Chapter 6
Hierarchical Structures in Sol, Gel and Coacervate Phases of Gelatin

6.1 Introduction

A macromolecule can remain in equilibrium with its solvent at room temperature in three physically distinct states namely; solution [1-3], gel [4-9] and coacervates [10-11]. Gelatin forms physical gels in hydrogen bond friendly solvents above a concentration larger than the gelation concentration $c_g (~2\% \text{ w/v})$ that relates to the intrinsic viscosity [$\eta$] as $c_g = 1/\eta$. The gelatin sol undergoes a first-order thermo-reversible gelation transition for temperatures $T < T_g$, where $T_g (~30 \degree C)$ is the gelation temperature. The schematic representation of sol-gel transition is described in section 1.2 (see Chapter 1).

Normally, high molecular weight (long chain) polymers are chemically crosslinked in the solvent medium to generate a chemical gel, which are rigid and can sustain significant amount of compressional and shear deformations. These gels are generally understood and described through percolation theory. On the other hand, physical gels are formed and stabilized in the solvent mostly through secondary forces like hydrogen bonds, van der Waal forces, hydrophobic interactions etc. Physical gels are fragile unlike chemical gels that are formed due to the prevalence of primary forces. However, as the incipient gel phase is approached, the system gradually develops an equilibrium modulus $G_e$ that increases with crosslink density. The second common feature that binds the two is the evolution of an infinitely large and interconnected network with a characteristic correlation length $\xi$ (mesh size) at the gelation point. In the percolation model, the correlation length $\xi$ and mass $M$ of the network diverge at the gelation point (defined by temperature $T_g$) following the scaling relation: $M \sim \varepsilon^{-\gamma}$ and $\xi \sim \varepsilon^{-\nu}$ for $\varepsilon = (T/T_g)-1$. The 3-d Ising model predicts $\gamma = 1.24$ and $\nu = 0.63$, which is consistent with the description of critical phase transitions observed in ferro-magnets, liquid-gas phase transitions and binary fluid mixtures. The percolation theory predicts similar scaling features with different numerical values, for example $\gamma = 1.80$. There is considerable
debate in the literature pertaining to the applicability of such universal scaling laws to physical gel systems because of the significant difference in their structures. Unlike chemical gels that have a single characteristic length scale given by $\xi$, the physical gels can have a multitude of length scales. It has been shown that gelatin gel is associated with the existence of a hierarchy in characteristic length scales like the mesh size ($\sim 5$nm), triple-helix length size ($\sim 200$nm), size of embedded heterogeneity ($\sim 15$nm) etc [12]. Such observations have been made for other bio-gels too. The propensity of triple helices offer topological hindrance to the motion of individual unbound chains and a variety of complex phenomena have been observed in gelatin gels [12-14]. Unlike gelation phenomenon, coacervation is a specific type of polyelectrolyte complex formation. On the contrary very little is known about the structure of gelatin coacervates. Simple coacervates are formed through sequential self-charge neutralization of gelatin molecules that are mostly intermolecular. This is described in excellent details in Chapters 2, and 4.

In the present chapter, I have reported CD, UV absorption, SANS, rheology, DSC and AFM experiments were performed on gelatin coacervates in order to provide some understanding to its microscopic structure and to deduce the hierarchy of length scales present. The comparative microscopic structural study of gelatin sol ($60 ^\circ$C) and gel ($25 ^\circ$C) samples (13% w/v in aqueous medium) are characterized by small-angle neutron scattering (SANS), and rheology techniques. The concentrations of the polypeptide in sol and gel phases were intentionally kept at 13% (w/v) to allow comparison between the three states, which constitutes the main objective of this paper. Such information is not available in the literature even for simple systems. Considering the application potential of coacervates the necessity of such studies can be hardly stressed. It has been shown in the past that hierarchies of length scales exist in single and multiphase systems close to spinodal decomposition [15]. A clear manifestation is the wave vector ($q$) dependence of Onsager kinetic coefficient ($\Lambda$) at distances ($r$) less than typical size ($R_g$) of a polymer chain, which has been discussed in the past. The length scale hierarchy is discussed within the purview of spinodal decomposition models [15,16].
6.1.1 Phenomenology of alcohol induced coacervation:

The phenomenology of alcohol induced coacervation (see Figure 6.1), which was discussed earlier is refined with inclusion of additional supporting data obtained from CD, UV absorption and SANS measurements. Addition of alcohol creates marginal solvent environment for gelatin molecules largely because of the rupture of hydrogen bonds between water molecules and the polion. These results in the reduction of the overall spatial extension of the polyelectrolyte chain, thereby, bringing the complementary charged segments closer (see Chapter 4).

As the ethanol concentration is increased the physical parameters like refractive index, dielectric constant, relative mass density, relative viscosity and surface tension of the water-ethanol mixture undergo significant change. This data is shown in Figure 6.2, which reveals that apart from surface tension and relative viscosity other parameters change in a monotonous manner and are structureless. It is shown (see Figure 6.2) that the dielectric constant of the medium continuously falls as added alcohol volume increases facilitating stronger electrostatic interactions and, hence growth in aggregate size. The relative viscosity maximum seen at ethanol concentration $\approx V_t$ is consistent with the infinite-shear viscosity maxima and thixotropy minima data observed exactly at this ethanol concentration [11]. This reveals that the ethanol-water binding is maximum at $V_t$.

At a critical volume of added alcohol ($V_t$) the liquid-liquid phase separation ensues (see Figure 6.1). The surface tension data shown in Figure 6.2 corroborates this. The surface tension of the gelatin solutions as function of ethanol concentration was measured by pendant drop method [17].
Figure 6.1: A schematic of the titration profile of a 1% (w/v) aqueous gelatin solution titrated with ethanol at 25 °C. The turbidity measured at 450nm remains almost invariant up to ethanol concentration, Vt = 49% (v/v) when it sharply rises to give a maximum at ethanol concentration, Vp = 52% (v/v). The insets depict the folding of gelatin chains initiated due to intra-molecular charge neutralization. At Vt, coacervation ensues giving rise to a liquid-liquid phase separation. The supernatant at the top remains in thermodynamic equilibrium with the dense coacervate phase located at the bottom. Beyond Vp precipitation occurs. See ref. [10] for details.

Figure 6.2: Dependence of physical parameters of a 1% (w/v) aqueous gelatin solution as function of ethanol concentration at 25 °C. The relative viscosity and surface tension depict a maximum at ethanol concentration, Vt = 49% (v/v) which signals the onset of coacervation. Thixotropy of the solution shows a minimum at Vt (see ref.[11]). The collective picture indicates the maximum binding of water with ethanol molecules at Vt (see ref. [18]). The concomitant conversion of a good to marginal solvent facilitates coacervation (see ref. [11]).
6.1.2 Dynamically asymmetric systems and coacervation:

The dynamics of phase separation in a dynamically symmetric binary mixture is normally characterized by non-linear time-dependent Landau-Ginzburg theory that treats the local space-time concentration fluctuations \( \delta \phi(r, t) \) through appropriate Onsager transport coefficient \( \Lambda \) and a suitable chemical potential \[15\]. A simpler description pertains to the situation where the local concentration fluctuations are small and the hydrodynamic interactions are negligible. This linearizes the Landau-Ginzburg equation and results in the so called Cahn-Hillard-Cook equation, which predicts the temporal evolution of structure factor \( I(q,t) \sim \langle \delta \phi(r,t) \rangle^2 \) at phase separation to follow

\[
I(q,t) \sim \exp[2R(q)t]
\]

(6.1)

where the growth parameter, \( R(q) \) is given by

\[
R(q) = \Lambda(q)q^2(r_0 - Cq^2)
\]

(6.2)

where \( C \) is a positive constant related to non-locality of interactions and \( r_0 \) is a parameter describing the thermodynamic driving force for phase separation which is positive in the unstable region and increases with quench depth. For a polymer solution, when the wave vector, \( q << R_g^{-1} \), \( R_g \) being the radius of gyration of the polymer chains, \( \Lambda(q) \) describes the centre of mass motion of chains, so that \( \Lambda(q) \) becomes independent of \( q \). Such a situation prevails in the early stage of spinodal decomposition, which is well confirmed \[22-28\]. Semi-dilute polymer solutions and gels can be adequately described through Cahn-Hillard-Cook model.

