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Supplementary Materials for:

Effect of Trimethylamine N-oxide on the Phase Separation of Aqueous Polyethylene Glycol-600-Dextran-75 Two-Phase Systems

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Methods

Light Scattering

DLS Analysis

Dynamic Light scattering (DLS) characterizes the time evolution of Brownian-motion-driven fluctuations in the intensity of light scattered by a specific sample. Brownian motion causes solute particles to move. When particles change positions, the light paths of the scattered waves are altered. DLS analyzes fluctuations in intensity of this scattered light through a correlation function:

$$S(q,\tau) = \int_0^T I(q,\tau)I(q, t + \tau)dt \qquad (S\ 1)$$

Where q is the magnitude of the scattering vector, τ is the delay time, and T is the duration of the experiment. The magnitude of the scattering vector is determined by:

$$q = \frac{4\pi n}{\lambda}\sin\theta/2$$

(S 2)

Where λ is the in-vacuo wavelength of the light source, θ is the scattering angle, and n is the refractive index of the solvent. The spectrum is analyzed using a field correlation function $g^{(1)}(q,t)$ which is determined by the position/motion of scattering molecules. Under normal circumstances, the collected data $S(q,\tau)$ is related to $g^{(1)}(q,t)$ using the Siegert equation:

$$S(q,\tau) = B\left(1 + f\left|g^{(1)}(t)\right|^2\right)$$

(S 3)

Where B is the baseline and f is an instrument parameter. Spectra are analyzed by converting the intensity correlation function to a field correlation function (stretch exponentials using a nonlinear least-squares simplex algorithm):

$$g^{(1)}(q,t) = \sum_{i=1}^{N} A_i \exp(-\theta_i t^{\beta_i})$$

(S 4)

Where N is the number of observed relaxation modes labeled by i, θ_i is the decay pseudorate, β_i is the stretching parameter, and A_i is the amplitude of the corresponding mode. Spectral moments analysis is also used is also used for multimodal data to isolate each mode of $g^{(1)}$ and yield a result for the diffusion coefficient (D_T) and spectral decay rate (Γ). For stretched exponential relaxations, the spectral time moments can be written as:

$$M_{oi} = \int_0^{\infty} \exp\left(-\theta_i t^{\beta}\right) = \frac{\gamma\left(1 + \frac{1}{\beta_i}\right)}{\theta_i^{\frac{1}{\beta_i}}}$$

(S 5)

Where γ is the Gamma function and M_o is the mean relaxation (Γ^{-1}) for each mode. This yields the averaged diffusion coefficient $(D_T^{-1})^{-1} = (M_0 q)^{-1}$. For monodisperse spherical particles we would also expect $\Gamma = Dq^2$. The diffusion coefficient is converted to R_h using the Stokes-Einstein equation:

$$R_h = \frac{k_B T}{6\pi\eta D_T}$$

(S 6)

Where k_B is the Boltzmann constant, T is the Kelvin temperature, and η is the solvent viscosity.

SLS Analysis

Static light scattering (SLS) is a powerful non-invasive experimental method to study that yields the molecular weight (weight average molecular weight, M_w), the size (radius of gyration, R_g), and interaction between the scatters (the second virial coefficient, A2). The proper SLS measurements also require determination of the specific refractive index increment (dn/dc) for the samples studied. By plotting Kc/R as a function of the concentration of the sample and scattering vector:

$$\frac{Kc}{R} = M_W^{-1}\left(1 + \frac{q^2 R_g^2}{3}\right) + 2A_2 c + \ldots$$

(S 7)

Here, c is the concentration of the solution being tested, R is the Rayleigh factor, which is related to scattered intensity, and K is an optical coefficient, which is expressed as:

$$K = \frac{4\pi^2 n^2 (dn/dc)^2}{\lambda^4 N_A}$$

(S 8)

where n is the index of refraction of the solvent, λ is the in-vacuo wavelength of the laser, N_A is Avogadro's number, and dn/dc is a very important parameter for SLS, called the specific refractive index increment, which shows how the refractive index changes with concentration. Zimm analysis, plot of Kc/R vs q^2+kc where k is a scaling factor, is used to extrapolate data at two important extremes. Linear trends of c=0 and q=0 extrapolations are used to determine the structural properties of the scatterer where M_w is dependent on the intercepts of both trends, R_g is dependent on the slope and intercept of the c=0 trend, and A2 is dependent on the slope of the q=0 trend. As an alternative to the Zimm plot, a Berry plot of $Kc/R^{1/2}$ vs q^2+kc can be used as a higher order fit if the extrapolations are not linear on the Zimm plot.

