Ion Entropy in Phase-Separated Aqueous Mixtures of Polyelectrolyte and Neutral Polymer

Mark Vis,*^{,†} Vincent F. D. Peters,[†] Ben H. Erné,^{*,†} and R. Hans Tromp^{†,‡}

[†]Van 't Hoff Laboratory for Physical and Colloid Chemistry, Debye Institute for Nanomaterials Science, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

[‡]NIZO food research, Kernhemseweg 2, 6718 ZB Ede, The Netherlands

Supporting Information

ABSTRACT: The macroscopic phase separation of aqueous mixtures of a neutral polymer and a polyelectrolyte is well described by a modified blob model, taking into account the entropy of ideal ions under the restriction of macroscopic charge neutrality. This is demonstrated by detailed measurements on aqueous mixtures of a neutral polymer (dextran) and a polymer whose charge is adjustable via the pH (nongelling fish gelatin). The critical point of the phase diagram of demixing, the asymmetric distribution of the solvent, and the interfacial electric potential difference all depend on polyelectrolyte charge and background salt concentration in a manner that is consistent with a dominant role for ion entropy.



1. INTRODUCTION

Mixtures of different polymers tend to demix because the resulting loss in entropy is small with respect to the reduction in enthalpy. This is true both for polymer blends and for mixtures with solvent. In a solvent, phase separation can be associative or segregative,^{1,2} where, respectively, one phase is enriched in both polymers or both phases are enriched in one polymer each. Poor or marginal solvent conditions favor associative phase separation, as do attractive polymer-polymer interactions. The complex coacervation of oppositely charged polyelectrolytes is a classic example.³ Segregative phase separation takes place generally for polymer mixtures in a good solvent, because of a positive mixing enthalpy, that is, repulsive interactions between the polymers. Such demixing typically takes place already at low polymer concentrations, often below 10% by mass. In water, such systems are often named aqueous two-phase systems (ATPS), with a so-called water/water interface separating the two phases.

ATPS have a long history. Beijerinck observed already in 1896 the phase separation in aqueous mixtures of solubilized starch and gelatin.⁴ Since then, various other aqueous polymer mixtures have been investigated, such as dextran/methylcellulose,⁵ dextran/poly(ethylene glycol),^{6–8} and gelatin with various polysaccharides^{9–12} such as dextran.^{13,14} The water/water interface formed in such mixtures has peculiar properties. For instance, compared to typical oil/water interfaces, the tension of water/water interfaces is orders of magnitude lower, in the range of μ N/m.^{6,15–18}

The introduction of salts composed of cations and anions with different partitioning coefficients leads to the formation of an interfacial electric potential difference.^{19–22} A similar effect is observed when one of the polymers carries a charge. In both phases, the condition for electroneutrality needs to be fulfilled upon phase separation, spatially restricting the counterions of

the charged polymer to part of the volume. This leads to an inhomogeneous distribution of ions over the two phases, causing an electric potential difference. We have shown recently for aqueous mixtures of dextran (uncharged) and gelatin (pH-dependent charge) that this electric potential difference—a Donnan potential^{23–25}—follows the magnitude and sign of the charge on the gelatin.^{26,27} In addition, for gelatin that was more strongly charged, the critical point of demixing was shifted to higher concentrations, as seen before for other mixtures as well.²⁸

Much literature has been devoted to the theoretical understanding of the phase behavior of polymer mixtures in solution. Flory-Huggins type theory is very versatile in this regard and has been used to describe both associative and segregative phase separation.² For instance, Voorn and Overbeek^{29,30} described the complex coacervation observed by Bungenberg de Jong³ by combining Flory–Huggins theory with Debye-Hückel theory to account for the electrostatic interactions. Since then, more elaborate models have been proposed as well.³¹ However, while Flory-Huggins theory qualitatively describes the experimental trends and is useful to gain fundamental insights, it is not a quantitative theory. More quantitative models include the blob model for semidilute polymer solutions by Broseta and co-workers.³²⁻³⁴ It describes segregative phase separation and shows quantitative agreement with experiments, for instance regarding the interfacial tension. 32

Here, we study both experimentally and theoretically the behavior of aqueous mixtures of a neutral polymer with a charged polymer, respectively dextran and (nongelling) fish

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gelatin. Dextran is a branched polysaccharide; gelatin is a partly hydrolyzed form of collagen, a linear protein, and a (weak) polyelectrolyte with a pH-dependent charge. Aqueous mixtures of dextran and gelatin separate into a dextran- and a gelatin-rich phase when their concentration is increased above the critical point of demixing, which lies typically below total polymer mass fractions of 10%. Because of the requirements of macroscopic electroneutrality in each phase upon phase separation, the concentrations of the ions in the two phases are unequal. This not only leads to the formation of a Donnan potential,^{26,27} but it is also entropically unfavorable, thereby favoring mixing. We expect the entropy of ions to have a major influence on the phase behavior of mixtures of charged and uncharged polymers in solution. We also presume that the addition of salt reduces the relative concentration differences, thereby lowering Donnan potentials and entropic penalties and favoring demixing.

We will present detailed and quantitative experimental measurements on the phase behavior, critical points, distribution of solvent, and Donnan potentials as a function of the polyelectrolyte charge and background salt concentration. We will compare our measurements with theory. Our theory is based on the blob model for the behavior of mixed polymer solutions. In order to account for the effects of charges, we will derive analytical expressions for the ion entropy and the equilibrium salt distribution in the system, which we will combine with the blob model to describe our mixtures of polyelectrolyte and neutral polymer in solution.

This paper is organized as follows. First, the theoretical framework will be discussed: the blob model, the expressions for the ion entropy, the calculation of the equilibrium salt distribution, the calculation of the Donnan potential, and the expression for the total free energy of the system (blobs plus ions). Second, the experimental details will be discussed. Third, the results obtained from theory and experiment will be given, which will be discussed subsequently. Finally, the main conclusions will be summarized.