In dynamically asymmetric systems, the dispersed polymer chains have different self-diffusivity (mobility) values. A highly polydisperse polyelectrolyte solution with polymer volume fraction, \( \phi_0 \sim 1 \) can be treated as an asymmetric system per se. Phase separation dynamics in such systems involves stress-diffusion coupling \[15,16\] not observed in dynamically symmetric systems. A screening length, called the viscoelastic length \( \xi_{ve} \), has been introduced to account for the dynamics of viscoelastic relaxations. Physically, a homogeneous high-molecular weight semi-dilute polymer solution is associated with two characteristic parameters: the growth rate of thermally activated concentration fluctuations, \( \Gamma_r \) and the relaxation rate of entangled networks, \( \Gamma_e \) that is characterized by the viscoelastic properties of the material. Local stress inside the system develops in case the growth rate of concentration fluctuations is faster than the network entanglement.
relaxation rate, i.e. $\Gamma_r \gg \Gamma_c$. The spatial variation of the stress field will be manifested as an equivalent osmotic pressure field inside the system. This forms the basic formalism of Doi-Onuki model. Doi-Onuki incorporated the concept of dynamical coupling between stress and diffusion into the Landau-Ginzburg equation [21,29]. The resultant equation could again be linearized using the same approximations discussed earlier to give an alternative expression for $R(q)$ written as

$$R(q) = \Lambda_{\text{eff}}(q) q^2 (r_0 - Cq^2)$$

(6.3)

where the new Onsager coefficient $\Lambda_{\text{eff}}(q)$ is given by

$$\Lambda_{\text{eff}}(q) = \frac{\Lambda(0)}{1 + q^2 \xi_{ve}^2}$$

(6.4)

Thus, a new length scale enters into the problem though Onsager coefficient called the viscoelastic length, $\xi_{ve}$, which can be explicitly defined from the space-averaged polymer volume fraction, $\phi_0$ and zero-shear viscosity of the solution, $\eta_0$ by

$$\xi_{ve}^2 = \frac{4\Lambda(0)\eta_0}{3\phi_0^2}$$

(6.5)

which reduces to

$$\xi_{ve}^2 = \frac{(D_c - D)\eta_0}{G_N} = \frac{D_{\text{eff}}}{G_N} \eta_0$$

(6.6)

Estimation of viscoelastic correlation length, $\xi_{ve}$ is nontrivial which necessitates simultaneous estimation of rubbery plateau modulus, $G_N$, cooperative diffusion coefficient, $D_c$ and stress induced diffusivity, D. If $(D_c - D) = D_{\text{eff}}$, one can write $D_{\text{eff}} = k_B T/6\pi \eta_0 \xi_{ve}$ which resets Eq. 6.6 to $\xi_{ve}^2 \approx k_B T/G_N$ (where $k_B$ is the Boltzmann constant and temperature is $T$).

In a network of transiently connected chains, the shear modulus is proportional to the concentration of inter-molecular bonds. The value of the length of elastically active strands, $\xi_{ve}$, calculated from Eq. 6.6 is similar to the characteristic viscoelastic network size, $\zeta_{\text{el}}$, estimated from the low-frequency shear modulus, $G_0$. This is a measure of elastic free energy stored per unit volume of a characteristic viscoelastic network of size, $\zeta_{\text{el}}$. Hence,

$$G_0 \approx k_B T/\zeta_{\text{el}}^3$$

(6.7)

Experiments reveal that shear storage modulus of coacervate is weakly dependent on frequency (to be discussed later) which allows one to conjecture $\zeta_{\text{el}} \approx \xi_{ve}$ (for $G_0 \approx G_N$).
Thus, typical viscoelastic length scale prevalent in these materials becomes easily accessible from oscillatory rheology measurements. When the value of viscoelastic length exceeds the average mesh size of the network, $\xi_{ve} > \xi_0$, the system shows no stress-diffusion coupling. Viscoelastic length is also called screening length as it screens the coupling effect for distances, $\xi_{ve} < \zeta$.

The theoretical concepts recapitulated above necessitate three requirements: (i) the initial system has a wide size particle distribution (mobilities are different), (ii) the growth of cluster-size is non-exponential and (iii) the final state is a polymer-rich dense state where the stress-diffusion coupling occurs. These conditions are sufficiently met in our experiments performed on coacervates. Thus, the formalism described above is a suitable model for the analysis of the following experimental results. The particle size distribution observed in our sample was continuous and could be represented through a power law distribution function $P(R)$ given by (see section 5.2, Chapter 5)

$$P(R) = \begin{cases} \alpha R^{\alpha-1}; & R \leq R_{\text{max}} \\ 0; & R > R_{\text{max}} \end{cases}$$  \hfill (6.8)

This system has very large size polydispersity with the maximum size given by $R_{\text{max}}$. The geometrical structure of the resultant clusters evolving from a system with characteristics defined by particle size distribution as in Eq. 6.8 is fully governed by the spatial correlations inside the system, which is accounted for by the parameter $\alpha$ ($\alpha > 0$). As $\alpha$ increases from 0 to $\infty$, $d_f$ reduces from 2 to 1.305. For smaller $\alpha$ fractal clusters are formed while the Apollonian packing limit is reached as $\alpha \rightarrow \infty$.

Dynamic light scattering measurement of particle size distribution of samples, reported in Chapter 5, gave $\alpha = 4.01$ and polydispersity determined from normalized variance was $\approx 0.63$. We also found $I_s(q) \sim q^2$ with $z = 1/d_f$, giving a fractal dimension $d_f$ in $3-d \approx 2.6 \pm 0.2$. Thus, coacervation phenomenon cannot be described through Landau-Ginzburg equation in the dynamically symmetric framework. In addition, we have argued and proved the existence of syneresis in coacervates of gelatin. Syneresis drives the non-equilibrium dynamics in coacervate phase and moves the system towards equilibrium. As the system spontaneously releases solvent during this process, the material becomes gradually more solid like. The atomic force microscope pictures clearly establish equilibrium coacervates as a heterogeneous and dense polymer-rich phase [30]. Thus, we resort to dynamically
asymmetric system picture to explain the salient features of coacervation in polyelectrolyte solutions in the following discussions.

