COACERVATES : A NOVEL STATE OF SOFT MATTER — AN OVERVIEW

H. B. BOHIDAR
Polymer and Biophysics Laboratory, Nanomaterials and Nanocomposites Laboratory, School of Physical Sciences, Jawaharlal Nehru University, New Delhi-110 067, India
Email : bohi0700@mail.jnu.ac.in

Abstract —Coacervation is usually defined as the spontaneous formation of a dense liquid phase from a macromolecular solution of poor solvent affinity. In "coacervation", the loss of solvation arises from the interaction of complementary macromolecular species. The formation of such macromolecule-rich fluids is well-known in mixtures of complementary polyelectrolytes; it can also occur from mixtures of polyelectrolytes with colloidal particles, leading to condensed soft matter phases with interesting properties. Though the polymer solution and gel states are adequately studied, characterized and mostly understood, the same for the coacervate phase is not true. Coacervates are macro-ionic hydrated complexes of a pair (self or complementary) of charge-neutralized polymers. Such a condensed phase remains in thermodynamic equilibrium with its supernatant that mostly contains a dilute dispersion of smaller intermolecular aggregates of the constituent polymers. This article intends to elucidate the salient features of this novel soft matter with some specific examples.

Keywords : Polyelectrolytes, intermolecular complexation, phase transition, coacervation.

INTRODUCTION

We can define coacervation as a process during which a homogeneous aqueous solution of charged macromolecules, undergoes liquid-liquid phase separation, giving rise to a polyelectrolyte-rich dense phase. Following the pioneering work of Bungenberg De Jung, coacervates are specified as either simple or complex depending on the process that leads to coacervation [1]. In simple coacervation processes, addition of salt normally promotes coacervation. On the other hand, in complex coacervation, two oppositely charged macromolecules, e.g., proteins or
oppositely charged polyelectrolytes can undergo coacervation through associative interactions. The other liquid phase, the supernatant, remains in equilibrium with the coacervate phase [1]. These two liquid phases are immiscible and hence, are incompatible. Complex coacervation of polyelectrolytes can be achieved through electrostatic interaction with oppositely charged proteins or polymers. The charges on the polyelectrolytes must be sufficiently large to cause significant electrostatic interactions, but not so large to cause precipitation. The features associated with coacervation have been summarized in details in several references [2-5]. For example, the solubilization efficiency of micelles is undiminished in micelle-polyelectrolyte associative phases [6] and protein structure appears to be unaltered in protein-polyelectrolyte coacervates, as evidenced most dramatically by the retention of enzymatic activity [7]. Since the properties of such self-assembled coacervates can be varied continuously from those of free-flowing fluids, to viscous gel-like materials, and finally to amorphous solids [8], without protein denaturation [9]; they offer the possibility of preparation of novel biocompatible and bio-active materials for a wide range of applications, extending well beyond current utilization in micro-encapsulation [10]. For example, protein-polyelectrolyte coacervates are a novel state of matter where the concentration of bound protein can reach a level of 150 g/L normally not sustainable in aqueous solutions. Coacervate formation by SDS and CTAB in aqueous solution in the presence of additives such as nonionic surfactants, PEG, glucose, urea, salts, etc., had also been studied [11].

Potential applications of coacervates are many starting from protein purification, drug encapsulation to treatment of organic plumes. This calls for better understanding of the coacervate structure and the transport of macromolecules inside this phase. Several questions pertaining to the structure of the coacervates can arise. The foremost of these is, is it a gel-like or a solution-like phase?

Liquid-Liquid Phase Transition

In a polyelectrolyte solution, the phase transition is driven by the electrostatic solute-solvent interactions which results in a gain in the configurational entropy and the formation of an amorphous randomly mixed polymer-rich phase (called "coacervates") remaining in equilibrium with the dilute supernatant. We have provided a rigorous proof to the empirical condition proposed by Dubin et al. [12] though we deal with a single polyelectrolyte undergoing self-charge neutralization, which is comparable to the complexation between oppositely charged polyelectrolytes described by Dubin et al. [12]. Physical conditions for phase separations was deduced explicitly which revealed that $\sigma^2/\sqrt{T} \geq \text{a constant}$, consistent with the experimental observations. In
the lattice model, $r$ is the number of sites occupied by the polymer having a critical volume fraction, $\varphi_{2c}$; it was found that phase separation would ensue when

$$
\sigma^3 r \geq \left(\frac{64}{9\alpha^2}\right) \frac{\varphi_{2c}}{(1-\varphi_{2c})^2}
$$

