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Kinetics of phase separation in systems exhibiting simple coacervation

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The kinetics of phase separation of a homogeneous polyelectrolytic solution into a dense polymer-rich coacervate and the dilute supernatant phase is discussed through statistical thermodynamics. It has been shown that the coacervate phase is associated with higher internal pressure, consequently giving rise to syneresis. Physical conditions for phase separations has been deduced explicitly which reveals that $\sigma^2/\sqrt{I} \geqslant$ constant (where σ is polyelectrolyte charge density and I is solution ionic strength), consistent with experimental observations. In the lattice model, r is the number of sites occupied by the polymer having a volume critical fraction φ_{2c}, it was found that phase separation would ensue when $\sigma^3 r \geqslant (64/9\alpha^2)[\varphi_{2c}/(1-\varphi_{2c})^2]$, which reduces to $(\sigma^3 r/\varphi_{2c}) \geqslant (64/9\alpha^2) \approx 0.45$ at 20 °C for $\varphi_{2c} \ll 1$. The separation kinetics mimics a spinodal decomposition process. Rate of release of supernatant due to syneresis was found to be independent of the initial coacervate mass. Syneresis results are discussed in the context of temporal evolution of self-organization in polymer melts through Avrami model.

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I. INTRODUCTION

Coacervation is a process during which a homogeneous solution of charged macromolecules, undergoes liquid-liquid phase separation, giving rise to a polymer rich dense phase [1]. Coacervation has been classified into simple and complex processes depending on the number of participating macromolecules [2]. In simple polyelectrolyte coacervation, addition of salt or alcohol normally promotes coacervation through self-charge neutralization [1]. In complex coacervation, two oppositely charged macromolecules (or a polyelectrolyte and an oppositely charged colloid) can undergo coacervation through associative interactions [3]. The charges on the polyelectrolytes must be sufficiently large to cause significant electrostatic interactions, but not so large 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.

In the past, the experimental data obtained from experimental studies on gelatin coacervates were used to test the theoretical models proposed by (i) Overbeek-Voorn [4], (ii) Veis-Aranyi [5,6], (iii) Nakajima-Sato [7], and (iv) Tainaka [8]. The Overbeek-Voorn model [4] estimates the total free energy of the system as a sum of electrostatic free energy (between pairs of charged sites of gelatin molecules) and free energy of mixing estimated on the basis of Flory-Huggins solute-solvent interaction. This model downplays the role of solute-solvent interactions and yields a coacervation condition given by $\sigma^3 r \geqslant 0.53$ for two component systems. Here σ is the charge density of the polyelectrolyte and r is the number of sites occupied by the gelatin molecule (basically equivalent to molecular weight). Thus both high molecular weight and large charge density facilitate coacervation. For three component systems (in presence of micro-ions) $\sigma^3 r \geqslant 1.06$.

Veis-Aranyi [5] proposed a model for coacervation between Type-A and Type-B gelatin molecules, popularly referred to as *Dilute phase aggregation model* which undermines the electrostatic interactions and postulates the formation of complex coacervates through solute-solvent interactions characterized by the Flory-Huggins interaction parameter χ assuming nonzero heat of mixing. In the Flory-Huggins picture χ has only an enthalpy term in it whereas Veis-Aranyi argue the presence of an additional configurational entropy term. Correspondingly, χ is not proportional to $1/T$, where T is the solution temperature. The proportionality is valid only in the absence of entropic contribution to χ. In this model, the heat of mixing, ΔH_m is proportional to χ and one observes coacervation even when $\sigma^3 r \leqslant 0.53$ contrary to Overbeek-Voorn prediction.

In the Nakajima-Sato model [7], the Overbeek-Voorn [4] treatment was formalized by inclusion of the solute-solvent interactions in the calculations through Flory-Huggins prescriptions. This model was applied to the data obtained from coacervation of nearly symmetrical polyvinyl alcohol molecules of high charge density [7]. Regardless, the model rests on the Overbeek-Voorn presumption that the polyelectrolyte charges are distributed uniformly both in the dilute and concentrated phase. In the Tainaka model [8] complementary charge pairing between polyelectrolyte molecules in the dilute phase occurs following Veis-Aranyi proposition. The main assertion of this model is that the aggregates are symmetrical in charge distribution and size regardless of the same of polyions. Tainaka used the virial coefficient expansion procedure, which Veis [6] applied in the dilute phase, for both phases. This model is not restricted to low charge density polyions, although the Veis-Aranyi critical condition for phase separation had to be met. However, at large charge density the polyion becomes too stiff to form chargeneutralized complexes. All these models agree on the suppression of coacervation at high ionic strength. Unfortunately, none of these models adequately explain the underlying dynamics of liquid-liquid phase separation preceding complex coacervation. Burgess [2], through a series of experiments performed

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on gelatin-acacia and alginic acid-albumin systems, has shown the relative merits of various models. In gelatin coacervation studies, the coherent picture that had emerged was [9] (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 liquid-liquid phase separation of the coacervate phase from the dilute supernatant is a dehydration 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 entropy gain achieved by random mixing of polyions in the coacervate phase. In summary, the coacervation proceeds in two steps, first the selective charge neutralization of polyions dictated by electrostatic interactions and second, the gain in entropy through random mixing of polyions in the dense phase plus the gain in entropy due to release of counter ions to the solvent.

A survey of literature confirms fewer systematic studies on simple coacervate systems. Gelatin, a polyampholyte obtained from denatured collagen, is a polypeptide and is an ideal case for such studies. The aqueous solutions properties of gelatin have been well studied and characterized in the past [10,11]. Depending on the process of recovery the gelatin molecules bear different physical characteristics. Type-A gelatin is acid processed, has an isoelectric pH, $p\mathrm{I} \approx 9$ whereas the alkali processed type-B gelatin has $p\mathrm{I} \approx 5$ [17]. In the past all the coacervation studies on gelatin involved complexation between type-A and type-B, or gelatin and acacia molecules [12–16].

