OH stretching of water has been studied by Giguere and Pigeon-Gosselin [5] and most recently by Hare and Sorenson [6, 7] but the stretching of -OH oscillators in H_2O–D_2O, H_2O_2–H_2O systems was studied a long time before by Green et al. [8–10]. Although many reports are available on the structure-breaking of water by introducing electrolytes such as KCl, NaCl, etc., the polymer–water system with surfactant as a third component has been sparsely studied notwithstanding the fact that surfactants have the ability to deform the tetrahedral network of the water structure. Reports on chemically cross-linked polymer gels with water as a solvent and on gelatin [11] are readily available, but to our knowledge physical gels with surfactants that form fragile cross-links still remain largley unexplored. Here we have studied the effect of sodium dodecyl sulfate (SDS) on the gelation dynamics of gelatin, which is a biopolymer, using water as a solvent at different temperatures (gel, sol-I and sol-II) tuning the surfactant concentration as an additional parameter.

Gelatin is a polypeptide obtained from denatured collagen comprising all 20 amino acid residues such as glysine, proline, glutamic acid, aspartic acid, lysine and arginine, etc., in different proportions [11]. The lysine and arginine groups comprise about 7.5% of the residues and are positively charged, and these form complexes with the polar head group of anionic surfactants such as SDS. Glutamic and aspartic acid constituting about 12.5% of the residues give the negative nature to the chain. Another 6% of the residues are strongly hydrophobic in nature leaving 58% of the chain to be neutral. In the gel state the individual chains are interlocked with each other by H bonds forming triple helices at junction points, and in the sol state these helices break up to assume random coil conformations. The gelatin concentration for the gelatin used, is typically around 2% w/v [12, 13]. The polar sites of the gelatin chains attract the oppositely polarized site of water molecules and these sites on the chain are screened off from interactions with other -OH oscillators. These water molecules which are attached to the polymer chains are hydration water or associated water and induce a defect in the tetrahedral network of water molecules. The strength of the collective motion is destroyed due to these defects and decreases as $(1\text{-}\rho_G)^2$, where ρ_G is the mole fraction of positive and negatively charged gelatin residues.

Gelatin in the gel state forms a triple-helix network that can accommodate a huge amount of water. The water molecules in the water pools inside the gel feel reduced effects from surrounding gelatin chains because most of the polar sites are screened off, but their motions are restricted due to the presence of the polymeric network which ultimately produces a deformation in the continuous network structure of the water molecules. This water is termed interstitial water or bound water and that which is totally out of the influence of gelatin chains is called bulk water. Any sort of attachment changes the environment of the -OH oscillators which correspondingly changes the vibrational frequency, peak center, peak amplitude and the collective feature value of the -OH oscillators making it possible for it to be observed by Raman spectroscopy as a shifted Raman band.

Experimental

Materials

Gelatin was purchased from M/S Loba-Chemie (Indo-Astranal, India) with a sharp molecular weight (M_w) distribution ($\simeq 10^6\,g\,mol^{-1}$). An aqueous solution was prepared by soaking the sample for nearly 45 min at about 55 °C to remove the history effects. The solvent was double-distilled Milli-Q (Millipore, USA) grade water. To prevent bacterial contamination 0.1 mM sodium azide was added. The details of the sample preparation have been discussed by Maity and Bohidar [14]. The SDS used from SISCO Research Laboratories Pvt. Ltd. (Bombay, India) was from batch no. TP/419331. The samples were excited with an argon ion (1 W) laser light source of wavelength 488 nm. The incident light was linearly polarized (using a polarizer) and focussed to a spot size of

about 10 μm on the sample. The laser power at the sample site was about 25 mW so that the sample in the experimental time window did not get bleached. The excited light from the sample was collected by an elliptic mirror and was then passed through an analyzer whose optic axis was set parallel or perpendicular to the optic axis of the polarizer depending on the requirement of the scattering geometry. The light was then passed through a SPEX-1877E triplemate spectrophotometer. A charge-coupled device (CCD) camera located at the exit slit of the spectrophotometer measured the intensity of the emitted light at different wavenumbers. The CCD camera was connected to a PC for display and data were stored using previously loaded DM3000R software. The parallel and perpendicular intensity components of the luminescence for each sample were scanned separately with a time duration of 1 min. The gelation temperature of 4% w/v gelatin solution has been reported to be $T_{gel} \sim 30$ °C [12].

