This Chapter:

Polarised static laser light scattering experiments have been reported on dilute sols, semi-dilute sols and gels of gelatin in the concentration range of 0.05% to 10% (w/v) at various ionic strengths. The static structure factor \( S(q) \) and the helix contents \( \chi \) of the gelling solutions have been deduced from the measured intensity of scattered light, \( I_{VV}(q) \) data and compared with optical rotatory dispersion (ORD) measurements. Below the threshold gelation concentration \( c^* \), \( S(q) \sim q^{-d} \) with \( d = 2.15 \pm 0.08 \) in conformity with Guinier results. Above this concentration at any temperature \( S(q) \) could be empirically described as \( S(q) = (1 - \chi) \exp(-q^2 R_g^2/3) + \chi \exp(-q^2 L^2) \); for \( R_g \) being the radius of gyration of gelatin chain and \( L \) being the length of the triple helices formed. In the gel state, this yielded two characteristic length scales; one corresponding to \( R_g \) (at large \( q \)) and another corresponding to \( L \) (at small \( q \)) as opposed to the single length scale \( R_g \) observed in all sol states.
Chapter 3

Polarised Light Scattering Study from Gelatin Solutions and Gels

3.1 Introduction to Polarised Scattering

In the treatment of scattering described in the subsection 2.1.5, it was considered that a periodic plane polarised wave incident upon a molecule induces a forced oscillation of bound charges synchronous with the applied field. Since the bound charges oscillated in the same direction as the electric vector of the incident light, the transversely scattered light should be completely polarised in the vertical direction. However, the light scattered at 90° from solutions, as well as gases and pure liquids, is found in general to be incompletely polarised, that is, there is both a horizontal and vertical component, the ratio of which is called the depolarisation value. The quantitative consideration of this effect is necessary for two reasons. First, all the horizontally polarised light and a small amount of the vertically polarised light originate, not at the expense of the scattered light previously considered, but from a new effect. Consequently, the absolute intensity of scattered light is greater than that calculated from Eq. 2.5, and is not directly proportional to the square of the polarizability. It is clear that this effect must be taken into account when measured turbidities are used for molecular weight determinations. Second, since the explanation of depolarisation must lie in the fact that the scattering centers, the molecules, are asymmetric and anisotropic, it is reasonable to expect that, if the relation between cause and effect can be determined, depolarisation measurements will contribute to our knowledge of the size and shape of molecules. These two aspects of depolarisation of scattered light will now be discussed in details.
Depolarisation due to molecular anisotropy

From the theoretical point of view, the scattered intensity is proportional to the square of the induced moment, which, in turn proportional to the square of the polarizability for a given field. \( \mathbf{E} \) i.e., \( \mathbf{p} = \alpha \mathbf{E} \). The induced moment will have the same direction as the incident electric vector only if the polarizability is independent of the direction; this situation can only occur in the case of small spherical, non-interacting, symmetric and isotropic molecules. But due to the asymmetry factor and multiple scattering [see section 2.1.5] depolarisation effect comes into picture. In general case, \( \alpha \) depends on the direction and actually a tensor. The induced dipole moment depends in general on the orientation of the molecule with respect to the incident electric field of the light. Because a molecule continuously reorients, the magnitude and direction of its induced dipole moment fluctuates which leads to a change in the polarization and the electric field strength of the light emitted by the fluctuating induced dipole moments. Therefore the equation for induced dipole moment is written as follows,

\[
\mathbf{p}_i = \sum_j \alpha_{ij} \mathbf{E}_j
\]

(3.1)

where, \( i \) symbolizes for \( x, y \) and \( z \). It will always be possible to find a rectangular coordinate system for which the nondiagonal terms of the tensor vanish. The three axes of such coordinate system correspond to the three principal polarizabilities of the molecule. Due to this anisotropic scattering, the scattered light has two components \( I_{VV} \) and \( I_{VH} \) though the incident beam was polarised. \( I_{VV} \) is the intensity of the Vertically polarised scattered light when incident beam is Vertically polarised and \( I_{VH} \) is the intensity of the Horizontally polarised scattered light when incident beam is Vertically polarised.