2. THEORY

In this section, the theoretical framework describing the experimental system is established. First, the blob model is discussed, which describes mixtures of two different polymers in solution. Then, in order to capture the effect of charges on one of the polymers, an expression for the entropy of the ions in the system is derived for an arbitrary distribution of ions. Using this expression, the equilibrium ion distribution is found by maximizing the ion entropy. This resulting ion distribution is used to calculate the Donnan potential. Finally, the blob model is combined with the model of the ion entropy in order to account for the phase behavior of aqueous mixtures of a neutral and a charged polymer. A list of symbols is given at the end of the paper.

2.1. Blob Model. The blob model is a convenient way to describe a mixture of two polymers in solution, as it reduces a three-component system to an effective two-component system. In the blob model,^{18,26,32-34} a polymer of N segments forms blobs with typical size ξ and consists of N_b blobs. The blobs form an ideal chain, whereas the excluded volume effects are restricted to the volume inside one blob. We assume that our polyelectrolyte and uncharged polymer have the same number of segments, blob size, and interaction with the solvent. The two have an interaction with each other characterized by u(c), a concentration dependent interaction parameter. The blobs of the polyelectrolyte (at volume fraction ϕ) and

uncharged polymer (at volume fraction $1 - \phi$) together fill the whole volume. The solvent is taken into account implicitly.

The parameters ξ , $N_{\rm b}$, and u all depend on the total monomer concentration c (number of monomers per unit volume). The free energy density $f^{\rm blob}$ is given by $^{18,26,32-34}$

$$f^{\text{blob}}(c,\phi) \equiv \frac{F^{\text{blob}}(c,\phi)}{VkT}$$
(1)

$$= \frac{1}{\xi^{3}(c)} \left[\frac{\phi}{N_{b}(c)} \ln \phi + \frac{1 - \phi}{N_{b}(c)} \ln(1 - \phi) + u(c)\phi(1 - \phi) + K \right]$$
(2)

where $F^{\text{blob}}(c,\phi)$ is the free energy, *V* is the volume, *k* is the Boltzmann constant, *T* is the absolute temperature, and *K* = 0.024 is an additional constant related to the free energy of mixing of monomers within a blob.³³

The following scaling relations apply:^{18,26,33}

$$\xi(c) \simeq 0.43 R_{g} \left(\frac{c}{c^{*}}\right)^{-\nu/(3\nu-1)}$$
(3)

$$N_{\rm b}(c) = \frac{N}{c \,\xi^3(c)} \tag{4}$$

$$u(c) \simeq u_{\rm crit} \left(\frac{c}{c_{\rm crit}}\right)^{\chi/(3\nu-1)}$$
(5)

where the scaling exponents are $\nu = 3/5$ and $\chi = 0.22$ for a good solvent. The overlap concentration c^* is defined as

$$c^* = \frac{N}{\frac{4}{3}\pi R_g^3} \tag{6}$$

with the number of segments N defined by the ratio of molecular mass of the polymer and the segments:

$$N = \frac{M_{\rm w,polymer}}{M_{\rm w,monomer}}$$
(7)

Furthermore, we define the interaction energy $u(c) = u_{crit}$ at $c = c_{crit}$. In order to make the connection to the experimental system, we take c_{crit} as the monomer concentration where experimentally phase separation is just observed. u_{crit} is chosen such that the total polymer mass fraction at the critical point found from the theory matches that found from experiments at pH values near the isoelectric point of the polyelectrolyte. For comparison with experiments, the monomer concentration c is converted to the mass fraction w using the equations in section B of the Supporting Information. Other parameters are either found from experiments or obtained from theory. An overview is given in Table 1.

Knowing the free energy density of mixing, one can write down the change in the blob free energy upon demixing, $\Delta f^{\text{blob}}(c_0,\phi_0)$, where c_0 is the global monomer concentration and ϕ_0 the global volume fraction of polyelectrolyte blobs (therefore, the global volume fraction of neutral polymer blobs is $1-\phi_0$). With monomer concentrations and polyelectrolyte blob volume fractions $(c_{\alpha}\phi_{\alpha})$ and (c_{β},ϕ_{β}) in phases α and β , respectively, one obtains:

Table 1. Values of the Parameters Used in the Calculations

parameter	value	description
ν	3/5	scaling parameter ³³
χ	0.22	scaling parameter ³³
Κ	0.024	constant related to free energy of mixing within ${\rm blob}^{33}$
R _g	9.3 nm	radius of gyration, taken to be $R_{\rm g}$ of dextran ³⁵
M _{w,polymer}	100 kg/mol	average molecular mass of dextran and gelatin
$M_{ m w,monomer}$	0.1 kg/mol	approximate molecular mass of a monomer
$ ho_{ m polymer}$	1496 kg/m ³	average aqueous densities of dextran and gelatin
$\rho_{\rm solvent}$	998 kg/m ³	density of water at 20 °C ³⁶
$c_{\rm crit}$	$c(w_{\rm crit})$	calculated using eq S1 in the Supporting Information
w _{crit}	0.063	experimental mass fraction of phase separation
u _{crit}	0.03	interaction at c_{crit} ; fit parameter

$$\Delta f^{\text{blob}}(c_0, \phi_0) = \theta f^{\text{blob}}(c_\alpha, \phi_\alpha) + (1 - \theta) f^{\text{blob}}(c_\beta, \phi_\beta) - f^{\text{blob}}(c_0, \phi_0)$$
(8)

where $\theta \equiv V_{\alpha}/V$, the relative volume of phase α , for which an expression will be derived in section 2.5, under the conditions that the total amounts of polyelectrolyte and neutral polymer in the system are conserved.

2.2. Entropy of the lons. The change in entropy of the ions when the system phase separates, assuming ideal behavior, is given by

$$\Delta S^{\text{ions}} = N_{\alpha}^{+} k \ln \left(\frac{c_{0}^{+}}{c_{\alpha}^{+}} \right) + N_{\beta}^{+} k \ln \left(\frac{c_{0}^{+}}{c_{\beta}^{+}} \right) + N_{\alpha}^{-} k \ln \left(\frac{c_{0}^{-}}{c_{\alpha}^{-}} \right)$$
$$+ N_{\beta}^{-} k \ln \left(\frac{c_{0}^{-}}{c_{\beta}^{-}} \right)$$
(9)

Here N_j^i is the number of ions of type *i* in phase *j*, and $c_j^i = N_j^i/V_j$, with V_j the volume of phase *j*. The subscript 0 again refers to the global concentrations, i.e., before phase separation.