Methods

We studied the parallel ($I_{\|}$) and perpendicular ($I_{\perp}$) components of the intensity of the scattered light by rotating the analyzer in front of the CCD camera. The collective intensity I_c was extracted from

$$I_c(\omega) = I_{\|}(\omega) - I_{\perp}(\omega)/\rho \ , \quad (1)$$

where ρ is degree of depolarization. Each measurement of $I_{\|}$ and $I_{\perp}$ was taken at least 3 times and all these spectra were seen to be reproducible. The stored spectra were decomposed into individual peaks by using a fitting algorithm which applies a linear combination of Gaussian and Lorentzian functions to the composite spectra. The detailed procedure is described elsewhere [9]. We normalized the collective band and retrieved the C value which reflects the collective nature of the -OH oscillators as follows

$$C = \int I_c(\omega)\mathrm{d}\omega \Big/ \int I_{\|}(\omega)\mathrm{d}\omega \ , \quad (2)$$

where ω is the wave number.

Results and discussion

The assignment of Raman bands in different frequency regions is well defined. The high frequency band around 3450 cm^{-1}, ω_1, is due to the symmetric stretching of -OH oscillators and the low frequency band is the first overtone of the bending mode ($2\omega_2$), while the weak shoulder around 3585 cm^{-1} is the asymmetric stretching of -OH oscillators. In a water molecule the oxygen atom has two pairs of electrons which make the oxygen atom more electronegative while the two hydrogen atoms are electropositive; so these free electron pairs can couple weakly with an electropositive site. This may be intramolecular, i.e., water–water or intermolecular, i.e., water with an electropositive site of a foreign atom. The degree of coupling depends strongly on the distance between the two atoms and also on the electropositivity of the other atom. As the temperature increases, the translational and rotational motion of the atom increases its amplitude which ultimately decreases the strength of the H bonds and the ordering is decreased.

Gelatin, which has positive and negative sites in its backbone, can strongly couple with the -OH oscillators of water and make it more ordered or structured by reducing the degree of freedom of the -OH oscillators; thus, gelatin can inhibit the ordering of -OH oscillators in solution. Again by introducing an anionic surfactant such as SDS which has a negative polar head one can screen off the polar sites of gelatin and observe the impact on the ordering of -OH oscillators below, at and above the cmc of SDS. In next three sections, these effects are discussed for three different physical situations relevant to our studies: (1) pure gelatin, (2) gelatin with SDS below the cmc and (3) gelatin with SDS above the cmc.

Pure gelatin

Typical spectra taken below and above T_{gel} are shown in Fig. 1. Both spectra were normalized to the same baseline. It is seen from the figure that for $T > T_{gel}$ (i.e., 40 °C) and for $T < T_{gel}$ (i.e., 25 °C) the spectra have two distinct peaks centered around 3250 cm^{-1} and 3450 cm^{-1} with a shoulder centered around 3650^{-1}. These values agree well with previously reported features [15]. The shoulder at 3585.5 cm^{-1} in the gel state was observed to be shifted towards the lower value of 3563 cm^{-1} in the sol-I state. The lower frequency peak (i.e., around 3250 cm^{-1}) which is due to the in-phase collective motion of -OH oscillators was observed to be highly polarized compared to the peak centered around 3450 cm^{-1}. The peak amplitude of the $I_{\perp}(\omega)$ spectra at 25 °C was 13% stronger than that at 40 °C, implying that on increasing the temperature (gel melting) the attachment of -OH oscillators with gelatin chains was

