Langmuir

# Donnan Potentials in Aqueous Phase-Separated Polymer Mixtures

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

[†]Utrecht University, Utrecht, The Netherlands
[‡]NIZO food research, Ede, The Netherlands

ABSTRACT: A promising approach to texturize water is by the addition of mutually incompatible polymers, leading to phase separation. Here, we demonstrate that the phase stability of aqueous polymer solutions is affected not only by chemical differences between the polymers but also by their electric charge. Direct electrochemical measurements are performed of the electric potential difference between two coexisting phases in aqueous solutions of the charged protein fish gelatin (nongelling) and the uncharged polysaccharide dextran. Charge counteracts demixing because of the entropic cost of confining the counterions to one phase, resulting in a strong shift of the critical point upon an increase of the charge on one of the polymers. Upon phase separation, the charged polymer is spatially confined, and due to the Donnan effect, an interfacial electric potential is developed. A direct proportionality is found between this Donnan potential and the difference in gelatin concentration in the two phases, for which we propose a theoretical explanation. The electrostatics may provide a new handle in the development of stable water-in-water emulsions.
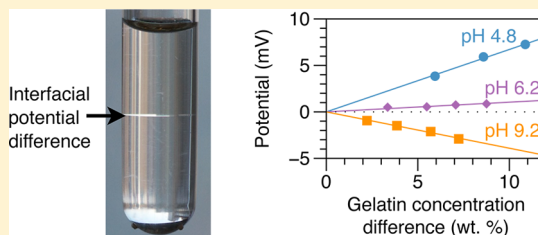
INTRODUCTION

It is well-known that mixtures of two different polymers, so-called polymer blends, tend to phase separate. In general, this is because the mixing entropy is too low to compensate for the positive mixing enthalpy. The same applies when polymers A and B are present in solution: the mixture phase separates above a critical demixing concentration. The result is either one phase rich in A and another rich in B (segregative phase separation) or one phase enriched in both polymers and one phase poor in both (associative phase separation).[1] Whether the phase separation is segregative or associative depends on the interactions between the two polymers and on the quality of the solvent for the two polymers.

Aqueous mixtures of two polymers that show segregative phase separation are often referred to as aqueous two-phase systems. Many examples exist,[2] such as PEG/dextran,[3,4] dextran/gelatin,[5−7] starch/gelatin,[8] alginate/caseinate,[9] and polystyrenesulfonate/poly(acrylic acid).[10] This type of system is, for instance, used to separate biomolecules without denaturing them,[11] but it has also been proposed as a method to obtain water-in-water emulsions.

Kinetically stable water-in-water emulsions might be used as a fat-free way to texturize food and as a way to encapsulate sensitive material such as proteins or cells. Well-defined water-in-water droplets can be prepared using microfluidics,[12−14] although the formation of the droplets is still not quite well-understood[15] and they eventually coalesce, unless stabilized by cross-linking.[16] Recent efforts to realize stable water-in-water emulsions include stabilizing the water/water interface with triblock copolymers.[17] Another route would be through the adsorption of solid particles at the water/water interface to create so-called Pickering emulsions.[18−20] This is difficult, however, because the interfacial tension of water/water interfaces is orders of magnitude lower than for regular oil/water interfaces.[21−23] Nevertheless, Nguyen et al.[24] were recently able to obtain stable emulsions in this way.

In order to understand the physics underlying the stabilization of water-in-water Pickering emulsions, we argue that it is essential to study the phase behavior of aqueous polymer mixtures and the physical properties of water/water interfaces. As already mentioned, forces underpinning the phase behavior are the affinities of the polymers for water, the nonideal mixing behavior, and differences in chain statistics. When one of the polymers is a polyelectrolyte, an additional factor that plays a role is electric charge. If one of the polymers is charged, not only will each polymer be restricted to its own phase upon phase separation but also the counterions of the charged polymer will have to be confined to one phase in order to ensure electroneutrality. This leads to a greater entropic cost for phase separation: the critical point of mixing is expected to shift to higher concentrations as the charge on one of the polymers is increased.

As the polyelectrolyte is spatially restricted, the phase-separated mixture is expected to carry an interfacial electric potential difference. This potential difference has the same origin as the membrane potential that develops when a salt reservoir is separated by a dialysis membrane from a dispersion of charged colloids or polyelectrolytes. It is due to the Donnan equilibrium,[25,26] and the associated interfacial or membrane potential is called the Donnan potential. Donnan potentials in aqueous two-phase systems have been studied before and have even been used for the separation of charged biomolecules.[27,28]

However, the Donnan potentials in these systems are due to the addition of salts composed of cations and anions with different partitioning coefficients and not due to charge on one of the polymers.[29,30] The relationships between polymer charge, Donnan potential, and phase behavior of aqueous polymer mixtures have to our knowledge not been studied before.