Assuming a polyelectrolyte with a number of positive charges z and global number of *chains* per unit volume c_0^p , the ion concentrations before phase separation are given by

$$c_0^+ \equiv c_s \tag{10}$$

$$c_0^{-} = c_s + z c_0^{\rm p} \tag{11}$$

taking into account electroneutrality and using c_s to denote the background salt concentration.

The concentrations after phase separation for phase α are

$$c_{\alpha}^{+} = c_{\rm s} - \frac{N_{\rm ex}}{V_{\alpha}} \tag{12}$$

$$=c_{\rm s}-\frac{c_{\rm ex}}{\theta} \tag{13}$$

$$c_{\alpha}^{-} = c_{\rm s} + z c_{\alpha}^{\rm p} - \frac{c_{\rm ex}}{\theta}$$
(14)

where the system has the freedom to move $N_{\rm ex}$ ions from phase α to β . The expelled salt concentration $c_{\rm ex}$ is defined as the number of transferred ions divided by the total volume, i.e., $c_{\rm ex} \equiv N_{\rm ex}/V$, $\theta \equiv V_{\alpha}/V$, and $c_{\alpha}^{\rm p}$ is the number of polyelectrolyte chains per unit volume in phase α . Similarly, for phase β :

$$c_{\beta}^{+} = c_{\rm s} + \frac{c_{\rm ex}}{1 - \theta} \tag{15}$$

$$c_{\beta}^{-} = c_{\rm s} + zc_{\beta}^{\rm p} + \frac{c_{\rm ex}}{1 - \theta}$$
(16)

Defining $\Delta s^{\text{ions}} \equiv \Delta S^{\text{ions}}/(Vk)$, inserting the expressions for c_j^{\pm} into eq 9, and using $c_0^{\text{p}} = c_{\alpha}^{\text{p}}\theta + c_{\beta}^{\text{p}}(1 - \theta)$, one finds

$$\Delta s^{\text{ions}} = [c_{s}\theta - c_{ex}] \ln \left(\frac{c_{s}}{c_{s} - \frac{c_{ex}}{\theta}}\right) + [(c_{s} + zc_{\alpha}^{p})\theta - c_{ex}] \ln \left(\frac{c_{s} + zc_{\alpha}^{p}\theta + zc_{\beta}^{p}(1 - \theta)}{c_{s} + zc_{\alpha}^{p} - \frac{c_{ex}}{\theta}}\right) + [c_{s}(1 - \theta) + c_{ex}] \ln \left(\frac{c_{s}}{c_{s} + \frac{c_{ex}}{1 - \theta}}\right) + [(c_{s} + zc_{\beta}^{p})(1 - \theta) + c_{ex}] \ln \left(\frac{c_{s} + zc_{\alpha}^{p}\theta + zc_{\beta}^{p}(1 - \theta)}{c_{s} + zc_{\beta}^{p} + \frac{c_{ex}}{1 - \theta}}\right)$$
(17)

Note that since we are assuming ideal behavior, the free energy change per VkT is just given by

$$\Delta f^{\rm ions} \equiv \frac{\Delta F^{\rm ions}}{VkT} = -\frac{\Delta S^{\rm ions}}{Vk} = -\Delta s^{\rm ions} \tag{18}$$

A different form of eq 17 can be found by replacing the concentrations c_j^p by the fraction of the total amount of polyelectrolyte that resides in phase α , defined as $\eta^p \equiv n_{\alpha}^p/(n_{\alpha}^p + n_{\beta}^p) = c_{\alpha}^p \theta/c_0^p$, with n_j^p the number of moles of polyelectrolyte in phase *j*. This form and its usage to obtain the approximations given in the following are described in the Supporting Information, section C.

2.3. Calculation of Equilibrium Salt Distribution. In order to find the equilibrium salt distribution in the system, we need to minimize the free energy of the ions with respect to c_{ex} . As ideal behavior is assumed, this is equivalent to maximizing the entropy of the ions. Taking the derivative of eq 17 to c_{ex} equating the resulting expression to zero, and solving for c_{ex} results in

$$c_{\text{ex}} = c_{\text{s}}\theta \frac{1-\theta}{1-2\theta} \Biggl\{ 1 + \frac{z[c_{\alpha}^{\text{p}}(1-\theta) + c_{\beta}^{\text{p}}\theta]}{2c_{\text{s}}} - \sqrt{1 + \frac{z[c_{\alpha}^{\text{p}}\theta + c_{\beta}^{\text{p}}(1-\theta)]}{c_{\text{s}}} + \Biggl[\frac{z[c_{\alpha}^{\text{p}}(1-\theta) + c_{\beta}^{\text{p}}\theta]}{2c_{\text{s}}} \Biggr]^{2}} \Biggr\}$$
(19)

where we have discarded the unphysical root that follows from solving the quadratic equation (using the condition that for $c_{\alpha}^{p} = c_{\beta}^{p}$, c_{ex} must be zero). It should be noted that eq 19 is indeterminate for $\theta = 1/2$, although this point does not have a special physical meaning. Instead, the following solution is valid for $\theta = 1/2$:

$$c_{\rm ex} = \frac{z(c_{\alpha}^{\rm p} - c_{\beta}^{\rm p})}{4} \frac{1}{2 + \frac{z(c_{\alpha}^{\rm p} + c_{\beta}^{\rm p})}{2c_{\rm s}}}$$
(20)

Thus, by inserting the appropriate expression for c_{ex} into Δs^{ions} , one obtains a complicated but analytic expression for the entropy change of the ions upon phase separation. The resulting expressions are given in the Supporting Information,

section C. A compact approximation for the change in ion entropy is given by

$$\Delta s^{\text{ions}} \simeq -\frac{\theta(1-\theta)}{2} \frac{(zc_{\alpha}^{\text{p}} - zc_{\beta}^{\text{p}})^2}{2c_{\text{s}} + z[c_{\alpha}^{\text{p}}\theta + c_{\beta}^{\text{p}}(1-\theta)]}$$
(21)

which is valid, roughly, until the polyelectrolyte charge density is comparable to the salt concentration.

