

Salt-dependent phase behavior of aqueous dextran
sulfate/polyethylene glycol mixtures

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Abstract

Aqueous two-phase systems of neutral polymers with polyelectrolytes were investigated. Aqueous solutions of dextran sulfate (DS) and polyethylene glycol (PEG) were found to phase separate at sufficiently high concentrations. The critical point of phase separation was observed to depend on the salt concentration, as was expected from theory. Construction of phase diagrams of the system at different NaCl concentrations was attempted with polarimetry and density measurements to determine DS and PEG concentrations respectively. While the DS concentrations obtained by polarimetry are reliable, PEG determination by density measurements proved to be problematic due to a high sensitivity to random and systematic error.

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1 Introduction

Polymers are frequently immiscible and thus, in a mixture of two polymers, phase separation can occur. For polymers the entropy gained by mixing is generally not large enough to compensate for the positive mixing enthalpy, due to the large molecular size[1]. Phase separation can also happen when two or more polymers are present in an (aqueous) solution, yielding an aqueous two-phase system (ATPS). These ATPS have much in common with conventional oil/water two phase systems, but also have peculiar properties due to the fact that both phases share a common solvent.

Emulsions of ATPS could have interesting applications in the food industry, providing a novel way of food texturing while avoiding the high caloric content associated with oil-water systems[2]. The creation of these aqueous emulsions is difficult, however, because of the low liquid/liquid interfacial tension, facilitating coagulation and thereby greatly reducing emulsion stability. Furthermore, adsorption of stabilizing agents on the interface is non-trivial due to this low interfacial tension and the small differences in chemical constitution of the two phases. If one of the polymers is charged, these difficulties could possibly be alleviated through Coulombic interactions coming into play and through the emergence of an electric potential difference, the Donnan potential, between the phases, as Mark Vis et al. have recently demonstrated[2]. Knowledge of the behavior of these charged systems is therefore essential for the development of stabilized water-in-water emulsions.

When phase separation confines a polyelectrolyte in a single phase, it also confines its counterions in one of the phases due to the requirement of charge neutrality. Due to this spatial restriction of the counterions phase separation is even more entropically unfavorable. Therefore, the higher the net charge on the polyelectrolyte, the more difficult phase separation will become and the critical point of phase separation (the minimal concentrations at which phase separation will occur) will shift to higher polymer concentrations.

Whether a polymer mixture phase separates is also determined by a number of other factors. Salt concentration plays a major role: presumably the presence of additional ions greatly decreases the entropy cost of spatial confinement of the polyelectrolyte and thus facilitates phase separation. As the additional ions introduced by adding salt can be freely exchanged for the counterions of the polyelectrolyte, confining ions in one phase requires a less strict decrease of the number of available configurations for the system.

Another important factor is the molecular weight of the polymers that are used. Polymers of low enough molecular weight will in general tend to mix well because of the higher entropy gain from mixing: thus, phase separation is difficult[1]. Polymers of high molecular weight will generally phase separate more easily, but can also tend to precipitate out of solution or raise viscosity problems.

The aim of this bachelor thesis is to find an ATPS with a neutral polymer and a highly charged polyelectrolyte that will phase separate at high salt, and then to characterize the salt dependence of the critical point of phase separation. Firstly the theory underlying phase separation of polymer solutions in general and of systems of two polymers (one of which is a polyelectrolyte) in particular is discussed. Then, after some exploratory experiments have been described, the preparation of aqueous two-phase systems containing polyethylene glycol and dextran sulfate and the determination of the phase properties of the dextran sulfate/polyethylene glycol system are discussed in more detail, after which some concluding remarks and suggestions for follow-up experiments are given.

2 Theory

2.1 Aqueous two-phase systems

Mixtures of two polymers (without solvent) can be described semi-quantitatively by the Flory-Huggins model[3]. From this model the following expression for the free energy can be derived:

$$\frac{F(\phi)}{VkT} = \frac{1}{a^3} \left[\frac{\phi}{N} \ln \phi + \frac{1-\phi}{N} \ln(1-\phi) + \chi\phi(1-\phi) \right], \quad (1)$$

in which χ is a dimensionless enthalpic parameter, describing the interaction between the two polymers, ϕ is the volume fraction of one of the polymers with respect to the other polymer (whose volume fraction, consequentially, is $1-\phi$), a is the length of a polymer segment, N is the number of segments (assumed equal for both polymers), V is the total volume of the mixture, k is Boltzmann's constant and T is the temperature.

The model can be extended to include solvent implicitly with the so-called 'blob model', by describing the polymer not in terms of monomers or polymer segments, but as a collection of spheres (blobs) and by making the number of blobs, the blob volume and the enthalpic interaction parameter dependent on total polymer concentration. A full discussion of the blob model is beyond the scope of this work and the predictions of this model are qualitatively equivalent to the predictions of Flory-Huggins theory for the present purposes.

The free energy from expression 1 can be plotted with respect to the volume fraction of one of the two polymers. In figure 1 such plots are shown for $\chi = 2$ and for $\chi = 3$ with all other parameters set to 1.

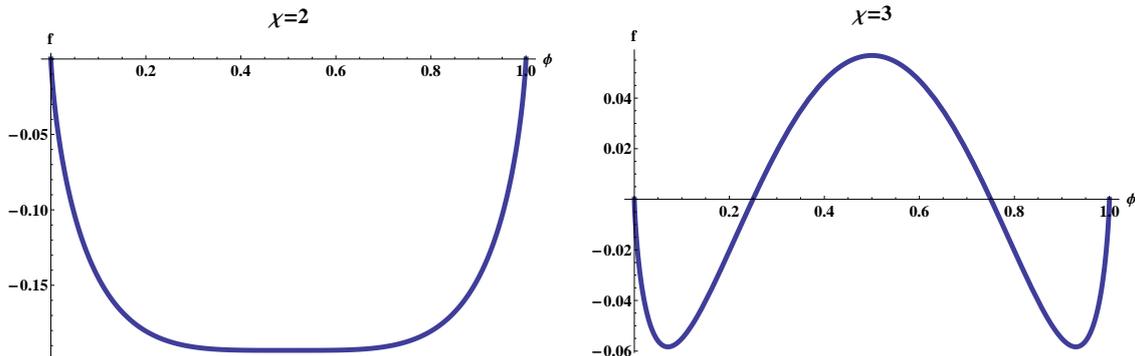


Figure 1: Plots of the free energy of a solution of two polymers as calculated with eq. 1, with $\chi = 2$ and $\chi = 3$.

In reality, the free energy curve will be asymmetrical and dependent on other factors. For example, the length of the polymers will in general not be equal. Qualitatively, however, the same general concepts apply.

As the enthalpic contribution χ increases, an energy maximum appears at $\phi = 0.5$, together with two energy minima around this maximum. A system with ϕ between the two minima can minimize its free energy by demixing into two phases with energies at the two minima. The contribution of the enthalpy of polymer-polymer interactions to the free energy of the system increases with polymer concentration. Thus, there is a critical point of polymer concentrations above which the system will spontaneously phase-separate, provided that the enthalpic penalty can be made large enough with respect to the entropy of mixing.