Results

Water subpopulation	PEG-600	Dextran-70
I	8.30 ± 0.049	8.27 ± 0.060
II	-4.73 ± 0.033	-4.65 ± 0.035
III	-2.10 ± 0.013	-2.17 ± 0.016
IV	-1.419 ± 0.004	-1.47 ± 0.012

Table S1. Differences between the fractions of water subpopulations I-IV in solutions of PEG-600 and Dextran-70 in the presence and absence of 7.2 wt.% TMAO

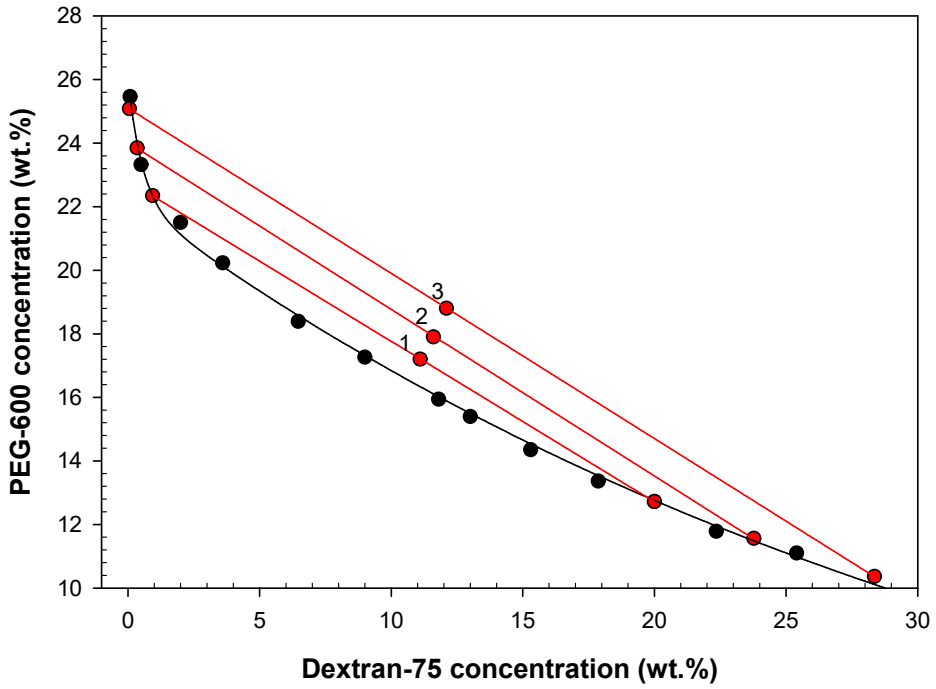

Figure S1. Full phase diagram for PEG-600/Dextran-75 ATPS containing 2 wt.% TMAO and 0.15M NaCl in 0.01M sodium-potassium phosphate buffer, pH 7.4. Slope of the tie lines (STL) are as follows: 1) STL = -0.52; 2) STL = -0.50; 3) STL = -0.52.

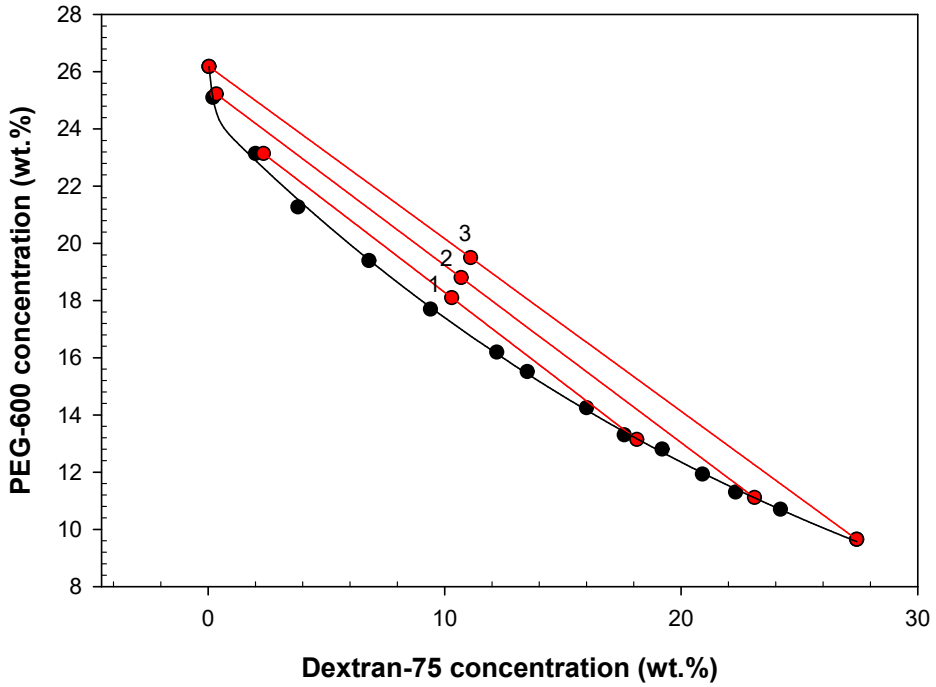

Figure S2. Full phase diagram for PEG-600/Dextran-75 ATPS containing 7.2 wt.% TMAO and 0.15M NaCl in 0.01M sodium-potassium phosphate buffer, pH 7.4. Slope of the tie lines (STL) are as follows: 1) STL = -0.63; 2) STL = -0.62; 3) STL = -0.60.