2.4. Donnan Potential. In the classical Donnan equilibrium, ^{23,24,37} a compartment α containing a solution of polyelectrolyte at concentration c_{α}^{p} with number of charges z is in equilibrium with an infinitely large salt reservoir (β) with salt concentration c_{s} . The two are separated from each other by a membrane impermeable to polyelectrolyte, but permeable to small ions. In that scenario, the Donnan potential $\psi_{D} \equiv \psi_{\alpha} - \psi_{\beta}$ is given by

$$\psi_{\rm D} = \frac{RT}{F} \operatorname{arcsinh}\left(\frac{zc_{\alpha}^{\rm P}}{2c_{\rm s}}\right)$$
(22)

$$\simeq \frac{RT}{F} \frac{z c_a^P}{2 c_s} \tag{23}$$

where *R* is the gas constant and *F* is the Faraday constant. However, in our system both phases contain polyelectrolyte to some degree. Additionally, phase β is of a size comparable to phase α , instead of being infinitely large. Nevertheless, if the distribution of ions between the two phases is known, the Donnan potential can still be calculated. Assuming a Boltzmann (i.e., ideal) distribution of the ions over the two phases with potential difference ψ_{D} , one can find a general expression for the Donnan potential:

$$\psi_{\rm D} = \frac{RT}{F} \ln \left(\frac{c_{\beta}^+}{c_{\alpha}^+} \right) \tag{24}$$

$$= \frac{RT}{F} \ln \left(\frac{c_{\rm s} + \frac{c_{\rm ex}}{1 - \theta}}{c_{\rm s} - \frac{c_{\rm ex}}{\theta}} \right)$$
(25)

where the definitions $c_{\alpha}^{+} = c_{s} - c_{ex}/\theta$ and $c_{\beta}^{+} = c_{s} + c_{ex}/(1 - \theta)$ were used.

In the following, this expression will be evaluated for two situations, where the volumes of the phases are either equal or unequal.

2.4.1. Equal Phase Volumes. For the situation where $\theta = 1/2$, the expression for c_{ex} is relatively simple and given by eq 20. After some algebra, one finds:

$$\psi_{\rm D} = \frac{RT}{F} \ln \left(\frac{1 + \frac{z \iota_{\mu}^{\rm P}}{2c_{\rm s}}}{1 + \frac{z \iota_{\beta}^{\rm P}}{2c_{\rm s}}} \right)$$
(26)

It is worth noting that, upon exchanging the concentrations of the polyelectrolyte between the two phases, the sign of ψ_D reverses, but its magnitude remains the same, as expected.

A good approximation (for $|\psi_D| \lesssim 20 \text{ mV}$) can be found to be

$$\psi_{\rm D} \simeq \frac{RT}{F} \frac{z(c_{\alpha}^{\rm p} - c_{\beta}^{\rm p})}{2c_{\rm s} + \frac{1}{2}z(c_{\alpha}^{\rm p} + c_{\beta}^{\rm p})}$$
(27)

Comparing eq 27 with the classic expression, eq 23, one sees that the absence of an infinitely large salt reservoir leads to a reduction of the Donnan potential, even if there is no polyelectrolyte in phase β .

2.4.2. Unequal Phase Volumes. In the situation where the volumes of the two phases are not equal, the Donnan potential is found by inserting eq 19 into eq 25:

$$\begin{split} \psi_{\rm D} &= \frac{RT}{F} \ln \left\{ \frac{c_{\rm s}}{c_{\rm s} + zc_{\beta}^{\rm p}(1-\theta)} \left[\frac{z[c_{\alpha}^{\rm p}(1-\theta) - c_{\beta}^{\rm p}\theta]}{2c_{\rm s}} \right. \\ &+ \sqrt{1 + \frac{z[c_{\alpha}^{\rm p}\theta + c_{\beta}^{\rm p}(1-\theta)]}{c_{\rm s}} + \left[\frac{z[c_{\alpha}^{\rm p}(1-\theta) + c_{\beta}^{\rm p}\theta]}{2c_{\rm s}} \right]^2} \right] \right\} \end{split}$$
(28)

An approximation can be found for low $\psi_{\rm D}$:

$$\psi_{\rm D} \simeq \frac{RT}{F} \frac{z(c_{\alpha}^{\rm p} - c_{\beta}^{\rm p})}{2c_{\rm s} + z[c_{\alpha}^{\rm p}\theta + c_{\beta}^{\rm p}(1-\theta)]}$$
(29)

$$=\frac{RT}{F}\frac{z\Delta c^{\rm p}}{2c_{\rm s}+zc_0^{\rm p}}\tag{30}$$

where $\Delta c^{\rm p} \equiv c^{\rm p}_{\alpha} - c^{\rm p}_{\beta}$ and $c^{\rm p}_{0}$ is the global polyelectrolyte concentration. Therefore, to first order, the reduction of the Donnan potential due to the absence of a salt reservoir is equivalent to an increase of the salt concentration by $(1/2)zc^{\rm p}_{0}$ in the classic Donnan equilibrium. Additionally, it is not the absolute concentration of polyelectrolyte in one of the phases that is important, but rather the concentration difference between the two phases.

If zc_0^p is negligible with respect to c_s , this further approximates to

$$\psi_{\rm D} \simeq \frac{RT}{F} \frac{z\Delta c^{\rm p}}{2c_{\rm s}} \tag{31}$$

This expression closely resembles the classical expression: the concentration difference of the polyelectrolyte now replaces the absolute polyelectrolyte concentration.

Equation 28 can be shown to reduce to the classic Donnan equilibrium, if one takes the limit where phase β is infinitely large. To do so, one takes $\theta \rightarrow 0$ and assumes $c_{\beta}^{p} = 0$, in order to find that

$$\psi_{\rm D} = \frac{RT}{F} \ln \left[\frac{zc_{\alpha}^{\rm P}}{2c_{\rm s}} + \sqrt{1 + \left(\frac{zc_{\alpha}^{\rm P}}{2c_{\rm s}}\right)^2} \right]$$
(32)

which is indeed identical to eq 22, because $\ln[x + (1 + x^2)^{1/2}]$ is equal to arcsinh *x*.

