Small-Angle Neutron Scattering Studies of Chemically Cross-Linked Gelatin Solutions and Gels

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ABSTRACT: Small-angle neutron scattering measurements are reported for experiments performed on mixtures of gelatin and glutaraldehyde (GA) in the aqueous phase, where the gelatin concentration was fixed at 5% (w/v) and the GA concentration was varied from 1.25×10^{-4} to 3×10^{-3}% (w/v). Two length scales associated with this system were identified in both sol (60° C) and gel state (28° C). One of these, the correlation length (ξ) or mesh size of the cross-linked network showed negligible dependence on temperature, but a scaling $\xi \sim [GA]^{\nu}$, where [GA] is the concentration of cross-linker GA, was observed with $\nu = 0.33 \pm 0.04$ in the gel state. Other length scale associated was the size of inhomogeneities ζ which showed no [GA] dependence but showed appreciable increase in size as the system transformed from a sol to gel state for all [GA]. Observations are discussed in the context of the results obtained from dynamic light scattering experiments performed earlier.

Introduction

Native collagen consists of triple helices which are held together by intermolecular hydrogen bonds. These bonds are destroyed during hydrolysis. Gelatin is a biopolymer obtained from the partial hydrolysis of collagen. At temperatures above 32 °C, gelatin forms homogeneous solution in aqueous or any hydrogen bond friendly solvent. Above an overlap concentration of ∼1% (w/v), such a solution undergoes a first-order phase transition to the gel state and forms a physical gel when cooled to room temperature.[1−4] During this process a three-dimensional interconnected network connecting most of the gelatin chains is formed in the dispersion medium, thereby reverting back to triple-helical structure. Though the kinetics and thermodynamics of the gel transition of this polypeptide are rather well studied and documented,[5−7] there is inadequate exploration of the same where the gelatin molecules are chemically cross-linked through the addition of small amount of cross-linker. Thus, one has a gel where both physical and chemical cross-links are present. When a cross-linker such as glutaraldehyde (GA) is added to a gelatin solution, such a possibility (presence of both physical and chemical cross-links) can arise.

In our earlier work, we have studied the sol−gel transition behavior of gelatin above overlap concentration where glutaraldehyde was present as a cross-linker in a small amount.[8] DLS (dynamic light scattering) measurements carried out on this system revealed two relaxation modes identified as fast and slow which had relaxation times of order 10−20 μs and 1−10 ms, respectively, and such features existed in the entire range of temperature studied when the system transformed from sol to gel state. Recent experiments have shown yet another mode, the intermediate mode, to exist along with the fast and slow modes in both the sol and gel states of gelatin solutions.[9−11] For a chemically cross-linked gelatin gel, Oikawa and Nakanishi[12] observed that the fast-mode diffusivity, $D_f \sim C^{0.79}$, C being the gelatin concentration, and the correlation length derived from the slow mode showed no dependence on C. On the basis of the static and dynamic light scattering data, it was concluded[12] that the presence of GA did not alter the size of the crystalline triple-helical structures but suppressed the number of nucleation sites, thus lowering the total cross-linking density. In the absence of chemical cross-linking, gelatin gels showed the fast-mode diffusivity scaling with gelatin concentration as[2] $D_f \sim C^{0.6}$.

The structural characterization of gelatin in the solution phase at 50 °C has been reported by Pezron et al.,[1] using light scattering and small-angle neutron scattering (DLS and SANS) techniques. They identified different length scales and reported a persistence length of 20 Å and the radius of gyration $R_g \sim 350$ Å in the dilute regime. However, experiments performed on a similar system in the semidilute regime at 50 °C revealed two length scales identified as the correlation length ξ of the order of $\sim R_g/10$ and inhomogeneities of the size $\sim R_g/3$. They found[2] the scaling relationship $\xi \sim C^{-\nu}$, and the exponent ν had a value in the range 0.5−0.7 as predicted by the scaling theory for homopolymers.[13]

In the present work, we have reported SANS experiments performed on GA cured gelatin solutions and gels. The different length scales of the system spanning the entire regime from sol to gel state have been studied and identified and the features discussed within the purview of existing models and earlier dynamic light scattering data.

Materials and Methods

SANS experiments were carried out in 5% (w/v) gelatin solutions and gels in D_2O for glutaraldehyde (GA) concentrations, $c = 0$, 1.25×10^{-4}, 2×10^{-4}, 1×10^{-3}, and 3×10^{-3}% (w/v), as it cooled from 60 °C (sol state) to 28 °C (gel state). The chemicals used were gelatin (bovine origin) from M/S Loba

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chemie (Indo-Astranal Co. India) containing nominal impurities (sulfate ash = 1.5%, $SO_2 = 2 \times 10^{-4}$%, and heavy metals (Zn, Cu, Pb) in concentrations lower than the SO_2 concentrations), and this preparation was devoid of any *E. coli* and liquifier presence. Aqueous GA stock solution (25% (w/w) from S. D. Fine-chem was used as received. The gelatin sample used had a narrow molecular weight distribution with M_w peak at about 1.5×10^5. The pH of the solutions were maintained at 6.8 using a sodium phosphate buffer ($Na_2HPO_4 + NaH_2PO_4$: $2H_2O$). Sample preparation was done by dissolving gelatin in the buffer followed by heating for nearly 1 h at about 50 °C to remove history effects. When the gelatin dissolved completely, the required amount of GA was added, and the mixture was stirred well for nearly 30 min. This solution was directly used for SANS measurements without centrifugation. All the samples looked transparent with a light yellow hue. Prior to SANS measurements, the solutions were allowed to equilibrate for 10−15 h at room temperature. During this time gelatin solutions formed homogeneous gels.

