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Polyampholytes: From Single Chains to Solutions

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ABSTRACT: We discuss multichain effects in salt-free polyampholyte solutions of finite concentration and find that the existing single-chain theories are limited to exponentially small concentrations, if the sample contains chains with net charges of both signs. We show that charged polyampholytes have a strong tendency to form neutral complexes and to precipitate. Neutral samples, where the *average* net charge of the chains is exactly zero, phase separate at exponentially small concentrations. The supernatant is dominated by neutral unimers or clusters that form *spherical* globules and its polymer concentration $c_{\mathrm{dil}} \sim \exp(-\tilde{N}^{2/3})$ is independent of the total polymer concentration. For non-neutral samples, on the other hand, the free counterions accumulate in the supernatant together with the most strongly oppositely charged unimers, if the system is concentrated beyond the onset of phase separation. The dilute phase then consists of *elongated* globules and has a concentration *proportional* to the total polymer concentration.

1. Introduction

Polyampholytes are polymers comprising neutral, positively charged, and negatively charged monomers. Such molecules are often water-soluble and offer numerous applications.[1] In addition, they can be regarded as simple model systems for electrostatic interactions in proteins and other biopolymers. Over the last years much theoretical effort has been devoted to understanding the conformations of intrinsically flexible, isolated, quenched random polyampholytes in solution.[2−11] For this special class of polyampholytes the charges are randomly distributed along the chain and insensitive to changes of the pH: they are predetermined by the chemistry.

The conformations of dissolved polyampholyte chains result from a competition between attraction due to fluctuations in the density of oppositely charged monomers and repulsion between excess charges. Higgs and Joanny[2] showed that *neutral* random polyampholytes collapse into spherical, dilute globules, while Kantor and Kardar demonstrated that already the typical net charge of order $\sqrt{N}$ for a random copolymer of length N is sufficient to stretch the chains.[4,5] They suggested that the averages for quantities such as the hydrodynamic radius can be dominated by the extended chains in the wings of the charge distribution and therefore grow like N as opposed to $N^{1/3}$ for the majority of collapsed chains. Although there are still open questions concerning the behavior of polyampholyte chains in solution (see, e.g., the necklace model of Kantor and Kardar[6,7]), the essential features seem to be captured in the elongated globule model introduced by Gutin and Shakhnovich.[1,9,10]

Even though the single chain picture is valid only at infinite dilution, the results have been tentatively applied to experiments on quenched random polyampholyte solutions.[1] Candau et al.[12] use copolymerization in a microemulsion consisting of varying compositions of neutral and charged monomers and non-polymerizable inorganic counterions to synthesize quenched random polyampholytes with controlled charge and net charge densities. Typically, already the inclusion of a few percent of charged monomers is sufficient to induce phase separation and precipitation in dilute polymer solutions, even if the quality of the solvent is good for the neutral backbone of the polymer.[13,14] As for polyelectrolytes, the solubility increases with the *average* net charge of a sample. But mixtures of oppositely charged chains precipitate, even if the average net charge is much higher than for a perfectly soluble sample with a narrower net charge distribution.[14] Moreover, Candau et al. reported an increase of the concentration of the supernatant with the *total* polyampholyte concentration[13] and apparently conflicting evidence for the predominance of either spherical[14] or elongated chains[13] in the dilute phase.

The purpose of the present paper is the investigation of polyampholyte solutions of finite concentration. Our analysis of the phase equilibrium between a homogeneous dense phase and a dilute supernatant is based on the description of polyampholytes in solution as elongated globules.[1,9,10] Up to now the solubility of polyampholytes has been estimated from analogies to polymers in a poor solvent and to polyelectrolytes (i.e., polymers that carry charges of only one sign), implying (i) that solutions precipitate under conditions where individual chains collapse and (ii) that charged chains are more soluble than neutral chains. These arguments are, however, questionable, as the water solubility of polyelectrolytes is mostly due to the gain in translational entropy of the counterions in the water phase.[15] The polymers are dissolved *in spite* of their high electrostatic self-energies, which they minimize by adopting stretched conformations. In contrast, a sample of polyampholyte chains can be globally neutral, even if individual molecules carry net charges. The situation therefore resembles mixtures of oppositely charged polyelectrolytes.[16] Indeed, we find[17] that multichain effects such as complexation and selective precipitation play an important role and that the solubility of polyampholytes is mostly due to the presence of the free counterions. Neutral samples, where the net charges on the chains cancel each other exactly, phase separate at exponentially small concentrations. The supernatant is dominated by neutral unimers or clusters which form *spherical* globules, and its polymer concentration $c_{\mathrm{dil}} \sim \exp(-\tilde{N}^{2/3})$ is independent of the total polymer concentration. For non-neutral samples, on the other hand,

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the free counterions accumulate in the supernatant together with the most strongly oppositely charged unimers, if the system is concentrated beyond the onset of phase separation. The dilute phase then consists of *elongated* globules and has a concentration *proportional* to the total polymer concentration.

Some of our results have already appeared as a short note.[17] Here we provide more details, in particular for the case of randomly copolymerized samples. The paper is organized as follows: In the second section we introduce the notations, review the elongated globule model, and outline the treatment of association and phase equilibria. In the third section we consider polyampholyte samples with uni- and bimodal net charge distributions, for which many of the effects can be worked out by simple arguments. Sections 4 and 5 deal with the more realistic case of randomly copolymerized samples. We conclude by a discussion of the interpretation of experiments on polyampholyte samples containing chains with net charges of both signs. The Appendix discusses the validity of the main assumption we made in order to calculate the composition of the phases.

2. Theoretical Background

In the following we review the scaling picture of the conformation of a random polyampholyte with a certain net charge density in solution. We introduce the model polyampholyte samples with idealized net charge distributions that we study in this paper and formally discuss association and phase equilibria in polyampholyte solutions of finite concentration.

2.1. Properties of the Dense Phase. Our model polyampholytes are flexible chains of N monomers of diameter b. A fraction $f = f_+ + f_-$ of the monomers at quenched random positions is charged. The net charge density of a particular chain is $\delta f = f_+ - f_-$. The strength of the electrostatic interactions is characterized by the Bjerrum length, $l_B = e^2/\epsilon k_B T$. We do not treat the case of ionomers[15] where oppositely charged groups on the chains are tightly bound to each other. By assuming that $b \gtrsim l_B$, we ensure that the attraction between any two charges is smaller than k_BT. Concentrations are always given as *monomer* concentrations. We use small (capital) letters to indicate concentrations in the dilute (dense) phase. The respective chain concentrations of molecules with net charge density δf are then $c_{\delta f}/N$ ($C_{\delta f}/N$).

We focus on the generic case of a neutral ensemble of polyampholytes in a Θ-solvent with no added salt. For a homogeneous dense solution of concentration C_{dense}, the contribution of the electrostatic interactions to the free energy density is given by the Debye–Hückel expression $f_{\text{DH}}/k_BT = -(1/12\pi)\kappa^3$, where $\kappa^2 = 4\pi l_B f C_{\text{dense}}$ is the inverse squared screening length.[8] Higgs and Joanny[2] have argued that the dense phase can be viewed as a liquid of blobs of $g_a = b^2/l_B{}^2 f^2$ monomers.[18] We usually measure the chain length in units of these polyampholyte blobs: $\tilde{N} \equiv N/g_a \gg 1$. The monomer density is of the order of $C_{\text{dense}} \sim g_a/\xi_a{}^3 = g_a/b^3 g_a{}^{3/2} = l_B f/b^4$ with a free energy $F_{\text{dense}} \approx -\tilde{N} k_B T$ per chain independent of the chain net charge.

In the Appendix we discuss the inflation of the dense phase due to the osmotic pressure of free counterions and the formation of mesophases. Throughout the paper we assume that the counterion concentrations are so small that these effects can be safely neglected.

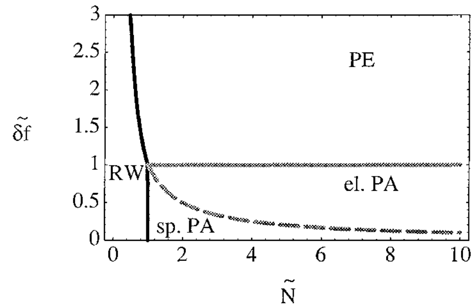

Figure 1. Diagram of states for polyampholyte chains.

2.2. Single-Chain Properties. To a first approximation the conformation of a polyampholyte molecule in solution depends on its length $\tilde{N}$ and its excess charge density δf. For $\tilde{N} \gg 1$, three characteristic regimes can be distinguished for the conformations of isolated molecules in solution (Figure 1):[1,10] (i) chains which are overall neutral collapse into spherical globules (polyampholyte effect), while (ii) highly charged chains are stretched (polyelectrolyte effect). The crossover between the two extremes was investigated by Kantor and Kardar.[3,4,5,6,7] Using RG arguments and Monte Carlo simulations, they showed that (iii) the typical net charge of order $\sqrt{fN}$ for a random copolymer of length N is sufficient to elongate the globule.

The essential features of these extended states seem to be captured in the elongated globule model. It was introduced by Gutin and Shakhnovich[9] and worked out as a Flory theory by Dobrynin and Rubinstein.[10] Here we summarize the formulation as a scaling theory by Candau and Joanny.[1] In the globular states the internal monomer concentration is still approximately given by C_{dense} so that three-body repulsion and polarization contribute F_{dense} to the free energy of the globule. The shape is determined from a competition between surface tension and electrostatic repulsion between the excess charges. The surface energy of a spherical globule is of the order of k_BT times the number of blobs on the surface: $F_{\text{surf}} \sim N^{2/3} k_B T$. In addition the globule contains $\delta f\, N$ excess charges at a typical distance of the order of the globule radius $\xi_a \tilde{N}^{1/3}$, which repel each other. The electrostatic energy is thus of the order $F_{\text{coul}} \sim (\delta f\, N)^2 (l_B/\xi_a \tilde{N}^{1/3}) k_B T$. It exceeds the surface energy for $N > g'_e = f/\delta f^2$. In this case the molecule can be viewed as a linear sequence of N/g'_e electrostatic blobs, each consisting of g'_e/g_a densely packed polyampholyte blobs. Note that for an ensemble of random polyampholytes $f/\langle \delta f^2 \rangle = N$. As a consequence, for *all* chain lengths a finite fraction of the chains form elongated globules with a size proportional to N.

Up to logarithmic corrections[19] the total surface and Coulomb energy of an elongated globule is N/g'_e times that of an electrostatic blob and can be written as $F_{\text{surf}} + F_{\text{coul}} \sim \tilde{N} \delta\tilde{f}^{2/3} k_B T$ with

$$\delta\tilde{f} = \delta f \sqrt{\frac{g_a}{f}} \tag{2.1}$$

The crossover to the polyelectrolyte regime occurs at $g'_e \equiv g_a/\delta\tilde{f}^2 = g_a$ or $\delta\tilde{f} = 1$. For chains with $\delta\tilde{f} > 1$, the polarization energy can be neglected and the chains form a linear sequence of N/g_e electrostatic blobs each containing $g_e = g_a/\delta\tilde{f}^{4/3}$ monomers. The free energy is $F_{\text{coul}} = (N/g_e) k_B T = \tilde{N}\, \delta\tilde{f}^{4/3} k_B T$. In summary the excess free energy of a single chains in solution relative to the dense phase may be approximated as

$$\frac{F_{\mathrm{ex}}(\delta\tilde{f},\tilde{N})}{k_{\mathrm{B}}T} = \begin{cases} \tilde{N}^{2/3}\,(1+\delta\tilde{f}^{\,2}N) & \delta\tilde{f}^{\,2} < 1/\tilde{N} & \text{(spherical globules)} \\ 2\delta\tilde{f}^{\,2/3}\tilde{N} & 1/\tilde{N} < \delta\tilde{f}^{\,2} < 1 & \text{(elongated globules)} \\ 2\delta\tilde{f}^{\,4/3}\tilde{N} & \delta\tilde{f}^{\,2} > 1 & \text{(polyelectrolytes)} \end{cases} \tag{2.2}$$

The Debye–Hückel approximation for the description of dense states and the elongated globule model for chains in dilute solution capture the essential physics and therefore provide a sensible base for the following discussion. Nevertheless, the resulting expressions for the free energies of polyampholytes in the dense and in the dilute phase can be refined (see the Appendix and refs 6, 8, and 16). For example, future work on the necklace model[6] may allow us to go beyond the rather crude characterization of a polyampholyte chain only by its net charge.

2.3. Model Polyampholyte Samples. For simplicity we only consider net charge polydispersity and treat the chain length N and the fraction f of charged monomers as constants. Polyampholyte samples are then characterized by a normalized distribution $p(\delta f)$ for the excess charge per monomer δf. For non-neutral samples with a non-vanishing average net charge density $\delta f_0 \equiv \int \delta f\, p(\delta f)\, \mathrm{d}\delta f \neq 0$, the net charge on the chains is balanced by a counterion density $c_{\mathrm{i,tot}} = \delta f_0 c_{\mathrm{tot}}$. We consider three different model distributions.

(I) Biased Random Copolymerization. Chemically, it is very difficult to find a pair of monomers with opposite residual charges and equal reactivities in the polymerization process. For this reason, there often is a surplus of, e.g., positive monomers in polyampholyte molecules formed in the early stages of the reaction. As long as the depletion of monomers of this type in the reaction bath is not felt, the reaction can still be described as a random process, however, with a bias toward the formation of non-neutral chains:

$$p(\delta\tilde{f}) = \sqrt{\frac{\tilde{N}}{2\pi}}\, \mathrm{e}^{-[(\delta\tilde{f}-\delta\tilde{f}_0)^2\tilde{N}/2]} \tag{2.3}$$

The Gaussian distribution eq 2.3 is a good approximation for the binomial distribution for $(\delta\tilde{f} - \delta\tilde{f}_0)^2\tilde{N} < \sqrt{Nf}$. Note that the unbiased case with $\delta\tilde{f}_0 = 0$ is an exception rather than the rule and that monomer depletion during the synthesis leads to broader net charge distributions.[12]

(II) Unimodal Samples. We briefly discuss the case when all chains have the same net charge density $\delta\tilde{f}_0$. This is a reasonable approximation for eq 2.3, if $\delta\tilde{f}_0$ is larger than the width $1/\sqrt{\tilde{N}}$ of the distribution.

(III) Bimodal Samples. Most finite concentration effects can be worked out qualitatively by considering a mixture of two types of polyampholytes with different net charges and otherwise identical characteristics. We discuss in detail the case of opposite net charges $\pm\delta\tilde{f}$ of equal magnitude and of a mixture of neutral and charged chains. In the former case, the total concentrations of the species in the sample are given by c_+^{tot} and $(^1/_2 + \epsilon)c^{\mathrm{tot}}$ and $c_-^{\mathrm{tot}} = (^1/_2 - \epsilon)c^{\mathrm{tot}}$. If $\epsilon \neq 0$, the sample also contains counterions to ensure charge neutrality.

2.4. Outline of the Problem: Association and Phase Equilibria. At infinite dilution, all molecules are dissolved as unimers. In a dilute phase of finite concentration, we have to consider an *association* equilibrium between unimers, dimers, and higher order aggregates. Formally, this corresponds to a chemical equilibrium. In addition, solutions of polyampholytes establish over a wide range of the total concentration c_{tot} a *phase* equilibrium between a dense phase of precipitated molecules (or precipitate) and a dilute supernatant.

Neglecting the interaction between the dissolved molecules and clusters (see the Appendix) the free energy density of the dilute phase can be obtained by minimizing the free energy

$$\begin{aligned} \frac{f_{\mathrm{dil}}(\{c_{\delta f}^{\mathrm{dil}}\},c_{\mathrm{i}})}{k_{\mathrm{B}}T} = {} & c_{\mathrm{i}}(\log(c_{\mathrm{i}}b^3) - 1) + \\ & \sum_{\delta f}\frac{c_{\delta\tilde{f}}}{N}\left(\log\frac{c_{\delta\tilde{f}}\,b^3}{N} - 1 + F_{\mathrm{dense}}(\tilde{N}) + F_{\mathrm{ex}}(\delta\tilde{f},\tilde{N})\right) + \\ & \sum_{\delta f,\delta f'}\frac{c_{\delta\tilde{f}\delta\tilde{f}\,'}}{N}\left(\log\frac{c_{\delta\tilde{f}\delta\tilde{f}\,'}\,b^3}{2N} - 1 + F_{\mathrm{dense}}(2\tilde{N}) + \right. \\ & \left. F_{\mathrm{ex}}\left(\frac{1}{2}(\delta\tilde{f}_+ + \delta\tilde{f}_-),2N\right)\right) + \ldots \end{aligned} \tag{2.4}$$

under the constraint of mass conservation:

$$c_{\delta\tilde{f}} + \frac{1}{2}\sum_{\delta f'} c_{\delta\tilde{f}\delta\tilde{f}\,'} + \ldots = c_{\delta f}^{\mathrm{dil}} \tag{2.5}$$

Here, $c_{\delta f}^{\mathrm{dil}}$ indicates the total monomer concentration of molecules with net charge density $\delta\tilde{f}$ in the dilute phase, $c_{\delta\tilde{f}}$ is the concentration of such molecules dissolved as unimers, $c_{\delta\tilde{f}\delta\tilde{f}'}$ is the concentration of dimers composed of molecules with net charge densities $\delta\tilde{f}$ and $\delta\tilde{f}\,'$, and c_{i} is the counterion concentration. The dots stand for higher order clusters that usually do not play an important role. Note that for a globular state the dimer free energy equals the free energy of a neutral unimer of twice the unimer length.

The free energy density of a phase-separated system

$$f = \Phi\, f_{\mathrm{dense}}(\{C_{\delta f}\},C_{\mathrm{i}}) + (1 - \Phi)\, f_{\mathrm{dil}}(\{c_{\delta f}^{\mathrm{dil}}\},c_{\mathrm{i}}) \tag{2.6}$$

depends on the volume fraction Φ of the dense phase and the compositions $\{C_{\delta f}\}$ of the precipitate and $\{c_{\delta f}^{\mathrm{dil}}\}$ and the supernatant. Since we are dealing with *multicomponent* systems, the composition of the phases is *not* fixed by establishing a phase equilibrium. It often turns out to be useful to use

$$\Phi = \frac{c_{\mathrm{tot}} - c_{\mathrm{dil}}}{C_{\mathrm{dense}} - c_{\mathrm{dil}}} \tag{2.7}$$

instead of the total concentration $c_{\mathrm{tot}} = \Phi C_{\mathrm{dense}} + (1 - \Phi)c_{\mathrm{dil}}$ as an auxiliary variable in the treatment of phase-separated systems. If c_{tot} is not too close to the onset of phase separation and c_{dil} is negligible compared to C_{dense}, $\Phi = c_{\mathrm{tot}}/C_{\mathrm{dense}}$.

Quite similarly to the association equilibrium (which needs to be solved as a subproblem), the phase equilibrium is given by the minimization of the free energy f subject to the constraints of mass conservation and charge neutrality:

$$\Phi C_{\delta f} + (1 - \Phi) c_{\delta f}^{\mathrm{dil}} = p(\delta f) c_{\mathrm{tot}} \tag{2.8}$$

$$\Phi C_{\mathrm{i}} + (1 - \Phi) c_{\mathrm{i}} = \delta f_0 c_{\mathrm{tot}} \tag{2.9}$$

$$\int \delta f\, C_{\delta f}\, \mathrm{d}\delta f = C_{\mathrm{i}} \tag{2.10}$$

$$\int \delta f\, c_{\delta f}^{\mathrm{dil}}\, \mathrm{d}\delta f = c_{\mathrm{i}} \tag{2.11}$$

The constraints are conveniently included in the minimization procedure as Lagrange multipliers related to the osmotic pressure and the chain and counterion (electro-) chemical potentials. The calculation of the monomer density in the precipitate in section 2.1 implicitly made the assumption that the osmotic pressure of the dilute phase and the ideal gas contribution of the chains in the dense phase can be neglected. This reduces the osmotic pressure equilibrium to the condition

$$\int C_{\delta f}\, \mathrm{d}\delta f = C_{\mathrm{dense}} \tag{2.12}$$

where $C_{\mathrm{dense}} = l_{\mathrm{B}} f / b^4$ is a constant that is independent of the sample composition. This point is further discussed in Appendix A.1.