2.5. Total Free Energy. Since we now know both the free energy change upon demixing for the blobs and for the ions, we write the total free energy change (scaled to VkT) of the system as follows:

$$\Delta f(c_0, \phi_0) = \Delta f^{\text{blob}}(c_0, \phi_0) - \Delta s^{\text{ions}}(c_\alpha^{\text{p}}, c_\beta^{\text{p}}, \theta)$$
(33)

$$= \theta f^{\text{blob}}(c_{\alpha}, \phi_{\alpha}) + (1 - \theta) f^{\text{blob}}(c_{\beta}, \phi_{\beta}) - f^{\text{blob}}(c_{0}, \phi_{0}) - \Delta s^{\text{ions}}(c_{\alpha}^{\text{p}}, c_{\beta}^{\text{p}}, \theta)$$
(34)

An expression for $\theta \equiv V_{\alpha}/V$ can be found from the conservation of mass. Suppose that the system is composed of $n^{\rm p}$ moles of polyelectrolyte and $n^{\rm u}$ moles of uncharged polymer. $x_0 = n^{\rm p}/(n^{\rm p} + n^{\rm u})$ is the global mole fraction of

polyelectrolyte with respect to the total amount of polymer, x_{α} and x_{β} similarly are the mole fractions of polyelectrolyte in each phase. The amounts of polyelectrolyte and uncharged polymer in the total system are given by $n^{p} = n^{p}_{\alpha} + n^{p}_{\beta}$ and $n^{u} = n^{u}_{\alpha} + n^{u}_{\beta}$, respectively.

For the polyelectrolyte with degree of polymerization N^p , the total amount of polyelectrolyte can be expressed as $n^p = (c_0/N^p)x_0V$. Likewise, $n^p_\alpha = (c_\alpha/N^p)x_\alpha V_\alpha$ and $n^p_\beta = (c_\beta/N^p)x_\beta V_\beta$. From this, the following expression for θ can be found:

$$\theta = \frac{c_0 x_0 - c_\beta x_\beta}{c_\alpha x_\alpha - c_\beta x_\beta} \tag{35}$$

Similarly, for the uncharged polymer

$$\theta = \frac{c_0(1 - x_0) - c_\beta(1 - x_\beta)}{c_\alpha(1 - x_\alpha) - c_\beta(1 - x_\beta)}$$
(36)

For θ to be uniquely defined, eqs 35 and 36 need to be equated and solved, which we choose to do for c_{β} , yielding

$$c_{\beta} = \frac{c_{0}c_{\alpha}(x_{0} - x_{\alpha})}{c_{0}(x_{0} - x_{\beta}) + c_{\alpha}(x_{\beta} - x_{\alpha})}$$
(37)

In other words, from the three concentrations c_0 , c_{α} , and c_{β} , we can only freely choose two due to the coupling of the conservation of mass of the two polymers via the volume of the phases. In the following, we will therefore only keep c_0 and c_{α} as independent variables. Inserting eq 37 into eq 35 or 36 gives

$$\theta = \frac{c_0(x_0 - x_\beta)}{c_\alpha(x_\alpha - x_\beta)}$$
(38)

The question now is how the mole fraction *x* relates to the volume fraction of polyelectrolyte blobs ϕ . The volume fraction ϕ_j in phase j ($j = 0, \alpha, \beta$) is given by the volume occupied by blobs of polyelectrolyte, divided by the volume occupied by blobs of polyelectrolyte and uncharged polymer:

$$\phi_{j} = \frac{N_{b,j}^{p} n_{j}^{p} \xi_{j}^{p3}}{N_{b,j}^{p} n_{j}^{p} \xi_{j}^{p3} + N_{b,j}^{u} n_{j}^{u} \xi_{j}^{u3}}$$
(39)

For generality, it is assumed that the number of blobs per polymer chain $N_{b,j}^k$ and blob size ξ_j^k depend on both the type of polymer (k = p, u) and the phase *j*. For a degree of polymerization N^k , using $N_{b,j}^k = N^k/(c_j\xi_j^k)$, $n_j^p = n_jx_j$, and $n_j^u = n_j(1 - x_j)$:

$$\phi_{j} = \frac{N^{p} x_{j}}{N^{p} x_{j} + N^{u} (1 - x_{j})}$$
(40)

We assume the degree of polymerization of the two polymers is the same, so

$$\phi_j = x_j \tag{41}$$

Therefore, $\theta = \theta(c_0, c_\alpha, \phi_0, \phi_\alpha, \phi_\beta)$, and it is given by

$$\theta = \frac{c_0(\phi_0 - \phi_\beta)}{c_\alpha(\phi_\alpha - \phi_\beta)} \tag{42}$$

The polyelectrolyte chain concentrations c^{p}_{α} and c^{p}_{β} , needed for the calculation of Δs^{ions} , can be found as follows:

$$c_{\alpha}^{\rm P} = c_{\alpha} \phi_{\alpha} / N \tag{43}$$

(44)

with c_{β} given by eq 37.

 $c_{\beta}^{\rm p} = c_{\beta} \phi_{\beta} / N$

Now including explicitly the dependences on the variables, the free energy of demixing can be expressed as

For a given global concentration c_0 and blob volume fraction ϕ_0 , the coexisting phases can be found by numerically minimizing eq 45 with respect to c_{α} ϕ_{α} and ϕ_{β} . Doing this for various concentrations enables the calculation of phase diagrams, while the application of eq 28 allows calculation of the Donnan potential. In our calculations, the global blob volume fraction will be kept constant at $\phi_0 = 0.5$, which represents a 1:1 ratio of polyelectrolyte and uncharged polymer. Changing ϕ_0 to different values would (for z > 0) shift the calculated binodal, because this would change θ , which in turn changes the salt expulsion and the associated entropy penalty. The background salt concentration c_s is taken to be either 10 mM, which will be named "low" ionic strength, or 50 mM, which will be called "high" or "increased" ionic strength.