6.2 Results and Discussion

6.2.1 Study of soluble charge neutralized aggregates

6.2.1.1 Circular dichroism and UV absorption:
The CD data is shown in Figure 6.3 for increasing ethanol concentrations up to the coacervation concentration, \( V_t = 49\% \) (v/v). The data shows a peak at \( \lambda \approx 212 \) nm and a dip at \( \lambda \approx 230 \) nm consistently for all ethanol concentrations. It is known that unless an unusual fraction of aromatic amino acid is present, optical activity in the region \( \lambda \approx 190-230 \) nm is dictated by the peptide backbone of proteins [31]. This region is devoid of any aliphatic side chain contributions. This allows the structural description of the polypeptide backbone to be expressed as weighted linear sum of contribution from \( \alpha \)-helix, \( \beta \)-turns and random coils and the measured molar ellipticity [\( \theta \)] will be

\[
[\theta] = \chi_r [\theta]_r + \chi_\alpha [\theta]_\alpha + \chi_\beta [\theta]_\beta \tag{6.9}
\]

where the fractional contributions of the individual structures corresponding to random coil, \( \alpha \)-helix and \( \beta \)-turns are \( \chi_r, \chi_\alpha \) and \( \chi_\beta \) respectively. The corresponding molar ellipticity are [\( \theta \)]_r, [\( \theta \)]_\alpha and [\( \theta \)]_\beta respectively. More details can be found in section 3.6.7 (see Chapter 3). Our data at 212nm revealed \( \chi_r << 1 \), which allows the degree of helicity \( \chi_{CD} \) to be defined from Eq. 6.9 as

\[
\chi_{CD} = \frac{[\theta] - [\theta]^{100}_r}{[\theta]^{100}_\alpha - [\theta]^{100}_r} \tag{6.10}
\]

where [\( \theta \)] is the measured ellipticity at a particular ethanol concentration. The superscripts refer to values of [\( \theta \)] with 100% of the structures belonging to that category. The calculated helicity (triple helices) values are shown in Figure 6.4, which reveals a significant growth of ordered structures inside the solution. Due to the nature of normalization chosen in Eq. 6.10, \( \chi_{CD} \) exhibits 100% helicity at \( V_t \), which is merely indicative. The UV absorbance (A) was measured at the same wavelength (212nm) and the degree of helicity \( \chi_{UV} \) was defined parallel to Eqs. 6.9 and 6.10 yielding

\[
\chi_{UV} = \frac{[A] - [A]^{100}_r}{[A]^{100}_\alpha - [A]^{100}_r} \tag{6.11}
\]
where \([A]\) is the measured absorbance at a particular ethanol concentration. Other terms have identical definitions as described above for the CD data.

\[
\text{where } [A] \text{ is the measured absorbance at a particular ethanol concentration. Other terms have identical definitions as described above for the CD data.}
\]

**Figure 6.3:** Circular dichroism data for 1% (w/v) gelatin solution at various ethanol concentrations at 25 °C. The secondary structure content is determined from the peaks at 212nm which is clearly shown in Figure 6.4.

Figure 6.5 depicts the degree of helicity determined through UV spectroscopy and CD technique with fairly good correlation. A linear least-squares fitting yields

\[
\chi_{\text{CD}} = a + b\chi_{\text{UV}} \tag{6.12}
\]

with \(a = 18\) and \(b = 0.735\). Here the helicity values are defined in percentage. The UV absorption data could also be fitted directly, which yielded

\[
[A] = [A]_r^{100} (1 - 0.5 \chi_{\text{CD}}) \tag{6.13}
\]

which shows excellent agreement with the results predicted by Busnel et al. [32]. They have shown that the helicity \((\chi_{\text{ORD}})\) determined from optical rotatory dispersion (ORD) and UV spectroscopy can be related as \([A] = [A]_r^{100} (1 - 0.4 \chi_{\text{ORD}})\) at \(\lambda \approx 227\text{nm}\), unlike in Eq. 6.12 the helicity values (normalized to 100% at Vt) in Eq. 6.13 (normalized to 1 at Vt) are not expressed in percentage but as fractions in consistence with the units used by Busnel et al. [33].
Figure 6.4: The secondary structure content determined from the peaks at 212nm shown in Figure 6.3. Notice the rapid growth in secondary structures at the expense of primary random coils. These are at maximum at ethanol concentration, $V_t = 49\% \ (v/v)$ where coacervation ensues. All measurements were done at 25 °C.

Figure 6.5: The degree of helicity estimated from circular dichroism data is plotted as function of UV absorbance measured at 212nm at 25 °C. The data were least-squares fitted to Eq. 6.13 enabling the establishment of a linear dependence consistent with Busnel et al. results [33].
The CD data clearly indicated the conformational transition of gelatin molecules from random coil to triple helix state as coacervation point was approached, which is corroborated by UV-absorbance data. The increase in helicity was almost 10-fold as the ethanol concentration increased from 0 to \( V_t \). As alcohol is added to water, the water molecules bind selectively with alcohol molecules through hydrogen bonding and the resultant binary mixture becomes a marginal solvent for gelatin molecules [11]. Secondly, the dielectric constant decreases significantly (see Figure 6.2) facilitating stronger electrostatic interactions between charged segments (both intra and inter) of gelatin molecules. It has been argued earlier [10,11] that the strength of electrostatic interactions between two oppositely charged particles increases with dielectric constant (\( \varepsilon \)) as \( \varepsilon^{-3/2} \) at a given temperature as per the Debye-Huckel theory [34]. The poor solvent quality compels the gelatin molecule to reduce its spatial expansion thereby bringing charged segments to each other’s vicinity through electrostatic interactions. This results in the collapse of some of the single gelatin molecules through intra-molecular interactions yielding a typical hydrodynamic radius \( \approx 20 \) nm whereas most other molecules associate through inter-molecular electrostatic interactions to form large aggregates of radius that are ten times larger and these eventually constitute the coacervate phase. It should be realized that when two oppositely charged segments join together, some amount of counter ion is always released into the solvent, thereby increasing the entropy of the solution. This will assist the process to move towards coacervation. This will assist the process to move towards coacervation. The gradual evolution of the coacervate phase from a homogeneous solution does involve a disorder-order transition through an aggregation mechanism. This was captured through circular dichroism (CD) experiments performed in the far-UV region. Alcohol induced denaturation of proteins sets in for ethanol concentrations larger than \( V_t \) value, which is discussed in details by Shimizu and Shimizu [18].

**6.2.1.2 Small angle neutron scattering:**

The small angle neutron scattering (SANS) experiments were performed at the Swiss neutron source at Paul Scherrer Institute, Switzerland for the studies of coacervation. The first objective was to probe the evolution of coacervation from a homogeneous solution. Eq 3.20 (see Chapter 3) was simplified by making \( S(q) = 1 \) valid for a diluted solution and thus the scattering intensity is governed by \( P(q) \). The concept remains invariant by
substituting the differential scattering cross-section $d\Sigma/d\Omega(q)$ with $I(q)$ which is the measured scattering intensity.

The kinetics of coacervation was analyzed in low $q$ (Guinier regime) as the scattering is dominated by incoherent scattering background at high-$q$ values (mainly due to the scattering of protons as ethanol was partially deuterated). In the Guinier regime ($q < 3R_g^{-1}$ and in absence of interactions) the scattering function is approximated by [35]

$$I(q) = \exp\left(-\frac{q^2R_g^2}{3}\right) \quad (6.14)$$

where $R_g$ is the radius of gyration of the particles that are soluble charge neutralized intermolecular aggregates of gelatin. The presence of soluble charge neutralized intramolecular gelatin particles is not ruled out here.

