

# Macromolecular hydration compared with preferential hydration and their role on macromolecule-osmolyte coupled diffusion†‡

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Hydration and preferential hydration of macromolecules are two distinct properties of their multicomponent aqueous solutions. We have experimentally investigated ternary diffusion in a macromolecule–osmolyte–water system in order to characterize and compare these two independent quantities and to experimentally establish their role on the phenomenon of coupled diffusion. Specifically, we report the four diffusion coefficients for the poly(ethylene glycol)–di(ethylene glycol)–water system at 25 °C using Rayleigh interferometry. In this work, the molecular weight of poly(ethylene glycol) (PEG) is 200-fold higher than that of di(ethylene glycol) (DEG). This ratio is comparable to that between proteins and low molecular-weight osmolytes. This system has been selected because both solutes are neutral hydrated species with similar chemical properties and very different size. Hence, the observed behavior of coupled diffusion can be directly related to solute hydration and size ratio and is not complicated by other factors such as ionic interactions usually encountered in protein systems. Using our multicomponent diffusion coefficients, we have found that PEG hydration is slightly smaller than its preferential hydration. The observed difference can be attributed to PEG–DEG excluded-volume interactions. Our experimental results also enable us to reveal that Onsager cross-transport coefficients are large and negative. This implies that this transport coefficient should not be neglected in multicomponent-diffusion theoretical models even when ionic interactions or chemical association between the solute species are absent. This work provides the basis for understanding coupled diffusion in more complex aqueous systems such as those containing charged proteins or nucleic acids in the presence of salts or osmolytes.

## Introduction

Aqueous solutions containing macromolecules typically contain other solutes such as salts, osmolytes and organic solvents.<sup>1–6</sup> These additives are necessary for modulating the thermodynamic state (chemical potential) of macromolecules in water so that processes such as crystallization,<sup>7,8</sup> aggregation,<sup>9</sup> conformational changes<sup>10–12</sup> and enzymatic activity<sup>13</sup> may be either promoted or inhibited.

Another important aspect of macromolecular solutions is their dynamic behavior in the presence of concentration gradients. This is described by mutual-diffusion (or inter-diffusion).<sup>14,15</sup> Diffusion coefficients are employed for modeling controlled release of macromolecular drugs,<sup>16</sup> drying behavior of polymers in mixed solvents,<sup>17</sup> *in vivo* transport processes involving biomacromolecules,<sup>18,19</sup> protein crystallization<sup>20</sup> and mixing inside microfluidic devices.<sup>21</sup> Clearly, additives also play a crucial role on the diffusion

properties of macromolecular aqueous mixtures. Interestingly, they not only change the diffusion coefficients of macromolecules in solution but also introduce complexity in the transport processes because additive diffusion is coupled to that of the macromolecule.<sup>22–27</sup>

Macromolecular hydration, *i.e.* the number of water molecules bound to a macromolecule, and preferential hydration, *i.e.* the excess of water molecules surrounding a macromolecule compared to bulk composition, are two important quantities that shape the thermodynamic and diffusion behavior of macromolecule–additive–water solutions.<sup>1–4</sup> Although these two properties bear obvious similarities, they should be regarded as independent entities, not directly related.<sup>1</sup> In this paper, we experimentally examine macromolecular hydration, preferential hydration and their distinct roles on macromolecule–osmolyte coupled diffusion for a model macromolecule–additive–water system.

For macromolecule (1) + additive (2) + water ternary systems, diffusion is described by the extended Fick's first law:<sup>23</sup>

$$-J_1 = D_{11}\nabla C_1 + D_{12}\nabla C_2 \quad (1a)$$

$$-J_2 = D_{21}\nabla C_1 + D_{22}\nabla C_2 \quad (1b)$$

where  $C_1$  and  $C_2$  are the molar concentrations of macromolecule and additive respectively,  $J_1$  and  $J_2$  are the corresponding molar fluxes, and the four  $D_{ij}$ 's (with  $i, j = 1, 2$ )

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are the multicomponent diffusion coefficients. Main-diffusion coefficients,  $D_{11}$  and  $D_{22}$ , describe the flux of macromolecule and additive due to their own concentration gradients, while cross-diffusion coefficients,  $D_{12}$  and  $D_{21}$ , describe the flux of a solute due to the concentration gradient of the other solute and are responsible for coupled diffusion. Multicomponent diffusion coefficients have been experimentally determined for lysozyme, a positively-charged protein, in the presence of chloride salts and neutral molecules as additives using interferometric methods.<sup>25,26,28–31</sup> These studies were then extended to poly(ethylene glycol) with a molecular-weight comparable to that of proteins in the presence of aqueous potassium chloride.<sup>27</sup>

