Chapter Seven

Effect of temperature on Surfactant induced softening in gelatin hydrogels

(Communicated)

7.1 Abstract

Dynamic light scattering (DLS) and oscillatory rheology experiments were performed to study temperature dependence (T=10-25°C) of the interactions in hydrogels of gelatin with AOS (alpha olefin sulfonate, anionic surfactant) for surfactant concentrations in the range 25-100mM, chosen larger than cmc (~8mM). The network mesh size ($\xi$) values deduced from fast mode diffusivity ($D_f$) data obtained from dynamic structure factor, $S(q,t) \sim \exp(-D_f q^2 t)$ (for $t \leq 1$ms and $q$ being the scattering wave vector), of micelle bound gelatin gels was analyzed within the framework of Flory-Rhener theory of crosslinking, which revealed a temperature dependence, $\xi \sim (0.5 - \chi)^{1/2} \exp(-\Delta G_{\text{rot}} / RT)$ where $\chi$ is the Flory-Huggins interaction parameter, the free energy of the gel-surfactant complex is $\Delta G_{\text{rot}}$ and $R$ is universal gas constant. The low-frequency isochronal storage, $G'$ and loss, $G''$ modulii revealed an universal transition from the rigid to a softened gel state occurring at surfactant concentration $\approx 55$mM independent of the temperature. The free energy of interaction, $\Delta G_{\text{int}} \approx (\Delta G_{\text{Total}} - \Delta G_{\text{gel}} - \Delta G_{\text{micelle}})$, $\Delta G_{\text{gel}}$ is free energy for pure gel and $\Delta G_{\text{micelle}}$ is same for micelles, between gel and surfactant deduced from Arrhenius plots obtained from temperature dependent rheology and light scattering data support this observation.
7.2 Introduction

Gelatin, the denatured product of collagen, a polypeptide forms gels in hydrogen bond friendly solvents above a threshold concentration. In physical gels, like that of gelatin, a three-dimensional interconnected network of polymer chains is formed in the dispersion medium, which is held together by intermolecular hydrogen bonding [1]. The physical gels achieve solution stability through an array of possible secondary forces, like hydrogen bonds, van der Waal forces, dipolar interactions and hydrophobic interactions etc. The fluidity and elasticity of such gels become a matter of time scales of the observations relative to the lability of these interactions, which are amenable to modifications through selective binding to micelles among other possibilities [2]. Since gelatin is a polyampholyte, it has the affinity to bind to anionic and cationic surfactants modifying its gel state properties [3-5]. On the other hand, formation of micelles is a strictly free energy driven process. These are formed of surfactant molecules through self-organization and these interact with polyelectrolytes in a characteristic manner, which are discussed at length in the past though largely for sols [6-9]. The complexation of micelles with a polyampholyte molecule like gelatin, which is a biopolymer with a sizable hydrophobic domain, necessitates a closer look because of the extensive use of gelatin in pharmaceutical, cosmetic, photographic and food industries.

In a recent work, we investigated the binding of three surfactants, alpha olefin sulfonate (AOS, anionic), Triton-X100 (TX-100, non-ionic) and cetyl trimethyl ammonium bromide (CTAB, cationic) to the hydrogels of gelatin at room temperature [10]. It was shown that binding of micelles significantly changed the structural properties of gelatin hydrogels in a gradual manner. A physically distinguishable surfactant concentration $\approx 55\text{mM}$ defined this cross-over point. Below this surfactant
concentration the hydrogen bonded triple-helix physical network is in abundance, but as AOS concentration increased the numbers of such physical crosslinks reduce and are replaced by micellar bridges. The formation of micellar bridges ensues even at 25mM of surfactant concentration but there is still a propensity of triple helices inside the gel structure to give it considerable rigidity. Beyond the cross-over concentration the density of micellar crosslinks increase exponentially and a softening –like behaviour of the gel phase is encountered. Since the rigidity of the gel owes its origin to the existence of intermolecular hydrogen bonds between peptide linkages of adjacent helix units, the loss of rigidity could be attributed to the loss of these linkages, which in turn manifests as the gain in micelle-bridged linkages. These are immediately replaced by micelles-bridged crosslinks though not in one to one ratio. In fact, though micelle induced softening starts at surfactant concentrations as low as 25mM the intensity of this is really felt only when a significant number of the original crosslinks are destroyed and replaced by transient ones, which occurs at surfactant concentration $\approx55$ mM.

In this work, we explore the temperature dependence of the softening phenomena described above pertaining to anionic surfactant, AOS. The results are discussed within the framework of Flory-Rehner theory of crosslinking of equilibrium networks. We are able to evaluate the free energy of interaction between the gel and surfactant from our data, which is in qualitative agreement with the argument of surfactant induced gel softening we are proposing.