![Figure 6.6](image)

**Figure 6.6:** The helicity measured by CD in the far UV-region and the size of helices measured in terms of radius of gyration ($R_g$) by SANS for a 1% (w/v) aqueous gelatin (Type-B) solution titrated with ethanol with pH = 5 performed at 25 °C. Plot of scattering intensity in the low $q$-range ($q < 3R_g^{-1}$). The data were fitted to Eq. 6.14.
Figure 6.6 shows the size of these soluble aggregates measured in terms of radius of gyration \( R_g \) for a 1% (w/v) aqueous gelatin solution titrated with ethanol with pH = 5 performed at 25 °C in low q-range \((0.03 \text{ nm}^{-1} \leq q \leq 0.1 \text{ nm}^{-1})\). It was seen that the size of the aggregates increased slowly from 56 nm to 69 nm with the increase in the concentration of ethanol. Beyond a concentration of 45±2% (v/v), the size values reduced with further increase in ethanol concentration. The size of the soluble aggregates at coacervation was \( \approx 69 \text{ nm} \). Assuming a rigid triple-helix of length L to have a cross-sectional size much less than L, one can equate \( R_g^2 = L^2/12 \), which immediately gives \( L \approx 239 \text{ nm} \), a size comparable to the size of triple-helix found in gelatin gels [29,36]. On the other hand, DLS experiments, reported previously, revealed two-particle sizes \( \approx 20 \text{ nm} \) and \( 200 \text{ nm} \) [10]. The smaller particles (consequence of charge neutralization through intra-particle interactions) had a size of circa 20 nm whereas the aggregates (formed due to inter-particle interaction driven charge neutralization) grew in size to reach a value of several hundred nanometers. SANS experiments could not resolve and detect these two sizes, and what was measured appears to be a geometric mean of the two.

The combined SANS and CD data are shown in the Figures 6.4, 6.5 and 6.6 for increasing ethanol concentrations up to the coacervation point. The calculated degree of helicity (triple helices) values is shown in Figure 6.4 and Figure 6.6 (inset), which reveals a significant growth of ordered structures inside the solution. The degree of helicity exhibits 100% helicity at coacervation point, which is merely indicative and is an artifact of normalization used. The CD data clearly indicated the conformational transition of gelatin molecules from unordered to triple-helix state as coacervation point was approached. Though the CD signal of denature proteins have identified short stretches of polyproline II helical conformation (Pn) previously considered less regular [37], the regions of polypeptide chain not belonging to α-helix, β-sheet, or β-turn conformations are referred to as unordered, unstructured, other, remainder, or random coil [37,38]. We have shown that a radius of gyration of 69 nm does translate into the equivalent rigid-rod length \( \approx 239 \text{ nm} \). Thus, there is a broad unanimity in the qualitative results yielded by these two techniques.
6.2.2 Hierarchical structure

6.2.2.1 Small angle neutron scattering:

The SANS experimental data were examined from gelatin (13% w/v) in sol (60 °C), gel (25 °C) and coacervate (25 °C) states to structurally discriminate these phases. The coacervate sample was initially carried at the G.T laboratory, Dhruva reactor (Bhaba Atomic Research Centre, Trombay, India). Further details of the SANS spectrometer at Dhruva are discussed in section 3.6.2.2 (see Chapter 3). Often it is impossible to probe low q-domain of the structure factor because of the instrumental limitations of SANS spectrometers. The structure factor I(q) determined from the SANS scattering profile data for coacervate samples is shown in Figure 6.7. Eq. 3.16 was simplified by making P(q) = 1 valid for a concentrated solution and thus scattering intensity I(q) governed by S(q).

![Figure 6.7: The neutron scattering intensity profile from a gelatin coacervate sample prepared at 25 °C from 1% (w/v) D_2O solution (pH=5). The data was fitted to a Ornstein-Zernike expression given by the theory of small angle neutron scattering (see section 3.6.2.2, Chapter 3). This yields a length parameter \( \xi = 2.7 \pm 2 \text{ nm} \) which is close to the persistence length of gelatin (2.5 nm).](image)

A least-square fit of the structure factor data in the q-range of \( 7.2 \times 10^{-1} \leq q \leq 2.0 \text{ (nm)}^{-1} \) simply gives the scattering length or correlation length \( \xi \) of the entangled network of supra molecular complex coacervate in the Ornstein-Zernike model (\( S_L(q) \)). For coacervates we found \( \xi = 2.7 \pm 2 \text{ nm} \). This length compares well with the known persistence length of gelatin, which is \( \approx 2.5 \text{ nm} \). At this stage it is not clear if the
coacervate phase has network like structures of correlation length so small. However, the fitting was found to be adequate with acceptable chi-squared values. The excess scattering normally observed at low q-regions could not be resolved satisfactorily. So the information hidden in long wavelength fluctuations could not be unearthed. This could be attributed to the artifacts associated with this low resolution SANS spectrometer. It is essential to experimentally identify the q-cutoff point on I(q) versus q data profile and resolve the low and long wavelength scattering regions. This will help in resolving the confusion pertaining to the small value of correlation length observed. It was possible to study low-q domain of the structure factor because of the high instrumental resolution of SANS spectrometers available at Paul Scherrer Institute (PSI), Switzerland. Further details of the SANS spectrometer at PSI are discussed in section 3.6.2.2 (see Chapter 3).

![Logarithmic plot of total structure factor I(q) versus scattering vector q](image.png)

**Figure 6.8**: Logarithmic plot of total structure factor I(q) versus scattering vector q for sol, gel and coacervate measured by SANS with zero ionic strength performed at 25 °C for gel and coacervate and 60 °C for sol sample. The concentration of gelatin sol was kept 13% (w/v) in all phases.
The experimental data is presented in Figure 6.8 which reveals remarkably identical structure factor profile for all the three states except in the asymptotic region of \( q \) that owes its origin to excess incoherent scattering. In fact, for sol and gel samples the scattering profiles are indistinguishable. Theoretically, an O-Z type of behavior (Eq. 3.21, see Chapter 3) has been predicted for the scattered intensities of the polymer solutions in good solvent. Accordingly, it was attempted fitting of neutron scattering data to a Lorentzian behavior (O-Z function) as given in Eq. 3.21, Chapter 3. A plot of \( I'(q) \) (contribution from structure factor \( S_L^{-1}(q) \)) versus \( q^2 \) was made in the scattering vector range \( 0.04 \text{ nm}^{-1} \leq q \leq 0.7 \text{ nm}^{-1} \) which is shown in Figure 6.9.

![Figure 6.9: Inverse scattering structure factor \( I'(q) \) versus \( q^2 \) for sol, gel and coacervate measured by SANS with zero ionic strength performed at 25 °C for gel and coacervate and 60 °C for sol sample. The data were fitted to Eq. 3.21, Chapter 3. A linear dependence is observed whose slope allows one to calculate the network size. The values extrapolated to the vertical axis determine the amplitude of the lorentzian component, \( I(0) \). At small \( q \) vectors, an excess scattering appears (downward curvature of data).](image-url)
Although, it showed a good linear fit to the data in the q-range of $0.2683 \text{ nm}^{-1} \leq q \leq 0.7 \text{ nm}^{-1}$, it showed deviations from linear fit in the range of scattering vector $q < 0.27 \text{ nm}^{-1}$ (the downward curve indicates deviation).

