Polyelectrolyte polypeptide scaling laws via mechanical and dielectric relaxation measurements

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Abstract
Experimental results from mechanical viscoelastic as well as dielectric relaxation times were compared to theoretical expectations utilizing polymer scaling theory. Viscoelastic relaxation of a hydrogel at 33% relative humidity fabricated from co-poly-L-(glutamic acid₄, tyrosine₁) [PLEY(4:1)] crosslinked with poly-L-lysine scaled with concentration according to reptation dynamics. High frequency dielectric relaxation of aqueous copolymer PLEY(4:1) scaled with concentration as an ideal chain and aqueous Poly-L-glutamic acid scaled as an extended chain. The study shows that two seemingly different measurement methods can yield information about the state of polymer chain conformation in situ.

1. Introduction
Currently, there has been wide interest in utilizing aqueous polypeptide solutions to electrospin nanofibers for various types of medical applications [1]. Recent studies have electrospun several different synthetic polypeptides from water [2]. One of the tenets of efficient electrospinning is that polymer chains must be highly overlapped [3]. Typical nanofiber research with aqueous synthetic polypeptide melts relies on concentrations near the solubility limit to ensure chain overlap. However, high concentration does not necessarily lead to spinnability [4]. It is still somewhat of a mystery why polypeptide melts at high concentrations do not necessarily lead to spinnable solutions [5–7]. The analysis presented here seeks to understand the state of polymer chain conformation through analysis of scaling laws. It is assumed that polymer chain conformation could affect the spinnability of a solution. For example, the conformation of an ideal chain could entangle at a critical concentration. However, neither the extended chain conformation nor the spherical conformation of a polymer in a poor solvent might ever entangle at high concentrations.

Here we find that molecular relaxation data provide insight into the state of polymer chain conformation through a relation of scaling laws to the state of solvent quality. Solution concentration scaling exponents give information about the solvent quality state in which molecular relaxation occurs whether it be due to mechanical strain or to aqueous dielectric high frequency probing.

There are three types of solvents: poor solvents, Θ-solvents and good solvents. In poor solvents, individual monomers in the polymer chain try to minimize contact with solvent molecules. Consequently, polymer chains collapse into a spherical configuration, which presumably would inhibit polymer chain entanglement. Θ-solvents allow for a balance of interaction forces between polymer chain and solvent. Thus, the polymer settles into an ideal, non-perturbed chain configuration. In a good solvent, chain monomers try to maximize interactions with solvent. Therefore, the chain becomes expanded.

Polymer is known to be fractal objects [8–10]. Down to a limit, dimensionality of the polymer varies with scale due to self-similarity. Theory due to Flory established a scaling exponent given by \( \nu = 1/d_p \), where \( d_p \) is a fractal dimension [11]. For an ideal chain with random walk, \( d_p = 2 \), therefore, \( \nu = 1/2 \). Thus, a Θ-solvent has \( \nu = 1/2 \) and a good solvent has \( \nu = 3/5 \). It has been found that a more accurate number for a good solvent is \( \nu = 0.588 \) [12].

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With regards to scaling of relaxation times as a function of polymer concentration, Rubinstein and Colby present the following for neutral polymers

\[ \begin{align*}
&\text{Good solvent} \quad (\nu = 0.588) : \quad \tau \sim N^2c^{(2-3\nu)/(3\nu-1)} \quad \Rightarrow \quad \tau_{pe} \sim N^2c^{0.31} \quad (1) \\
&\text{Θ - solvent} \quad (\nu = 1/2) : \quad \tau \sim N^2c^{(2-3\nu)/(3\nu-1)} \quad \Rightarrow \quad \tau_{Θs} \sim N^2c \\
&\text{Reptation} \quad (\nu = 0.588) : \quad \tau \sim N^3c^{3(1-\nu)/(3\nu-1)} \quad \Rightarrow \quad \tau_{rgs} \sim N^3c^{1.6} \\n\end{align*} \]

where \( N \) is the degree of polymerization, \( c \) is polymer concentration, and \( \nu \) is the Flory scaling exponent. Equations (1)–(3), respectively, give relaxation times as: \( \tau_{pe} \) for unentangled neutral polymers in a good solvent; \( \tau_{Θs} \) for unentangled neutral polymers in a \( Θ \)-solvent; and polymer relaxation according to reptation dynamics \( \tau_{rgs} \) for entangled neutral polymers in a good solvent.

The polymers involved in this study are polyelectrolyte polypeptides. Neutral polymers’ scaling differs significantly from scaling laws for semi-dilute, polyelectrolyte solutions which are given as [12–15]:

\[ \begin{align*}
&\text{Unentangled} \quad : \quad \tau_{pe} \sim N^2c^{-1/2} \\
&\text{Entangled} \quad : \quad \tau_{dpe} \sim N^2c^0. \\
\end{align*} \]

It will be seen that the samples in this study, though polyelectrolyte polypeptides, behaved as given by equations (1), (2) and (3) rather than equations (4) and (5). This was due to the high concentrations utilized.