The above reduces to

$$
(\sigma^3 r/\varphi_{2c}) \geq \left(\frac{64}{9\alpha^2}\right) = 0.45 \text{ at } 20^\circ\text{C for } \varphi_{2c} < < 1
$$

where $\alpha$ is the electrostatic interaction parameter [13,14]. The separation kinetics was observed to mimic a spinodal decomposition process. Our model supported generation of a simple coacervate from a homogeneous solution that could be extended to describe phase transition leading to complex coacervation too. Fig. 1 depicts two pictures, one representing a coacervating solution and another showing a solution undergoing precipitation. As far as thermodynamics of liquid-liquid phase transition leading to coacervation is concerned not everything is known. The phase separation models proposed by Veis-Aranyi, Tainaka, Overbeek-Voorn and Nakajima-Sato do not address all the possible interactions adequately in their treatments [15-18]. However, there is unanimity on the following : (i) that a homogeneous solution containing $N_1$ molecules of solvent and $N_2$ molecules of solute at temperature $T$ and pressure $P$, will remain stable as long as the free energy of the solute $F_2$ in solution obeys the thermodynamic condition \((\partial^2 F_2/\partial N_2^2)_{N_1,T,P} > 0\), (ii) that the phase

---

\[\text{Coacervate} \hspace{1cm} \text{Precipitate} \]

Fig. 1. Coacervation is a liquid-liquid phase transition, and precipitation is a liquid-solid phase separation, process.
separation of the coacervate phase from the dilute supernatant is a dehydration (of the individual polyion) process, (iii) that charge neutralization of polyion segments precedes phase separation and (iv) that the polyions do not precipitate out of the solvent because of an entropy gain achieved by random mixing of the polyions in the coacervate phase. In summary, the coacervation proceeds in two steps, first, the selective charge neutralization of the polyions dictated by electrostatic interactions and second, the gain in entropy through random mixing of the polyions in the dense phase plus the gain in entropy due to release of counter ions to the solvent.

Synthesis of coacervates

It has been already mentioned that self-charge neutralization achieved in polyampholyte solutions can lead to coacervation. Such coacervates are called simple coacervates as opposed to complex coacervates formed from intermolecular complexation of a pair of complementary polyelectrolytes in solution.

(a) Simple coacervates

Gelatin is a random coil polymer carrying positive and negative charge-sites in almost 1:1 ratio. At the same time, it is associated with a small persistence length $\approx 2$ nm. The zeta potential curves shown in Fig. 2 imply an isoelectric pH $= 5$, though a small concentration dependence in its value could be clearly seen. This clearly prescribes the recipe that would lead to coacervation. Specifically, a gelatin solution prepared close to pH=5 is required to be turned into a poor solvent for gelatin molecules which will ensure chain collapse facilitating intramolecular electrostatic interactions leading to charge neutralization, and finally, coacervation (Fig. 3). The simple coacervates of gelatin were prepared in the following manner.

Typically 50 ml of stock solution (1% w/v aqueous gelatin) was taken in a beaker kept on a magnetic stirrer and was stirred at moderate speed with the stir bars throughout the titration process. The change in transmittance of the solution was monitored continuously using a turbidity meter (Brinkmann-910, Brinkmann Instruments, USA) operating at 450 nm. Alcohol was taken in a calibrated burette and added in drops to the reaction beaker and the volume of alcohol added to produce the first occurrence of turbidity was measured (B in Fig. 4) and the process was continued until a turbidity maximum was noticed (C in Fig. 4). Addition of more alcohol drove the system towards precipitation point. The reference points B and C characterized the initiation of intramolecular folding and intermolecular aggregate formation of the charge-neutralized gelatin molecules, and the subsequent micro coacervate droplet formation. The pH of the reaction mixture was monitored
Fig. 2. Determination of iso-electric pH (pl) of alkali processed type-B gelatin in aqueous solutions, pl was found to be 5.5±0.5. All measurements were done at 25°C. Solid lines are guide to eye only.