From equation 1 it can also directly be concluded that the entropy of mixing decreases with increasing polymer length, and thus the length (or, equivalently, the molecular weight) of a polymer is also a factor that will decide whether phase separation will occur.

2.2 Effects in polyelectrolyte phase separation

When one of the two polymers in an ATPS is a polyelectrolyte, counterions are also introduced to the system. Assuming that the polyelectrolyte itself behaves like a normal polymer, the most important additional factor is the entropy cost associated with the confinement of these counterions in a single phase. This entropy cost will hinder phase separation and will thus presumably shift the critical point towards higher polymer concentrations.

The entropic cost for ion confinement can be calculated by relating the entropy of the ions before and after phase separation. This yields the following formula[4], with N_n^i the number of ions of sign i in phase n :

$$\frac{\Delta S}{k} = N_\alpha^+ \ln \left(\frac{N_0^+}{N_\alpha^+} \right) + N_\beta^+ \ln \left(\frac{N_0^+}{N_\beta^+} \right) + N_\alpha^- \ln \left(\frac{N_0^-}{N_\alpha^-} \right) + N_\beta^- \ln \left(\frac{N_0^-}{N_\beta^-} \right). \quad (2)$$

The fractions inside the logarithms can equivalently be expressed as fractions of concentrations and then the concentrations can be expressed in terms of the concentrations of the individual components:

$$\begin{aligned} \frac{\Delta S}{k} = & N_\alpha^+ \ln \left(\frac{c_s}{c_s - \frac{c_{\text{ex}}}{v}} \right) + N_\beta^+ \ln \left(\frac{c_s}{c_s + \frac{c_{\text{ex}}}{1-v}} \right) \\ & + N_\alpha^- \ln \left(\frac{c_s + zc_0^{\text{p}}}{c_s + zc_\alpha^{\text{p}} - \frac{c_{\text{ex}}}{v}} \right) + N_\beta^- \ln \left(\frac{c_s + zc_0^{\text{p}}}{c_s + zc_\beta^{\text{p}} + \frac{c_{\text{ex}}}{1-v}} \right) \end{aligned} \quad (3)$$

where c_s is the background salt concentration, c_0^{p} is the polyelectrolyte concentration before phase separation, c_j^{p} is the polyelectrolyte concentration in phase $j (= \alpha, \beta)$ after phase separation, z is the charge per molecule of polyelectrolyte, c_{ex} is a factor that takes the possibility of an unequal distribution of salt over the two phases into account and v is the volume of phase α with respect to the total volume.

As the salt concentration c_s goes up, the fractions in the logarithms in equation 3 become increasingly dominated by the background salt concentrations and the entropic contribution of other ions (such as the polyelectrolyte counterions) vanishes:

$$\frac{c_s + \dots}{c_s + \dots} \rightarrow 1 \Rightarrow \frac{\Delta S}{k} \rightarrow 0. \quad (4)$$

Therefore, the entropic penalty for the counterions associated with phase separation is predicted to be reduced by background salt.

2.3 Determination of optically active compounds by polarimetry

Chiral compounds in solution rotate the polarization direction of light. The magnitude of the optical rotation depends on the chemical species of the optically active solute, the path length of the light through the sample and the wavelength of the incident light beam. Most importantly, the effect depends linearly on the concentration of solute present in the sample. It is assumed that, at least in the dilute regime, the concentration can also be specified as a mass fraction. Then the optical rotation of a solution of an optically active species in a measurement cell is given by:

$$\alpha = [\alpha]wl \quad (5)$$

with α the optical rotation of the sample, $[\alpha]$ the specific rotation of the solute, w the mass fraction of optically active solute in the sample and l the path length of light through the sample.

By regression analysis of a reference concentration series the unknown concentration of the same solute in a sample can be determined.

2.4 Determination of polymer concentrations by density measurements

We assume that the density of a solution of polymers is additive in good approximation, or equivalently, the total volume of a sample depends only on the respective volumes of the constituent components, without additional volume contraction. In such cases, the density of a solution with components a and b in solvent s (including background salt) is given by:

$$\rho = \frac{m_a + m_b + m_s}{V_a + V_b + V_s} \quad (6)$$

with m_x and V_x the mass and volume of component x respectively. This can be rewritten as:

$$\rho = \frac{1}{\frac{V_a}{m_{\text{tot}}} + \frac{V_b}{m_{\text{tot}}} + \frac{V_s}{m_{\text{tot}}}} = \frac{1}{\frac{w_a}{\rho_a} + \frac{w_b}{\rho_b} + \frac{1-w_a-w_b}{\rho_s}} \quad (7)$$

with w_x the mass fraction of component x with density ρ_x . For the mass fraction of the polymer components this ultimately yields:

$$w_a = \frac{\rho_a \rho_b (\rho - \rho_s) + w_b \rho \rho_a (\rho_s - \rho_b)}{\rho \rho_b (\rho_a - \rho_s)} \quad (8)$$

When the mass fraction of one of the polymers in a solution of two polymers is known, this formula provides a means of determining the mass fraction of the other polymer.

The (effective) densities of the polymers can be extracted by regression analysis from a concentration series of solutions of that polymer. This can be shown by considering equation 7 where the mass fraction of one of the components is zero. Then, the expression can be rewritten to:

$$\rho = \frac{1 - \rho_s}{\rho_a} w_a \rho + \rho_s. \quad (9)$$

When the measured ρ is plotted against ρw_a , the density of the solvent (including salt) is the y-intercept of the fitted curves. Furthermore, the density of the polymer ρ_a is given by the following expression:

$$\rho_a = \frac{\rho_s}{1 - a_p} \quad (10)$$

with a_p the slope of the fitted curve.

The density of the solvent can also be measured by simply measuring the density of pure salt stock.

3 Experimental

Salt stock solutions were prepared by weighing the appropriate amount of salt on an analytical balance and dissolving this in MQ water in a volumetric flask, resulting in solutions with a maximum deviation of 1% of the specified molarity.

3.1 Exploratory experiments

Samples were prepared by weighing polystyrene sulfonate (70 kDa), dextran (500 kDa), polyethylene glycol (6 kDa, 35 kDa), polyethylene oxide (600 kDa) or dextran sulfate (500 kDa) in glass vials and adding the appropriate amount of salt stock.

3.2 Semiquantitative determination of the salt dependence of the critical point

10% polymer stocks were prepared by weighing the polymer in a glass vial or bottle on an analytical balance and adding the appropriate amount of salt stock.

Samples were prepared by weighing the appropriate amounts of polymer stocks in small centrifuge tubes on an analytical balance and diluting with salt stock. The samples were then centrifuged if necessary and left overnight.

3.3 Construction of a dextran sulfate/polyethylene glycol phase diagram

Polymer stocks were prepared by weighing the contents in a glass vial or bottle on an analytical balance and adding the appropriate amount of salt stock.