Here, we study the quantitative effect of charge on the phase behavior of aqueous mixtures of dextran and gelatin in terms of the Donnan potential. Dextran is an uncharged, branched polysaccharide, while gelatin is a partially hydrolyzed form of the protein collagen with a pH-dependent charge. We use acid-extracted nongelling fish gelatin, which has an isoelectric point at approximately pH 8.[31] Below this pH, the gelatin is positively charged, whereas at higher pH, it is negatively charged. Besides the possibility to vary the charge on one of the polymers, other reasons to choose this system are relevance in the food industry, absence of complexation, and well-defined phase separation, as water is a good solvent for both polymers.

Measuring the electric potential difference between the two phases is a challenge because the Donnan potential cannot be measured directly. If one would insert an identical reversible electrode in each phase, the potential difference between the two electrodes must be zero, as a system in thermodynamic equilibrium cannot do work. A common approach to circumvent this problem is to use reference electrodes, connected via a salt bridge to each phase. As will be explained later, this method measures a quantity related to the Donnan potential: the Donnan electromotive force (emf). However, under our experimental conditions, it can be shown that the Donnan emf is equivalent to the Donnan potential.[32,33] We did not measure the ζ-potential of droplets of one phase suspended in the other since it is the Donnan potential that is directly related to the equilibrium phase behavior, whereas the ζ-potential can even have a different sign, depending on the position of the shear plane.[29]

The presence of a Donnan potential in aqueous two-phase systems is expected to be important for the stability of water-in-water emulsions. This interfacial electric potential may have an impact on droplet coalescence, the interfacial tension, and the adsorption of charged colloidal particles for the preparation of water-in-water Pickering emulsions.

This paper is organized as follows. First, we present our theory for the Donnan potential at the liquid/liquid interface of phase-separated polymer mixtures. Second, experimental details are given regarding the preparation of samples, the determination of phase compositions and construction of phase diagrams, and the measurement of the Donnan potential. Third, measured Donnan potentials, phase behavior, and their correlation are presented. The paper ends with a general discussion and concluding remarks.

■ THEORY

Classically, an expression for the Donnan potential is derived for a system that consists of a solution of a polyelectrolyte in equilibrium with a salt reservoir.[25,26,34] The polyelectrolyte solution with concentration c^P and number of charges z (sign included) and the reservoir containing a monovalent salt with concentration c_s are separated from each other by a semipermeable membrane. The membrane is permeable to the small ions and solvent but impermeable to the polyelectrolyte, such that the salt reservoir does not contain any polyelectrolyte. For such systems, the Donnan potential is given by

$$\psi_D = \frac{RT}{F}\operatorname{arcsinh}\left(\frac{zc^P}{2c_s}\right) \tag{1}$$

$$\simeq \frac{RT}{F}\frac{zc^P}{2c_s} \tag{2}$$

with R the gas constant, T the absolute temperature, and F the Faraday constant.

This model does not apply directly to our system, which consists of two phases that both contain the polyelectrolyte gelatin at different concentrations. Both phases also contain dextran to varying degree, but since that is an uncharged polymer, it does not affect the Donnan equilibrium. In the following, the derivation of the Donnan potential will be briefly revisited for our system.

Suppose that there are two phases α and β, where α is rich in the polyelectrolyte. The phases have a potential difference $\psi_D = \psi_\alpha - \psi_\beta$. In equilibrium, the concentration of the monovalent cations and anions in phase i, $c_i^\pm$, will be given by

$$c_\alpha^+ = c_\beta^+ \exp\left(-\frac{\psi_D F}{RT}\right) \tag{3a}$$

$$c_\alpha^- = c_\beta^- \exp\left(+\frac{\psi_D F}{RT}\right) \tag{3b}$$

For simplicity, y is defined as $y \equiv \exp(+\psi_D F/RT) \equiv \exp(\Phi)$, with Φ the dimensionless Donnan potential.

The concentration of polyelectrolyte in phase i is denoted as c_i^P. Let us assume that the polyelectrolyte has a positive number of charges z per polymer chain. Overall, each phase should be electrically neutral, thus $c_\alpha^+ + zc_\alpha^P = c_\alpha^-$ and $c_\beta^+ + zc_\beta^P = c_\beta^-$. This can be combined with eqs 3a and 3b to yield

$$c_\beta^+ y^{-1} + zc_\alpha^P = (c_\beta^+ + zc_\beta^P)y \tag{4}$$

Solving for y gives

$$y = \frac{zc_\alpha^P}{2(c_\beta^+ + zc_\beta^P)} + \sqrt{\left(\frac{zc_\alpha^P}{2(c_\beta^+ + zc_\beta^P)}\right)^2 + \frac{c_\beta^+}{c_\beta^+ + zc_\beta^P}} \tag{5}$$