However, to our knowledge, no multicomponent diffusion study has been previously performed on aqueous systems in which both macromolecule and additive are neutral species. Experimental investigations on this type of system are needed because the corresponding observed behavior of coupled diffusion can be directly related to hydration, preferential hydration and size ratio between the two solutes, and is not complicated by the presence of ionic interactions. Hence, we report here multicomponent diffusion coefficients on the poly(ethylene glycol)–di(ethylene glycol)–water ternary system measured at 25 °C using Rayleigh interferometry. Poly(ethylene glycol) is a hydrophilic nonionic polymer used in many processes based on aqueous solutions, including the partitioning and the precipitation of biomacromolecules from aqueous systems and for drug-delivery applications.<sup>32–34</sup> In our work, the molecular weight of poly(ethylene glycol) (PEG, 20 kg mol<sup>-1</sup>) is about 200 times higher than that of the osmolyte di(ethylene glycol) (DEG, 106 g mol<sup>-1</sup>). This difference in molecular weight between PEG and DEG is comparable with that between proteins and low molecular-weight osmolytes.

Our goal is to use the four experimental diffusion coefficients of a ternary system in which both solute components are neutral species to characterize and compare macromolecular hydration with preferential hydration. We will also examine their distinct roles on the quotients:  $(D_{12}/D_{11})/C_1$  and  $(D_{21}/D_{22})/C_2$ , which are used to characterize coupled diffusion. A theoretical basis on the relation of these two properties with coupled diffusion has been previously reported<sup>35</sup> and is applied for the first time in this paper to aqueous systems in which both macromolecule and additive are neutral species.

We note that multicomponent diffusion studies have been previously reported on ternary aqueous mixtures of poly(ethylene glycol) pairs.<sup>36–39</sup> However, the molecular weights of the previously investigated polymers were at least one order of magnitude lower than that of the PEG macromolecule in our work. Thus, our experimental results complement previous diffusion data on binary<sup>40</sup> and ternary<sup>36–39</sup> aqueous mixtures containing poly(ethylene glycol). Moreover, it is important remark that our experimental conditions (chosen system, compositions and experimental setup) has enabled us to discuss general fundamental aspects related to macromolecular hydration, preferential hydration and coupled diffusion that could not be examined in the aqueous mixtures of ethylene-glycol oligomers previously investigated.

## Theoretical background

In this section, we summarize the fundamental equations needed for understanding and examining multicomponent diffusion in the PEG–DEG–water ternary system.

Ternary diffusion coefficients in eqn (1a),(b) can be described relative to different reference frames.<sup>41</sup> In the volume-fixed frame, the fluxes of the components of a ternary system satisfy the condition:  $(J_0)_V \bar{V}_0 + (J_1)_V \bar{V}_1 + (J_2)_V \bar{V}_2 = 0$ ; in the solvent-fixed frame, we have  $(J_0)_0 = 0$ . Here,  $J_i$  and  $\bar{V}_i$  are the molar flux and partial molar volume of component  $i$ , respectively. The subscript “V” denotes the volume-fixed frame. The subscript “0” denotes the solvent component when appended directly to a flux, and denotes the solvent-fixed frame when appended outside the parentheses to an already-subscripted flux or diffusion coefficient.

The solvent-fixed frame coefficients,  $(D_{ij})_0$ , are related to the volume-fixed frame coefficients  $(D_{ij})_V$  by the following relations:<sup>42,43</sup>

$$(D_{11})_0 = (D_{11})_V + [(C_1/(1 - C_1\bar{V}_1 - C_2\bar{V}_2))] \times [\bar{V}_1 (D_{11})_V + \bar{V}_2 (D_{21})_V] \quad (2a)$$

$$(D_{12})_0 = (D_{12})_V + [(C_1/(1 - C_1\bar{V}_1 - C_2\bar{V}_2))] \times [\bar{V}_1 (D_{12})_V + \bar{V}_2 (D_{22})_V] \quad (2b)$$

$$(D_{21})_0 = (D_{21})_V + [(C_2/(1 - C_1\bar{V}_1 - C_2\bar{V}_2))] \times [\bar{V}_1 (D_{11})_V + \bar{V}_2 (D_{21})_V] \quad (2c)$$

$$(D_{22})_0 = (D_{22})_V + [(C_2/(1 - C_1\bar{V}_1 - C_2\bar{V}_2))] \times [\bar{V}_1 (D_{12})_V + \bar{V}_2 (D_{22})_V] \quad (2d)$$

