Influence of gradient strength and composition profile on the onset of the cloud point transition in hydroxyethyl methacrylate/dimethylaminoethyl methacrylate gradient copolymers

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1. Introduction

Lower Critical Solution Temperature (LCST) behavior has widely been observed in aqueous solutions containing polymers that are capable of hydrogen bonding with water [1]. Polymers that exhibit LCST-type behavior are soluble in solution at low temperatures; they precipitate out of solution at higher temperatures when thermal energy overcomes hydrogen bonding. The cloud point is the temperature at which the polymers begin to precipitate and the solution becomes cloudy. Thermodynamically, the cloud point is represented by the binodal, whereas the LCST is the cloud point temperature at which the binodal and spinodal intersect [2]. The existence of the cloud point was first predicted using Flory theory [3], with substantial research subsequently conducted to elucidate this transition [3–11]. More recently, polymeric materials that exhibit cloud points have been investigated for a number of different applications, including drug delivery [12], protein separation [13], nanofiltration [14,15], adaptive lenses [16], and tissue scaffolding [17]. Accordingly, understanding the parameters that influence the cloud point is necessary to properly engineer the desired temperature response in these materials. The polymer molecular weight [18–20], pH [20–22], ion concentration [23], and copolymer composition [21,24] have all been previously demonstrated to influence the cloud point.

Since copolymer composition influences the observed cloud point, one expects changes in the instantaneous composition along polymer chains to impose differences in the local cloud point, thereby affecting the macroscopically observed clouding phenomenon. Whereas dilute solutions of homopolymers, and compositionally statistical random copolymers exhibit bipolar-type transitions between clear and turbid states as the solutions are heated across their cloud points — and diblock copolymers transition from unimers to micelles as dilute solutions comprising them are heated across their cloud points — dilute solutions of gradient copolymers have been reported to gradually form micelles whose size and solution viscosities are temperature dependent [25–28]. This temperature sensitivity stems from the presence of an interfacial region having intermediate compositions between those of the insoluble core and the soluble corona. With LCST-type comonomers, a small temperature rise above the local cloud point that is imposed by the composition of this interface can make this region insoluble, thus causing it to precipitate into the core and resulting in micelle shrinkage. Alternatively, a small temperature drop below the local cloud point can make this interfacial region more soluble, causing it to dissolve into the corona, which results in micelle expansion. These studies demonstrated that a composition gradient in the copolymer can impart a gradual and continuous

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Abstract

We examined the onset of the cloud point in dilute aqueous solutions of gradient copolymers of 2-hydroxyethyl methacrylate and dimethylaminoethyl methacrylate. Dynamic Light Scattering (DLS) and transmittance measurements both indicate that the copolymer solution cloud point decreases with increasing copolymer gradient strength, defined as the largest difference in the instantaneous composition along the copolymer. While transmittance measurements suggest that macroscopic clouding does not set in until 30% of the polymer chain becomes insoluble, DLS experiments, which are more sensitive to the onset of aggregation, indicate that the onset of aggregation occurs at the point where the least soluble polymer chain end becomes insoluble. Collectively, our data indicate that the macroscopic cloud point transition is highly sensitive to co-monomer sequence distribution of the copolymers, with its onset and transition breadth tunable through copolymer gradient strength.
thermoresponse to dilute solutions of gradient copolymers undergoing the cloud point transition, as opposed to the binary-type response observed in dilute solutions of homopolymers, random- and block copolymers.

While these studies have demonstrated that the introduction of a composition gradient along the polymer chain can broaden the thermoresponse of the copolymer over a substantial temperature window [25–29], there remains a lack of quantitative understanding of the influence of monomer sequence on the cloud point transition of dilute copolymer solutions, partially due to the difficulty in introducing pre-specified composition profiles. While gradient copolymers have been synthesized as “tapered block” copolymers for many years using anionic polymerization [30–33], the development of controlled radical polymerization techniques has substantially increased the number of monomers that can be polymerized. With a considerably wider library of co-monomers to choose from, researchers are now able to select monomer pairs having reactivity ratios near unity to increase the ability to tune the chemical composition profiles of the resulting gradient copolymers. We have, for example, previously demonstrated a simple method to quantitatively control the composition profile in gradient copolymers of 2-hydroxyethyl methacrylate (HEMA) and 2-(dimethylamino)ethyl methacrylate (DMAEMA) [34]. Polymers containing DMAEMA are known to exhibit a cloud point [35–38], and can therefore be used to probe the influence of co-monomer sequence distribution on the clouding phenomenon.

