Chapter 6

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

6.1 Introduction

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

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

SANS experiments were carried out in 5% (w/v) gelatin solutions and gels in $D_2O$ for glutaraldehyde (GA) concentrations, $c = 0, 1.25 \times 10^{-4}, 2 \times 10^{-4}, 1 \times 10^{-3}$ and $3 \times 10^{-3}\%$, (w/v) as it cooled from $60^\circ C$ (sol state) to $28^\circ C$ (gel state). The chemicals used were gelatin (bovine origin) from M/S Loba chemie (Indo-Astranal Co. India) containing nominal impurities (sulphate 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%) 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 $1.5 \times 10^5$. 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 1hr at about $50^\circ C$ 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 minutes. 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 $10-15$hr 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 is discussed in Ref. [3]. The wavelength of the neutrons used covered the scattering vector range

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

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

The gelatin gels were heated to melt and reproduce gelatin solutions. The liquid samples were transferred to quartz cells and heated up to $60^\circ C$ and the scattered intensity was measured as function of scattering vector $q$. Now, the sol was allowed to spontaneously cool to $28^\circ C$ and similar measurements were carried out in the gel.

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state. The measured intensity were corrected for the background and the empty cell contributions and the data were normalized to get the structure factors. Details of the normalization procedure is discussed in Ref. [3].

6.3 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 [4] given by

$$S_L(q) = \frac{S_L(0)}{1 + q^2 \xi^2} ; \quad q \xi \ll 1$$  \hfill (6.1)

where $S_L(0)$ is the extrapolated structure factor at zero wavenumber and $\xi$ being the correlation length of the concentration fluctuations. Physically, $S_L(0)$ is related to the crosslink density and longitudinal osmotic modulus.

Although Okano et al. [5] and others [6] have found full agreement with the (O-Z) behavior, experiments carried out in the semi-dilute 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, 7, 8, 9, 10, 11, 12] 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. [11] 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. They adapted the Debye-Bueche theory of scattering by a heterogeneous solid [13] to fit the data of polymer solutions. If the spatial scale of the density fluctuations due to inhomogeneities is large compared to the correlation length $\xi$, then the two contributions can be treated separately and added to give the total structure factor as

$$S(q) = S_L(q) + S_{ex}(q)$$  \hfill (6.2)

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

$$S_{ex}(q) = \frac{S_{ex}(0)}{1 + q^2 \xi^2}$$  \hfill (6.3)
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 spectrometer. A better proposition is 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.

6.4 Results and Discussion

We have examined neutron scattering from glutaraldehyde (GA) cured gelatin solutions and gels at various concentrations of the crosslinker by varying the temperature from 60°C (sol state) to 28°C (gel state). The experiments were performed on five different samples having a fixed concentration of gelatin, 5% (w/v) but varying amount of GA.
As gelatin sol evolves to a gel phase, the structure factor $S(q)$ does not seem to change significantly, as shown in Fig. 6.1. This is consistent with our earlier experimental observation done through light scattering studies [14], 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 [15]. Theoretically, an (O-Z) type of behavior (Eq. (6.1)) has been predicted for the scattered intensities of the polymer solutions in a good solvent. Accordingly, we attempted fitting our neutron scattering data of Fig. 6.2 to a lorentzian behavior as in Eq. (6.1). To fit the data a plot of $S^{-1}(q)$ vs. $q^2$ was made in the range of the scattering vector $1.8 \times 10^{-2} \leq q \leq 2.0 \times 10^{-1}\AA^{-1}$ in Fig. 6.3. Although it showed a good linear fit to the data in the $q$ range of $3.2 \times 10^{-2} < q < 2.0 \times 10^{-1}\AA^{-1}$, it showed deviations from linear fit in the range of scattering vector $q < 3.2 \times 10^{-2}\AA^{-1}$ (the downward curve indicates deviations). A typical situation is shown in Fig. 6.4 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.
Figure 6.3: Inverse structure factor $S^{-1}(q)$ vs. $q^2$ for a GA added concentration of $2 \times 10^{-4}\% \text{ (w/v)}$ in 5\% (w/v) gelatin sample at 60$^\circ$C. In the range of $3.2 \times 10^{-2} \leq q \leq 2.0 \times 10^{-1} \AA^{-1}$, a linear dependence is observed and the slope allows one to calculate $\xi$. At small $q$ vectors in the range of $q < 3.2 \times 10^{-2} \AA^{-1}$, an excess scattering appears (downward curvature) as deviation from the linear fit. Solutions were prepared in phosphate buffer (0.1M) maintained at a pH of 6.8 in $D_2O$.