Rheology measuring instrument was a strain controlled AR 500 Rheometer (TA instruments, UK). Measurements were made in the dynamic oscillation mode with appropriate inertial corrections at temperature $T = 25, 20, 15$ and $10^\circ C$. 

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7.3 Results and Discussions

(i) DLS studies

The DLS experiments were mostly carried out at a fixed scattering angle of 90° though for elucidation of q-independence of diffusivity (fast mode) some representative experiments were performed with varying scattering angles. The issue of non-ergodic scattering from gels does cause concern at smaller scattering volumes. In our experiments, we did not focus the laser beam onto the sample deliberately to maintain a larger scattering volume, thus ensuring ergodicity in all DLS measurements. The normalized intensity correlation function, g2(q, t), obtained from the gel state scattering can be related to the structure factor, S(q, t) which is described in details elsewhere [11, 12]. The fast mode diffusion coefficient (Df) was deduced from the fitting of measured S(q, t) data to S(q, t) ~ exp.(-Dfqt) for t ≤1ms, where a single exponential fitting was found to be adequate. Same behavior for this mode was noted at all temperatures. Figure (7.1) illustrates Df as a function of surfactant concentration at 25°C and Figure 7.2 shows the same as function of temperature for surfactant bound gel. A comparison of the temperature dependence of Df (between surfactant bound gel in Figure 7.2 and pure gel in Figure 7.3) shows decrease in the diffusivity value with increase in temperature. The fast mode measures the relaxation of local concentration fluctuations, which can be attributed to the cooperative movement of entangled networks of polymer chains. The mesh size ξ values of the network can be deduced from Stokes-Einstein relation \( D_f = k_B T / (6\pi \eta_0 \xi) \), where \( k_B \) is Boltzmann’s constant and \( \eta_0 \) is solvent viscosity at T. One gets a value for \( \xi \) of the order of ~200 nm at T=25°C and ~80 nm at T=10°C and for pure gel its varied from 244 nm to 220 nm at same temperature thus observed temperature dependence

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was significant. The value of $\xi \approx 200$ nm at $T=25^\circ C$ is comparable to the size of the triple-helices present in gelatin gels [13]. Figure (7.4) shows a plot of $\log \xi$ versus $1/T$ for gel samples containing various amount of surfactant, which implies shrinking of the network at lower temperatures. An increase in the number of crosslink density with reduction in temperature has also been reported earlier [14, 15]. No significant concentration dependence of the mesh size was observed in our measurements. However, this contention does not preclude the presence of other networks comprising smaller correlation lengths.

Figure 7.1: Plots of low frequency isochronal (3.5 rad/s) storage and loss modulii ($G'$ and $G''$), dynamic viscosity $|\eta^*|$ and gel mode diffusivity $D_r$ as function of concentration of anionic surfactant AOS measured at $25^\circ C$. The gel concentration was fixed at 5% (w/v) in water. Notice the change in slope close to AOS concentration $\approx 55$ mM, which signals the micelle induced melting of the gel network and its transformation to a soft gel. The smooth lines are guide to eye only.
Figure 7.2: Plots of low frequency isochronal (3.5 rad/s) storage and loss modulii (G' and G'"), dynamic viscosity |\eta^*| and gel mode diffusivity D_f as function of temperature for pure gel with [AOS] at 25Mm. The gel concentration was fixed at 5% (w/v) in water. The smooth lines are guide to eye only.

Figure 7.3 Plots of low frequency isochronal (3.5 rad/s) storage and loss modulii (G' and G'"), dynamic viscosity |\eta^*| and gel mode diffusivity D_f as function of temperature for pure gel. The gel concentration was fixed at 5% (w/v) in water. The smooth lines are guide to eye only.
Figure 7.4: Arrhenius fitting (solid lines) of $\ln \xi$ versus $(1/T)$ for a gel with various AOS concentrations. The symbols refer to: 25mM (●); 50mM (○); 75mM (▼) and 100mM (△). Average mesh size $\xi$ was determined from DLS experiments. Representative experimental error present in data is shown for a data point.