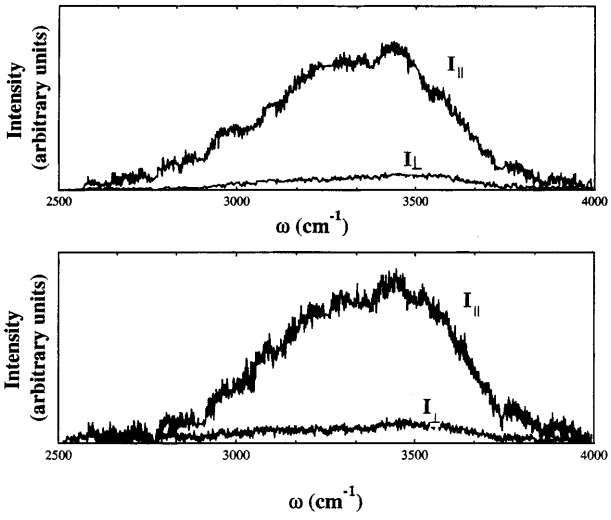

Fig. 1 (*Top*) $I_{\|}(\omega)$ and $I_{\perp}(\omega)$ Raman intensity profiles of -OH stretching in the gelatin gel state (25 °C). (*Bottom*) $I_{\|}(\omega)$ and $I_{\perp}(\omega)$ in the gelatin sol state (40 °C). Notice that the $I_{\perp}(\omega)$ component at all temperatures is highly polarized

destroyed due to temperature-mediated weakening of H bonds which resulted in a decrease in the peak amplitude.

In Fig. 2 we have shown all $I_{\|}(\omega)$ spectra taken at three different temperatures in pure gelatin. The peak amplitude of the symmetric stretching bands at 3450 cm^{-1} first decreases from the gel to the sol-I state and then increases in the sol-II state. It is interesting to observe that the band at 3250 cm^{-1} decreases from the gel to the sol-I state at 40 °C but increases again to a higher value in the sol-II state, i.e., 60 °C. The results displayed in Fig. 1 are confirmed in Fig. 3 where we plotted $I_c(\omega)$ versus ω. If we compare the C values given in Table 1, it is observed that from the gel state at 25 °C to the sol state at 40 °C they decrease by 14% and from 40 °C to 60 °C they increase by 13% which again substantiates the features shown in Figs. 2 and 3.

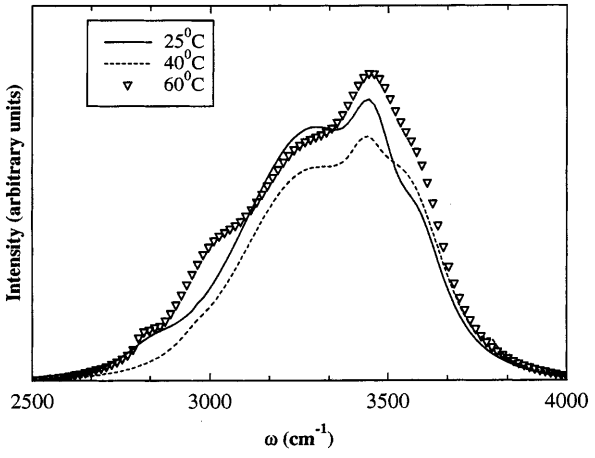

Fig. 2 $I_{\|}(\omega)$ Raman spectra of gelatin in gel (25 °C) and sol states (40 and 60 °C). Here the peak amplitude at 60 °C is almost equal to that at 25 °C

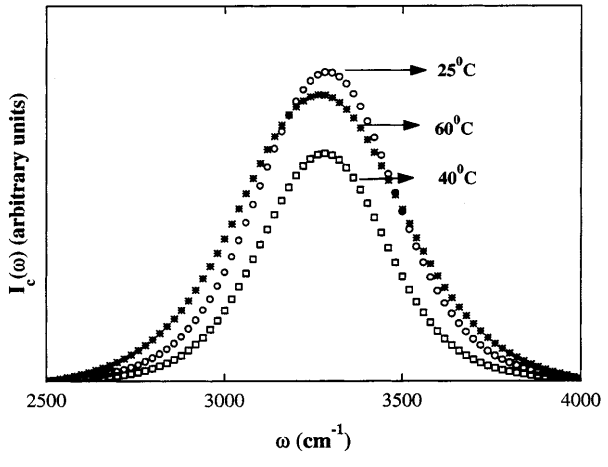

Fig. 3 $I_C(\omega)$ of gelatin in gel (25 °C) and sol states (40 and 60 °C) plotted against frequency shift

It could be interpreted that when the gel melts to the sol state, the triple-helix structures of gelatin are broken exposing more charged sites for -OH oscillators. These induce deformations in the tetrahedral network structure of water through attractions and the interstitial water inside the gelatin network in the gel state decreases in volume due to the fact that the gel network turns out to be a pseudonetwork comprised of transient entanglements of chains which eventually decrease the C value. At 60 °C the scenario changes and due to the thermal vibration of the gelatin chains the probability of constituting the pseudonetwork decreases. Ultimately interstitial water which was trapped in the pseudonetwork is released adding to the bulk water and consequently some -OH oscillators are released from the pseudonetwork, thus contributing to the increase in the C value by about 13%.

