Macromolecule Diffusiophoresis Induced by Concentration Gradients of Aqueous Osmolytes

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ABSTRACT: Diffusiophoresis is the migration of a particle in a fluid induced by the concentration gradient of another solute. We have experimentally investigated diffusiophoresis of a neutral macromolecule, poly(ethylene glycol) (PEG; molecular weight, 20 kg mol⁻¹), in water induced by concentration gradients of osmolytes. Three osmolytes were examined: trimethylamine-N-oxide (TMAO), diethylene glycol (DEG), and urea. PEG diffusiophoresis coefficients were obtained from measurements of multicomponent-diffusion coefficients at 25 °C using Rayleigh interferometry. Osmotic diffusion coefficients, characterizing osmolyte diffusion from high to low PEG concentration, were also extracted. PEG diffusiophoresis was found to occur from high to low osmolyte concentration in all cases, with magnitude increasing in the order urea < DEG < TMAO. This ranking is consistent with that of osmolyte effectiveness in stabilizing protein native state. Osmotic diffusion coefficients, which allowed us to determine preferential-interaction coefficients, revealed that TMAO and DEG are preferentially excluded from the vicinity of PEG whereas urea was found to preferentially bind to this macromolecule. A novel model for macromolecule diffusiophoresis, which allowed us to examine the roles of preferential hydration, hydration, solute binding, and frictional dragging in this transport process, was developed. Our experimental results suggest that TMAO concentration gradients may be exploited to direct the motion of PEG and PEG-functionalized particles such as micelles, PEGylated proteins, and PEG-coated inorganic nanoparticles with potential applications to separation and adsorption technologies.

INTRODUCTION

In aqueous mixtures, a concentration gradient of a solute may induce the net migration of a colloidal rigid particle. This phenomenon, which is known as diffusiophoresis, has been investigated not only for large particles but also for relatively smaller macromolecules, such as synthetic polymers and proteins. Diffusiophoresis has attracted much attention because the manipulation of particle motion by solute concentration gradients may be exploited for applications in microfluidics, self-assembly, separation technologies, and adsorption processes. Related experimental investigations (e.g., spreading of colloidal solutions in microfluidics) have focused on salt concentration gradients. For charged colloidal particles at low salt concentration (e.g., 0.1 M NaCl or less), salt-induced diffusiophoresis can be mostly described by invoking an electrophoretic mechanism. In the protein case, relatively high salt concentrations can be employed and, in these electrostatically screened conditions, diffusiophoresis is mainly caused by preferential exclusion of the salt solute at the protein–solvent interface (protein preferential hydration). This implies that a macromolecule need not be charged for its diffusiophoresis to occur. Indeed, salt-induced diffusiophoresis of poly(ethylene glycol) (PEG), a neutral hydrophilic polymer, has been experimentally observed, with diffusiophoresis magnitude following the Hofmeister series.

PEG is one of the most extensively employed synthetic macromolecule. It is either directly used as a polymer coil in aqueous formulations (e.g., crowding agent) or as a solvent-interacting component of relatively more complex systems, such as micelles, PEGylated proteins, and PEG-coated metal and oxide nanoparticles. Thus, studies on PEG diffusiophoresis provide the basis for exploiting the above-mentioned diffusiophoresis-based applications in the pharmaceutical, biotechnological, and polymer industries.

As in the case of salts, neutral cosolutes such as osmolytes may also induce macromolecule diffusiophoresis in aqueous systems. Osmolytes, which have widespread applications in aqueous solutions, are historically discussed in the context of protein aqueous solutions, where they are divided into stabilizer and destabilizer of the protein native state. The two most representative osmolyte cases of protein stabilizer and destabilizer are trimethylamine-N-oxide (TMAO) and urea, respectively. Interestingly, the stabilizing effect of
an osmolyte is found to be independent of protein chemical nature, a property also encountered in the case of salts, following the Hofmeister series. To our knowledge, no diffusiophoresis study has been previously reported on these well-known osmolytes. Hence, the goal of this paper is to characterize osmolyte-induced PEG diffusiophoresis coefficients. We specifically employ precision Rayleigh interferometry\(^7,22\) to experimentally determine multicomponent-diffusion coefficients for the ternary PEG–TMAO–water and PEG–urea–water systems at 25 °C, with PEG nominal molecular weight of 20 kg mol\(^{-1}\). For completeness, we also extract and examine PEG diffusiophoresis coefficients from previously reported\(^{23}\) multicomponent-diffusion data on the ternary PEG–diethylene glycol (DEG)–water system.