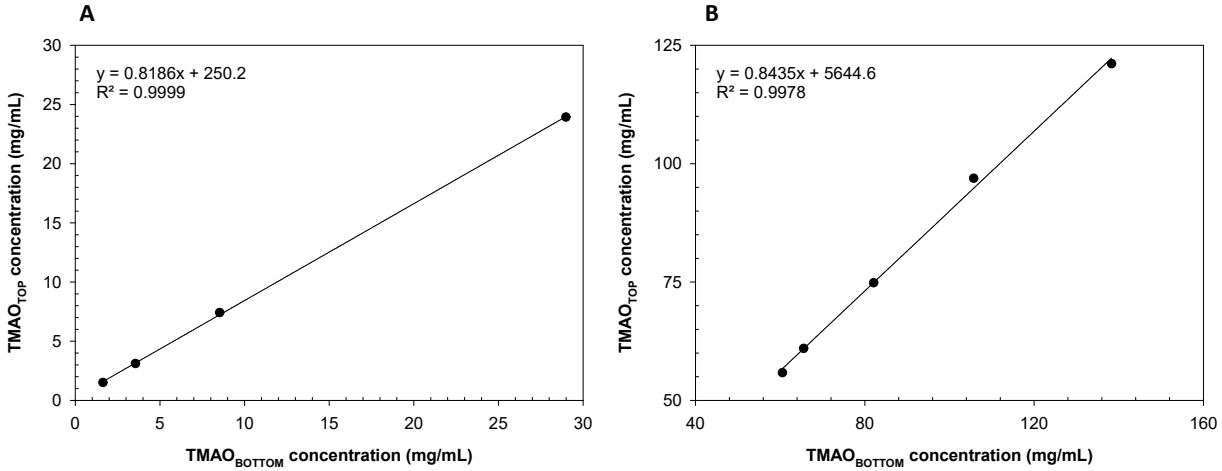

Figure S3. The distribution of TMAO between top and bottom phases in dilutions of systems containing (a) 2 wt.% TMAO and (b) 7.2 wt.% TMAO as measured via total nitrogen assay with chemiluminescent nitrogen detector (CLND).

In DLS, the spectral time moment analysis explained above, yields Γ at various angles. Plotting Γ vs q2 and using a linear trend, determines translation diffusion DT for the studied system. Figure S4a-c show results for Systems 1-3.

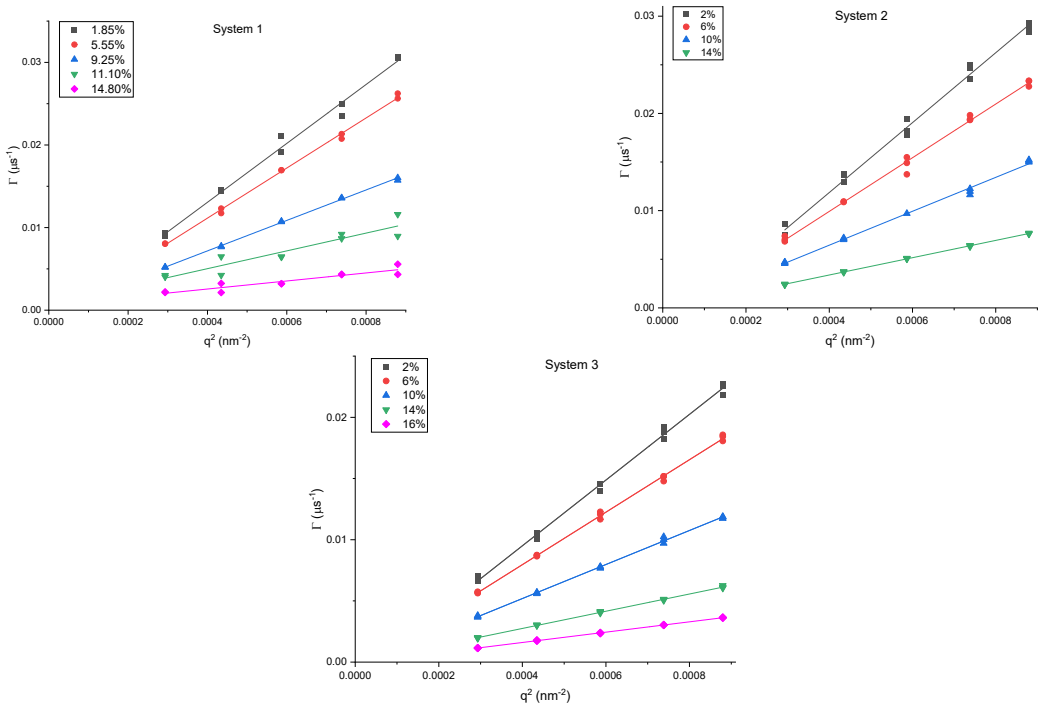

Figure S4: G vs q^2 graph for system 1 (a, upper left), system 2 (b, upper right) and system 3 (c, bottom) at 5 different mixture concentrations

Figures S5 compare the diffusion coefficients obtained at each angle with the diffusion coefficient obtained from the slopes of Γ vs q^2 (Γ/q^2 solid lines).