The temperature dependence of the peak center of the collective bands has been plotted as a function of SDS concentration in Fig. 4. Two important observations can be made from here. In the gel state ($T < T_{gel}$; 25 °C), the peak center for pure gelatin observed at around 3286 cm^{-1} gradually reduced as the SDS concentration was raised systematically to a concentration of 15 mM. Similarly the transformation of gel to sol-I

Table 1 C values of the system at different sodium dodecyl sulphate (*SDS*) concentrations (maximum error ±2%)

Conc. of SDS (mM)	Gel state	Sol state	
	25 °C	40 °C	60 °C
0	0.323	0.276	0.310
0.5	0.296	0.281	0.313
1	0.312	0.310	0.313
3	0.303	0.351	0.301
10	0.292	0.359	0.295
15	0.281	0.372	0.292

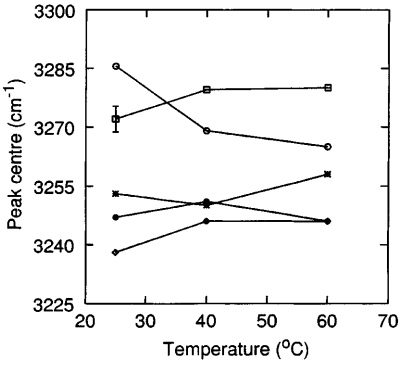

Fig. 4 Peak center versus temperature plotted for gelatin at different molarities of sodium dodecyl sulfate (*SDS*): (○) 0 mM, (■ 0.5 mM, (*) 1 mM, (●) 5 mM and (◇) 15 mM. For the sake of clarity the error bar is shown for only one datum point

and sol-II states was observed to produce a decrease in the peak center values. This shows that on increasing the temperature the stretching frequency of -OH oscillators lies outside the span of the vibron density of states and decoupling of -OH oscillators takes place which shifts the peak center towards lower values.

In Figs. 5 and 6 we have plotted the peak amplitude and the full width at half-maxima (FWHM) versus temperature as functions of surfactant concentrations. The peak amplitude and FWHM both decreased from the gel to the sol state. The peak amplitude which is proportional to the nearest neighbor density is expected to decrease from the gel to the sol state as the gelatin chains induce more decoupling of -OH oscillators from the tetrahedral network, but at 60 °C due to thermal agitations some of the -OH oscillators are released from the charged and interstitial sites which again reconstruct the networks to some extent. Note that in Fig. 6 the bandwidth of the collective band decreases with temperature, which is a measure of the disorder in the network.

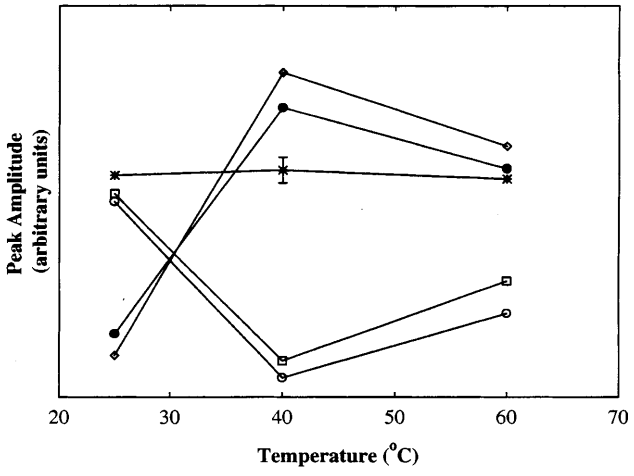

Fig. 5 Peak amplitude of the in-phase collective band at 3250 cm^{-1} of gelatin with (○) 0 mM, (□) 0.5 mM, (*) 1 mM, (●) 10 mM and (◇) 15 mM SDS plotted versus temperature. Notice the temperature independence of the peak amplitude at an SDS concentration equal to its critical micelle concentration value