Depolarisation due to molecular size

It is clear from the previous description that the depolarisation of light from molecules is intimately connected with their asymmetry and anisotropy. In general, the depolarisation is a measure of the deviation of the scattering particles from isotropic spheres [1]. However, if the scattering particles are about one-tenth of the wavelength of light or more in their largest dimension, the effect of size, independent of anisotropy, will give rise to depolarisation. Consequently, depolarisation measurements on polymer solution are likely to reflect a combined effect due to size and anisotropy. Mie solved this problem considering a spherical particle of higher refractive index as shown in Fig. 3.1. Refraction causes the incident electric vector to pass through the particle at an angle so that the light scattered transversely in the plane of the paper will possess a horizontal component, \( I_{VH} \) as well as vertical component \( I_{VV} \). Complete interference is not possible because the scattering from
different parts of the plane shown in figure will not be in phase because of its large size. So by measuring $I_{VV}$ and $I_{VH}$, it is possible to get the size and anisotropy of the examined molecule.

### 3.2 Importance of helices in gelation dynamics

Our sample is gelatin gels, which are considered to be a novel state of matter in view of their ability to trap a large amount of solvent in the core. The structural characterization of chemically cross-linked gels and physical gels is of primary importance for understanding the mechanism of phase transitions. As opposed to the chemical gels that are formed as a result of primary forces (like covalent bonding), the physical gels are formed and stabilized in the solvent mostly through secondary forces (like hydrogen bonding, Van der waals forces, hydrophobic interactions etc.). The chemical gels have been generally understood and described through percolation theory [2, 3, 4]. Since the dynamics of formations of physical gels are completely different, need arises to treat them differently.

Gelatin which is denatured collagen, undergoes thermoreversible gelation in hydrogen-bond friendly environment, when the polypeptide concentration is higher than typically
1% (w/v). A lot of effort have been devoted in the past to study the kinetics of gelation of this polypeptide. Considering the fact that this is the most abundantly found polypeptide in mammals and its wide use in food, pharmaceutical and cosmetic industries, the importance of such studies can be hardly stressed. Despite this, the exact conformational path undertaken by this protein in transforming the fluid sol state to the solid-like gel state has not been established conclusively. Djabourov and co-workers reported excellent studies on the conformation of gelatin chains in aqueous solutions and the gelation mechanism of gelatin in various thermodynamic environments [5, 17, 18, 13]. Busnel [1] et al. have interpreted the renaturation kinetics of gelatin solutions using data from a variety of experimental techniques and have discussed the observed features through model Monte Carlo simulations. They proposed renaturation of gelatin to collagen through bimolecular and trimolecular inter and intra-molecular conjugation models.

The renaturation of gelatin to collagen occurs due to the formation of interconnected triple helices through intermolecular (for higher concentrations) and intramolecular interactions (for lower concentrations) [1]. The dispersion medium, which in most cases is aqueous provides a hydrogen bond friendly environment. For gelatin, the initial helix formation step is known to be associated with a high activation energy barrier, hence is very slow. The propagation of these helices has been modelled through various schemes guided by experimental observations. The propagation is controlled by cis-proline residues present in the backbone of gelatin molecule. Busnel et al. have studied various nucleation models to provide a comprehensive answer to the triple helix "Zipping" rate in gelling solutions of gelatin, but with limited success [1, 11, 11]. The earlier "fringed micelle" model could not explain the experimental data either [12]. The generally accepted model seems to be the formation of junction zones between helical segments and random coil gelatin molecules. Each molecule participates in the formation of numerous such junction zones. In the gel state, the triple helices continuously reorganize themselves to increase the propagation of hydrogen bonds in the helical conformations and thus minimize the free energy.