Fig. 3. A polyampholyte molecule like gelatin can undergo intramolecular or intermolecular folding. In a controlled phase transition process the latter leads to coacervation. However, small amount of intramolecular folded molecules are observed to exist in the supernatant of the coacervate.
continuously during this process. A typical titration curve is shown in Fig. 4. The general observation has been that as one proceeds from methanol to t-butanol both the neutralization points B and C occur at lower concentrations of the added alcohol. As one moves from methanol to t-butanol the hydrophobicity increases. It appears that higher hydrophobicity facilitates formation of the charge-neutralized aggregates of gelatin molecules that drive the system towards coacervation. Typically, coacervates are collected in the window between B and C. After achieving a cloudy solution during titration, the stock solution was allowed to stand for 30 minutes. Subsequently, this was centrifuged at 10,000 rpm for 15 minutes, the supernatant was decanted and the remaining solution was centrifuged again. This was continued 3-4 times which yielded an optically clear polymer-rich phase (coacervate) that collected at the bottom.

Fig. 4. A schematic representation (100–% Transmission (%T) vs. % (v/v) ethanol added) of the titration profile of a 0.01% (w/v) aqueous gelatin solution titrated with ethanol at 25°C close to iso-electric pH=5. The turbidity measured at 450 nm remains almost invariant up to ethanol concentration, 49% (v/v) when it sharply rises to give a maximum at ethanol concentration, 52% (v/v). The insets depict the folding of gelatin chains initiated due to intra-molecular charge neutralization. Coacervation transition initiates at B and continues until C beyond which phase separation leads to precipitation.
of the centrifuge tube.

Fig. 5 implies that as coacervation point is approached, the zeta potential of the aggregates that are formed due to associative interactions, tends to a very low value indicating effective charge neutralization achieved due to strong electrostatic binding between oppositely charged segments of the polymer. In fact the turbidity maxima corresponds to minimum zeta potential which is in complete agreement with the requirement dictated by the model of phase transition discussed earlier [14]. It must be realized here that gelatin is insoluble in ethanol, thus addition of ethanol to water creates a marginal solvent environment for the polymer that induces chain collapse. The experimental data presented, indicate the interplay of at least three different types of interactions that precede coacervation; (i) electrostatic interactions between charged segments of this polion, (ii) hydrophobic interactions between hydrophobic patch of gelatin molecule with aliphatic hydrocarbon tail of alcohols and (iii) solute-solvent interactions. It should also be realized that when two oppositely charged segments join together, some amount of counter ion is always released into

![Fig. 5. Titration profile shown for ethanol/water system for a 0.01% (w/v) aqueous gelatin (Type-B) solution performed at 25°C. Notice the coacervation transition occurring at volume fraction=0.47±0.2 coincides with minimum zeta potential and mobility. Solid lines are guide to eye only.](image-url)
the solvent, thereby increasing the entropy of the solution. This can also assist the process to move towards coacervation [19].

(b) Complex Coacervates

In complex coacervation, associative interaction between a pair of oppositely charged polyelectrolytes causes a thermodynamic condition identical to what was discussed earlier in the context of simple coacervation. The electrophoresis data shown in Fig. 6 imply polyanionic character of chitosan (a biopolymer and a polysaccharide) and gelatin-A below pH=9. Thus, it may appear that there will be hindrance to electrostatic interactions between these two molecules. However, the polyampholytic nature of gelatin-A makes associative electrostatic interactions possible through what is called ‘surface patch” binding [20]. The following paragraph describes how such coacervates are formed.