In a typical simple coacervation process, a homogeneous solution of $1\%(w/v)$ gelatin is driven toward a liquid-liquid phase separation, giving rise to a polymer dense phase remaining in equilibrium with its supernatant. Coacervates are formed through sequential self-charge neutralization of gelatin molecules that are mostly intermolecular. This is described in excellent details elsewhere [17,18]. Gelatin is a polyampholyte with typically the positive and negatively charged segments occurring in its backbone in the ratio 1:1 which constitute about 22% of chain length together. Addition of a nonsolvent like alcohol facilitates the process of inducing chain collapse and the positively charged segments interact with the negatively charged segments through screened Coulomb interactions, the details of this and phase diagrams are discussed elsewhere [17].

In the recent past, we have systematically studied the coacervation phenomenon in gelatin system by using an array of experimental techniques like light and neutron scattering, turbidimitry, rheology, circular dichroism, UV spectroscopy, etc. The kinetics of liquid-liquid phase separation of polyelectrolytic solutions into coacervate and supernatant phases has been discussed in this paper in specific context to gelatin system [19]. The theoretical study on the kinetics of complex coacervation has not attracted much attention in the past. Very little is known about the kinetics of simple coacervation occurring in gelatin solutions. And systematic and comprehensive theoretical approach that encapsulates the understanding of kinetics of phase separation, spinodal decomposition and syneresis is still missing. The present theoretical treatment is an attempt in that direction and our model is parallel to Veis model with the exception that we include contribution from electrostatic interactions. Existence of syneresis in coacervates has been established conclusively, though qualitatively.

II. THEORETICAL TREATMENT

A. Phase transition in polyelectrolytic solutions

In a binary system comprising a flexible polyelectrolyte chain and a solvent, the larger entropy of the solution arises from the greater number of spatial arrangements accessible to the polymer chain. The long polyelectrolytic chain may be split into r number of chain segments each of which is comparable to the size of the solvent molecule. The parameter r may be conceived as the ratio of the molar volumes of the solute and the solvent. Such a description is adaptable to the lattice model where the solvent and the polymer segment can trade locations *per se*. The total number of lattice points is, $n_0 = n_1 + rn_2$, where n_1 and n_2 are number of lattice sites occupied by solvent and solute (polymer) molecules, respectively. Let z be the coordination number (first nearest neighbors) to a given cell. Suppose i polyelectrolyte molecules have been inserted at random to the lattice. This will leave $(n_0 - ri)$ vacant and available for others. The first segment of $(i+1)$th polyelectrolyte molecule may go to any of these vacant cells. The second segment must be assigned to any of the z neighbors of the cell already occupied by the first cell.

Let f_i represent the expectancy that a given cell adjacent to a previously vacant site is occupied. The expected number of cells available for the second segment will be $z(1-f_i)$. Same for the third segment will be $(z-1)(1-f_i)$. Thus the expected number of r contiguous sites available to the polyelectrolyte molecule is

$$v_{i+1} = (n_0 - ri)z(z-1)^{r-1}(1-f_i). \qquad (1)$$

If each of the n_2 sites available to the polymer segments are distinguishable from one another, the number of ways in which all of these can be arranged in the lattice will be $\prod_{i=1}^{n_2} v_i$. So the total number of ways (Ω) in which n_2 sets of r consecutively adjacent sites may be chosen from the lattice is

$$\prod_{i=1}^{n_2} v_i = n_2!\,\Omega \quad \text{or} \quad \Omega = (1/n_2!)\prod_{i=1}^{n_2} v_i. \qquad (2)$$

Also,

$$1 - f_i = 1 - (ir/n_0). \qquad (3)$$

Thus Eq. (1) can be written as

$$v_{i+1} = (n_0 - ir)^r \left(\frac{(Z-1)}{n_0}\right)^{r-1}. \qquad (4)$$

In the Flory and Huggins model the free energy of mixing, $\Delta F_m = \Delta H_m - T\Delta S_m$ was given as $\Delta F_m = k_B T[(n_1 \ln \varphi_1 + n_2 \ln \varphi_2) + \chi n_1 \varphi_2]$. Such an expression is appropriate for nonionic polymer solutions where the excluded volume interactions adequately describe the solution stability through Flory-Huggins interaction parameter χ.

The entropy of mixing ($\Delta S_m = S - S^*$) of a polyelectrolyte and the solvent in presence of finite electrostatic interactions will be given as

$$\Delta S_m = -k_B(n_1 \ln \varphi_1 + n_2 \ln \varphi_2) + \alpha k_B(n_1 + rn_2)(\sigma\varphi_2)^{3/2}. \tag{5}$$

The star corresponds to values in the pure state and the volume fractions of the solvent and solute components are $\varphi_1 = n_1/(n_1 + rn_2)$ and $\varphi_2 = r\, n_2/(n_1 + rn_2)$, respectively. In the absence of the second term on the right-hand side ΔS_m represents only the configurational entropy change due to mixing. The second term represents the electrostatic contribution and the positive sign implies gain in entropy due to interactions. The electrostatic interaction parameter in a solution of dielectric constant, ε at temperature T will be given by $\alpha = (e^2/3\varepsilon)(4\pi e^2/\varepsilon k_B v T)^{1/2}(1/k_B T)$, where v is the volume of a lattice site in solution. Correspondingly the mixing energy $(U - U^*)$ is given in terms of Flory-Huggins interactions parameter χ as

$$(U - U^*) \approx k_B T n_0 \chi \varphi_1 \varphi_2. \tag{6}$$

In our treatment the number of sites occupied by the solvent and solute molecules are $n\varphi_1$ and $n\varphi_2$, respectively, with n_0 being the total number of sites available. The number of solvent and solute molecules are $n_1 = n_0\varphi_1$ and $n_2 = n_0\varphi_2/r$, respectively. From Eqs. (2) and (4),

$$\Omega = (1/n_2\,!)\prod_{i=1}^{n_2} v_i = \left(\frac{n_0!}{(n_0 - rn_2)\,!\, n_2!}\right)\left(\frac{(z-1)}{n_0}\right)^{n_2(r-1)}. \tag{7}$$