3.3 Sample Preparation and Experimental Procedure

Gelatin was purchased from M/S Loba Chemie (Indo-Astranal Co. India) batch No. 3920 with maximum nominal impurities as follows: Sulphate ash = 1.5%, SO₂ = 2 × 10⁻⁴% and heavy metals (Zn, Cu, Pb) in concentrations lower than SO₂ concentration. This preparation is devoid of any E-coli and liquifier presence. This material was used without further purification. The solvent was double distilled water. The ionic strength of the solvent was fixed to 0.1M NaCl and NaN₃ was added with a concentration of 1mM
to prevent bacterial growth. The solvent was now ultracentrifuged for 1 hr. to remove dust and the clean solvent was used for dissolving the gelatin powder. The pH was adjusted to 7.0 in a tris buffer. The stock solution had a prepared concentration of 10% (w/v) and three other solutions were prepared by suitably diluting the stock solution. The solutions were prepared at $T = 55^\circ C$. All the solutions were transparent when prepared. Next, the solutions were transferred to previously cleaned glass test tubes. These were now stored in a low temperature bath with the temperature set at $T = 5^\circ C$ for about one month. No noticeable cracks or fungal developments were observed in the gels after this period. This set of four solutions had concentrations of $c = 4\%, 6\%, 8\%$ and 10% (w/v). The dilute solutions had concentrations 0.05%, 0.07%, 0.15% and 0.30% (w/v). These did not gel upon cooling below 15°C over extended period of time (~1 month). The scattering measurements were carried out by using a Homemade scattering set-up. It comprised of a standard goniometer arrangement. The scattering source was an Aerotech (He:Ne) laser (randomly polarised) delivering a power of 15 mW. The detection system was comprised of a highly sensitive photodiode (Hamamatsu S-2281-01) and a photosensor amplifier (Hamamatsu C-1837). The amplified photocurrent was measured by a standard digital multimeter. The temperature regulated sample chamber located at the centre of the goniometer had a design identical to that described in ref. [14]. The intensity of the scattered light was measured at $20^\circ \leq \theta \leq 140^\circ$ in all cases. The samples held inside the regulated temperature bath were slowly heated from 10°C to 60°C over a period of 160 minutes. Care was taken to ensure that all the samples were subjected to an identical heating path. In this sample chamber the temperature could be held fixed to ±0.1°C of the desired temperature. In actual experiments the laser light was rendered linearly polarised by using a polariser and the detection optics was fitted with an analyzer, the $I_{VV}(q)$ components of the scattered light were detected by suitably adjusting the mutual alignment of these two. The system was first calibrated by using benzene as the test sample. Data were collected over the scattering angles corresponding to wave vectors $6.8 \times 10^4 < q < 2.5 \times 10^5$ cm$^{-1}$. The experiments were carried out in two steps: First the solvent was loaded into the sample holder and the scattered intensities (polarised) were measured at different scattering angles. In the second step gelatin samples were used and the experiments were repeated. The reported $I_{VV}(q)$ values used in data analysis are the solvent subtracted values. Thus the contribution from the solvent to the scattering intensities has been minimized if not eliminated altogether. The temperature of the samples was monitored both at the bottom and the top of the sample cell to ensure the absence of thermal gradients. All the reported parameters like $T$, $I_{VV}(q)$ etc., were averaged over at least five independent measurements.

The optical rotation angle $\theta$ was measured automatically with a Perkin Elmer 241 MC polarimeter operating at a wavelength of 589.3 nm, with a precision of ±0.001°. The cells had an optical path of 10 cm and a volume of 5 ml. The temperature was controlled by an external bath circulating into the jacketed cells and measured with a thermocouple inserted into the cell, with an accuracy of ±0.1°C. The helix content was deduced
from [14]

\[ \chi = \frac{[\theta]_\lambda^m - [\theta]_\lambda^{\text{collagen}}}{[\theta]_\lambda^{\text{collagen}} - [\theta]_\lambda^{\text{coil}}}; \quad \lambda = 589.3 \text{ nm} \]

\([\theta]_\lambda^m\) was the measured specific rotation corresponding to rotation angle \(\theta^m\) and was \(= \theta^m/c.l\); \(c\) was the concentration of gelatin and \(l = 10\) cm. \([\theta]_\lambda^{\text{collagen}}\) was taken as \(= (800\pm10)\) from ref.(20) and \([\theta]_\lambda^{\text{coil}}\) corresponded to the rotation angle \(\theta\) for the gelatin sol at 55°C = \((118\pm10)\). Further discussions as ORD can be found elsewhere [15].

