Small-angle neutron and dynamic light scattering study of gelatin coacervates

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Abstract. The state of intermolecular aggregates and that of folded gelatin molecules could be characterized by dynamic laser light and small-angle neutron scattering experiments, which implied spontaneous segregation of particle sizes preceding coacervation, which is a liquid-liquid phase transition phenomenon. Dynamic light scattering (DLS) data analysis revealed two particle sizes until precipitation was reached. The smaller particles having a diameter of $\sim$50 nm (stable nanoparticles prepared by coacervation method) were detected in the supernatant, whereas the inter-molecular aggregates having a diameter of $\sim$400 nm gave rise to coacervation. Small-angle neutron scattering (SANS) experiments revealed that typical mesh size of the networks exist in polymer dense phase (coacervates) [1]. Analysis of the SANS structure factor showed the presence of two length scales associated with this system that were identified as the correlation length or mesh size, $\xi = 10.6$ Å of the network and the other is the size of inhomogeneities $= 21.4$ Å. Observations were discussed based on the results obtained from SANS experiments performed in 5% (w/v) gelatin solution at 60°C ($\xi = 50$ Å, $\zeta = 113$ Å) and 5% (w/v) gel at 28°C ($\xi = 47$ Å, $\zeta = 115$ Å) in aqueous phase [2] indicating smaller length scales in coacervate as compared to sol and gel.

Keywords. Gelatin; coacervates; light scattering; neutron scattering.

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1. Introduction

Coacervation is a process in which a homogeneous solution of charged macromolecules undergoes liquid–liquid phase separation, giving rise to a polymer rich dense phase. Coacervation has been classified into simple and complex processes depending on the number of participating macromolecules. In simple polyelectrolyte coacervation, addition of salt or alcohol normally promotes coacervation. In complex coacervation, two oppositely charged macromolecules (or a polyelectrolyte and an oppositely charged colloid) could undergo coacervation through associative interactions. The charges on the polyelectrolyte must be sufficiently large as to cause significant electrostatic interactions, but not so large as to cause precipitation. The dilute liquid phase, usually the supernatant, remains in equilibrium with

the coacervate phase. These two liquid phases are incompatible and immiscible. Coacervates are a novel state of matter where the concentration of bound protein can reach a level of $\approx$150 g/l normally unsustainable in aqueous solutions. The investigations of basic aspects of coacervation of polyelectrolyte complexes provide a foundation not only for the basic understanding of these supramolecular structures but also for their practical applications to protein-related industrial process. In the present work, we have undertaken a systematic study on simple coacervation of Type-B gelatin (a low charge density molecule) in aqueous environment through turbidimetry, DLS and SANS to understand the kinetics and structure of simple coacervation. Such an understanding may serve as a precursor to the mapping of the complexities of complex coacervation.

2. Materials and methods

Ethanol was obtained from Merck, Germany. Gelatin (Type-B, molecular weight $\approx$90 $\pm$ 10 KD, microbiology grade devoid of *E. coli* and liquefier presence) and sodium chloride were bought from E. Merck, India. The solvent used was deionized water (for DLS), D_2O (for SANS), and pH (using 0.1 M HCl) and ionic strength of the solvent was first set as per the experimental requirement and the gelatin solutions (1% w/v) were prepared by dispersing gelatin in this medium at 60°C. The macromolecules were allowed to hydrate completely; this took 30 min to 1 hour. Then the system was titrated with ethanol at pH $= 5 = pI$ with ionic strength $I = 0.1$ M NaCl. The titration profiles clearly established the transition points in terms of the percentage of volume of ethanol added relative to that of solvent corresponding to the first occurrence of turbidity ($V_t = 47 \pm 2$ (v/v)%) and a sharp point of inflection ($V_p = 52 \pm 2$ (v/v)%), where the turbidity attained its maximum value observed through transmittance measurements by colorimeter (figure 1). The values of V_t and V_p characterized the initiation of intermolecular folding and intramolecular aggregate formation of the charge neutralized gelatin molecules, and the subsequent micro coacervate droplet formation [2]. As V_p is reached, the system follows a high centrifugation (relative centrifugal force (RCF) $\sim$6637 g), which allows the aggregates to form coacervate phase while the folded gelatin molecules mostly stay in the dispersed supernatant. The state of intermolecular aggregates and that of folded gelatin molecules form nanoparticles in the supernatant phase. The particle sizing measurements were done by dynamic laser light scattering (DLS) technique, using a Brookhaven-9000AT digital auto-correlator (Brookhaven Instruments, USA) and a home-made goniometer. The excitation source was a diode pumped solid-state laser (Model-DPY 305-II, Adlas, Germany) emitting 50 mW of power at 532 nm in linearly polarized single frequency mode. The scattering angle was fixed at 90° and the data analysis was done using CONTIN software provided by Brookhaven Instruments. The static structure factor of coacervate has been measured by small-angle neutron scattering. SANS experiments were performed on the spectrometer at the G.T. Laboratory, Dhruva reactor, Bhabha Atomic Research Centre, India. The wavelength of the neutrons used covered the scattering vector (q) range