![Electrophoresis data for 0.01% (w/v) aqueous chitosan, gelatin and coacervating solutions at different solution pH. The data confirms the polyanionic behaviour of chitosan and polyampholyte nature of gelatin. Note that intermolecular complexation occurs even when the net charge of both the biopolymers are similar.](image-url)
Complex coacervate samples were prepared by mixing these two solutions (each of concentration, 1% w/v) in volumetric ratios, [chitosan] : [gelatin] = 1 : 10. The mixture was titrated with 0.1 N NaOH to increase the pH and the cloudiness was monitored through turbidity measurements. The titration profile is shown in Fig. 7 where the first occurrence of turbidity and precipitation are designated by pH\(_C\) and pH\(_\Phi\), respectively. Since, the electrostatic interactions are anomalous, the two transition pHs were observed to be independent of Debye screening length (see Fig. 7). The turbid samples were subjected to centrifugation at 10 thousand rpm for 30 minutes, which separated the turbid solution into two liquid phases, namely, the coacervate at the bottom and the supernatant at the top. The polymer-rich phase at the bottom was collected after decanting the supernatant. This was repeated at least three times, which yielded the coacervate. This is the normal procedure of extracting the coacervate from the reacted solutions.

Fig. 8 depicts the apparent Stokes radius (R\(_h\) = k\(_B\)T/6\(\pi\)\(\eta\)D) determined from the
translational diffusion coefficient, D for the polymer aggregates dispersed in the solvent having viscosity, η at the absolute temperature, T. k_B is the Boltzmann constant. A 256-channel digital correlator (Photocor Inc., USA) was used to obtain the Stokes radius data. For the non-interacting solution, the effective Stokes radius should correspond to chitosan which was ~340 nm. The window between B and C designates the interaction zone, and it is clearly seen that the size of the aggregates is smaller in this region. As chitosan binds to gelatin, the cluster attains an overall size less than that of chitosan due to charge neutralization that immediately alters its electrostatic persistence length. However, beyond C, the presence of coacervate droplets will contribute to an increase in the measured apparent Stokes radius as is seen in Fig. 8.

MICROSCOPIC STRUCTURE

Coacervate material is a novel and poorly understood state of matter. Some coacervates exhibit syneresis due to strong internal stress developed inside the
material. However, the pertinent question that arises is related to the nature of inter-
molecular assembly present inside the material. The following reports the results
obtained from three experimental techniques: rheology, small angle neutron scattering
(SANS) and atomic force microscopy (AFM). Mass transport inside the coacervate
material is of considerable importance which reveals the porosity of the material.

**Rheology**: The visco-elastic properties of the condensed materials can be best studied
by this technique [21]. The stress in this experiment is referred to as the complex
stress, $\sigma^* = \sigma' + i\sigma''$. The complex stress can be separated into two components. An
elastic stress ($\sigma'$) is in phase with the strain $\sigma' = \sigma^* \cos \delta$, where $\sigma'$ is the degree
to which material behaves like a elastic solid and a viscous stress ($\sigma''$) is in phase
with the strain rate $\sigma'' = \sigma^* \sin \delta$ where $\sigma''$ is the degree to which material behaves
like an ideal liquid. The parameter $\delta$ is the phase angle or phase shift between the
deformation and the response that is measured. As far as dynamic behavior is
concerned, a large number of experimental studies performed on visco-elastic systems
have shown that the complex shear modulus $G(t)$ follows a power law behaviour as
$G(t) \sim t^n$, which in the angular frequency, $\omega$ gives

$$G'(\omega) \sim G''(\omega) \sim \omega^n$$

(1)

This can be generalized to

$$G'(\omega) \sim \omega^{n'}$$

(2)

and

$$G'(\omega) \sim \omega^{n''}$$

(3)

Knowledge of the values of the exponents, $n'$ and $n''$ is of importance. As regard
the values of $n$, it is now accepted that a wide variety of values can be obtained
experimentally. Scalan and Winter [22], in particular, have shown that $n$ 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 poly(dimethylsiloxane) by a tetra functional cross-linking agent,
they obtained values varying from 0.2 to 0.92 depending on the experimental
conditions. These experimental results seem to show that there is no universal value
for $n$.