### 3.4 Light Scattering from Semi-dilute Gelatin Solutions and Gels

When the concentration \((c)\) of a polymer solution is increased beyond a threshold value \((c^*)\), the chains start to overlap. This can result in the formation of cross-linked networks for solutions having \(c>c^*\). These crosslinks can be formed through the mediation of covalent bonds or through secondary forces like hydrogen bonds, Van der waals interactions etc. The overlap concentrations can be deduced from the measurement of intrinsic viscosity [25] [\(\eta\)] of the solution. And

\[ c^* \approx \frac{1}{[\eta]} \quad (3.2) \]

For flexible polymers of equivalent hydrodynamic radius \(R_h\)

\[ [\eta] = \frac{10\pi N_A}{3M_w} R_h^2 \quad (3.3) \]

The ratio \(R_g/R_h\) is a shape dependent constant. Hence, \(c^*\) is a function of effective size of the chain and its molecular weight. In gelatin \(c^*\) is often defined as the concentration above which the sol yields a gel upon cooling. The typical values of \(R_h \approx 22\) nm for gelatin molecules having molecular weight of \(M \approx 2 \times 10^5\) Daltons measured by us earlier [17]. This implied that \(c^* \approx 1\% \text{ (w/v)}\). Our semi-dilute samples had concentrations much higher than this \((4\% \text{ to } 10\% \text{ (w/v)}\). It has been observed earlier that gelatin molecules manifest themselves as either random coils or helices in the solution phase. The onset of gelation occurs when the helix formation reaches a threshold value [14].

The polarised scattered light for such a system can be written as

\[ I_{VV}(q) = K_c c M_e S_c(q) + K_h c_h M_h S_h(q) \quad (3.4) \]
where $K_c$ and $K_h$ are contrast factors for the coil or helix solutions. The concentrations and molecular weight of coils and helices are designated as $c_c$, $M_c$ and $c_h$, $M_h$ respectively and coil and helix structure factors are $S_c(q)$ and $S_h(q)$. It is not very meaningful to assign a molecular weight parameter $M_h$ to the helix since these are interconnected through intermolecular hydrogen bonds. Nonetheless for the analysis that follows this assumption bears no specific anomaly.

In the sol state much above the gelation temperature, $T \gg T_{gel}$ the molecules are most likely to have random coil conformation and helix structures are mostly absent. Similarly close to and below the gelation temperature $T_{gel}$, there is a propensity of helices in the solutions, hence Eq. 3.4 reduces to

$$I_{VV}(q) = \begin{cases} K_c c_c M_c S_c(q) & ; T \gg T_{gel} \\ K_h c_h M_h S_h(q) & ; T \leq T_{gel} \end{cases}$$  \hspace{1cm} (3.5)

This implies as $q \rightarrow 0$,

$$I_{VV}(q) \bigg|_{q \rightarrow 0} = \begin{cases} K_c c_c M_c & ; T \gg T_{gel} \\ K_h c_h M_h & ; T \leq T_{gel} \end{cases}$$  \hspace{1cm} (3.6)

For semi-dilute sol state $S_c(q)$ is normally expressed as

$$S_c(q) \sim \exp(-q^2 R_g^2/3)$$  \hspace{1cm} (3.7)

Deduction of $S_h(q)$ from the structure factor equation for interconnected triple helices is not trivial. But from Eqs. (3.6) and (3.7) one obtains

$$S_x(q) = \frac{I_{VV}(q)}{I_{VV}(q) \bigg|_{q \rightarrow 0}} = \frac{I_{VV}(q)}{K_x c_x M_x} ; \text{ for } x = c, h$$  \hspace{1cm} (3.8)

Again the degree of helicity ($\chi$) can be inferred from the ratio

$$\chi = \frac{\text{Fraction of molecules in helix state}}{\text{Fraction of molecules in coil state}} = \frac{I_{VV}^{M}(q) - I_{VV}^{coil}(q)}{I_{VV,10\%}^{gel}(q) - I_{VV,10\%}^{coil}(q) \bigg|_{q \rightarrow 0}}$$  \hspace{1cm} (3.9)

where $I_{VV}^{coil}(q)$ and $I_{VV}^{gel}(q)$ are scattered intensities at temperatures far above (55°C) and far below (≈ 15°C) the gelation temperature of gelatin, $T_{gel} \sim 25^0C$. $I_{VV}^{M}(q)$ is the measured intensity at temperature T. Based on Eqs. (3.7), (3.8) and (3.9) it is possible to define the structure factor of any gelling solution empirically as

$$S(q) = (1 - \chi) \exp(-q^2 R_g^2/3) + \chi \exp(-q^2 L^2)$$  \hspace{1cm} (3.10)