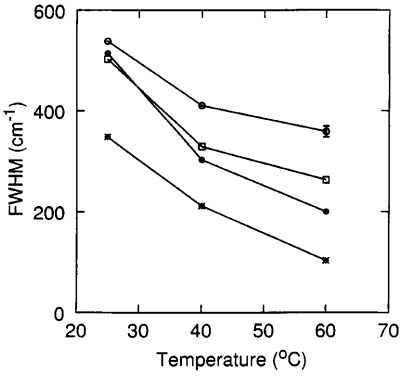

Fig. 6 Full width at half-maxima for the in-phase collective band at 3250 cm^{-1} of -OH oscillators in gelatin plotted versus temperature for different concentrations of SDS. (○) 0 mM, (□) 0.5 mM, (*) 1 mM and (●) 5 mM

Gelatin with SDS below and at the cmc of SDS

The cmc of SDS is known to be about 1 mM [16]. Let us look at the system below the cmc (0.5 mM) and at the cmc. In these systems, SDS displaces the -OH oscillators from the nearby regions to the interior regions of gelatin chains. So by increasing the SDS concentration the peak amplitude of the collective band should increase as clearly seen in Fig. 5 for the gel states. When we heated the system from the gel to the sol-I state the amount of interstitial water and the peak amplitude decreased. From the sol-I to the sol-II state it increased again due to thermal agitation confirming that the network structure produces the phenomenon as was observed in the case of pure gelatin. If we compare this system with the case of pure gelatin, it is observed that due to the SDS molecules more -OH oscillators are freed from the hydration layer which increases the peak amplitude; however, we notice anomalous behavior at the cmc where spherical micelles of SDS molecules start forming. At this concentration the amplitude remains constant within experimental error at all temperatures. This behavior has not been reported before. Hence, providing a comparison at this stage is impossible yet a plausible explanation can be attributed to this. At the cmc, in the gel state, the charged sites on the gelatin backbone are completely occupied by SDS molecules and the extra SDS molecules that are in solution try to form spherical micelles around the gelatin chains or in the intermediate space. When we allow the system to transform from gel to sol states though the extra charged sites are exposed to the -OH oscillators by denaturation of gelatin they are nullified by the SDS molecules which were engaged in micelle formation earlier in the gel state. The peak amplitude should, therefore, not increase much at the cmc. The peak center (Fig. 4) changes only by 7–8 cm^{-1}, which is within experimental error. When we look at Fig. 6, the bandwidth of the collective band decreases when the SDS concentration is increased and it reaches its lowest value at the cmc at 60 °C. The decrease in bandwidth means more ordering and coupling of -OH oscillators in the network structure at a particular temperature. So if we take a closer look it may be inferred that the polymer coupled with surfactant can interact strongly on the network structure of water while increasing the temperature. If we look at the C value (Table 1) then it is seen to increase by at least 3–4% compared that for pure gelatin. Tsukida et al. [11]