The chemical potentials are given by

$$\mu_{\mathrm{dense}} = F_{\mathrm{dense}} + k_{\mathrm{B}} T \log\left(\frac{C_{\delta f}\, b^3}{N}\right) \tag{2.13}$$

$$\mu_{\mathrm{uni}}(\delta\tilde{f}, N, c_{\delta\tilde{f}}) = F_{\mathrm{dense}}(\tilde{N}) + F_{\mathrm{ex}}(\delta\tilde{f}, \tilde{N}) + k_{\mathrm{B}} T \log \frac{c_{\delta\tilde{f}}\, b^3}{N} - \delta\tilde{f}\, N \tilde{\Psi} \tag{2.14}$$

$$\mu_{\mathrm{dim}}(\delta\tilde{f}, \delta\tilde{f}', \tilde{N}, c_{\delta\tilde{f}\delta\tilde{f}'}) = F_{\mathrm{dense}}(2\tilde{N}) + F_{\mathrm{ex}}\left(\frac{1}{2}(\delta\tilde{f} + \delta\tilde{f}'), 2\tilde{N}\right) + k_{\mathrm{B}} T \log \frac{c_{\delta\tilde{f}\delta\tilde{f}'} b^3}{2N} - (\delta\tilde{f} + \delta\tilde{f}')\tilde{N}\tilde{\Psi} \tag{2.15}$$

Equating the chemical potentials leads to relations like

$$\frac{c_{\delta\tilde{f}}}{C_{\delta\tilde{f}}} \equiv X_{\delta\tilde{f}} = \exp(-F_{\mathrm{ex}}(\delta\tilde{f}, \tilde{N}) + \delta\tilde{f}\, \tilde{N} \tilde{\Psi})$$

$$\frac{c_{\delta\tilde{f}\delta\tilde{f}'}}{c_{\delta\tilde{f}} c_{\delta\tilde{f}'}} = \frac{2b^3}{N} \exp\left(-F_{\mathrm{ex}}(\delta\tilde{f}, \tilde{N}) - F_{\mathrm{ex}}(\delta\tilde{f}', \tilde{N}) + F_{\mathrm{ex}}\left(\frac{1}{2}(\delta\tilde{f} + \delta\tilde{f}'), 2\tilde{N}\right)\right)$$

In the general case, the chemical potentials have to be chosen in such a way that the mass balances are fulfilled. In addition, for asymmetric net charge distributions, an electrostatic potential difference[20] $\Psi = \tilde{\Psi}/\sqrt{f g_{\mathrm{a}}}$ develops between the dense and the dilute phase, which has to be determined from the condition of charge neutrality of both phases. In section 3 we consider simple cases where these tedious procedures can be avoided by considering solubility products and laws of mass action for the association.

A limiting case, which greatly simplifies the calculations, is a supernatant in equilibrium with an infinite dense phase: the composition of the dense phase is identical with the sample composition and the chemical potentials are known from eq 2.13. For a given system, the assumption of an infinite dense phase is justified, if there is no depletion, i.e., if for all $\delta\tilde{f}$ the *number* of molecules in the supernatant is negligible compared to the dense phase. If the supernatant is dominated by unimers, this amounts to the condition $(1 - \Phi) c_{\delta\tilde{f}} = (1 - \Phi) C_{\delta\tilde{f}} X_{\delta\tilde{f}} \ll \Phi C \delta\tilde{f}$ for any $\delta\tilde{f}$. In this case, we can also write $(1 - \Phi) c_{\mathrm{dil}} + \Phi C_{\mathrm{dense}} \approx \Phi C_{\mathrm{dense}} = c_{\mathrm{tot}}$, so that the total concentration has to exceed

$$\frac{c_{\mathrm{tot,min}}}{C_{\mathrm{dense}}} = \frac{1}{(\max(X_{\delta\tilde{f}}))^{-1} + 1} = \begin{cases} \max(X_{\delta\tilde{f}}) & \text{if} \quad \max(X_{\delta\tilde{f}}) \ll 1 \\ 1 - (\max(X_{\delta\tilde{f}}))^{-1} & \text{if} \quad \max(X_{\delta\tilde{f}}) \gg 1 \end{cases} \tag{2.16}$$

Equation 2.16 is straightforward to generalize for more complicated compositions of the supernatant.

3. Simple Model Distributions

In general, the calculation of the chemical and phase equilibria introduced in the preceding section is a difficult task. To gain some insight, we investigate in this section simple model distributions. We start by considering the bimodal sample (III). In solution, the unimers are in chemical equilibrium with neutral dimers and, already for very low total concentrations c_{tot}, the sample phase separates. We show that in the symmetric case the supernatant is dominated by *spherical* globules, formed either from neutral or from pairs of charged polyampholytes. In section 3.2 we discuss the formation of higher order clusters; they become important for overall neutral, but asymmetric bimodal net charge distributions, where the positive and negative chains have net charges of different magnitudes.

We then turn to non-neutral ensembles, where the charges on the polymers do not exactly cancel each other but are neutralized by free counterions. We discuss two cases: the bimodal sample (III) with a small asymmetry in the fraction of chains with positive and negative net charge and a mixture of neutral chains with a small proportion of charged chains. In both cases the counterions and excess chains accumulate in the supernatant, if the sample is concentrated beyond the onset of phase separation.

3.1. Dimerization and Precipitation. We start by discussing dimerization and precipitation for the bimodal sample (III) with net charges of equal magnitude but opposite signs on the polyampholyte chains. We write the equations for the general case, where we allow for a small asymmetry ϵ in the fraction of chains with positive and negative net charge. The discussion of the results for $\epsilon \neq 0$ is, however, postponed to section 3.3.

Within the dilute phase there is an association or chemical equilibrium between unimers and dimers. Using eqs 2.14 and 2.15, the condition $\mu_{\mathrm{dim}} = \mu_+ + \mu_-$ leads to the law of mass action:

$$\frac{c_{\mathrm{dim}}}{c_+ c_-} = \frac{2b^3}{N}\, \mathrm{e}^{2F_{\mathrm{ex}}(\delta\tilde{f}, \tilde{N}) - F_{\mathrm{ex}}(0, 2\tilde{N})} \equiv K_{\mathrm{A}} \tag{3.1}$$

where $c_\pm = c_{\pm,\mathrm{tot}} - c_{\mathrm{dim}}/2$. The dimer concentration is given by

$$c_{\mathrm{dim}} = c_{\mathrm{tot}} + \frac{2}{K_{\mathrm{A}}}\left(1 - \sqrt{\epsilon^2 K_{\mathrm{A}}{}^2 c_{\mathrm{tot}}{}^2 + (1 + K_{\mathrm{A}}\, c_{\mathrm{tot}})}\right) \tag{3.2}$$

Thus, for $c_{\mathrm{tot}}K_{\mathrm{A}} \ll 1$ the degree of association is very small: $c_{\mathrm{dim}}/c_{\mathrm{tot}} \sim K_{\mathrm{A}}c_{\mathrm{tot}}^2 \rightarrow 0$. On the other hand, dimers dominate if $c_{\mathrm{tot}} > 1/K_{\mathrm{A}}$ before the onset of precipitation. In equilibrium with a dense phase, the chemical potentials have to fulfill $\mu_{\mathrm{dim}} = \mu_+ + \mu_- = 2\mu_{\mathrm{dense}}$. As a consequence, the unimer concentrations in the dilute phase are related to each other by a solubility product[21]

$$c_+^{\mathrm{coex}}\, c_-^{\mathrm{coex}} = \frac{1}{4}\, C_{\mathrm{dense}}{}^2\, \mathrm{e}^{-2F_{\mathrm{ex}}(\delta\tilde{f},\tilde{N})} = L_{\mathrm{P}} \qquad (3.3)$$

the dimer concentration is given directly by

$$c_{\mathrm{dim}}^{\mathrm{coex}} = \frac{b^3}{2N}\, C_{\mathrm{dense}}{}^2\, \mathrm{e}^{-F_{\mathrm{ex}}(0,2\tilde{N})} = K_{\mathrm{A}}L_{\mathrm{P}} \qquad (3.4)$$

Note, that $c_{\mathrm{dim}}^{\mathrm{coex}}$ is independent of ϵ and $\delta\tilde{f}$.

At coexistence, the solubility product eq 3.3 has to be solved for $c_\pm = c_{\pm,\mathrm{tot}} - c_{\mathrm{dim}}/2 - \Phi C_{\mathrm{dense}}/2$, where Φ is the volume fraction of the precipitate. The result is

$$\Phi = \frac{c_{\mathrm{tot}} - K_{\mathrm{A}}L_{\mathrm{P}} - 2\sqrt{\epsilon^2 c_{\mathrm{tot}}{}^2 + L_{\mathrm{P}}}}{C_{\mathrm{dense}}} \qquad (3.5)$$

Phase separation sets in at a total concentration of

$$c_{\mathrm{phase\ sep}}^{\mathrm{tot}} = \frac{\mathrm{K}_{\mathrm{A}}L_{\mathrm{P}} + 2\sqrt{L_{\mathrm{P}} + \epsilon^2(K_{\mathrm{A}}{}^2L_{\mathrm{P}}{}^2 - 4L_{\mathrm{P}})}}{(1 - 4\epsilon^2)} \qquad (3.6)$$

At this point we only consider the case $\epsilon = 0$. If unimers dominate the dilute phase at the onset of phase separation $c_{\mathrm{dim}} = {}^1\!/_4 K_{\mathrm{A}} c_{\mathrm{phase\ sep}}^{\mathrm{tot}}{}^2 = K_{\mathrm{A}}L_{\mathrm{P}}$ or $c_{\mathrm{phase\ sep}}^{\mathrm{tot}} = 2\sqrt{L_{\mathrm{P}}}$. In the opposite limit where dimers dominate $c_{\mathrm{dim}} = c_{\mathrm{phase\ sep}}^{\mathrm{tot}} = K_{\mathrm{A}}L_{\mathrm{P}}$. In the general case, eq 3.6 reduces to $c_{\mathrm{phase\ sep}}^{\mathrm{tot}} = K_{\mathrm{A}}L_{\mathrm{P}} + 2\sqrt{L_{\mathrm{P}}}$. For symmetry reasons the composition of the dilute phase remains unchanged beyond the onset of phase separation: $c_\pm^{\mathrm{coex}} = \sqrt{L_{\mathrm{P}}}$. As a consequence, dimers dominate in the supernatant for $\sqrt{L_{\mathrm{P}}} > 1/K_{\mathrm{A}}$, or, up to logarithms,

$$\delta\tilde{f}^{\,2} > \frac{1}{\tilde{N}} \qquad (3.7)$$

The result is quite suggestive. As soon as the net charge forces unimers to elongate they start to form dimers. Note that the domination of dimers over charged unimers is a consequence of the reduced unimer solubility. Figure 2 illustrates the change in the composition of the dilute phase for symmetric bimodal distributions of polyampholytes with different net charges. The different curves end on a line which indicates the total concentration at coexistence. For neutral chains $c_{\mathrm{phase\ sep}}^{\mathrm{tot}}$ is of order $\exp(-\tilde{N}^{2/3})C_{\mathrm{dense}}$ and decreases for highly charged chains where the dilute phase consists predominately of neutral dimers to $c_{\mathrm{tot}}^{\mathrm{coex}} \sim \exp(-(2\tilde{N})^{2/3})C_{\mathrm{dense}}$. Thus, a symmetric ensemble of polyampholytes with high reduced chain length $\tilde{N}$ is practically insoluble. Experimentally, such a system always consists of a supernatant in coexistence with a precipitate. The dilute phase contains only *spherical* globules, either formed from neutral or pairs of charged polyampholytes.

3.2. Formation of Higher Order Clusters. In general, the tendency toward association increases with concentration. In view of the low solubility of neutral samples, we only consider the case of a phase equilibrium where the vast majority of the polyampholytes is precipitated into a dense phase of known composition $p(\delta\tilde{f})$. The chemical potentials of the polyampholytes in the dense phase are given by eq 2.13.

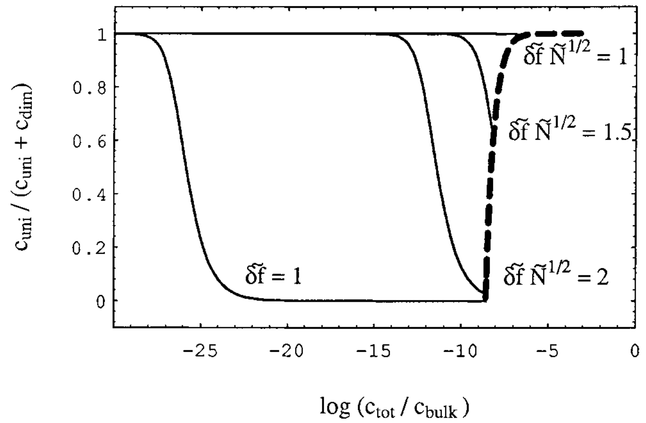

Figure 2. Fraction of unimers in the dilute phase versus total concentration for symmetric bimodal distributions of chains with $\tilde{N} = 20$, $g_{\mathrm{a}} = 10$ and different net charges. For polyampholytes in the polyelectrolyte regime ($\delta\tilde{f} \geq 1$) the solution contains practically no unimers. The dashed line indicates the onset of phase separation (eq 3.6). For $\mathrm{c}_{\mathrm{tot}} > c_{\mathrm{phase\ sep}}^{\mathrm{tot}}$ the ratio $c_{\mathrm{uni}}/(c_{\mathrm{uni}} + c_{\mathrm{dim}})$ remains constant.

A polyampholyte cluster in solution can be characterized by a set of numbers $\{\nu_{\delta\tilde{f}}\}$, which indicate the number of polyampholyte molecules with net charge $\delta\tilde{f}$ that it contains. The cluster size, the net charge density, and the chemical potential are $\nu = \sum\nu_{\delta\tilde{f}}$, $\Delta\tilde{f} = (1/\nu)\sum\delta\tilde{f}\,\nu_{\delta\tilde{f}}$, and $\mu(\{\nu_{\delta\tilde{f}}\}) = F_{\mathrm{dense}}(\nu\tilde{N}) + F_{\mathrm{ex}}(\Delta\tilde{f},\nu\tilde{N}) + k_{\mathrm{B}}T \log(\mathrm{c}(\{\nu_{\delta\tilde{f}}\})b^3/\nu\tilde{N}) + \nu\tilde{N}\Delta\tilde{f}\tilde{\Psi}$. In general, the electrostatic potential difference $\tilde{\Psi}$ does *not* vanish for samples with an asymmetric net charge density distribution. At coexistence $\mu(\{\nu_{\delta\tilde{f}}\}) = \sum\nu_{\delta\tilde{f}}\mu_{\mathrm{dense}}(\delta\tilde{f})$, so that the concentration in the dilute phase is given by

$$c^{\mathrm{coex}}(\{\nu_{\delta\tilde{f}}\}) = \frac{\nu N}{b^3}\left(\frac{C_{\mathrm{dense}}b^3}{N}\right)^{\nu} \times \left(\prod p(\delta\tilde{f})^{\nu\delta\tilde{f}}\right) \mathrm{e}^{-F_{\mathrm{ex}}(\Delta\tilde{f},\nu\tilde{N}) - \nu\tilde{N}\Delta\tilde{f}\tilde{\Psi}} \qquad (3.8)$$

For symmetric net charge distributions $\tilde{\Psi} = 0$ and for dimers eq 3.8 reduces to eq 3.4. Since the excess internal free energy of a cluster relative to the dense phase is at least equal to its surface energy, and therefore of order $(\nu N)^{2/3}$, there is no association beyond the formation of neutral dimers. This may, however, change for strongly asymmetric net charge distributions.

Asymmetric net charge distributions occur when equal amounts of positive and negative monomers are copolymerized, but the reaction constants for the two types differ. Polyampholytes produced in the early stages of the reaction contain a surplus of, e.g., positively charged groups with the consequence that polymers formed toward the end of the reaction predominantly consist of the remaining negatively charged monomers. Here we consider again globally neutral bimodal distributions with $-\delta\tilde{f}_- \geq \delta\tilde{f}_+ \geq 0$. Charge neutrality determines the concentration ratio of the two types of molecules in the dense phase:

$$\frac{C_+}{C_{\mathrm{dense}}} = \frac{1}{1 - \delta\tilde{f}_+/\delta\tilde{f}_-} \qquad (3.9)$$

$$\frac{\mathrm{C}_-}{C_{\mathrm{dense}}} = \frac{1}{1 - \delta\tilde{f}_-/\delta\tilde{f}_+} \qquad (3.10)$$

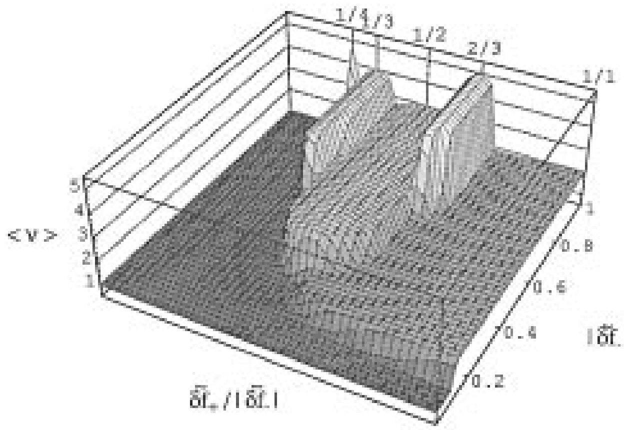

Figure 3. Average degree of association $\langle \nu \rangle$ in the dilute phase at coexistence for an asymmetric bimodal distribution with $\tilde N = 100$, $g_a = 20$, and different combinations of $0 < -\delta\tilde f_- < 1$ and $0 < \delta\tilde f_+/|\delta\tilde f_-| < 1$. Integral values correspond to the predominance of unimers, dimers, trimers, etc.

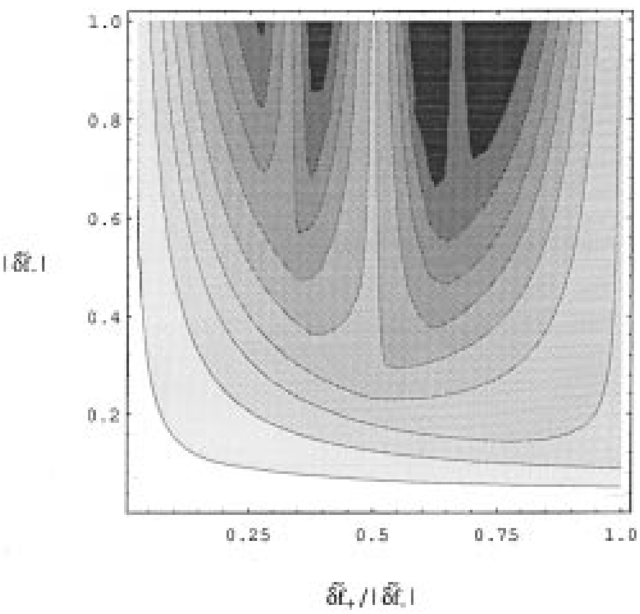

Figure 4. Total concentration of the dilute phase at coexistence for an asymmetric bimodal distribution with $\tilde N = 100$, $g_a = 20$, and different combinations of $0 < -\delta\tilde f_- < 1$ and $0 < \delta\tilde f_+/|\delta\tilde f_-| < 1$.