![Logarithmic plot of I(q) versus scattering vector q measured by SANS with zero ionic strength performed at 25 °C for gel and coacervate and 60 °C for sol sample. The solid lines represent the Lorentzian behavior (Eq. 3.21, Chapter 3) and the broken curve is the contribution of the inhomogeneities (Eq. 3.23, Chapter 3).](image)

**Figure 6.10:** Logarithmic plot of I(q) versus scattering vector q measured by SANS with zero ionic strength performed at 25 °C for gel and coacervate and 60 °C for sol sample. The solid lines represent the Lorentzian behavior (Eq. 3.21, Chapter 3) and the broken curve is the contribution of the inhomogeneities (Eq. 3.23, Chapter 3).

A typical situation is shown in Figure 6.10 where experimental data was fitted to O-Z and D-B functions and the conclusion is obvious. Thus, the excess scattering from samples at low wave numbers had to be dealt with separately. Two contributions to the structure factor, the O-Z component and the excess scattering component, can be visualized from the plot shown in Figure 6.10. The excess scattering data have been analyzed within the framework of Debye-Bueche model (see section 3.6.2.2, Chapter 3). By subtracting the O-Z component from the total structure factor data (through visual estimation of q-cut off...
≈ 0.27 nm⁻¹, one can easily get the contribution arising from excess scattering, \( S_{\text{exe}}(q) \). Next, the \( I(q) \) data were fitted to Debye-Buche structure factor (\( S_{\text{exe}}(q) \)) described in Eq. 3.23 (see Chapter 3). A plot of this was made (see Figure 6.10) and the slope gave the value of the parameter \( \zeta \). Inhomogeneities of size, \( \zeta \approx 20 \pm 2 \text{ nm} \) were found in case of sol, gel and coacervate samples (a).

A linear fit of the structure factor data in the q-range \( 0.2683 \text{ nm}^{-1} \leq q \leq 0.7 \text{ nm}^{-1} \) (see Figure 6.9) gives the scattering length or correlation length (mesh size) of the entangled network, normally designated as \( \xi \). The slope of the plot enables one to easily calculate the value of \( \xi \) for sol and gel samples (identified as \( \xi_0 \)). For coacervate samples, we found the correlation length, \( \xi_{0s} \), given by \( \xi_{0s} \approx 1.2 \pm 0.2 \text{ nm} \), which was 50% less than the correlation length obtained for sol and gel states, \( \xi_0 \approx 2.6 \pm 0.2 \text{ nm} \) (b). It is clear from this data that there is a decrease in the correlation length in coacervates as compared to sol and gel phase indicating strong interactions inside the coacervate phase. Secondly, the measured correlation length (for sol and gel samples) is comparable to that of persistence length of gelatin, which is \( \approx 2.5 \text{ nm} \).

The correlation length for sol and gel phases calculated from scaling formula [35] is called average mesh size (\( \xi_0 \)), which adequately fits data in the intermediate-q range to O-Z function. But, the value of correlation length or mesh size in coacervate samples at small length scale concentration fluctuations was observed to be smaller than \( \xi_0 \) indicating the unique structure of coacervates. Though it is an asymmetric system, as already discussed, there is no stress-diffusion coupling, which confirms the existence of two mesh sizes: one is smaller, \( \xi_{0s} \), than the average mesh size (present in polymer dense region, \( \xi_{0s} < \xi_0 \)), and another is larger, \( \xi_{0l} \), than the same (present in polymer poor region, \( \xi_{0l} > \xi_0 \)) [16]. In our experiments, we could measure only the value of the smaller mesh size existing in the polymer dense region. The higher mesh size, \( \xi_{0h} \) prevailing in polymer-poor region remained elusive to our measurements. In an earlier experiment, analysis of the structure factor data in the limited q-range of \( 0.72 \text{ nm}^{-1} \leq q \leq 2.0 \text{ nm}^{-1} \) measured at Bhabha Atomic Research Centre (BARC), India only gave the correlation length of the entangled network of coacervate in the Ornstein-Zernike model. The value obtained was \( \approx 2.7 \pm 2 \text{ nm} \). The limited q-range available questioned the reliability of this data, which prompted us to repeat this experiment with a high resolution SANS spectrometer at PSI [39]. It is clear from PSI data that the coacervate phase has network...
like structures of correlation length $\xi_{os} \approx 1.2 \pm 0.2$ nm, which is $\approx 50\%$ small than the previously measured value, but is more reliable. According to the scaling laws [35], $\xi$ varies with the concentration as

$$\xi_0 = R_g (c / c^*)^{v/(1-3v)}$$ (6.15)

From the above scaling law, the value of $\xi$ for sol and gel using $R_g = 33$ nm, $v = 3/5$, $c = 13\%$ (w/v) and $c^* \sim 0.5\%$ (w/v) was $\sim 2.8$ nm, which was comparable to that obtained from the fitting of $I(q)$ data to Ornstein-Zernike function (see Figures 6.9 and 6.10). Eq. 6.15 cannot be applied to the coacervate phase because of its unique microscopic structure, which attributes a dense phase state to this matter.

In asymptotic range, for swollen coils, scaling laws [35] and computer simulations have shown that for $q \rightarrow \infty$

$$I(q) \sim q^{-1v}$$ (6.16)

where $v$ is the excluded-volume parameter; $v = 3/5$ in a good solvent. Strictly speaking, this prediction is valid for infinitely long chains. Experimentally, the asymptotic behavior requires a large enough $q$-range and very precise measurement [40] conditions that are satisfied by our experiments (see Figure 6.11). The asymptotic region in the far $q$-domain is expected to reveal the local rigidity whereby the chain cross-section makes finite contribution to measured structure factor. According to the Kratky-Porod formalism, for $q \rightarrow \infty$ and degree of polymerization, $N \rightarrow \infty$, one has

$$I(q) = \frac{c}{q} \exp(-R_c^2 q^2 / 2) + const.$$ (6.17)

where $c$ is gelatin concentration and chain cross-sectional radius is $R_c$. The fitting of our data shown in Figure 6.11 in the $q$-range, $0.7 < q < 3.5$ nm$^{-1}$, yields $R_c = 0.35 \pm 0.04$ nm.

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Figure 6.11: Scattering intensities $I(q)$ versus $q$ in asymptotic $q$-range for sol, gel and coacervate measured by SANS with zero ionic strength performed at 25 °C for gel and coacervate and 60 °C for sol sample. An exponential dependence of (Eq. 6.17) was observed which measures the cross sections of the chains ($R_c$). The $I(q)$ data for coacervate sample (inset figure) has 5-10% error as it contains partially deuterated ethanol.

This compares well with the literature value of 0.32nm [35] (c). The measured cross-sectional radii of the coils is in agreement with the molecular composition of this protein chain. The cross-sectional radius of the triple-helix strands in sol and gel states has been found to be equal, $R_c \approx 0.6$nm [41]. It may appear intriguing that SANS could probe local rigidity of gelatin molecules in a dense coacervate material. However, it has been shown in the past that in the asymptotic region, the scattering profile remains invariant of the polymer concentration.
6.2.2.2 Rheology and Differential scanning calorimetry (DSC):
Most concentrated structured liquids have shown strong viscoelastic effects at small
deformations, and their measurement is very useful as a physical probe of the
microstructure. However at large deformations such as steady-state flow, the
manifestation of viscoelastic effects - even from the systems that show large effects - can
be different. If viscoelastic forces manifest themselves on a grand scale, large changes
can occur in flow and force patterns. Measurements performed in the flow mode, in the
shear rate ($\gamma$) ranging from 0.005 to 100s$^{-1}$, yielded the shear rate dependent viscosity ($\eta$)
of sol, gel and coacervate samples. The sol phase showed completely Newtonian behavior
while the gel and coacervate phases exhibited non-Newtonian features beyond a shear
rate $\approx 0.1s^{-1}$ as shown in Figure 6.12.