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

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

The dense polymer phase coacervate was transformed to a quartz cell and the scattered intensity was measured as a function of scattering vector. The measured intensity was corrected for the background and the empty cell contribution and the data were normalized to get the structure factors.

For SANS studies one should follow mean field theory which reveals that the polymers in a good solvent at equilibrium has led to a form of structure factor of concentration fluctuations at high-q, known as the Ornstein–Zernike (O–Z) function given by

$$S_L(q) = S_L(0)/1 + q^2\xi^2; \quad q\xi \gg 1, \tag{1}$$

where $S_L(0)$ is the extrapolated structure factor at zero wave number and ξ is the correlation length associated with the size of the entangled network. Physically, $S_L(0)$ is related to the cross-link density and longitudinal osmotic modulus. An 'excess scattering' [1] has been reported at low wave numbers from polymeric solutions. This is caused by the enhanced long wavelength concentration fluctuations in the system. It is not clear so far as to what causes this excess scattering. If the spatial scale of density fluctuations due to the presence of inhomogeneities is large compared to the correlation length ξ, then the two contributions can be treated separately and added to give the total structure factor as

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

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

$$S_{\text{ex}}(q) = S_{\text{ex}}(0)/(1 + q^2\zeta^2)^2, \tag{3}$$

where $S_{\text{ex}}(0)$ is the extrapolated structure factor at zero wave vector and ζ is the inhomogeneities in the system. As $S_{\text{ex}}(q) \propto 1/q^4$, the D–B contribution dominates over O–Z function at low-q, whereas at high-q ($S_L(q) \propto 1/q^2$), O–Z function contributes the most to the scatter. Further details can be found in [1].

3. Result and discussion

Turbidimitric data reveals the transition points of coacervate droplets formation (figure 1). During the titration process, a few milliliters of the sample was drawn from the reaction beaker and loaded into borosilicate cylindrical cell (volume = 5 ml) and the DLS experiment was performed. The data analysis revealed two particle sizes throughout until V_p was reached. The smaller particles had a size of $\approx$50 nm whereas the aggregates grew in size to reach a value of several hundred nanometers at V_p. This is shown in figure 2. For comparison the value of dielectric constant of the ethanol–water mixture is shown in the same plot. Gelatin is not soluble in alcohols whereas water is a good solvent. As ethanol is added to water, the water
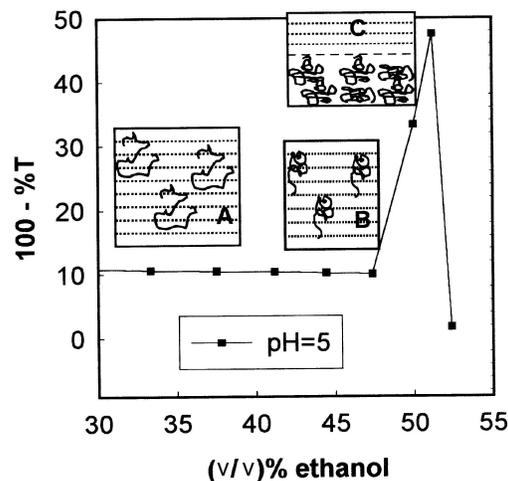
Figure 1. Transition profiles of V_t and V_p (both are in terms of (v/v)% of ethanol) vs. turbidity (100-% T, T is transmittance) data for a 1% (w/v) aqueous gelatin (Type-B) solution with $I = 0.1$ M performed at 25°C.

molecules will bind with ethanol molecules through hydrogen bonding and the resultant binary mixture becomes a poor solvent for gelatin molecules. Secondly, the dielectric constant decreases significantly facilitating stronger electrostatic interactions between charged segments (both intra and inter) of gelatin molecules. As per the Debye–Huckel theory, the strength of electrostatic interactions between two oppositely charged particles increases as $\varepsilon^{-3/2}$ increases at a given temperature. 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 intramolecular interactions yielding a typical hydrodynamic radius $\approx$25 nm (stable nanoparticles in supernatent) whereas most other molecules associate through intermolecular electrostatic interactions to form large aggregates of radius that are eight times larger.