Rheology measurements were carried out using a stress controlled rheometer
operating in the oscillation mode (T.A. Instruments, UK). Fig. 9 presents the storage
and loss modulus curves, $G'(\omega)$ and $G''(\omega)$ in linear scale. Values $n'$ and $n''$ were
obtained by fitting the power-law functions given by Eq. 2 and 3 to the experimental
data for $G'$ and $G''$ of the coacervate samples. This yielded $G' \sim \omega^{0.25}$ and $G'' \sim \omega^{0.78}$. From definitions it is possible to determine the zero-shear viscosity, $\eta_0$ and
the corresponding creep compliance, $J_c^0$. These are given by

$$J_c^0 = \frac{1}{\eta_0^2} \lim_{\omega \to 0} G'/\omega^2 \tag{4}$$
$$\eta_0 = \lim_{\omega \to 0} G''/\omega \tag{5}$$

Numerical analysis of $G'$ and $G''$ as a function of $\omega$ leads directly to the values of $\eta_0$ and $J_c^0$ which were found to be 705 Pa and 1280 Pa$^{-1}$, respectively, for the coacervate phase. For comparison, rheology measurements were performed on a 5% (w/v) gelatin hydrogel (at 25°C) and its sol (at 60°C). The results obtained were $n' = 2.1$ for gel, and $n'' = 1.1$ for both the hydrogel and the sol. $\eta_0$ and $J_c^0$ were found to be 0.8 cP and 0.011 Pa$^{-1}$ for the sol, and 50 cP and 1.12 Pa$^{-1}$ for the gel sample, respectively. The concentration of the coacervate was estimated from the measurement of its dried mass and it was found to be $\approx 130$ g/L. Here the comparison is aimed at giving an idea of the magnitude of the parameters involved. The rheology data implied that the coacervate phase comprised a solvated polymer

![Image of frequency dependence of storage and loss modulli (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.](image)
phase that was highly dense with a 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 phase.

Here it is worth while to note that the semi-opaque nature of the material did not permit light scattering experiments to be performed. Dynamic light scattering (DLS) can probe network/mesh size of the interconnected domains inside the system. The osmotic swelling and de-swelling equilibrium is counter balanced by the excluded volume and the network entropic forces arising from the chain connectivity. The network topology permits statistical description of the system in terms of a well defined correlation length uniquely characterizing the network. However, the mesh size determined from DLS is different from the same inferred from rheology.

**Small Angle Neutron Scattering**: SANS is a diffraction technique and in order to improve contrast, the solvent is deuterated. These experiments were carried out at neutron scattering facility of Dhruv reactor, BARC, Trombay, India. The wavelength of the neutrons used covered the scattering vector (q) range,

\[ 7 \times 10^{-3} \leq q \leq 3 \times 10^{-1} \text{(Å)}^{-1} \]

Mean field theory applied to SANS studies reveal that the polymers in a good solvent at equilibrium yield a form of structure factor describing concentration fluctuations at low wave vector region known as the Ornstein-Zernike (O-Z) function given by

\[ S_L(q) = S_L(0)/(1 + q^2 \xi^2); q \xi << 1 \] (6)

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

An "excess scattering" 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. However, it has been observed that the random inhomogeneities with correlation length many times larger than the radius of gyration of the dissolved polymer, cause this excess scattering at low wave numbers domain of the scattering function. If the spatial scale of density fluctuations due to the presence of the inhomogeneities, is large compared to the correlation length, \( \xi \), then the two contributions can be treated separately and added to give the total structure factor as

\[ S(q) = S_L(q) + S_{ex}(q) \] (7)

where \( S_L(q) \) is the Ornstein-Zernike function [23], and the Debye-Bueche [24]
The structure factor has the form $S_{\text{ex}}(q)$ given by

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

(8)

where $S_{\text{ex}}(0)$ is the extrapolated structure factor at zero wave vector and $\xi$ is the correlation length. Often it is impossible to probe low $q$-domain of the structure factor because of the instrumental limitations of SANS spectrometers.

The structure factor $S(q)$ determined from the SANS scattering profile data for the coacervate samples is shown in Fig. 10. A least-square fit of the structure factor data in the $q$-range of $7.2 \times 10^{-2} \leq q \leq 2.0 \times 10^{-1} \text{(Å)}^{-1}$, simply gives the scattering length or the correlation length, $\xi$ of the entangled network of supra molecular complex coacervate in the Ornstein-Zernike model. For coacervates, we found $\xi = 27 \pm 2$ Å. This length compares well with the known persistence length of gelatin, which is $25$ Å. 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 the S(q) versus q data profile and resolve the low and the long wavelength scattering regions. This will help in resolving the confusion pertaining to the small value of correlation length observed. For qualitative comparison, we measured the values of \( \xi \) and \( \zeta \) for 5 % (w/v) gelatin sol (at 60°C) and gel (at 25°C); the values were \( \xi = 50 \) Å and \( \zeta = 115 \) Å for both the samples. The coacervate concentration was 13 % (w/v), hence the comparison is aimed at giving an idea of the magnitude of the parameters involved with an objective to understand these at micro level.