In fact, the same ratios also hold in the dilute phase for $c_\pm^{\text{dil}}/c_{\text{dil}}$. However, the actual polymer concentration, c_{dil}, and the nature of the formed clusters depend on the net charge densities of the polyampholyte molecules. To determine them, one has to calculate the electrostatic potential difference $\tilde\Psi$ between the dense and the dilute phase, which is implicitly determined by the condition

$$\sum_{\{\nu_{df_t}\}} \Delta\tilde f\, c^{\text{coex}}(\{\nu_{\delta\tilde f}\}) = 0 \qquad (3.11)$$

of charge neutrality. Equation 3.11 is difficult to evaluate in general, since it extends over all types of clusters.

We solved eqs 3.8 and 3.11 numerically for molecules with $\tilde N = 100$ and $g_a = 20$. Figures 3 and 4 show the variation of the average degree of association $\langle \nu \rangle$ and the total concentration c_{dil} of the dilute phase for different combinations of $0 < -\delta\tilde f_- < 1$ and $0 < \delta\tilde f_+/\delta\tilde f_- < 1$. The plateaus in Figure 3 at values of $\langle \nu \rangle = 1, 2, 3, \ldots$ correspond to unimer, dimer, trimer, etc. dominance. The clusters form at charge ratios $\delta\tilde f_+/|\delta\tilde f_-| \approx 1$ for dimers, $\delta\tilde f_+/|\delta\tilde f_-| \approx 1/2$ for trimers, etc.; i.e., charge commensurability plays an important role. In general, the solubility of the sample decreases for higher charges on the unimers. Note, however, that the solubility of *neutral* clusters is independent of the charge densities of their constituents. At intermediate charge ratios the concentration c_{dil} of the dilute phase drops by several orders of magnitude. In general, larger clusters are disfavored due to their surface energy and the reduced gain in translational entropy in the dilute phase. For this reason, they dominate the supernatant only, if the electrostatic energy of any two fragments is too high. The arguments are the same as in the preceding section, where we found that unimers only form dimers if $\delta\tilde f^2 > 1/\tilde N$.

Unimers dominate not only for weakly charged polyampholytes with $\delta\tilde f^2 < 1/\tilde N$ but also for very asymmetric samples. Whenever the solution is dominated by only two components, one can evaluate a solubility product and avoid the calculation of the potential difference $\tilde\Psi$. For unimers with different charge densities eq 3.3 takes the form

$$\frac{c_+^{|\delta\tilde f_-|}\, c_-^{\delta\tilde f_+}}{C_+^{|\delta\tilde f_-|}\, C_-^{\delta\tilde f_+}} = \exp(-|\delta\tilde f_-|F_{\text{ex}}(\delta\tilde f_+) - \delta\tilde f_+ F_{\text{ex}}(\delta\tilde f_-)) \qquad (3.12)$$

and with eqs 3.9 one obtains for the concentration of the supernatant

$$c_{\text{dil}} = C_{\text{dense}} \exp\left(-\frac{|\delta\tilde f_-|F_{\text{ex}}(\delta\tilde f_+) + \delta\tilde f_+ F_{\text{ex}}(\delta\tilde f_-)}{|\delta\tilde f_-| + \delta\tilde f_+}\right) \qquad (3.13)$$

For weakly charged chains in the spherical globule regime the solubility corresponds to that of a symmetrical sample with $\delta\tilde f^2 = \delta\tilde f_+|\delta\tilde f_-|$.

The case of an almost symmetrical sample where the unimers like to form dimers ($\delta\tilde f_\pm{}^2 > 1/\tilde N$) can be treated likewise. The slight negative excess charge of dimers in solution is compensated for by a corresponding amount of positively charged unimers. The solubility product and the polymer concentration in the supernatant are now given by

$$\frac{c_+^{|\delta\tilde f_+ + \delta\tilde f_-|}\, c_{+,-}^{\delta\tilde f_+}}{C_+^{|\delta\tilde f_-|}\, C_-^{\delta\tilde f_+}} \left(\frac{\tilde N}{2b^3}\right)^{\delta\tilde f_+} = \exp\left(-\left|\delta\tilde f_+ + \delta\tilde f_-\right| F_{\text{ex}}(\delta\tilde f_+) - \delta\tilde f_+ F_{\text{ex}}\left(\frac{1}{2}(\delta\tilde f_+ + \delta\tilde f_-), 2\tilde N\right)\right) \qquad (3.14)$$

$$c_{\text{dil}} = C_{\text{dense}} \left(\frac{\tilde N}{b^3 C_{\text{dense}}}\right)^{\delta\tilde f_+/\delta\tilde f_-} \frac{|\delta\tilde f_-|(\delta\tilde f_+ - \delta\tilde f_-)^{\delta\tilde f_+/\delta\tilde f_-}}{|\delta\tilde f_+ + \delta\tilde f_-|^{1+\delta\tilde f_+/\delta\tilde f_-}} \times \left(-\frac{\delta\tilde f_+ + \delta\tilde f_-}{\delta\tilde f_-} F_{\text{ex}}(\delta\tilde f_+) + \frac{\delta\tilde f_+}{\delta\tilde f_-} F_{\text{ex}}\left(\frac{1}{2}(\delta\tilde f_+ + \delta\tilde f_-), 2\tilde N\right)\right) \qquad (3.15)$$

Equations 3.13 and 3.15 faithfully reproduce the numerical results in the regions of unimer and dimer dominance, respectively. A similar analysis can be performed for the plateaus corresponding to higher order clusters. However, since the values for c_{dil} easily become astronomically low, there seems little point in elaborating on these effects. The main conclusion is that higher order complexes may play a role for asym-

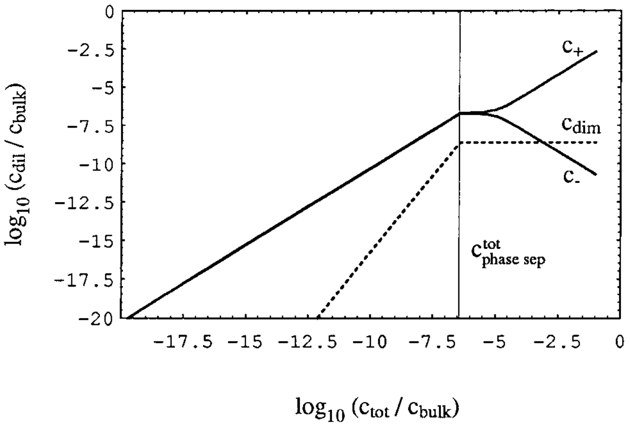

Figure 5. Composition of the dilute phase versus total concentration for asymmetric ($\epsilon = 0.01$) bimodal distributions of chains with $\tilde{N} = 20$, $g_a = 10$, and net charge $\delta\tilde{f}\tilde{N}^{1/2} = 1$.

metric net charge distributions without, however, enhancing the solubility of a globally neutral polyampholyte sample.

3.3. Non-neutral Charge Distributions: Symmetric Charges. We now discuss non-neutral samples containing free counterions that cannot be removed by dialysis. We assume that there are so few counterions that they modify the properties neither of the dense phase nor of the dissolved globules. These points are further addressed in the Appendix. In this section we return to the example of section 3.1, a bimodal sample with net charges of equal magnitude on all chains, but a slightly higher proportion of positively charged polyampholytes ($\epsilon > 0$). In the following section, we consider a mixture of neutral and charged chains.

Qualitatively, the association equilibrium in the dilute phase expressed via the law of mass action (eq 3.1) is not affected by the presence of the counterions. The behavior of a phase-separated sample is, however, fundamentally different. In a three- or more-component system the composition of the phases is not fixed by a phase rule at phase equilibrium. In the present case, for example, the unimer concentrations are not limited individually but are limited by a solubility *product* [22] (eq 3.3) and become dependent on the *total* concentration.

We first discuss the limit $\sqrt{L_P} < 1/K_A$, where dimers may be neglected. Before the onset of phase separation there are no significant changes compared to the $\epsilon = 0$ case (Figure 5): $c_+ - c_- = 2\epsilon c_{tot}$ and the changes in c_{dim} and $c^{tot}_{phase\ sep}$ are of order ϵ^2. At coexistence, however, the unimer concentrations are limited by the solubility product eq 3.3. For large c_{tot} the volume fraction of the precipitate is $\Phi = (1 - 2\epsilon)c_{tot}/C_{dense}$, so that the unimer concentration in the supernatant grows like $c_+ = c_{+,tot} - C_{dense}\Phi/2 = 2\epsilon c_{tot}$, while $c_- = L_P/c_+ \rightarrow 0$.

In the opposite limit, where the unimers are elongated globules, the solution is dominated by dimers for $K_A c_{tot} > 1$ (Figure 6). For $K_A c_{tot} > 1/4\epsilon^2$ one finds $c_{dim} = (1 - 2\epsilon)c_{tot}$ and $c_+ = c_{+,tot} - c_{dim}/2 = 2\epsilon c_{tot}$. The latter results also hold at coexistence, but now the dimers start to precipitate and their concentration in the supernatant is independent of c_{tot}. The concentration of the minority unimers can be calculated from the law of mass action, $c_- = c_{dim}/c_+K_A$. Before phase separation, $c_- = (1/2\epsilon - 1)/K_A$, i.e., independent of c_{tot}, and at coexistence, $c_- = L_P/2\epsilon c_{tot} \rightarrow 0$.

In both cases the composition and concentration of the supernatant is no longer fixed with the onset of phase separation. Rather, the "impurities", i.e., the additional polyampholyte molecules with their counterions, accumulate in the dilute phase and dominate its properties for high enough total concentrations. For net charges of $\delta\tilde{f}\tilde{N}^{1/2} \gg 1$ on the polyampholyte chains there are three different regimes: (i) for $c_{tot} < 1/K_A$ the dilute phase predominantly consists of unimers of both charges which form elongated globules, (ii) around the onset of phase separation for $1/K_A < c_{tot} < K_A L_P/2\epsilon$ one finds neutral dimers which form spherical globules, and (iii) finally, at even higher concentrations the supernatant again contains mostly elongated globules, but this time only the majority unimers.

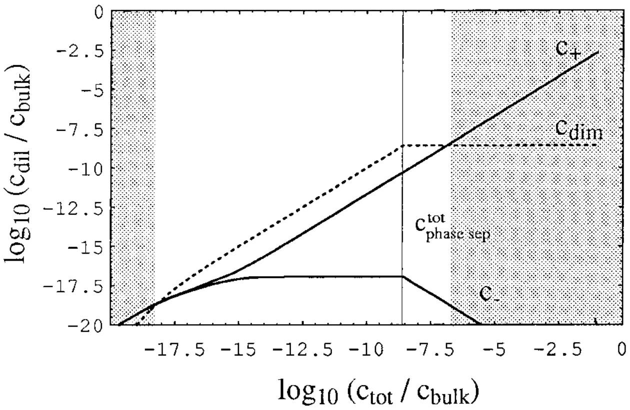

Figure 6. Composition of the dilute phase versus total concentration for asymmetric ($\epsilon = 0.01$) bimodal distributions of chains with $\tilde{N} = 20$, $g_a = 10$, and net charge $\delta\tilde{f}\tilde{N}^{1/2} = 3$. The gray shaded areas indicate concentrations for which the dilute phase is dominated by elongated globules.

Of course, the concentration of the supernatant cannot increase indefinitely as the extra chains can precipitate together with their counterions. The respective concentrations are related by a solubility product

$$\frac{c_+(c_+ - c_-)^{N\delta f}}{C_+(C_+ - C_-)^{N\delta f}}\, e^{-F_{ex}(\delta\tilde{f},\tilde{N})} \qquad (3.16)$$

where we have used the fact that $c_i = \delta f(c_+ - c_-)$, $C_i = \delta f(C_+ - C_-)$, and the properties of the precipitate remain unaffected for small enough ϵ. Asymptotically, $c_+ - c_- \approx c_+$, $C_+ = (1/2 + \epsilon)C_{dense}$, and $C_- = (1/2 - \epsilon)C_{dense}$ so that

$$c_+^{max} = 2\epsilon C_{dense}\, e^{-F_{ex}/(N\delta f+1)} \qquad (3.17)$$

Only for $c_{tot} > C_{dense} \exp(-F_{ex}/(N\delta f + 1))$ does the composition of the supernatant become independent of the total concentration. The effect is quite dramatic. For $\delta fN = 1$, i.e., a single counterion per chain, F_{ex} is effectively reduced by a factor of 2 and c_+^{max} corresponds to the c_+^{coex} of neutral chains which are 3 times shorter. For larger $\delta\tilde{f}_0$, c_+^{max} becomes independent of chain length and reaches a maximum of order C_{dense} for chains at the crossover between the elongated globule and the polyelectrolyte regimes. Qualitatively, one would thus expect that for a sample that consists of polyampholyte chains with different charge densities those with the highest charge accumulate in the supernatant.

3.4. Non-neutral Charge Distributions: Impurities and Fractionation. In this section we consider a model sample consisting of neutral polyampholytes plus a small fraction ϵ of charged chains together with their counterions. We write the respective concentrations as $(1-\epsilon)c_{\rm tot}$ for the neutral chains, $\epsilon c_{\rm tot}$ for the charged chains, and $\delta f\epsilon c_{\rm tot}$ for the counterions. At infinite dilution the solution is dominated by the neutral chains. With increasing concentration, a point is reached where the neutral chains start to precipitate and the sample phase separates. We are interested in the composition of the supernatant, i.e., in the precipitation of the charged chains with the counterions into a large dense phase of neutral chains.

Since we have only two charged components, the counterion concentration is fixed by the neutrality constraint: $c_{\rm i} = \delta f c_{\delta\bar f}$ and $C_{\rm i} = \delta f C_{\delta\bar f}$. From the solubility product of charged chains and counterions $c_{\rm i}^{\delta fN}\, c_{\delta f} = C_{\rm i}^{\delta fN} C_{\delta f} \exp(-F_{\rm ex}(\delta\tilde f,\tilde N))$ one obtains the ratio $c_{\delta f}/C_{\delta f} = X_{\delta f} = \exp(-F_{\rm ex}(\delta\tilde f)/(N\delta f + 1))$ of the concentrations of the charged chains in the two phases. For the neutral chains, the usual relation $c_0/C_0 = X_0 = \exp(-F_{\rm ex}(0))$ holds. In a phase separated system, the mass balances and the condition for phase equilibrium take the forms

$$(1-\epsilon)\,c_{\rm tot} = \Phi c_0\, X_0^{-1} + (1-\Phi)c_0 \tag{3.18}$$

$$\epsilon c_{\rm tot} = \Phi c_{\delta f}\, X_{\delta f}^{-1} + (1-\Phi)c_{\delta f} \tag{3.19}$$

$$C_{\rm dense} = c_0\, X_0^{-1} + c_{\delta f}\, X_{\delta f}^{-1} \tag{3.20}$$

The concentrations c_0 and $c_{\delta f}$ in the dilute phase are written in terms of ϵ, $c_{\rm tot}$, and Φ:

$$c_0 = \frac{(1-\epsilon)c_{\rm tot}}{1 + \Phi(X_0^{-1} - 1)} \tag{3.21}$$

$$c_{\delta f} = \frac{\epsilon c_{\rm tot}}{1 + \Phi(X_{\delta f}^{-1} - 1)} \tag{3.22}$$

and the condition eq 3.20 for phase equilibrium can be used to eliminate Φ:

$$\Phi = \frac{1}{2}\left(\frac{\epsilon\,\dfrac{c_{\rm tot}}{C_{\rm dense}} - {\rm X}_{\delta f}}{1 - X_{\delta f}} + \frac{(1-\epsilon)\,\dfrac{c_{\rm tot}}{C_{\rm dense}} - X_0}{1 - X_0}\right) + \left[\frac{1}{2}\left(\frac{\epsilon\,\dfrac{c_{\rm tot}}{C_{\rm dense}} - X_{\delta f}}{1 - X_{\delta f}} + \frac{(1-\epsilon)\,\dfrac{c_{\rm tot}}{C_{\rm dense}} - X_0}{1 - X_0}\right)^2 - 4\,\frac{X_0X_{\delta f} - \dfrac{c_{\rm tot}}{C_{\rm dense}}((1-\epsilon)X_{\delta f} + \epsilon\, X_0)}{(1 - X_{\delta f})(1 - X_0)}\right]^{1/2} \tag{3.23}$$

with $\Phi = 0$ at $C_{\rm dense}/c_{\rm tot} = (1-\epsilon)X_0^{-1} + \epsilon X_{\delta f}^{-1}$.

While Figure 7 shows a plot of the concentrations in the dilute phase obtained from these equations, the main features can be deduced from very simple arguments: At the onset of precipitation $\Phi = 0$ and $c_{\rm tot} \approx X_0C_{\rm dense}$, while in the opposite limit, when the system is almost dense, $\Phi = 1$ and $c_{\rm tot} \approx C_{\rm dense}$. Inserting these two limits into eqs 3.21 and 3.22 yields $c_0 = (1-\epsilon)X_0C_{\rm dense}$ independent of the total concentration, while $c_{\delta f}$ can be seen to increase from $\epsilon X_0C_{\rm dense}$ to $\epsilon X_{\delta f}C_{\rm dense}$. As a consequence, the composition $c_{\delta f}/c_0$ of the supernatant varies between the sample average $\epsilon/1-\epsilon$ and $(\epsilon/1-\epsilon)X_{\delta f}X_0^{-1}$, while its concentration $c_{\rm dil} = c_{\delta f} + c_0$ increases from $c_{\rm dil} \approx X_0C_{\rm dense}$ to $c_{\rm dil}/C_{\rm dense} = (1-\epsilon)X_0 + \epsilon X_{\delta f}$. Since we showed in the preceding section that, due to the counterion translational entropy, $X_{\delta f}$ can be orders of magnitude larger than X_0, the effect is quite drastic.

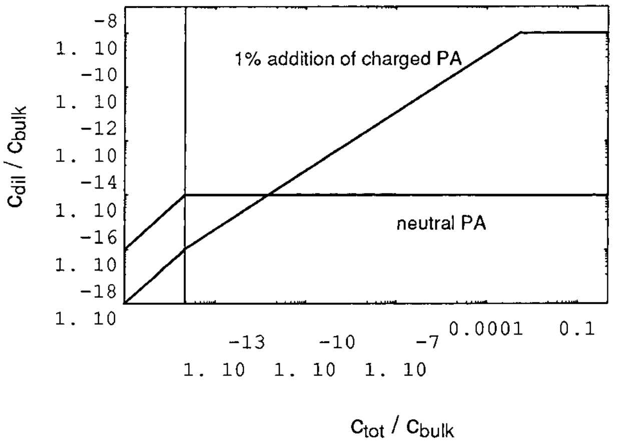

Figure 7. Concentration of charged and neutral chains in the supernatant versus total concentration.

In order to estimate the composition of the dilute phase between these two limits, we replace the exact solution eq 3.23 for Φ by the expression $\Phi_0 = (c_{\rm tot}/C_{\rm dense} - X_0)/(1 - X_0) \approx c_{\rm tot}/C_{\rm dense}$ obtained for $\epsilon = 0$. This yields

$$c_0 \approx (1-\epsilon)X_0C_{\rm dense} \qquad c_{\delta f} \approx \frac{\epsilon c_{\rm tot}}{1 + \dfrac{c_{\rm tot}}{C_{\rm dense}X_{\delta f}}} \tag{3.24}$$

In agreement with Figure 7 this ansatz predicts that the concentration of the neutral chains remains constant after phase separation sets in, while the concentration of charged chains $c_{\delta f} = \epsilon c_{\rm tot}$ keeps growing until $c_{\rm tot} \approx X_{\delta f}C_{\rm dense}$, i.e., until they reach their solubility product with the counterions. At higher total concentration, $c_{\delta\bar f}$ saturates at a value $c_{\delta\bar f} = \epsilon X_{\delta f}C_{\rm dense}$. Thus, not only is the composition of the supernatant different from the composition of the sample, but it also changes significantly with the total concentration. In the present case, the supernatant is dominated by neutral chains only up to a total concentration of X_0/ϵ. Beyond that, most of the dissolved chains are charged.