Assuming that in both phases the charge density due to the polyelectrolyte is smaller than that of the other ions (i.e., $zc_\alpha^P \ll c_\beta^+$ and $zc_\beta^P \ll c_\beta^+$), eq 5 can be simplified. The first term can be approximated to first order as $zc_\alpha^P/[2(c_\beta^+ + zc_\beta^P)] \simeq zc_\alpha^P/(2c_\beta^+)$. When approximating the square root term to first order, the term $\{zc_\alpha^P/[2(c_\beta^+ + zc_\beta^P)]\}^2$ gives zero, while $c_\beta^+/(c_\beta^+ + zc_\beta^P) \simeq 1 - zc_\alpha^P/c_\beta^+$. Thus

$$\sqrt{\left(\frac{zc_\alpha^P}{2(c_\beta^+ + zc_\beta^P)}\right)^2 + \frac{c_\beta^+}{c_\beta^+ + zc_\beta^P}} \simeq \sqrt{1 - \frac{zc_\beta^P}{c_\beta^+}} \tag{6}$$

$$\simeq 1 - \frac{zc_\beta^P}{2c_\beta^+} \tag{7}$$

Combining the two approximations gives the following linearized expression for y:

$$y \simeq 1 + \frac{z(c_\alpha^{\mathrm{P}} - c_\beta^{\mathrm{P}})}{2c_\beta^+} \tag{8}$$

However, as $\Phi \equiv \ln y$

$$\Phi = \ln\left(1 + \frac{z(c_\alpha^{\mathrm{P}} - c_\beta^{\mathrm{P}})}{2c_\beta^+}\right) \tag{9}$$

which can be linearized again using the relation $\ln(1 + x) \simeq x$:

$$\Phi \simeq \frac{z(c_\alpha^{\mathrm{P}} - c_\beta^{\mathrm{P}})}{2c_\beta^+} \tag{10}$$

Defining $\Delta c_{\mathrm{Gt}} \equiv c_\alpha^{\mathrm{P}} - c_\beta^{\mathrm{P}}$ and making the approximation $c_\beta^+ \approx c_\beta^- = c_s$, the Donnan potential ψ_{D} can be expressed as

$$\psi_{\mathrm{D}} \simeq \frac{RT}{F} \frac{z \Delta c_{\mathrm{Gt}}}{2c_s} \tag{11}$$

This expression is remarkably similar to eq 2. By plotting eqs 5 and 11, it can be shown that eq 11 describes the Donnan potential within 1 mV even if the polyelectrolyte charge density in the polyelectrolyte-rich phase is as high as the salt concentration in the polyelectrolyte-poor phase, that is, $zc_\alpha^{\mathrm{P}} \approx c_\beta^+$, resulting in Donnan potentials of the order of 10 mV. Our experiments fall well within this limit.

From eq 11, it can be concluded that if, for samples with increasing concentrations, both the Donnan potential and gelatin concentration difference were measured, a plot of ψ_{D} versus Δc_{Gt} should give a straight line through the origin, with a slope proportional to the charge of the gelatin. In addition, eq 11 also predicts that the Donnan potential should carry the same sign as the charge z of the gelatin.

■ EXPERIMENTAL SECTION

Sample Preparation. Stock solutions of dextran (Dx) were prepared by weighing the desired amounts of dextran (Sigma-Aldrich, from *Leuconostoc* spp., 100 kDa) and Milli-Q water. The dextran was dissolved by shaking the mixture at room temperature. Stock solutions of gelatin (Gt) were prepared similarly, except that the gelatin (Multiproducts Amersfoort, fish gelatin type A, gelling temperature 8−10 °C, approximately 100 kDa) was dissolved using a warm water bath of 60 °C under magnetic stirring for approximately 30 min. This resulted in a solution with a pH of 6.2. For experiments at a different pH, part of the Milli-Q water in the gelatin stock solution was replaced by a dilute solution of HCl or NaOH. No buffer was used, as the gelatin itself already acts as a buffer. No preservatives were added because an agent like sodium azide might affect the electrochemical measurements. For experiments at increased ionic strength, the dextran and gelatin were dissolved in a KCl solution instead of Milli-Q water. For samples without additional salt, the ionic strength was approximately 5−10 mM due to salt present in (mainly) the gelatin. The polymer content of solutions will be, unless otherwise mentioned, expressed as mass fractions. For instance, the mass fraction of dextran is defined as $w_{\mathrm{Dx}} = m_{\mathrm{Dx}}/\sum_i m_i$, with m_i the total mass of component i. Typically, stock solutions had a polymer fraction of 10−20%.

Samples were prepared by adding weighed amounts of the stock solutions and diluting (if necessary) with Milli-Q water, followed by vortex mixing. The pH and conductivity of the freshly mixed samples, which were turbid due to the onset of phase separation, were measured using a double junction pH electrode (Hanna Instruments pH 210 pH meter, HI 1043B electrode) and a four-electrode conductivity probe (WTW inoLab Cond Level 1, TetraCon 325 electrode). The ionic strengths of the samples were then deduced from conductivities measured on KCl solutions of known concentrations. The samples were centrifuged overnight at 20 °C at 100−200g (with the higher speeds for the more concentrated, viscous samples) to obtain fully

phase-separated samples. For each sample, the bottom phase was denoted as α and the top phase as β. Which of the two phases was dextran-rich and which one was gelatin-rich depends on the circumstances such as pH, salt, and even polymer concentration. The overall dextran and gelatin mass fractions were kept equal to each other in all samples within an absolute deviation of 0.05%.