Flory-Rehner equation estimates the crosslinking density associated with a semi-dilute gel at a given temperature. This defines the ratio of the volume of the unswollen network to that of the swollen network at equilibrium ($v_e = 1/q_m$) as a characteristic cross-linking density parameter. The equation of Flory-Rehner for a good solvent is [16].

$$q_m^{5/3} = (\bar{v}_c) (1-2M_c/M) (0.5-\chi)/V_1$$  \hspace{1cm} (7.1)

where $\bar{v}$ partial specific volume of the polymer, $M$ polymer molecular weight, $\chi$ is the Flory-Huggins interaction parameter defining the solvent quality, $V_1$ molar volume of solvent. Flory defined $(1-2M_c/M)$ as the network imperfection factor with upper cut-
off value of 1 for \( M \to \infty \) in Eq. (1) and, \( M_e \) is the mass of the polymer between two entanglement points. Approximately, all the parameters except \( \chi \) are independent (or weakly dependent) on temperature in Eq. (7.1). This allows us to write

\[
q_m \sim (0.5 - X)^{3/5}
\]  

(7.2)

If \( \xi_0 \) and \( \xi \) represent the mesh size of unswollen and swollen networks respectively,

\[
q_m \sim (\xi /\xi_0)^3
\]  

(7.3)

Hence, combining Eq. (7.2) and Eq. (7.3), one would get

\[
\xi \sim \xi_0 (0.5 - X)^{1/5}
\]  

(7.4)

or correspondingly, in terms of diffusion coefficients

\[
D_r \sim D_0 (0.5 - X)^{1/5}
\]  

(7.5)

Assuming an Arrhenius temperature dependence for \( D_0, \ D_0 \sim \exp(\Delta G_{\text{Total,D}} /RT) \), \( \Delta G_{\text{Total,D}} \) being the appropriate total activation energy for the gel-surfactant network probed by DLS. Thus Eq. (7.5) reduces to

\[
D_r \sim (0.5 - X)^{1/5} \exp. (\Delta G_{\text{Total,D}} /RT)
\]  

(7.6)

Correspondingly, the mesh size would show a temperature dependence given by

\[
\xi \sim (0.5 - X)^{1/5} \exp. (-\Delta G_{\text{Total,D}} /RT)
\]  

(7.7)

Consequently, a plot of \( \ln \xi \) versus \( 1/T \) would yield a straight line as shown in Figure 7.4. The implication of this will be discussed later.
In the long time scale regime, the dynamic structure factor $S(q,t)$ exhibited a stretched exponential behavior (called the slow mode relaxation) for $1 \text{ms} \leq t \leq 1 \text{s}$, which can be represented by

$$S(q,t) \sim \exp \left(-\frac{t}{\tau_s}\right)^\beta$$  \hspace{1cm} (7.8)

With a characteristic time scale $\tau_c$ and width parameter, $\beta$. The slow mode time constant $\tau_s$ given by

$$\tau_s = \frac{\tau_c}{\beta} \Gamma(1/\beta)$$  \hspace{1cm} (7.9)

Where $\Gamma(1/\beta)$ is the gamma function, $\beta$ was found $\beta=0.85\pm0.04$ showing no surfactant concentration or temperature dependence. The $\tau_s$ values are plotted in Figure 7.6 showing decreasing in the values of $\tau_s$ with increase in temperature. Relaxation time, $\tau_s$ undergoes a smooth transition close to surfactant concentration $\approx 55 \text{ mM}$ at all temperatures.

The exact origin of the slow mode relaxation is still unresolved. Amis et al [17, 18] and Herning et al [19] attributed the presence of this slowly relaxing mode to the self-diffusion of a few polymer clusters through the rest of solution. Such a description would be incompatible in a chemically cross-linked gel phase. Cho and Sakasita [20] observed the slow mode relaxation frequency showing sharp discontinuity at the gelatin temperature $T_{g\beta}$. It can be described through the inter-mode coupling prevailing in constrained or interacting system. The coupling model allows dynamic constraints to come into play at long time scales and it manifests itself in giving rise to fractional decay of dynamic structure factor though this does not identify the specificity of the mechanism of interactions responsible for coupling. It can be qualitatively argued that a pure gelatin gel is more solid-like because of the presence of strong excluded volume interactions, which will make the viscous relaxations
difficult because of topological constraints and hence, would reveal a slower relaxation. On the contrary, in a gel that is devoid of such interactions and topological constraints will permit rapid viscous relaxation of the network. So when a gel undergoes softening the network relaxation time will decrease and since with decrease in temperature the network becomes more rigid, which has been observed in these studies. The gelatin network is largely comprised of inter and intra molecular hydrogen bonding, which appears to be less susceptible to temperature change in the range $100^\circ C \leq T \leq 25^\circ C$. This explains the invariance of the AOS concentration ($\approx 55\text{mM}$) where the softening ensues with temperature. We shall not discuss the slow mode relaxation any further.

