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# Molar mass fractionation in aqueous two-phase polymer solutions of dextran and poly(ethylene glycol)

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ABSTRACT

Dextran and poly(ethylene glycol) (PEG) in phase separated aqueous two-phase systems (ATPSs) of these two polymers, with a broad molar mass distribution for dextran and a narrow molar mass distribution for PEG, were separated and quantified by gel permeation chromatography (GPC). Tie lines constructed by GPC method are in excellent agreement with those established by the previously reported approach based on density measurements of the phases. The fractionation of dextran during phase separation of ATPS leads to the redistribution of dextran of different chain lengths between the two phases. The degree of fractionation for dextran decays exponentially as a function of chain length. The average separation parameters, for both dextran and PEG, show a crossover from mean field behavior to Ising model behavior, as the critical point is approached.



## 1. Introduction

Polymeric aqueous two-phase systems (ATPSs) can be formed by mixing aqueous solutions of two different polymers, such as dextran and poly(ethylene glycol) (PEG), above a certain concentration. Two immiscible phases largely containing water are obtained, with one phase rich in dextran and the other one rich in PEG. Such ATPSs are widely employed for the separation and purification of proteins, nucleic acids, viruses and cells [1,2], due to their biocompatibility and the unique feature to provide a mild environment with extremely low interfacial tension. ATPSs can be employed in microfluidic setups [3], as well as in large scales [4], for the separation and purification of biological materials. The partitioning of biomolecules or cells in ATPS is not only controlled by their own physico-chemical affinities to the two phases or the liquid-liquid interface, but also influenced by the properties of the ATPS such as the tie line length and the molar mass of polymers, etc [5–7]. The interfacial tension between the coexisting phases, which depends on the composition and molar mass of polymers in the phases, also plays an important role in the partitioning of cells in ATPS [8]. It is therefore important to study the phase behaviors of ATPS and find their correlations with the partitioning of specific samples.

Accurate determination of the tie lines of dextran-PEG system is not trivial. To measure the polymer concentrations in each phase, usually one has to measure two physical properties, such as optical rotation and refractive index, of both phases [9]. The dextran concentration in the phase is determined by a polarimeter, while the PEG concentration is obtained after subtracting the dextran contribution to the refractive index. A gravimetric method was introduced to determine the tie lines of ATPS containing PEG and salt, by measuring the weight of two coexisting phases and forcing the tie line end points on a predetermined binodal fitted with an empirical equation [10]. One must be careful to apply this method for the dextran-PEG system considering the polydispersity of generally available dextran, because the tie line end points do not exactly match the binodal of ATPS for polymers with broad molar mass distributions. Recently, we introduced a simple density method to determine the tie lines of the dextran–PEG system by measuring the density of the coexisting phases [11]. The approach was

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based on the assumption that end points of the tie lines lie on the binodal, which is a good approximation when polymers with narrow molar mass distributions are employed. Phase separation in polydisperse polymer solutions has been considered both theoretically and experimentally [12–18]. It has been shown that during phase separation of aqueous mixtures of dextran with either gelatin or poly(ethylene oxide), all having broad molar mass distributions, fractionation of both species occurs [19,20]. While in ATPSs containing dextran with a broad molar mass distribution and PEG with a narrow molar mass distribution, the molar mass of dextran in the dextran-rich phase is much larger than that in the PEG-rich phase, and no significant molar mass difference of PEG in the two phases is found [21,22]. A number of different theoretical models for phase equilibria of ATPS have been reported in the literature [23,24], such as the model based on osmotic viral expansions [25,26], the lattice model based on Flory-Huggins theory [27] and the UNIQUAC model [18,28]. An effective excluded volume model has also been developed using methods of statistical geometry for the calculation of the binodal curves of ATPS [29]. Zaslavsky et al. proposed that phase separation in ATPS is due to the incompatibility of the polymer-modified water structures [30]. These models describe the experimental phase diagram reasonably well, but a comprehensive theory is still lacking. Additionally, the coexisting phases of ATPS offer distinct physical and chemical environments which allow for the selective partitioning of solutes such as proteins. Protein partitioning in ATPS is related to the polymer concentration difference between the phases via parameters such as the molar mass of polymers, protein-polymer interaction parameters, and the electrostatic potential difference between the phases [31,32]. Very recently, it was shown that the protein partitioning in ATPS is governed by the solvatochromic solvent properties of the coexisting phases [33,34], which depends on the composition and molar mass of the phase-forming polymers. Therefore, studying the phase diagram and molar mass fractionation of the dextran-PEG system, not only contributes to a better understanding of the liquid-liquid equilibria in ATPS, but also provides new insights into the mechanisms of biomolecules partitioning in ATPS.