After a sample was phase-separated, part of each phase was collected. First, as much as possible of the top phase was isolated using a syringe with hypodermic needle. Afterward, a fresh syringe with hypodermic needle was carefully lowered through the interface into the bottom phase, avoiding mixing of the phases. After the interface had recovered to its equilibrium position, part of the bottom phase was collected, as well.

Stock solutions and isolated phases were stored at 1 °C. Before reuse, they were heated again in a water bath of 60 °C for 15 min and subsequently allowed to cool to room temperature for the gelatin to lose its helical gel structure.[35] Stock solutions and isolated phases were kept for a maximum of 1 week.

Determination of Phase Composition and Construction of Phase Diagrams. Since dextran and gelatin are both optically active, polarimetry was used to measure the mass fractions of dextran and gelatin in each phase of a phase-separated sample. As the optical rotation was measured at more than one wavelength, the mass fractions of dextran and gelatin could be found simultaneously in the coexisting phases, thus enabling the construction of phase diagrams.[7,36]

Part of an isolated phase, obtained as described before, was diluted by a known factor with water to contain approximately 2% polymer. The optical rotation was measured at four different wavelengths (365, 436, 546, and 589 nm) on an Anton Paar MCP 500 polarimeter thermostated at 20.00 °C in a sample cell with a length of 1.0000 dm. The angular accuracy of this instrument is better than ±0.002°.

The optical rotation α^{λ_i} measured at wavelength λ_i is the sum of the optical rotations contributed by both dextran and gelatin:

$$\alpha^{\lambda_i} = [\alpha]_{\mathrm{Dx}}^{\lambda_i} l w_{\mathrm{Dx}} + [\alpha]_{\mathrm{Gt}}^{\lambda_i} l w_{\mathrm{Gt}} \tag{12}$$

where $[\alpha]_{\mathrm{Dx}}^{\lambda_i}$ and $[\alpha]_{\mathrm{Gt}}^{\lambda_i}$ are the specific rotations per mass fraction of dextran and gelatin with units of deg/dm at wavelength λ_i; w_{Dx} and w_{Gt} are the mass fractions of dextran and gelatin, and $l = 1$ dm is the length of the sample cell.

Under the condition that the ratio between the specific rotations of dextran and gelatin changes sufficiently as a function of wavelength, the mass fractions of dextran and gelatin can be determined from the optical rotation measured at a pair of wavelengths (λ_i and λ_j with $i \neq j$) as follows:

$$w_{\mathrm{Dx}}(\lambda_i, \lambda_j) = \frac{1}{l}\left(\frac{\alpha^{\lambda_j}[\alpha]_{\mathrm{Gt}}^{\lambda_i} - \alpha^{\lambda_i}[\alpha]_{\mathrm{Gt}}^{\lambda_j}}{[\alpha]_{\mathrm{Dx}}^{\lambda_j}[\alpha]_{\mathrm{Gt}}^{\lambda_i} - [\alpha]_{\mathrm{Dx}}^{\lambda_i}[\alpha]_{\mathrm{Gt}}^{\lambda_j}}\right) \tag{13a}$$

$$w_{\mathrm{Gt}}(\lambda_i, \lambda_j) = \frac{1}{l}\left(\frac{\alpha^{\lambda_i}[\alpha]_{\mathrm{Dx}}^{\lambda_j} - \alpha^{\lambda_j}[\alpha]_{\mathrm{Dx}}^{\lambda_i}}{[\alpha]_{\mathrm{Dx}}^{\lambda_j}[\alpha]_{\mathrm{Gt}}^{\lambda_i} - [\alpha]_{\mathrm{Dx}}^{\lambda_i}[\alpha]_{\mathrm{Gt}}^{\lambda_j}}\right) \tag{13b}$$

As we have measured the optical rotation at four wavelengths, there are six sets of two different wavelengths. The mass fractions were computed for each pair, and the average and standard deviations are calculated for each phase, taking into account the dilution. The difference in the mass fraction of gelatin in the two phases α and β of a sample is defined as $\Delta w_{\mathrm{Gt}} = |w_{\mathrm{Gt}}^\alpha - w_{\mathrm{Gt}}^\beta|$. Its standard deviation is given by $s_{\Delta w_{\mathrm{Gt}}} = (s_{w_{\mathrm{Gt}}^\alpha}^2 + s_{w_{\mathrm{Gt}}^\beta}^2)^{1/2}$, where $s_{w_{\mathrm{Gt}}^\alpha}$ and $s_{w_{\mathrm{Gt}}^\beta}$ are the standard deviations of the gelatin mass fractions for phases α and β.