SANS experiments were performed on the spectrometer at the G.T. Laboratory, Dhruva reactor (Bhabha Atomic Research Centre, India). Further details of the SANS spectrometer at Dhruva are discussed in ref 14. The wavelength of the neutrons used covered the scattering vector (q) range

$$1.8 \times 10^{-2} \leq q \leq 3 \times 10^{-1}\ \text{Å}^{-1}$$

given by $q = (4\pi/\lambda)\sin(\theta/2)$, λ being the wavelength of neutron and θ the scattering angle.

Chemically cross-linked gelatin gels were heated to ca. 50 °C to reproduce hot gelatin solutions comprising gelatin and very small concentration of cross-linker GA dispersed in the medium. GA molecules provide the sites for formation of chemical bonds, thus supplementing the physical cross-links that gelatin forms normally in a solution. In all experiments GA concentration was kept very small, so the cross-links were mostly physical, and these melted at temperatures >32 °C. The density of chemical cross-links is inadequate to maintain the gel structure on its own. The liquid samples were transferred to quartz cells and heated to 60 °C, and the scattered intensity was measured as a function of scattering vector. Now, the sol was allowed to cool spontaneously to 28 °C, and similar measurements were carried out in the gel state. The measured intensity was corrected for the background, and the empty cell contributions and the data were normalized to get the structure factors. Details of the data normalization procedure are the same as discussed in ref 15.

Theoretical Background

Mean-field theory of polymers in a good solvent, at equilibrium, has led to a form of structure factor of concentration fluctuations at low wavevector, known as the Ornstein−Zernike (O−Z) function[16] given by

$$S_L(q) = \frac{S_L(0)}{1 + q^2\xi^2}; \quad q\xi \ll 1 \tag{1}$$

where $S_L(0)$ is the extrapolated structure factor at zero wavenumber and ξ is the correlation length of the concentration fluctuations. Physically, $S_L(0)$ is related to the cross-link density and longitudinal osmotic modulus.

Although Okano et al.[17] and others[18] have found full agreement with the O−Z behavior, experiments carried out in the semidilute regime of polymer solutions have shown deviations from the Ornstein−Zernike function. An "excess scattering" has been reported at low wavenumbers from polymeric solutions[1,19−24] which is caused by the enhanced long wavelength concentration fluctuations in the system. It is not clear so far as to what causes this excess scattering. However, Koberstein et al.[23] have suggested long-range random inhomogeneities, with correlation length many times larger than the radius of gyration of the dissolved polymer to cause this excess scattering at low wavenumbers. If the spatial scale of the density fluctuations due to the presence of inhomogeneities is large compared to the correlation length ξ, then the two contributions can be treated separately and added to give the total structure factor as[25]

$$S(q) = S_L(q) + S_{ex}(q) \tag{2}$$

where $S_L(q)$ is the Ornstein−Zernike function, and the Debye−Bueche structure factor has the form $S_{ex}(q)$ given by

$$S_{ex}(q) = \frac{S_{ex}(0)}{(1 + q^2\xi^2)^2} \tag{3}$$

where $S_{ex}(0)$ is the extrapolated structure factor at zero wavevector. Often it is impossible to probe low-q domain of the structure factor because of the instrumental limitations of SANS spectrometers. A better proposition would be to perform light scattering studies in this domain and, by suitable normalization, combine it with the SANS data. However, such a procedure often leads to large statistical errors and hence is not popular.

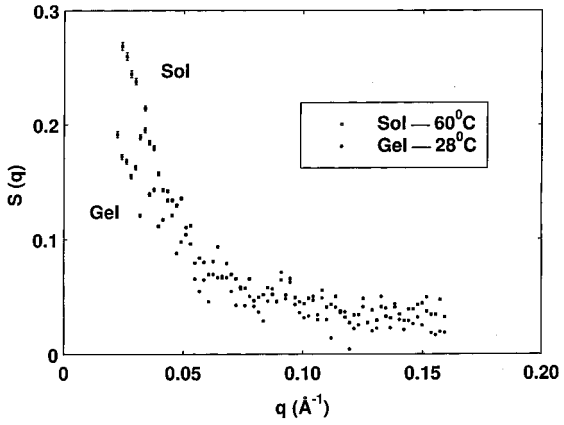

Figure 1. Plot of the structure factor $S(q)$ vs scattering vector q at two different temperatures above (sol) and below (gel) the gelation temperature (~32 °C) for a sample of 1×10^{-3}% (w/v) GA in 5% (w/v) gelatin prepared in phosphate buffer (0.1 M) maintained at a pH of 6.8 in D_2O. Notice the overall similarity between these two curves.