![Figure 7.5: Plots free energy interaction $\Delta G_{\text{int,R}}$ and $\Delta G_{\text{int,D}}$ for different concentrations of AOS which shows gradual increase in the values of $\Delta G_{\text{int,D}}$ and $\Delta G_{\text{R,int}}$ with increase in [AOS]. The gel concentration was fixed at 5% (w/v) in water. The smooth lines are guide to eye only.](image-url)

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Figure 7.6: Plot of the scaling exponent, $\beta$ and slow mode relaxation time $\tau_s$ as a function of concentration of surfactant and temperature. The gel concentration was fixed at 5% (w/v) in water. Notice the change in slope of $\tau_s$ close to AOS concentration $\approx 55$ mM, which signals the micelle induced melting of the gel network and its transformation to a soft gel. The scaling exponent $\beta$ remained invariant of this transition. The smooth lines are guide to eye only.

(ii) Rheological studies

Owing to instrumental limitations the frequency range scanned was limited which did not permit exploration of detailed fundamental structure of the gel network. Thus the discussion of rheological data is largely qualitative. For a thermally reversible gel probed on a time-scale much shorter than the network re-arrangement time, one expects linear viscoelastic behaviour implying $G'' \sim \omega$, which was not observed in our studies. The isochronal values of $G'$ and $G''$ at low frequency (3.5 rad/sec) are plotted in Figures 7.7 and 7.8 for various temperatures of the samples. The theory of rubber elasticity extended to transient networks predicts that magnitude $G'$ at low and moderate frequency is related to the density of mechanically active and reversible physical cross-links [13, 21, 22] and that $G''$ represents the effective volume occupied...
by the network in the continuous medium. Figure (7.1) illustrated there is gradual transition in the value of $G'$ near surfactant concentration $\approx 55$ mM that was found to be invariant of sample temperature to a high configurational entropic state which has lower dynamic viscosity, low storage and loss modulii. The dynamic viscosity, $|\eta^*| = [G^{12}(\omega) + G^{12}(\omega)]^{0.5/\omega}$ reflects the combined effect of both $G'$ and $G''$ as far as the evolution of a more liquid-like phase from a solid- like gel is concerned as seen in the present case. Values of dynamic viscosity shown in Figure 7.1 decrease with increase in temperature implying the possible rupture of some hydrogen bonded networks due to the presence of micelles inside the gel. From data shown in Figures 7.7 and 7.8 it can be inferred that beyond the AOS concentration ($\approx 55$ mM ) the micelle-bound gel phase gains significantly in fluidity which resembles a melting process. A comparison of Figures (7.2) and (7.3) shows that $G'$ drops by almost 40% at AOS concentration as little as 25 mM at a given $T$ indicating that the presence of surfactant had profound effect on gel rigidity.

![Figure 7.7: Plots of low frequency isochronal storage and loss modulii ($G'$) function of concentration of anionic surfactant AOS as function of temperature. The gel concentration was fixed at 5% (w/v) in water. Notice the change in slope in all temperatures close to AOS concentration $\approx 55$ mM, which signals the micelle induced melting of the gel network and its transformation to a soft gel. The smooth lines are guide to eye only.](image-url)
Figure 7.8: Plots of isochronal loss modulus (G'') as function of concentration of anionic surfactant AOS, at various sample temperatures. The gel concentration was fixed at 5% (w/v) in water. Notice the sharp decrease in magnitude of G'' between AOS concentration =50mM and 75mM, which signals the micelle induced melting of the gel network and its transformation to a soft gel. The smooth lines are guide to eye only.

(iii) Free energy of gel surfactant interactions

In order to characterize the system better, we have attempted to estimate the typical value for gel-surfactant interaction free energy from a simple calculation. The total free energy for a gel-surfactant system can be expressed as

\[ \Delta G_{\text{total},D} = \Delta G_{\text{gel}} + \Delta G_{\text{micelles}} + \Delta G_{\text{int},D} \]  

(7.10)

where the suffices indicate free energy of corresponding states to be evaluated from DLS data which is indicated by second suffix “D”. The cmc for AOS is \( \approx 8 \text{mM} \) [12] which gives \( \Delta G_{\text{micelles}} \approx RT \ln \text{cmc} \approx -12 \text{ KJ/mol} \) (in a solvent), a gross underestimation because in a sol (a crowded solvent) a simple relation like the one used may not hold due to the presence of polymeric molecules. However, we shall ignore this for a qualitative study of the problem. The typical value estimated for
\( \Delta G_{\text{gel}} \) from gel melting temperature data analyzed within the framework of Ferry-Eldridge model [23] yields a value for \( \Delta G_{\text{gel}} \approx -130 \text{ KJ/mol} \) [21] implying the value of \( \Delta G_{\text{micelles}} \) is typically 10\% of the value of \( \Delta G_{\text{gel}} \). The values for \( \Delta G_{\text{Total,D}} \) for gels with various AOS concentrations were obtained from the least-squares fitting of data shown in Figure 7.4 to Eq. (7.11). Thus \( \Delta G_{\text{int,D}} \) can be deduced which is plotted in Figure 7.5.