Phase separating mixtures of differing total polymer content in 1 M NaCl and in 0.1 M NaCl were obtained by transferring the appropriate amounts of polymer stocks with a Pasteur pipette to large centrifuge tubes in a 2:1 weight ratio (dextran sulfate:polyethylene glycol) and diluting with NaCl(aq) stock to the desired concentrations. After vortex mixing the samples were equilibrated by leaving them overnight (for the samples in 1 M NaCl) or by overnight centrifugation (for the samples in 0.1 M NaCl).

After phase separation, the upper phases were transferred to separate vials with a Pasteur pipette and the lower phases were carefully transferred with a syringe. Samples were diluted with the appropriate salt stock when the volume obtained was insufficient for density measurements.

Density measurements were performed with an Anton Paar DMA 5000 density meter thermostated to 20.00 °C.

Small volumes of the samples (as diluted for density measurement if applicable) were taken and diluted with MQ water to adjust the concentration to the concentration range that is desirable for polarimetric measurements. Some of the PEG-rich phases were not diluted further as the expected DS concentration was already low and the available sample volume was sufficient. Optical rotation measurements were performed with an Anton Paar MCP 500 polarimeter thermostated to 20.00 °C.

4 Results & discussion

4.1 Exploratory experiments

Solutions of different polymer systems were prepared. The polymers utilized were polystyrene sulfonate (PSS), dextran (DX), dextran sulfate (DS) and polyethylene glycol/polyethylene oxide (PEG). The choice of polymer systems was dictated by availability of the polymers and partially by ease of analysis: PSS concentrations can readily be determined by UV-VIS spectroscopy and DX and DS can be quantitatively determined by optical rotation measurements.

A highly concentrated (20%/20%, all percentages are weight percentages) solution of PSS (70 kDa) and dextran (500 kDa) was prepared in 2 M KCl (aq), resulting in a yellow, viscous, homogeneous solution. No phase separation was observed. As these salt and polymer concentrations were already fairly extreme, no phase separation was expected under any feasible circumstances in the polystyrene sulfonate/dextran system. Therefore, this system was abandoned for further research.

A highly concentrated (20%/20%) sample with 1:1 dextran (500 kDa) and dextran sulfate (500 kDa) was also prepared in 2 M KCl, yielding a turbid, viscous mixture containing undissolved polymer material. More samples at lower polymer and salt concentrations were prepared, but the solubility problem persisted. In all cases no phase separation was observed, but when a sample containing approximately 5% of both dextran and dextran sulfate was viewed under a microscope the mixture turned out to contain small bubbles that slowly coalesced, implying that some form of phase separation was taking place, which was unexpected due to the chemical similarity of the two polymers. A photograph is shown in Figure 2.

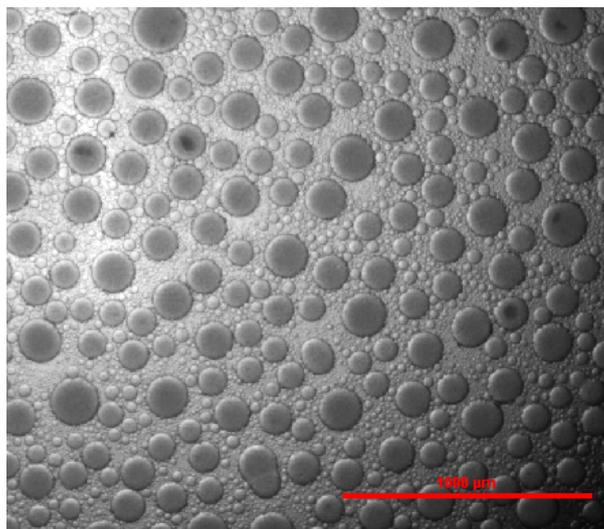


Figure 2: Dextran (5%) and dextran sulfate (5%) in 0.25 M KCl viewed under a microscope.

This could be liquid-liquid phase separation or precipitation of solid dextran or dextran sulfate. Due to the problematic viscosity of the solutions, the observed solubility issues and the lack of well-observed large scale phase separation, this system was also abandoned.

A series of aqueous solutions of dextran sulfate (500 kDa) and polyethylene glycol (6000 Da, 35 kDa) or polyethylene oxide (600 kDa) in water was prepared at several concentrations of KCl between 0.1 M and 2 M and polymer concentrations between 1% and 5%. At KCl concentrations of around 0.5 M and higher, dextran sulfate ceased to dissolve fully at polymer concentrations of approximately 5%. Phase separation was observed in some instances at 0.25 M KCl when

PEG of 35 kDa molecular weight was used, but this solubility limit of dextran sulfate reduced the usefulness of the system. When NaCl was substituted for KCl, this problem was resolved. Reduced solubility of dextran sulfate in aqueous KCl solutions was found to be described in literature[5]. Also, as the Na salt of dextran sulfate was used, using NaCl instead of KCl as the background salt has the advantage of not introducing an additional species of cations to the system, thereby ruling out the possibility of any effects caused by cation exchange.

When PEG was used with a molecular weight of 6000 Da, no phase separation was observed. This has to do with the fact that polymers of low molecular weight generally have a high entropy of mixing, which impedes phase separation. Samples containing 600 kDa PEG were of troublesome viscosity and were hard to homogenize. Samples with 35 kDa PEG readily phase separated and PEG with this molecular weight was used for all further experiments.

4.2 Semiquantitative determination of the salt dependence of the critical point

Four series of aqueous solutions of PEG and DS were prepared, with NaCl concentrations of 0.1 M, 0.25 M, 0.5 M, and 1 M respectively.

Photographs were made of the phase separated samples. These photos are shown in Figures 3, 4, 5, and 6.

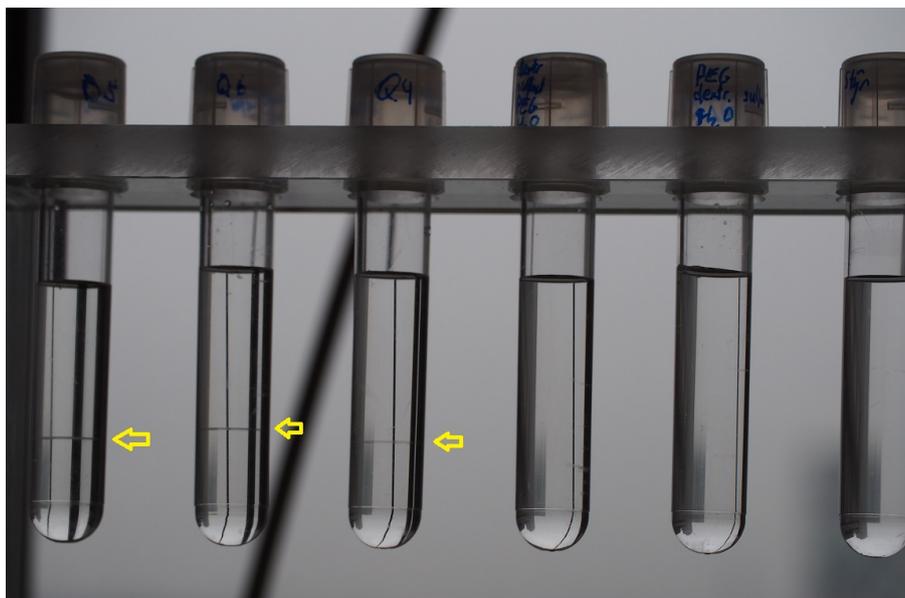


Figure 3: Equilibrated samples at a NaCl concentration of 0.1 M. DS and PEG concentrations from left to right: 10.1%/10.2%, 7.7%/7.3%, 6.0%/6.1%, 5.0%/6.0%, 4.2%/4.3%, 3.1%/3.1%. Interfaces are marked with a yellow arrow.