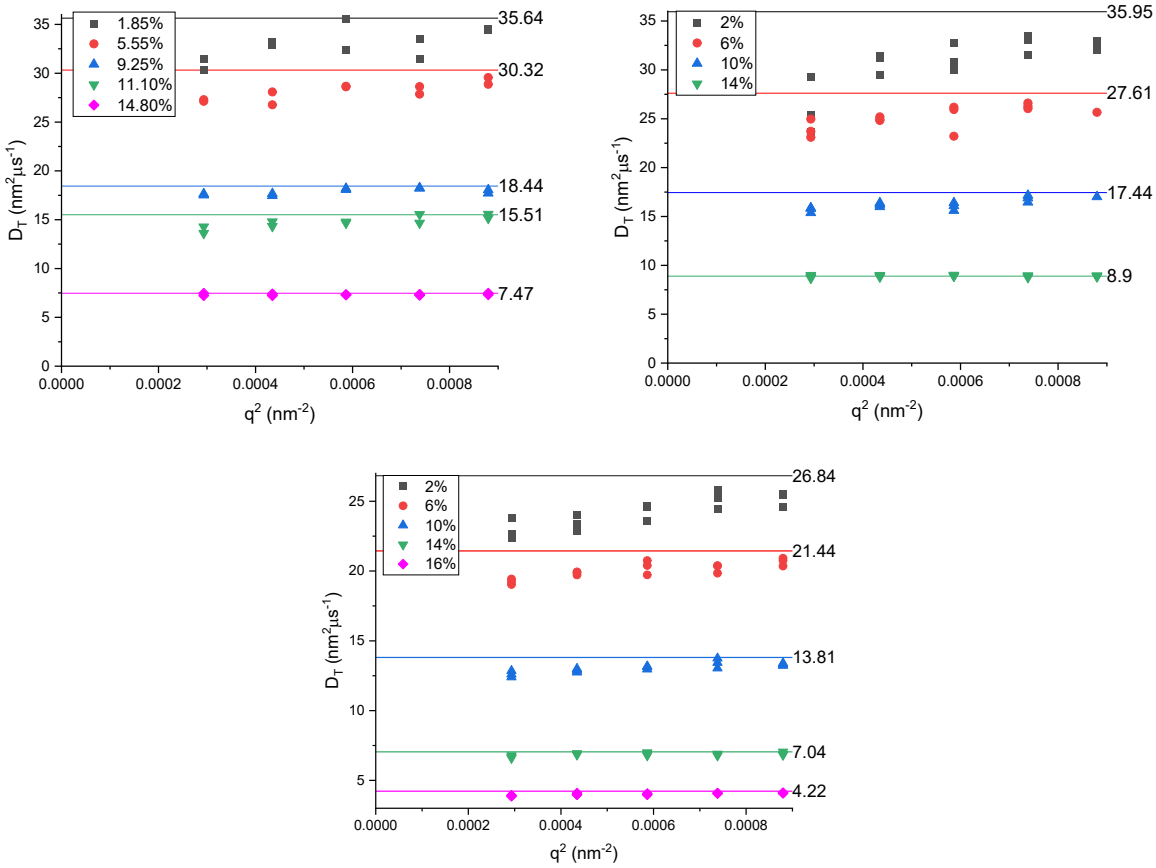

Figure S5: D_T vs q^2 graph for system 1 (a, upper left), system 2 (b, upper right) and system 3 (c, bottom) for all concentrations.