In our previous work, we found that the cloud points of gradient copolymers having linear composition profiles to decrease linearly with increasing gradient strength, defined as the largest difference in the instantaneous composition along the copolymer [34]. Given the symmetry about the overall composition in these linear gradient copolymers, the instantaneous composition of the least soluble end – by definition – varies linearly with gradient strength. Our previous results thus imply that the onset of clouding occurs when the least soluble end becomes insoluble. Previous studies [26–28] on gradient copolymers of methoxylvinyl vinyl ether (MOVE) and ethoxylvinyl vinyl ether (EOVE) seem to confirm this hypothesis, showing clouding to begin at the cloud point of EOVE, ~23 °C. The solution becomes progressively more cloudy as the temperature is raised until the solution reaches the cloud point of MOVE, ~60 °C, when it becomes completely opaque. Since the composition profile of the copolymers range from pure EOVE at the least soluble end of the copolymer to pure MOVE at the other chain end, these studies of the EOVE/MOVE system also imply that clouding begins when the least soluble chain end (consisting of pure EOVE) becomes insoluble. Once the least soluble end becomes insoluble, inter-chain aggregation occurs until the aggregates are large enough to scatter light, and the solution becomes cloudy. This mechanism implies that only the very tip of the copolymer chain needs to become insoluble before large-scale clouding sets in. Consequently, the cloud point of a gradient copolymer will be independent of its composition profile so long as the instantaneous compositions at the least soluble end of the polymer chain are the same. Accordingly, if we knew the local cloud point associated with the instantaneous composition at the least soluble end, we should be able to predict the onset of clouding for any gradient copolymer.

The ability to pre-specify the monomer sequence distribution of gradient copolymers, and synthesize accordingly, presents a unique opportunity for us to explore and verify this hypothesis.

2. Experimental

2.1. Materials

Dimethyl sulfoxide (DMSO, Acros, 99.7%), ethyl bromoisobutyrate (EBiB, Acros, 98%), N, N’, N”, N” pentamethyldiethylentriamine (PMDETA, Acros, 99%), 1,2,4 trimethoxybenzene (TMB, Acros, 97%), copper (II) chloride (CuCl2, Acros, 97%), glycine (Fisher, 99.7%), and sodium hydroxide (Acros, 99%) were used as-received. 2-Hydroxyethyl methacrylate (HEMA, Acros, 97%) was distilled under reduced pressure (150 mTorr, 70 °C) to remove ethylene glycol dimethacrylate and methyl ether hydroquinone (MEHQ) and stored at ~20 °C prior to use. 2-(Dimethylamino)ethyl methacrylate (DMAEMA, Acros, 99%) was passed through a column of basic alumina to remove MEHQ and used immediately. Copper (I) chloride (CuCl, Acros, 97%) was used as-received and stored in dry nitrogen after the container was initially opened.

2.2. Synthesis

The details of the synthesis of HEMA/DMAEMA random [39–42] and gradient [34] copolymers having pre-specified composition profiles have been previously reported. Briefly, HEMA and DMAEMA were copolymerized at 50 °C in DMSO using atom transfer radical copolymerization (ATRP). The initiator was EBiB, the ligand was PMDETA, and CuCl and CuCl2 were used as the activator and deactivator, respectively. The rate of copolymerization was extracted as a function of time and monomer composition from a series of random copolymerizations in a batch reactor. With the rate of copolymerization, a reaction model was constructed that enabled us to predict the instantaneous co-monomer feed rate for the generation of gradient copolymers in a semi-batch reactor with pre-specified composition profile, gradient strength (ΔfD), overall composition, molecular weight, and fraction of chain over which the composition gradient exists (ξ). These five parameters uniquely define the monomer sequence distribution in a gradient copolymer. We define the gradient strength as the maximum difference in instantaneous compositions along the polymer chain. For all gradient copolymers studied here, the gradient strength is the difference between the instantaneous compositions at the two polymer chain ends. Thus, if fD(L) is a mathematical function that describes the instantaneous DMAEMA content along the polymer chain length, L, i.e., the composition profile, then the gradient strength, ΔF0 is simply |fD(L = 0) – fD(L = 1)|.