![Viscosity measurement graph](image)

**Figure 6.12:** Viscosity, $\eta$(dyne.cm$^{-2}.s$) of sol, gel and coacervate as a function of shear
rate, $\gamma$ (1/s) performed at 25 °C for gel and coacervate, and 60 °C for sol sample,
measured in flow mode by Rheology. The data was fitted to Correau model (Eq. 6.18, see
page 166) to find out the relaxation time and viscosity.
The non-Newtonian behavior observed in gel samples may be attributed to high polyelectrolyte concentration. We were able to fit the data obtained for gel and coacervate samples to Carreau model expression [42]

\[ \eta = \eta_0 \left[ 1 + (\tau_m \dot{\gamma})^2 \right]^k \]  

(6.18)

using \( \eta_0, \tau_m, \) and \( k \) as floating parameters, where \( \tau_m \) and \( k \) are, respectively, the longest relaxation time and an arbitrary power-index. The Newtonian viscosity of sol state was measured to be 0.4/\text{dyne.cm}^2.s whereas same for gel and coacervate samples was \( 8 \times 10^4/\text{dyne.cm}^2.s \) and \( 10^5/\text{dyne.cm}^2.s \) respectively. The higher viscosity observed in coacervate state indicated that the electrostatic interactions were the considerably stronger in this phase state as compared to the other two states. For coacervate sample, the relaxation time determined was \( \approx 2.2s \) whereas for gel sample the same was \( \approx 9.7s \). The relaxation time was found to be higher in gel compared to the same in coacervate sample which may have arisen from the statistical error incurred during the fitting of data to Carreau expression.

In qualitative terms the oscillatory curves in rheology give a fingerprint of the state of the microstructure in the same way as does an NMR or an infrared spectrum. As has been discussed earlier, coacervate is dynamically asymmetric mixture of polymer molecules and the coacervate material is viscoelastic. Thus, the structural dynamics must be affected by viscoelastic relaxation features that involves a new characteristic length scale, the so-called viscoelastic length \((\xi_{ve})\), as elaborated by Doi and Onuki [15,16]. If the growth rate of thermally activated concentration fluctuations \((\Gamma_r)\) is faster than the relaxation rate of the entangled polymer networks \((\Gamma_e)\), the concentration fluctuations will build up local stress. The built-up stress and its spatial variation is relaxed at rate \((\Gamma_e)\) and at a length scale \((\xi_{ve})\). The storage and loss modulii data are shown in Figures 6.13 and 6.14 (see page 167).
Figure 6.13: Storage modulus (G'/Pa) of gel and coacervate as a function of the angular frequency (rad.s⁻¹) measured at a constant oscillation frequency of 1 Hz, a constant strain of 10%, performed at 25 °C, in oscillation mode by rheology.

Figure 6.14: Loss modulus (G''/Pa) of sol, gel and coacervate as a function of the angular frequency (rad.s⁻¹) measured with a constant oscillation frequency of 1 Hz, a constant strain of 10%, performed at 25 °C for gel and coacervate, and at 60 °C for sol sample, in oscillation mode.
As far as dynamic behavior is concerned (see section 3.6.5, Chapter 3), a large number of experimental studies [43-46] have shown that the complex shear modulus $G(t)$ follows a power law behavior as $G(t) \sim t^\Delta$, which in turn gives

$$G' (\omega) \sim G''(\omega) \sim \omega^\Delta$$  \hspace{1cm} (6.19)

This can be generalized to

$$G' (\omega) \sim \omega^{\Delta'}$$  \hspace{1cm} (6.20)

and

$$G''(\omega) \sim \omega^{\Delta''}$$  \hspace{1cm} (6.21)

Knowledge of the values of exponent $\Delta'$ and $\Delta''$ is of importance. As regard the values of $\Delta$, it is now accepted that a wide variety of values can be obtained experimentally [47-49]. Scalan and Winter [47], in particular, have shown that $\Delta$ depends not only on the stoichiometric ratio but also on the initial molar mass of the monomers and on their concentration when the reaction takes place in a solution. In the case of cross-linking of polydimethylsiloxane by a tetra functional cross-linking agent, they obtained values varying from 0.2 to 0.92 depending on the experiment conditions. These experimental results seem to show that there is no universal value of $\Delta$.

![Figure 6.15: The frequency dependence of storage and loss modulii ($G'$ and $G''$) of a gelatin coacervate sample prepared at 25 °C from 1 % (w/v) aqueous solution (pH = 5). The coacervation was ethanol induced. The modulii data could be fitted to power law expressions given by Eqs. 6.20 and 6.21.](image)
Figure 6.15 presents the storage and loss modulus curves $G'(\omega)$ and $G''(\omega)$ in linear scale. Values $\Delta'$ and $\Delta''$ were obtained by fitting power-law function given by Eqs. 6.20 and 6.21 to the experimental data for $G'$ and $G''$ of coacervate samples shown in Figure 6.13. This yielded $G' \sim \omega^{0.38}$ and $G'' \sim \omega^{0.75}$. From definitions [46] it is also possible to determine the zero-shear viscosity $\eta_0$ and corresponding creep compliance $J_c^0$. These are given by

\begin{align*}
J_c^0 &= \frac{1}{\eta_0} \lim_{\omega \to 0} G'/\omega^2 \\
\eta_0 &= \lim_{\omega \to 0} G''/\omega
\end{align*}

(6.22)
(6.23)

Numerical analysis of $G'$ and $G''$ as a function of $\omega$ leads directly to values of $\eta_0$ and $J_c^0$. The values of $\eta_0$ and $J_c^0$ were found to be $\approx 705$ Pa and $\approx 0.03$ Pa$^{-1}$ for the coacervate, which almost same for gel phase. But the rheology data (Figs 6.12 and 6.13) implied that the coacervate phase comprised a solvated polymer phase that was highly dense with a high viscosity and large storage modulus. Such a large storage modulus is unlike of a polymer solution, which implies the existence of highly crosslinked networks inside the coacervate medium. The gelling solution of a polyelectrolyte can be described through Muthukumar’s theoretical model [50] based on the assumption that variations in the strand length between cross-linking points of the incipient gel network give rise to changes of the excluded volume interactions, to rationalize values of the relaxation exponent $\Delta$ in the whole physically accessible range ($0 < \Delta < 1$). In this approach, a relationship between $\Delta$ and fractal morphology of the incipient gel network was established [50]

$\Delta = d (d+2-2d_f) / 2(d+2-d_f)$

(6.24)

where, $d$ ($d=3$) is a spatial dimension and $d_f$ is the fractal dimension, which relates the mass ($M$) of a molecular cluster to its radius of gyration ($R$) by $R_{df} \sim M$. In the framework of Eqs. 6.20 and 6.21, all values of the relaxation exponent $0 < \Delta < 1$ are possible for a fractal in the physically realizable domain $1 \leq d_f \leq 3$. Though the coacervate system is not exactly comparable to a gelling system it is tempting to use Eq. 6.24 to evaluate the value of $d_f$. The value of the fractal dimension obtained for the present gel and coacervate system is $2.29$ ($\Delta' = 0.23$) and $2.14$ ($\Delta' = 0.38$), which suggests a heterogeneous network structure present in both the system [51-52]. A lower fractal dimension means that the
molecular weight grows slower with radius, i.e. \( M \sim R^{df} \). It shows that coacervates is less heterogeneous than gel. This is somewhat surprising since we are expecting the cross-linking density to be higher in cocervates attribute to the heterogeneous character of the system [50].