Results and Discussion

Correlation Length. We have examined neutron scattering from glutaraldehyde (GA) cured gelatin solutions and gels at various concentrations of the cross-linker by varying the temperature from 60 °C (sol state) to 28 °C (gel state). The experiments were performed on five different samples using a fixed gelatin concentration, 5% (w/v), but with varying amounts of GA. As gelatin sol evolves to a gel phase, the structure factor $S(q)$ does not seem to change significantly, as shown in Figure 1. This is consistent with our earlier experimental observation done through light scattering,[26] a fact that was thought to be consistent with Flory's original proposal of a first order thermodynamic transition for the sol-to-gel (coil-to-helix) transition of gelatin.[27] Theoretically, an O−Z type of behavior (eq 1) has been predicted for the scattered intensities of the polymer solutions in a good solvent. Accordingly, we attempted

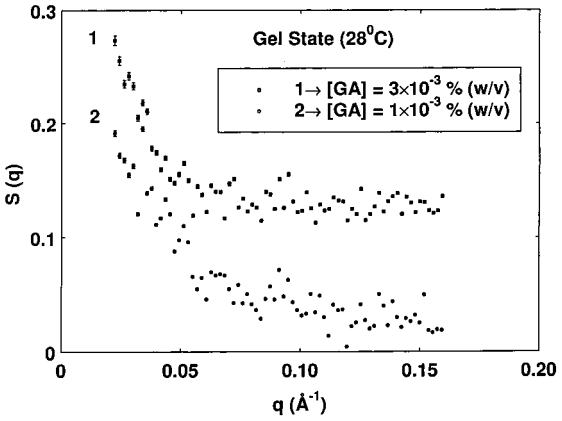

Figure 2. Plot of the structure factor $S(q)$ vs scattering vector q at two different concentrations of glutaraldehyde (GA), viz., 1×10^{-3} and 3×10^{-3}% (w/v), respectively, in 5% (w/v) gelatin sample at a fixed temperature of 28 °C (gel state). All the solutions were prepared in phosphate buffer (0.1 M) maintained at a pH of 6.8 in D_2O.

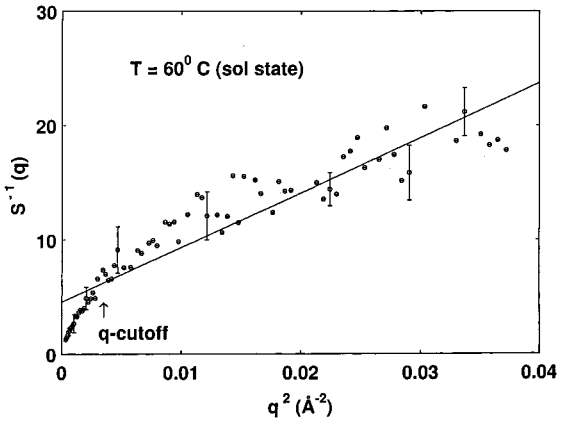

Figure 3. Inverse structure factor $S^{-1}(q)$ vs q^2 for GA concentration of 2×10^{-4}% (w/v) in 5% (w/v) gelatin sample at 60 °C. In the range of $3.2 \times 10^{-2} \leq q \leq 2.0 \times 10^{-1}$ Å^{-1}, a linear dependence is observed, and the slope allows one to calculate ξ. At small q vectors in the range of $q < 3.2 \times 10^{-2}$ Å^{-1}, an excess scattering appears (downward curvature) as a deviation from the linear fit. Solutions were prepared in phosphate buffer (0.1 M) maintained at a pH of 6.8 in D_2O.

fitting our neutron scattering data of Figure 2 to a Lorentzian behavior as given by eq 1. To fit the data, a plot of $S^{-1}(q)$ vs q^2 was made in the range of the scattering vector $1.8 \times 10^{-2} \leq q \leq 2.0 \times 10^{-1}$ Å^{-1} in Figure 3. Although it showed a good linear fit to the data in the q range of $3.2 \times 10^{-2} < q < 2.0 \times 10^{-1}$ Å^{-1}, it showed deviations from linear fit in the range of scattering vector $q < 3.2 \times 10^{-2}$ Å^{-1} (the downward curve indicates deviations). A typical situation is shown in Figure 4 where experimental data is fitted to O−Z and D−B functions, and the conclusion is obvious. Thus, the excess scattering from the sample at low wavenumbers has to be dealt separately.