The specific rotations of dextran and gelatin were obtained by measuring the optical rotations of aqueous solutions containing only dextran or gelatin of various mass fractions and performing a least-squares fit to the equation $\alpha_j^{\lambda_i} = [\alpha]_j^{\lambda_i} l w_j$. The resulting specific rotations are given in Table 1. We verified that, under our experimental conditions, the specific rotation is not affected by the addition of salt or a change in pH. Further, by measuring the mass fractions in dilute non-phase-separating aqueous mixtures of dextran and gelatin of known composition at various pH values and ionic

Table 1. Measured Specific Rotations $[\alpha]_j^{\lambda_i}$ of the Aqueous Dextran and Gelatin Used in This Study and Their Ratio $[\alpha]_{Dx}^{\lambda_i}/[\alpha]_{Gt}^{\lambda_i}$ at Various Wavelengths λ_i

	specific rotation (deg/dm)		
λ_i (nm)	dextran	gelatin	ratio
365	+544	−369	−1.47
436	+358	−226	−1.59
546	+216	−129	−1.68
589	+183	−108	−1.70

strengths, we verified that the mass fractions that we find in this way are indeed the actual mass fractions of dextran and gelatin, within the margin of error indicated by the standard deviation.

Donnan Potential Measurements. Donnan potential measurements were carried out in a U-shaped glass tube, which was filled with the α-phase from one side and subsequently with the β-phase from the other side, after having isolated the phases as described before. Two identical Ag/AgCl reference electrodes were used in order to measure the potential difference. The key aspect here is that reference electrodes have, in principle, a well-defined and fixed potential difference with their surrounding solution. The potential difference between the two phases could be found by immersing one reference electrode in each phase, as schematically shown in Figure 1a, and comparing the electric potential difference to a baseline recorded when both electrodes were immersed in the same 100 mM KCl solution.

Radiometer Analytical REF200 Ag/AgCl electrodes were used. These electrodes consist of a silver wire coated with silver chloride, immersed in a saturated potassium chloride solution. The solution is kept saturated by the presence of solid potassium chloride. The electrochemical half reaction is $Ag(s) + Cl^-(aq) \rightleftarrows AgCl(s) + e^-$. Because the concentration of Cl^- is constant, the electrochemical potential of the electrons is fixed with respect to the KCl solution. This KCl solution, in turn, was in contact via a small porous plug with the phase in which the electrode was immersed and leaked at about 10 μL per hour. Because the transport numbers of K^+ and Cl^- are nearly equal,[37] the liquid junction potential with the polymer solution is close

to zero. Additionally, because the saturated KCl solution was much more concentrated than the sample solution, the liquid junction potential is dominated by the electrode solution and therefore nearly independent of the sample composition.[38] The effect of streaming potentials, caused by liquid flow through the porous plug, was minimized by keeping the flow rates of the two reference electrodes as similar as possible. This was accomplished by having both electrodes filled to the same height with the saturated KCl solution and having the electrodes immersed at the same depth in both phases. In order to check that the measured potential would be symmetric with respect to the baseline, during each measurement the electrodes were swapped between the two phases. An asymmetric response to swapping would point to a failure of the earlier discussed assumptions. Before the electrodes were immersed in a solution, they were cleaned by rinsing with plentiful amounts of Milli-Q water and dabbed dry with a tissue.

It should be noted that, strictly speaking, measurements performed in this way by definition only yield the Donnan emf, which includes, for instance, the contributions of the liquid junction potentials. However, it can be shown that for systems that behave ideally (e.g., low charge density of the polymer chain, not too low salt concentration in the system, high salt concentration in the reference electrodes, identical transport numbers for K^+ and Cl^-) the Donnan emf is the same as the Donnan potential.[32,33] This method has been employed successfully before in the determination of Donnan potentials in a system of sedimenting charged silica spheres in ethanol.[39] An alternative approach would be with two capillary reference electrodes, one of which would cross the liquid/liquid interface,[27−29] but we found that puncturing the interface perturbs it and causes mixing in a way that can lead to lengthy equilibration times.

The electric potentials were recorded as a function of time at an interval of 0.2 s using a Metrohm Autolab PGSTAT100 setup. One reference electrode (A) was connected to both the working electrode (WE) and sense (S) leads, whereas the other reference electrode (B) was connected to the counter electrode (CE) and reference electrode (RE) leads. All measurements were carried out in a grounded Faraday cage, connected to the ground of the instrument, which is crucial to obtain reliable measurements. The experimentally determined Donnan potentials will be reported with respect to the dextran-rich phase.
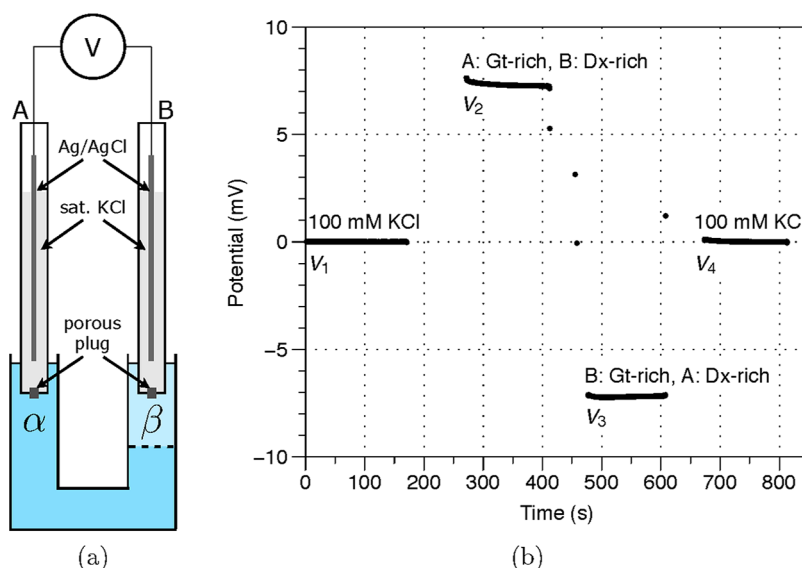