In a pure gel state much below $T_{gel}$, only the second term on the right would prevail. Similarly, for hot sols, $T > T_{gel}$, $\chi \sim 0$ and Eq. 3.10 would converge to Eq. 3.7. For all intermediate states Eq. 3.10 would empirically define the structure factor of the solution.
3.5 Results and Discussions

3.5.1 Dilute solutions (c < c*)

The polarised, $I_{VV}(q)$ component of scattered light was measured for four different concentrations of gelatin (0.05%, 0.07%, 0.15% and 0.30% (w/v)). These were well below the known gelation concentration of gelatin. The double logarithmic plot of $S(q)$ vs. q is shown in Fig. 3.2. The slope of the linear part comes out to $-(2.15 \pm 0.08)$. This compares well with the theoretical value of $-2$. These solutions did not form gels upon cooling below 15°C. The radius of gyration of the molecules were derived by fitting Eq. 3.7 to the $S(q)$ versus q data for these four samples. The measured $R_g(c)$ values were plotted as function of concentration ‘c’ and the extrapolation to $c \to 0$ yielded $R_g = 51 \pm 4$ nm. It has been shown in the past that gelatin manifests itself as random coils below the overlap concentrations [7,17]. For $R_g < q^{-1}$ one would expect the Guinier regime to be prevailing and the structure factor $S(q)$ would scale as $q^{-d}$ with $d = 2$. Our results come very close to this. Below the Kratky and Porod regime [18, 19] of high q ($q \to \infty$), $S(q)$ is known to scale as above for a variety of physical situations. For fractals, ‘d’ becomes the fractal dimension of the chain. The dilute state behaviour of gelatin solutions has been studied in greater details earlier and hence will not be discussed further [7,17].

3.5.2 Semi-dilute solutions and gels

Figure 3.3 shows the typical plot of $I_{VV}(q)$ vs. q for both the sol and gel states of gelatin. The lower q portions of the data are plotted separately in Fig. 3.4 and these have been extrapolated to $q \to 0$ to determine ($K_c c_c M_c$) and ($K_h c_h M_h$) from the intensity axis intercepts. The inset of Fig. 3.3 shows the evolution of the static structure factor as the sol evolves to the gel state. Figure 3.3 also indicates the variation of $I_{VV}(q)$ as function of concentration of the polypeptide both in the sol and gel states. In the sol state these curves appear parallel to each other with solutions having higher concentration of gelatin scattering more light implying that $I_{VV}(q) \sim c$, $c$ being the net gelatin concentration. This further indicated that $M_c$ changed very little as gelatin concentration was raised by more than two fold from 4% (w/v) to 10% (w/v) at 55°C. The contrast factor $K_c$ depends on the solution parameters as

$$K_{c,n} \simeq n_o^2 \left( \frac{\partial n}{\partial c} \right)_T^2$$ (3.11)

where $n_o$ and n are the refractive indices of the solvent and solution respectively. Earlier measurements [17] gave $(\partial n/\partial c)_T = (0.142 \pm 0.04)$ measured at $\lambda = 6328$ Å.
Figure 3.2: Plot of static structure factor log $S(q)$ vs. log $q$ for 0.3% (w/v) solution of gelatin at $T = 30^\circ$C. The slope of the least squares fitted line to the data points is $(2.15 \pm 0.08)$ as predicted by Eqn. 2.44.

Figure 3.3: Plot of scattered intensity $I_{VV}(q)$ as function of scattering vector $q$ for various gels and sols of gelatin. The sols were at $T = 55^\circ$C and gels were at $T = 15^\circ$C. The inset shows the structure factor of the sol state (filled squares) and gel state (filled circles) determined as per Eq. 3.8. Notice the broadening of $S(q)$ as the sol evolves to gel. Solid lines are least squares fitting to the data points, fitted to Eq. 3.7 for sols and Eq. 3.10 for gels, plotted in Fig. 3.6(a) and (b).
Hence, for all the four concentrations $K_e$ was almost a constant. A plot of $K_eeM_e$ as a function of $c_e$ would yield a straight if our contention of $M_e$ remaining independent of $c_e$ were to be correct. Figure 3.5 shows a similar plot, which provided an excellent least, squares fitting to a straight line, giving support to our claim of $M_e$ remaining independent of gelatin concentration in the sol state far above the gelation temperature $T \gg T_{gel}$.