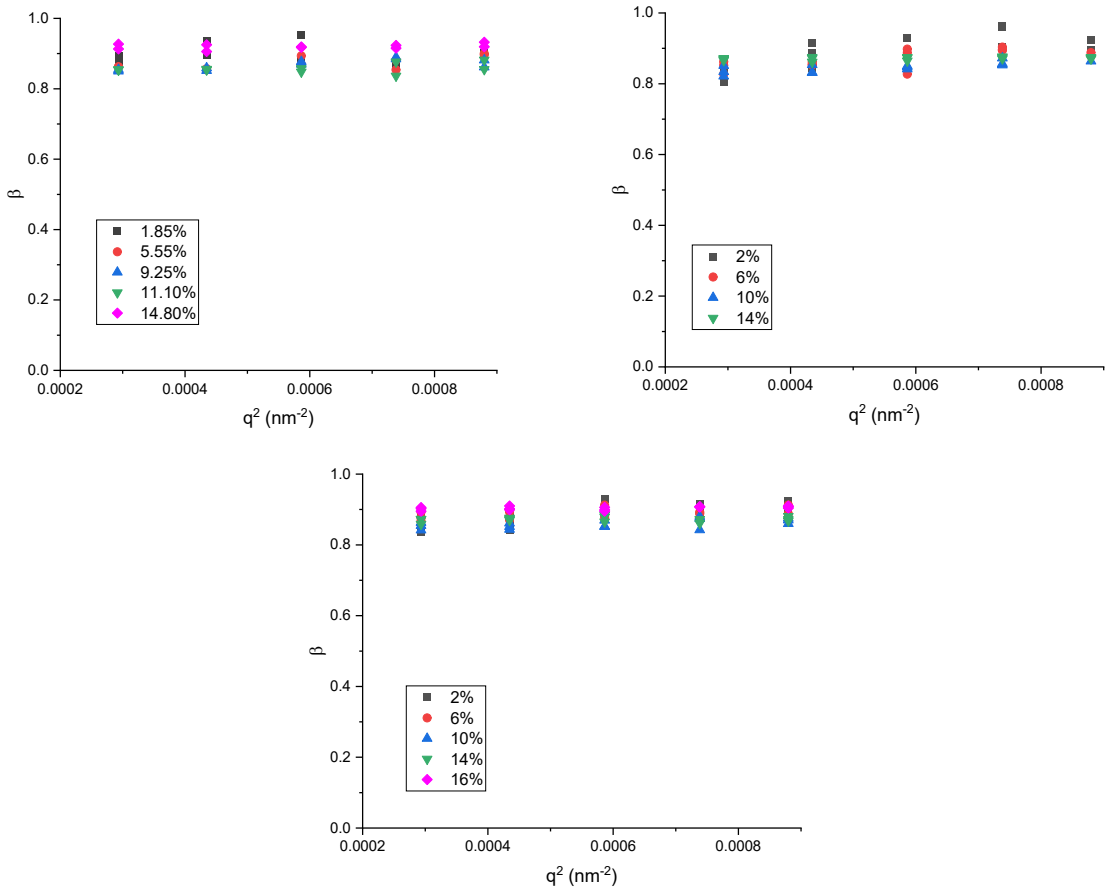

Figure S6: β vs q^2 graph for system 1 (a, upper left), system 2 (b, upper right) and system 3(c, bottom) for all concentrations.

In the SLS experiments, the intensity data was collected and analyzed in the form of Zimm/Berry plot for each system. dn/dc values needed for calculating optical constant K in the Zimm/Berry plots were directly measured for each system and found to be in the range of 0.144-0.184mL/g. Figure S7-S9 show the example of the SLS results for the three systems. The colored horizontal lines on this Zimm show multi-angle data at single concentrations while the colored vertical lines show multi-concentration data at single angles. All data sets appear to follow linear trends. Concentration and angle trendlines run parallel to each other and are used to find the extrapolated values at q=0 (orange points on the left) and at c=0 (orange points at the bottom). These extrapolated values are then being fit with linear trends themselves yielding the apparent molecular weight (Mw), radius of gyration (R_g), and second virial coefficient (A_2).

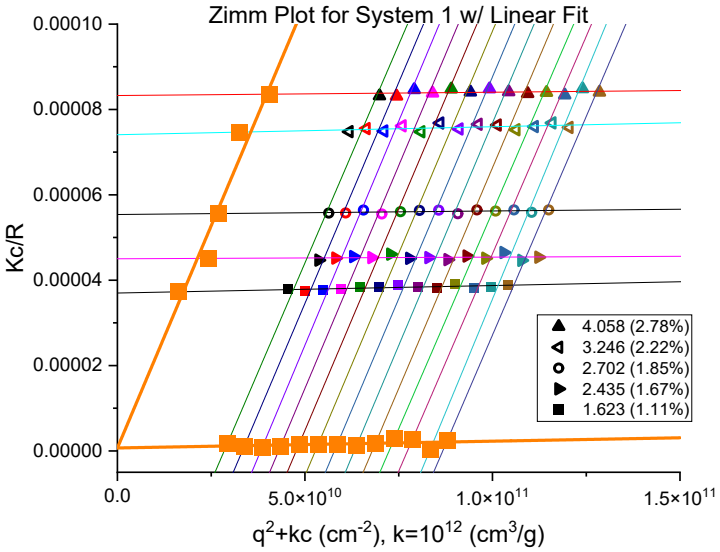

Figure S7: Zimm plot for System 1 (18.5 wt.% PEG-600/8.5 wt.% Dextran/0.15 M NaCl/0.01M NaPB, pH 7.4) studied at five concentrations between 6 and 15%. Orange lines and points are the c=0 and q=0 extrapolations. All other colored lines show linear trends of single concentrations and angles.