Figure 1. (a) Schematic representation of the setup for the measurement of the Donnan potential at the interface of demixed aqueous solutions of dextran and gelatin. A U-shaped tube is first filled with the heaviest phase (α), and then the lighter phase (β) is added. The Donnan potential is determined by inserting silver/silver chloride reference electrodes A and B at each end of the tube and measuring the potential difference $V_A − V_B$. (b) Example of a Donnan potential measurement on a phase-separated sample containing 7.5% dextran and 7.5% gelatin at pH 4.8 and a salt concentration of 9.4 mM. At $t = 0$ s, the two reference electrodes are immersed in a solution of 100 mM KCl; at $t \approx 275$ s, one reference electrode is immersed in each phase; at $t \approx 480$ s, the electrodes are reversed; and finally at $t \approx 680$ s, the electrodes are immersed again in 100 mM KCl. After each equilibration, the potential reaches a stable value, used for calculation of the Donnan potential.
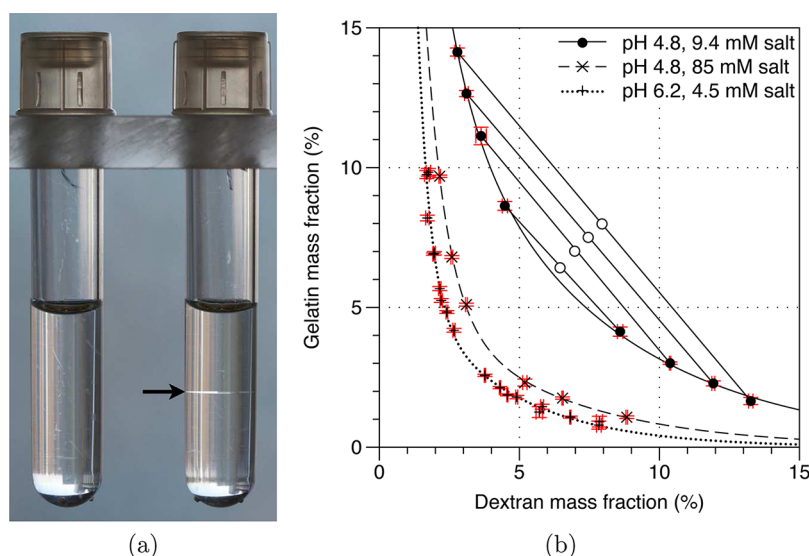
Figure 2. Phase behavior of aqueous mixtures of dextran and gelatin. The critical point depends strongly on pH and salt concentration. (a) Photograph of samples below and above the critical point, with dextran/gelatin mass fractions of (left) 6.0/5.9% and (right) 6.5/6.4% at pH 4.7 and a salt concentration of approximately 10 mM. The position of the water/water interface is indicated by an arrow. (b) Phase diagrams. At low salt concentrations (~5−10 mM), the critical point shifts to higher concentrations when the charge of the gelatin is increased by lowering the pH. Addition of salt brings the critical point back to lower concentrations. For pH 4.8, the overall composition of the samples is shown (open circles) as well as the corresponding tie lines. The curves are a guide for the eye.

■ RESULTS

In this section, first an example of a measurement of the Donnan potential will be described. Next, the influence of the pH and ionic strength on the phase behavior will be presented. Finally, measurements of the Donnan potential as a function of polymer concentration for three different pH values will be shown in order to illustrate the relation between the phase behavior and the Donnan potential.

An example of a Donnan potential measurement is shown in Figure 1b, for a system consisting of 7.5% dextran and 7.5% gelatin at pH 4.8 and a salt concentration of 9.4 mM. The electric potential is recorded as a function of the time t and the measurement consists of four parts:

- $t = 0$ s: The reference electrodes are immersed in the same 100 mM KCl solution to record the baseline potential $V_1 = \langle V_A - V_B \rangle = 0.0121 \pm 0.0018$ mV.
- $t \approx 275$ s: Electrode A is immersed in the gelatin-rich phase and electrode B in the dextran-rich phase to obtain $V_2 = \langle V_A - V_B \rangle = +7.31 \pm 0.04$ mV.
- $t \approx 480$ s: The electrodes are reversed, with electrode B now immersed in the gelatin-rich phase and A in the dextran-rich phase, which gives $V_3 = \langle V_A - V_B \rangle = -7.204 \pm 0.019$ mV.
- $t \approx 680$ s: A final baseline is recorded by immersing both electrodes in 100 mM KCl, $V_4 = \langle V_A - V_B \rangle = +0.019 \pm 0.018$ mV.