In the remaining part of this section, how the framework of multicomponent diffusion can be used to extract the macromolecule diffusiophoresis coefficient and another transport coefficient denoted as osmolyte osmotic diffusion is outlined. This second transport property, which characterizes osmolyte diffusion induced by a macromolecule concentration gradient, is directly related to osmolyte preferential exclusion or enrichment (preferential binding) near the macromolecule surface.\(^7,11\) For a ternary macromolecule (P)–osmolyte (L)–water (W) system, multicomponent diffusion can be described by extended Fick’s first law \(^{7,9}\):

\[
\begin{align*}
-J_p &= D_{pp} \nabla C_p + D_{pl} \nabla C_L \\
-J_L &= D_{lp} \nabla C_p + D_{ll} \nabla C_L
\end{align*}
\tag{1a,1b}
\]

where \(C_i\) (with \(i = \text{P}, \text{L}, \text{W}\)) is the molar concentration of component \(i\), \(J_i\) is the corresponding molar flux, and the four \(D_{ij}\)’s (with \(i, j = \text{P}, \text{L}\)) are the multicomponent-diffusion coefficients. Here, molar fluxes are defined with respect to the solvent-fixed reference frame with solvent molar flux: \(J_0 = 0\).

Main-term diffusion coefficients, \(D_{pp}\) and \(D_{ll}\), describe the flux of macromolecule and additive due to their own concentration gradients, whereas cross-term diffusion coefficients, \(D_{pl}\) and \(D_{lp}\), describe the flux of a solute due to the concentration gradient of the other solute, with \(D_{pl}\) describing macromolecule diffusiophoresis and \(D_{lp}\) describing osmolyte osmotic diffusion. It is convenient to introduce the following reduced (and unitless) diffusiophoresis \(\hat{D}_{LP}\) and osmotic diffusion \(\hat{D}_{LP}\) coefficients \(^{7,9}\):

\[
\begin{align*}
\hat{D}_{PL} &\equiv \left(\frac{C_L}{y_L}\right) \lim_{C_p \to 0} \frac{D_{pl} C_p}{D_{pp}} \left(\frac{C_L}{y_L}\right) \lim_{C_p \to 0} \frac{D_{pl} C_p}{D_{pp}} \\
\hat{D}_{LP} &\equiv \lim_{C_L \to 0} \frac{D_{lp} C_L}{D_{ll}}
\end{align*}
\tag{2a,2b}
\]

where \(y_L\) is a known \(^{7,9,24–26}\) thermodynamic factor characterizing thermodynamic nonideality of the binary osmolyte–water system with \(\lim_{C_L \to 0} y_L = 1\) and \(D_P \equiv \lim_{C_L \to 0} D_{pl}\) is the macromolecule Brownian mobility. Finally, we observe that \(\lim_{C_L \to 0} D_{ll}\) is the solvent-fixed diffusion coefficient of the binary osmolyte–water system, which will be denoted henceforth as \(D_L\).

These two reduced coefficients have a well-defined physical interpretation in associated transport and equilibrium processes. Macromolecule diffusiophoresis coefficient, \(\hat{D}_{PL}\), describes the relative effect of the osmolyte chemical-potential gradient (thermodynamic driving force) on the net diffusion rate, \(r_p = J_p/C_p\), of a macromolecule \(^{8}\):

\[
r_p = -D_p \left(\frac{\partial \ln C_p}{\partial C_p} + \frac{\partial (\mu_L/R)}{\partial T} \right)
\tag{3}
\]

where \(\mu_L\) is the osmolyte chemical potential, \(R\) is the ideal-gas constant, and \(T\) is the absolute temperature. Note that eq 3 is analogous to other transport laws in which the osmolyte "chemical field", \(-\partial \mu_L/\partial T\), is replaced by other type of fields (e.g., electrical or sedimentation). The osmolyte chemical potential gradient can be rewritten as \(\nabla \mu_L = RT \partial \ln C_L\) where \(y_L\) is the previously mentioned thermodynamic factor.

The osmolyte osmotic diffusion coefficient, \(\hat{D}_{LP}\), is linked to the equilibrium distribution coefficient of osmolyte along a macromolecule concentration gradient.\(^9\) Specifically, in the hypothetical case in which the mobility of a macromolecule is infinitely low compared to that of the osmolyte molecules, we can write

\[
\lim_{C_L \to 0} \left(\frac{\partial C_L}{\partial C_p}\right)_{pl} = -\lim_{D_{LP} \to 0} \hat{D}_{LP}
\tag{4}
\]

where temperature and pressure subscripts, normally appended to partial derivatives, are omitted for simplicity.