Two series of gradient copolymers were synthesized for this study to investigate the influence of gradient strength and composition profile on the cloud point. In addition, a series of random copolymers with varying overall compositions but similar molecular weights was also synthesized; the cloud point characteristics of dilute solutions comprising these random copolymers form a basis for comparison with those of dilute solutions of gradient copolymers. Between the two series of gradient copolymers, the variable is composition profile; here we focused on gradient copolymers having composition profiles that can be described as linear and hyperbolic tangent (i.e., “tanh”) functions of the chain lengths. We intentionally kept the molecular weight and the overall composition constant among all the gradient copolymers. Despite our efforts, there is some variability in the molecular weight. This variability, however, should translate to cloud point deviations of less than 1 °C per prior studies on poly(DMAEMA) [19]. We chose these composition profiles because the linear composition profile is expected to maximize the influence of composition gradient, while the hyperbolic gradient copolymers exhibit composition profiles that are very similar to those of previous “tapered block” type gradient copolymers. Within each series of gradient copolymers we varied the gradient strength. The gradient copolymers were compared against a random copolymer, PHD65, having the same overall composition (xo = 0.65) and a comparable molecular weight of 39 kg/mol. A complete list of the copolymers used in this study is given in Table 1.
2.3. Characterization

The monomer concentrations were tracked during copolymerizations using an Agilent 6850 Gas Chromatograph equipped with a HP-1 poly(dimethyl siloxane) capillary column (30 m × 0.25 mm × 0.25 μm) and using hydrogen gas as the mobile phase. Absolute molecular weights were measured by size exclusion chromatography on a system that included a Waters 515 solvent pump, a Waters 717 Autosampler, a Waters 410 differential refractometer with a Precision Detectors PD2020 light scattering unit operating at 680 nm. A Viscotek I-series guard column, a Viscotek low molecular weight I-series column, and a Viscotek mid-molecular weight I-series column completed the assembly. DMF with 0.02 M ammonium acetate was used as the eluent. Absolute molecular weights were calculated using a dn/dc of 0.06 mL/g. The dn/dc of both poly(HEMA) and poly(DMAEMA) in DMF are approximately 0.06 mL/g, thus no correction was applied to the random or the gradient copolymers. For cloud point measurements, buffered aqueous solutions were used as solvents for HEMA/DMAEMA copolymers instead of pure water to prevent pH drifts upon addition of the copolymer (DMAEMA is basic). We prepared a 1.0 M buffer solution by adding 15.0 g ammonium acetate to 200 mL of deionized water (18.2 MΩ), and adjusted its pH to 8.46 or 8.31 using sodium hydroxide. Dilute solutions of copolymers (at 1 wt%) were prepared by adding the copolymers to the buffer solution and allowing them to dissolve at 5 °C overnight. Turbidity measurements were taken on a home-built system equipped with a 2.0 mW He-Ne laser (JDS Uniphase 1122) operating at 633 nm. Buffered copolymer solutions were injected into demountable cells comprising two quartz windows embedded in an aluminum water jacket. The solution temperature inside the cell was controlled using an internal Peltier plate set to 12.9 °C/hr. Measurements were taken every 0.7 °C. With DLS measurements, we define the aggregation temperature as the midpoint between the adjacent two temperatures at which the unimers are visible and absent, respectively. The error on the aggregation temperature measured by DLS is ±0.35 °C given our step size.