Linear laws of non-equilibrium thermodynamics in isothermal conditions can be used to obtain a relation between solvent-frame diffusion coefficients and solute chemical potentials,  $\mu_i$ :<sup>42–44</sup>

$$(D_{11})_0 = (L_{11})_0 \mu_{11}^{(c)} + (L_{12})_0 \mu_{21}^{(c)} \quad (3a)$$

$$(D_{12})_0 = (L_{11})_0 \mu_{12}^{(c)} + (L_{12})_0 \mu_{22}^{(c)} \quad (3b)$$

$$(D_{21})_0 = (L_{21})_0 \mu_{11}^{(c)} + (L_{22})_0 \mu_{21}^{(c)} \quad (3c)$$

$$(D_{22})_0 = (L_{21})_0 \mu_{12}^{(c)} + (L_{22})_0 \mu_{22}^{(c)} \quad (3d)$$

where  $(L_{ij})_0$  are the Onsager transport coefficients in the solvent-fixed frame,  $\mu_{ij}^{(c)} \equiv (\partial\mu_i/\partial C_j)_{T,p,C_k,k \neq j}$  are the molarity-based chemical-potential derivatives,  $T$  is the temperature and  $p$  the pressure. The  $(L_{ij})_0$  matrix is symmetric and  $(L_{12})_0 = (L_{21})_0$  represents the Onsager reciprocal relation.<sup>45,46</sup>

We note that volume-frame diffusion coefficients are obtained experimentally.<sup>41</sup> However, solvent-frame diffusion coefficients are more directly related to solute chemical potentials. Hence, eqn (2a)–(d) are often used to convert the experimentally obtained  $(D_{ij})_V$  into  $(D_{ij})_0$ .

The thermodynamics of macromolecule-additive-solvent ternary mixtures is often described using molality-based chemical-potential derivatives:  $\mu_{ij}^{(m)} \equiv (\partial\mu_i/\partial m_j)_{T,p,m_k,k \neq j}$ , where  $m_j$  is the molality of the  $j$ -th solute component.<sup>1,2,47,48</sup> The  $\mu_{ij}^{(m)}$  matrix is symmetric and  $\mu_{12}^{(m)} = \mu_{21}^{(m)}$  represents the

Euler reciprocal relation.<sup>49</sup> The  $\mu_{ij}^{(m)}$  are related to the  $\mu_{ij}^{(c)}$  by the following linear relations:<sup>46</sup>

$$\begin{aligned} (C_1/m_1)(1 - C_1\bar{V}_1 - C_2\bar{V}_2)\mu_{11}^{(c)} \\ = (1 - C_2\bar{V}_2)\mu_{11}^{(m)} + C_2\bar{V}_1\mu_{12}^{(m)} \end{aligned} \quad (4a)$$

$$\begin{aligned} (C_1/m_1)(1 - C_1\bar{V}_1 - C_2\bar{V}_2)\mu_{12}^{(c)} \\ = (1 - C_1\bar{V}_1)\mu_{12}^{(m)} + C_1\bar{V}_2\mu_{11}^{(m)} \end{aligned} \quad (4b)$$

$$\begin{aligned} (C_2/m_2)(1 - C_1\bar{V}_1 - C_2\bar{V}_2)\mu_{21}^{(c)} \\ = (1 - C_2\bar{V}_2)\mu_{21}^{(m)} + C_2\bar{V}_1\mu_{22}^{(m)} \end{aligned} \quad (4c)$$

$$\begin{aligned} (C_2/m_2)(1 - C_1\bar{V}_1 - C_2\bar{V}_2)\mu_{22}^{(c)} \\ = (1 - C_1\bar{V}_1)\mu_{22}^{(m)} + C_1\bar{V}_2\mu_{21}^{(m)} \end{aligned} \quad (4d)$$

Eqn (3a)–(d) and eqn (4a)–(d) can be combined together yielding the following expression for  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$ :<sup>27</sup>

$$\frac{\mu_{12}^{(m)}}{\mu_{22}^{(m)}} = \frac{(D_{21})_0(1 - C_1\bar{V}_1) - (D_{22})_0C_2\bar{V}_1 - (\mu_{11}^{(m)}/\mu_{22}^{(m)})[(D_{12})_0(1 - C_2\bar{V}_2) - (D_{11})_0C_1\bar{V}_2]}{(D_{22})_0(1 - C_2\bar{V}_2) - (D_{11})_0(1 - C_1\bar{V}_1) + (D_{12})_0C_2\bar{V}_1 - (D_{21})_0C_1\bar{V}_2} \quad (5)$$