It is clearly seen that (from estimation of \( \tan \delta = G''/G' \)) the gel state is associated with more fluidity as compared to the coacervate state. We have estimated the value of \( \xi_{ve} (= 14 \pm 2 \text{ nm (e)}) \) by applying Eq. 6.7 to the data given in Figure 6.16. The existence of syneresis in gelatin coacervate system has been mentioned earlier [53].

Figure 6.16: Storage modulus (G'/Pa) coacervate as a function of the angular frequency (rad.s\(^{-1}\)) measured at a constant oscillation frequency of 1 Hz, a constant strain of 10%, performed at 25 °C in oscillation mode. Syneresis effect was observed with respect to time and corresponding storage modulii and viscoelastic lengths were measured.
A manifestation of this is shown in Figure 6.16 depicts the temporal evolution of storage modulus that increases by 400% over a period of one hour. During this process, $\xi_{ve}$ evolves from a value $\approx 14$nm at $t = 5$min to $\approx 10$nm at $t = 60$min. Thus, there is a significant loss of fluidity of the sample due to syneresis. Isochronal temperature sweep measurements were performed on gel and coacervate samples to measure their phase melting behavior.

![Graph](image)

**Figure 6.17**: Storage modulus ($G'/\text{Pa}$) and loss modulus ($G''/\text{Pa}$) of gel and coacervate samples plotted as function of temperature. Temperature ramp was $\approx 0.3 \, ^\circ\text{C/min}$, a constant oscillation frequency of 1 Hz was used, and a constant strain of 10% was maintained during experiments.

Figure 6.17 reveals that when temperature was increased, the value of storage modulus $G'$ and viscous modulus $G''$ decreased because of melting of the junction zones. Junction zones were found to be stable in the temperature range of 25-30 °C and a shoulder gradually appeared at $\approx 34 \pm 2 \, ^\circ\text{C}$, this temperature corresponded to the melting temperature for gel and coacervate. A typical plot of $\tan\delta$ ($G''/G'$) with temperature gave a melting transition, which was approximately equal for both gel and coacervate states, is shown in Figure 6.18. The heat flow variation detected by DSC corresponds to the
energy required to melt the junction zones and to achieve the helix-to-coil transformation. Figure 6.18 gives a typical thermogram obtained by heating a gelatin coacervate, originally at a temperature 25 °C to 45 °C at a heating rate of 1 °C/min. Note the gradual melting of the coacervate material with the increase in temperature. The melting transition temperature ($T_m$) was observed at $\approx 33 \pm 3$ °C in the DSC data, which showed excellent matching with that determined from rheology.

![Figure 6.18: Heat flow (W/g) and $\tan\delta$ of coacervate as a function of temperature was measured by differential scanning calorimeter and rheology. Notice that the melting temperature $\approx 33 \pm 2$ °C deduced through with the techniques provides excellent melting.](image)

Theoretical treatment of this melting transition, for coacervate samples, is not trivial mostly due to the absence of any relevant modeling of the problem. Here, we adopt models developed for polymer blends in order to understand the phase separation phenomenon occurring in our system. The following discussion is focused on the effect of phase separation kinetics on scattering and rheology data. For highly concentrated polymeric systems the behavior of $G'$ and $G''$ reflect interaction dynamics operating inside the system. One can follow the results obtained from mean-field theory to predict
evolution of the dynamic storage and loss modulii in terminal one phase region near the melting transition temperature [54,55], which are given by

$$G'(\omega) = \frac{k_B T \omega}{1920 \pi} \left[ \frac{1}{3} \left( \frac{R_{g1}^2}{\phi N_1} + \frac{R_{g2}^2}{(1-\phi) N_2} \right) \right]^{1/2} \left[ \frac{1}{\phi a_1^2 W_1} + \frac{1}{(1-\phi) a_2^2 W_2} \right]^{1/2} \left( 2(\chi_s - \chi) \right)^{-5/2}$$

(6.25)

$$G''(\omega) = \frac{k_B T \omega}{240 \pi} \left[ \frac{1}{3} \left( \frac{R_{g1}^2}{\phi N_1} + \frac{R_{g2}^2}{(1-\phi) N_2} \right) \right]^{-1/2} \left[ \frac{1}{\phi a_1^2 W_1} + \frac{1}{(1-\phi) a_2^2 W_2} \right]^{-1/2} \left( 2(\chi_s - \chi) \right)^{-1/2}$$

(6.26)

where $a_1$ and $a_2$ are the statistical segment length of two species and $W_1$ and $W_2$ are the rate of orientation of segment of polymer, $\chi$ is the value of interaction parameter and $\chi_s$ is the value of interaction parameter at the spinodal. $R_g$ is the radius of gyration defined as $R_g = N \pi a_i^2 / 6$, $N_i$ is the number of segments of different species and $\omega$ is the angular frequency. Applicability of Eqs. 6.25 and 6.26 is confined to high molecular weight polymers to avoid complications arising from the temperature dependence of non-mean-field behaviour. Several conclusions can be derived from these equations: (a) $G' \sim (\chi_s - \chi)^{-5/2}$; (b) $G'' \sim (\chi_s - \chi)^{-1/2}$; (c) these equations permit elimination of the interaction parameter dependence. The ratio $G'/G''$ is given by

$$\frac{G'}{G''} = 30 \pi \left[ \phi(1-\phi) \right]^3 \left[ \frac{\alpha'}{6} \right] \left[ \phi(1-\phi)(\chi_s - \chi) \right]^{-1/2}$$

(6.27)

Then we can write

$$\frac{G'}{G''} = \frac{30 \pi}{k_B T} \left[ \frac{a_1^2}{36 \pi} + \frac{a_2^2}{36(1-\phi)} \right] \left( \chi_s - \chi \right)^{-3/2}$$

(6.28)

However, the point must be emphasized that such an expression is valid only for the terminal response near the melting region. This equation leads to an important observation when a linear dependence of $(G''^2/G'T)^{2/3}$ versus $1/T$ is predicted for concentrated systems, assuming $\chi = A + B/T$ behavior of the interaction parameter ($A$
and $B$ are temperature independent constants). The correlation length of blends, extended to concentrated systems, is given by

$$\xi_T = \frac{a'}{6} \left\{ \phi(1-\phi) (\chi_s - \chi) \right\}^{-1/2}$$  \hspace{1cm} (6.29)

which implies the frequency independent expression:

$$\frac{G'}{G''} = \frac{30\pi}{k_B T} \left[ \phi(1-\phi) \right]^3 \xi_T^3$$  \hspace{1cm} (6.30)

This permits the evaluation of correlation length close to the critical region from rheological measurements:

$$\xi_T = \left[ \frac{k_B T G''}{30\pi G'} \right]^{1/3} \phi(1-\phi)$$  \hspace{1cm} (6.31)

The frequency independence behaviour has been transmitted to the expression for correlation length. Close to the critical point ($T_s$), the correlation length increases drastically as a result of concentration fluctuations, giving rise to an anomalous contribution to the viscoelastic properties. The data presented in Figure 6.17 can be used to evaluate the value of $\xi_T$ which comes to $\approx 500$ nm at transition temperature. The explicit temperature dependence of $\xi_T$, determined from Eq. 6.31, is revealed in Figure 6.19 for gelatin coacervates.