2. Analysis and results

2.1. Polyelectrolyte polypeptides

Here we have studied polyelectrolyte polypeptides (PEPs), which are polypeptides with electrolyte repeating groups. Polyelectrolyte solutions have solution properties unlike that of neutral polymers [9, 16]. In stark contrast to most neutral polymers PEPs are soluble in water because of amino acid side chain ionization. Ions remain on the side chain while counter-ions surround them in solution. Increasing the PEP concentration increases counterion concentration, which in turn increases the ionic strength. This results in a large charge screening so strong that PEPs lose their unique properties and behave as neutral polymer chains [9]. As this study was conducted on PEPs at high concentrations, close to solubility limits, polymers turned out to behave as neutral polypeptides.

Figures 1(a), (b) show the polyelectrolyte structures of Poly-(L-Glutamic Acid) [PLE] and Poly-L-(Glutamic Acid, Tyrosine) [PLEY(4:1)], respectively. The glutamic acid residue is a weak polycarboxylic acid in solution. The tyrosine amino acid remains neutral but is a polar molecule with hydrophobic tendencies.

2.2. Scaling parameter analysis

Concentration scaling parameters shown on figures 2(a), (b) were obtained from a non-linear regression convex power law fit with two parameters using a Gauss-Newton algorithm. The two-parameter model was of the form, \( \tau = k_1c^{k_2} \), where \( \tau \) is relaxation time, \( k_1 \) is an irrelevant curve fitting parameter, \( c \) is the polymer concentration, and \( k_2 \) is the concentration scaling parameter. Table 1 presents \( k_2 \) parameter values and associated standard errors (SE) as well as the number of iterations for model convergence and mean standard error of the fit (MSE).

2.3. Mechanical relaxation scaling results

Previous studies showed that a hydrogel of poly-L-(glutamic acid, tyrosine) crosslinked with poly-L-lysine (PLL) is highly viscoelastic at 33% relative humidity [17]. Table 2 presents the relaxation times at high strain for
samples at three different PLEY nominal concentrations. Data is taken from Monreal et al.\cite{17}. In that study, PLEY(4:1) was crosslinked with PLL via a carbodiimide crosslinker. The hydrogel was allowed to reach equilibrium at 33\% relative humidity.

It would be expected that the force applied to the crosslinked polymer network leads to chain relaxation according to reptation dynamics. Indeed such relaxation was found through the study here. Strain-induced relaxation studies of PLEY(4:1) viscoelastic material showed that relaxation at high strain scales as an entangled neutral polymer in good solvent according to the reptation model of equation \eqref{equation}. Figure 2(a) shows that the high strain curve scales close to $\tau \sim \varepsilon^{1.7}$.

### 2.4 Dielectric relaxation scaling results

Useful insights were also obtained from dielectric relaxation studies of PLE and PLEY(4:1) in aqueous solutions. Data for this study was obtained from Monreal et al.\cite{18} as shown in Table 3. There, dielectric relaxation of PLE and PLEY aqueous solutions were studied in the radio frequency range of 50–800 MHz with an impedance analyzer. Dielectric relaxation peaks appeared at certain frequencies dependent on the PEP at its concentration. The points shown in figure 2(b) represent peak relaxation times for each PEP and related concentration.

![Figure 2](image)

**Figure 2.** Relaxation times versus concentration (% w/v). (a) Mechanical relaxation time of PLEY(4:1) viscoelastic material versus concentration taken from table 2.; (b) Dielectric relaxation times of PLE and PLEY(4:1) aqueous solutions versus concentration taken from table 3.

### Table 1. Concentration scaling parameter, $k_2$, for all four curves shown on figure 2 with associated standard error (SE) as well as the number of iterations for convergence and overall mean standard error (MSE) of each model. The subscript V in PLEY stands for viscoelastic relaxation data and D for dielectric relaxation data.

| Material | $k_2$ | SE  | Iterations | MSE  |
|----------|-------|-----|------------|------|
| PLEY$_V$ | 1.7   | 0.04| 7          | 78   |
| PLE      | 0.33  | 0.11| 23         | 442  |
| PLEY$_1D$| 0.98  | 0.22| 11         | 1030 |
| PLEY$_2D$| 0.82  | 0.09| 10         | 236  |

### Table 2. Relaxation times for three different concentrations of PLEY(4:1) crosslinked hydrogel samples at 33\% relative humidity at high strain (Adapted from Monreal et al.\cite{17}).

| Nominal concentration (% w/v) | $\tau_2$ (s) |
|-------------------------------|--------------|
| 30                            | 470          |
| 40                            | 770          |
| 50                            | 1100         |
interpenetrate. That concentration is known as the overlap limit and is given by

\[ f^* = \frac{N}{R_F^3}, \]  

where \( N \) is the degree of polymerization and \( R_F \) is the Flory radius, which is a measure of the volume radius that encloses an expanded polymer chain. The polymer volume fraction, \( f^* \), at the critical overlap concentration, \( f^*_m \), is \( f^*_m = \frac{v_m c_m}{v} \), where \( v_m \) is the monomer volume. For a neutral polymer in good solvent typically \( f^*_m < N^{-4/5} \).