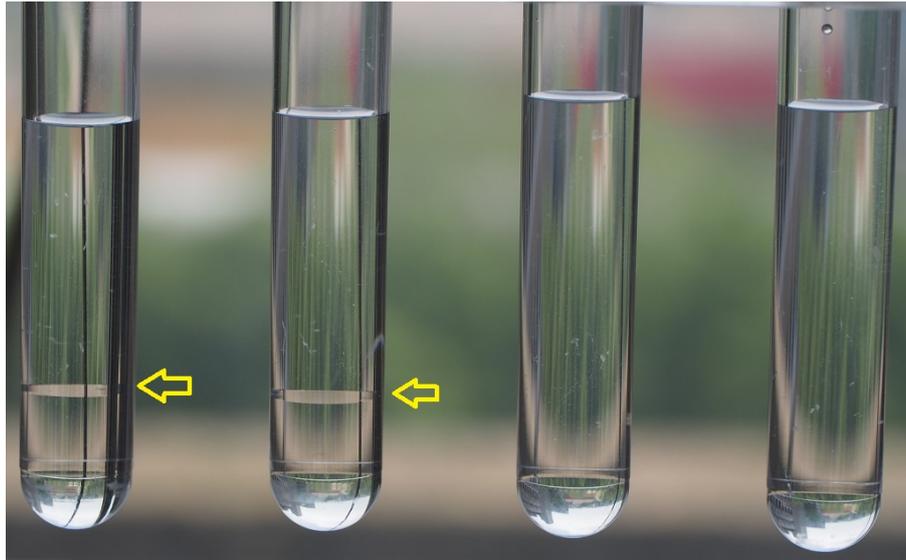


Figure 4: Equilibrated samples at a NaCl concentration of 0.25 M. DS and PEG concentrations from left to right: 3.9%/4.2%, 3.0%/3.1%, 2.0%/2.1%, 0.9%/1.0%. Interfaces are marked with a yellow arrow.

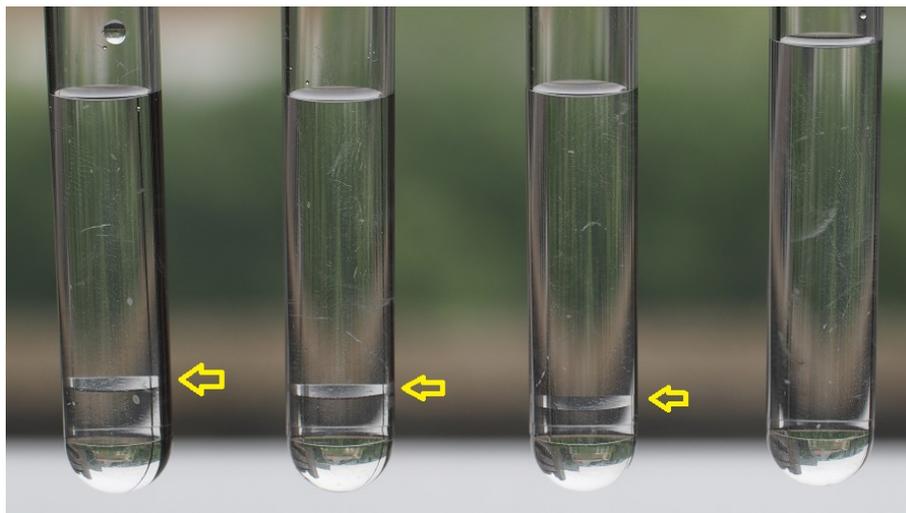


Figure 5: Equilibrated samples at a NaCl concentration of 0.5 M. DS and PEG concentrations from left to right: 4.1%/4.0%, 3.2%/3.0%, 2.1%/2.1%, 1.0%/1.0%. Interfaces are marked with a yellow arrow.

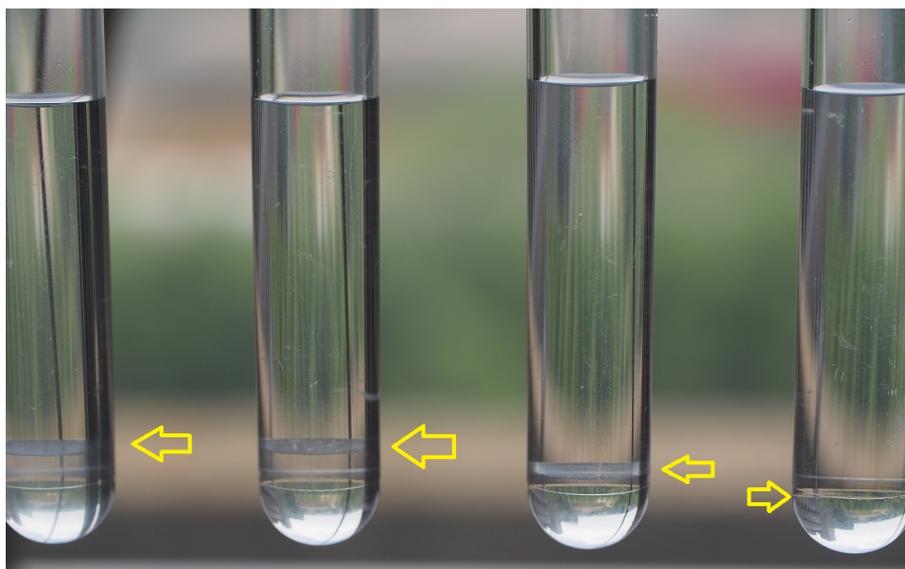


Figure 6: Equilibrated samples at a NaCl concentration of 1 M. DS and PEG concentrations from left to right: 3.9%/3.8%, 3.1%/2.8%, 1.9%/1.8%, 1.0%/1.2%. Interfaces are marked with a yellow arrow.

The critical point shifts from around 6% (of both polymers) at 0.1 M NaCl to around 2-3% at 0.25 M NaCl to around 1-2% at 0.5M NaCl to below 1% at 1 M NaCl. This shift to lower concentrations at higher salt concentration is what was expected from theory. Due to the small sample volumes, the ratio of DS to PEG could not be kept as constant as would be desirable for these experiments, but the trend is clear and the effects observed are large enough to ascertain the salt dependence of the critical point.