A linear fit of the structure factor data in the $q$ range of $3.2 \times 10^{-2} < q < 2.0 \times 10^{-1} \AA^{-1}$, simply gives the screening length or the correlation length $\xi$ of the entangled network of the chemically crosslinked gels. Slope of the fit enables one to easily calculate the value of $\xi$. Values of $\xi$ obtained from such a fit are listed in the Table 6.1 for various concentrations of GA at different temperatures. The temperature dependence of correlation length for varying concentrations of GA is shown in Fig. 6.5. As clear from this figure, there is a decrease in $\xi$ 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 the table). The magnitude of this decrease however, is very small. Hence, through SANS measurements, we cannot say with certainty whether this decrease is actually taking place. However, earlier dynamic light scattering (DLS) studies [16] further substantiates this decrease in the value of $\xi$ with decrease in temperature in the above case but the value of $\xi$ measured through DLS was much higher. The values of $\xi$ obtained from DLS are listed in the Table 6.1 as $\xi_{fast}$. The mesh size showed a temperature dependence given by [16]

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

(6.4)
Experimental data
Debye - Bueche
Ornstein-Zernike

Figure 6.4: Plot of the reconstructed structure factor $S(q)$ vs. scattering vector $q$ obtained by adding the two contributions of scattered intensity in the different $q$ ranges of the fitting (for Eq. (6.2)) for a sample of $2 \times 10^{-4}\%$ (w/v) GA in 5% (w/v) gelatin sample at $60^\circ$C. The broken curve represents the Lorentzian behavior with $\xi = 48\text{Å}$ (Eq. (6.1)), the smooth curve is the contribution of the inhomogeneities (Eq. (6.3)) with $\xi = 64\text{Å}$, and the full curve is the total structure factor (Eq. (6.2)) obtained directly from the experimental data.

where $\chi$ is the Flory-Huggins interaction parameter defining the solvent quality and $A$, the appropriate activation energy of diffusion for the unswollen network. An increase in the number of crosslink density with reduction in temperature has also been reported earlier [17]. This possibly causes $\xi$ to decrease as has been observed in the present study. However, for a fixed temperature, a variation in the value of $\xi$ was observed with change in the concentration of the crosslinker 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 (Fig. 6.6). The length scale of $\xi$ follows a power law behavior of $\xi \sim [GA]^\nu$ where $[GA]$ is the concentration of GA and the exponent $\nu$ has a value of $0.33 \pm 0.04$ in the gel state. No 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 variation in the GA concentration was small in the DLS experiments. The highest concentration of GA used in case of SANS is almost three times higher than the same in DLS.
Figure 6.5: Temperature dependence of the correlation length $\xi$ (SANS) for different concentrations of GA in 5% (w/v) gelatin sample. At a fixed concentration of GA, $\xi$ decreases with the decrease in temperature at low concentrations of GA. The DLS value of mesh size $\xi_{fast}$ as 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 $\xi$ (SANS) and $\xi_{fast}$ (DLS) for different concentrations of GA and at different temperatures are