4. Randomly Copolymerized Polyampholytes

A simple model for a continuous net charge density distribution are randomly copolymerized polyampholytes (I). We first discuss the symmetric case where charges of both types are included with equal probability so that the sample is globally neutral. The case of biased copolymerization, where the polyampholytes have an average net charge, is treated along similar lines in section 5.

We begin with the simplest, and as it turns out, also the most important concentration regime: a supernatant in equilibrium with a precipitate that is large enough to have the same composition as the sample. In section 4.1 we show that this regime is reached for total concentrations exceeding $C_{\rm dense}\exp(-\tilde N^{2/3})$ and that the supernatant contains practically no unimers outside the spherical globule regime. Sequential dimerization in extremely dilute solutions and the onset of precipitation are discussed in sections 4.2 and 4.3, respectively.

4.1. Composition of a Supernatant in Equilibrium with a Large Precipitate. In this section, we

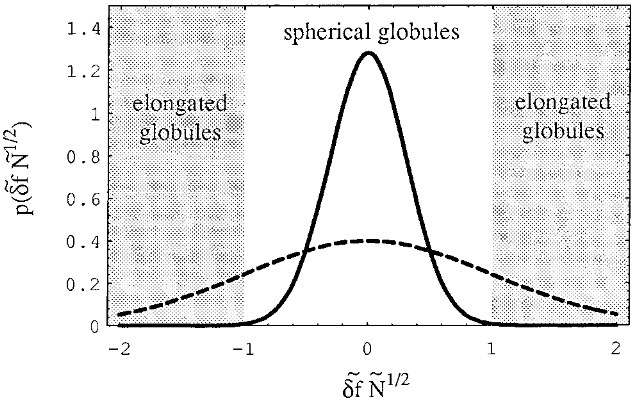

Figure 8. Probability distribution for the excess charge on the unimers: in the supernatant when most of the chains are precipitated (—); at infinite dilution (- - -).

assume that for a sample of randomly copolymerized polyampholytes the vast majority of the chains has precipitated into a dense phase. The composition of this dense phase is therefore $C(\delta\tilde{f}) = C_{\text{dense}}p(\delta\tilde{f})$ with the sample composition given by eq 2.3 for $\delta f_0 = 0$. In sections 4.1.1 and 4.1.2 we discuss the unimer and dimer content of the supernatant. We conclude this section by discussing the limit of validity of the assumption that the dense phase can be considered as infinite.

4.1.1. Unimers. Equating the chemical potentials of the polyampholytes in the dense phase (eq 2.13) to the unimer chemical potentials in the supernatant (eq 2.14) one obtains

$$c(\delta\tilde{f}) = \sqrt{\frac{\tilde{N}}{2\pi}}C_{\text{dense}} \times \exp\begin{pmatrix} -\dfrac{\delta\tilde{f}^{\,2}\tilde{N}}{2}(1 + 2\tilde{N}^{\,2/3}) - \tilde{N}^{\,2/3} & \text{if} \quad \delta\tilde{f}^{\,2} < 1/\tilde{N} \\ -\dfrac{\delta\tilde{f}^{\,2}\tilde{N}}{2} - 2\delta\tilde{f}^{\,2/3}\tilde{N} & \text{if} \quad \delta\tilde{f}^{\,2} < 1 \\ -\dfrac{\delta\tilde{f}^{\,2}\tilde{N}}{2} - 2\delta\tilde{f}^{\,4/3}\tilde{N} & \text{if} \quad \delta\tilde{f}^{\,2} > 1 \end{pmatrix} \tag{4.1}$$

The net charge density distribution for the dissolved unimers is therefore

$$p_{\text{uni}}(\delta\tilde{f}) \sim \left(-\frac{\delta\tilde{f}^{\,2}\tilde{N}}{2}(1 + 2\tilde{N}^{\,2/3})\right) \quad \delta\tilde{f}^{\,2} < 1/\tilde{N}$$

Compared to the dense phase (or to the case of infinite dilution), the net charge density distribution is $\tilde{N}^{1/3}$ times narrower (Figure 8); i.e., its width is $\tilde{N}^{-5/6}$ compared to $\tilde{N}^{-1/2}$. For large $\tilde{N}$ *there are practically no dissolved unimers outside the spherical globule regime.*

4.1.2. Dimers. Besides the unimers, the supernatant also contains polyampholyte clusters. For example, the concentration of a particular type of dimers is given by

$$c(\delta\tilde{f},\delta\tilde{f}\,') = C_{\text{dense}}\frac{2p(\delta\tilde{f})\,p(\delta\tilde{f}\,')}{\tilde{N}g_{\text{a}}^{\;3/2}} \times \exp\begin{pmatrix} -\left(\dfrac{\delta\tilde{f} + \delta\tilde{f}\,'}{2}\right)^2 (2\tilde{N})^{5/3} - (2\tilde{N})^{2/3} & \text{if} \quad \left(\dfrac{\delta\tilde{f} + \delta\tilde{f}\,'}{2}\right)^2 < 1/2\tilde{N} \\ -2\left(\dfrac{\delta\tilde{f} + \delta\tilde{f}\,'}{2}\right)^{2/3}(2\tilde{N}) & \text{if} \quad \left(\dfrac{\delta\tilde{f} + \delta\tilde{f}\,'}{2}\right)^2 < 1 \end{pmatrix} \tag{4.2}$$

The net charge distribution for the dimers can be obtained by fixing $\delta\tilde{f}\,' = 2\delta\tilde{f}_{\text{dim}} - \delta\tilde{f}$ and integrating out $\delta\tilde{f}$ in the previous expression

$$c_{\text{dim}}(\delta\tilde{f}_{\text{dim}}) = C_{\text{dense}}\sqrt{\frac{2}{\pi\tilde{N}g_{\text{a}}^{\;3}}} \times \exp\begin{pmatrix} -\dfrac{\delta\tilde{f}_{\text{dim}}^{\;2}(2\tilde{N})}{2}(1 + 2(2\tilde{N})^{2/3}) - (2\tilde{N})^{2/3} & \text{if} \quad \delta\tilde{f}_{\text{dim}}^{\;2} < 1/2\tilde{N} \\ -\dfrac{\delta\tilde{f}_{\text{dim}}^{\;2}(2\tilde{N})}{2} - 2\delta\tilde{f}_{\text{dim}}^{\;2/3}(2N) & \text{if} \quad \delta\tilde{f}_{\text{dim}}^{\;2} < 1 \end{pmatrix} \tag{4.3}$$

and has the same form as for the unimers. The actual concentrations are, however, much smaller due to the prefactor $\exp(-(2\tilde{N})^{2/3})$. The total concentration of polyampholytes with net charge $\delta\tilde{f}$ dissolved in the dilute phase as *part of a dimer* is thus given by

$$c_{\text{dim}}(\delta\tilde{f},*) = \frac{1}{2}\int \mathrm{d}\delta\tilde{f}\,'\; c(\delta\tilde{f},\delta\tilde{f}\,')$$

$$\approx C_{\text{dense}}\frac{2^{1/6}\sqrt{\pi}}{\tilde{N}^{11/6}g_{\text{a}}^{\;3/2}}\,p(\delta\tilde{f})\,p(-\delta\tilde{f})\,\mathrm{e}^{-(2\tilde{N})^{2/3}} \tag{4.4}$$

Comparing this to the unimer concentration

$$\frac{c_{\text{dim}}(\delta\tilde{f},*)}{c(\delta\tilde{f})} = \frac{2^{1/6}\sqrt{\pi}}{N^{11/6}g_{\text{a}}^{\;3/2}}\,p(-\delta\tilde{f})\,\mathrm{e}^{-(2\tilde{N})^{2/3} + F_{\text{ex}}(\delta\tilde{f},\tilde{N})} \tag{4.5}$$

we recover the result from section 3.1: for large $\tilde{N}$, polyampholyte molecules with a net charge density $\delta\tilde{f}$ larger than the critical value $\tilde{N}^{-1/2}$ are preferentially dissolved as part of neutral dimers. However, these aggregates play no role for the properties of the supernatant: according to eq 4.2 there are more dimers formed by pairs of neutral chains than by pairs of oppositely charged chains, and even the former are by a factor of the order of $\exp(-\tilde{N}^{2/3})$ less frequent than neutral unimers. Thus, the supernatant is dominated by unimers in the spherical globule regime. The determination of the total concentration of charged chains in the supernatant may nevertheless be of interest for experiments, where the supernatant is first separated from the precipitate and afterward diluted so strongly that the dimers split into (elongated) unimers.

4.1.3. Condition for a Quasi-Infinite Precipitate. Our assumption that we know the composition of the precipitate requires that for any charge $\delta\tilde{f}$, the amount of dissolved polymer in the dilute phase is much smaller than the amount in the dense phase. In the present case, the supernatant is dominated by unimers and the neutral chains have the highest solubility: $\max(X_{\delta\tilde{f}}) = X_0 \ll 1$. Depletion effects due to dimer formation can be neglected, since, according to eq 4.4, it is again the neutral chains that have the highest solubility as dimers but with $c_{\text{dim}}(0,*) \sim X_0^{\,2}$. For strictly symmetric random copolymerization the dense phase can therefore be regarded as infinite as soon as the total concentration exceeds $c_{\text{tot,min}} = C_{\text{dense}}\exp(-\tilde{N}^{2/3})$ (see section 2.4).

4.2. Sequential Dimerization. After having discussed the limit of total concentrations exceeding $c_{\text{tot,min}} = C_{\text{dense}}\exp(-\tilde{N}^{2/3})$ we now turn to even more dilute systems, where none or only a small portion of the material has precipitated. To calculate the composition of a solution of polyampholytes of different excess charge, one has to consider the formation of all types of dimers and higher order clusters. In the preceding section this task was relatively simple due to our assumption that a large dense phase of known composition acted as a reservoir for the chains and thereby fixed their chemical potentials. In contrast, in a very dilute system, one has to include explicitly the conservation of the total concentrations of the various species. We first consider the case of a homogeneous solution (prior to phase separation) where molecules can either be dissolved as unimers or dimers.[23] In principle, all the concentrations are coupled in the mass balances:

$$c_{\delta\tilde{f}} + \frac{1}{2}\int \mathrm{d}\delta\tilde{f}\,'\; c_{\delta\tilde{f}\delta\tilde{f}\,'} = p(\delta\tilde{f})c_{\mathrm{tot}} \tag{4.6}$$

Due to the chemical equilibrium between dimers and unimers their concentrations are related by the law of mass action:

$$\frac{c_{\delta\tilde{f}\delta\tilde{f}\,'}}{c_{\delta\tilde{f}}c_{\delta\tilde{f}\,'}} = \frac{2}{C_{\mathrm{dense}}\tilde{N}{g_{\mathrm{a}}}^{3/2}}\,\mathrm{e}^{F_{\mathrm{ex}}(\delta\tilde{f},\tilde{N})+F_{\mathrm{ex}}(\delta\tilde{f}\,',\tilde{N})-F_{\mathrm{ex}}((\delta\tilde{f}+\delta\tilde{f}\,')/2,2\tilde{N})} \tag{4.7}$$

Inserting eq 4.7 into eq 4.6 and evaluating the integral by the method of steepest decent show that neutral dimers are predominantly formed:

$$\frac{1}{2}\int \mathrm{d}\delta\tilde{f}\,'\; c_{\delta\tilde{f}\delta\tilde{f}\,'} = \frac{\sqrt{2^{1/6}\pi}}{\tilde{N}^{11/6}{g_{\mathrm{a}}}^{3/2}}\,\frac{c_{\delta\tilde{f}}\;c_{-\delta\tilde{f}}}{C_{\mathrm{dense}}}\,\mathrm{e}^{2F_{\mathrm{ex}}(\delta\tilde{f},\tilde{N})-(2\tilde{N})^{2/3}} \tag{4.8}$$

Thus, using this approximation, the concentrations of unimers with excess charge $\pm\delta\tilde{f}$ and of *all* dimers containing at least one of the chains are related by a law of mass action with association constant

$$K_{\delta\mathrm{f}} \equiv \frac{\frac{1}{2}\int \mathrm{d}\delta f\,'\; c_{\delta f\delta f\,'}}{c_{\delta f}c_{-\delta f}} \tag{4.9}$$

The mass balances eq 4.6 now couple only the concentrations of molecules with opposite net charge density $\pm\delta\tilde{f}$ and reduce to the same quadratic equations as in the case of bimodal distributions, i.e.

$$c_{\delta\tilde{f}} \overset{p(\delta f)=p(-\delta f)}{=} \frac{-1+\sqrt{1+4K_{\delta\tilde{\mathrm{f}}}\,p(\delta\tilde{f})\,c_{\mathrm{tot}}}}{2K_{\delta\tilde{\mathrm{f}}}} \tag{4.10}$$

The interpretation of these equations remains unchanged. At infinite dilution all molecules are dissolved as unimers. At finite concentrations, however, those for which $K_{\delta\tilde{f}}\,p(\delta\tilde{f})c_{\mathrm{tot}} \gg 1$ form dimers.

4.3. Sequential Precipitation. The composition of the dilute phase changes between the onset of phase separation and the limit where most of the polymer is precipitated. After phase separation has occurred, the following relations hold between the volume fraction Φ of the precipitate, the total monomer concentration c_{tot}, the polyampholyte concentrations in the precipitate $C(\delta\tilde{f})$, the unimer and dimer concentrations in the supernatant $\mathrm{c}(\delta\tilde{f})$ and $K_{\delta\tilde{\mathrm{f}}}\,c(\delta\tilde{f})^2$, and the ratio $X_{\delta f} = \mathrm{e}^{-F_{\mathrm{ex}}(\delta\tilde{f})}$ of the unimer concentrations in the dilute and the dense phase:

$$c_{\mathrm{tot}}\,p(\delta\tilde{f}) = \Phi C(\delta\tilde{f}) + (1-\Phi)(c(\delta\tilde{f}) + K_{\delta\tilde{f}}\,c(\delta\tilde{f})^2) \tag{4.11}$$

The unimer concentration $c(\delta\tilde{f})$ can be written in terms of $p(\delta\tilde{f})$, c_{tot}, and Φ:

$$c(\delta\tilde{f}) = \frac{1}{2K_{\delta\tilde{\mathrm{f}}}}\left(\frac{\Phi}{\Phi(1-\Phi)X_{\delta\tilde{f}}} + 1\right)\left(-1 + \sqrt{1 + 4\,\frac{p(\delta\tilde{f})\,c_{\mathrm{tot}}\,K_{\delta\tilde{f}}\,(1-\Phi)}{(\Phi {X_{\delta\tilde{f}}}^{-1} + (1-\Phi))^2}}\right) \tag{4.12}$$

The relation between the parameter Φ and the total concentration c_{tot} follows from the constraint that the individual concentrations in the dense phase have to add up to C_{dense}. It can either be expressed directly in terms of the concentrations in the dense phase or indirectly via eq 4.11 in terms of the concentrations in the supernatant:

$$\int \mathrm{d}\delta\tilde{f}\;C(\delta\tilde{f}) = C_{\mathrm{dense}} \tag{4.13}$$

$$\frac{c_{\mathrm{tot}}}{\Phi} - \frac{1-\Phi}{\Phi}\int \mathrm{d}\delta\tilde{f}\;c(\delta\tilde{f}) + K_{\delta\tilde{f}}\,c(\delta\tilde{f})^2 = C_{\mathrm{dense}} \tag{4.14}$$

For $1 \gg \Phi \gg X_0 = \exp(-\tilde{N}^{2/3})$ we recover the case treated in section 4.1: eq 4.12 simplifies to $C(\delta\tilde{f}) = p(\delta\tilde{f})c_{\mathrm{tot}}/\Phi$, since $K_{\delta\tilde{f}}X_{\delta\tilde{f}}{}^2/\Phi^2 \sim \exp(-(2\tilde{N})^{2/3})/\Phi^2 \ll 1$ and the condition for phase equilibrium, eq 4.13, yields $\Phi = c_{\mathrm{tot}}/C_{\mathrm{dense}}$.

The total concentration where phase separation sets in can be obtained by setting $\Phi = 0$ in eq 4.12. At this point, the unimer concentrations in the dilute phase are

$$c(\delta\tilde{f}) = \frac{1}{2K_{\delta\tilde{f}}}\,(-1 + \sqrt{1 + 4p(\delta\tilde{f})\;c_{\mathrm{tot}}^{\mathrm{phase\ sep}}K_{\delta\tilde{f}}}) \tag{4.15}$$

Weakly charged polyampholytes are predominantly dissolved as unimers and, accordingly, have a concentration of

$$c(\delta\tilde{f}) = p(\delta\tilde{f})\;c_{\mathrm{tot}}^{\mathrm{phase\ sep}} \qquad p(\delta\tilde{f})\;c_{\mathrm{tot}}^{\mathrm{phase\ sep}}K_{\delta\tilde{f}} \ll 1$$

Highly charged polyampholytes are predominantly dissolved as dimers, and the unimer concentration are strongly reduced:

$$c(\delta\tilde{f}) = \sqrt{p(\delta\tilde{f})\;c_{\mathrm{tot}}^{\mathrm{phase\ sep}}\;{K_{\delta\tilde{f}}}^{-1}} \qquad p(\delta\tilde{f})\;c_{\mathrm{tot}}^{\mathrm{phase\ sep}}K_{\delta\tilde{f}} \gg 1$$

The total concentration, $c_{\mathrm{tot}}^{\mathrm{phase\ sep}}$, at which phase separation sets in, is determined by the condition eq 4.13 for the polymer density in the dense phase. We calculate the individual polyampholyte concentrations in the dense phase from the *unimer* concentrations in the dilute phase, $C(_{\delta\tilde{f}}) = c(_{\delta\tilde{f}})X_{\delta\tilde{f}}{}^{-1}$, and approximate eq 4.15 by the two limiting expressions; i.e., we assume that molecules with a lower (higher) net charge density than a certain $\delta\tilde{f}^*$ are exclusively dissolved as unimers (dimers) and calculate the two contributions to the density of the precipitate separately. Rather than characterizing the onset of phase separation by the total concentration $c_{\mathrm{tot}} = (p(\delta\tilde{f}^*)K_{\delta\tilde{f}}{}^*)^{-1}$ we use the parameter $\delta\tilde{f}^*$. The contribution of precipitated unimers to the dense phase density, $C(\delta\tilde{f}) = p(\delta\tilde{f})c_{\mathrm{tot}}X_{\delta\tilde{f}}{}^{-1}$, is monotonically increasing for $\delta\tilde{f} < 1$, while the contribution of precipitated dimers, $C(\delta\tilde{f}) = \sqrt{p(\delta\tilde{f})c_{\mathrm{tot}}{K_{\delta\tilde{f}}}^{-1}}\,X_{\delta\tilde{f}}{}^{-1} \sim \sqrt{p(\delta\tilde{f})}$, is largest at $\delta\tilde{f} = 0$. The polyampholyte concentration in the dense phase has a sharp maximum at $\delta\tilde{f}^*$ and the two approximations match at this point. As a consequence, the precipitate is dominated by chains with a net charge density around $\delta\tilde{f}^*$ contributing, with logarithmic accuracy, $C(\delta\tilde{f}^*) = K_{\delta\tilde{f}}^{-1}X_{\delta\tilde{f}}^{-1}$ to the polymer concentration. Up to prefactors, eq 4.13 therefore reads $K_{\delta\tilde{f}}^{-1}X_{\delta\tilde{f}}^{-1} = C_{\mathrm{dense}}$ or $F_{\mathrm{ex}}(\delta\tilde{f}^*) \approx (2\tilde{N})^{2/3}$. The result $\delta\tilde{f}^* \sim \tilde{N}^{-1/2}$ implies that phase separation occurs for $c_{\mathrm{tot}}^{\mathrm{phase\ sep}} \sim \exp(-2\tilde{N}^{2/3})$ with the precipitate mainly consisting of chains with net charges around the crossover to the elongated globule regime. Note that neglecting dimer formation leads to a qualitatively different result. Phase separation is then predicted to start with chains in the polyelectrolyte regime with a net charge of $\delta\tilde{f} = ({}^{8}\!/_{3})^{3/2}$ and to occur at much lower concentrations of the order of $\exp(-\tilde{N})C_{\mathrm{dense}}$.