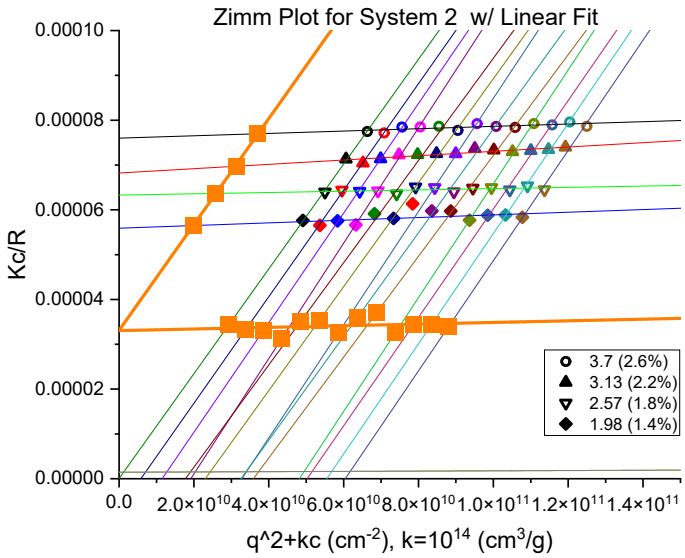

Figure S8: Zimm plot for System 2 (20 wt.% PEG-600/8.5 wt.% Dextran/2 wt.% TMAO/0.15 M NaCl/0.01M NaPB, pH 7.4) studied at four different concentrations between 7 and 13%. Orange lines and points are the c=0 and q=0 extrapolations. All other colored lines show linear trends of single concentrations and angles.

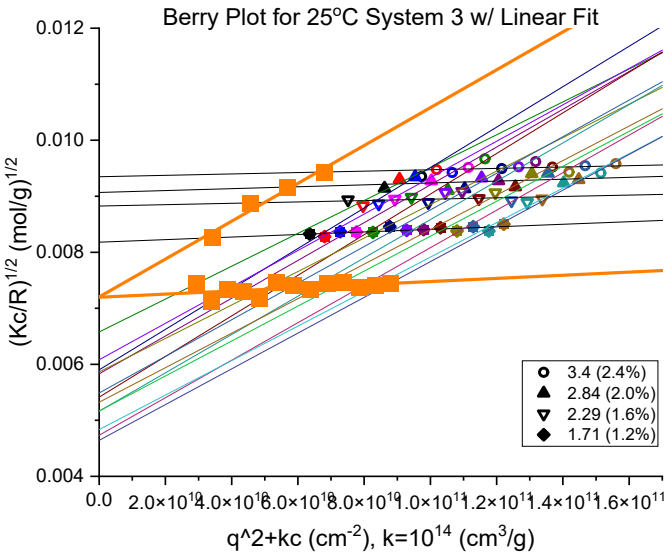

Figure S9: Berry plot of System 3 (20 wt.% PEG-600/8.5 wt.% Dextran/7.2 wt.% TMAO/0.15 M NaCl/0.01M NaPB, pH 7.4) studied at four different concentrations between 6 and 12%. Orange lines and points are the c=0 and q=0 extrapolations. All other colored lines show linear trends of single concentrations and angles.