The entropy of mixing of the perfectly ordered pure polymer and pure solvent will be

$$S = k_B \ln \boldsymbol{\Omega} = -k_B\{n_1 \ln[n_1/(n_1 + rn_2)] + n_2 \ln[n_2/(n_1 + rn_2)] - n_2(r-1)\ln[(Z-1)/e]\}. \tag{8}$$

The formation of a homogeneous solution occurs in two steps (i) disorientation of polymer molecules and (ii) mixing of the disoriented polymer with solvent. The first step can be ensured by setting $n_1 = 0$ then

$$\Delta S_{\text{disorient}} = k_B n_2[\ln r + (r-1)\ln\{(Z-1)/e\}]. \tag{9}$$

If r is large then the first term can be neglected. So,

$$(1/rn_2)\Delta S_{\text{disorient}} \approx k_B \ln[(Z-1)/e]. \tag{10}$$

The entropy of the disoriented polymer chain will be greater for more flexible (smaller persistence length) chains and more disordered the packing of segments in the solution. The free energy of mixing, $\Delta F_m = (F - F^*)$ will be given from Eqs. (5) and (6) as

$$\begin{aligned}\frac{(F - F^*)}{k_B T} &= \frac{(U - U^*)}{k_B T} - \frac{(S - S^*)}{k_B} = \Delta F_m = \Delta H_m - T\Delta S_m \\ &= (n_1 \ln \varphi_1 + n_2 \ln \varphi_2) + \chi n_1 \varphi_2 - \alpha(n_1 + rn_2) \\ &\quad \times (\sigma\varphi_2)^{3/2}.\end{aligned} \tag{11}$$

Veis *et al.* [5,6] derived the most general form for free energy of a multicomponent system. However, they have not shown the explicit critical condition for phase separation. In their model, introduction of the solute-solvent interaction yielded unrealistic values for $r(\approx 10^7)$. They concluded unless $r \geqslant 10^7$ coacervation transition was not possible if σ were independent of the environment. As contrast to this we shall show that for a simple two-component system (solute and solvent) their contention is not correct and it is possible to arrive at explicit phase separation conditions, which are experimentally verifiable.

Taking derivative with respect to n_1 and n_2,

$$\begin{aligned}\frac{\partial(F - F^*)/k_B T}{\partial n_1} &= \frac{(\mu_1 - \mu_1{}^*)}{k_B T} = \ln \varphi_1 + 1 - \varphi_1 - (\varphi_2/r) + \chi\varphi_2{}^2 \\ &\quad + (\alpha/2)(\sigma\varphi_2)^{3/2}\end{aligned} \tag{12}$$

and

$$\begin{aligned}\frac{\partial(F - F^*)/k_B T}{\partial n_2} &= \frac{(\mu_2 - \mu_2{}^*)}{k_B T} = \ln \varphi_2 + 1 - \varphi_2 - (\varphi_1 r) + r\chi\varphi_1{}^2 \\ &\quad + (\alpha/2)(\sigma)^{3/2} r(\varphi_2{}^{3/2} - 3\varphi_2{}^{1/2}),\end{aligned} \tag{13}$$

the parameter μ stands for chemical potential. For phase separation,

$$\left(\frac{\partial\mu_1}{\partial\varphi_2}\right)_{n_2,T,P} \leqslant 0 \quad \text{and} \quad \left(\frac{\partial^2\mu_1}{\partial\varphi_2{}^2}\right)_{n_2,T,P} = 0 \tag{14}$$

which yields the following conditions:

$$-\frac{1}{(1 - \varphi_2)} + 1 - (1/r) + 2\chi\varphi_2 + (3\alpha/4)\sigma^{3/2}\varphi_2{}^{1/2} \leqslant 0, \tag{15}$$

$$-\frac{1}{(1 - \varphi_2)^2} + 2\chi + (3\alpha/8)\sigma^{3/2}\varphi_2{}^{-1/2} = 0. \tag{16}$$

Equations (15) and (16) give the condition for phase transition as

$$\frac{\varphi_{2c}{}^2}{(1 - \varphi_{2c})^2} + (3\alpha/8)\sigma^{3/2}\varphi_{2c}{}^{-1/2} = 1/r. \tag{17}$$

This equation, which is a fifth order polynomial in φ_2 interrelates the critical volume fraction (φ_{2c}), charge density (σ), electrostatic interaction constant (α) and molecular weight (r) of the polyelectrolyte. The critical values for volume fraction (φ_{2c}) that will ensure phase transition can be deduced from Eq. (15) these correspond to conditions where $f(\varphi_2)$ changes sign. In Fig. 1, $f(\varphi_2)$ vs φ_2 is plotted and two physical values for φ_2 are identified corresponding to $f(\varphi_2) = 0$ (other three solutions are complex and, hence not physical). The values of interaction parameter taken from literature are $\alpha = 4.073$, 4.148, and 4.236 at 20 °C, 30 °C, and 40 °C, respectively [5,6]. This combined with $\sigma \approx 0.01$ for gelatin gives $\phi_{2c} \approx 4.42 \times 10^{-5}, 3.43 \times 10^{-3}, 2.67 \times 10^{-2}$, and 8.90×10^{-2} for $r = 10^5, 10^4, 10^3$, and 10^2, respectively, which is plotted in Fig. 2.