\section*{EXPERIMENTAL SECTION}

\textbf{Materials.} Poly(ethylene glycol) (PEG) with nominal weights of 20 kg mol\(^{-1}\) was purchased from Sigma-Aldrich. For PEG, the certificate of analysis obtained from Sigma-Aldrich gives the number (\(M_n\)) and mass average (\(M_w\)) molecular weights based on size-exclusion chromatography: \(M_n = 22.2\) kg mol\(^{-1}\) and \(M_w = 26.7\) kg mol\(^{-1}\), with \(M_w/M_n = 1.20\). Urea (purity, 99.7%; molecular weight, 60.06 g mol\(^{-1}\)) and trimethylamine-N-oxide dihydrate (purity, 98%; molecular weight, 111.14 g mol\(^{-1}\)) were purchased from Fisher Scientific and Acros Organics, respectively. All chemicals were used without further purification. Deionized water was passed through a four-stage Millipore filter system to provide high-purity water for all experiments. All solutions for interferometric experiments were prepared by mass and included buoyancy corrections. Density measurements (Mettler-Paar DMA40 density meter) were performed to obtain molar concentrations. To remove polymer dissolution hurdles, PEG–water stock solutions were prepared by weight (uncertainty of 0.1 mg) and stirred overnight. Since TMAO is hygroscopic, TMAO–water stock solutions were also prepared. Corresponding TMAO weight fractions were determined by precise density measurements and using the available density–weight fraction relation at 25 °C.\(^7\) Stock solutions were passed through Nalgene 0.2 \(\mu\)m filter systems. Pure solid urea was directly employed in the preparation of solutions for diffusion experiments.

\textbf{Rayleigh Interferometry.} Multicomponent-diffusion coefficients were measured at 25.00 ± 0.01 °C, with the Gosting diffusiometer operating in the Rayleigh interferometric optical mode. The refractive-index profile inside a diffusion cell is measured as described in ref. 28 and references therein. The four ternary diffusion coefficients in the volume-fixed reference frame, \(D_{ij}\), were obtained by applying the method of the nonlinear least squares to the refractive-index profiles, as described in ref 29. Due to PEG molecular-weight polydispersity, a corrective procedure\(^{30}\) based on the experimental refractive-index profiles of binary PEG–water systems was applied to our ternary experiments to remove the contribution of polydispersity from the ternary refractive-index profiles. Values of \(D_{ij}\) obtained at the PEG concentration of \(C_p = 0.250\) mM (5.00 g L\(^{-1}\)) were calculated with respect to the nominal polymer molecular weight of 20 kg mol\(^{-1}\) as a function of osmolyte concentration, \(C_L\). Note that values of \(D_{ij}\) and \(D_{ij}\) scale inversely and directly with polymer molecular weight, respectively. At our low macromolecule concentration, experimental values of \((D_{LP})_{ij}/C_p\) and \((D_{LP})_{ij}/(C_L)_{ij}\) are independent of \(C_p\) within the experimental error.\(^{8,5}\) We therefore set them to be equal to \(\lim_{C_L \to 0}(D_{LP})_{ij}/C_p\) and \(\lim_{C_L \to 0}(D_{LP})_{ij}/(C_L)_{ij}\).
and preferential binding of the osmolyte to the macromolecule, yielding expected for protein-stabilization strength. In data. The dashed line, which will be discussed in the Results and Discussion section, represents: 

Figure 1A, we have also included the reference line, di molar volume. Positive and negative deviations of osmotic Plots of osmolyte osmotic di function of osmolyte concentration, C_L, for urea (circles), DEG (squares), and TMAO (diamonds). Solid curves are quadratic fits through the data. The dashed line, which will be discussed in the Results and Discussion section, represents: 

(A) Osmolyte osmotic diffusion coefficient, \( \hat{D}_{LP} \), as a function of osmolyte concentration, \( C_L \), for urea (circles), DEG (squares), and TMAO (diamonds). Solid curves are quadratic fits through the data. The dashed line, which will be discussed in the Results and Discussion section, represents: 

(B) PEG diffusiophoresis coefficient, \( \hat{D}_{PL} \), as a function of osmolyte concentration, \( C_L \), for urea (circles), DEG (squares), and TMAO (diamonds). Solid curves are linear fits through the data. The dashed line, which will be discussed in the Results and Discussion section, represents: 