Figure 3.3 (inset) shows the structure factor of the semi-dilute sol state and this could be least square fitted to a function like $S_e(q) \sim \exp(-q^2R_g^2/3)$ to a good accuracy. However for the gel state the data was fitted to a double exponential function defined by Eq. 3.10 and fitting was excellent. Here $R_g$ and $L$ were adjustable parameters with $\chi$ preset to our measured values; listed in Table-1. A typical set of results are shown in Fig. 3.6. At large q-values, one is probing the sample over a smaller length scale and is likely to observe centre of mass diffusion of free chains as dominating scattering centres. This gave the values of radius of gyration at various concentrations of gelatin. These are listed in Table-1.

<table>
<thead>
<tr>
<th>Concentration (w/v %)</th>
<th>Parameter</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_g$ (nm)</td>
<td>40±2</td>
<td>47±3</td>
<td>53±3</td>
<td>60±3</td>
</tr>
<tr>
<td></td>
<td>L (nm) (gel)</td>
<td>218±12</td>
<td>225±20</td>
<td>220±15</td>
<td>224±18</td>
</tr>
<tr>
<td>$\chi^{30^o C}$(%)</td>
<td>LS</td>
<td>0.26±0.22</td>
<td>0.29±0.20</td>
<td>0.52±0.14</td>
<td>0.65±0.10</td>
</tr>
<tr>
<td>$\chi^{30^o C}$(%)</td>
<td>ORD</td>
<td>0.17±0.01</td>
<td>0.20±0.01</td>
<td>0.27±0.01</td>
<td>0.35±0.01</td>
</tr>
<tr>
<td>$\chi^{15^o C}$(%)</td>
<td>LS</td>
<td>0.40±0.20</td>
<td>0.56±0.15</td>
<td>0.71±0.15</td>
<td>1.00±0.10</td>
</tr>
<tr>
<td>$\chi^{15^o C}$(%)</td>
<td>ORD</td>
<td>0.32±0.01</td>
<td>0.39±0.01</td>
<td>0.47±0.01</td>
<td>0.59±0.01</td>
</tr>
</tbody>
</table>

At smaller q-values, a much bigger length scale would yield the scattering originating from larger scattering entities and these are being identified as the triple helices of length $L$ formed in the gelling solutions. Similar arguments have been used earlier by
Figure 3.6: (a) Plot of $S(q)$ vs. $q$ for a 6% (w/v) gel at $T = 15^\circ C$. The inset shows $S^{-1}(q)$ vs. $q^2$ where two length scales are evident. At lower $q$, the triple helix length $L$ defines the characteristic length scale and at higher $q$ values the length scale is defined by radius of gyration $R_9$ of free gelatin chains. (b) Variation at $R_9$ and $L$ as function of gelatin concentration for gel state ($T = 15^\circ C$) open and filled squares; and for sol state ($T = 55^\circ C$) open circles. These were deduced from least squares fitting of Eqs. 3.7 and 3.10 to data of Fig. 3.3.
Coviello et al. [20] in their studies of thermoreversible gelation in a polysaccharide which formed intermolecular double helices upon renaturation. The variation of these parameters with concentration is shown in Fig. 3.7. Busnel et al. [1] studied the renaturation kinetics of gelatin using optical rotation and UV absorption techniques. The measured data were analysed using a linear sum of contributions from a constant rate intramolecular nucleation and a biomolecular nucleation. In the dilute regime \( \mathrm{c} \sim 1\% \, \mathrm{(w/v)} \); the gelation mechanism could be well described by the intramolecular nucleation. Quantitatively, their observation of increased intermolecular nucleation at higher concentrations of gelatin agrees with our measurements where we did see the \( I_{\mathrm{VV}}(q) \big|_{q=0} \) in the gel state increasing rapidly with the concentration, consistent with the Harrington model (Fig. 3.7).

Qualitatively \( S_h(q) \) does not appear any different from \( S_c(q) \) which implies that the sol to gel transition was a first order thermodynamic phase transition consistent with the Flory model [21]. The differential scanning calorimetry studies provide strong support to this argument. This is in spite of the fact that the relaxation dynamics of a gelling gelatin solution exhibits three hierarchically constrained relaxation regimes and the origin of these individual regimes is still under considerable debate. Harrington and coworkers have discussed several second order kinetic processes to describe the gelation of gelatin [22, 23].