Figure 6.6: Plot of the variation of correlation length $\xi$ as function of added concentration of GA in 5% (w/v) gelatin sample at two different temperatures representing the sol and gel state respectively. A scaling behavior of $\xi \sim [GA]^\nu$ was found where the exponent $\nu$ has a value 0.33 $\pm$ 0.04 in the gel state.
Figure 6.7: Fit of $S^{-1/2}(q)$ vs. $q^2$ to Debye-Bueche model (Eq. (6.3)) in the low $q$ regime of $q < 3.2 \times 10^{-2} \text{Å}^{-1}$ where excess scattering was observed. Slope of the linear fit allows one to calculate the inhomogeneity parameter $\zeta$. The typical data shown in the figure corresponds to case of $2 \times 10^{-4}\%$ (w/v) GA in 5% (w/v) gelatin sample at 60°C. Solutions were prepared in phosphate buffer (0.1M) maintained at a pH of 6.8 in $D_2O$.

Figure 6.8: Temperature dependence of the fitting parameter $\zeta$ for 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, $\zeta$ increases with the decrease in temperature. The values of $\zeta$ for different concentrations of GA and at different temperatures are listed in the table.
Figure 6.9: Plot of variation in the size of the inhomogeneity $\zeta$ vs. the added concentration of GA in a 5% (w/v) gelatin sample at two different temperatures of 60°C and 28°C representing the sol and gel state respectively.

Plot of $S^{-1}(q)$ vs. $q^2$ in Fig. 6.3 shows the excess scattering at low scattering vectors in the range of $q < 3.2 \times 10^{-2} \text{Å}^{-1}$ where a deviation from straight line fitting was observed (Fig. 6.3). Two contributions to the structure factor, the O-Z component and the excess scattering contribution can be realized from the plot of Fig. 6.4 where we have used the numerical value of $\xi$ from the Table 6.1. The excess scattering data has been analyzed within the framework of the Debye-Bueche model discussed earlier [13]. By subtracting the O-Z component from the total structure factor data (through visual estimation of q-cutoff $\approx 3.2 \times 10^{-2} \text{Å}^{-1}$), one can easily get the excess scattering contribution, $S_{\text{ex}}(q)$ (see Fig. 6.4). Next, this $S_{\text{ex}}(q)$ data was fitted to Eq. (6.3). A plot of $S_{\text{ex}}^{-1/2}(q)$ versus $q^2$ was made whose slope gives the parameter $\zeta$. A typical fit for the case of an added GA concentration of $2 \times 10^{-4}$% (w/v) in 5% (w/v) gelatin is shown in Fig. 6.7. The characteristic size of the inhomogeneities calculated from this fit are listed in the Table 6.1. An inhomogeneity of size $\approx 114 \text{Å}$ 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 $\approx R_g/3$. The temperature dependence of inhomogeneity size $\zeta$ for varying concentration of GA...
is shown in Fig. 6.8. The figure shows an increase in the value of $\zeta$, with decrease in temperature at a fixed concentration of GA which is again negligible in the case of pure gelatin. However, for a fixed temperature, a variation in the size of inhomogeneity is observed for change in the concentration of the crosslinker GA. Addition of GA to pure gelatin decreases the value of $\zeta$ on the first hand but the opposite happens if GA is added further (see the table). The behavior of parameter $\zeta$ with GA concentration is plotted in Fig. 6.9. 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. [9].