Figure 1. (A) Osmolyte osmotic diffusion coefficient, \( \hat{D}_{LP} \), as a function of osmolyte concentration, \( C_L \), for urea (circles), DEG (squares), and TMAO (diamonds). Solid curves are quadratic fits through the data. The dashed line, which will be discussed in the Results and Discussion section, represents: 

\[
(D_{L1})_{V_L} \text{ respectively. Moreover, } (D_{LP})_V/C_P \text{ and } C_P(D_{LP})_V \text{ are approximately independent of PEG molecular weight.}^8 \text{ This implies that the value of 20 kg mol}^{-1} \text{ is merely chosen as a reference molecular weight to calculate the values of } (D_{LP})_V \text{ and } (D_{LP})_V. \text{ The only diffusion parameter that appreciably depends on polymer molecular weight and helps in determining } D_{LP} \text{ only is the polymer tracer-diffusion coefficient, } D_p. \text{ We set } D_p = 0.0608 \times 10^{-9} \text{ m}^2 \text{s}^{-1}, \text{ which corresponds to a hypothetical monodisperse PEG in water at 25 °C, with molecular weight of 20 kg mol}^{-1} \text{ (see the Supporting Information).}^9 \text{ The dependence of } D_p \text{ on osmolyte concentration was calculated from available viscosity data on binary osmolyte-solvent systems using } D_P(C_L) = D_P(0)/\eta_C(C_L)^{\gamma}, \text{ where } \eta_C(C_L) \text{ is the value of relative viscosity at } C_L. \text{ To convert volume-fixed diffusion coefficients into the corresponding solvent-fixed values, } D_{LP}/C_P \text{ and } D_{LP}/D_{L1}, \text{ the terms } V_{L1}D_{L1} \text{ and } (C_LV_L/(1 - C_LV_L))[D_{LP}/D_{L1}] \text{ were added to } (D_{LP})_V/C_P \text{ and } (D_{LP})_V/(D_{L1})_V \text{ respectively,}^{25} \text{ where } V_L = 16.7 \text{ dm}^3 \text{ mol}^{-1} \text{ (0.835 cm}^3 \text{ g}^{-1} \text{) is the partial molar volume of PEG (20 kg mol}^{-1} \text{) and } V_{L1} \text{ is the partial molar volume in the binary osmolyte-water system.}^{23,27,34} \text{ Finally, } D_{LP}(C_L) \text{ and } D_{LP}(C_L) \text{ were calculated from } D_{LP}/C_P \text{ and } D_{LP}/D_{L1}, \text{ using eqs 2a and 2b. Experimental ternary diffusion coefficients, thermodynamic and transport properties of binary osmolyte-water systems obtained from this work (binary diffusion coefficients) and the literature, and } D_{LP}(C_L) \text{ and } D_{LP}(C_L) \text{ values are reported in the Supporting Information.}

RESULTS AND DISCUSSION

Plots of osmolyte osmotic diffusion coefficient, \( \hat{D}_{LP} \), and PEG diffusiophoresis coefficient, \( \hat{D}_{PL} \), as a function of osmolyte (urea, DEG, and TMAO) concentration, \( C_L \), are shown in Figure 1A,B. In all cases, \( \hat{D}_{LP} \) and \( \hat{D}_{PL} \) increase with \( C_L \) starting from \( \hat{D}_{LP} = \hat{D}_{PL} = 0 \) at \( C_L = 0 \). The corresponding slopes increase in the order: urea < DEG < TMAO. This is the same ranking expected for protein-stabilization strength.\(^{20,35}\) In Figure 1A, we have also included the reference line, \( \hat{D}_{LP} = V_P/C_P \),\(^{11}\) with \( V_P = 16.7 \text{ dm}^3 \text{ mol}^{-1} \) being the polymer partial molar volume. Positive and negative deviations of osmotic diffusion data from this line characterize preferential exclusion and preferential binding of the osmolyte to the macromolecule, respectively.\(^{11}\) Thus, \( \hat{D}_{LP} \) data in Figure 1A are also consistent with TMAO and DEG being protein stabilizers and urea being a protein destabilizer. This will be further examined in the Results and Discussion section. In all three cases, \( \hat{D}_{LP}(C_L) \) exhibits some curvature, with its slope tending to converge toward that of the reference line as \( C_L \) increases. This can be related to a partial reduction of hydration in the case of TMAO and DEG and ligand-binding saturation in the case of urea at high \( C_L \).

In Figure 1B, PEG diffusiophoresis, \( \hat{D}_{PL}(C_L) \), linearly increases with \( C_L \). Positive values of \( \hat{D}_{PL} \) imply that osmolyte gradients induce a PEG net migration from high to low \( C_L \). This occurs in all three cases, independent of osmolyte nature. Note that macromolecule migration from high to low osmolyte concentration is consistent with osmolyte preferential exclusion. However, urea preferential binding would suggest a PEG migration in the opposite direction (\( \hat{D}_{PL} < 0 \)), contrary to our experimental results. This will be further examined in the Results and Discussion section.