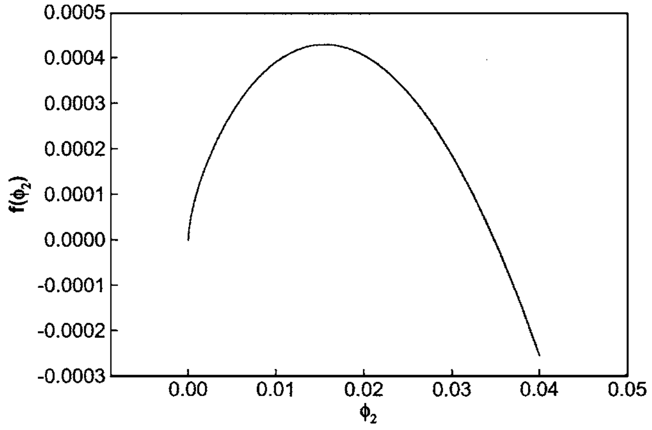

FIG. 1. Plot of $f(\varphi_2)$ vs polyelectrolyte volume fraction φ_2. Two physical values for φ_2 can be identified corresponding to $f(\varphi_2)=0$ (other three solutions are complex and, hence not physical). The values of interaction parameter are taken from the literature [5,6]. See text for details.

From Eq. (15),

$$(3\alpha/8)\sigma^{3/2}{\varphi_2}^{-1/2} \geqslant 1 + 2\varphi_2 - 2\chi. \tag{18a}$$

Further in the absence of excluded volume interactions, $\chi \approx 0.5$ and one has (for a given φ_2)

$$(3\alpha/8)\sigma^{3/2}{\varphi_2}^{-1/2} \geqslant 2\varphi_2, \tag{18b}$$

$$\sigma^{3/2}{\varphi_2}^{-1/2} \geqslant (16/9\alpha)\varphi_2, \tag{18c}$$

$$\sigma^2/\sqrt{\sigma\varphi 2} \geqslant (16/9\alpha)\varphi_2, \tag{18d}$$

$${\sigma_c}^2/\sqrt{I} \geqslant \text{const}. \tag{19}$$

Since, $\sigma\varphi_2$ is equivalent to the ionic strength of the solution, I. The factor $(16/9\alpha)\varphi_2$ is constant for a given polymer concentration. The typical values are ≈ 0.044 and ≈ 0.087 for $\varphi_2=0.1$ and 0.2, respectively, where we use $\alpha \approx 4.073$ as before.

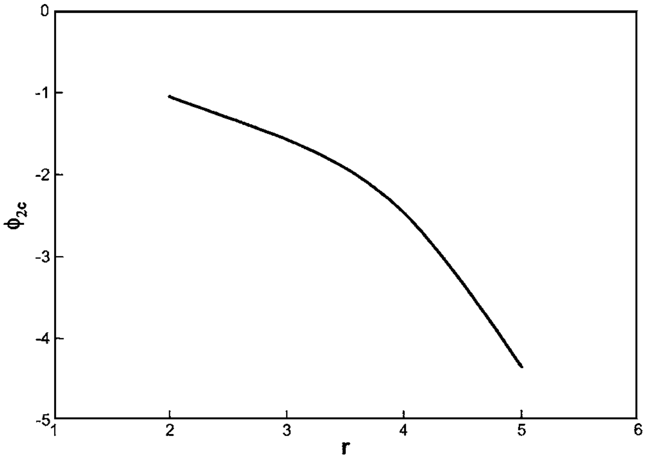

FIG. 2. Variation of critical polyelectrolyte volume fraction φ_{2c} as function of polyelectrolyte molecular weight (r) for a given linear charge density of the polymer ($\sigma \approx 0.1$) at 20 °C. Notice that the φ_{2c} decreases with increase in the polyelectrolyte molecular weight.

It has been shown that for a wide variety of experimental conditions [20,21], the onset of complexation between complementary macroions conforms to an empirical relation given by $\sigma v/\sqrt{I} \geqslant \text{const}$, where v is the charge density of complementary polyelectrolyte. Odijk [22] has argued that for this empirical relation to be valid the interactions in the system must adhere to the following requirements: (i) Debye-Huckel approximation must be valid, (ii) complexation is independent of polyelectrolyte chain statistics, and (iii) excluded volume effects are not significant. For self-charge neutralization v can be replaced by σ and Eq. (19) follows. We have provided a rigorous proof to the empirical condition proposed by Dubin *et al.* [23] 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.* [23]. Our system will generate a simple coacervate whereas a two-polyelectrolyte system yields a complex coacervate.

Equation (16) yields a general condition for phase separation

$$\sigma^{3/2} \geqslant \left(\frac{8}{3\alpha}\right)\left(\frac{{\varphi_2}^{3/2}}{(1-\varphi_2)}\right). \tag{20}$$

For the ideal case, $\varphi_2 \approx 1/r^{1/2}$ gives

$$\sigma^3 r \geqslant \left(\frac{64}{9\alpha^2}\right)\left(\frac{\varphi_2}{(1-\varphi_2)^2}\right) \tag{21}$$

normally, $\varphi_2 \leqslant 1$, hence

$$(\sigma^3 r/\varphi_{2c}) \geqslant \left(\frac{64}{9\alpha^2}\right) \approx 0.45. \tag{22}$$

Under the condition ($\chi \approx 0.5$ and ${\phi 2_{2c}}^2 \sim 1/r$), Eq. (16) gives a condition for phase separation (at 20 °C)

$$\sigma/\varphi_{2c} \geqslant (0.45)^{1/3} \approx 0.78. \tag{23}$$

Here the phase separation kinetics becomes independent of polymer size. The variation of φ_{2c} as function of excluded volume interaction is shown in Fig. 3, which predicts that no coacervation transition is possible below gelatin concentration $\approx 0.01\%(w/v)$ which has been experimentally verified. Similarly, Eq. (22) gives the dependence on polyelectrolyte molecular weight. It predicts absence of coacervation transition for polyelectrolytes having $r \leqslant 10\,000$. For low molecular weight (less than 10 kD) polymers self-charge neutralization seems difficult.