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 [16]. The relaxation time, $\tau_c$, 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. [18], Amis et al. [19, 20] and Herning et al. [21] among others. Amis et al. [19, 20] and Herning et al. [21] 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 crosslink sites of gelatin [22, 23] to be of a crystalline triple-helical structure like collagen [24]. A critical size in the range of $\sim 100$ nm has been reported after a number of investigations [25, 26, 27]. Considering these facts, we have attributed the slow-diffusive mode to be arising from the 'clusters' that may be the junction zones in gelatin and $\xi_{slow}$ is a measure of the length scale of these clusters. Assuming $\tau_c$ 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 $\eta$, the solvent viscosity, length scale $\xi_{slow}$ associated with the slow mode can be calculated. The value of $\xi_{slow}$ although had no GA concentration dependence in the sol state, showed a sharp increase in the value of $\xi_{slow}$ with increase in the concentration of crosslinker GA for the gel state. The average value of $\xi_{slow}$ in sol state is $\sim (400 \pm 50)$ nm, a value comparable to the size of a triple helix. It is worth debating why the mesh size obtained from SANS and DLS fast mode are different.
Table 6.1: Values of the correlation length $\xi$ and size of the inhomogeneities $\zeta$ obtained after fitting of the experimental data to Eq. (6.1) and (6.3) respectively. The values listed are above (sol state) and below (gel state) the gelation temperature ($\sim 32^\circ$C) for different added concentrations of GA in 5% (w/v) gelatin sample prepared in phosphate buffer (0.1M) maintained at a pH of 6.8 in $D_2O$. Comparative $\xi_{fast}$ values derived from DLS are also listed along with the SANS measurements.
6.5 Conclusion

Our investigation of the chemically crosslinked gelatin solutions and gels using SANS (small angle neutron scattering) reveals the existence of two kinds of length scales. One of them being the correlation length $\xi$, associated with the size of the entangled transient network and the other having a much higher length scale can be attributed to the long-range concentration fluctuations due to the inhomogeneities present in these solutions. A physical picture of gelatin gels chemically crosslinked in GA was provided by Oikawa and Nakanishi [17] where the total crosslink density, $\nu_e$, was assumed to be the sum of the physical crosslink density $\nu_{e,\text{phys}}$, and the chemical crosslink density, $\nu_{e,\text{chem}}$. They calculated the value of $\nu_{e,\text{chem}}$ assuming the chemical crosslinking sites to be tetra functional. Based on the assumption that crosslinking sites in case of GA are tetra functional and the length scales derived we have drawn a schematic structure of the chemically crosslinked gelatin gels with appropriate length scales as shown in Fig. 6.10. We have seen shrinking of the mesh size of the entangled network due to addition of little amount of croslinker GA to a pure solution of gelatin. A swelling effect, of course, was observed on further addition of the crosslinker which is clear from Fig. 6.5. This swelling is caused by the increase in the osmotic pressure because of increase in crosslinked density inside the gel network that makes it expand further. A similar effect was observed for the concentration dependence of the size of the inhomogeneities present in a solution at a fixed temperature (Fig. 6.8). However, an opposite behavior for parameter $\zeta$ was observed for a fixed concentration of GA with varying temperature i.e., the sizes of inhomogeneities increased 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, such effects of increase in the size of the entangled transient network or the mesh size was also observed in dynamic light scattering (DLS) experiments of the chemically crosslinked gelatin solutions and gels [16]. The mesh sizes measured from the DLS studies varied from $(210 \pm 20)\, \text{Å}$ to $(38 \pm 2)\, \text{Å}$ as solution moved from the sol to the gel state. Also, the presence of inhomogeneities in such solutions is not unique to SANS studies. Our DLS studies on similar samples have also substantiated the argument that chemically crosslinked gelatin solutions are not
homogenous. 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. However, length scale derivable from the slow mode relaxation time, $\xi_{\text{slow}}$ has been argued to represent the size of crystalline triple-helical junction zones that has structure like collagen. We observed negligible dependence of $\xi_{\text{slow}}$ on the GA concentration in the sol state whereas it increased sharply in the case of a gel as opposed to case of Oikawa and Nakanishi [17] where no observable dependence was found in both the cases of sol and gel. A Zimm dynamics was proposed for the motion of polymer chain in such solutions [16].

In essence, both the SANS and DLS studies have suggested the presence of inhomogeneities in the aqueous semi-dilute solution of chemically crosslinked gelatin gels. 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. [9] 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 [25, 28]. 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 micro-gels 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 [9]. In yet another experiment, it was found to depend on experimental conditions of sample preparation [11]. However, unanimity could not be achieved about the origin of excess scattering.
Bibliography