At 1 M NaCl, a series of samples was prepared in which approximately the same total polymer content of 6% was maintained, but the ratio of DS to PEG was varied from 1:5 to 5.5:0.5. After equilibration all samples had phase separated and the volume ratio of the fractions was found to shift from approximately 1:1 for a DS:PEG ratio of 5.6:0.5 to a system with a very large upper phase and a very small lower phase at a DS:PEG ratio of 1.0:5.1. This indicates that the upper phase is the PEG-rich phase and the lower phase is the DS-rich phase.

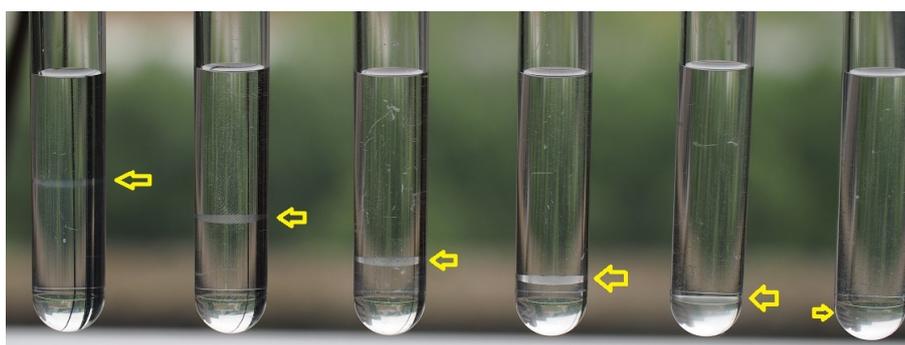


Figure 7: Equilibrated samples at a NaCl concentration of 1 M. DS and PEG concentrations from left to right: 5.6%/0.5%, 5.0%/1.0%, 4.0%/2.0%, 3.1%/2.8%, 2.0%/4.1%, 1.0%/5.1%. Interfaces are marked with a yellow arrow.

The asymmetric phase volume ratio, even at a 1:1 DS:PEG ratio, is due to the fact that the

molecular weights of the polymers are not equal.

It was also observed that the relative volume of the DS-rich phase increases somewhat with decreasing salt concentration. This could be due to an entropic effect, as the low salt concentration increases the entropic contribution of the counterions confined in the DS-rich phase and these ions strive to increase their entropy by obtaining as large a volume as possible.

4.3 Phase diagram construction by polarimetry and density measurements

Two series of phase-separating dextran sulfate/PEG samples, in 1 M NaCl(aq) and in 0.1 M NaCl(aq), were prepared, with a 2:1 DS:PEG mass ratio. The initial mass fractions are given in table 1.

	w_{DS} (%)	w_{PEG} (%)	DS.:PEG
1A	1.501	0.747	2.011
1B	2.002	0.995	2.006
1C	3.002	1.497	2.008
1D	3.982	1.983	2.011

Table 1: Mass fractions of DS and PEG in the phase separated samples in 1 M NaCl and the ratio of the two polymers.

	w_{DS} (%)	w_{PEG} (%)	DS:PEG
2A	9.989	4.999	1.998
2B	11.151	5.606	1.989
2C	12.255	6.110	2.005

Table 2: Mass fractions of DS and PEG in the phase separated samples in 0.1 M NaCl and the ratio of the two polymers.

After equilibration, the separate phases were collected and the DS and PEG concentrations were analyzed by polarimetry and density measurements respectively.

4.3.1 [DS] determination by polarimetry

From regression analysis of a concentration series of DS solutions in MQ water in a concentration range of 0.2 - 1.0 mass percent, a specific rotation of 0.8868 ± 0.0010 degrees per weight percent per dm was determined. The reference curve (shown in figure 8) has $R^2 = 0.999992$. This curve shows that the random error in the optical rotation is small. The full data of the reference curve are given in appendix B.

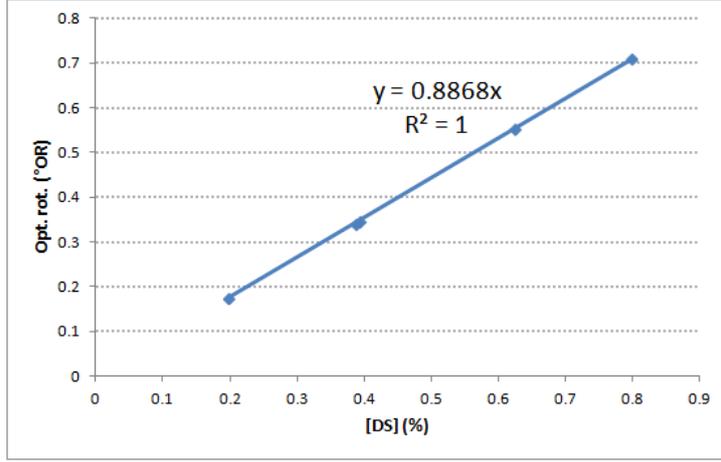


Figure 8: Curve fit for a reference concentration series for polarimetry ($R^2 = 0.999992$).

A few control samples in 1 M NaCl, containing a known concentration of DS, were analyzed to ensure that the presence of salt had no significant influence on the optical rotation. The concentration that was determined did not deviate significantly from the known concentration.

The samples of all phases were diluted first with the proper salt stock if the volume that was obtained was insufficient to work with. The density of these samples was determined as will be described later. The samples were then diluted further with MQ water to obtain samples suitable for polarimetry. From the obtained optical rotations, the phase DS mass fractions were calculated according to the following equation, derived from equation 5:

$$w_{\text{phase}} = \frac{\alpha}{[\alpha]l} \frac{m_{\text{samp},1} + m_{\text{H}_2\text{O},1}}{m_{\text{samp},1}} \frac{m_{\text{samp},2} + m_{\text{H}_2\text{O},2}}{m_{\text{samp},2}} \quad (11)$$

in which $m_{\text{samp},n}$ and $m_{\text{H}_2\text{O},n}$ are the masses of the sample and H_2O /salt stock respectively, for the n th dilution step.

The standard deviations of the mass fractions were calculated from:

$$\begin{aligned} \sigma_w^2 = & \left(\frac{\partial w}{\partial \alpha} \right)^2 \sigma_\alpha^2 + \left(\frac{\partial w}{\partial [\alpha]} \right)^2 \sigma_{[\alpha]}^2 + \left(\frac{\partial w}{\partial m_{\text{samp},1}} \right)^2 \sigma_{m_{\text{samp},1}}^2 + \left(\frac{\partial w}{\partial m_{\text{H}_2\text{O},1}} \right)^2 \sigma_{m_{\text{H}_2\text{O},1}}^2 \\ & + \left(\frac{\partial w}{\partial m_{\text{samp},2}} \right)^2 \sigma_{m_{\text{samp},2}}^2 + \left(\frac{\partial w}{\partial m_{\text{H}_2\text{O},2}} \right)^2 \sigma_{m_{\text{H}_2\text{O},2}}^2 \end{aligned} \quad (12)$$

where the terms corresponding to dilution steps were left out if necessary. The error in the cell length was neglected. The error in the specific rotation $[\alpha]$ was 0.0010 degrees per mass percent per dm, obtained from the regression statistics. The error in the dilution masses were estimated to be 0.0005 g and the error in the measured optical rotation was estimated to be 0.001 degrees.