Busnel et al. have measured the molecular weight of the helices in a gelling solution [11]. However in our analysis the apparent molecular weight of the helix \( M_h \) cannot be extracted from \( K_h c_h M_h \) data. For reasons cited earlier it is not a physical parameter since the triple helices are intertwined and no isolated triple helix exists in the gelling solution. Nonetheless the structure factor \( S_h(q) \) could be deduced using Eq. 3.10 and is plotted in Fig. 3.3.

### 3.5.3 Degree of helicity

The degree of helicity \( \chi \), determined according to Eq. 3.12 for different concentrations of gelatin gels are listed in Table-1. The corresponding values measured using ORD technique are listed in the same table for comparison. These are plotted in Fig. 3.7. The helix content in the gel phase as a function of concentration of the protein has been plotted in this figure. The data concurs with the ORD measurements. The concentration dependence of helix content in the gel phase could be expressed as

\[
\chi = \chi_0 \left[ 1 + \left( \frac{\partial \chi}{\partial c} \right)_T (c - c^*) \right] \quad (3.12)
\]

Least square fitting of data in Fig. 3.5 yielded \( (\partial \chi / \partial c)_T = 1.2 \pm 0.1 \) and \( \chi_0 = 0.08 \pm 0.005 \). The helix content at concentration \( c = c^* \) has been defined as \( \chi_0 \). These results qualita
Figure 3.7: Degree of helicity $\chi$ vs. gelatin concentration measured at $T = 30^\circ C$ and $15^\circ C$. The filled symbols corresponds to LS data (Eq. 3.9) and open symbols corresponds to ORD data. The LS data is symmetrically $\sim 50\%$ higher than $\chi$ values from ORD data due to reasons cited in the text.

Figure 3.8: Dependence of $R_g$, L and $\chi$ on the ionic strength of the solution for 6% (w/v) sample of gelatin in gel state at $15^\circ C$ and the gelling solution at $30^\circ C$. Open squares corresponds to values of L, open circles to $R_g$ (both left scale) and filled squares correspond to gel state values of $\chi$ at $T = 15^\circ C$ and filled circles to $T = 30^\circ C$ (both right scale).
tively agree with the general observations of Djabourov et al. performed on thermally quenched and annealed samples [14]. The helix content reflects the degree of renatured structure and its degree of thermal stability. Based on these studies Djabourov et al. argued that the distribution of length of the helical sequence, local distortions along the helix with respect to their equilibrium positions and crystallization effects were responsible for inducing instability into the gels containing higher fraction of helices. A gel with high $\chi$ is less stable then a gel with lower $\chi$ value. However either a conclusive experimental or theoretical evidence supporting this contention is lacking. The degree of helicity measured by LS was found to be systematically higher than the corresponding ORD measured values by approximately 50%. This can be attributed to the way Eq. 3.12 has been normalized. Ideally, the normalization should be with respect to the $I_{VV}(q)$ of collagen. Since this value was not available, hence normalization was done with respect to the highest concentration data of $I_{VV}(q)$ available with us.

3.5.4 Effect of NaCl concentration

Effect of ionic strength on the degree of helicity ($\chi$) was studied by taking measurements on 6%(w/v) gelatin sol prepared at three different NaCl concentrations, namely 0.01M, 0.1M and 1M. The values of $\chi$ measured remained well within the uncertainty value of $\chi$ listed in Table-1 for a 6% gelatin sample both in sol and gel states. These are plotted in Fig. 3.8 along with the corresponding $R_g$ and L values of the gel state. The fact that change in NaCl concentrations did not change the degree of helicity $\chi$ implied that electrostatic interactions were not playing an active role in formation of these helices (see Fig. 3.8). This has been observed earlier too [13]. Hence, the secondary forces alike hydrogen bonding, Van der waals interactions and hydrophobic interactions seem to be dynamically stabilizing these structures. Also, it has been clearly established in the past that coil-helix conformational transitions is a necessary condition for the onset of gelation in this biopolymer [24, 25].
Bibliography