3. EXPERIMENTAL SECTION

The preparation of samples, construction of phase diagrams, and measurement of Donnan potentials were discussed extensively in an earlier $\operatorname{article}^{27}$ and will be briefly recapitulated here.

Samples were prepared by mixing aqueous stock solutions of the uncharged polymer dextran (Sigma-Aldrich, from *Leuconostoc spp.*, 100 kDa) and the polyelectrolyte gelatin (kindly provided by Norland Products through FIB Foods, fish gelatin type A, gelling temperature 8-10 °C, high molecular weight, approximately 100 kDa) and diluting with Milli-Q water if necessary. The polymer concentrations will be reported as mass fractions. Dextran and gelatin were always mixed in a 1:1 mass ratio and, because of their nature, are both polydisperse.³⁸ The pH was adjusted if necessary using dilute hydrochloric acid or sodium hydroxide. Potassium chloride was added to a concentration of 50 mM to explore increased ionic strengths. Without the additional salt, the ionic strength of the samples was on the order of 5-10 mM as deduced from conductivity measurements. The situation with added KCl will be referred to as "high" or "increased" ionic strength, while the situation without added KCl will be named "low" ionic strength.

Fully phase separated samples were obtained by centrifuging vortexmixed solutions overnight at 100–200g. Phase-separating samples typically contained in total 10–20% polymer (by mass). The composition of each phase was determined by measuring the optical rotation at multiple wavelengths using an Anton Paar MCP 500 polarimeter.^{27,39} The gelatin-rich phase is denoted α , the dextran-rich phase β . The Donnan potential was measured electrochemically using reference electrodes.^{27,40}

The charge of the gelatin was deduced from a titration. For this titration, 1-2 g of gelatin was dissolved in approximately 80 mL Milli-Q water and, while modestly stirring, slowly titrated with dilute hydrochloric acid or sodium hydroxide from a buret while measuring the pH (Hanna Instruments pH 210 pH meter, HI 1043B electrode). Blank titrations of pure solvent were subtracted and, assuming a molecular weight of 100 kDa, the number of H⁺ or OH⁻ entities added

per polymer chain were calculated. Each added H⁺ or OH⁻ is assumed to change the number of charges per chain z by +1 or -1, respectively. As the isoelectric point (pI) of acid extracted gelatins typically lies in the pH-range 7-9,^{41,42} we assume $z \approx 0$ at pH 7.5, the inflection point of the obtained titration curve. This allows for the calculation of the absolute number of charges as a function of pH. While this approach may neglect effects such as ion condensation, it does provide an upper bound to the charge of gelatin.

4. RESULTS

In this section, first the results of the titration of the polyelectrolyte gelatin will be described. Then, experimentally measured phase diagrams will be compared with calculated phase diagrams. From the phase diagrams, the total polymer mass fraction at the critical point and the distribution of water between the phases as a function of polyelectrolyte charge will be deduced. Finally, the experimentally measured Donnan potentials published before²⁷ will be compared to the calculated Donnan potentials.

The charge of the polyelectrolyte gelatin as a function of pH, as deduced from titration, is shown in Figure 1, where it is



Figure 1. Charge of gelatin as derived from titration. The number of charges per polymer chain z was taken to be zero at pH 7.5. The solid line is a guide to the eye. The dashed vertical lines indicate the experimentally studied pH values, for which the charges are listed in Table 2.

assumed that the pI of gelatin is located at pH 7.5. The titration curve is fairly flat in the region of pH 6.5–8.5, thus the number of charges *z* obtained from the titration is not very sensitive to the precise choice of the pI. Additionally, this indicates that not many of the chargeable groups have a p K_a of around 7.5, in line with the amino acid composition of gelatin.⁴³ Outside this region, the number of charges increases more rapidly. However, given the degree of polymerization $N \approx 1000$, even for z = 20(pH 4.8) only roughly 2% of the monomers is charged, which still is a low charge density. The pH values at which the phase behavior of our system was studied experimentally are given in Table 2, together with the number of charges per gelatin chain derived from the titration.

 Table 2. Charge of Gelatin As Derived from Titration at the

 Experimentally Studied pH Values

рН	charge z
4.8	+20
6.2	+5
8.3	-1.6
8.8	-3.5
9.2	-6

Measured and calculated phase diagrams are shown in Figure 2 for various values of z. It is evident from both that, the larger the absolute number of charges |z| is, the further the coexistence line is shifted away from the origin. For small z_i only a minor shift in the binodal is observed, whereas for $z \ge 8$ the shift becomes much more pronounced. In the calculations, the shift of the binodal with z is solely due to the effects of ion entropy. The calculations also show a strong asymmetry in the phase diagram for higher z, but this is not seen as clearly in the experiments. Additionally, for higher z_i the calculations show a "gap" in the binodal, which is not seen in the experiments. The gap originates from the fact that at higher z_i , the critical value $\phi_{\rm crit}$ of the polyelectrolyte blob volume fraction is no longer equal to the global value $\phi_0 = 0.5$. Since changing ϕ_0 leads to a significant shift in the binodal for higher z, we choose to maintain $\phi_0 = 0.5$ in our calculations. (See Figures S1–S3 of the Supporting Information for calculations with different values of ϕ_{0} .) This and other observations will be addressed in more detail in the discussion.

On the basis of the phase diagrams, the total polymer mass fraction at the critical point can be obtained. For experimental phase diagrams, a straight line is fitted through the midpoints of the tie-lines and a smooth curve (using the sum of two exponentials) is fitted through the coexisting points forming the binodal. Finally, the total mass fraction at the critical point is given by the intersection of the two. For the theory, the total mass fraction at the critical point is defined as the lowest total mass fraction where demixing into two phases did lower the free energy given the set values of z, c_s , and ϕ_0 . The resulting critical mass fractions are plotted in Figure 3 as a function of |z| for low and high ionic strength. The experiments and theory show very similar trends: at low ionic strength, the critical point depends strongly on |z|, whereas at increased ionic strength this dependence is suppressed.