In the present study, we performed gel permeation chromatography (GPC) measurements on the coexisting phases of the aqueous mixtures of dextran and PEG. Tie lines were constructed based on the GPC data and compared with a previous method based on density measurements of the phases. Molar mass fractionation of dextran and PEG during phase separation was studied and compared with theory.

## 2. Material and methods

### 2.1. Materials

Dextran from the bacterium Leuconostoc mesenteroides (molar mass between 400 and 500 kg/mol, lot number BCBG1982V) and PEG (molar mass 8 kg/mol, lot number MKBD4398V) were purchased from Sigma-Aldrich; they were desiccated in vacuum until no further reduction in mass was observed before use. All other reagents were of analytical grade. All solutions were prepared using ultrapure water from Sartorius water purification system with a resistivity of 18.2 MΩ cm.

### 2.2. Phase separation

Cloud-point titration was employed to determine the binodal and the critical point of dextran and PEG aqueous solution at $25 \pm 1$ °C [11]. Concentrated stock solutions of dextran and PEG (10–20% by weight) were prepared by dissolving polymers in water. To establish the binodal curve, a certain concentration of dextran (or PEG) solution was prepared by adding water to the dextran (or PEG) stock solution in a 10 mL vial. Then PEG (or dextran) stock solution was added dropwise into the vial followed by shaking. The titration was continued until the solution became turbid. The mass of each stock solution and water was measured by a balance to establish the binodal curve. The critical point, at which the phase volumes are equal as one approaches the binodal from the two-phase region, was determined by titration as described elsewhere [11]. To construct the tie lines, mixtures of dextran and PEG solutions were prepared in the two-phase region in 50 mL separating funnels by keeping the weight ratio between dextran and PEG equal to that at the critical point. The solutions were shaken by hand to ensure good mixing of the polymers. The samples were kept at a temperature of $25 \pm 1$ °C for one week to ensure complete phase separation before the PEG-rich phase was taken from the upper outlet and the dextran-rich phase was collected from the lower one. The density of each separated phase was measured at $25 \pm 0.01$ °C by a density meter (DMA4500, Anton Paar) with a resolution of $5 \times 10^{-5}$ g/mL.

### 2.3. Gel permeation chromatography

GPC measurements were performed on separated phases to obtain both composition and molar mass distribution of dextran and PEG in each phase [22]. The GPC system was equipped with two PL aquagel-OH mixed-H columns (7.5 × 300 mm, Polymer Laboratories Ltd.) and a 2414 differential refractive index (RI) detector (Waters Corporation). The eluent of water containing 0.02 wt% $NaN_3$ was delivered by a Waters 515 HPLC pump at a constant flow rate of 1.0 mL/min. The separated phases were diluted 10–100 times with the eluent and 100 μL polymer solution was injected to the system via a Waters 717plus autosampler. The concentration of dextran and PEG in each separated phase was obtained from the area of the eluting peaks for dextran and PEG using calibration curves relating the RI peak area to the injected polymer concentration. The molar mass of dextran and PEG in each separated phase was determined after calibrating the columns with narrow PEG and poly(ethylene oxide) (PEO) standards obtained from Polymer Laboratories Ltd. Universal calibration was applied to obtain the molar mass of dextran [35–37], which was validated by coupling GPC with a DAWN HELLEOS II multi-angle laser light scattering detector (Wyatt Technology Corporation).

## 3. Results and discussion

### 3.1. Binodal and critical point

The binodal of the aqueous solution of dextran and PEG is shown in Fig. 1. It should be noted that for data points with large weight fraction of PEG ($w_p > 0.06$), the weight fraction of dextran is very small with $w_d < 0.001$. Even a small amount of high molar mass dextran is immiscible with a concentrated PEG solution, due to the unfavorable interaction between the long dextran chains and the PEG molecules. Following the protocol in Ref. [11], a series of polymer solutions in the two-phase region at a certain weight ratio $w_d/w_p$ between dextran and PEG were prepared, and the volume fraction of each phase was measured when gradually approaching the binodal by adding water. The volume fraction $\Phi^D$ of the dextran-rich phase in the vicinity of the phase boundary is plotted as a function of $w_d/w_p$ in the inset of Fig. 1. The volumes of the dextran-rich and PEG-rich phases are equal ($\Phi^D = 0.50$) at the weight ratio $w_d/w_p = 1.23$. Carefully studying the phase behavior of solutions close to the binodal with $w_d/w_p$ fixed at 1.23 gave the composition of the critical point of the system with a total polymer weight fraction of $w_{cr} = 0.0811 \pm 0.0002$. The phase dia-