The measured optical rotations for the diluted samples, the dilution and the calculated original DS concentrations in all phases with their standard deviation are given in table 3. The DS concentrations in the phase separated samples are displayed graphically in figures 9 and 10.

		OR ($^{\circ}$)	Dilution	$w_{\text{DS}} \pm \sigma_w$
1A	(upper)	1.0774	1.00	1.2149 ± 0.0015
	(lower)	0.3592	21.00	8.506 ± 0.015
1B	(upper)	0.9523	1.00	1.0738 ± 0.0013
	(lower)	0.5151	15.13	8.787 ± 0.013
1C	(upper)	0.6167	1.00	0.6954 ± 0.0010
	(lower)	2.5516	4.23	12.170 ± 0.014
1D	(upper)	0.1540	2.95	0.512 ± 0.0018
	(lower)	1.4363	10.54	17.074 ± 0.020
2A	(upper)	0.0661	10.46	0.779 ± 0.006
	(lower)	1.5000	10.36	17.522 ± 0.021
2B	(upper)	0.0711	10.53	0.845 ± 0.006
	(lower)	1.7193	10.33	20.018 ± 0.024
2C	(upper)	0.1683	10.05	1.908 ± 0.006
	(lower)	0.3154	10.78	22.077 ± 0.026

Table 3: Optical rotations (in degrees), dilution (sample weight divided by original weight) and phase DS concentration for all phases of the two series of phase separated mixtures.

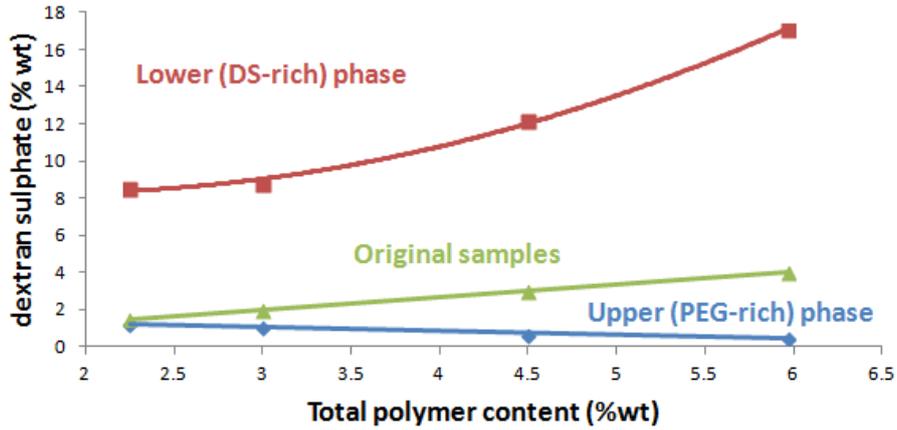


Figure 9: DS concentrations of the phase separated samples at 1 M NaCl as measured by polarimetry.

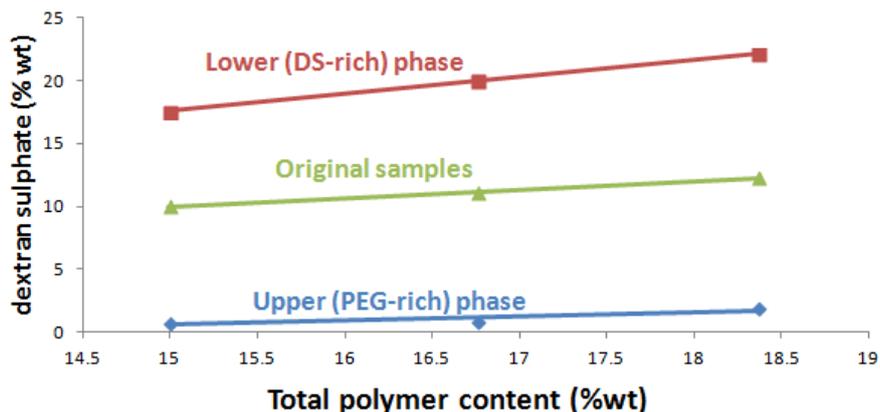


Figure 10: DS concentrations of the phase separated samples at 0.1 M NaCl as measured by polarimetry.

These results are as expected, with DS concentrations increasing with increasing total polymer content in the lower (DS-rich) phases, and decreasing in the upper (PEG-rich) phases, except in the upper phases of the 0.1 M NaCl series, where the DS concentration apparently increases together with the PEG concentration, which is anomalous for an equilibrated system. These deviations could be caused by experimental inaccuracies, such as small disturbances of the equilibrium with the lower phase upon collection of the samples from the systems, having a large effect on the small initial DS concentration. It is also possible that the results are caused by a real physical effect, due to the low NaCl concentration and the higher total polymer concentration. The exact cause of these results is not known.

In the other results, no peculiarities are found. Systematic error could arise if either the polarimeter is improperly gauged or the dextran sulfate used is impure in the first place. As the concentration series still yields an accurately fitted reference curve and the optical rotation itself is not of interest, the first situation would not be problematic. The use of impure DS would, however, cause a systematic overestimation of the DS concentrations in the system due to the samples used to obtain the reference curve containing less DS than nominally reported. The DS which was used has a reported minimal purity of 95%, which is acceptable in this context.

4.3.2 [PEG] determination by density measurements

Four concentration series of polymers in aqueous NaCl solutions were prepared; two series of PEG samples, one in 0.1 M NaCl ($R^2 = 0.999974$) and one in 1 M NaCl ($R^2 = 0.999980$), and two series of DS samples, one in 0.1 M NaCl ($R^2 = 0.999536$) and one in 1 M NaCl ($R^2 = 0.999863$). Curves were fitted to the measured densities and from these fitted curves the densities of DS, PEG, 0.1 M NaCl (aq) and 1 M NaCl (aq) were determined.

From the series in 0.1 M NaCl:

- $\rho_s = 1.002 \pm 0.001 \text{ g cm}^{-3}$
- $\rho_{\text{PEG}} = 1.202212 \text{ g cm}^{-3}$
- $\rho_{\text{DS}} = 2.051812 \text{ g cm}^{-3}$

From the series in 1 M NaCl:

- $\rho_s = 1.0385 \pm 0.0001 \text{ g cm}^{-3}$

- $\rho_{PEG} = 1.194561 \text{ g cm}^{-3}$
- $\rho_{DS} = 2.011547 \text{ g cm}^{-3}$

The solvent densities were also measured directly from pure salt stock. This yielded values of $\rho_s = 1.002279 \text{ g cm}^{-3}$ for 0.01 M NaCl and $\rho_s = 1.038456 \text{ g cm}^{-3}$ for 1 M NaCl.

The DS-rich phases in 1 M NaCl were diluted with 1 M NaCl stock, to increase the small working volume. Then the densities of the samples of all phases were measured and the results calculated back to PEG concentrations in the original phases, using the mass fractions of DS, as determined previously by polarimetry, corrected for dilution wherever necessary. The results are given in table 4 and displayed graphically in figure 11.