3. Results and discussion

Fig. 1 shows the cloud points from turbidity measurements of dilute solutions of PHD55, PHD65, PHD75, and poly(DMAEMA) in 1.0 M glycine buffer at pH 8.31 and 8.46. The random copolymers are compositionally uniform along the polymer chain because the copolymerization conditions were selected such that the reactivity ratios of HEMA and DMAEMA are near unity [40]. Given the compositional uniformity along the chain length, the similarity in molecular weight, and the narrow molecular weight distribution, changes in the cloud point must solely reflect differences in the overall composition. Previous reports have shown that the cloud points of random copolymers vary linearly with copolymer composition [21,43]. Our data in Fig. 1 are consistent with this finding thus allowing us to extrapolate this composition dependence to lower DMAEMA content. This extrapolation is necessary, since copolymers with overall DMAEMA mole fraction (x_D) below x_D = 0.50 do not readily dissolve in water or buffer solutions at the pH of choice (HEMA is water swellable but not soluble in water). Fig. 1 also indicates that the cloud points for DMAEMA-containing copolymers are higher at lower pHs, as the fraction of DMAEMA-mers that are ionized increases with decreasing pH. The slope of the fit, however, does not change with the pH of the buffer solution; we merely observe a shift along the temperature axis with the pH of the solution. Fitting the cloud point data yields CP(x_D) = 37.5x_D + 2.9 °C at pH 8.31 and CP(x_D) = 37.2x_D − 3.4 °C at pH 8.46.

For the gradient copolymers used in this study, the composition at the least soluble polymer chain end (x_D,LSE) is given as a function of the gradient strength by:

\[ x_{D,LSE} = x_{D,AVG} - \frac{\Delta D}{2} \]  

In Eq. (1) x_{D,AVG} is the overall composition, and ΔD is the gradient strength. The local cloud point at any point along the gradient copolymer depends on the instantaneous composition at that point. It seems likely that the local cloud point at a point on

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Table 1

Parameters of Copolymers Used in This Study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Composition Profile</th>
<th>Overall DMAEMA Mole Fraction</th>
<th>λ</th>
<th>ΔD</th>
<th>M₀ (g/mol)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHD55</td>
<td>Random</td>
<td>0.54</td>
<td>–</td>
<td>–</td>
<td>40,000</td>
<td>1.06</td>
</tr>
<tr>
<td>PHD65</td>
<td>Random</td>
<td>0.64</td>
<td>–</td>
<td>–</td>
<td>34,000</td>
<td>1.06</td>
</tr>
<tr>
<td>PHD75</td>
<td>Random</td>
<td>0.75</td>
<td>–</td>
<td>–</td>
<td>39,000</td>
<td>1.06</td>
</tr>
<tr>
<td>PDMAEMA</td>
<td>Homo</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>41,000</td>
<td>1.04</td>
</tr>
<tr>
<td>L1</td>
<td>Linear</td>
<td>0.65</td>
<td>1</td>
<td>0.15</td>
<td>38,000</td>
<td>1.07</td>
</tr>
<tr>
<td>L2</td>
<td>Linear</td>
<td>0.65</td>
<td>1</td>
<td>0.31</td>
<td>33,000</td>
<td>1.05</td>
</tr>
<tr>
<td>L6</td>
<td>Linear</td>
<td>0.65</td>
<td>1</td>
<td>0.39</td>
<td>35,000</td>
<td>1.03</td>
</tr>
<tr>
<td>L7</td>
<td>Linear</td>
<td>0.67</td>
<td>1</td>
<td>0.49</td>
<td>42,000</td>
<td>1.05</td>
</tr>
<tr>
<td>H2</td>
<td>Hyperbolic</td>
<td>0.67</td>
<td>0.16</td>
<td>0.11</td>
<td>42,000</td>
<td>1.04</td>
</tr>
<tr>
<td>H3</td>
<td>Hyperbolic</td>
<td>0.67</td>
<td>0.16</td>
<td>0.26</td>
<td>39,000</td>
<td>1.04</td>
</tr>
<tr>
<td>H4</td>
<td>Hyperbolic</td>
<td>0.64</td>
<td>0.16</td>
<td>0.39</td>
<td>39,000</td>
<td>1.04</td>
</tr>
<tr>
<td>H1</td>
<td>Hyperbolic</td>
<td>0.68</td>
<td>0.20</td>
<td>0.52</td>
<td>36,000</td>
<td>1.07</td>
</tr>
</tbody>
</table>
a gradient copolymer will be the same as the cloud point of a truly statistical random copolymer whose overall composition is equal to the instantaneous composition of the point on the gradient copolymer. With this assumption, we can substitute our expression for the composition at the least soluble end, given by Eq. (1), into the equation that describes the composition dependence of cloud points extracted from turbidity measurements on dilute solutions of HEMA/DMAEMA random copolymer at pH 8.46 in Fig. 1:

$$CP(\Delta f_D) = 37.2 \left[ x_{D, AVG} - \frac{\Delta f_D}{2} \right] - 3.4$$

Eq. 2 can be readily tested against the experimental data collected on dilute solutions of HEMA/DMAEMA gradient copolymers at pH 8.46. Fig. 2 shows the observed cloud points of gradient copolymers with both linear and hyperbolic composition profiles. Of particular note is that the cloud points of gradient copolymers with hyperbolic composition profiles exhibit the same dependence on gradient strength as those of gradient copolymers having linear composition profiles, suggesting the composition profile to not be a variable that influences the clouding phenomenon of these gradient copolymers. The cloud points predicted by Eq. (2) for gradient copolymers having variable gradient strength and an overall composition of 0.65 are shown as a solid line in Fig. 2. This prediction assumes that the macroscopic cloud point is the same as the local cloud point corresponding to the instantaneous composition of the least soluble end, and it clearly under-predicts the observed cloud point by a significant margin.

In order to understand why Eq. 2 under-predicts the observed macroscopic cloud point, we have to look at the monomer sequence distribution of the gradient copolymers. For a gradient copolymer that is at the temperature corresponding to the local cloud point of the least soluble chain end, most of the chain is still soluble. Only the portion of the chain having the composition of the least soluble chain end will be insoluble at this temperature. In order for macroscopic clouding to occur, this insoluble portion must aggregate with the insoluble portions of other chains. This insoluble portion, however, is likely to be sterically hindered from inter-chain aggregation since the remaining portion of the chain remains soluble. Large-scale clouding is thus not likely to occur and the solution remains transparent at the local cloud point of the least soluble chain end. Nonetheless, the fact that the cloud points of gradient copolymers with both hyperbolic and linear composition profiles exhibit the same dependence on gradient strength suggests that the onset of the cloud point transitions in gradient copolymers is correlated with the local cloud point and must depend on the composition of the least soluble chain end. In light of these observations, it seems likely that a larger portion of the copolymer must become insoluble in order for macroscopic clouding to occur. In the scenario that a portion of the chain, $i_0$, must become insoluble, as opposed to just the chain end, before clouding can occur, the theoretical macroscopic cloud point at pH 8.46 should be given by:

$$CP(\Delta f_D, i) = 37.2 \left[ x_{D, AVG} - \frac{\Delta f_D (1 - i_0) - 0.5}{2} \right] - 3.4$$

for gradient copolymers having linear composition profiles, and

$$CP(\Delta f_D, i) = 37.2/A' + B'Tanh[C'\pi((1 - i_0) - D')] - 3.4$$

for gradient copolymers having hyperbolic composition profiles where $A'$ is related to the overall composition (and is equal to the overall composition if the gradient copolymer is symmetric), $B'$ is $\Delta f_D/2$, $C'$ relates to the interfacial width ($\lambda$), and $D'$ is the location of the inflection point in the composition profile. We note that if $i_0 = 0$, we recover the simplest case wherein the macroscopic cloud point is set by the local cloud point dictated by the instantaneous composition at the least soluble chain end, and Eqs. 3 and 4 simplify to Eq. 2. Using Eqs. 3 and 4, we can estimate the value of $i_0$ by fitting the equations to the cloud point data derived from the linear and hyperbolic gradient copolymers in Fig. 2, respectively. Fig. 3a shows the cloud points of the series of linear gradient copolymers from Fig. 2 plotted against gradient strength. The solid line represents a fit of Eq. (3) to the data. The best fit to the data yields $i_0 = 0.27$; in other words, the temperature must be raised such that 27% of the chain becomes insoluble in order to observe macroscopic clouding of gradient copolymers with linear composition profiles. Fig. 3b shows the analogous plot for the hyperbolic series of gradient copolymers. In this case, the best fit of Eq. (4) to the data yields $i_0 = 0.35$; hence, 35% of the chain must become insoluble for the copolymer solution to exhibit macroscopic clouding. Consistent with the idea that a critical portion of the polymer chain needs to become insoluble prior to the onset of macroscopic clouding, the temperature range over which clouding takes place broadens with gradient strength [34]. A larger gradient strength implicates a larger spread in the local compositions corresponding to a larger range of local cloud points being accessed over which macroscopic clouding takes place.