To study scaling behavior of the material, it was first necessary to estimate the critical overlap volume fraction using \( f^*_m \sim N^{-4/5} \).

For both PLE and PLEY we assume 50% of glutamic acids are ionized at neutral pH and lyophilized in sodium salt. For PLEY in particular we assume tyrosine is neutral. Haynie, thus, obtained for PLEY (4:1) of \( M_w \approx 30 \text{ kDa} \) \( \rho \approx 1.54 \text{ g cm}^{-3} \) and for PLE of \( M_w \approx 30 \text{ kDa} \) \( \rho \approx 1.52 \text{ g cm}^{-3} \) \( N \approx 214 \) [20]. As an approximation we can estimate \( N \approx 200 \) for both PLE and PLEY. Thus, the critical overlap volume fraction \( f^* \sim (200)^{-4/5} = 0.014 \). We can compare \( f^*_m \) to an experimental volume fraction via

\[ f^* = \frac{N}{R_F^3} \approx \frac{\phi c}{\rho} \],

where \( \phi = \frac{\phi}{\rho} \) is polymer chain density and \( \phi \) is polymer volume fraction in w/v (g cm\(^{-3}\)) and \( \rho \) is the polymer density. We, thus, obtain the following volume fractions: PLEY (\( \phi_{40\%} = 0.26 \); \( \phi_{20\%} = 0.33 \)) which are about 14 to 23 times \( f^* \); and PLE (\( \phi_{15\%} = 0.07 \); \( \phi_{15\%} = 0.10 \); \( \phi_{20\%} = 0.13 \)) which are about 5 to 9 times \( f^* \). So we find that \( f^*_m \) and \( f^*_m \) for PLEY and PLE.

These estimates show that in general, both PLE and PLEY are in the regime of chain overlap. PLEY is in the concentrated regime. But, it is possible that the lowest concentration of PLE could be in the semi-dilute regime and only reaches the concentrated regime at 20% w/v. Regardless, figure 2(b) shows that PLE scales as a neutral polymer not a semi-dilute polyelectrolyte. That is it scales with \( \tau \sim \phi^{0.5} \) not \( \tau \sim \phi^0 \).

Table 3. Dielectric relaxation times of PLE and PLEY (4:1) aqueous solutions at various concentrations obtained from capacitance measurements. Relaxation times are in picoseconds (ps). (Adapted from Monreal et al [18]).

| Material | Nominal concentration (%w/v) | \( \tau \) (ps) |
|----------|-------------------------------|---------------|
| PLE      | 10                            | 362           |
|          | 15                            | 388           |
|          | 20                            | 455           |
| PLEY\(_1\) | 30                           | 309           |
|          | 40                            | 455           |
|          | 50                            | 522           |
| PLEY\(_2\) | 30                           | 370           |
|          | 40                            | 490           |
|          | 50                            | 568           |

For polymer solutions, there exists a concentration at which polymer chains begin to interact and start to interpenetrate. That concentration is known as the overlap limit and is given by [19]

\[ f^*_m \sim N \frac{1}{R_F^3}, \]

where \( N \) is the degree of polymerization and \( R_F \) is the Flory radius, which is a measure of the volume radius that encloses an expanded polymer chain. The polymer volume fraction, \( f^* \), at the critical overlap concentration, \( f^*_m \), is \( f^*_m = \frac{v_m c_m}{v} \), where \( v_m \) is the monomer volume. For a neutral polymer in good solvent typically \( f^*_m < N^{-4/5} \).

To study scaling behavior of the material, it was first necessary to estimate the critical overlap volume fraction using \( f^*_m \sim N^{-4/5} \).