To elucidate this further, we have plotted \( \log G' \) versus inverse of temperature (1/T) \( ^0 \text{K}^{-1} \) for different concentrations of AOS in Figure 7.9, which follows a Arrhenius type temperature dependence for \( G' \) given by

\[
G'(T) \sim G_{0,R} \exp \left( \frac{\Delta G_{\text{Total,R}}}{RT} \right)
\]

And, we can find the values of \( \Delta G_{\text{Total,R}} \) (the suffix R refers to rheology data) which is the appropriate activation energy from the least-squares fitting of the rheology data shown in Figure 7.9 to Eq.(7.11). Again assuming the validity of Eq. (7.10), one can evaluate the gel surfactant interaction free energy term \( \Delta G_{\text{int,R}} \). Figure 7.5 illustrates the dependence of this parameter as function of AOS concentration pertaining to the DLS and rheology data \( \Delta G_{\text{int,D}} \) and \( \Delta G_{\text{int,R}} \) respectively, which is quite revealing. The value of \( \Delta G_{\text{int,R}} \) was found to be systematically different than \( \Delta G_{\text{int,D}} \) by about \( \pm 15\% \).

The DLS evaluated interaction free energy term was found to be less sensitive to changes in AOS concentration as compared to that of rheology data, which can be attributed to the probe length scale. In DLS, the sample gets probed on a length scale \( \approx 1/q \), which is typically \( \sim 300 \text{ nm} \) comparable to the combined size of a few network units. These units are driven by local concentration fluctuations and deformations are governed by longitudinal osmotic modulus. It seems over such length scales the
changes in free energy involved in network relaxation $\Delta G_{\text{int,D}}$ is weakly dependent on the presence of surfactants. On the other hand a macroscopic sample is sheared in the rheology experiment that enables probing of the sample globally. Thus $\Delta G_{\text{int,R}}$ provides a more comprehensive picture of the sample behaviour. The $\Delta G_{\text{int,R}}$ data presented in Figure 7.5 clearly shows gradual increase in its value from $\approx 135$ KJ/mol at $[\text{AOS}] = 25$ mM to a high positive value $\approx 170$ KJ/mol at $[\text{AOS}] = 100$ mM. This unambiguously indicates the gel surfactant interactions becoming gradually unfavorable as AOS concentration increases leading to the softening of the gel system.

![Figure 7.9: Arrhenius fitting of ln G' versus inverse of temperature (1/T)°K⁻¹ for different concentrations of AOS. The gel concentration was fixed at 5% (w/v) in water. See text for details.](image)

7.4 Conclusion

The data for $G'$ could not be obtained at smaller frequency values because of instrumental limitations, which did not permit evaluation of an anticipated terminal response $G_c$. Nevertheless, a significant 50% decrease in the value of $G'$ was observed
(Figures 7.1-7.2) even at [AOS]=25mM that increased to a 10-fold decrease at [AOS]=100mM. The general feature for dynamic structure factor at different temperature shows two relaxation modes. The fast mode relaxation is related to the gel mode diffusion process where micelle bound gel medium represent soft gel, the mesh size shows strong temperature dependence. When the temperature is decreased from T=25 °C to T=10 °C the value of $\xi$ decreased by ~60% for all AOS concentrations. The second mode, the stretched exponential relaxation mode, can be described as a Gaussian diffusion of scattering entities in the dispersion medium. Rheology data indicated the presence of universal relaxation and melting behavior and a cross-over surfactant concentration ≈ 55 mM. Below this concentration triple-helix networks are in propensity and dominate the structure whereas beyond this concentration the density of micellar crosslink increase exponentially leading to the occurrence of melting like behavior of the gel phase. A simple interaction free energy calculation revealed this feature even more explicitly. The simultaneous shift in the relaxation times could indicate monitoring unequal responses in $G'$ and $G''$ data, which would consequently have bearing on the reported $|\eta^*|$ and $\tan\delta$ values. This we visualize as the major problem associated with our rheological data. Regardless, the DLS and rheology data together do imply a structural change inside the gel closely associated with increasing surfactant concentration.

7.5 References