A linear fit of the structure factor data in the q range of $3.2 \times 10^{-2} < q < 2.0 \times 10^{-1}$ Å^{-1} simply gives the screening length or the correlation length ξ of the entangled network of cross-linked gels. The slope of the fit enables one to easily calculate the value of ξ. Values of ξ obtained from such a fit are listed in Table 1 for various concentrations of GA at different temperatures. For pure gelatin we found $\xi = 47 \pm 3$ and 50 ± 4 Å for sol and gel states, respectively. These values are within 15% of the ξ values reported in ref 2. The temperature

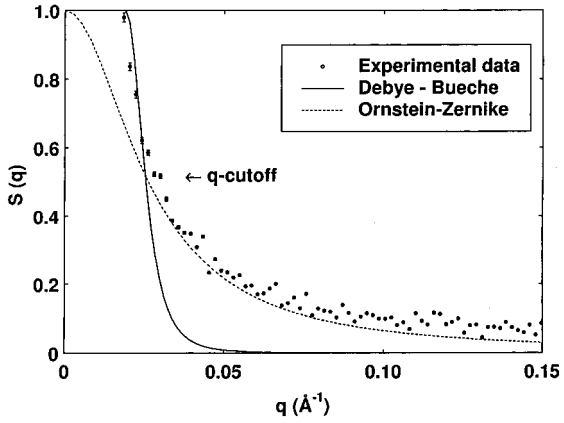

Figure 4. Plot of the reconstructed structure factor $S(q)$ vs scattering vector q obtained by adding the two contributions of scatterd intensity in the different q ranges of the fitting (for eq 2) for a sample of 2×10^{-4}% (w/v) GA in 5% (w/v) gelatin at 60 °C. The broken curve represents the O−Z function (eq 1) behavior with $\xi = 48$ Å (eq 1), the smooth curve is the contribution of the inhomogeneities (eq 4) with $\zeta = 64$ Å, and the full curve is the total structure factor (eq 2) obtained directly from the experimental data. Solutions were prepared in phosphate buffer (0.1 M) maintained at a pH of 6.8 in D_2O.

dependence of correlation length for varying concentrations of GA is shown in Figure 5. As is clear from this figure, there is decrease in ξ as the solution is cooled to the gel state at a relatively low concentration of GA, which is almost negligible in the case of pure gelatin 5% (w/v) (see Table 1). The magnitude of this decrease, however, is very small. However, earlier dynamic light scattering (DLS) studies[8] showed similar decrease in the value of ξ with lowering of temperature though the numerical value of ξ measured through DLS was much higher. The values of ξ obtained from DLS are listed in the Table 1 as ξ_{fast}. The mesh size showed a temperature dependence given by[8]

$$\xi_{fast} \sim (0.5 - \chi)e^{-A/RT} \tag{4}$$

where χ is the Flory−Huggins interaction parameter defining the solvent quality and A is the appropriate activation energy of diffusion for the unswollen network. An increase in the number of cross-link density with reduction in temperature has also been reported earlier.[12] This possibly causes ξ to decrease as has been observed in the present study. However, at a fixed temperature, a variation in the value of ξ was observed with change in the concentration of the cross-linker GA. Addition of GA to pure gelatin caused shrinking of the mesh size at a constant temperature. However, on further addition of GA at the same temperature causes the mesh size to increase (Figure 6). The correlation length ξ showed a power law behavior, $\xi \sim [GA]^{\nu}$ where [GA] is the concentration of GA and the exponent ν has a value of 0.33 ± 0.04 in the gel state. No GA concentration dependence of the mesh size was observed in our DLS measurements as opposed to the SANS case. This discrepancy can be attributed to the fact that the range of the GA concentrations chosen was small in the DLS experiments. The highest concentration of GA used in the case of SANS is almost 3 times higher than the same in DLS.

Inhomogeneities and Size of Triple Helices. Plot of $S^{-1}(q)$ vs q^2 in Figure 3 shows the excess scattering at low scattering vectors in the range of $q < 3.2 \times 10^{-2}$ Å^{-1} where a deviation from straight line fitting was

Table 1. Values of the Correlation Length ξ and Size of the Inhomogeneities ζ Obtained after Fitting of the Experimental Data to Eqs 1 and 3, Respectively[a]

	SANS				DLS	
	correlation length ξ (Å)		inhomogneeity size ζ (Å)		correlation length ξ (Å)	
concn of GA % (w/v)	sol state (60 °C)	gel state (28 °C)	sol state (60 °C)	gel state (28 °C)	sol state (60 °C)	gel state (28 °C)
0	50 ± 4	47 ± 3	113 ± 10	115 ± 11		
1.0×10^{-5}					200 ± 20	37 ± 2
5.0×10^{-5}					212 ± 20	38 ± 2
1.25×10^{-4}	45 ± 3	41 ± 2	56 ± 5	74 ± 6		
2.0×10^{-4}	48 ± 4	45 ± 3	64 ± 6	80 ± 7	211 ± 20	39 ± 2
1.0×10^{-3}	64 ± 4	71 ± 6	70 ± 4	85 ± 6	200 ± 18	40 ± 3
3.0×10^{-3}		110 ± 8		77 ± 5		

[a] The values listed are above (sol) and below (gel) the gelation temperature (~32 °C) for different concentrations of GA in 5% (w/v) gelatin sample prepared in phosphate buffer (0.1 M) maintained at a pH of 6.8 in D_2O. Comparative ξ_{fast} values derived from DLS are also listed along with the SANS data.