For both PLE and PLEY we assume 50% of glutamic acids are ionized at neutral pH and lyophilized in sodium salt. For PLEY in particular we assume tyrosine is neutral. Haynie, thus, obtained for PLEY (4:1) of \( M_w \approx 30 \text{ kDa} \) \( \rho \approx 1.54 \text{ g cm}^{-3} \) and for PLE of \( M_w \approx 30 \text{ kDa} \) \( \rho \approx 1.52 \text{ g cm}^{-3} \) \( N \approx 214 \) [20]. As an approximation we can estimate \( N \approx 200 \) for both PLE and PLEY. Thus, the critical overlap volume fraction \( f^* \sim (200)^{-4/5} = 0.014 \). We can compare \( f^*_m \) to an experimental volume fraction via \( f^* = \frac{\phi c}{\rho} \), where \( c \) is polymer concentration in w/v (g cm\(^{-3}\)) and \( \rho \) is the polymer density. We, thus, obtain the following volume fractions: PLEY (\( \phi_{40\%} = 0.20 \); \( \phi_{20\%} = 0.26 \); \( \phi_{20\%} = 0.33 \)) which are about 14 to 23 times \( f^* \); and PLE (\( \phi_{15\%} = 0.07 \); \( \phi_{15\%} = 0.10 \); \( \phi_{20\%} = 0.13 \)) which are about 5 to 9 times \( f^* \). So we find that \( f^*_m \) and \( f^*_m \) for PLEY and PLE.

These estimates show that in general, both PLE and PLEY are in the regime of chain overlap. PLEY is in the concentrated regime. But, it is possible that the lowest concentration of PLE could be in the semi-dilute regime and only reaches the concentrated regime at 20% w/v. Regardless, figure 2(b) shows that PLE scales as a neutral polymer not a semi-dilute polyelectrolyte. That is it scales with \( \tau \sim \phi^{0.5} \) not \( \tau \sim \phi^0 \).

Figure 2(b) also shows that PLEY (4:1) scales as \( \tau \sim \phi^{0.8-1.0} \), which is close to the scaling law predicted for neutral polymers in a \( \theta \)-solvent. Deviations appear due to experimental variations. Nonetheless, PLEY(4:1) evidently behaves as an ideal chain with \( \nu = 1/2! \) This is expected for neutral polymers. As de Gennes explains, in concentrated polymer melts each polymer chain experiences opposing forces throughout so that there are no net expansive forces [8]. The result is polymer conformation as an ideal chain. Additionally, the chemical structure of PLEY (figure 1(b)) contains a hydrophilic amino acid residue, due to glutamic acid, that wants to maximize contact with the solvent. Opposing that tendency is the hydrophobic tyrosine amino acid residue. These two phenomena seem to result in PLEY behaving as an ideal chain at the concentrations studied.

A surprising result was the scaling trend for PLE. As shown in figure 2(b), PLE scaled as \( \tau \sim \phi^{0.3} \). This scaling changes with concentration analogous to a neutral polymer in good solvent of equation (1)! This means the PEP chain is in an extended conformation. The possibility that PLE solutions could have been in the dilute regime was discounted by the estimate that \( f^* \) is greater than \( f^* \). In good solvent a polymer exhibits chain swelling due to excluded volume effects. Given that PLE is a polyanion there seems be excluded volume effects possibly due to monomer-monomer charge repulsion. As a consequence, PLE polymer chains might not entangle in a manner conducive to electrospinning even at higher concentrations. A main reason could be due to chain stiffness. Monomer charge repulsion results in stiffening of a polymer chain. It has been found that polymer chain elasticity is an essential component for the fabrication of nano-fibers by electrospinning [21].

Given that the extended chain conformation of PLE found through this analysis would point to a lack of PEP chain entanglement, it would not be expected that PLE can be electrospun. Indeed, Khadka et al found that PLE did...
particularly given the high cost of the raw materials involved. Scaling laws analysis performed here shows that at the concentrations studied, PLEY(4:1) behaves as a neutral polymer in a \( \Theta \)-solvent. This means that the PEP chain has an ideal chain conformation. Theory would expect this PEP to produce nano-fibers through electrospinning as polymer chains are likely to entangle. Experiments by Khadka et al. indeed confirm such expectation [2]. A third confirmation of the adequacy of this analysis is the result that neither PLEY nor PLE scaled as polyelectrolytes such as \( \tau \sim c^{0.5} \), for semi-dilute solutions or \( \tau \sim \xi^4 \) for concentrated entangled solutions. Rather, both scaled as neutral polymers. The fact that highly concentrated solutions of PLEY and PLE, both of which are polyelectrolyte polypeptides, behave as neutral polymers is supported by the theory of de Gennes as previously noted.

3. Conclusion

We have provided a method to analyze aqueous polyelectrolyte polypeptide solutions via scaling laws. The results would provide a new method to predict whether or not a PEP aqueous solution would lead to a spinnable melt. If the polymer concentration scaling exponent indicates scaling as a polymer in a \( \Theta \)-solvent, the PEP solution might be spinnable. Conversely, if the concentration scaling exponent indicates scaling as a good solvent, the solution might not be spinnable due to an extended chain conformation that cannot entangle.

We hope this type of analysis will aid in the efficient conduct of research into electrospun polypeptides. Particularly given the high cost of the raw materials involved.

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