For each part of the measurement, the potential is first allowed to stabilize before it is averaged over at least 1 min. Using the differences between the values of V_i, the Donnan potential can be computed in three different ways: $\psi_1 = V_2 - V_1$, $\psi_2 = (V_2 - V_3)/2$, and $\psi_3 = V_4 - V_3$. Ideally, these measurements should be identical, whereas a strong asymmetry would point to, for instance, unstable liquid junction potentials. In practice, nearly symmetrical behavior is observed in all our experiments. For the present example, $\psi_1 = +7.30$ mV, $\psi_2 = +7.26$ mV, and $\psi_3 = +7.22$ mV were found. The Donnan

potential ψ_D is calculated as the average and standard deviation of ψ_1, ψ_2, and ψ_3, so in this case $\psi_D = +7.26 \pm 0.04$ mV.

Phase diagrams for aqueous mixtures of dextran and gelatin were measured at pH 6.2 (c_s = 4.5 mM) and pH 4.8 (c_s = 9.4 mM and 85 mM) and are shown in Figure 2, together with a photograph of a homogeneous and phase-separated mixture. At pH 6.2, close to the isoelectric point of the gelatin (pI ∼ 8), the gelatin is expected to be weakly positively charged. From the phase diagram, it can be found that the critical point is at a total polymer mass fraction of 6.3%.

A large shift of the binodal is observed when the pH is decreased to 4.8, farther away from the isoelectric point of gelatin. This is in line with expectations, as the gelatin is now more strongly positively charged, which makes phase separation more difficult due to the entropy of the counterions. The critical point in this situation is shifted to 12.3%. When in addition the salt concentration is increased by an order of magnitude, the binodal shifts back close to the situation at pH 6.2 with low ionic strength. This is also reflected in the critical point, now at 7.5%.

Measurements of the Donnan potential were carried out as a function of the total polymer mass fraction at pH 4.8, 6.2, and 9.2 at salt concentrations of on average 9.4, 4.5, and 6.7 mM. The results are depicted in Figure 3, where the approximate position of the critical point (determined from phase diagrams such as shown in Figure 2b) is indicated by the points at 0 mV. At pH 6.2, a positive Donnan potential that increases from 0.5 to 0.9 mV with increasing polymer content is found. When the gelatin becomes more strongly charged by changing the pH to 4.8, the Donnan potential increases by an order of magnitude. When the pH is increased above the isoelectric point of gelatin, a negative Donnan potential is observed, as expected from the change in sign of the charge of the gelatin. While the phase separation is "delayed" to much higher concentrations if the gelatin is more strongly charged, these measurements show that the Donnan potential actually increases much more rapidly as a function of concentration under these circumstances.
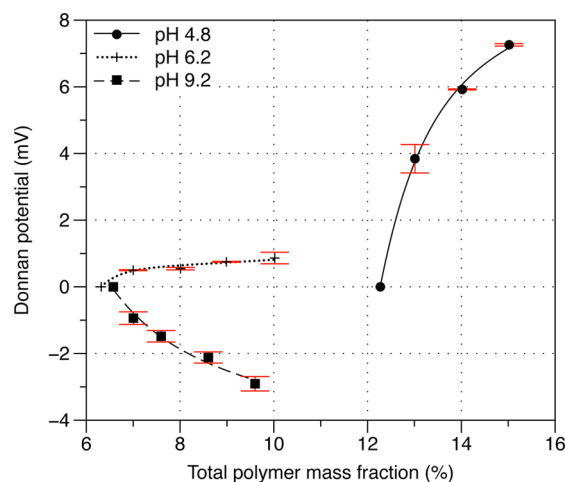
Figure 3. Measurements of the Donnan potential at low salt (∼5−10 mM) as a function of the total polymer fraction for various pH values. Below the isoelectric point (pI) of gelatin, a positive Donnan potential is observed, whereas above the pI, a negative Donnan potential is found. For each curve, the point at $\psi_D = 0$ mV is based on the position of the critical point. The lines are to guide the eye.

DISCUSSION

The main initial premise of our study was that there should be an electric potential difference at the spontaneously formed interface obtained by demixing of aqueous solutions of dextran and gelatin. The charged polymer is spatially confined, so that an electric potential difference is expected on the basis of the Donnan equilibrium. Our measurements confirm that this is indeed the case. Here we discuss the relation with the observed phase behavior and the implications for the stabilization of water/water interfaces.