5. Biased Random Copolymerization

We now discuss to the most general case of a polyampholyte sample with a continuous net charge distribution with non-vanishing mean. As a concrete example, we consider samples synthesized by biased random copolymerization ((I) with $0 < \delta f_0$, i.e., a bias toward the inclusion of positively charged monomers into the chains with the consequence that the sample also contains negatively charged counterions), but the results should not sensitively depend on this choice. After a brief outline of the problem, we show in section 5.1 that only for exponentially small δf_0 is the asymptotic composition of the phases reached for finite volume fractions $1 - \Phi$ of the supernatant. As a consequence, most experimental systems should be in a crossover regime between the onset of precipitation and the asymptotic regime corresponding to $\Phi = 1$, which is investigated in section 5.2. Before we discuss the consequences for the solubility of a sample of randomly copolymerized polyampholytes in section 5.4, we briefly consider complexation before the onset of precipitation in section 5.3.

We have already shown in section 3 that counterions strongly influence the composition of the phases. Qualitatively, this is due to the fact that their behavior is largely governed by translational entropy, since their energy gain in precipitating into the dense phase is minute (of the order F_{dense}/fN) and negligible compared to the polymer free energies. The counterions have therefore the tendency to remain in the dilute phase. In contrast to the simple cases discussed before, the system now contains more than one type of charged chains. The main question to be addressed is which chains precipitate and which accumulate together with the counterions in the supernatant to ensure electroneutrality.

Formally, the electroneutrality constraint leads to an electrostatic potential difference $\Psi = \tilde{\Psi}/\sqrt{fg_{\mathrm{a}}}$ (eq 2.14), which modifies the concentration ratios between the phases:

$$X_{\delta\tilde{f}} \equiv \frac{c(\delta\tilde{f})}{C(\delta\tilde{f})} = \mathrm{e}^{-F_{\mathrm{ex}}(\delta\tilde{f})+\delta\tilde{f}\tilde{N}\tilde{\Psi}} \tag{5.1}$$

$$X_{\mathrm{i}} \equiv \frac{c_{\mathrm{i}}}{C_{\mathrm{i}}} = \mathrm{e}^{F_{\mathrm{dense}}^{\mathrm{ion}}-\Psi} \approx 1 \tag{5.2}$$

In addition, the concentrations are coupled by mass balances eqs 2.8 and 2.9. Dimers and higher order aggregates can be neglected, because (i) beyond the onset of phase separation practically all unimers in the supernatant have net charges of equal sign and (ii) for $c_{\mathrm{tot}} \gg X_0 C_{\mathrm{dense}}$ the dissolution of predominately neutral aggregates (see section 5.3) cannot significantly reduce the concentrations in the dense phase. In this case

$$C(\delta f) = \frac{p(\delta\tilde{f})\ c_{\mathrm{tot}}}{\Phi + (1-\Phi)X_{\delta\tilde{f}}} \tag{5.3}$$

$$c(\delta f) = \frac{p(\delta\tilde{f})\ c_{\mathrm{tot}}}{\Phi X_{\delta\tilde{f}}^{-1} + (1-\Phi)} \tag{5.4}$$

$$C_{\mathrm{i}} = \frac{\delta f_0\ c_{\mathrm{tot}}}{\Phi + (1-\Phi)X_{\mathrm{i}}} \approx \delta f_0 c_{\mathrm{tot}} \tag{5.5}$$

$$c_{\mathrm{i}} = \frac{\delta f_0\ c_{\mathrm{tot}}}{\Phi X_{\mathrm{i}}^{-1} + (1-\Phi)} \approx \delta f_0 c_{\mathrm{tot}} \tag{5.6}$$

Usually one can neglect the small difference between the ion concentrations in the precipitate and in the supernatant: $F_{\mathrm{dense}}^{\mathrm{ion}} = -(3/2^{11/3})(1/fg_{\mathrm{a}}) \ll 1$ and $\Psi < 1/fg_{\mathrm{a}} \ll 1$, since $\tilde{\Psi}$ is often of order one or smaller. In fact, Φ has to be larger than X_{i} before the ion concentration in the supernatant is appreciably reduced due to ion accumulation in the dense phase. We have checked that in the numerical examples in section 5.2 deviations occur only in the physically less interesting case of an almost dense system with Φ of order 1. Furthermore, these effects do not qualitatively change the behavior, but considerably complicate the discussion of an already quite intricate system. For the sake of simplicity we therefore assume in the following that $c_{\mathrm{i}} = \delta f_0 c_{\mathrm{tot}}$.

For the following discussion it is useful to introduce the polyampholyte monomer and charge densities in the supernatant:

$$c_{\mathrm{dil}} = \int \mathrm{d}\delta\tilde{f}\ c(\delta\tilde{f}) \tag{5.7}$$

$$c_{\mathrm{pc}} = \int \mathrm{d}\delta\tilde{f}\ \delta\tilde{f}\ c(\delta\tilde{f}) \tag{5.8}$$

For a given volume fraction Φ of the dense phase the potential difference Ψ can be calculated from the electroneutrality condition (eq 2.11), since both c_{pc} and c_{i} are proportional to c_{tot}:

$$c_{\mathrm{pc}} = \delta\tilde{f}_0\ c_{\mathrm{tot}} \tag{5.9}$$

In a second step, the volume fraction Φ of the dense phase can be related to the total concentration c_{tot} using the condition that the concentrations in the dense phase have to add up to C_{dense} (eqs 4.13 or 4.14).

5.1. Composition of a Supernatant in Equilibrium with an Infinite Dense Phase. As in section 4 we start with the case of a dense phase composition that is identical with the sample composition eq 2.3. This reduces the problem to finding the electrostatic potential difference $\tilde{\Psi}$, for which the polyampholyte charge density in the supernatant neutralizes the counterion density. Figure 9 illustrates the influence of $\tilde{\Psi}$ on the

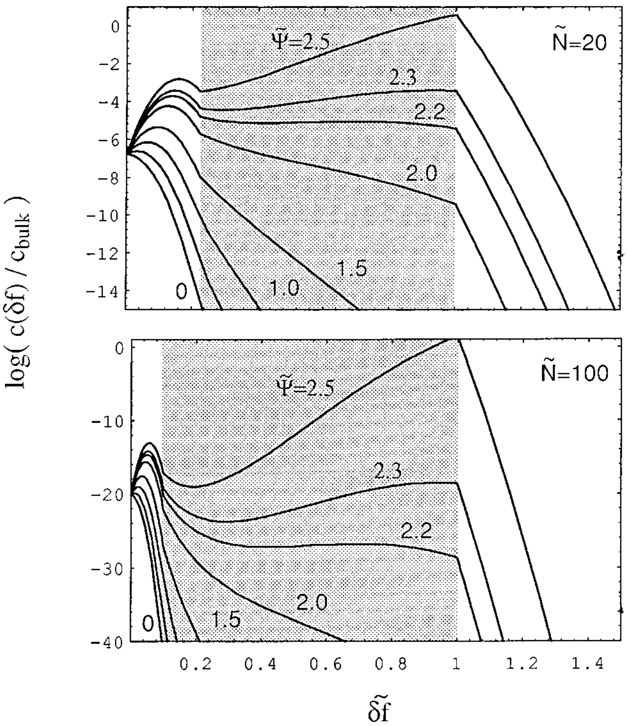

Figure 9. Concentration $c(\delta\tilde{f})$ of polyampholyte unimers in the supernatant (eq 5.1) imposing different electrostatic potentials $\tilde{\Psi}$ and a composition of the dense phase corresponding to a randomly copolymerized sample (eq 2.3 with $\delta\tilde{f}_0 = 0$). Shifting the composition to a nonzero $0 < \delta\tilde{f}_0 \ll 1/\sqrt{\tilde{N}}$ has negligible effects on the drawn curves. Note the different scales on the y-axis for chain lengths of $\tilde{N} = 20$ and $\tilde{N} = 100$, respectively. The gray shaded area indicates the elongated polyampholyte regime.

unimer concentrations in the supernatant. In the plot we used a symmetric Gaussian net charge distribution with $\delta\tilde{f}_0 = 0$. The deviations for small $0 < \delta\tilde{f}_0 \ll 1/\sqrt{\tilde{N}}$ are negligible. For all values of $\tilde{\Psi}$, where the supernatant is dominated by chains from a well-defined peak, one can approximate the polyampholyte (charge) density by the method of steepest decent. Up to prefactors, $c_{\rm pc} = \delta\tilde{f}_{\rm peak} c_{\rm dil}$ and $c_{\rm dil} = c(\delta\tilde{f}_{\rm peak})$. From the neutrality condition eq 5.9 one obtains the simple result

$$c_{\rm dil} = \frac{\delta\tilde{f}_0}{\delta\tilde{f}_{\rm peak}} C_{\rm dense} \tag{5.10}$$

Compared to the precipitate, the polyampholyte concentration in the supernatant is smaller by a factor of $\delta\tilde{f}_0/\delta\tilde{f}_{\rm peak}$. At the same time, the net charge density on the dissolved chains is higher by a factor of $\delta\tilde{f}_{\rm peak}/\delta\tilde{f}_0$. Both phases have therefore the comparable polyampholyte charge densities, which are required to neutralize the nearly homogeneous counterion charge density.

In general, an electrostatic potential difference $\tilde{\Psi} > 0$ leads to an increase of the concentrations of positively charged polyampholytes. The details of this process are, however, quite intricate, since the concentrations depend on both the gain in electrostatic potential energy $\delta\tilde{f}\tilde{\Psi}\tilde{N}$ and the free energy penalty $F_{\rm ex}(\delta\tilde{f},\tilde{N})$ for dissolved chains relative to the dense phase. Most importantly, the interplay between these two terms is different in the spherical and the elongated globule regimes.

The concentrations in the supernatant show a peak in the spherical polyampholyte regime, which dominates for values of $\tilde{\Psi}$ up to order 1 (see Figure 9). The height and the position of this peak increase slowly with the potential $\tilde{\Psi}$. However, the net charges on spherical globules can only neutralize an exponentially small counterion content. Using $\tilde{\Psi}_{\rm max} = 5/2$ (see below) one can estimate that $\delta\tilde{f}_0$ has to be smaller than $\exp(-\tilde{N}^{2/3} + {}^{25}/_{16}\tilde{N}^{1/3})$. As a consequence, elongated globules play an important role in samples containing counterions.

For values of $\tilde{\Psi}$ larger than ${}^7/_3$ a second maximum in the unimer concentrations $c_{\delta\tilde{f}}$ appears independent of $\tilde{N}$ at the crossover between the elongated polyampholyte and the polyelectrolyte regimes at $\delta\tilde{f}_{\rm peak} = 1$. This is due to the *sublinear* dependence of the excess free energy on the chain net charge density in the elongated globule regime (eq 2.2). The maximum therefore exists quite generally, although its particular shape and position are an artifact of our free energy function. Note that the solubility of elongated globules *increases* with their net charge for $1 > \delta\tilde{f} > ({}^3/_4\tilde{\Psi})^{-3}$ and that for values of the potential beyond $\tilde{\Psi} = 2$ the concentrations in the supernatant of chains with net charge density $1 > \delta\tilde{f} > ({}^1/_2\tilde{\Psi})^{-3}$ *exceed* those in the precipitate. In this range, small changes of $\tilde{\Psi}$ have an enormous effect on the concentrations in the dilute phase. While $\tilde{\Psi} = 1$ corresponds to an exponentially dilute supernatant dominated by spherical globules, the other extreme is reached for $\tilde{\Psi}_{\rm max} = {}^5/_2$, where $c(\delta\tilde{f} = 1) \approx C_{\rm dense}$. According to eq 5.10, the density of the supernatant for $\delta\tilde{f}_{\rm peak} = 1$ is $c_{\rm dil} = \delta\tilde{f}_0 C_{\rm dense}$ and therefore independent of $\tilde{N}$. Since $c_{\rm dil} \sim c(\delta\tilde{f}_{\rm peak}) = C_{\rm dense}\, p(\delta\tilde{f}_{\rm peak}) \exp(-F_{\rm ex}(\delta\tilde{f}_{\rm peak},\tilde{N}) + \delta\tilde{f}_{\rm peak}\tilde{N}\tilde{\Psi}) = C_{\rm dense} \exp((\tilde{\Psi} - {}^5/_2)\tilde{N})$ one finds for the electrostatic potential difference $\tilde{\Psi} = {}^5/_2 + (1/\tilde{N}) \log(\delta\tilde{f}_0)$. Finally, we compare the polyampholyte charge density in the supernatant due to the dissolution of chains from the two peaks. The crossover to the dominance of strongly elongated chains occurs for $\tilde{\Psi} = {}^5/_2 + {}^{25}/_{16}\tilde{N}^{-2/3} - \tilde{N}^{-1/3}$, corresponding to the exponentially small bias of $\delta\tilde{f}_0 \sim \exp(-\tilde{N}^{2/3} + \tilde{N}^{1/3}) \ll 1/\sqrt{\tilde{N}}$ we had already estimated in the preceding paragraph.

With respect to the applicability of the results to experiments, one has to realize that in a realistic sample the total concentration of chains with $\delta\tilde{f} = 1$ is of order $c_{\rm tot}\exp(-\tilde{N}/2)$. Consequently, the above considerations can only hold if these chains accumulate in an exponentially small volume fraction of the system. To be more specific, we have to evaluate the criterion eq 2.16. The assumption of an infinite dense phase is justified, if there is no depletion, i.e., if the number of molecules with the highest solubility in the supernatant is negligible compared to the dense phase. In the present case $(\max(X_{\delta\tilde{f}})) = X_1 = \exp((\tilde{\Psi} - 2)\tilde{N})$. Since $\tilde{\Psi} > {}^7/_3 > 2$, one needs $1 > c_{\rm tot}/C_{\rm dense} > 1 - \exp((2 - \tilde{\Psi})\tilde{N})$, corresponding to the expected exponentially small volume fractions of the supernatant. Nevertheless, the considerations illustrate the fact that the dissolution of highly charged globules offers the best compromise between the gain in counterion translational entropy and the increase in the polyampholyte free energy. In the following section we discuss the balance between this tendency to dissolve highly charged globules and the restrictions on their total number.

5.2. Selective Precipitation. We now consider precipitation for a sample with $\tilde{N}\delta\tilde{f}_0^2 \ll 1$ where the dense phase may be treated as infinite only for exponentially small volume fractions of the supernatant. We mostly restrict ourselves to the experimentally relevant concentration range $X_0 C_{\rm dense} \ll c_{\rm tot} \ll C_{\rm dense}$ where $\Phi \approx c_{\rm tot}/C_{\rm dense}$. In this limit eqs 5.3 and 5.4 for the polyam-

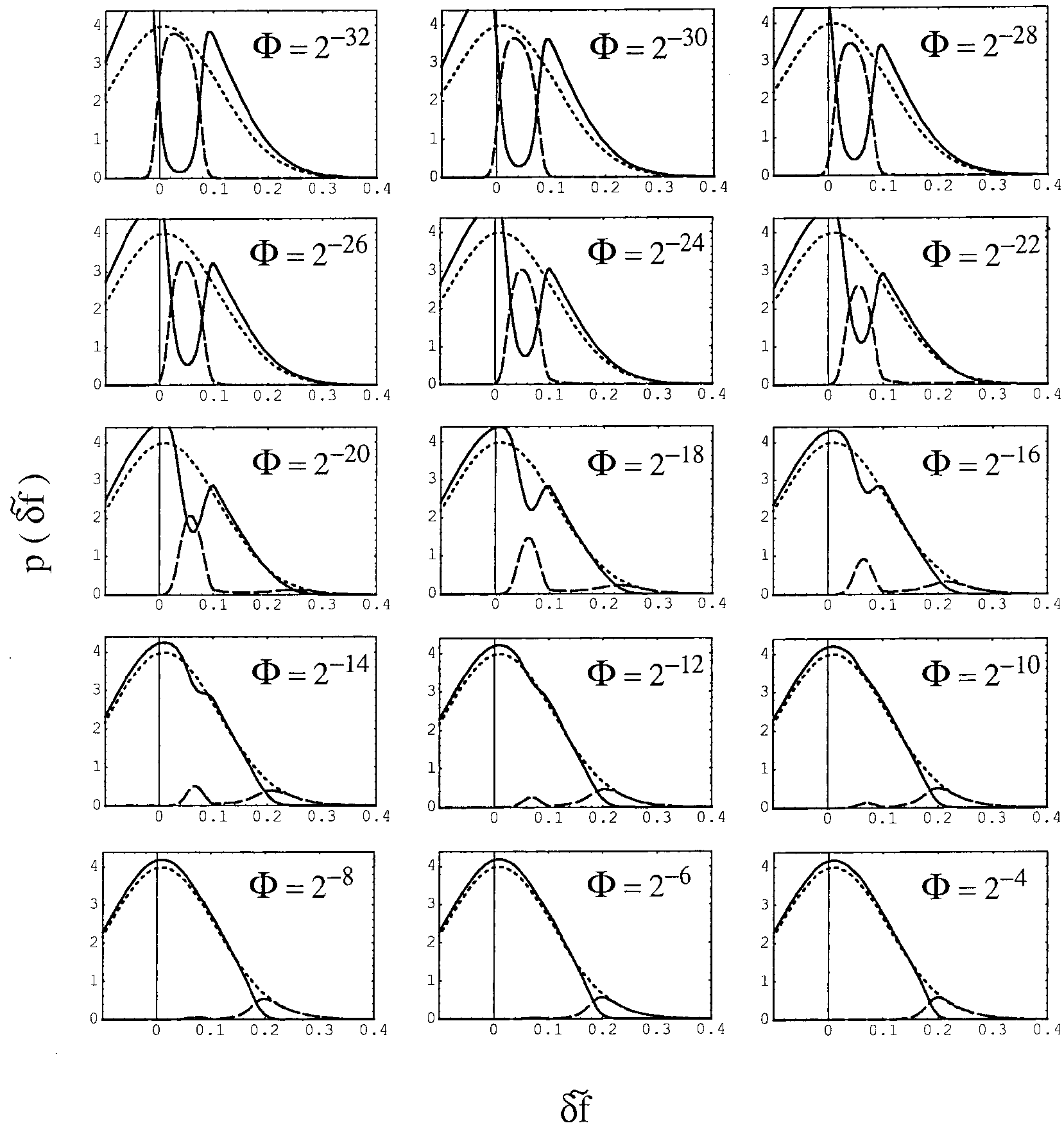

Figure 10. Composition of the phases for a sample with $\tilde{N} = 100$, $\delta\tilde{f}_0 = 0.01$, and volume fractions of the dense phase $e^{-\tilde{N}^{2/3}} \approx 2^{-32} \leq \Phi \approx c_{tot}/C_{dense} \leq 2^{-4}$ from a numerical solution of eqs 5.1−5.9 setting $c_i = \delta\tilde{f}_0 c_{tot}$. We show the sample composition $p(\delta\tilde{f})$ (- - -, eq 2.3), the composition of the dense phase $C(\delta\tilde{f})/C_{dense}$ (—, eq 5.3), and the polyampholyte concentrations $c(\delta\tilde{f})/c_{tot}$ in the supernatant (− − −, 5.4).

pholyte concentrations can be simplified to

$$C(\delta\tilde{f}) \overset{X_0 \ll \Phi \ll 1}{=} \frac{p(\delta\tilde{f})}{{C_{dense}}^{-1} + {X_{\delta\tilde{f}} c_{tot}}^{-1}} = \begin{cases} p(\delta\tilde{f})\, {X_{\delta\tilde{f}}}^{-1}\, c_{tot} & \text{if } c_{tot} \ll X_{\delta\tilde{f}}\, C_{dense} \\ p(\delta\tilde{f})\, C_{dense} & \text{if } c_{tot} \gg X_{\delta\tilde{f}}\, C_{dense} \end{cases} \quad (5.11)$$

$$c(\delta f) \overset{X_0 \ll \Phi \ll 1}{=} \frac{p(\delta\tilde{f})}{{C_{dense}}^{-1}\, {X_{\delta\tilde{f}}}^{-1} + {c_{tot}}^{-1}} = \begin{cases} p(\delta\tilde{f})\, c_{tot} & \text{if } c_{tot} \ll X_{\delta\tilde{f}}\, C_{dense} \\ p(\delta\tilde{f})\, X_{\delta\tilde{f}}\, C_{dense} & \text{if } c_{tot} \gg X_{\delta\tilde{f}}\, C_{dense} \end{cases} \quad (5.12)$$

showing that chains start to precipitate as soon as $c_{tot} \approx X_{\delta\tilde{f}} C_{dense}$. Using this argument, one has, of course, to keep in mind that $\tilde{\Psi}$ and therefore the $X_{\delta\tilde{f}}$ depend on the total concentration c_{tot}.