Fig. 4 plots the instantaneous DMAEMA composition as a function of chain length, $f_D(L)$, for two hypothetical gradient copolymers, one having a linear and the other having a hyperbolic composition profile with identical gradient strengths. The insoluble fraction at the cloud point is shown as dotted lines and the points that separate the soluble from insoluble portions along the polymer chains are shown as open symbols at $L = 0.27$ and 0.35 for the gradient copolymer with linear and hyperbolic composition profiles, respectively. From Fig. 4, it appears that the gradient copolymer with the hyperbolic composition profile requires the entire least soluble “block” to become insoluble before clouding occurs. The observation that the hyperbolic gradient copolymer requires a larger critical insoluble fraction ($i_0$) than the linear gradient copolymer suggests that the soluble portion of the chain more effectively hinders inter-chain aggregation than the soluble portion of the linear gradient copolymer, likely because the soluble portion of the hyperbolic gradient copolymer has a higher overall DMAEMA content compared to that of the linear gradient copolymer. Our analysis thus suggests that while the details of the composition profile do not affect the cloud point temperature in dilute solutions of gradient copolymers, they do dictate the fraction of chain that needs to be insoluble before macroscopic clouding occurs.
The cloud point data in Fig. 2 also allow us to examine how the fraction of polymer chain over which gradient takes place, \( \lambda \), affects the macroscopic clouding phenomenon. For gradient copolymers with linear composition profiles, the local composition changes continuously across the entire copolymer chain, or \( \lambda = 1 \). On the other hand, the change in the local composition in gradient copolymers with hyperbolic composition profiles mostly occurs over a small region of the copolymer chain, so \( \lambda \) is substantially less than 1. These hyperbolic gradient copolymers thus resemble diblock copolymers with a segment having intermediate local compositions inserted between the two blocks. We define this intermediate segment by the points at which the tangent from the inflection point intersects the composition of the chain ends. Table 1 lists \( \lambda \) for all the copolymers examined in this study; \( \lambda \) for hyperbolic gradient copolymers is substantially smaller than that for linear copolymers. Yet, examining the data in Fig. 2 suggests that the macroscopic cloud point only correlates with gradient strength and does not correlate with \( \lambda \).

The turbidity data suggest that a critical portion of the chain, \( l_0 \), must become insoluble in order to observe macroscopic clouding. This mechanism makes one final prediction that can be tested. At temperatures above the local cloud point of the least soluble end, but below the temperature corresponding to the cloud point of the critical insoluble fraction, a small portion of the copolymer is insoluble. This fraction, however, is not sufficient to induce macroscopic clouding. Under these conditions, this portion of the chain is attracted more strongly to itself than to the solvent, and should coil upon itself in order to minimize unfavorable segment-solvent contacts. Due to the insolubility of this small portion of the chain, a small amount of inter-chain aggregation is expected to occur due to the inability to sterically hinder all inter-chain aggregation. Given the sensitivity of Dynamic Light Scattering (DLS) to smaller sizes, we have opted to probe the early stages of the cloud point transition before macroscopic clouding via DLS.

Using DLS we can measure the hydrodynamic sizes of objects in dilute polymer solutions as a function of temperature. DLS measurements on dilute gradient copolymer solutions indicate two populations, one corresponding to a hydrodynamic radius, \( R_h \), of ~4 nm that we attribute to dissolved unimers, and another corresponding to an \( R_h \) of ~300 nm that we attribute to aggregates. Since the scattered intensity is proportional to the square of volume, and volume is proportional to cube of the radius, the scattering from an aggregate is \( \approx 12 \) orders of magnitude larger than the scattering due to a unimer, allowing us to easily detect small changes in the concentration of the aggregates during the temperature ramp. A small change in the concentration of aggregates will not likely induce turbidity, but DLS, which is much more sensitive, should allow us to probe the onset of this aggregation.