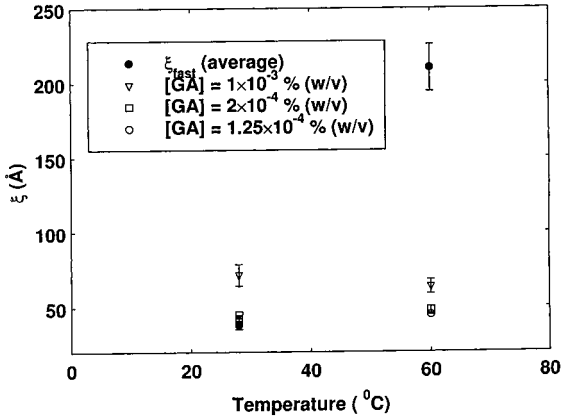

Figure 5. Temperature dependence of the correlation length ξ for different concentrations of GA in 5% (w/v) gelatin sample. At a fixed concentration of GA, ξ decreases with the decrease in temperature at low concentrations of GA. The DLS value of mesh size ξ_{fast} is an average over the entire range of GA concentration (since no concentration dependence was observed) is also plotted along with SANS data. The values of ξ (SANS) and ξ_{fast} (DLS) for different concentrations of GA and at different temperatures are listed in Table 1.

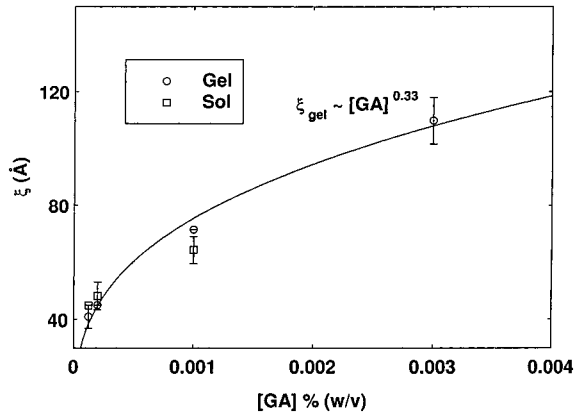

Figure 6. Plot of the variation of correlation length ξ as a function of concentration of GA in 5% (w/v) gelatin at two different temperatures representing the sol and gel state. A scaling behavior of $\xi \sim [GA]^{\nu}$ was found where the exponent ν has a value 0.33 ± 0.04 in the gel state.

observed (Figure 3). Two contributions to the structure factor, the O−Z component and the excess scattering component, can be visualized from the plot shown in Figure 4 where we have used the numerical value of ξ from Table 1. The excess scattering data have been analyzed within the framework of the Debye−Bueche model discussed earlier.[25] By subtracting the O−Z component from the total structure factor data (through

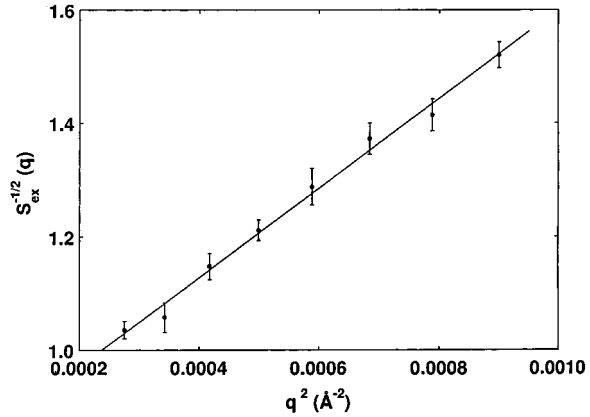

Figure 7. Fit of $S_{ex}^{-1/2}(q)$ vs q^2 to Debye−Bueche model (eq 3) in the low-q regime of $q < 3.2 \times 10^{-2}$ Å^{-1} where excess scattering was observed. Slope of the linear fit allows one to calculate the inhomogeneity size ζ. The typical data shown in the figure correspond to case of 2×10^{-4}% (w/v) GA in 5% (w/v) gelatin at 60 °C. Solutions were prepared in phosphate buffer (0.1 M) maintained at a pH of 6.8 in D_2O.

visual estimation of q cutoff $\simeq 3.2 \times 10^{-2}$ Å^{-1}), one can easily get the excess scattering contribution, $S_{ex}(q)$ (see Figure 4). Next, these $S_{ex}(q)$ data were fitted to eq 3. A plot of $S_{ex}^{-1/2}(q)$ vs q^2 was made, and the slope gave the parameter ζ. A typical fitting of the experimental data obtained from a sample with GA concentration $= 2 \times 10^{-4}$% (w/v) following the above-mentioned strategy is shown in Figure 7. The characteristic sizes of the inhomogeneities calculated from this fit are listed in Table 1. An inhomogeneity of size ~114 Å has been measured for both sol and gel state of pure gelatin (5% (w/v)), which is close to the value measured by Pezron et al.[1] in the sol state (50 °C), where they reported inhomogeneity size $\sim R_g/3$. The temperature dependence of inhomogeneity size ζ for varying concentration of GA is shown in Figure 8. This figure shows an increase in the value of ζ with decrease in temperature at a fixed concentration of GA, which was again negligible in the case of pure gelatin. However, for a fixed temperature, the size of inhomogeneity was observed to be dependent on the concentration of the cross-linker GA in the sol state, but the same was absent in the gel state. Addition of GA to pure gelatin decreases the value of ζ on the first hand, but the opposite happens if GA is added further (see Table 1). The behavior of parameter ζ with GA concentration is plotted in Figure 9. A power law scaling of $\zeta \sim C^{-1}$, where C is the total concentration of the polymer solvent mixture, has been reported by Gan et al.[21]