The phase diagrams also inform about the distribution of solvent between the two phases. In the calculations, for z = 0 the total concentration of polymer in phase α and β is the same, so that also the amount of solvent in the two phases is the same. For z > 0, the solvent starts to distribute unevenly across the two phases. In the experiments, the solvent content of the two phases is different close to the pI of the polyelectrolyte, owing to slightly different solvent affinities of the two polymers, and the solvent distribution changes when moving further away from the pI.

In order to quantify and compare this, we define the water uptake $\Delta_{water}(L,z)$ as the amount of water gained by the polyelectrolyte-rich phase at fixed tie-line length *L* with respect to a reference situation. The tie-line length, reported as a mass fraction, is defined as $L \equiv [(w_{\alpha}^{p} - w_{\beta}^{p})^{2} + (w_{\alpha}^{u} - w_{\beta}^{u})^{2}]^{1/2}$, with w_{j}^{k} the mass fraction of polyelectrolyte (p) or uncharged polymer (u) in phase α or β .

First, the difference in the total polymer mass fractions between two phases is calculated using $\Delta w \equiv w_{\alpha} - w_{\beta}$, where w_{α} and w_{β} are the total polymer mass fractions in the two phases. The water uptake Δ_{water} is then defined as $\Delta_{water} \equiv -[\Delta w(L,z) - \Delta w_{ref}(L,z_{ref})]$. Here, $\Delta w_{ref}(L,z_{ref})$ is a function given by a linear fit through the origin of $\Delta w(L,z_{ref})$ versus *L*. For the theory, $z_{ref} = 0$. As the theoretical phase behavior is perfectly symmetrical for z = 0, $\Delta w_{ref}(L,z_{ref}) = 0$. Experimentally, the phase behavior is not perfectly symmetrical close to the pI, but by taking $z_{ref} = -1.6$ (pH 8.3, the lowest experimentally studied absolute charge), we correct for this and can still visualize the net effect of charge on the uptake of water.



Figure 2. (a) Experimentally and (b) theoretically ($\phi_0 = 0.5$) determined phase diagrams at low ionic strength (5–10 mM for experiments, 10 mM for calculations). The solid lines are a guide to the eye. The open circles denote a few initial compositions, with the tie-lines drawn as dashed lines.



Figure 3. Total mass fraction at the critical point of demixing at low ionic strength (5-10 mM for experiments, 10 mM for calculations) and high ionic strength (50 mM additional KCl for experiments, 50 mM salt for calculations) as a function of the absolute charge |z| as derived from (a) experimentally measured and (b) theoretically calculated phase diagrams. The dashed lines are a guide to the eye.



Figure 4. Mass fraction of water taken up by the gelatin-rich phase as a function of the tie-line length for various charges *z* at low ionic strength (5–10 mM for experiments, 10 mM for calculations). (a) Experimentally measured water uptake taken with respect to the situation at z = -1.6 (pH 8.3). (b) Theoretically calculated uptake of water.

The water uptake calculated in this way is shown in Figure 4 for a low ionic strength. Indeed, both in experiments and theory, the polyelectrolyte-rich phase takes up more water as the absolute charge on the polyelectrolyte is increased. At high ionic strength, this effect would disappear again (not shown).

Additionally, our theory also provides Donnan potentials, which can be compared with the experimentally measured Donnan potentials published previously.²⁷ Experimental and theoretical Donnan potentials are shown in Figure 5. Again,



Figure 5. Absolute Donnan potential $|\psi_D|$ at low ionic strength (5–10 mM for experiments, 10 mM for calculations) as a function of the difference in the polyelectrolyte mass fraction for various polyelectrolyte charges *z*: (a) measured experimentally²⁷ and (b) calculated theoretically. The solid lines are a fit to eq 31.

experiment and theory follow the same trend, but the exact values are clearly different.

5. DISCUSSION

The detailed measurements of the phase diagrams of our experimental system presented in the previous section allow us to pursue a quantitative comparison with our calculations, in order to gain a deeper fundamental insight into the effect of charge and salt on the behavior of mixtures of polyelectrolyte and neutral polymer in solution.

Our theoretical description consists of the blob model to describe the behavior of polymer mixtures in solution, which is paired with the entropy of ideally behaving ions under the restriction of charge neutrality. The latter results in an entropic penalty for the ions upon phase separation, which can be calculated analytically together with the salt distribution in the system. In contrast to the classic Donnan equilibrium, our model accounts for the finite size of the system by conserving the number of ions in the system.

The theoretical model describes the trends seen experimentally rather well. The trends in the mass fraction at the critical point, the uptake of water by the polyelectrolyte-rich phase, and the Donnan potentials as a function of charge and ionic strength are certainly captured qualitatively, although there are quantitative differences.

In all cases, the effects predicted by theory are larger than observed in experiments. For instance, theory predicts a larger increase of the mass fraction at the critical point upon an increase in z than observed experimentally, which may in part be attributed to ion condensation at the polyelectrolyte, reducing its effective charge.

Another intriguing effect seen in the experiments is that, for low z, an increase in the *ionic strength* actually increases the total polymer mass fraction at the critical point, as evident from Figure 3a. This can be attributed to a change in the solvent quality (e.g., polarity) for the polymers, leading ultimately to slightly different interactions between the polymers. Our theory supposes a charge- and salt-independent interaction between the polymers.

The theoretical phase diagram shows a "gap" in the binodal for higher values of *z*. An explanation for this is that the global ratio between polyelectrolyte and neutral polymer is not the same as their ratio in the critical point for set values of *z*, *c*_s, and ϕ_0 . Therefore, the initial compositions do not intersect the binodal in the critical point and a gap in the binodal results. For z = 0, there is a 1:1 ratio of polyelectrolyte and neutral polymer (i.e., $\phi = 1/2$) in the critical point, which is equal to the global ratio used, enabling us to calculate the complete binodal. However, for z > 0, this ratio appears to be different at the critical point, so that we cannot calculate the complete binodal as we maintain a 1:1 ratio of polyelectrolyte and neutral polymer. The latter is important, because changing this ratio also changes the relative volumes of the phases. For z > 0, this change in volume would result in a change in the distribution and entropy of the ions, and therefore shift the complete binodal to different concentrations (see Supporting Information, section A). It is interesting to note that these effects of charge resemble those of polydispersity, which also results in a dependence of the binodal on the global ratio between the two polymers.⁴⁴ Experimentally, gaps in the binodal have not been observed directly and may even be masked by the polydispersity. However, it was observed experimentally that short tie-line lengths were inaccessible at high z. This is evidenced by Figure 4a, where the shortest tie-line lengths are around 2% at low z and at 5% for the highest z.