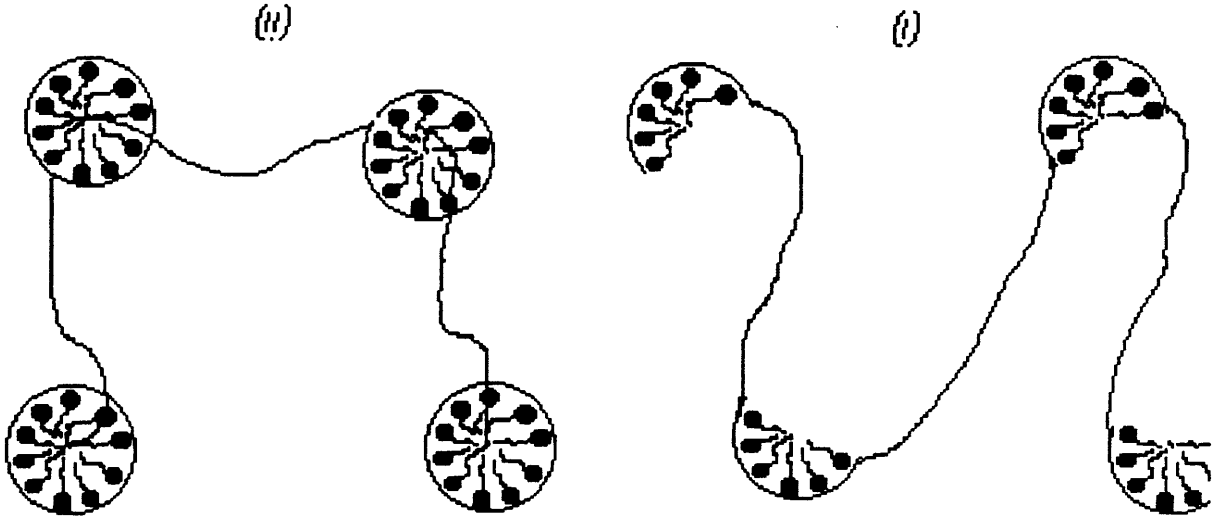

Fig. 7 Two possible "necklace-bead" structures. (*i*) Gelatin wraps around surfactant molecules and (*ii*) surfactants aggregate around the gelatin chain and form micelles (see Refs. [16, 17])

described the same type of behavior with only gelatin and got the same kind of result that the network construction was made by increasing the temperature of the system; however, their interpretation of the formation of the clathrate cage around the gelatin chain could not be established though the first interpretation could be made applicable to the sol state.

Above the cmc of SDS

From Fig. 5, it is observed that for SDS concentrations above 1 mM, the peak amplitude increases from the gel to the sol state while it decreases for concentrations below the cmc. In the gel state at the cmc, the gelatin chains are saturated with SDS molecules, and almost all the positive polar sites of the gelatin chains are neutralized by the opposite polar sites of the SDS molecules and the neutral sites which comprise about 58% of the total chain length act as the hydrophobic core of the micelle. No extra polar sites are exposed upon heating because the system is full of SDS molecules. The extra positive sites due to heating the gelatin chains are also neutralized by SDS molecules present in the system: these are not sufficient in the cases of systems at and below the cmc. This ultimately increases the volume of interstitial water in the network pool. At the neutral position micelles start growing according to the necklace-bead model [16, 17] (Fig. 7) and the polar sites of these micelles are exposed to the interior of the network pool and repel each other increasing the network size (~75 Å) by about 5–7%. The boundary of interstitial water is also displaced further into the pool and the thickness and number of hydrating water molecules that are in contact with gelatin chains decreases thus increasing the coordination number of the coupled -OH oscillators. For this reason the peak amplitude increases. When the temperature increases further due to molecular agitation some decoupling occurs and the peak amplitude decreases by about 16% from the value at 40 °C.

In the gel state just beyond the cmc a very complex phenomenon was observed. The peak amplitudes decrease abruptly by about 20% at approximately 10 mM and then they suddenly increase. In the gel state gelatin chains are interlocked with each other and make triple helices. Beyond the cmc two mechanisms may occur: firstly, due to the micellar interaction the network size may increase as in our case (~5–7%) and, secondly, the association of polar head groups of the micelles with the -OH oscillators may occur. Here according to our proposition the second cause dominates the scenario because in the gel state micelle–micelle interaction is not sufficient to increase the coordination number of the water molecules by having more interstitial water in its increased area. As we keep on increasing the concentration of SDS, the population of SDS molecules increases and the polar head groups of these are attached with more and more -OH oscillators which ultimately decouple some of the oscillators from the network.

Conclusion

Tsukida et al. [11] concluded in their report on pure gelatin that at 60 °C the water molecules are more structured due to the cancellation of the influence of disordered bulk water by relatively ordered hydration water. However, according to our observation it may be due to the thinning of the hydration water around the gelatin molecules due to strong thermal agitation. According to Fig. 4, the peak center of the collective

bands of water decreases linearly with SDS concentration in the gel state which suggests that as we increase the concentration of SDS, the -OH oscillators gradually lose their attachment to the gelatin chains and are replaced by SDS molecules. This ultimately thins the hydration layer around the gelatin and the oscillation frequency of the -OH oscillators moves towards 3250 cm^{-1} at 1 mM SDS concentration resulting in increased coupling of -OH oscillators to form the tetrahedral network at the cmc of SDS (Fig. 4).

A very interesting anomaly is observed in Fig. 5. The variation in the peak amplitudes and the systematic reversal of their trend about the cmc axis is surprising: there must be an anomaly in the region above the cmc of SDS. At 40 °C the amplitude of the peak at 3250 cm^{-1} increased drastically due to a possible coil expansion of about 7–8% which accommodated more interstitial water into the pseudonetwork leading to an increase in the number of nearest neighbors and an about 6% increase in the *C* value. A more exhaustive detailed study of this system is called for.

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