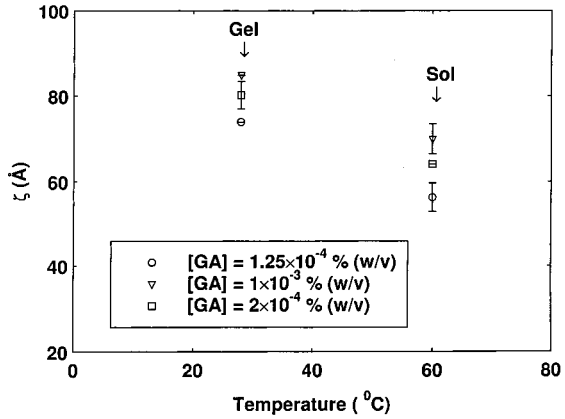

Figure 8. Temperature dependence of the parameter ζ derived from the excess scattering in the low-q regime for different concentrations of GA in 5% (w/v) gelatin sample. At a fixed concentration of GA, ζ increases with the decrease in temperature in the sol state but not in the gel state. The values of ζ for different concentrations of GA and at different temperatures are listed in Table 1.

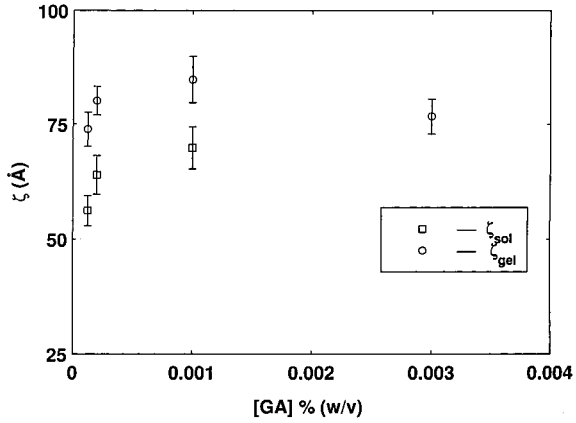

Figure 9. Plot of variation in the size of the inhomogeneity ζ vs the concentration of GA in a 5% (w/v) gelatin sample at two different temperatures of 60 and 28 °C representing the sol and gel state, respectively.

Let us discuss the effect of these inhomogeneities on DLS data.[8] In addition to the normal fast-mode relaxation which can be attributed to the incipient gel mode arising due to short-range monomer or blob motion of the polypeptide chains, a yet another "slow" mode relaxation was observed in the entire range of sol and gel states studied by DLS.[8] The relaxation time, τ_c, derived from the slow mode, showed a strong dependence on the concentration of GA in the gel state whereas no observable dependence was found in the sol state of the system. The physical origin of the slow mode continues to be a matter of controversy. The existence of slow mode in the sol phase has been pointed out by Borsali et al.,[28] Amis et al.,[29,30] and Herning et al.[2] among others. Amis et al.[29,30] and Herning et al.[2] attributed the presence of this slow relaxing mode to the self-diffusion of a few polymer clusters through the rest of the solution. A number of studies from different authors have reported the size of junction zones or the physically cross-link sites of gelatin[31,32] to be of a crystalline triple-helical structure like collagen.[33] A critical size in the range of ~1000 Å has been reported by a number of investigators.[34−36] In view of these observations, we have attributed the slow-diffusive mode to be arising from the "clusters" that may be the junction zones in gelatin, and ξ_{slow} is a measure of the

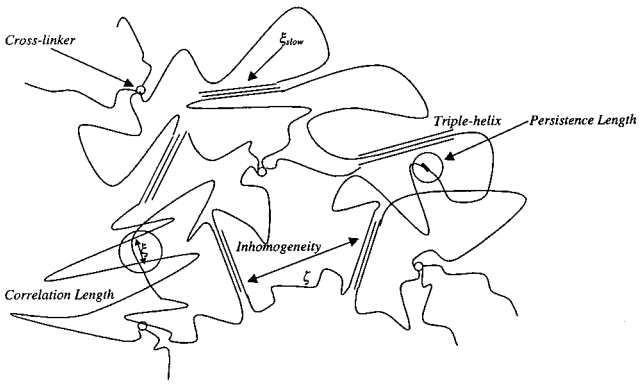

Figure 10. Schematic representation of the existing length scales in a chemically cross-linked gelatin network swollen in an aqueous medium (not to scale). The typical sizes are ξ = 50 Å, ζ = 100 Å, persistence length = 20 Å, and $\xi_{slow} \simeq$ 3000−4000 Å.

typical physical length of these clusters. Assuming τ_c to be arising from a diffusive process, governed by the Stoke−Einstein's relation, $D_{slow} = (k_B T)/(6\pi\eta\xi_{slow})$ where the terms have their usual meaning and η, the solvent viscosity, length scale ξ_{slow} associated with the slow mode can be calculated. The value of ξ_{slow}, although had no GA concentration dependence in the sol state, showed a sharp increase in the value with increase in the concentration of GA for the gel state. The average value of ξ_{slow} in sol state was $\simeq$4000 ± 500 Å, a value comparable to the size of a triple helix. However, ξ_{slow} can also be attributed to the size of the inhomogeneities as observed by Mallam et al.[37] and Mendes et al.[38] in synthetic polymers.