To quantitatively characterize diffusiophoresis magnitude, we write: \( \hat{D}_{PL} = bV_W/C_L \) where the slope \( b \) is reported as a multiple of the molar volume of water, \( V_W = 18.07 \text{ cm}^3 \text{ mol}^{-1} \), which is also approximately equal to the reciprocal of the water molar concentration, \( C_W \). We obtain: \( b = 53 \pm 5 \) (urea), 186 ± 5 (DEG), and 262 ± 6 (TMAO), which can be compared with the slope values of 130 ± 3 (NaCl) and 384 ± 3 (Na_2SO_4) extracted from salt-induced PEG diffusiophoresis.\(^8\)

According to eq 3, the macromolecule diffusiophoretic migration is directly proportional to \( b \) and not \( C_L \), after noticing that this concentration variable in \( \hat{D}_{PL} = bV_W/C_L \) disappears when combined with \( V_{\mu_L} \); i.e., \( C_LV_{\mu_L}/RT = y_LV_VC_L \). The thermodynamic factor, \( y_L \), is also important, as it contributes to the overall thermodynamic driving force. Since we know that \( y_L = 0.97 \) (urea),\(^{25,26}\) 1.17 (DEG),\(^{25,26}\) and 1.55 (TMAO)\(^{24}\) at \( C_L = 1.0 \text{ M} \), we can conclude that TMAO exhibits relatively large values of both \( b \) and \( y_L \).

To examine the experimental behavior of \( \hat{D}_{LP}(C_L) \) and \( \hat{D}_{LP}(C_L) \), we start from the following relationships based on nonequilibrium thermodynamics\(^8\):

\[
\hat{D}_{PL} = \gamma - \lambda 
\]

\[
\hat{D}_{LP} = [(1-V_L/C_L)\gamma + V_P/C_L] - a\lambda
\]

In eq 5a, macromolecule diffusiophoresis coefficient, \( \hat{D}_{PL} \), is written as the difference between a thermodynamic coefficient (\( \gamma \)) and a transport coefficient (\( \lambda \)). The thermodynamic
coefficient, γ_j, is a preferential-interaction coefficient^{36} defined by γ ≡ lim_{C→0} (μ_jL/μ_jLL), where μ_jL ≡ (∂μ_j/∂C_j) and μ_jLL ≡ (∂μ_j/∂C_j)C_j = RTγ_j/C_j are chemical-potential derivatives, μ_j the chemical potential of component i, and γ_j is the previously introduced nonideality factor. The transport coefficient, λ, is a frictional-interaction coefficient defined by λ ≡ -(C_j/C_k)-lim_{C→0} (D_{j,k}D_{k,j}),^{6} where D_{i,j} (with i, j = P, L, W) is a Stefan—Maxwell diffusion coefficient,^{37} with D_{i,j}^{-1} describing the apparent frictional interaction between i and j. The value of D_{i,j} is straightforward related to osmolyte diffusion in water, D_{j,L}(C_j) in the solvent-fixed reference frame, with D_{i,j} → D_{i,j} in the limit of C_j → 0. The use of the negative sign in the definition of λ is motivated by the values of D_{i,j} being found to be negative for PEG and lysozyme in the presence of salting-out salts.\(^{7-9}\) In eq 5b, osmolyte osmotic diffusion coefficient, \(\hat{D}_{i,j}\), is also a function of y and λ. However, the contribution of the transport coefficient, αλ, in eq 5b is small as α ≡ D_{i,j}/D_{i,i} is less than 10% and may also be approximately neglected for large particles. This implies that \(\hat{D}_{i,j}\) is approximately equal to the thermodynamic quantity, \([(1 – V_j/C_j)Y_j + \hat{V}_j C_j]\), where \(\hat{V}_j\) is the osmolyte partial molar volume and \(V_j ≡ \hat{V}_j \div \hat{V}_j / γ_j\). This thermodynamic quantity is the opposite of the partitioning coefficient, \((∂C_i/∂C_j)_μ\), as can also be appreciated after comparing eqs 5b and 4. Values of \(γ(C_j)\) and \(λ(C_j)\) were extracted from eqs 5a and 5b using the known values of \(α, \hat{V}_j,\) and \(\hat{V}_j\) as a function of \(C_j\). All data are reported in the Supporting Information. Note that \(\hat{V}_j ≡ \hat{V}_j\) is an excellent approximation because the osmolyte molecular weight is significantly lower than that of the macromolecule. We shall employ this approximation in the discussion of \(γ(C_j)\) below.