**Atomic Force Microscopy**

The control parameters (alcohol concentration or pH) drive the solution to a point where turbidity shows a sharp rise implying coacervation transition as is seen in Fig. 4,5 and 7. Such a situation corresponds to a state where very large number of charge-neutralized inter and intra molecular clusters of biopolymer molecules, is formed in a cooperative manner. The corresponding AFM pictures do show the presence of typically 1 \( \mu \)m clusters along with the larger ones (see Fig. 11). The polymer particles and their complexes could be visualized in the solvent environment because the hydration solvent has a refractive index different from that of the bulk solvent, which was responsible for providing the necessary optical contrast. Most of these are intermolecular 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.

![Atomic force microscopy picture (5 \( \mu \)m × 5 \( \mu \)m) of a gelatin coacervate sample prepared at 25°C from 1%(w/v) aqueous solution (pH=5) in the con-contact mode. The picture shows lumps of dense matter with immense heterogeneity spread in space having no definite geometric structures.](image)
and we attribute this to instrumental inadequacy. Equilibrium images of the coacervates (Fig. 11) show lumps of dense matter with immense heterogeneity spread in space having no definite geometric structures.

**Transport inside coacervate**

Diffusion of probe particles in cross-linked gels and concentrated polymer solutions are known to obey different dynamics. Here it would be worthwhile to discuss the formalisms of transport in polymer solutions and gels commonly used. In a normal diffusion process, the mean-square displacement of the particle is directly proportional to the time, whereas in an anomalous process, this dependence is highly nonlinear.

Let us discuss the solution features first. In normal fluids, the Stokes-Einstein (S-E) relation \( D = \frac{k_B T}{6 \pi \eta R} \) assumes that the dispersing medium is continuous with viscosity \( \eta \). This equation is a manifestation of equilibrium between the concentration fluctuation driven Fickian diffusion motion and the opposing viscous drag acting on a particle of hydrodynamic radius \( R \) diffusing with a diffusion coefficient \( D \) at temperature \( T \). Assuming that there is no adsorption on the probe particle, the product \( D\eta \) should be constant at a fixed temperature for various dispersion media. If the corresponding product in the solvent phase is \( D_o\eta_o \), it is customary to define a ratio \( D\eta/D_o\eta_o \) as an indicator to classify a (Newtonian) medium. If this ratio is 1, S-E equation is obeyed by the medium. Both positive and negative deviations are observed in a number of solvents and polymer solutions. A positive deviation implies that the micro-viscosity is larger than the macroscopic viscosity. These features have been discussed in details in several reports.

However, one needs to exercise caution in making arbitrary conclusions from the \( D\eta/D_o\eta_o \) ratio data. In any such comparison it must be ensured in advance that both the dispersion media are Newtonian. Another approach to this problem has been largely empirical and the polymer molecules dispersed in the solvent are basically treated as crowding agents creating obstructions to the diffusing particle. Here one expresses the diffusivity ratio as a function of polymer volume fraction \( \phi \), as

\[
D/D_o = \exp(-a \phi^\alpha)
\]

where, \( a \) and \( \alpha \) are constants. Eq.(9) would revert to S-E equation in the limit of \( \alpha = 1 \) and \( a=1 \), implying that both the dispersion media are Newtonian. This has been successfully applied to many polymeric solutions to describe macromolecular diffusion.