B. Coalescence of coacervate droplets

Formation of coacervate droplets precedes the phase separation during coacervation. If we visualize the phase separation as a spinodal decomposition process [24–26] which ensures that the derivative of the free energy with respect to the volume fraction of the polymer (solute) is same for both supernatant and the coacervate giving $(\mu_1-\mu_1^0)^{\mathrm{I}}=(\mu_1-\mu_1^0)^{\mathrm{II}}$ and $(\mu_2-\mu_2^0)^{\mathrm{I}}=(\mu_2-\mu_2^0)^{\mathrm{II}}$. Superscripts II and I are for coacervate and dilute phase, respectively. The subscripts 1 and 2

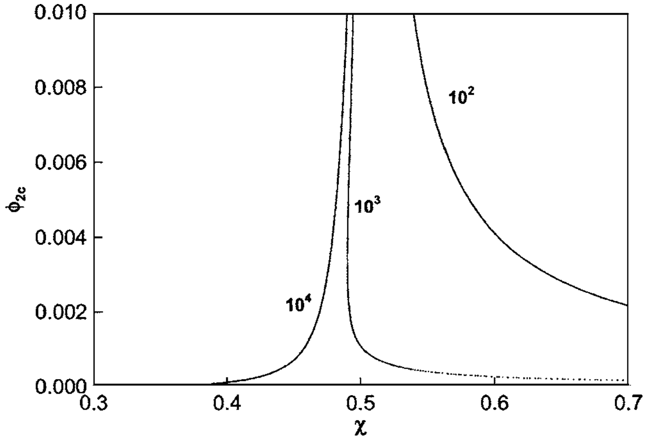

FIG. 3. Dependence of φ_{2c} on the solute-solvent interaction parameter χ for different values of polyelectrolyte molecular weight (r). Attractive interactions dominate at large r values facilitating coacervation.

are for supernatant and coacervate phase, respectively. During the late stage of spinodal decomposition, it has been shown that the dynamical scale invariance at different times can be related to a single characteristic length scale (L), which is typically the droplet size of a coacervating solution. During extraction of coacervate from the phase separated solution, it was necessary to subject the solution to low speed centrifugation at ≈4000 rpm to collect the coacervate material. This allows the droplets fuse together to yield the dense polymer-rich phase and often to facilitate phase separation within a short time. Here, we will discuss the kinetics of coacervate droplet fusion through coalescence of droplet theory. The characteristic length (typical droplet size) grows as

$$L \sim t^n. \tag{24}$$

There are three competing forces that dictate the equilibrium situation. These are the surface tension force, torque or shear force and the dissipative or restoration force. The surface tension force facilitates coalescence, shear force favors droplet disintegration and the restoration force enforces an equilibrium droplet shape. It must be realized that as the kinetic energy associated with the droplets grow so is the shearing force. This brings the Reynold number into the picture. The droplets can follow one of the two possible paths of size evolution, (i) two droplets can collide and fuse to form a droplet of bigger volume taking the process towards mixing, (ii) a droplet can disintegrate through hydrodynamic shearing and split into many thus driving the system toward a demixing path.

For coacervating solutions it is appropriate to apply coalescence droplet theory applicable to viscoelastic liquids. Here the surface energy density is comparable to the viscous stress. The process is strongly time dependent. Initially viscous regime prevails strongly and dissipative force is active giving rise to a growth process $L \sim t$. After this the inertial regime takes over and $L \sim t^{2/3}$. Finally for long times the asymptotic regime is encountered with the size growth given by $L \sim t^{1/3}$. Correspondingly, the Reynolds number (Re) evolves as

$$\mathrm{Re} \sim t^{2n-1} \quad \text{for } n = 1, 2/3, 1/3. \tag{25}$$

At the onset of formation of droplets, Re scales linearly with time (viscous domain), in the intermediate regime (inertial regime) this scaling change to 1/3 and it approaches an asymptotic value of −1/3. For gelatin solutions we have estimated (surface tension ≈75 dyne/cm, viscosity =0.8 cP, density =1.4 g/cc) that the prefactor on the right-hand side orf Eq. (25) is typically 20. Realize that initially Reynold number is directly proportional to time thus the Re value will increase. As the inertial regime takes over this rate of increase is significantly retarded, and as the system moves to asymptotic regime Re starts falling. Because of this small prefactor, the coacervating solution escapes the fate of mixing-demixing oscillation observed in turbulent solutions and coacervation follows.

C. Syneresis effect in coacervates

Let us consider a homogeneous solution (volume V) of N_+ ions of valency Z_+ and N_- ions of valency Z_- with charge neutrality demanding $N_+Z_+ = N_-Z_-$. If the local charge density of each species is $N_\pm / V = \Sigma_{i=1}^{N_+} \delta(r-r') = n_\pm(r)$. It can be shown that for point particles, the partition function can be written as [27,28]

$$Z = (V^{(N_+ + N_-)})/(N_+!\,N_-!)\prod_k |1 + VG|^{-1/2} \times \exp((1/2V)\sum \mu G^2 \mu), \tag{26}$$

where G is given by the matrix

$$G = \begin{pmatrix} n_+ g_+ & 0 \\ 0 & n_- g_- \end{pmatrix}, \tag{27}$$

and, the chemical potential $\mu(r)$ is given by

$$\mu(r) = \begin{pmatrix} \mu_+(r) \\ \mu_-(r) \end{pmatrix}. \tag{28}$$

Equation (26) is central to the estimation of free energy (F) per unit volume ($F/V = f$) of the system at temperature T. It has been shown that in the thermodynamic limit, $V \to \infty$,