To examine the thermodynamic coefficient, \(γ(C_j)\), we consider the following thermodynamic relation\(^{10}\)

\[
γ \equiv -ν_L \equiv ν_W C_j/C_W
\]

where \(ν_L \equiv \lim_{C→0} (\partial (n_i/n_w))/\partial (n_j/n_w)\) and \(ν_W \equiv \lim_{C→0} (\partial (n_i/n_j))/\partial (n_j/n_w)\) are, respectively, the thermodynamically linked preferential-binding and preferential-solvation coefficients, with \(n_i\) being the number of moles of component i. These thermodynamic coefficients may be interpreted by considering a two-domain model,\(^{38}\) in which a local domain, represented by the osmolyte–water layer surrounding a macromolecule, is in chemical equilibrium with a bulk domain, representing the osmolyte–water remaining solution. Since the macromolecule interacts with the osmolyte and water molecules in their vicinity, the osmolyte concentration in the local domain is different from that of the unperturbed bulk domain. If the osmolyte concentration in the local domain is higher than in the bulk domain, \(ν_L > 0\) is the number of osmolyte molecules in excess compared to that in the bulk-domain composition. Correspondingly, osmolyte depletion implies that \(ν_L < 0\). In this case, the preferential-interaction parameter, \(ν_W > 0\), characterizes the excess of water molecules near the macromolecule. Clearly, \(ν_L\) and \(ν_W\) are just two equivalent ways to characterize the magnitude of PEG preferential binding to urea or PEG preferential hydration in the other osmolyte cases. Values of \(ν_L\) and \(ν_W\), extracted from eq 6, are shown in Figure 2A,B as a function of \(C_j\). To quantitatively rank the three osmolytes, we specifically consider the values of \(ν_W\) at \(C_j = 0\) M. We obtain the following: ~$-$580 ± 50 (urea, −1.3 water molecules per ethoxy unit), 1350 ± 50 (DEG, 3.0 water molecules per ethoxy unit), and 2600 ± 50 (TMAO, 5.7 water molecules per ethoxy unit). For comparison, we have: 1090 ± 50 (NaCl, 2.4 water molecules per ethoxy unit) and 3540 ± 50 (Na_2SO_4, 7.8 water molecules per ethoxy unit). These values represent a scale in which some of the most common aqueous additives are quantitatively ranked with respect to their preferential-hydration strength.

We now turn our attention to the transport coefficient, λ. In Figure 2C, values of the ratio, \(λ/γ\), are shown. These are positive in all three cases. Values of \(λ/γ\) for TMAO are about 10% larger than those for DEG. This change is small compared to the corresponding 2-fold increase in the value of \(ν_W\). Furthermore, the values of \(λ/γ\) ≈ 0.8–0.9 < 1 observed in the DEG and TMAO cases are in the same range as those extracted in the NaCl and Na_2SO_4 cases.\(^{7}\) This implies that compared to \(ν_W\) the ratio \(λ/γ\) is a relatively weak function of preferential-hydration strength. Interestingly, this analysis cannot be extended to the urea case. In Figure 2C, the corresponding values of \(λ/γ\) ≈ 1.1 larger than one originate from \(D_{PL}\) being positive even if \(γ\) is negative. In the final part of this section, we introduce a model that will allow us to derive mathematical expressions of \(γ\) and \(λ\) explaining why (1) \(λ/γ\) is a weak function of preferential-hydration strength in the TMAO and DEG cases and (2) \(D_{PL}\) remains positive in the urea case.
As a starting point, we consider the equilibrium two-domain model discussed above and modify it to take into account diffusioosmophoresis, a slip surface boundary surrounding the particle becomes operational. As shown in Figure 3, it is expected that the local domain of a macromolecule extends beyond its slip surface because only osmolyte and solvent molecules strongly interact with the macromolecule can be dragged. We therefore split the local domain into an inner local domain (I), enclosed by the slip surface, and an outer local domain (II), characterizing the fraction of local domain beyond the slip surface. The composition of the inner (I) and outer (II) local domains are linked to the composition of the bulk domain by formally introducing partitioning coefficients, $K^{(i)}$ and $K^{(II)}$, with $N^{(i)}_{W}/N^{(II)}_{W} = K^{(i)}C_{I}/C_{W}$ and $N^{(II)}_{W}/N^{(II)}_{W} = K^{(II)}C_{I}/C_{W}$, where $N^{(i)}_{W}$ and $N^{(II)}_{W}$ and $N^{(II)}_{W}$ are the osmolyte and water number of molecules in domains I and II, respectively. Preferential osmolyte (water) binding implies that both $K^{(i)}$ and $K^{(II)}$ are larger (smaller) than one. In any case, we expect that $1 - K^{(II)} < 1 - K^{(i)}$ as interactions with the macromolecule are relatively weak in the outer local domain.