In the following we study the phase equilibrium numerically for several cases. We also employ a simple approximation where chains with a particular net charge density $\delta\tilde{f}$ are assumed to be either completely precipitated or dissolved depending on the above criterion. This ansatz has the advantage that the monomer concentration c_{dil} and the charge concentration c_{pc} can be expressed in terms of simple functions and allows us to rationalize the numerical results.

To illustrate the general behavior, we have solved eq 5.9 numerically for $\tilde{N} = 100$, $\delta\tilde{f}_0 = 0.01$, and total concentrations c_{tot} that vary over 10 orders of magnitude. Figures 10−12 show how the composition of the phases, the electrostatic potential difference between them, and the concentration of the dilute phase depend on the total concentration. We find the following:

(i) At low concentrations with precipitate volume fractions around $\Phi \approx X_0$ the situation is very similar to the unbiased case with $\delta\tilde{f}_0 = 0$ treated in section 4: the supernatant is composed of spherical globules, while most of the chains with higher net charges are precipitated. The difference is that now on average the spherical globules in the supernatant are charged. Close to the onset of phase separation the composition of the phases can be approximated by saying that all chains with net charge density $\delta\tilde{f} < 0$ and $\delta\tilde{f} > \delta\tilde{f}^*$ are precipitated, while the dilute phase consists of those chains with $0 < \delta\tilde{f} < \delta\tilde{f}^*$, i.e. $c(\delta\tilde{f}) = c_{tot} p(\delta\tilde{f})\ \Theta(\delta\tilde{f})$

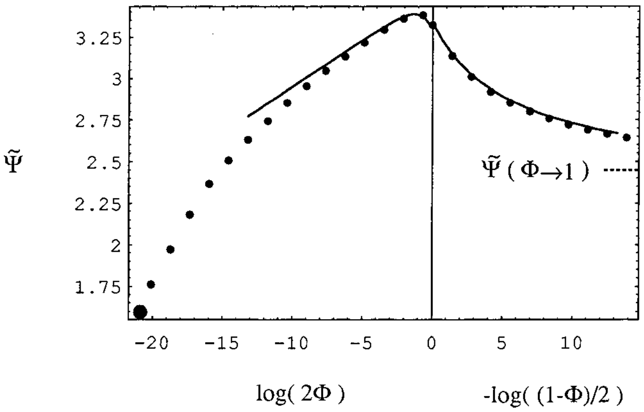

Figure 11. Electrostatic potential difference between the phases for the same system as in Figure 10. The small dots in the left half of the figure correspond to the plots in Figure 10, while the large dot and the solid line indicate our approximations for $\Phi = e^{-\tilde N^{2/3}}$ and for large Φ.

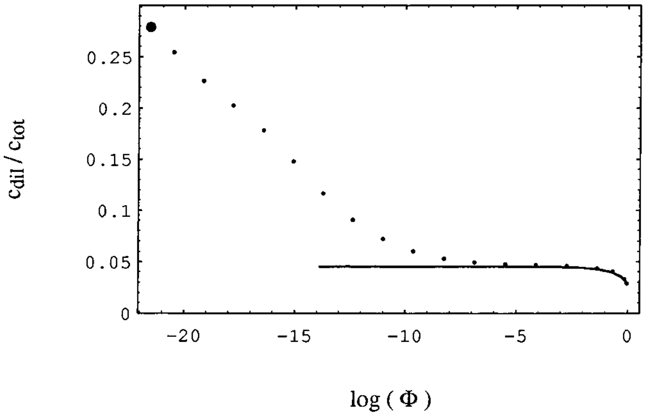

Figure 12. Ratio of the supernatant and the total concentration for the same system as in Figure 11. The small dots correspond to the plots in Figure 10, while the large dot and the solid line indicate our approximations for $\Phi = e^{-\tilde N^{2/3}}$ and for large Φ.

$\Theta(\delta\tilde f^* - \delta\tilde f)$. To be specific, we have chosen $\Phi = X_0 \ll 1$. (For a neutral sample with $\delta\tilde f = 0$ both phases contain at this point roughly equal amounts of polyampholyte.) For $\delta\tilde f_0, \delta\tilde f^* \ll 1/\sqrt{\tilde N}$ the condition of charge neutrality eq 5.9 requires $\delta\tilde f^* = \sqrt{\delta\tilde f_0}(8\pi/\tilde N)^{1/4}$ and

$$c_{\mathrm{dil}}/c_{\mathrm{tot}} = \left(\frac{\tilde N}{2\pi}\delta\tilde f_0^{\,2}\right)^{1/4} \qquad (5.13)$$

The potential difference $\tilde\Psi$ follows from the condition $X_{\delta\tilde f^*} = \Phi/(1 - \Phi)$ that both phases contain equal amounts of the chains with $\delta\tilde f^*$: $\tilde\Psi = \delta\tilde f^* \tilde N^{2/3}$.

(ii) With increasing concentration and in spite of a growing potential difference between the phases, c_{tot} exceeds $X_{\delta\tilde f}C_{\mathrm{dense}}$ for the spherical globules, i.e. these chains precipitate and their concentration in the supernatant becomes independent of the total concentration. As a consequence, their contribution to the polyampholyte charge density in the supernatant can no longer balance the counterion charge density $\delta\tilde f_0 c_{\mathrm{tot}}$. (Otherwise, one would reach the asymptotic compositions discussed in the previous section.)

(iii) For $\tilde\Psi$ of order 1 the most strongly charged chains redissolve as $X_{\delta\tilde f}$ grows faster than c_{tot} and finally exceeds $c_{\mathrm{tot}}/C_{\mathrm{dense}}$—initially for chains at the crossover between the elongated polyampholyte and the polyelectrolyte regimes, for larger $\tilde\Psi$ also for chains with progressively smaller $\delta\tilde f$. The potential difference $\tilde\Psi$ adapts itself to such a value that the charge density in the tail of the distribution cancels the counterion charges. To approximate this behavior, we assume that *all* chains with a net charge density larger than a certain $\delta\tilde f^*$ are in the dilute phase, while all other chains are precipitated. The polyampholyte concentrations in the supernatant are then given by $c(\delta\tilde f) = c_{\mathrm{tot}}/(1 - \Phi)\, p(\delta\tilde f)\, \Theta(\delta\tilde f - \delta\tilde f^*)$. For small $\delta\tilde f_0$ eq 5.9 yields $\sqrt{\tilde N/2}(\delta\tilde f^* = \delta\tilde f_0) = -\sqrt{\log(\sqrt{2\pi\tilde N}(1 - \Phi)\delta\tilde f_0}$). As long as $\Phi \ll 1$ the supernatant has a concentration

$$c_{\mathrm{dil}} = \frac{c_{\mathrm{tot}}}{1 - \Phi}\frac{1}{2}\mathrm{Erfc}(\sqrt{\tilde N/2}(\delta\tilde f^* - \delta\tilde f_0)) \qquad (5.14)$$

$$\approx c_{\mathrm{tot}}\frac{\sqrt{\tilde N/2}\,\delta\tilde f_0}{\sqrt{-\log(\sqrt{2\pi\tilde N}(1 - \Phi)\delta\tilde f_0)}} \qquad (5.15)$$

proportional to $\delta\tilde f_0$ and the total concentration c_{tot}. The composition of the supernatant is, however, only weakly dependent on c_{tot}. The potential difference $\tilde\Psi$ follows from the condition $X_{\delta\tilde f^*} = \Phi/(1 - \Phi)$ that both phases contain equal amounts of the chains with $\delta\tilde f^*$:

$$\tilde\Psi = \frac{\log\left(\frac{\Phi}{1 - \Phi}\right) + F_{\mathrm{ex}}(\delta\tilde f^*)}{\tilde N\delta\tilde f^*} \qquad (5.16)$$

(iv) For total concentrations of order of the concentration of the dense phase and net charges where $(1 - c_{\mathrm{tot}}/C_{\mathrm{dense}})X_{\delta\tilde f} \gg 1$, eq 5.4 for the concentrations in the supernatant reduces to $c(\delta\tilde f) = p(\delta\tilde f)c_{\mathrm{tot}}/(1 - c_{\mathrm{tot}}/C_{\mathrm{dense}})$. The relative increase occurs because chains with a total concentration $p(\delta\tilde f)c_{\mathrm{tot}}$ become confined to the small volume $(1 - c_{\mathrm{tot}}/C_{\mathrm{dense}})$ of the supernatant. Due to this "leverage", the potential difference can actually decrease toward the value calculated in the preceding section as smaller and smaller parts of the tail are sufficient to balance the counterion charges. These effects can also be understood in the framework of our simple approximation. For $\Phi \rightarrow 1$, $\delta\tilde f^*$ starts to grow. Due to the higher average charge on the dissolved chains, c_{dil} decreases. In eq 5.15 $c_{\mathrm{tot}} \approx C_{\mathrm{dense}}$ and $c_{\mathrm{dil}} \sim \delta\tilde f_0/\delta\tilde f^* C_{\mathrm{dense}}$. The aymptotic case of a quasi-infinite dense phase is reached for $\delta\tilde f^* = 1$. Inserting this into eqs 5.15 and 5.16 yields $c_{\mathrm{dil}} = \delta\tilde f_0 C_{\mathrm{dense}}$ and $\tilde\Psi = {}^5\!/_2 + (1/\tilde N)\log(\sqrt{2\pi\tilde N}\delta\tilde f_0)$ in agreement with what we found in the preceding section.

5.3. Complexation before the Onset of Precipitation.

In very dilute solutions with $c_{\mathrm{tot}} \ll \exp(-\tilde N^{2/3})$ charged polyampholyte molecules have a tendency to form neutral complexes. In section 3.1 we considered bimodal distributions of polyampholytes with opposite net charges $\pm\delta\tilde f$ of equal magnitude. We found that chains with charge densities $\delta\tilde f > \tilde N^{-1/2}$ have a tendency to form dimers at concentrations $c_{\mathrm{tot}} \sim K_{\delta\tilde f}^{\;-1}$ much lower than the onset of phase separation, where $K_{\delta\tilde f}$ is the association constant in the law of mass action eq 3.1. Beyond $K_{\delta\tilde f}^{\;-1}$ the dimer and unimer concentrations were found to converge to $\boldsymbol{c}_{\mathrm{dim}} = 2p(-\delta\tilde f)c_{\mathrm{tot}}$, $c_{\delta\tilde f} = \Delta p_{\delta\tilde f}c_{\mathrm{tot}} = (p(\delta\tilde f) - p(-\delta\tilde f))c_{\mathrm{tot}}$, and $c_{-\delta\tilde f} = 2p(-\delta\tilde f)/K_{\delta\tilde f}\Delta p_{\delta\tilde f}$, where we have assumed an excess of positively charged chains. In section 4.2 we showed that for a slowly varying distribution of polyampholyte net charges the dimer and unimer concentration were still related to each other by a law of mass action with an effective association constant eq 4.9. This result, and the predominance of neutral dimers, is not affected by a small asymmetry in eq 2.3. Since for a symmetric ensemble it is sufficient to consider dimers (section 4.2, one might hope that a small asymmetry $\delta\tilde{f}_0$ does not fundamentally change this result.

Unfortunately, the relative excess of positively charged chains $\Delta p_{\delta\tilde{f}}/p_{\delta\tilde{f}}$ approaches unity for $\delta\tilde{f} > \delta\tilde{f}_0^{-1}$ and is largest for highly charged chains which, in spite of their high excess free energy, are predicted to be dissolved as unimers. This suggests that the chains might instead prefer to form trimers by associating with two chains of half the opposite charge.[24] The law of mass action for these trimers[25] has the form

$$\frac{c_{\delta\tilde{f},-\delta\tilde{f}/2,-\delta\tilde{f}/2}}{c_{\delta\tilde{f}}c_{\delta\tilde{f}/2}^{\;2}} = L_{\delta\tilde{f}} \sim \mathrm{e}^{F_{\mathrm{ex}}(\delta\tilde{f})+2F_{\mathrm{ex}}(\delta\tilde{f}/2)-(3\tilde{N})^{2/3}} \qquad (5.17)$$

and the trimer formation competes with the formation of dimers from chains with charge $\delta\tilde{f}/2$ as well as $\delta\tilde{f}/2$. If we imagine concentrating the sample from infinite dilute, we can calculate threshold concentrations for the formation of these different complexes:

$$1 = \frac{c_{\delta\tilde{f},-\delta\tilde{f}}}{c_{\delta\tilde{f}}} \approx K_{\delta\tilde{f}}\, p(-\delta\tilde{f})\, c_{\mathrm{tot}} \qquad (5.18)$$

$$1 = \frac{c_{\delta\tilde{f},-\delta\tilde{f}/2,-\delta\tilde{f}/2}}{c_{\delta\tilde{f}}} \approx L_{\delta\tilde{f}}\, p(-\delta\tilde{f}/2)^2\, c_{\mathrm{tot}}^{\;2} \qquad (5.19)$$

Comparison shows that the dimerization occurs first and that the formation of trimers of the type $\{\delta\tilde{f}, -\delta\tilde{f}/2, -\delta\tilde{f}/2\}$ competes with that of dimers composed of chains with $\pm\delta\tilde{f}/2$. The problem becomes solvable if $\Delta p_{\delta\tilde{f}} \ll p(-\delta\tilde{f}/2)$, i.e., if the trimers do not deplete the $-\delta\tilde{f}/2$ unimers. At the onset of phase separation, the dilute phase would then, as in the symmetric case, consist of unimers with charges between $\pm\tilde{N}^{-1/2}$ with a concentration of $c_{\delta\tilde{f}} = p(\delta\tilde{f})c_{\mathrm{tot}}$, those with a negative charge between $-\tilde{N}^{-1/2}$ and $-{}^1\!/_2\tilde{N}^{-1/2}$ slightly depleted due to the formation of trimers. All polyampholytes with higher charges were dissolved in complexes with $c_{\delta\tilde{f},-\delta\tilde{f}} = 2p(-\delta\tilde{f})c_{\mathrm{tot}}$ and $c_{\delta\tilde{f},-\delta\tilde{f}/2,-\delta\tilde{f}/2} = \Delta p_{\delta\tilde{f}}c_{\mathrm{tot}}$. Unfortunately, this condition is not so obviously fulfilled, which will probably lead to the formation of higher order clusters, although it seems likely that dimers and trimers should dominate.

5.4. Solubility of Polyampholyte Samples. In the experiments of Candau et al.[13,14] solutions appeared perfectly transparent and homogeneous even though phase separation had, in fact, occurred. Only if the solutions were subjected to several hours of centrifugation, the precipitate—representing roughly half of the total polymer content—became visible. Thus, for practical purposes, exponentially small volume fractions Φ of the dense phase are of little consequence for the question of the solubility of a sample. In the following, we define solubility as the total polymer concentration c_{tot}, where a precipitate becomes *observable*. As a threshold, we use quite arbitrarily a volume fraction $\Phi = 10^{-3}$.

As an example, we consider samples synthesized by biased random copolymerization, but now with a bias $\sqrt{\tilde{N}}\delta\tilde{f}_0$ of order 1 or larger. Note that this case is an idealization where the width of the net charge density distribution can be controlled by varying the chain length. In experiments other factors certainly play a role as well.

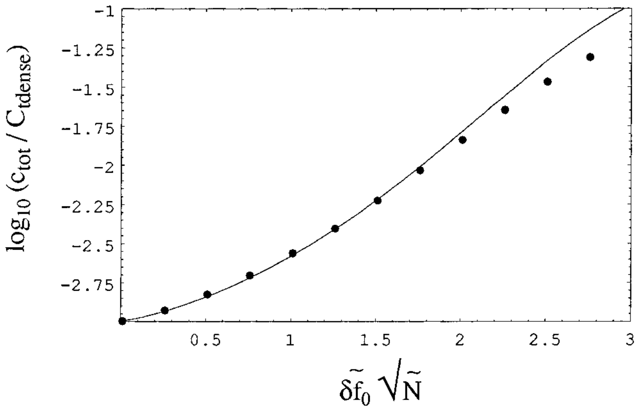

Figure 13. Total polymer concentration c_{tot} of a solution with a volume fraction $\Phi = 10^{-3}$ of the precipitate versus the scaled bias $\delta\tilde{f}_0\sqrt{\tilde{N}}$ for randomly copolymerized polyampholytes. The solid line represents the numerical solution of the approximate scheme, while the points were generated by solving numerically eqs 5.1−5.9 including the differences in the counterion concentrations in the two phases for $fg_{\mathrm{a}} = 10$.

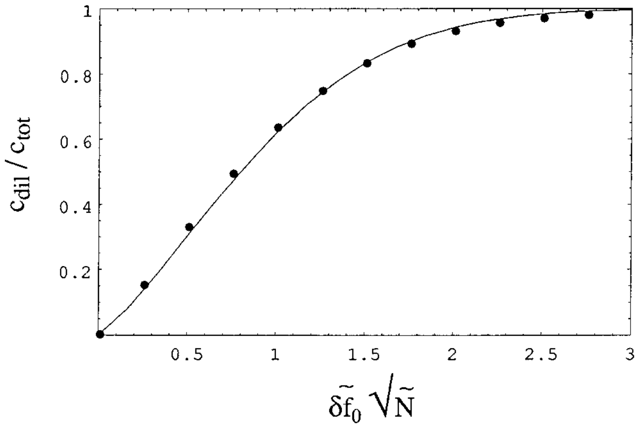

Figure 14. Ratio of the polyampholyte concentration in the dilute phase c_{dil} and the total polymer concentration for the same systems as in Figure 13.

For the example in Figures 13 and 14 we numerically solved eqs 5.1−5.9 including the differences in the counterion concentrations in the two phases for $fg_{\mathrm{a}} = 10$. The results are in very good agreement with what we obtain from the approximation introduced in section 5.2, indicating that the relevant mechanism is the precipitation of polyampholyte chains with mutually canceling net charge. We therefore discuss this aspect first, before considering the precipitation of polyampholyte chains together with their counterions.