		ρ	w_{PEG} (%)	dilution	w_{PEG} (%)
1A	(upper)	1.045705	0.73	1	0.73 ± 0.15
	(lower)	1.045675	-0.97	5.10	-5.0 ± 0.8
1B	(upper)	1.045803	1.32	1	1.32 ± 0.15
	(lower)	1.05511	-0.38	2.63	-1.0 ± 0.5
1C	(upper)	1.045436	2.46	1	2.46 ± 0.14
	(lower)	1.085964	-1.58	1.29	-2.03 ± 0.4
1D	(upper)	1.045886	3.46	1	3.46 ± 0.14
	(lower)	1.075413	-0.95	2.33	-2.21 ± 0.6
2A	(upper)	1.027152	12.14	1	12.14 ± 0.12
	(lower)	1.100778	-2.60	1	-2.6 ± 0.8
2B	(upper)	1.028285	12.58	1	12.58 ± 0.12
	(lower)	1.11575	-3.38	1	-3.3 ± 0.9
2C	(upper)	1.035714	13.37	1	13.37 ± 0.14
	(lower)	1.128242	-3.95	1	-3.9 ± 1.0

Table 4: Density (g cm^{-3}), sample PEG concentration (%) as measured, dilution (sample weight divided by original weight) and phase PEG concentration (%) with standard deviation for all phases of the two series of phase separated mixtures.

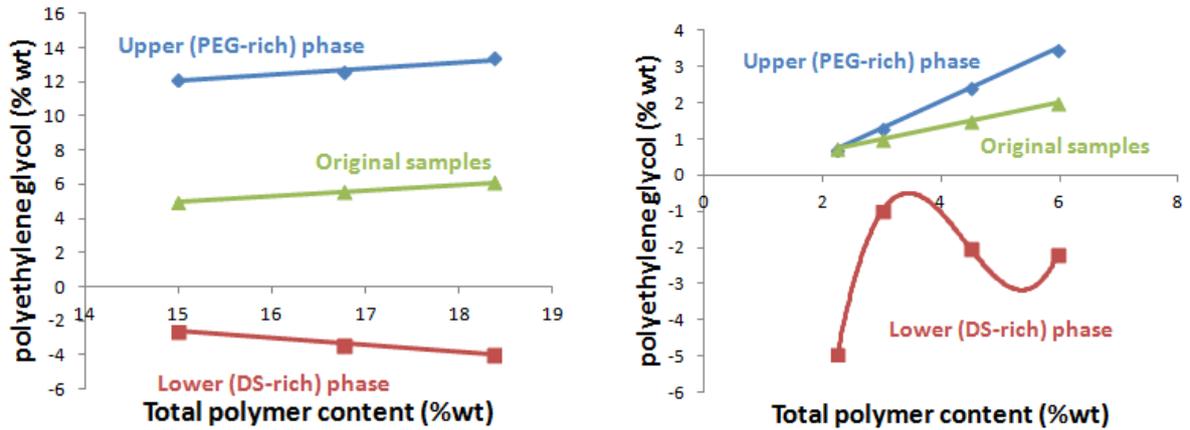


Figure 11: PEG concentrations in phase separated samples at 0.1 M (left) and 1 M (right) as determined by density measurements.

The determined concentrations look plausible for the PEG-rich phases. However, the negative PEG concentrations determined in the DS-rich phases are clearly unphysical. These results

can only stem from experimental inaccuracies, systematic errors or very large random statistical errors. As the PEG concentrations for the DS-rich phases are all negative in a fairly limited range, it seems that the results are at least partially due to systematic errors, as a large random scatter without systematic error would also result in values *above* the expected concentration range.

The standard deviations of w_{PEG} for each of the phases was calculated by standard error propagation through expression 13, with the additional factor to take into account the dilution step from the undiluted phase as collected to the samples for density measurement:

$$w_{\text{PEG}} = \frac{\rho_{\text{PEG}}\rho_{\text{DS}}(\rho - \rho_{\text{s}}) + w_{\text{DS}}\rho\rho_{\text{PEG}}(\rho_{\text{DS}} - \rho_{\text{DS}})}{\rho\rho_{\text{DS}}(\rho_{\text{PEG}} - \rho_{\text{s}})} \frac{m_{\text{samp}}}{m_{\text{samp}} + m_{\text{H}_2\text{O}}}. \quad (13)$$

Summing the squared derivatives of this expression with respect to all parameters multiplied by the errors in the parameters yielded the standard deviations.

To obtain the error in the DS mass fractions in the density measurement samples the same calculations were performed as before (eq. 12) with one dilution step less taken into account.

The error in ρ is simply the statistical error of the measuring apparatus. This error is of the order of $0.00001 \text{ g dm}^{-3}$.

The uncertainty in the polymer densities can be calculated from error propagation of the regression statistics of the fitted curves used to obtain these densities. As the density of the polymers is given by equation 10, the error in these densities is given by:

$$\sigma_{\rho_{\text{p}}} = \sqrt{\left(\frac{\partial\left(\frac{\rho_{\text{s}}}{1-a_{\text{p}}}\right)}{\partial\rho_{\text{s}}}\right)^2 \sigma_{\rho_{\text{s}}}^2 + \left(\frac{\partial\left(\frac{\rho_{\text{s}}}{1-a_{\text{p}}}\right)}{\partial a_{\text{p}}}\right)^2 \sigma_{a_{\text{p}}}^2} \quad (14)$$

$$= \sqrt{\left(\frac{1}{1-a_{\text{p}}}\right)^2 \sigma_{\rho_{\text{s}}}^2 + \left(\frac{\rho_{\text{s}}}{(1-a_{\text{p}})^2}\right)^2 \sigma_{a_{\text{p}}}^2}. \quad (15)$$

With this expression the errors can be reported together with the polymer densities:

From the series in 0.1 M NaCl:

- $\rho_{\text{PEG}} = 1.2022 \pm 0.0009 \text{ g cm}^{-3}$
- $\rho_{\text{DS}} = 2.05 \pm 0.03 \text{ g cm}^{-3}$

From the series in 1 M NaCl:

- $\rho_{\text{PEG}} = 1.1946 \pm 0.0006 \text{ g cm}^{-3}$
- $\rho_{\text{DS}} = 2.012 \pm 0.015 \text{ g cm}^{-3}$

Not only do the two values for the density of DS have a rather high statistical uncertainty, the densities of DS as well as the densities of PEG from both concentration series differ significantly with respect to each other, for PEG even some ten standard deviations. If this is a statistical effect, the uncertainty in these values is underestimated and the polymer densities are a source of systematic errors. The effect could also be physically real and this could be a sign that the assumptions underlying the analysis are invalid in this case, as the apparent densities of the polymers could also depend on the presence of other polymers.

For the DS-rich phases, the DS concentrations were calculated from the densities of the diluted samples (assuming no PEG in the phases as an approximation), to check whether these are in agreement with the DS concentrations as measured by polarimetry (and corrected for dilution). The results are shown in table 5. Note that these are the DS concentrations of the samples that were prepared for the density meter, not the concentrations of the original phases.