If we apply the thermodynamic definition of $\lambda$, and eq 6, we can derive (see the Supporting Information)

$$\gamma = N^{(i)}_{W}(1 - K^{(i)})\left[1 + \frac{N^{(II)}_{W}}{N^{(i)}_{W}} \frac{1 - K^{(II)}}{1 - K^{(i)}} \frac{C_{I}}{C_{W}}\right]$$

(7)

Note that the sign of $\gamma$ is the same as that of $1 - K^{(i)}$ because the factor inside square brackets is expected to be always positive.

To derive the corresponding expression of $\lambda$, we rewrite eq 3 in terms of the macromolecule chemical potential gradient, $\nu_{\mu_{P}}$, and the transport coefficient, $\lambda$. Specifically, we first consider the thermodynamic relation, $\nu_{\mu_{P}} = RT \nu_{C_{P}} + \gamma \nu_{\mu L}$, and replace $\nu_{C_{P}}$ with $\nu_{\mu_{P}}$ in eq 3. We then replace $D_{PL}$ with $\lambda$ based on eq 5a. This yields

$$-r_{P} = D_{P}\left(\frac{\nu_{\mu_{P}}}{RT} - \lambda \frac{\nu_{\mu_{P}}}{RT}\right)$$

(8)

We now remark that the diffusing particle is $\{P\} \equiv P(W)N^{(i)}_{W}(1)N^{(i)}_{W}$, which includes the osmolyte and solvent molecules within the slip surface (inner local domain). This will have an impact on the sign and physical meaning of $\lambda$. Indeed, we can rewrite eq 8 in terms of the chemical potential gradient, $\nu_{\mu_{(P)}} = \nu_{\mu_{P}} + N^{(i)}_{W}\nu_{\mu_{W}} + N^{(II)}_{W}\nu_{\mu_{L}}$, of the diffusing particle, $\{P\}$

$$-r_{P} = D_{P}\left(\frac{\nu_{\mu_{P}}}{RT} + f_{PL} \frac{C_{I}}{C_{W}} \frac{\nu_{\mu_{L}}}{RT}\right)$$

(9)

where we have also introduced the reduced frictional coefficient, $f_{PL} \equiv \lim_{C_{P} \to 0} (D_{PL}/D_{PL})$, with $D_{PL}$ being the Stefan–Maxwell diffusion coefficient describing the frictional interaction between osmolyte and $\{P\}$. Within the framework of the proposed model, $f_{PL}$ is assumed to be positive, as $D_{PL}^{-1} > 0$ describes the actual frictional interaction experienced by the diffusing macromolecule. Specifically, the diffusion of osmolyte molecules from high to low $C_{I}$ exerts a frictional dragging effect\(^{39,40}\) on the macromolecule along the same direction. To link $\lambda$ to $f_{PL}$, we equate eqs 8–9. After using $\nu_{\mu_{P}} = \nu_{\mu_{P}} + N^{(i)}_{W}\nu_{\mu_{W}} + N^{(II)}_{W}\nu_{\mu_{L}}$, together with the Gibbs–Duhem equation, $C_{W}\nu_{\mu_{W}} = -C_{I}\nu_{\mu_{P}} - C_{I}\nu_{\mu_{L}} (1 - N^{(II)}_{W}/C_{W})\nu_{\mu_{P}} \to -\nu_{\mu_{P}}$ and $N^{(II)}_{W}/N^{(II)}_{W} = K^{(II)}C_{I}/C_{W}$ we derive

$$\lambda = [N^{(II)}_{W}(1 - K^{(II)})] - f_{PL} \frac{C_{I}}{C_{W}}$$

(10)

Note that the same expression of $\lambda$ can be also derived from the osmolyte diffusion rate, consistent with Onsager Reciprocal Relations (see the Supporting Information)\(^{41}\).