Our calculations assume that the blob size ξ depends only on concentration and not directly on charge, which is a simplification,⁴⁵ especially because the Debye length κ^{-1} is similar to the blob size ξ and electrostatic interactions between blobs are therefore not screened. However, given the very low charge densities in our system, we expect our model to be relatively unaffected by this. Further assumptions in our theory include that the polymers are identical in their uncharged state, except for their interaction with one another, and that they are monodisperse, while in fact both dextran and gelatin are highly polydisperse. Polydispersity will affect at least the shape of the phase diagram, because of the presence of low molecular weight material that does not "participate" in phase separation. Ions are assumed to behave ideally and ion condensation is neglected. It is also assumed that at the isoelectric point the gelatin is completely free of charges, while in fact there are just equal amounts of positive and negative charges, giving rise to counterions.

A relatively large difference is observed between the calculated and measured Donnan potentials. Part of these differences may be attributed to the fundamental difficulties encountered in measuring Donnan potentials,²⁷ such as due to the liquid junction and streaming potentials. Additionally, ions present in the system may have a preference for one of the

phases or even adsorb onto the polymers, affecting the experimental Donnan potentials.

Yet, despite our assumptions and simplifications, the theory captures the essential features of the experiments quite well, illustrating the dominant effect of ion entropy on the behavior of mixtures of polyelectrolytes and neutral polymers in solution. Furthermore, both in theory and experiment, the pHdependent critical point, water uptake, and Donnan potential all appear to be minimal near the isoelectric point of the polyelectrolyte, underlining their common origin.

6. CONCLUSIONS

We have presented measurements and theory on aqueous mixtures of a charged and an uncharged polymer. The behavior not only depends on the chemistry of the polymers, but also on their charge. Because of the Donnan equilibrium, the restriction of charge neutrality in each phase leads to an inhomogeneous distribution of salt over the two phases, which is entropically unfavorable. An increase in the charge of the polyelectrolyte leads therefore to a shift of the critical point to higher mass fraction, an uptake of water by the polyelectrolyte-rich phase, and an electrical potential difference at the interface, the Donnan potential. An increase in the ionic strength weakens the relative differences in the salt concentration, and thereby the entropic penalty and the observed effects. Our combination of the blob model to describe the interactions of polymers in solution and maximized ion entropy of the phase separated system works well in reproducing the experimental observations.

ASSOCIATED CONTENT

S Supporting Information

Additional expressions, derivations, and data. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Authors

*(M.V.) E-mail: M.Vis@uu.nl. *(B.H.E.) E-mail: B.H.Erne@uu.nl.

Notes

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■ LIST OF SYMBOLS

С	total monomer concentration (number per
	unit volume)
c _i	total monomer concentration in <i>j</i> (number per
,	unit volume)
c_i^k	number of chains of polymer k per unit
,	volume in <i>j</i>
Δc^{p}	$c^{\rm p}_{\alpha} - c^{\rm p}_{\beta}$, difference in number of polyelectrolyte
	chains per unit volume between α and β
$c_{\rm crit}$	critical monomer concentration, calculated
	from the experimental critical mass fraction
	of phase separation w_{crit}
c_i^i	concentration of ions of type <i>i</i> in <i>j</i>
ć,	background salt concentration

$c_{\rm ex}$	number of ion pairs transferred from phase α
	to β divided by total volume
W	mass fraction
w _i	total polymer mass fraction in <i>j</i>
w_i^k	mass fraction of polymer k in j . (Calculated
,	using eq S3 in the Supporting Information
	from c_{i} .)
Warit	experimental critical mass fraction of phase
cin	separation close to isoelectric point
Δ142	$w - w_0$ difference in total polymer mass
A <i>W</i>	$w_{\alpha} = w_{\beta}$, unreference in total polymer mass fractions between α and β
T	$[(u^{p} - u^{p})^{2} + (u^{u} - u^{u})^{2}]^{1/2}$ tig ling length
	$[(w_{\alpha} - w_{\beta}) + (w_{\alpha} - w_{\beta})]$, the interaction
n Ni	number of moles of polymer chains κ
N_j^i	number of ions of type i in j
x_j	mole fraction of polyelectrolyte in j
$\eta^{ m p}$	fraction of total amount of polyelectrolyte
	residing in phase α
ϕ	volume fraction of polyelectrolyte blobs
z	number of charges on polyelectrolyte
Ν	degree of polymerization
R _o	radius of gyration
M _{w polymer}	molar mass of polymer
M	molar mass of monomer
u(c)	interaction parameter between polyelectro-
<i>u</i> (<i>c</i>)	lyte/neutral polymer in units of kT
11	interaction parameter at c , fit parameter
u _{crit}	number of blobs nor polymor choin
ε IN _b	han de la biobs per porymer cham
ς	biod size
ν	scaling parameter
X	scaling parameter
K	constant related to free energy of mixing
	within blob
$ ho_{ m polymer}$	mass density of polymer (mass per unit
	volume)
$ ho_{ m solvent}$	mass density of solvent (mass per unit
	volume)
<i>i</i> (superscript)	type of ions, + or –
j (subscript)	in what phase (α or β), or 0 for global
k (superscript)	type of polymer (p for polyelectrolyte, u for
	uncharged polymer)
α	polyelectrolyte-rich phase
ß	neutral polymer-rich phase
r f	free energy per VkT
J	entropy per Vk
3 17	volume
v A	V/V relative volume of polyclastralists with
v	$v_{\alpha'}v_{\gamma}$ relative volume of polyelectrolyte-ficm
1	pnase P l
ĸ	Boltzmann constant
1	absolute temperature
R	gas constant
F	Faraday constant

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