![Figure 6.19: The correlation length is shown as function of melting temperature of coacervate sample.](image-url)
The correlation lengths obtained from rheological data (Eq. 6.31) has been compared with those from SANS, in the past for polymer blends [55], that showed excellent agreement. Our SANS experiments were carried out at temperatures far away from the transition temperature, which did not permit appropriate data comparison.

6.2.2.3 Atomic Force Microscopy:
As the alcohol concentration reached Vt (%v/v), the turbidity showed a sharp rise as seen in Figure 6.1. Such a situation corresponds to a state where very large number of charged neutralized inter and intra-molecular clusters of gelatin molecules are formed in a cooperative manner. The corresponding AFM pictures do show presence of typically 1μm clusters along with larger ones. Most of these are inter molecular clusters. These large clusters are expected to contribute excess scattering to the SANS data at low-q, which could not be observed in our experiments and we attribute this to instrumental deficiency. The images of the coacervates (see Figure 6.20) show lumps of dense matter with immense heterogeneity spread in space having no definite geometric structures.

Figure 6.20: Atomic force microscopy picture (5μm x 5μm) of a gelatin coacervate sample prepared at 25 °C from 1%(w/v) aqueous solution (pH=5) in the non-contact mode. The picture shows lumps of dense matter with immense heterogeneity spread in space having no definite geometric structures.
6.3 Conclusions

The microscopic structure of gelatin coacervates prepared in water was investigated by an array of experimental techniques for the first time. The short persistence length of 2.5 nm associated with gelatin molecules indicates that the Type-B gelatin chains are less flexible than polystyrene, but much more flexible than other biopolymers, like polysaccharides, DNA etc. which are known to form gels. The circular dichroism (CD) and UV absorption data revealed the definite existence of helical structures inside the coacervate medium. We have successfully evaluated and identified the length scale hierarchy existing in coacervate phase of gelatin. The values ranged from 1.4nm to 500nm, which makes the system rich in dynamics, summarized in Table 6.1.

<table>
<thead>
<tr>
<th>Length scales</th>
<th>Techniques</th>
<th>Sol</th>
<th>Gel</th>
<th>Coacervate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Inhomogeneities (ξ)</td>
<td>SANS (low q)</td>
<td>20 ± 1 nm</td>
<td>20 ±1 nm</td>
<td>20 ± 1 nm</td>
</tr>
<tr>
<td>(b) Mesh size (ξ₀)</td>
<td>SANS (intermediate q)</td>
<td>2.6 ± 0.2 nm</td>
<td>2.6 ± 0.2 nm</td>
<td>1.2 ± 0.2 nm (ξ₀)</td>
</tr>
<tr>
<td>(c) Chains cross-sectional radius (Rc)</td>
<td>SANS (asymptotic range)</td>
<td>0.35 nm</td>
<td>0.33 nm</td>
<td>0.34 nm</td>
</tr>
<tr>
<td>(d) Triple helix length (L)</td>
<td>SANS (Guinier range)</td>
<td>-</td>
<td>-</td>
<td>239 nm</td>
</tr>
<tr>
<td>(e) Viscoelastic length (ξᵥ)</td>
<td>Rheology (frequency sweep)</td>
<td>-</td>
<td>-</td>
<td>14 ± 2 nm</td>
</tr>
<tr>
<td>(f) Correlation length at melting region (ξ₇)</td>
<td>Rheology (temperature sweep)</td>
<td>-</td>
<td>-</td>
<td>500 nm</td>
</tr>
</tbody>
</table>

Table 6.1: Characteristic length scales of gelatin sol gel and coacervates deduced from small angle neutron scattering (SANS) and rheology experiments, gelatin concentration was 13% (w/v) in aqueous medium, T=25 °C.

In the study of microscopic structure of coacervates, results obtained from the techniques used were in good mutual agreement. SANS investigation of coacervate phase revealed the existence of two length scales – one is the correlation length ξ₀ ≈ 1.2 ± 0.2 nm, associated with the size of the entangled network and the other having a higher length that can be attributed to long-wavelength concentration fluctuations or presence of inhomogeneities ζ ≈ 20 ± 1 nm associated with these systems. The size of inhomogeneities associated with sol and gel states were found to be the same with that of the coacervate phase. The correlation length or mesh size of the cross-linked network
measured in coacervate state was half of the size found in sol and gel states (≈ 2.6 ± 0.2 nm). The reason for observing such a short correlation length may be attributed to the presence of strong electrostatic interactions inside the coacervate medium. Similar observations have been made in polymer blends earlier. A schematic representation of coacervate phase microstructure has been depicted in Figure 6.21 (see page 178) which is an adaptation from references [15,16]. Figure 6.21(a) is a 2-d AFM picture of coacervate material [12]. A small portion (shown as a circle) is zoomed to reveal the polymer-rich and poor regions, Figure 6.21(b). There is a propensity of triple-helices in the polymer-rich domains. The interfacial region is zoomed to produce Figure 6.21(c) that shows the smaller length scale hierarchy. Realize that the characteristic length scale in polymer-poor region remained elusive to our experimental observations. Rheology results showed only the rubbery or plateau region for gel and coacervate material. The \( G' \) and \( G'' \) data did not show any crossover. The instrumental limitation did not permit the complete exploration of the low frequency region as a result of which the terminal or equilibrium modulus \( G_e \) remained elusive. The large storage modulus value associated with the coacervates implied the existence of crosslinked networks beyond doubt. Gelatin gels are known to have a propensity of intertwined triple helix networks [33,56]. Thus the combination of rheology and CD data indicates a similar microscopic structure to be prevailing inside the coacervate medium but the density of these physical crosslink is too high. Hence, it is tempting to refer to it as a high density structural liquid and not a gel because the network does not pervade the entire space homogeneously as evidenced from our experimental results. The gelatin coacervate samples were so dense that it was impossible to perform dynamic light scattering experiments and study various relaxation modes.

Simple coacervate is expected to release solvent and exhibit syneresis due to which the system shows sponge-like structure. It was visually observed that with the loss of solvent through syneresis, the coacervate material developed an elastomeric texture. It must be mentioned in passing that we were unable to perform DLS experiments on coacervate samples due to the opaque texture of the scattering material. Regardless, the results presented provides an insight into the distinctive microstructure of the three phase states of gelatin-water system: the sol, gel and coacervate. This chapter answer all the questions related to structure of coacervates and it makes an attempt to give a good foundation to its understanding.
Figure 6.21: Schematic representation of the concept of stress diffusion coupling and the viscoelastic effects in gelatin coacervate: (a) Global picture observed by Atomic Force Microscopy, (b) Concentration fluctuations at short and large length scales, the shaded regions designate polymer-rich phase, while the un-shaded portions designate polymer poor regions at the interface (where there was no measurement of experimental length scale) and (c) local picture with smaller mesh size $\xi_{os}$ and $\zeta$ is the size of inhomogeneities for the concentration fluctuations, and $L$ is the length of helix.
6.4 References

(b) M. Muthukumar, Macromolecules, 22, 4656, 1989.