$$f = k_B T (n_+^0 \ln n_+^0 + n_-^0 \ln n_-^0 - \xi^3/12\pi), \tag{29}$$

where ξ is given by

$$\xi^2 = 4\pi e^2 (n_+^0 Z_+^2 + n_-^0 Z_-^2)/\varepsilon k_B T, \tag{30}$$

where k_B is Boltzmann constant and dielectric constant of the medium is ε. The terms with superscript 0 refer to initial values of the parameters concerned. Correspondingly, the osmotic pressure (P) of an electrolytic solution will be

$$P = k_B T (n_+^0 + n_-^0 - \xi^3/24\pi). \tag{31}$$

When a homogeneous polyelectrolytic solution undergoes coacervation through charge neutralization, alike in the aqueous gelatin system, the coacervate phase is observed to exhibit syneresis effect. We have observed this in gelatin coacervates prepared under different pH, ionic strength and temperature conditions. However, from Eq. (31), it can be argued that coacervates are expected to release solvent and exhibit syneresis. It is well known, that the supernatant phase is an extremely dilute polymer solution compared to its partner, the coacervate. Let V_1, N_1 and V_2, N_2 represent the volume and polymer chain density in the supernatant and the coacervate phase, respectively. In coacervates, $N_2 \gg N_1$ and $V_2 \ll V_1$. Hence, $N_2/V_2 \gg N_1/V_1$. The osmotic pressure of the polyelectrolytic solution can be expressed from Eq. (31) as

$$\frac{PV}{Nk_BT} = 1 - \frac{\sqrt{\pi}}{3}e^3\frac{1}{(k_BT)^{3/2}}\left(\frac{N}{V}\right)^{1/2}. \tag{32}$$

The osmotic pressure difference between the coacervate and the supernatant $(P_2 - P_1)$ will be

$$P_2 - P_1 = k_BT\left(\frac{N_2}{V_2} - \frac{N_1}{V_1}\right) - \frac{\sqrt{\pi}}{3}e^3\frac{1}{(k_BT)^{1/2}} \times\left[\left(\frac{N_2}{V_2}\right)^{3/2} - \left(\frac{N_1}{V_1}\right)^{3/2}\right]. \tag{33}$$

Since, $k_BT \gg (\sqrt{\pi}/3)e^3[1/(k_BT)^{1/2}]$ and $N_2/V_2 \gg N_1/V_1$.

It immediately follows that $P_2 \gg P_1$, which establishes the cause for syneresis rather explicitly.

Another consequence of Eq. (32) is the setting up of a concentration gradient of polyelectrolyte chains increasing downwards from the supernatant-coacervate interface. Let us use the bottom of the cell as reference with $z=0$ with polymer chain density $n(0)$. Using $N/V = n(z)$, Eq. (32) can be expressed as [28]

$$P = n(z)k_BT - \frac{\sqrt{\pi}}{3}e^3\frac{1}{(k_BT)^{1/2}}[n(z)]^{3/2}. \tag{34}$$

The corresponding force, $F_{\text{osmotic}} = -(dP/dz)$ and force per unit volume due to gravity, $F_{\text{garvity}} = m_{\text{eff}}gn(z)$, where m_{eff} is effective polymer chain mass inside the coacervate and g is acceleration due to gravity. Balancing these two forces

$$m_{\text{eff}}gn(z) = -k_BT\frac{dn(z)}{dz} + \frac{\sqrt{\pi}}{3}e^3\frac{1}{(k_BT)^{1/2}}\left(\frac{d[n(z)]^{3/2}}{dz}\right) \tag{35}$$

which integrates with the boundary condition $z=0, n(z) \to n(0)$ giving

$$n(z) = n(0)\exp(-m_{\text{eff}}gz/k_BT) - \sqrt{\pi}\frac{e^3}{(k_BT)^{3/2}}\left(\sqrt{n(z)} - \sqrt{n(0)}\right). \tag{36}$$

The second term on the right-hand side has a prefactor that is very small. So the concentration profile is largely governed by gravitational effects yielding

$$n(z) = n(0)\exp(-m_{\text{eff}}gz/k_BT) \tag{37}$$

with the passage of time a concentration gradient of polymer chains will be established with the concentration decreasing gradually with increasing z from the bottom of the cell and reaching the dilute concentration limit at the interface separating the coacervate from supernatant. This interface can be imagined as a semipermeable membrane that permits transport of the solvent molecules from the dense phase into the supernatant.

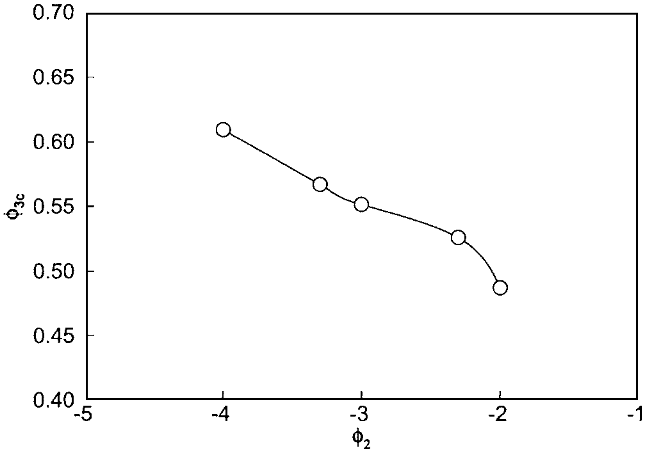

FIG. 4. This plot shows that the critical volume fraction (ϕ_{3c}) of the nonsolvent to induce phase separation φ_3 is strongly dependent of gelatin concentration φ_2. Our model predicts minimum $(\varphi_2 \approx 10^{-5})$ and the experimental verification is established up to $\varphi_2 \approx 10^{-4}$.