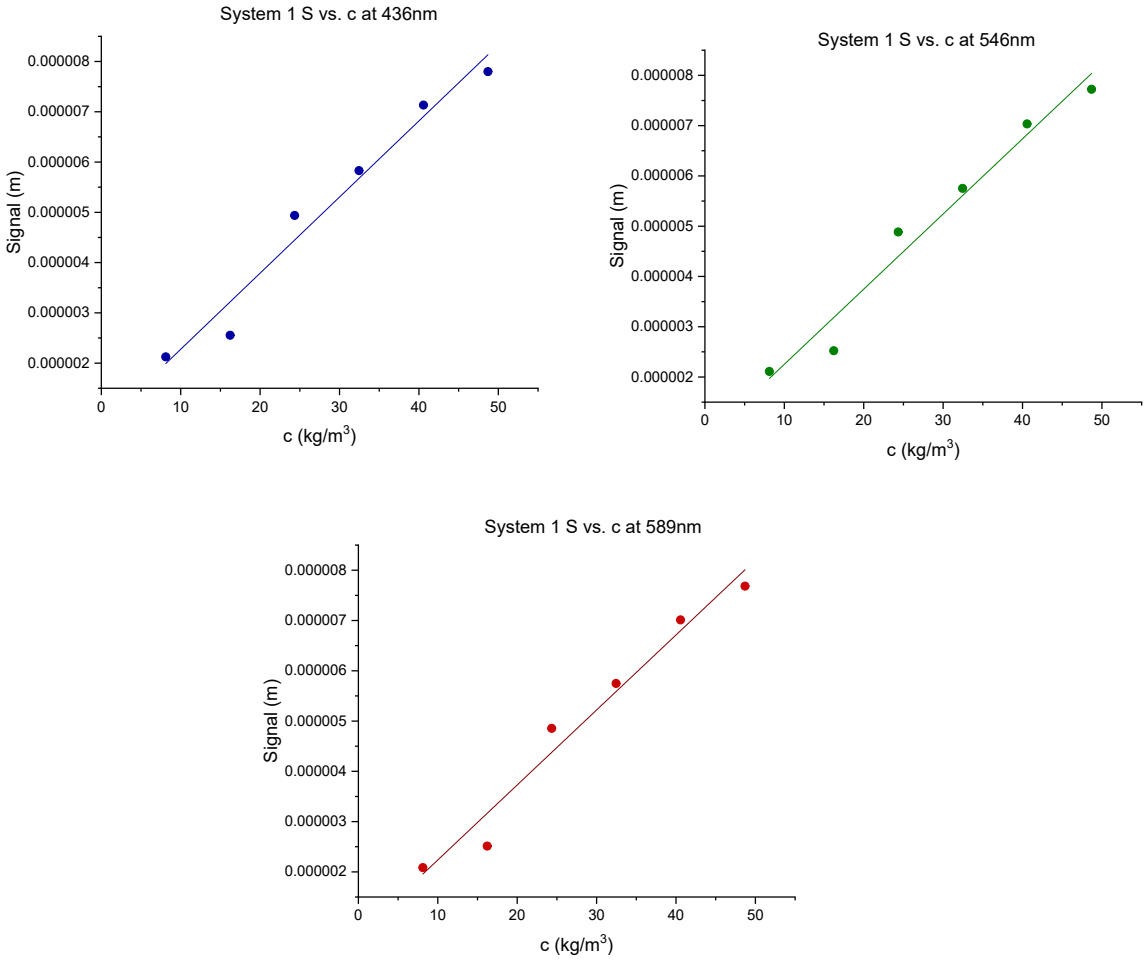

Figure S10: Signal vs concentration results for system 1 using wavelengths of 436nm (a, upper left), 546nm (b, upper right), and 589nm (c, bottom) to determine dn/dc.