We assume that *all* chains with a net charge density larger than a certain $\delta\tilde{f}^*$ are dissolved, while all other chains are precipitated. The polyampholyte concentrations in the supernatant are then given by $c(\delta\tilde{f}) = c_{\mathrm{tot}}/(1 - \Phi)p(\delta\tilde{f})\ \Theta(\delta\tilde{f} - \delta\tilde{f}^*)$, so that

$$c_{\mathrm{dil}} = \frac{c_{\mathrm{tot}}}{1-\Phi}\frac{1}{2}\mathrm{Erfc}(\sqrt{\tilde{N}/2}(\delta\tilde{f}^* - \delta\tilde{f}_0)) \qquad (5.20)$$

$$c_{\mathrm{pc}} = \frac{c_{\mathrm{tot}}}{1-\Phi}\left(\frac{\delta\tilde{f}_0}{2}\mathrm{Erfc}(\sqrt{\tilde{N}/2}(\delta\tilde{f}^* - \delta\tilde{f}_0)) + \frac{1}{\sqrt{2\pi\tilde{N}}}\exp(-\tilde{N}/2(\delta\tilde{f}^* - \delta\tilde{f}_0)^2)\right) \qquad (5.21)$$

and, using $c_{\mathrm{tot}} = \Phi C_{\mathrm{dense}} + (1 - \Phi)c_{\mathrm{dil}}$,

$$c_{\mathrm{tot}} = \frac{\Phi C_{\mathrm{dense}}}{1 - {}^1\!/_2\mathrm{Erfc}(\sqrt{\tilde{N}/2}(\delta\tilde{f}^* - \delta\tilde{f}_0))} \qquad (5.22)$$

Assuming a homogeneous counterion distribution throughout the system, $\delta\tilde{f}^*$ is determined from the condition $c_i = \delta\tilde{f}_0 c_{tot} = c_{pc} = \int_{\delta\tilde{f}^*}^{\infty} d\delta\tilde{f}\ \delta\tilde{f}\ p(\delta\tilde{f}) c_{tot}$. Here we discuss three points:

$\delta\tilde{f}_0 = 0$: For comparison we repeat the results for the unbiased case (see section 4). Such a sample is practically insoluble as the concentration of the dilute phase is limited to $c_{dil} = \exp(-\tilde{N}^{2/3})C_{dense} \approx 0$. For finite volume fractions Φ of the dense phase, practically all molecules are precipitated: $c_{tot} = \Phi C_{dense}$ and $c_{dil}/c_{tot} = 0$.

$\delta\tilde{f}^* = \delta\tilde{f}_0$: The net charge density distribution is cut at its peak for $\sqrt{\tilde{N}}\delta\tilde{f}_0 = \sqrt{2/\pi}(1 - \Phi)/(1 - 2\Phi) \approx \sqrt{2/\pi}$. In this case, already half of the polymers are dissolved $c_{dil}/c_{tot} = {}^1/_2$, but the solubility is still extremely small $c_{tot} = 2\Phi C_{dense}$.

$\sqrt{\tilde{N}}\delta\tilde{f}_0 > 1$: There is a dramatic increase in solubility, if the bias is larger than the width of the net charge density distribution. For $\tilde{N}\delta\tilde{f}_0^2 > -2 \log(2\sqrt{\pi}\Phi)$ precipitation becomes observable only for solutions with a total polymer concentration of the order of $c_{tot} = c_{dil} \sim C_{dense}$. Thus, if we compare systems at the onset of phase separation with $\Phi = 10^{-3}$, then by changing the bias $\delta\tilde{f}_0$ from zero to 3 times the standard deviation $1/\sqrt{\tilde{N}}$, the concentration of the dilute phase increases by $\tilde{N}^{2/3}$ orders of magnitude! In the same way, the solubility of a sample with fixed non-zero average net charge density $\delta\tilde{f}_0$ *increases* with chain length as a consequence of the reduced width of $p(\delta\tilde{f})$ (Figure 15).

It is instructive to compare this scenario to a *uniform* sample, where all chains have the same net charge density $\delta\tilde{f}_0$. In this case, one can obtain the concentration ratios in the two coexisting phases from a solubility product for the chains and their counterions (see section 3.4):

$$X_{\delta\tilde{f}_0} = \exp\left(-F_{ex}(\delta\tilde{f},\tilde{N})/(\delta\tilde{f}\tilde{N}\sqrt{fg_a} + 1)\right) \quad (5.23)$$

$$c_{dil} = C_{dense} X_{\delta\tilde{f}_0} \quad (5.24)$$

$$c_{tot} = C_{dense}\,(\Phi + (1 - \Phi)X_{\delta\tilde{f}_0}) \quad (5.25)$$

For $\delta\tilde{f}_0\sqrt{\tilde{N}} < 1$ the solubility increases much faster than for a randomly copolymerized sample. Not surprisingly, the uniform distribution is only a reasonable model for samples where the mean of the net charge density distribution $p(\delta\tilde{f})$ is much larger than the width, i.e., in the present case for $\delta\tilde{f}_0\sqrt{\tilde{N}} \gg 1$. In this limit, one obtains an upper bound for the solubility due to the precipitation of polyampholyte chains with their counterions:

$$c_{tot} = C_{dense} \exp\left(-\frac{2\delta\tilde{f}_0^{\ -1/3}}{\sqrt{fg_a}}\right) \quad (5.26)$$

where we have taken $\delta\tilde{f}_0 < 1$ to be in the elongated polyampholyte regime. Note that around $\delta\tilde{f}_0 = 3\tilde{N}^{-1/2}$ the exponential takes the form $\exp(-2(3\tilde{N})^{1/6}/\sqrt{fg_a})$, becoming much smaller than 1 in the limit of large $\tilde{N}$. This sheds some doubt on our earlier remark that randomly copolymerized samples should become soluble as soon as their mean is larger than a few standard deviations. However, as the number of charges per polyampholyte blob, fg_a, is typically of order 10, the chains need to have a length exceeding $\tilde{N} = 10^3$ blobs for the exponential factor to become relevant. Note that this implies a chain length of $N = (fg_a)^4/f$ and a bias as small as $\delta f_0 = f/(fg_a)^2$. For all practical purposes, the precipitation of polyampholyte chains together with their counterions requires concentrations of the dilute phase of the order of C_{dense}. In this sense, a sample can be said to be soluble, if the width of the net charge density distribution $p(\delta\tilde{f})$ is much smaller than the mean and vice versa.

6. Discussion and Conclusion

To summarize, our results suggest that care has to be taken in the interpretation of experiments on samples which contain polyampholyte chains with net charges of both signs. Pairs of oppositely charged chains have a strong tendency to form neutral complexes and to precipitate. At finite concentrations it is therefore *not* possible to identify the composition of the dilute phase with the composition of the sample. In addition we find a striking contrast in the behavior of neutral and non-neutral samples. In the first case, samples are almost insoluble with a dilute phase consisting of spherical globules. In the second case, the systems behave like a mixture of a non-soluble, neutral part with a soluble part consisting of the most strongly charged chains and the counterions. If the system is concentrated beyond the onset of phase separation, the soluble part accumulates in the dilute phase where the dissolved polyampholyte chains have the form of elongated globules.

It is worthwhile to illustrate the consequences using the example of randomly charged chains (case I with $\delta\tilde{f}_0 = 0$). Kantor and Kardar[4,5] showed that at infinite dilution the averages for quantities such as the hydrodynamic radius are dominated by the extended chains in the wings of the *sample* charge distribution. To illustrate the effect, they presented a set of simulations of randomly charged, but overall neutral chains, which were subsequently cut in the middle: The neutral chains collapsed into spherical globules, while the halves, when *separated from each other*, stretched on average due to their non-vanishing net charge. However, our analysis in section 4 shows that the least charged, elongated halves start to form dimers for $c_{tot} > c_{dense} \exp(-2\tilde{N}^{2/3})$. Neutral samples of polyampholytes are for large $\tilde{N}$ practically insoluble. For total concentrations as low as $C_{dense} \exp(-\tilde{N}^{2/3})$ most of the material is precipitated, so that the compositions of the *precipitate* and the sample coincide. The supernatant has a concentration $c_{dil} = C_{dense} \exp(-\tilde{N}^{2/3})$ *independent* of the total concentration and contains, except for highly asymmetric samples, practically *no* elongated globules.

Nevertheless, we find that elongated globules play an important role in experiments. The reason is (i) the *sublinear* dependence of the excess free energy on the chain net charge in the elongated globule regime (eq 2.2) and (ii) the fact that samples are never *perfectly* self-neutralizing and therefore contain free counterions. The behavior of these counterions is dominated by translational entropy, and the condition of electroneutrality (via an electro-static potential difference between the phases) leads to the dissolution of charged globules. At coexistence, the composition of the supernatant is determined by the interplay between the the gain in electrostatic potential energy and the free energy penalty for dissolved chains.

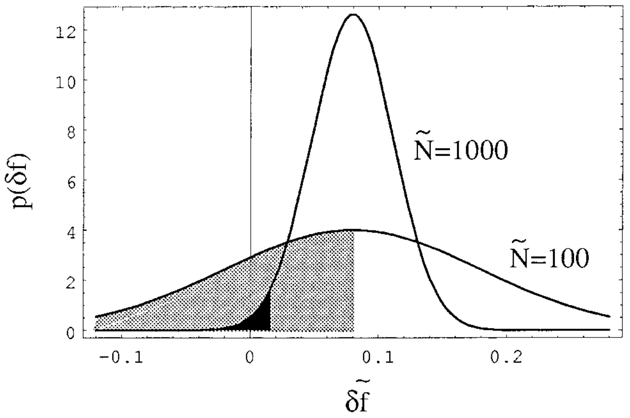

Figure 15. Net charge density distribution for two randomly copolymerized polyampholyte samples with $\delta\tilde{f}_0 = \sqrt{2/\pi 100}$ and chain lengths $\tilde{N} = 100$ and $\tilde{N} = 1000$, respectively. The shaded areas indicate precipitated chains.

We have shown (section 5), that in the case of random copolymerization an exponentially small bias leads to the accumulation of the most strongly charged chains in the supernatant, while the counterion concentration remains almost uniform throughout the system. To balance these charges, the potential difference adapts its value so that all chains from the tail of the sample net charge density distribution with $\delta\tilde{f} > \delta\tilde{f}^*$ are dissolved, where $\delta\tilde{f}^*$ is determined by the condition $\delta\tilde{f}_0 = \int_{\delta\tilde{f}^*}^{\infty} \mathrm{d}\delta\tilde{f}\, \delta\tilde{f}\, p(\delta\tilde{f})$. The arguments leading to this result are fairly general, and we therefore expect any non-neutral sample to behave like a mixture of a soluble and a non-soluble component (Figure 15). Solutions of finite concentration separate into an almost self-neutralizing dense phase (containing all chains with net charges of the same sign as the counterions plus a corresponding amount of weakly charged chains with net charges of the opposite sign) and a supernatant consisting of charged, elongated globules and counterions with a concentration $c_{\mathrm{dil}} \sim c_{\mathrm{tot}}$.

Clearly, our understanding of the effects discussed in this article can only be as good as our understanding of the properties of single chains in solution and of the dense phase. For example, we show in the Appendix that for a sample with on the average less than one counterion per polyampholyte blob, precipitated counterions do not change the properties of the dense phase. Higher values may, however, lead to an inflation of the dense phase and eventually to the formation of mesophases.

Our treatment of the dilute phase, on the other hand, is based on the elongated globule model. This description is rather crude as it characterizes a polyampholyte only by its net charge (nevertheless, the sublinear regime in the excess free energy seems, at least on a scaling level, rather robust). In addition, long-range electrostatic interactions between dissolved globules of a solution become relevant at fairly low concentrations. Our crude arguments in the Appendix show that the resulting corrections to the chemical potential of dissolved polyampholyte chains do not change the *composition* of the phases. However, as a consequence of the strong repulsion between the macroions the polyampholytes may arrange in structures similar to colloidal crystals. At the same time, the globules have due to their size relatively small surface potentials, so that the interaction with the counterions remains weak.

From an experimental point of view, it would be very interesting to extend the calculations to solutions containing salt, even though our considerations show that this is not necessary in order to prepare polyampholyte solutions of *finite* concentration. Including salt, chain stiffness, or solvent effects requires work on the single chain level before solutions can be discussed along the lines of the present paper. A theory for solutions of *rodlike* polyampholytes could, for example, be based on the results by Barrat and Joanny[26] for the pair interaction; salt and solvent effects have been discussed in the framework of the elongated globule model.[1,10] For the systems treated here, future work on the necklace model[6,7] will hopefully lead to a more detailed picture and, in combination with arguments along the lines of those given here, allow predictions on whether or not particular charge sequences lead to more soluble molecules than others.

In conclusion, we believe that the surprising properties of single polyampholyte chains in solution, which have been, at least partially, understood over the last years, give rise to equally interesting multichain effects in solutions of finite concentration. In this paper, we have mainly addressed the *composition* of dilute solutions. We have shown that pairs of oppositely charged chains have a strong tendency to form neutral complexes and to precipitate and that the solubility of polyampholytes is due to the translational entropy of counterions. The preferential dissolution of the most strongly charged chains in a sample turns out to be a direct consequence of the way single, dissolved polyampholyte chains deform into elongated globules in order to minimize the electrostatic repulsion between their excess charges and to preserve the energy gain from fluctuations in the charge density.

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Appendix

A. Dense Polyampholyte Phase Containing Higher Concentrations of Free Counterions.

Throughout the paper we have assumed that the concentrations of free counterions are too small to affect the properties of the dense or the dilute phase. We essentially treated the counterions as non-interacting particles whose distribution was coupled to the polyampholyte concentrations through the electroneutrality condition for the phases and otherwise dictated by translational entropy. In this section we discuss the possibilities of an inflation of the dense phase due to the counterion osmotic pressure and the formation of mesophases.

A.1. Inflation of the Dense Phase Due to the Osmotic Pressure of Free Counterions. Consider a dense phase of polyampholytes and counterions with concentrations $C_{\delta f}$ and C_{i}. Charge neutrality requires $C_{\mathrm{i}} = \int \delta f C_{\delta f}\, \mathrm{d}\delta f$, and we assume that the counterion concentration is much smaller than the polymer concentration $C_{\mathrm{dense}} = \int C_{\delta f}\, \mathrm{d}\delta f$. The free energy density consists of a Debye–Hückel term for the polarization energy, which is proportional to the total charge density $(fC_{\mathrm{dense}} + C_{\mathrm{i}})^{3/2}$, a term for the three-body repulsion, and the translational entropies of the different species:

$$\frac{f_{\rm dense}}{k_{\rm B}T} = -((fC_{\rm dense} + C_{\rm i})l_{\rm B})^{3/2} + b^6 C_{\rm dense}{}^3 + \sum_{\delta f} \frac{C_{\delta f}}{N}\left(\log\left(\frac{C_{\delta f} b^3}{N}\right) - 1\right) + C_{\rm i}(\log(C_{\rm i} b^3) - 1) \quad \text{(A.1)}$$

The chemical potential for chains with net charge δf is given by $\mu_{\delta f} = N(\partial f/\partial C_{\delta f})$:

$$\frac{\mu_{\delta f}}{k_{\rm B}T} = -\frac{3}{2}((fC_{\rm dense} + C_{\rm i})l_{\rm B})^{1/2} l_{\rm B} f N + 3b^6 C_{\rm dense}{}^2 N + \log\left(\frac{C_{\delta f} b^3}{N}\right) \quad \text{(A.2)}$$

while the counterion chemical potential is calculated from $\mu_{\rm ion} = \partial f/\partial C_{\rm ion}$:

$$\frac{\mu_{\rm ion}}{k_{\rm B}T} = -\frac{3}{2}((fC_{\rm dense} + C_{\rm i})l_{\rm B})^{1/2} l_{\rm B} + \log(C_{\rm i} b^3) \quad \text{(A.3)}$$

Finally, we obtain the osmotic pressure of the dense phase $\Pi = \sum_{\delta f} C_{\delta f}\mu_{\delta f} + C_{\rm i}\mu_{\rm i} - f_{\rm dense}$:

$$\frac{\Pi_{\rm dense}}{k_{\rm B}T} = -\frac{1}{2}((fC_{\rm dense} + C_{\rm i})l_{\rm B})^{3/2} + 2b^6 C_{\rm dense}{}^3 + \frac{C_{\rm dense}}{N} + C_{\rm i} \quad \text{(A.4)}$$

Throughout the paper we neglected the $O(1/N)$ chain and the counterion osmotic pressure contributions to eq A.4 as well as the osmotic pressure of the dilute phase. Under these assumptions eq A.4 becomes independent of the composition of the dense phase. The osmotic pressure equilibrium can then be solved independent of the phase equilibrium, yielding, up to prefactors, the result derived from scaling arguments in section 2.1: $C_{\rm dense,0} = 2^{-4/3} b^{-3} g_{\rm a}{}^{-1/2}$ and $F_{\rm dense,0} = -(3/2^{8/3})\tilde{N} k_{\rm B} T$.

We now include the counterion contribution to the osmotic pressure. Treating the dilute phase as an ideal gas and writing the density of the precipitate as $C_{\rm dense} = \gamma C_{\rm dense,0}$ give the pressure equilibrium in the form:

$$\gamma^{3/2} - \gamma^3 = \pi_{\rm i} = 2^{5/3} g_{\rm a} \frac{C_{\rm i} - c_{\rm i}}{C_{\rm dense,0}} \quad \text{(A.5)}$$

Thus, the relevant measure for the counterion osmotic pressure is the number $\pi_{\rm i}$ of excess counterions in the dense phase per blob. For $\pi_{\rm i} \ll 1$, γ tends to 1 (the branch of solutions converging to zero is unphysical). Since the osmotic pressure of the counterions tends to swell the dense phase, γ is in general smaller than 1.

The difference of the counterion densities in the two phases for a given volume fraction Φ can be calculated from the chemical equilibrium and the mass balance for the counterions.

$$c_{\rm i} = C_{\rm i} X_{\rm i} \equiv C_{\rm i} {\rm e}^{F^{\rm ion}_{\rm dense} - \Psi} \quad \text{(A.6)}$$

$$F^{\rm ion}_{\rm dense} \sim -\frac{1}{fg_{\rm a}}\gamma^{1/2} \ll 1 \quad \text{(A.7)}$$

$$C_{\rm i} - c_{\rm i} = \frac{\delta f_0 c_{\rm tot}}{\Phi + (1 - \Phi)X_{\rm i}}(1 - X_{\rm i}) \quad \text{(A.8)}$$

The counterion distribution in the system becomes nonuniform for $X_{\rm i} < 1$. Since the gain in polarization energy of the counterions in the dense phase is negligible (eq A.7), the ratio $c_{\rm i}/C_{\rm i}$ is controlled by the electrostatic potential barrier, Ψ, between the phases. The resulting excess osmotic pressure in the dense phase is proportional to the total counterion concentration, $\delta f_0 c_{\rm tot}$.

Since Ψ is a function of the sample composition and concentration, eq A.8 is difficult to discuss in detail. In general, Ψ is large, if the counterions are forced to stay in the dense phase for want of easily dissolvable polyampholytes with opposite charge. In that case it is justified to neglect the osmotic pressure of the dilute phase and

$$\pi_{\rm i} \overset{X_{\rm i} \ll 1}{\sim} \delta f_0 g_{\rm a} \gamma \frac{1}{1 + X_{\rm i}\Phi^{-1}} < \delta f_0 g_{\rm a} \quad \text{(A.9)}$$

In many cases, we expect polyampholyte solutions to be in the opposite limit, where the counterion concentrations in precipitate and supernatant are of the same order. In section 5 we saw that the *scaled* potential $\tilde{\Psi} = \sqrt{fg_{\rm a}}\Psi$ is usually of order 1. On the other hand, $fg_{\rm a}$, the number of charges per polyampholyte blob, is usually of order 10, so that $X_{\rm i} \approx (1 - \Psi)$ can be expanded. In this case

$$\pi {\rm i} \overset{\Psi < 1}{\sim} \delta f_0 g_{\rm a} \gamma \Phi \Psi < \delta f_0 g_{\rm a} \quad \text{(A.10)}$$

Independent of Ψ and Φ, the number $\pi_{\rm i}$ of excess counterions in the dense phase per blob is smaller than the ensemble average $\delta f_0 g_{\rm a}$. Thus, *for a sample with on the average less than one counterion per polyampholyte blob, precipitated counterions do not change the properties of the dense phase.* Note that this criterion is not related to the crossover between spherical and elongated globules.