According to eq 11, the magnitude of the Donnan potential should be directly proportional to the difference in gelatin concentration Δc_{Gt} in the two phases. As the composition of the coexisting phases has been analyzed for each sample, a plot of the Donnan potential versus the difference in the gelatin mass fraction Δw_{Gt} can be constructed. As shown in Figure 4, the experimentally measured Donnan potential is indeed directly proportional to the difference in the gelatin mass fraction, in agreement with our theoretical prediction.

From eq 11, the slopes $\partial \psi_D / \partial \Delta w_{Gt}$ in Figure 4 inform about the charge z of the gelatin. Taking into account the known salt concentration, the molecular weight of gelatin (100 kDa), and a density of the solutions of approximately 1 g/cm³, the calculated charge of gelatin for the three pH values is given in Table 2. Qualitatively, the results conform well to measurements of the electrophoretic mobility of this type of gelatin known from literature.[31] Quantitative comparisons are much more difficult, as it is not trivial to convert the electrophoretic mobility into the absolute charge of a particle or molecule.

Our measurements clearly show that the charge of gelatin has a strong influence on the phase behavior of aqueous mixtures of dextran and gelatin and that these effects largely disappear on the addition of salt. It also leads to a measurable interfacial potential difference, and the magnitude of this Donnan potential evidently correlates to the critical demixing concentration. In order to understand the phase behavior, one can consider the role of the counterions. If a mixture phase separates, the counterions associated with the charged gelatin
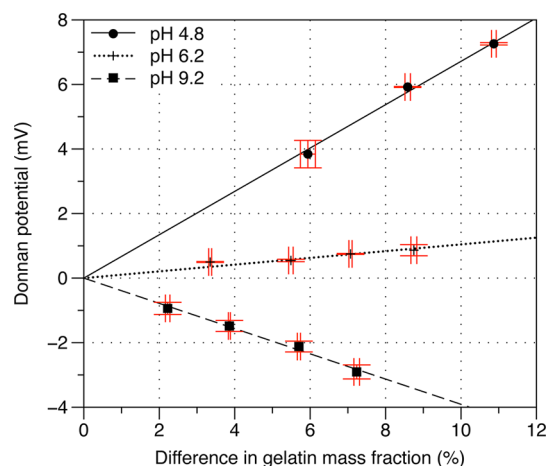

Figure 4. Measurements of the Donnan potential at low salt (∼5−10 mM) as a function of the difference in the mass fraction of gelatin in the two phases for various pH values. Below the pI, a positive Donnan potential is observed, whereas above the pI, a negative Donnan potential is found. The lines are linear fits through the origin.

Table 2. Charge of Gelatin As Calculated from Donnan Potential Measurements Using Equation 11 at Three pH Values[a]

pH	$\partial \psi_D / \partial \Delta w_{Gt}$ (mV)	$\langle c_s \rangle$ (mM)	z
4.8	67	9.4	4.9
6.2	10	4.5	0.4
9.2	−39	6.7	−2.0

[a]The charge is calculated from the slope $\partial \psi_D / \partial \Delta w_{Gt}$ of the fit through the experimental data in Figure 4 and the average salt concentration $\langle c_s \rangle$ for each concentration series.

also have to be confined in one phase, which leads to an entropically unfavorable difference in concentration of ions in the two phases. For an increased salt concentration, the relative concentration difference is smaller and the influence of charge on the phase behavior is greatly reduced. Therefore, it is the concentration ratio of counterions to background salt that is important for the phase behavior.

The presence of a Donnan potential may also have other implications, for instance, for the coalescence of droplets during phase separation. If droplets carry an interfacial electric potential due to the Donnan effect, their coalescence may be impeded. Even without an electrical potential difference, there will be an energy barrier for the fusion of two droplets. The presence of an electrical potential gradient will only increase this barrier.

One may also speculate whether the interfacial potential has an influence on the interfacial tension. On one hand, the interfacial potential may lead to an increase in the interfacial width, leading to smaller concentration gradients and thus a lower interfacial tension. On the other hand, the interfacial tension might increase because of the energy stored in the interfacial electrical double layer, which basically functions as a capacitor. Finally, the presence of an interfacial potential difference may affect the stiffness and preferential curvature of the interface[40] and consequently droplet stability.

CONCLUSIONS

We have shown that the phase separation of aqueous polymer mixtures depends not only on chemical differences between the

polymers but also on their charge. Important for the phase behavior is the concentration ratio of counterions to background salt. The influence of charge manifests itself through an interfacial electric potential difference, which develops spontaneously together with the interface and which can be described on the basis of thermodynamic equilibrium. The Donnan potential could be measured directly and scales as expected from theory with the difference in concentration of the charged polymer in the two phases. The interfacial potential difference may have an influence on various interfacial phenomena, such as droplet coalescence, particle adsorption, and the interfacial tension, and our results warrant future research in these areas.

■ AUTHOR INFORMATION

Corresponding Authors
*E-mail: m.vis@uu.nl.
*E-mail: b.h.erne@uu.nl.
Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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