III. COMPARISON WITH EXPERIMENTS

A. Materials and methods

Gelatin (type-B, microbiology grade devoid of E-coli and liquefier presence) was obtained from E.Merck (India). Ethanol was obtained from Merck, Germany and sodium chloride were brought from E.Merck. The polypeptide molecular weight was determined from SDS-PAGE and was found to be (110±10) kD. The solvent used was Millipore deionized water. The preparation of coacervate was done by dissolving 0.01–1 % gelatin and sodium chloride (0.1 M) in deionized water for turbidity studies. The gelatin stock solutions were titrated with ethanol and the turbidity profiles were recorded with a Brinkman Colorimeter (Model-910) at 450 nm wavelength. The ethanol concentration corresponding to the first occurrence of turbidity (φ_{3C}) was measured. Coacervates collected after removal of the supernatant through repeated centrifugation (4 000 rpm). Further details are given elsewhere [9]. All experiments were carried out at room temperature, $T = 20$ °C.

B. Dependence on polyelectrolyte volume fraction

The dependence of φ_{3C} on gelatin concentration (φ_2) reveals that the value of φ_{3C} decreases significantly with increase in gelatin concentration [9] (Fig. 4). The critical polymer volume fraction for phase separation φ_{2c} can be determined from this data. For ethanol induced coacervation, φ_{3C} lies between typically, 0.5–0.6 of alcohol concentration [9], which corresponds to φ_{2c} lying between 10^{-4} to 10^{-3}. It has been argued in the literature that linear charge density of

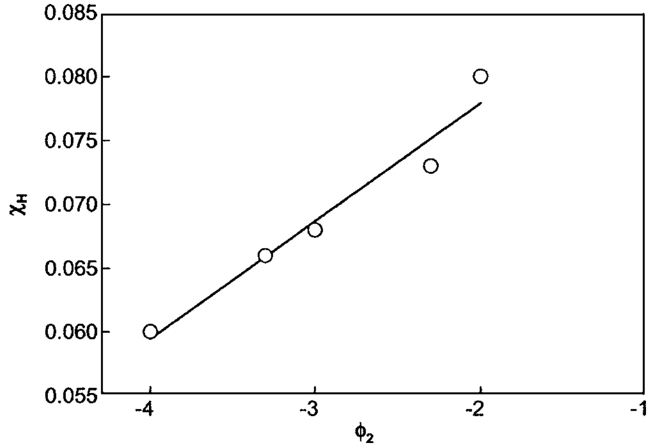

FIG. 5. Variation of enthalpic component of solvent-solute interaction parameter χ_H with the concentration (volume fraction) of the polyelectrolyte. The dependence is almost linear in the concentration range $10^{-4} \leqslant \varphi_2 \leqslant 10^{-2}$. See text for details.

gelatin of a specific type (alkali or acid processed) obtained from various sources is associated with same charge density. Taking the literature value of $\sigma \approx 3 \times 10^{-3}$ units of charge/site and $r \approx 10^5$, the ratio $(\sigma^3 r/\varphi_{2c})$ comes in the range ≈ 27 to 2.7 for φ_{2c} lying between 10^{-4} to 10^{-3}, which adequately satisfies the condition that this ratio must exceed 0.45. The phase separation experiments have been reported for a range of aliphatic alcohols from methanol to butanol and the φ_{3C} values have been measured explicitly. The condition imposed by Eq. (22) is satisfied for all these cases.

Incidentally, the same experimental data adequately supports the prediction made by Eq. (23), $\sigma/\varphi_{2c} \geqslant 0.78$, which implies that the phase separation occurs when a good solvent (water) is turned into a marginal one (ethanol-water mixture). The self-charge neutralization induced by the presence of a nonsolvent basically drives the polyelectrolyte toward desolvation. The interaction parameter χ will change continuously as the volume fraction of ethanol is increased. The polymer solvent interaction parameter χ comprises both entropic (χ_S) and enthalpic (χ_H) contributions, $\chi = \chi_H + \chi_S$. As ethanol concentration is increased the gelatin molecules and complexes undergo a series of conformational transitions induced by intermolecular interactions [18]. This makes any reasonable guess of χ_S difficult. However, it is possible to quantify the enthalpic contribution with some approximation. It will be assumed that the solvent mixture can be treated as a single solvent by appropriately determining the solubility parameter of this mixture. Second, we will apply the concept of interchange energy density of solvent-polymer pair to describe the solution [29]. Thus χ_H can be expressed as

$$\chi_H = \frac{V_1}{RT}({\delta_1}^2 + {\delta_2}^2 - 2 l_{12}\delta_1\delta_2), \qquad (38)$$

where V_1 is molar volume of the solvent, δ_1 and δ_2 are solubility parameters of water and ethanol, and l_{12} describes the intermolecular interactions. Since, $0.4 < \chi < 0.5$, it implies for our system $1.16 < l_{12} < 1.18$. Using $\delta_1 = 23.4(\text{cal/cc})^{1/2}$ for water [30] and $\delta_2 = 12.7(\text{cal/cc})^{1/2}$ for ethanol [30], χ_H would vary between 0.06 and 0.08 for our data. This is shown in Fig. 5. The results are quite revealing, for example, as the gelatin volume fraction was increased by two decades the enthalpy part of χ increased by about 30%. The χ_H parameter was found to be a linear increasing function of polyelectrolyte volume fraction, yielding $\chi_H(\varphi_2) \approx [\chi_H(0) + m\ \varphi_2]$ with $\chi_H(0) = 0.065$ and $m = 1.68$. It has been said earlier that exact estimation of χ_S is quite difficult, however if we assume a median value of 0.34 for this parameter, χ will show a change from 0.40 to 0.42 as φ_2 increases from 10^{-4} to 10^{-2}. This effectively means that χ is almost independent of gelatin concentration and it can be conjectured that intermolecular electrostatic interaction is primarily responsible for the phase separation leading to coacervation of this polyelectrolyte. The best possible way to resolve this is through direct measurement of χ.