Eqn (5) can be used to extract the thermodynamic parameter  $(\mu_{12}^{(m)}/\mu_{22}^{(m)})$  provided that  $(\mu_{11}^{(m)}/\mu_{22}^{(m)})$  is known with reasonable accuracy. Values of  $(\mu_{12}^{(m)}/\mu_{22}^{(m)})$  have been extracted from ternary diffusion coefficients of macromolecule–salt–water mixtures at low concentration of macromolecules.<sup>27–31</sup> For these systems, the term containing  $(\mu_{11}^{(m)}/\mu_{22}^{(m)})$  in eqn (5) is relatively small and the accuracy of  $(\mu_{11}^{(m)}/\mu_{22}^{(m)})$  need not be high. Adequate estimates of  $(\mu_{11}^{(m)}/\mu_{22}^{(m)})$  can be obtained by setting  $\mu_{11}^{(m)} = RT/m_1$  and calculating  $\mu_{22}^{(m)}$  from available activity-coefficient data for the additive–solvent binary system. If available, accurate values of  $\mu_{11}^{(m)}$  can be obtained from light-scattering or osmotic-pressure data on macromolecular solutions. The values of  $(\mu_{12}^{(m)}/\mu_{22}^{(m)})$  can be then used to quantitatively characterize the preferential interaction of macromolecule with either the solvent or the additive. We will use our experimental ternary diffusion coefficients of the PEG–DEG–water system to extract  $(\mu_{12}^{(m)}/\mu_{22}^{(m)})$  as a function of  $C_2$ . This quantity will be used to characterize PEG preferential hydration in the presence of DEG.

Onsager transport coefficients may be also extracted from the  $(D_{ij})_0$ , provided that the chemical-potential derivatives are known.<sup>28</sup> In this paper, we shall examine the ratio  $(L_{12})_0/(L_{11})_0$ . In this way, explicit dependence of  $(L_{12})_0$  and  $(L_{11})_0$  on macromolecule hydrodynamic radius and solution viscosity is removed since  $(L_{12})_0$  and  $(L_{11})_0$  are both directly proportional to the tracer diffusion coefficient of the macromolecule.<sup>35</sup> The following expression for  $(L_{12})_0/(L_{11})_0$  can be obtained by solving eqn (3a),(b):

$$\frac{(L_{12})_0}{(L_{11})_0} = \frac{(\mu_{11}^{(c)}/\mu_{22}^{(c)})[(D_{12})_0/(D_{11})_0] - (\mu_{12}^{(c)}/\mu_{22}^{(c)})}{1 - (\mu_{21}^{(c)}/\mu_{22}^{(c)})[(D_{12})_0/(D_{11})_0]} \quad (6)$$

where:

$$\frac{\mu_{11}^{(c)}}{\mu_{22}^{(c)}} = \frac{(1 - C_2\bar{V}_2)(\mu_{11}^{(m)}/\mu_{22}^{(m)}) + C_2\bar{V}_1(\mu_{12}^{(m)}/\mu_{22}^{(m)})}{(1 - C_1\bar{V}_1) + C_1\bar{V}_2(\mu_{12}^{(m)}/\mu_{22}^{(m)})} \quad (7a)$$

$$\frac{\mu_{12}^{(c)}}{\mu_{22}^{(c)}} = \frac{(1 - C_1\bar{V}_1)(\mu_{12}^{(m)}/\mu_{22}^{(m)}) + C_1\bar{V}_2(\mu_{11}^{(m)}/\mu_{22}^{(m)})}{(1 - C_1\bar{V}_1) + C_1\bar{V}_2(\mu_{12}^{(m)}/\mu_{22}^{(m)})} \quad (7b)$$

$$\frac{\mu_{21}^{(c)}}{\mu_{22}^{(c)}} = \frac{(1 - C_2\bar{V}_2)(\mu_{12}^{(m)}/\mu_{22}^{(m)}) + C_2\bar{V}_1}{(1 - C_1\bar{V}_1) + C_1\bar{V}_2(\mu_{12}^{(m)}/\mu_{22}^{(m)})} \quad (7c)$$

according to eqn (4a)–(d). Eqn (6) can be used to extrapolate  $(L_{12})_0/(L_{11})_0$  provided that both  $(\mu_{12}^{(m)}/\mu_{22}^{(m)})$  and  $(\mu_{11}^{(m)}/\mu_{22}^{(m)})$  are known. We will use our experimental  $(D_{12})_0/(D_{11})_0$  to extract  $(L_{12})_0/(L_{11})_0$  as a function of  $C_2$  and examine our results. This quantity will be used to characterize PEG hydration.