	$w_{\text{DS, pol}} (\%)$	$w_{\text{DS, dens}} (\%)$
1A	1.66	1.41
1B	3.34	3.24
1C	9.44	9.02
1D	7.33	7.08
2A	17.49	16.72
2B	19.98	18.97
2C	22.03	20.86

Table 5: DS concentrations of the density meter samples of the lower (DS-rich) phases, as determined by polarimetry and as determined by density measurements.

As can be seen in the table, the concentrations from density measurements and from polarimetry are in reasonable agreement with each other, but the correspondence is not perfect. If the PEG concentrations would be taken into account in the density measurement calculations, the discrepancy would be even larger, as the DS concentrations from density measurements would drop further below the values from polarimetry due to some of the density being occupied by PEG. The deviation to the lower end of the DS mass fractions determined this way could be due to an overestimation of the density of dextran sulfate, introducing systematic error in the calculations. This would also explain in part the negative concentrations that were found in the DS-rich phases: because the concentration of PEG in these phases is small with respect to the concentration of DS, a small error in the density of DS could lead to large deviations of the calculated PEG concentrations.

When all values for the parameters in the standard deviation of w_{PEG} were filled in, but not the errors in these parameters, numerical expressions for σw_{PEG} in terms of the errors in all parameters were obtained. Typically the errors in ρ_s , ρ and w_{DS} have more influence on the standard deviation of w_{PEG} than the errors in other parameters. To illustrate the relative weight of errors of the parameters, a typical expression for the upper phase of sample 1A is as follows:

$$\begin{aligned} \sigma_{w_{\text{PEG}}}^2 = & 52.90 (\text{g}^{-1}\text{cm}^3)^2 \sigma_r^2 + 0.002 (\text{g}^{-1}\text{cm}^3)^2 \sigma_{r_{\text{PEG}}}^2 \\ & + 0.0006 (\text{g}^{-1}\text{cm}^3)^2 \sigma_{r_{\text{DS}}}^2 + 52.20 (\text{g}^{-1}\text{cm}^3)^2 \sigma_{r_s}^2 + 13.52 \sigma_{w_{\text{DS}}}^2 \end{aligned} \quad (16)$$

Here, all mass fractions are fractions of 1. The error in ρ is generally small due to the measuring apparatus being accurate. If we assume a standard deviation of 0.0001 in ρ_s (a realistic value, seen the discrepancies between regression analyses and solvent densities as measured) and a standard deviation of 0.0005 in w_{DS} (which is quite broad), the standard deviation in w_2 will be 0.0020, or 0.20 weight percent. This is still by far not enough to account for the discrepancies that were observed. It thus seems that the results are due to some unknown cause of systematic and random error.

5 Conclusions

Solutions of dextran sulfate (500 kDa) and polyethylene glycol (35 kDa) in water in broad ranges of NaCl and polymer concentrations were found to phase separate into aqueous two-phase systems. The critical point of phase separation was dependent on the background salt concentration, as was expected from theory, shifting to lower critical polymer concentrations at higher salt concentration. An attempt was made to characterize the phase behavior of these systems quantitatively through concentration determinations by polarimetry and density measurements, but the methods employed were troubled by accuracy problems.

6 Outlook

Of interest are the Donnan electric potential differences between the phases in the system described here, recently researched in other systems (involving less charge density as well as less salt) by Vis et al.[2].

To obtain correct phase diagrams of the dextran sulfate/polyethylene glycol system, other methods than the techniques employed here need to be used.

High performance liquid chromatography (HPLC) can quantitatively determine polymer concentrations with good accuracy if a suitable column is used, with the disadvantage that the method is slow.

The refractive index of a solution is additive from the refractive indices of its components. If the concentration of dextran sulfate has been determined (i.e. by polarimetry) and the molar refractive index of the one remaining component is known, the concentration of polyethylene glycol could be determined by measuring the refractive index of the solution. It is not certain that this method will yield high accuracy, but it is a method to be tried.

Infrared spectroscopy can also be used. This method has the advantage of being fast and easy to use in principle, but it is not trivial to obtain quantitative results with this technique.

When a suitable method is chosen for the determination of the PEG concentration, full phase diagrams can be constructed at different NaCl concentrations. The salt dependence of the critical point can then be quantitatively evaluated and related to theory. Also, other properties of the system could be examined, such as the Donnan potential and the interfacial tension, and correlated with the salt concentration and critical point.

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Appendix A: chemicals

Polystyrene sulfonate (70 kDa, Na salt), dextran (500 kDa) and polyethylene oxide (600 kDa) were supplied by Aldrich. Dextran sulfate (500 kDa, Na salt, purity 95% by weight) was supplied by Fisher. Polyethylene glycol (35 kDa) was supplied by Merck. Polyethylene glycol (6 kDa) was supplied by Acros Organics.

Purified water was obtained from a Merck Millipore Synergy water purification unit.

Appendix B: reference concentration series for [DS] determination by polarimetry

A DS stock was prepared of 0.5008 g DS in 9.6047 g MQ water. From this DS stock samples were prepared, of which the optical rotation was measured.

Stock (g)	MQ (g)	[DS] (%)	OR (deg.)
0.8034	19.3542	0.1975	0.1741
1.5584	18.4624	0.3857	0.3413
1.6067	18.7283	0.3916	0.3463
2.5156	17.5024	0.6228	0.5517
3.2234	16.8274	0.7967	0.7081

Table 6: Composition and measured optical rotation of the DS concentration series used for polarimetry analysis.

Appendix C: reference concentration series for [PEG] determination by density measurements

Samples were prepared by weighing the contents on an analytic balance and homogenizing the contents.

DS (g)	MQ (g)	[DS] (%)	ρ (g cm ⁻³)
0.0995	10.1757	0.968	1.043441
0.1905	9.8436	1.899	1.048246
0.3050	9.9288	2.980	1.053808
0.3972	9.7967	3.896	1.058373

Table 7: Composition and measured density of the DS concentration series in 1 M NaCl used for density analysis.

DS (g)	MQ (g)	[DS] (%)	ρ (g cm ⁻³)
0.1945	9.7693	1.952	1.013149
0.4085	9.6024	4.081	1.025042
0.6225	9.5211	6.137	1.035490
0.8960	10.3627	7.958	1.045826

Table 8: Composition and measured density of the DS concentration series in 0.1 M NaCl used for density analysis.

PEG (g)	MQ (g)	[PEG] (%)	ρ (g cm ⁻³)
0.0996	9.9091	0.995	1.039830
0.2131	10.7995	1.935	1.041116
0.3111	9.7479	3.093	1.042707
0.4607	10.3103	4.277	1.044309

Table 9: Composition and measured density of the PEG concentration series in 1 M NaCl used for density analysis.

PEG (g)	MQ (g)	[PEG] (%)	ρ (g cm ⁻³)
0.2211	9.8149	2.203	1.005918
0.4142	9.6109	4.132	1.009142
0.5852	9.4713	5.819	1.012051
0.7974	9.2251	7.956	1.015657

Table 10: Composition and measured density of the PEG concentration series in 0.1 M NaCl used for density analysis.