C. Dependence on charge density and ionic strength

In the absence of any knowledge about the gelatin charge density at various *p*H's and ionic strengths of the solution, it will be improper to establish the condition, $\sigma^2/I^{1/2} \geqslant$ constant for phase separation in the gelatin system. However, it has been argued earlier that such an expression $(\sigma v/I^{1/2} \geqslant$ constant) was empirically proposed by Dubin *et al.* [21–23] based on complementary polyelectrolyte complexation data. This feature was observed in a wide variety of experimental situations. In the experimental plots of micellar surface charge density (v) and polymer linear charge density (σ) vs $I^{1/2}$, the intercepts at $I = 0$ were not strictly zero. But, it is clearly seen that in a typical ionic strength range of 0.01 M to 1 M the relation remains effective. The predictions of Eq. (19) are qualitatively consistent with Dubin *et al.* data [21–23].

D. Quantification of syneresis

A coacervate sample weighing 3.158 g was kept in a sealed test tube which was stored in an air-tight desiccator at 20 °C. The supernatant released was collected periodically by soaking it with a blotting paper without touching the surface of coacervate samples. Thus the contact effects are minimized.

This was weighed by a microbalance which gave amount of supernatant released. The data is shown in Fig. 6 pertaining to measurements carried in controlled conditions over a period of 300 hours. The amount of supernatant released as function of time has been expressed in percentage with respect to the initial weight of the coacervate sample. It was possible to make a least squares fitting of this data to a linear function which gave the rate of release as $\approx 0.05\%/\text{h}$ (chi-squared $\geqslant 0.94$). This is an interesting result which indicates that rate of syneresis is independent of initial coacervate mass. Though Eq. (33) inadequately predicts such a behavior, these results explicitly imply existence of syneresis in gelatin coacervates as dictated by this equation.

The temporal evolution of coacervate due to syneresis effect can be qualitatively compared to a self-organization process that can be qualitatively discussed within the framework

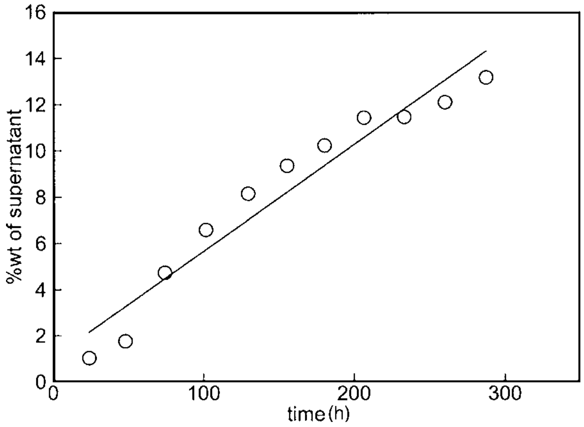

FIG. 6. Weight percentage of the supernatant released from a gelatin coacervate sample at 20 °C due to syneresis. The linear time dependence indicates invariance of rate of release with initial weight of the coacervate sample.

of polymer melts. The overall evolution of self-organization (like crystallinity) in polymer melts is discussed through Avrami equation, which enables the representation of experimental data without the explicit knowledge of the microscopic mechanisms driving the system toward an equilibrium [30]. This model accounts for isothermal nucleation (through equilibrium constant, K) and the subsequent rate of growth of these nuclei to microscopic dimensions (through a parameter, n. The Avrami exponent is the sum of the order of the rate process and the Euclidian dimension of the morphological unit formed. Rapid nucleation arises from a zero-order process arising from the simultaneous formation of growth centers. Sporadic nucleation, on the other hand, is a first-order process. Thus the general Avrami equation for temporal evolution of the coacervate can be given by [30]

$$1-\varphi_c=\exp(-Kt^n) \tag{39}$$

and for the early stage of coalescence, we can expand the exponential term which gives $\varphi_c=Kt^n$, which yields a value for $n\approx 5\pm 0.5$ upon fitting the data in Fig. 6 to this equation. For an isothermal three-dimensional growth process evolving with spherical morphology the predicted value for $n\approx 4$ which is within 20% of our value for n. This allows us to identify the syneresis akin to be a second-order rate process.

IV. CONCLUSION

We have determined explicit conditions for phase separation of a homogeneous polyelectrolyte solution into a supernatant and a dense polymer-rich (coacervate) phase. Though we have adopted the Veis *et al.* [5,6] framework for developing our model, we are able to quantify the phase separation conditions very explicitly. The system undergoes an interesting coalescence dynamics from the formation of microscopic coacervate droplets at phase separation threshold to the ultimate accumulation of the dense phase at the bottom of the reaction cell, which has been described within the framework of spinodal decomposition process. As far as the application of the model to simple coacervation of gelatin solutions is concerned, it was found that the nonelectrostatic interaction parameter is a function of temperature as well as initial concentration of gelatin. The critical concentration for formation of coacervates depends on the molecular weight (through r) and linear charge density of the polyelectrolyte. The phase separation is driven by the electrostatic and solute-solvent interactions which results in the gain in configurational entropy and the formation of an amorphous randomly mixed polymer-rich phase remaining in equilibrium with the dilute supernatant. The quantitative conditions of phase separation agree with the experimental data of Dubin *et al.* [21–23] though their results pertain to complex coacervation. Thus the model can selectively be applicable to phase separation induced by formation of complexes between oppositely charged macroions. Syneresis is commonly observed in coacervate systems though no systematic measurement has ever been reported. For gelatin coacervates the rate of syneresis was observed to be independent of initial coacervate mass. We had observed syneresis in complex coacervates made of bovine serum albumin (BSA) and poly-(dimethyldiallylammonium chloride) (PDAMDAC) [3] earlier though no systematic measurements were carried out. We have provided an explanation to this phenomenon. We also prove that there is a gravity induced concentration gradient in the coacervate phase.

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