We attempted fitting our neutron scattering data for coacervate to a Lorenzian behavior as given in eqs (1) and (3). To fit the data, a plot of $S(q)$ vs. q was made in the range of scattering vector $1.8 \times 10^{-2} \leq q \leq 2.0 \times 10^{-1}$ (Å)$^{-1}$. A typical situation is shown in figure 3 where experimental data is fitted to O–Z and D–B functions. A Ornstein–Zernike fit of the structure factor data in the q-range of $7.2 \times 10^{-2} \leq q \leq 2.0 \times 10^{-1}$ (Å)$^{-1}$ simply gives the scattering length or correlation length ξ of the entangled network of supra molecular complex coacervate. The slope of the fit enables one to easily calculate the value of ξ. For coacervate we found $\xi = 11 \pm 1$ Å. The excess scattering data have been analyzed within the framework of the Debye–Buche model. By subtracting the O–Z component from the total structure factor data (through visual estimation of q cut-off $\approx 7.2 \times 10^{-2}$ (Å)$^{-1}$), one can easily get the excess scattering contribution $S_{ex}(q)$. Next, these $S_{ex}(q)$ data were fitted to eq. (3) and the slope gave the parameter ζ. The characteristic sizes of the inhomogeneties (ζ) calculated from this fit is 21.4 Å. As it is clear from this
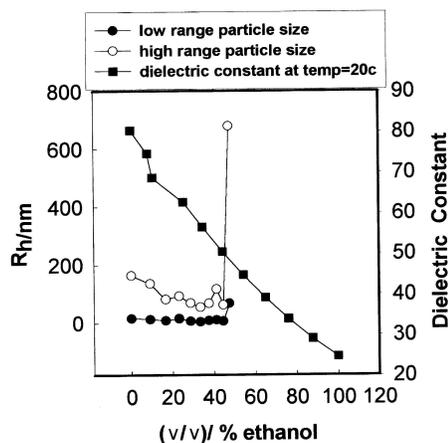
Figure 2. Size of various particles measured in terms of hydrodynamic radius (R_h) by DLS for a 1% (w/v) aqueous gelatin (Type-B) solution titrated with ethanol with $I = 0.1$ M and pH = 5 performed at 25°C (left-hand scale). The dielectric constant (ε) data of ethanol/water binary mixture is shown for comparison (right-hand scale).
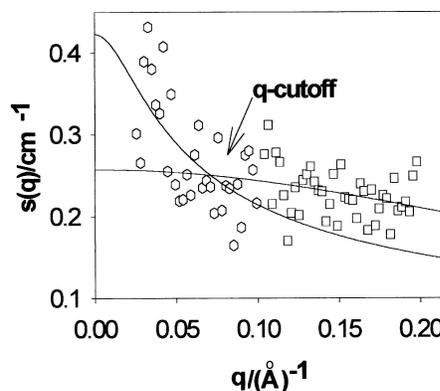
Figure 3. Structure factor ($S(q)/\text{cm}^{-1}$) of simple coacervates vs. wave-vector ($q/(\text{Å})^{-1}$) measured by SANS with $I = 0.1$ M performed at 25°C.

data, there is a decrease in ξ and ζ as we go from solution to gel and further to a coacervate phase.

The quality of data points in SANS was very poor because of incoherent scattering due to undeuterated ethanol present in the system. The reliability of estimation of the above two parameters can be tolerated with the instrumental limitations of SANS spectrometers.

4. Conclusion

Based on the above observation the gelatin coacervation phenomenon can be visualized as shown in figure 1. As it has been said earlier, addition of alcohol creates poor solvent environment for gelatin molecules largely because of the rupture of hydrogen bonds between water molecules and the polyion. This results in the reduction of the overall spatial extension of the polyelectrolyte chain, thereby, bringing the complementary charged segments closer. In a single chain this leads to self-charge neutralization and formation of gelatin particles of size $\approx$50 nm. These are mostly present in the supernatant. Simultaneously, intermolecular segments of complementary charge form aggregates of size $\approx$400 nm. Since, these aggregates may not be fully charge-neutralized, they can attract other gelatin molecules and thus, grow in size and forms coacervate.

Our investigation of coacervate state using SANS (small-angle neutron scattering) revealed the existence of two kinds of length scales – one of them being the correlation length ξ, associated with the size of the entangled transient network and the other having a higher length scale that can be attributed to long-range concentration fluctuations due to the inhomogeneities ζ present in these systems. It appears that the coacervate phase is a low dimensional heterogeneous material with possible gel-like networks, which owe to the existence of strong electrostatic interactions. Much of this need to be probed in greater details.

Acknowledgement

References

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