On the other hand, in a cross-linked polymer medium (i.e., a gel), the transport features become even more complex because the elasticity of the network can contribute to the transport process. This is an anomalous diffusion process and
necessitates the reformulation of the normal diffusion problem by invoking non-ergodicity concepts. In free solution phase the probe particle can explore the entire configurational space of the system and hence, the time averaged dynamic structure factor measured through dynamic light scattering (DLS) gives a good ensemble averaged picture of the system. However, in gels the probe particle gets confined due to geometrical constraints offered by the cross-linked networks, and can execute only local excursions about its mean position. Thus it cannot explore the entire configurational space and the time averaged dynamic structure factor no longer is a representative of the whole ensemble of the system. Here the data interpretation of DLS measurements becomes too ambiguous. Brownian motion of the probe particles in such systems was investigated by Ohbayashi et al. [25]. The probe particles were used to measure micro-viscosity and channel size of sickle-cell haemoglobin gels by Madonia et al. [26]. Allain et al. [27] studied the diffusion of probe particles in gelling copolymerization of acrylamide and bisacrylamide solutions and deduced the evolution of micro-viscosity. The wave vector dependence of the probe diffusion was explored by Nishio et al. [28] in polyacrylamide gels and the pore size of the gel network where the cross-link content was varied through the gelation threshold was deduced from the DLS data. Djabourov et al. [29] adopted a similar approach and explored colloidal probe diffusion in a physical gel. Subsequently, Reina et al. [30] reported observation of a complex dynamical behaviour ranging from the purely translational diffusion of the probe particles in the medium to a relaxational behaviour associated with the local motion of the probe particles in a confined space inside the gel. The dynamics of probe particles in a semidilute polymer solution was quantified through a set of phenomenological scaling laws by Phillies et al [31] The molecular weight dependence of probe diffusion in polyacrylamide gels was studied in details by Tokita et al. [32]. Pusey and Van Megen [33] proposed the ergodic to non-ergodic transition as seen by the probe particles during the sol to gel transition process. Several theoretical models have been proposed to map the specific dynamics of diffusion processes in non-Newtonian media. These have been reviewed extensively by Amsden [34].

Given this background, it would be interesting to study the transport of protein in coacervates. Realizing that this has direct bearing on transport of proteins through membranes, the importance of these studies can be hardly stressed. The system chosen was a complex coacervate of protein (BSA) and a polycationic polymer PDMDAC (polydimethyl diammonium chloride) [35]. As comparative models, we study the diffusion of Ficoll and BSA in Dextran medium with the concentration of Dextran tuned to produce the same macroscopic viscosity as that of coacervates. We have investigated a set of physical situations, namely, (a) diffusion of BSA in a
medium with significant electrostatic field (the coacervate phase), (b) diffusion of BSA in a neutral medium (in Dextran) with the same macroscopic viscosity as the coacervate (diffusion of a charged particle in neutral medium), (c) diffusion of Ficoll in coacervate medium (transport of a neutral particle in an electrostatically charged medium), (d) diffusion of neutral particle (Ficoll) of same size as BSA in a neutral medium (Dextran) (transport governed by hydrodynamics only) and (e) diffusion of BSA in a non interacting solution of the polyelectrolyte. Details of the results and conclusions are given in ref. [35].

CONCLUSION

Coacervation has long been postulated as an important mechanism for the concentration of bio-macromolecules necessary for the origin of cells [36]. Such coacervates could have been formed from a "thermal proteinoid" prebiological polymer by means of simple dehydration and rehydration [37]. Experiments that explored the formation of similar self-organized microscopic structures using nucleic acids were highly successful [38]. Because the physical chemistry of this phenomenon was largely ignored during the last 50 years, it is not known whether such self-assembled fluids are concentrated polymer solutions, transient physical gels or some intermediate state. Thus, while the phenomenology of coacervation might be considered "mature", the nature of coacervates remains essentially unexplored due to the lack of systematic studies with modern techniques. This array of methods allows us to examine coacervates on many length scales leading to a conclusive description of the microscopic structure and dynamics of these unique self-assembled materials. The results taken together reveal that the coacervate phase is a solution-like state in which homogeneous fluid-like domains coexist with denser and more nearly charge-neutralized domains which inhibit local protein diffusion and confer transient network rheology.

ACKNOWLEDGEMENTS

Most of the results cited in this paper owe their origin to the Ph.D. theses of two of my former students, Biswaranjan Mohanty and Amarnath Gupta. This research was partly funded by a grant from Department of Science and Technology, Govt. of India.

REFERENCES

Coacervates: A Novel State of Soft Matter — An Overview