Conclusion

Our investigation of the chemically cross-linked gelatin solutions and gels using SANS (small-angle neutron scattering) revealed the existence of two kinds of length scales—one of them being the correlation length ξ, associated with the size of the entangled transient network, and the other having a much higher length scale that can be attributed to the long-range concentration fluctuations due to the inhomogeneities present in these solutions. A physical picture of gelatin gels chemically cross-linked in GA was provided by Oikawa and Nakanishi[12] where the total cross-link density, ν_e, was assumed to be the sum of the physical cross-link density $\nu_{e,phys}$, and the chemical cross-link density, $\nu_{e,chem}$. They calculated the value of $\nu_{e,chem}$ assuming the chemical cross-linking sites to be tetrafunctional. On the basis of the assumption that cross-linking sites in the case of GA are tetrafunctional and the length scales derived, we have drawn a schematic structure of the chemically cross-linked gelatin gels with appropriate length scales shown in Figure 10.

We have seen shrinking of the mesh size of the entangled network due to addition of little amount of cross-linker GA to a pure solution of gelatin. An increase in the mesh size ξ, of course, was observed on further addition of the cross-linker which is clear from Figure 5. A similar effect was observed for the concentration dependence of the size of the inhomogeneities present in a solution at a fixed temperature (Figure 8). This shrinking is often caused by the increase in the osmotic pressure because of increase in cross-linked density inside the gel network for chemically cross-linked gels. However, the parameter ζ was observed to be indepen-

dent of temperature in the gel state; i.e., the sizes of inhomogeneities remained constant with the inception of gelation. This is due to participation of the chains originally belonging to the inhomogeneities to the gel network which were localized earlier.

In a related experiment, effects of increase in the size of the entangled transient network or the mesh size were also observed in dynamic light scattering (DLS) experiments of the chemically cross-linked gelatin solutions and gels.[8] The SANS and DLS experiments were performed on identical samples to the extent possible. At low concentrations of GA, the SANS spectrometer exhibited poor resolution and large error bars; thus, these results are not included in the analysis. We had to include one large GA concentration sample in the SANS experiments in order to establish the scaling relation without ambiguity. Apart from this, the DLS and SANS covered mostly the overlapping concentrations. The mesh sizes measured from the DLS studies varied from 210 ± 20 to 38 ± 2 Å as solution transformed from the sol to the gel state. Also, the presence of inhomogeneities in such solutions is not unique to SANS studies. DLS studies on similar samples have also substantiated the argument that chemically cross-linked gelatin solutions are not homogeneous. We found from DLS measurements that in addition to the "fast mode", which is attributed to the cooperative diffusion of the entangled network, a slow diffusive mode (the origin of which is not clear) was also present in such solutions above the overlap concentration. The length scale derivable from the slow mode relaxation time, ξ_{slow}, has been argued to represent the size of crystalline triple-helical junction zones.[12] We observed negligible dependence of ξ_{slow} on the GA concentration in the sol state whereas it increased sharply in the case of a gel as opposed to the case of Oikawa and Nakanishi[12] where no observable dependence was found in both sol and gel states. A Zimm dynamics was proposed for the motion of polymer chain in such solutions.[8] It is worth debating why the mesh sizes obtained from SANS and DLS fast mode are different.

In essence, both the SANS and DLS studies have suggested the presence of inhomogeneities in the aqueous semidilute solution of chemically cross-linked gelatin gels. The presence of inhomogeneities in the aqueous gelatin solutions has also been reported by Pezron et al.[1] A possible relationship between the excess scattering and the physical gelation mechanism has been pointed out by Gan et al.[21] where they suggested the formation of local complexes between the polymer and solvent to be the driving force behind the incipient gelation mechanism. The role of solvent molecules in the gelation mechanism is well-known as they provide the junction zones for gelation.[34,39,40] Although presence of inhomogeneities in the hot gelatin solution has little significance, it is assumed that these inhomogeneities may act as driving force for gelation. These could be microgels that are formed below the gelation threshold (temperature) on cooling. The origin of the excess scattering is still not clear though these excess scattering have been reported to be dependent on temperature.[21] In yet another experiment, it was found to depend on experimental conditions of sample preparation.[23] However, unanimity could not be achieved about the origin of excess scattering.