Fig. 5 shows the aggregation temperature of both hyperbolic and linear gradient copolymers measured using DLS, as well as that of PHD65. All solutions for DLS measurements were 1 wt% copolymer in 1.0 M glycine buffer at pH 8.31; the aggregation temperature was defined as the midpoint between the temperatures where unimers were visible and where unimers were not visible. Therefore the aggregation temperature represents the point at which the concentration of aggregates rises above the background level. Fig. 5 shows the aggregation temperature of both hyperbolic and linear gradient copolymers measured using DLS, as well as that of PHD65. All solutions for DLS measurements were 1 wt% copolymer in 1.0 M glycine buffer at pH 8.31; the aggregation temperature was defined as the midpoint between the temperatures where unimers were visible and where unimers were not visible. Therefore the aggregation temperature represents the point at which the concentration of aggregates rises above the background level.

![Fig. 4](image4.png)

**Fig. 4.** The estimated insoluble fraction (dotted) required for macroscopic clouding for linear and hyperbolic gradient copolymers. The region to the left of the symbol is insoluble at the cloud point.

![Fig. 5](image5.png)

**Fig. 5.** The aggregation temperatures of gradient copolymers with linear and hyperbolic composition profiles as measured by dynamic light scattering at pH 8.31. The solid line shows an upshifted theoretical curve based on the cloud point of random copolymers (Eq. 2) with the same composition as the least soluble end. The upshift is 9.8 °C.
clearly shows a linear relationship between the aggregation temperature and the gradient strength, and both series of gradient copolymers exhibit the same dependence on gradient strength. The DLS results thus mirror the turbidity measurements in this respect. The solid line in Fig. 5 represents the theoretical cloud points for pH 8.31, as predicted by Eq. 2, but shifted up by 9.8 °C. We observe that Eq. 2 describes the temperature dependence of this aggregation behavior very well. This observation suggests that aggregation starts at the onset of insolubility at the least soluble chain end, and that it occurs at temperatures well below the visible cloud point. The 9.8 °C upshift is systematic across all copolymer solutions examined and is attributed to the fact that the experimental temperature is read at the Peltier stage and not directly of the copolymer solutions.

Based on turbidity and DLS data, Fig. 6 schematizes the structural development that the polymer chains in these dilute solutions undergo as the temperature is raised. Below the cloud point of the least soluble end (TCP,LE), the polymers exist as dissolved unimers. Above the cloud point TCP,LE, but below the temperature at which the critical portion of the polymer chain becomes insoluble, or the observed cloud point temperature (TCPD), the insoluble portion of the chain coils upon itself while the soluble end extends into the solvent. While large-scale clouding is not observed in this temperature range, the insoluble chain ends can aggregate. This initial aggregation phenomenon can be detected by DLS. Finally, when the temperature increases beyond the temperature that induces the critical portion of chain to precipitate, TCPD, the unimers aggregate and precipitate, resulting in a cloudy appearance. Therefore, TCP,LE is the aggregation temperature detected by DLS and its dependence on gradient strength is well described by the composition at the least soluble end, while TCPD is the cloud point detected by turbidity and its dependence on gradient strength depends on the size of the critical insoluble fraction.

4. Conclusion

We have presented data indicating that gradient strength is the primary variable for determining the cloud point of gradient copolymers. Our data suggest that composition profile and fraction of gradient material (λ) play a secondary role in determining the cloud point of gradient copolymers. The macroscopic cloud point of gradient copolymers cannot be explained solely by aggregation of the least soluble end, but requires approximately 30% of the copolymer backbone to become insoluble before macroscopic clouding will begin. However, small levels of aggregation will occur at lower temperatures, when any portion of the copolymer becomes insoluble. These data demonstrate that the introduction of a gradient monomer sequence distribution results in an increased tunability over the observed cloud point and the breadth over which this transition takes place.

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References