In summary, we have obtained mathematical expressions for $\gamma$ (eq 7) and $\lambda$ (eq 10). To discuss our experimental results, we consider two limiting cases in eqs 7 and 10. In the first limiting case, we neglect the contribution of $f_{PL}$ in eq 10. Correspondingly, eqs 7 and 10 give

$$\lambda = \frac{N^{(II)}_{W}(1 - K^{(II)})}{N^{(II)}_{W}(1 - K^{(II)})}$$

(11)

This shows that $\lambda/\gamma < 1$. Furthermore, it is reasonable to expect that the ratios, $K^{(II)}/K^{(i)}$ and $N^{(II)}_{W}/N^{(i)}_{W}$, are weak functions of preferential-hydration strength. Thus, eq 11 successfully describes why the values of $\lambda/\gamma$ for TMAO, DEG, NaCl, and Na2SO4 are similar to each other even though the corresponding $\nu_{C_{P}}$ values may be quite different. However, in the case of urea, eq 11 incorrectly predicts that $\lambda/\gamma$ cannot be neglected in general. Thus, as a second limiting case, we retain $f_{PL}$ in eq 10 and neglect the contribution of the outer local domain in eq 7 for $\gamma$. Combination of eqs 7 and 10 then leads to $\lambda = \gamma - f_{PL}(C_{I}/C_{W})$, and eq 5a becomes

$$D_{PL} = f_{PL} \frac{C_{I}}{C_{W}}$$

(12)
Since $f_{PL}$ is positive, eq 12 is consistent with our $\hat{D}_{PL}$ results in the urea case. In other words, PEG–urea frictional interaction prevails over the extent of PEG–urea preferential binding occurring outside the slip surface of the diffusing macromolecule.

It remains to explain why the outer local domain is relatively more important for DEG and TMAO than for urea. It is reasonable to assume that the macromolecule hydration number, which is represented by $N^0_W$, is approximately the same in all osmolyte cases due to large excess of solvent compared with osmolyte molecules ($N^0_W \gg N^0_I$). This implies the thickness of the hydration layer (inner local domain) must also be approximately the same in all cases. However, the magnitude of $\nu_W$ for urea is significantly smaller than that found for DEG and TMAO. This indicates that PEG–urea preferential binding may be exhausted within the hydration shell of the macromolecule contrary to the other cases in which PEG preferential hydration extends well beyond the macromolecule slip surface.

According to eq 12, $\hat{D}_{PL}$ may be approximately described by the value of $f_{PL}$ alone in the urea case. Thus, we make an attempt to estimate this frictional parameter. If we assume that $D_i/D_k = \alpha_i/\alpha_k$ is independent of $i$, where the $\alpha_i$'s are parameters used to scale the ratios of frictional coefficients, we can deduce that $D_W/\hat{D}_{PL} = D_{NW}/\hat{D}_{PL} = D_W/\hat{D}_{PL}$ where $D_W$ is the water self-diffusion coefficient. After applying the definition of $f_{PL}$, we can then write

$$f_{PL} = \frac{D_W}{D_p}$$

We are now in a position to calculate $f_{PL} = 37.8$ by using $D_W = 0.0608 \times 10^{-9} \text{ m}^2 \text{s}^{-1}$ and $D_p = 2.30 \times 10^{-9} \text{ m}^2 \text{s}^{-1}$ for pure water at 25 °C. In Figure 1B, values of $\hat{D}_{PL}$ based on eqs 12 and 13 are shown as a reference line. We can see that the $\hat{D}_{PL}(C_I)$ data obtained in the urea case are close to this reference line, further supporting our analysis.

**SUMMARY AND CONCLUSIONS**

PEG diffusiophoresis in water was experimentally found to occur from high to low osmolyte concentration in all three osmolyte cases. The magnitude of diffusiophoresis increases in the order urea < DEG < TMAO. This ranking is the same as that expected for osmolyte effectiveness in stabilizing the protein native state. Related osmolyte osmotic diffusion coefficients were used to determine preferential-interaction coefficients ($\nu_W$) that provided a quantitative ranking of osmotolytes and salting-out salts. Values of $\nu_W$ were positive for TMAO and DEG (preferential exclusion) and negative for urea (preferential binding). A general model was developed to examine the contributions of preferential-interaction and frictional dragging in macromolecule diffusiophoresis. According to this model, preferential hydration is mainly responsible for PEG diffusiophoresis from high to low TMAO or DEG concentration. On the other hand, frictional dragging prevails in PEG diffusiophoresis from high to low urea concentration.

Our $\hat{D}_{PL}$ results on TMAO, together with the relatively large values of $\nu_W$, indicate that concentration gradients of this osmolyte can be potentially applied to induce migration of PEG or PEG-functionalized particles. Since TMAO solubility in water is also fairly high (6 M), large spatial differences of TMAO concentration, of the order of TMAO solubility, may be exploited to induce diffusiophoresis and develop new ways to drive and direct particle motion in solution.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.8b02065.

Tables reporting multicomponent-diffusion coefficients of the investigated ternary systems, related volumetric properties, thermodynamic and transport parameters of the binary osmolyte–water systems, PEG diffusion coefficient, expression of the preferential-interaction coefficient, and the validity of the Onsager (PDF)

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**Notes**

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