In the most general case, however, the electrostatic potential difference Ψ between the phases has to be determined from the condition of electroneutrality of the total system. Unfortunately, the polyampholyte concentration ratios between the phases also depend on the degree of inflation of the dense phase, since the free energies entering the chemical potential are changed:

$$F_{\rm dense}(\gamma) = F_{\rm dense,0} + \Delta F_{\rm dense}$$

$$\Delta F_{\rm dense} = -\frac{3}{2^{5/3}}(\gamma^{1/2} - 1)\tilde{N} + \frac{3}{2^{8/3}}(\gamma^2 - 1)\tilde{N} \quad \text{(A.11)}$$

Thus, the pressure and chemical equilibria cannot be solved independently.

A.2. Formation of Mesophases. It has been argued[27,28] that polyelectrolytes in a poor solvent may form mesophases, and this raises the question whether the effect should also occur in a dense phase of charged polyampholytes.

In the case of polyelectrolytes (with charge density f_{PE} per monomer) mesophases form as a result of the competing effects of counterion entropy and monomer–monomer attraction. The preferred lengthscale ξ_φ for density fluctuations $\delta\varphi$ can be estimated by equating the elastic energy $b^2(\delta\varphi)^2\xi_\varphi{}^3/\varphi\xi_\varphi{}^2$ to the electrostatic energy $l_{\mathrm{B}}(\delta\varphi)^2 f_{\mathrm{PE}}{}^2\xi_\varphi{}^6/\xi_\varphi$ (the counterion distribution is assumed to be undisturbed): $\xi_\varphi{}^{-4} \sim l_{\mathrm{B}} b^{-2} f_{\mathrm{PE}}{}^2\varphi$. While ξ_φ does not depend on the quality of the solvent for the uncharged backbone of the chains, the instability (corresponding to a divergence of the structure factor at $q = 2\pi/\xi_\varphi$) occurs only in poor enough solvents. It is important to note that the tendency to form mesophases is diminished in the presence of salt, i.e., screening of the electrostatic interactions. The effect vanishes if the Debye-length κ^{-1} becomes shorter than ξ_φ.

For the purpose of screening at a given density, there is no difference between free salt ions and the bound charges on random polyampholytes.[8] A dense phase of charged polyampholytes can therefore be mapped on a polyelectrolyte solution with $f_{\mathrm{PE}} = \delta f$, $\kappa^2 = l_{\mathrm{B}} f\varphi$, and $\varphi = \gamma C_{\mathrm{dense}}$. Mesophases can form only if $\delta\tilde{f}^2 > \gamma$, i.e., for a non-inflated dense phase only for chains that are on average in the polyelectrolyte regime.

B. More Concentrated Dilute Polyampholyte Phases Containing Free Counterions. So far we treated the dilute phase as an ideal gas composed of unimers, dimers, etc. and free counterions. We now discuss the leading corrections in more concentrated dilute phases as they can occur for samples containing free counterions. In particular, we address two questions (counterion condensation into dissolved globules and corrections to the ideal gas chemical potential due to electrostatic interactions) and find that these processes can be neglected for the purposes of the present paper.

B.1. Counterion Condensation into Dissolved Globules. One of the main conclusions of the paper is that even in dilute solutions oppositely charged polyampholytes have a strong tendency to associate. In principle, the electrostatic energy of charged globules can also be lowered by counterion condensation.

Formally, the following treatment is analogous to the dimer formation discussed in section 3.1; i.e., we consider a chemical equilibrium between dissolved globules, free counterions, and globules with a bound counterion. We need to determine the change in the globule free energy $\Delta F_{\mathrm{ex}}(\delta\tilde{f},\tilde{N})$ to be able to write down a law of mass action. In general, this quantity is not easy to determine. Here we assume that the main effect is the reduction of the net charge density of the globule from δf to $\delta f - 1/N$ or, in our scaled variables, from $\delta\tilde{f}$ to $\delta\tilde{f} - 1/\sqrt{fg_{\mathrm{a}}}/\tilde{N}$. This implies[29]

$$\frac{\Delta F_{\mathrm{ex}}(\delta\tilde{f},\tilde{N})}{k_{\mathrm{B}}T} = \frac{F_{\mathrm{ex}}(\delta\tilde{f} - 1/\sqrt{fg_{\mathrm{a}}}/\tilde{N},\tilde{N}) - F_{\mathrm{ex}}(\delta\tilde{f},\tilde{N})}{k_{\mathrm{B}}T} = \frac{1}{\sqrt{fg_{\mathrm{a}}}}\begin{cases} -2\delta\tilde{f}\tilde{N}^{2/3} & \delta\tilde{f}^2 < 1/\tilde{N} \quad \text{spherical globules} \\ -{}^4/_3\delta\tilde{f}^{-1/3} & 1/\tilde{N} < \delta\tilde{f}^2 < 1 \quad \text{elongated globules} \\ -{}^8/_3\delta\tilde{f}^{1/3} & \delta\tilde{f}^2 > 1 \quad \text{polyelectrolytes} \end{cases} \tag{B.1}$$

When the polyelectrolyte regime is disregarded, $\Delta F_{\mathrm{ex}}(\delta\tilde{f},\tilde{N})$ has a maximum of order $-\tilde{N}^{1/6}/\sqrt{fg_{\mathrm{a}}}$ for globules at the crossover between the spherical and the elongated polyampholyte regimes. Following the arguments in section 3.1 and assuming a uniform sample with $c_{\mathrm{i}} = \delta f_0 c_{\delta f}$ mean that the globule concentrations would have to be of the order of $c_{\mathrm{ion\ cond}} = C_{\mathrm{dense}}(\sqrt{f}/\delta\tilde{f}_0)\exp(-\tilde{N}^{1/6}/\sqrt{fg_{\mathrm{a}}})$ for counterion condensation to occur. This can be compared to the concentration where the sample phase separates (see section 5.4):

$$C_{\mathrm{dense}}\exp(-F_{\mathrm{ex}}(\delta\tilde{f},\tilde{N})/(\delta\tilde{f}\tilde{N}\sqrt{fg_{\mathrm{a}}}))$$

The exponent has, except for the surface energy which dominates F_{ex} in the spherical polyampholyte regime, the same scaling form as eq B.1, but a comparison of the prefactors suggests that phase separation occurs at *lower* concentrations than the condensation of counterions onto the globules. Although in a multicomponent system there is no simple upper limit for the concentrations in the dilute phase, our arguments in section 5.4 show that the argument of the exponential is usually smaller than 1. Furthermore, we neglected the inflation effects discussed in the preceding section as well as possible changes in the surface energy. Both would tend to destabilize the complex so that our estimate should be regarded as a lower bound on the concentration of the dilute phase where counterion condensation becomes relevant.

B.2. Electrostatic Interactions. The systems investigated in the paper were assumed to be salt-free, and as a consequence the deviations from the ideal gas behavior due to long-range electrostatic interactions between charged constituents become relevant at fairly low concentrations. Since the paper mostly discusses phase equilibria, we concentrate on corrections to the chemical potential of dissolved polyampholyte chains, which might influence the *composition* of the phases.

At sufficiently low concentrations of the dilute phase, one can again invoke the Debye–Hückel theory for point charges. At higher concentrations, the strong repulsion between the macro-ions leads to an ordered structure similar to charge-stabilized colloids, which we describe using a simple cell model. In both cases we find that dissolved charged globules are *stabilized* relative to the ideal gas. Within the Debye–Hückel theory, this is due to a gain in polarization energy, at higher concentrations due to the reduction of the electro-static self-energy of the chain as the central charge is neutralized by counterions over the distance of the cell radius.

In section 5 we have argued that for non-neutral samples the electrostatic potential difference between the precipitate and the supernatant is usually high enough to lead to the dissolution of the most strongly charged chains *in spite* of their high excess free energies. Since the corrections discussed in this Appendix reduce these excess free energies, our simple approximation for the composition of the phases remains valid: all chains that have a net charge density beyond a certain value $\delta\tilde{f}^*$ determined by charge neutrality are dissolved; the rest precipitate. In particular, the proportionality between c_{dil}, the concentration of the dilute phase, and c_{tot}, the total polymer concentration, remains unaffected. Only the estimate for the necessary potential barrier Ψ is lowered by the additional stabilization of the charged chains in solution.

B.2.1. Debye–Hückel Theory. Within Debye–Hückel theory the square of the inverse screening length

κ for a solution containing charged polyampholyte chains and counterions is given by

$$\kappa_{\mathrm{dil}}{}^2 = l_{\mathrm{B}} c_{\mathrm{i}} + l_{\mathrm{B}} \int \mathrm{d}\delta f \, (\delta f N)^2 \frac{c_{\delta f}}{N} + \ldots \qquad \text{(B.2)}$$

where the dots represent dimers and higher order clusters. Taking into account the polarization energy yields correction terms to the free energy density $f_{\mathrm{dil,DH}} = -\kappa_{\mathrm{dil}}{}^3$, the chain chemical potential $\mu_{\delta\tilde{f},\mathrm{DH}} = -{}^3/{}_2 \kappa l_{\mathrm{B}} f g_{\mathrm{a}} \delta\tilde{f}^2 \tilde{N}^2$, the ion chemical potential $\mu_{\mathrm{i,DH}} = -{}^3/{}_2 \kappa l_{\mathrm{B}}$, and the osmotic pressure of the dilute phase $\Pi_{\mathrm{dil,DH}} = -{}^1/{}_2 \kappa_{\mathrm{dil}}{}^3$.

It is instructive to calculate κ in the three characteristic concentration regimes for randomly copolymerized samples treated in the paper.

(1) At Infinite Dilution or for $\tilde{\delta} f_0 \sqrt{\tilde{N}} \gg 1$.

$$\kappa_{\mathrm{dil}}{}^2 = l_{\mathrm{B}} \delta f_0 c_{\mathrm{tot}} + l_{\mathrm{B}} f c_{\mathrm{tot}} \sqrt{\frac{\tilde{N}}{2\pi}} \int \mathrm{d}\delta\tilde{f} \, \delta\tilde{f}^2 \tilde{N} \mathrm{e}^{-(\delta\tilde{f} - \delta\tilde{f}_0)^2 \tilde{N}/2} = \left(1 + \delta\tilde{f}^2 \tilde{N} + \frac{|\delta\tilde{f}_0|}{\sqrt{f g_{\mathrm{a}}}}\right) l_{\mathrm{B}} f c_{\mathrm{tot}} \qquad \text{(B.3)}$$

Note, that one would expect the same result for the corresponding simple electrolyte solutions, where all the polymers are cut into monomers.

(2) At Coexistence for a Neutral Sample.

$$\kappa_{\mathrm{dil}}{}^2 = l_{\mathrm{B}} f C_{\mathrm{dense}} \mathrm{e}^{-\tilde{N}^{2/3}} \sqrt{\frac{\tilde{N}}{2\pi}} \int \mathrm{d}\delta\tilde{f} \, \delta\tilde{f}^2 \tilde{N} \mathrm{e}^{-\delta\tilde{f}^2 \tilde{N}^{5/3}} = \frac{l_{\mathrm{B}} f C_{\mathrm{dense}}}{\tilde{N}} \mathrm{e}^{-\tilde{N}^{2/3}} \qquad \text{(B.4)}$$

For volume fractions $\Phi \approx \exp(-\tilde{N}^{2/3})$ this result also holds approximately for a sample with $0 < \delta\tilde{f}_0 < 1/N^{1/2}$ and can then be written in the form $\kappa_{\mathrm{dil}}{}^2 = l_{\mathrm{B}} f c_{\mathrm{tot}}/\tilde{N}$.

(3) For $c_{\mathrm{tot}} \gg \exp(-\tilde{N}^{2/3})$ and for $\delta\tilde{f}_0 \sqrt{\tilde{N}} \ll 1$ where the most strongly charged chains of a non-neutral sample are dissolved together with the counterions:

$$\kappa_{\mathrm{dil}}{}^2 = \sqrt{2\pi\tilde{N}} \delta\tilde{f}_0 (1 - \Phi) \, l_{\mathrm{B}} f c_{\mathrm{tot}} \qquad \text{(B.5)}$$

In all cases, $\kappa_{\mathrm{dil}}{}^2$ is proportional $l_{\mathrm{B}} \, f c_{\mathrm{tot}}$. In the single chain regime at extremely low concentrations the prefactor is of order 1. If the sample contains polyampholytes with net charges of both signs, this value is strongly reduced due to the dimerization and precipitation of charged globules at small, but finite, concentrations and of order $1/\tilde{N}$ around the onset of phase separation. For higher total concentrations, when the most strongly charged chains are redissolved, the prefactor increases again to $\sqrt{\tilde{N}} \delta\tilde{f}_0$.

The results have to be compared to the validity range of the Debye–Hückel theory. For interaction energies between globules with a net charge density $\delta\tilde{f}^2 \sim 1/\tilde{N}$ to be smaller than $k_{\mathrm{B}}T$, the concentration of the dilute phase has to be smaller than $C_{\mathrm{dense}}/\tilde{N}^2$.[31] At this point, the corrections to the chain chemical potential $\Delta\mu_{\delta\tilde{f}} \sim -k_{\mathrm{B}}T$ are still negligible.

Although the Debye–Hückel theory is restricted to fairly low concentrations (the overlap concentration for the corresponding neutral polymer solution is $C_{\mathrm{dense}}/\sqrt{\tilde{N}}$), this is sufficient for neutral samples. The concentrations of the dilute phase of the order of $\exp(-\tilde{N}^{2/3})$ C_{dense} are so low that the polarization effects can be neglected altogether. However, for non-neutral samples the concentration of the dilute phase becomes proportional to the total polymer concentration and can exceed the validity range of the Debye–Hückel theory.

B.2.2. Cell Model. At concentrations ρ, where the interaction between the macroions at their mean distance $d = \rho^{-1/3}$ is much larger than $k_{\mathrm{B}}T$, the probability to find two macroions at distances smaller than d is strongly reduced. This situation is typical for charged-stabilized colloids, and we adopt a simple cell model[30,32] to calculate the mean-field free energy for a system of fixed globules and mobile counterions.

The spherical globules with diameter σ and a net charge Z homogeneously distributed over its volume are assumed to be located in the center of a spherical cell with diameter d together with Z mobile counterions to ensure charge neutrality. Instead of solving the nonlinear Poisson–Boltzmann equation, we use a simple variational ansatz along the lines of ref 32. We assume that $Z - Z^*$ counterions are inside the globule with the variational parameter Z^* being the renormalized central charge. Furthermore, we assume that the counterion distribution inside and outside the globule is uniform and that the globule radius remains unchanged.

The variational free energy per cell is then given as the sum of the counterion translational entropies in the two regions and the electrostatic field energy:

$$\frac{F_{\mathrm{var}}}{k_{\mathrm{B}}T} = \frac{3}{10} \frac{Z^{*2} l_{\mathrm{B}}}{\sigma} \frac{1 - {}^3/{}_2 \Phi^{1/3} + {}^1/{}_2 \Phi}{(1 - \Phi)^2} + Z \log\left(\frac{Z - Z^*}{\Phi}\right) + Z^* \log\left(\frac{Z^*}{Z - Z^*} \frac{\Phi}{1 - \Phi}\right) - Z \log\left(\frac{d^3}{b^3}\right) \qquad \text{(B.6)}$$

where $\Phi = \sigma^3/d^3$ is the globule volume fraction. Minimizing with respect to Z^* yields

$$\frac{Z - Z^*}{Z} = \begin{cases} \Phi \mathrm{e}^{\Lambda} & \Phi \ll \mathrm{e}^{-\Lambda} \\ \Phi & \Phi \rightarrow 1 \end{cases} \qquad \text{(B.7)}$$

Since $\Lambda \sim Z l_{\mathrm{B}}/\sigma$ is the surface potential and for polyampholytes $\Phi = c_{\mathrm{dil}}/C_{\mathrm{dense}}$, we actually recover the results from Appendix B.1 for the onset of counterion condensation at small Φ. There we argued that the surface potential is often small and in this case $Z^* = Z(1 - \Phi)$; i.e., the counterions remain uniformly distributed throughout the *entire* cell.

The chemical potential of the polyampholyte globules is nevertheless reduced relative to the case of infinite dilution or $\Phi = 0$, since the electric field outside the globule decays to zero at the cell boundary (Gauss' law). In section 2.2, we argued that the excess free energy of a dissolved globule relative to the dense phase is the sum of the globule's surface and electrostatic self-energies. In the elongated globule regime, both terms are of the same order and we can write:

$$F_{\mathrm{ex}}(\Phi,\delta\tilde{f},\tilde{N}) = F_{\mathrm{ex}}(\Phi = 0,\delta\tilde{f},\tilde{N})(1 - 2\Phi^{1/3} + \Phi) \qquad \text{(B.8)}$$

Equation B.8 implies that the globules become more spherical with increasing concentration and fully so at $\Phi = ({}^1/{}_2(1 - \tilde{N}\delta\tilde{f}^2)^{-1}))$. However, the above considerations certainly break down when the long axis of the globules is of the order of the cell diameter d, i.e., at the much smaller overlap concentration $\Phi^* = c^*_{\mathrm{dil}}/C_{\mathrm{dense}} = (\tilde{N}\delta\tilde{f}^2)^{-2}$. Addressing this point therefore requires a more careful approach. The cell model also breaks down if with increasing density the interactions between neighboring macro-ions become smaller than

k_BT. There are two possible mechanisms for this to happen: (i) the counterion screening length can reduce faster than the typical macroion distance and (ii) the interactions can be weakened by the *renormalization* of the central charges. Both limits can be expressed in terms of the surface potential $\Lambda = Zl_B/\sigma$ and the central charge Z: the screening becomes efficient for $\Phi > \Lambda^{-3}$ and charge renormalization for $\Phi > 1 - 1/\sqrt{Z\Lambda}$. For polyampholytes $\Lambda < 1 < Z\Lambda$, so that from this point of view the cell model remains valid for practically all concentrations of the dilute phase.

In summary, the present spherical cell model can be trusted upto the overlap concentration of the elongated globules $\Phi = \sqrt{c^*_{\text{dil}}/C_{\text{dense}}} = (\tilde{N}\delta\tilde{f}^{\,2})^{-2}$, i.e., for concentrations of the dilute phase that are a finite fraction of the concentration of the dense phase.

References and Notes

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(18) For example, one recovers consistent estimates for the screening length $\kappa^2 = 4\pi l_B c_{\text{ion}} z^2$ for a solution of ions with valence z by considering (i) the interaction between individual charges *inside* a blob ($c_{\text{ion}} = fC_{\text{dense}}$ and $|z| = 1$) and (ii) the interaction *between* blobs ($c_{\text{ion}} = c_{\text{dense}}/g_a$) with a typical net charge $|z| = \sqrt{fg_a}$.

(19) These correction terms become important, when one considers the formation of dimers from unimers with net charges of the *same* sign. In the framework of the formalism developed below (see, e.g., eq 3.1), the difference of the unimer and dimer free energies $2F_{\text{ex}}(\delta\tilde{f},\tilde{N}) - F_{\text{ex}}(\delta\tilde{f},2\tilde{N})$ would seem to vanish in the elongated globule regime. However, taking into account the $\log(\tilde{N})$ corrections, the difference is of the order of $-F_{\text{ex}}(\delta\tilde{f},\tilde{N})$, i.e., dimer formation is strongly prohibited.

(20) The product fg_a is the number of charges per blob and, in general, much larger than 1.

(21) Here we assume that the precipitate contains only a negligible fraction of counterions and therefore equal amounts of chains with positive and negative net charge. This is reasonable until the concentrations in the supernatant exceed the limit eq 3.17.

(22) Equation 3.3 is correct only as long as the counterions and the excess chains do not start to precipitate into the dense phase. In this section we estimate the validity range from the asymptotic case where the total concentration is so high that precipitate and sample composition become identical (see eq 3.17). The crossover will be treated in more detail for the example in the following section.

(23) In light of our results for bimodal distributions, it seems reasonable to neglect higher order clusters for (almost) symmetric charge distributions.

(24) It is not obvious that the negatively charged chains should have equal charges $\delta\tilde{f}/2$, but other combinations only make the problem more complicated without changing the basic physics.

(25) As in the case of dimers the trimer free energy dominates and leads to the formation of predominantly neutral complexes.

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