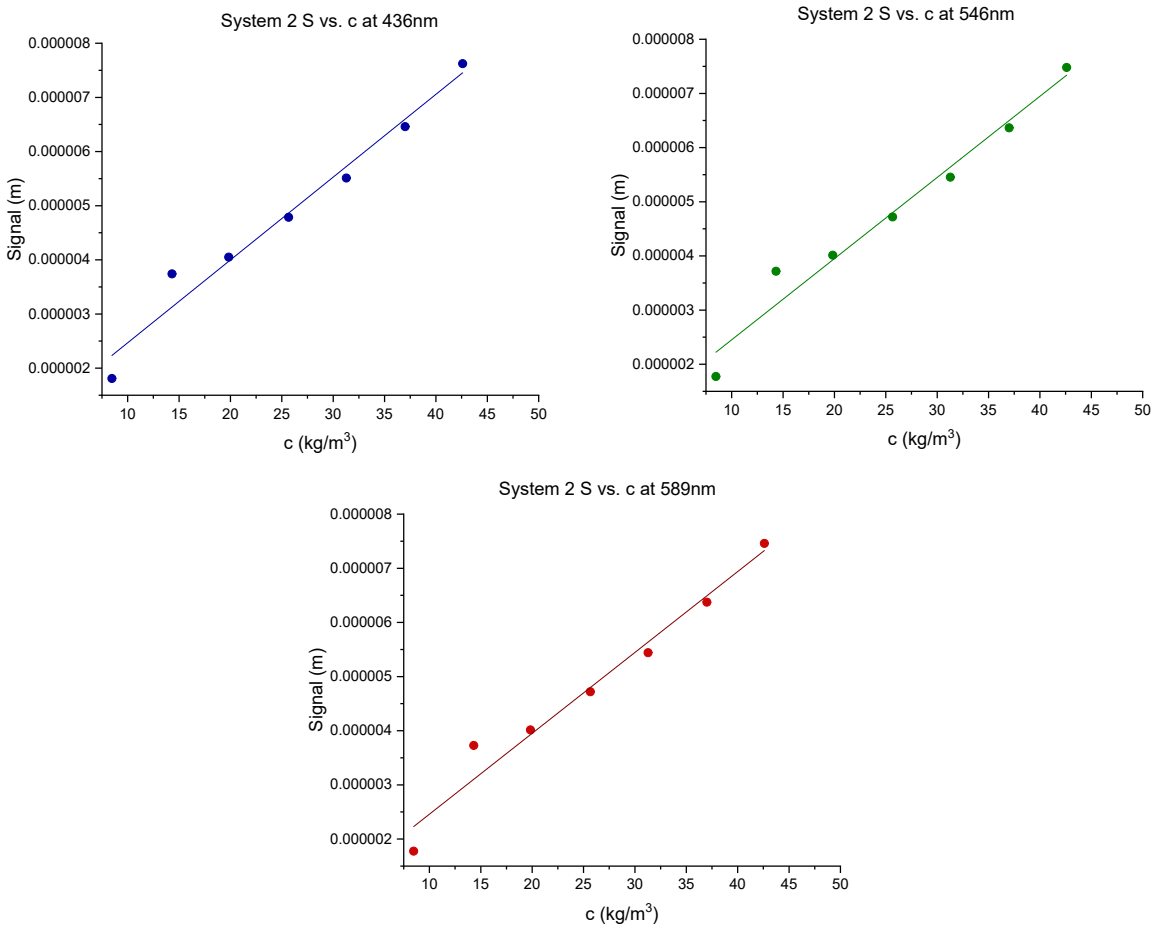

Figure S11: Signal vs concentration results for system 2 using wavelengths of 436nm (a, upper left), 546nm (b, upper right), and 589nm (c, bottom) to determine dn/dc.

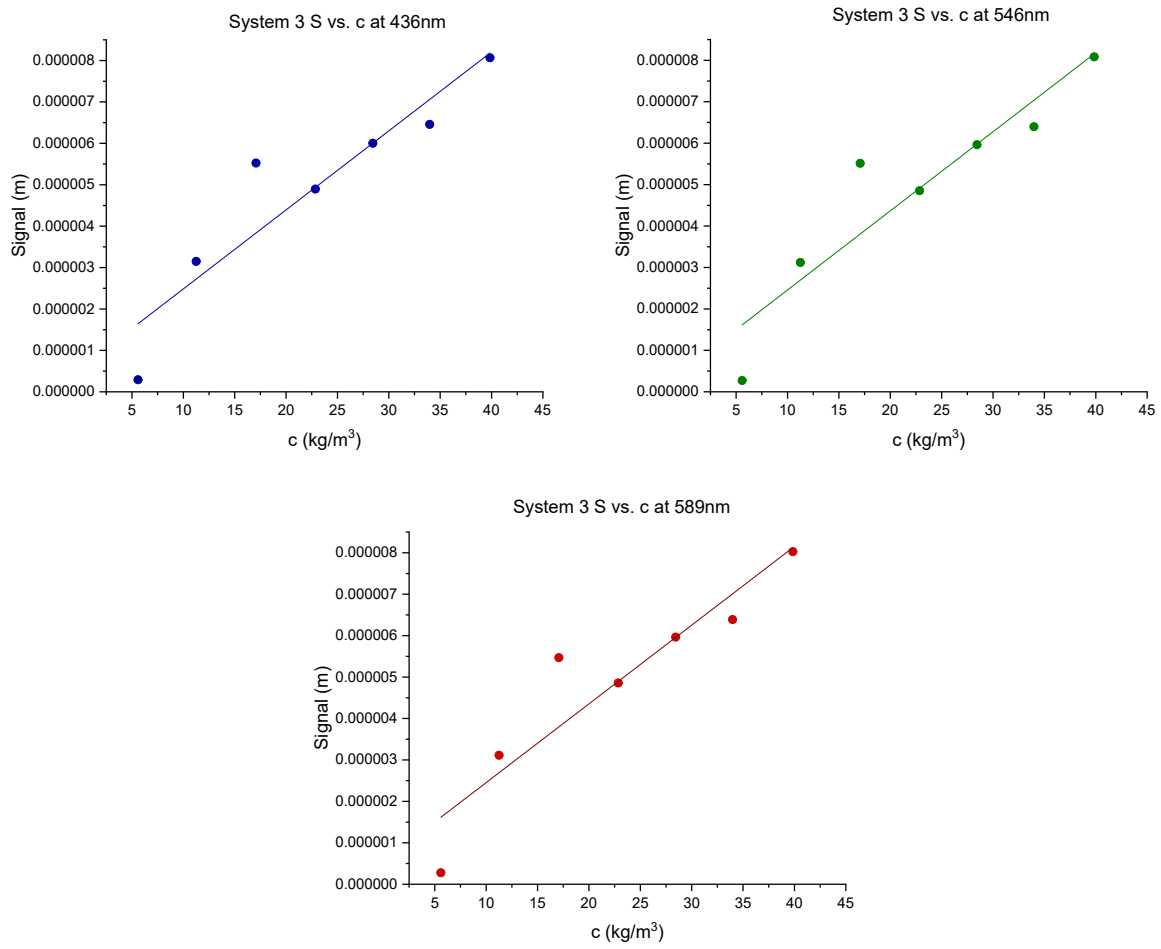

Figure S12: Signal vs concentration results for system 3 using wavelengths of 436nm (a, upper left), 546nm (b, upper right), and 589nm (c, bottom) to determine dn/dc.