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References and Notes

(1) Pezron, I.; Djabourov, M.; Leblond, J. *Polymer* **1991**, *32*, 3201.
(2) Herning, T.; Djabourov, M.; Leblond, J.; Takekart, G. *Polymer* **1991**, *32*, 3211.
(3) Pezron, I.; Herning, T.; Djabourov, M.; Leblond, J. In *Physical Networks, Polymers and Gels*; Burchard, W., Ross- Murphy, S. B., Eds.; Elsevier: London, p 231.
(4) Djabourov, M. *Polym. Int.* **1991**, *25*, 135.
(5) Bohidar, H. B.; Jena, S. S. *J. Chem. Phys.* **1993**, *98*, 3568.
(6) Bohidar, H. B.; Jena, S. S. *J. Chem. Phys.* **1993**, *98*, 8970.
(7) Bohidar, H. B.; Jena, S. S. *J. Chem. Phys.* **1994**, *100*, 6888.
(8) Sharma, J.; Bohidar, H. B. *Colloid Polym. Sci.* **2000**, *278*, 15.
(9) Ren, S. Z.; Sorensen, C. M. *Phys. Rev. Lett.* **1993**, *70*, 1727.
(10) Ren, S. Z.; Shi, W. F.; Zhang, U. B.; Sorensen, C. M. *Phys. Rev. A* **1991**, *45*, 2416.
(11) Maity, S.; Bohidar, H. B. *Phys. Rev. E* **1998**, *58*, 729.
(12) Oikawa, H.; Nakanishi, H. *Polymer* **1993**, *34*, 3358.
(13) Nagarajan, R.; Kalpakchi, B. *Microdomains in Polymer Solutions*; Dubin, P., Ed.; Plenum: New York, 1986; p 369.
(14) Goyal, P. S.; Aswal, V. K.; Joshi, J. V. *Curr. Sci.* **2000**, *79*, 947.
(15) Thiyagarajan, P.; Epperson, J. E.; Crawford, R. K.; Carpenter, J. M.; Klippert, T. E.; Wozniak, D. G. *J. Appl. Crystallogr.* **1997**, *30*, 280.
(16) De Gennes, P. G. *Scaling Concepts in Polymer Physics*, 2nd ed.; Cornell University Press: Ithaca, NY, 1985.
(17) Okano, K.; Wada, E.; Kurita, K.; Fukuro, H. *J. Appl. Crystallogr.* **1978**, *11*, 507.
(18) Cosgrove, T.; White, S. J.; Zarbakhsh, A.; Heenan, R. K.; Howe, A. M. *Langmuir* **1995**, *11*, 744.
(19) Dautzenberg, H. *J. Polym. Sci., Part C* **1972**, *39*, 123.
(20) Xie, Y.; Ludwig, K. F., Jr.; Bansil, R.; Gallagher, P. D.; Konak, C.; Morales, G. *Macromolecules* **1996**, *29*, 6150.
(21) Gan, J. Y. S.; Francois, J.; Guenet, J. M. *Macromolecules* **1986**, *19*, 173.
(22) Benoit, H.; Picot, C. *Pure Appl. Chem.* **1966**, *12*, 1271.
(23) Koberstein, J. T.; Picot, C.; Benoit, H. *Polymer* **1985**, *26*, 673.
(24) Guenet, J. M.; Willmott, N. F. F.; Ellsmore, P. A. *Polym. Commun.* **1983**, *24*, 230.
(25) Debye, P.; Bueche, A. M. *J. Appl. Phys.* **1949**, *20*, 518.
(26) Bohidar, H. B.; Maity, S. *Eur. Polm. J.* **1998**, *34*, 1361.
(27) Flory, P. J.; Weaver, E. S. *J. Am. Chem. Soc.* **1960**, *82*, 4518.
(28) Borsali, R.; Durand, D.; Fischer, E. W.; Giebel, L.; Busnel, J. P. *Polym. Networks Blends* **1991**, *1*, 11.
(29) Amis, E. J.; Janmey, P. A.; Ferry, J. D.; Yu, H. *Macromolecules* **1983**, *16*, 441.
(30) Amis, E. J.; Janmey, P. A.; Ferry, J. D.; Yu, H. *Polymer* **1981**, *6*, 13.
(31) Ward, A. G.; Courts, A. *The Science and Technology of Gelatin*; Academic Press: London, 1977.
(32) Mark, H. F.; Gaylord, N. G.; Bikales, N. M. *Encyclopedia of Polymer Science and Technology*; John Wiley: New York, 1977; Vol. 7.
(33) Djabourov, M.; Leblond, J.; Papon, P. *J. Phys. (Les Ulis, Fr.)* **1988**, *49*, 319 and references therein.
(34) Busnel, J. P.; Morris, E. R.; Ross-Murphy, S. B. *Int. J. Biol. Macromol.* **1989**, *11*, 119.
(35) Benguigui, L.; Busnel, J. P.; Durand, D. *Polymer* **1991**, *32*, 2680.
(36) Hwang, F. S.; Cummins, H. Z. *J. Chem. Phys.* **1983**, *79*, 5188.
(37) Horkey, F.; Hecht, A. M.; Mallam, S.; Geissler, E.; Rennie, A. R. *Macromolecules* **1991**, *24*, 2896.
(38) Mendes, E., Jr.; Linder, P.; Buzier, M.; Boue, F.; Bastide, J. *Phys. Rev. Lett.* **1991**, *66*, 1595.
(39) Busnel, J. P.; Ross-Murphy, S. B. *Int. J. Biol. Macromol.* **1988**, *10*, 121.
(40) Nakaoki, T.; Kobayashi, M. *J. Mol. Struct.* **1991**, *242*, 315.

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