## Materials and methods

### Materials

Poly(ethylene glycol) with average molecular weight of 20 kg mol<sup>−1</sup> (PEG) was purchased from Fluka and used without further purification. Diethylene glycol (DEG) (ReagentPlus, 99%) was purchased from Sigma-Aldrich and used without further purification. The molecular weight of PEG was taken as 20 000 g mol<sup>−1</sup>, and its density as 1.3 g cm<sup>−3</sup> for buoyancy corrections. The molecular weight of DEG was taken as 106.12 g mol<sup>−1</sup>, and its density as 1.114 g cm<sup>−3</sup> for buoyancy corrections. Deionized water was passed through a four-stage Millipore filter system to provide high-purity water for all the experiments.

All solutions were prepared by weight using a Mettler-Toledo AT400 analytical balance, with appropriate buoyancy corrections. Stock solutions of PEG and DEG were made by weight to 0.1 mg. Density measurements were made on the stock solutions for buoyancy corrections. For binary PEG–water and DEG–water experiments, precise masses of stock solutions were diluted with pure water to reach the final target solute concentrations. For ternary PEG–DEG–water solutions, precise masses of both stock solutions were added to flasks and diluted with pure water to reach the final target PEG and DEG concentrations. The densities of these solutions were measured to determine the corresponding molar concentrations and to calculate partial molar volumes.<sup>43</sup> All density measurements were made using a computer-interfaced Mettler-Paar DMA40 density meter, thermostated with water from a large, well-regulated ( $\pm 0.01$  °C) water bath.

### Diffusion experiments

Binary and ternary mutual diffusion coefficients were measured at 25.00 °C with the Gosting diffusometer operating

**Table 1** Ternary diffusion coefficients for the PEG–DEG–H<sub>2</sub>O system at 25 °C

$\bar{C}_1/\text{mM}$	0.2500	0.2500	0.2500	0.2500	0.2499	0.2492	0.2456
$\bar{C}_2/\text{M}$	0.0000	0.1000	0.2000	0.5001	0.9997	1.9938	3.9298
$\rho/\text{g cm}^{-3}$	0.99784 <sub>0</sub>	0.99926 <sub>9</sub>	1.00069 <sub>8</sub>	1.00496 <sub>6</sub>	1.01229 <sub>2</sub>	1.02701 <sub>8</sub>	1.05521 <sub>0</sub>
$\bar{V}_0/\text{M}^{-1}$	0.01807	0.01805	0.01806	0.01805	0.01805	0.01806	0.01804
$\bar{V}_1/\text{M}^{-1}$	16.7	16.8	16.7	16.6	16.7	16.6	16.8
$\bar{V}_2/\text{M}^{-1}$	—	0.0921	0.0913	0.0922	0.0920	0.0917	0.0919
$(D_{11})_V/10^{-9} \text{ m}^2 \text{ s}^{-1}$	0.06116 ± 0.00006	0.05941 ± 0.00006	0.05800 ± 0.00006	0.05337 ± 0.00010	0.04663 ± 0.00010	0.03588 ± 0.00010	0.02108 ± 0.00040
$(D_{12})_V/10^{-9} \text{ m}^2 \text{ s}^{-1}$	—	0.000026 ± 0.000002	0.000028 ± 0.000001	0.000027 ± 0.000002	0.000026 ± 0.000002	0.000023 ± 0.000002	0.000016 ± 0.000002
$(D_{21})_V/10^{-9} \text{ m}^2 \text{ s}^{-1}$	0	3.2 ± 0.2	6.8 ± 0.2	13.5 ± 0.2	25.0 ± 0.2	40.3 ± 0.2	49.6 ± 0.6
$(D_{22})_V/10^{-9} \text{ m}^2 \text{ s}^{-1}$	—	0.869 ± 0.001	0.857 ± 0.001	0.827 ± 0.001	0.777 ± 0.001	0.684 ± 0.001	0.511 ± 0.002
$(D_{11})_0/10^{-9} \text{ m}^2 \text{ s}^{-1}$	0.06142	0.05974	0.05841	0.05393	0.04748	0.03720	0.02298
$(D_{12})_0/10^{-9} \text{ m}^2 \text{ s}^{-1}$	—	3.3	7.1	14.6	28.4	50.8	80.0
$(D_{21})_0/10^{-9} \text{ m}^2 \text{ s}^{-1}$	0	0.000046	0.000048	0.000047	0.000046	0.000043	0.000035
$(D_{22})_0/10^{-9} \text{ m}^2 \text{ s}^{-1}$	—	0.877	0.873	0.867	0.857	0.839	0.804
$m_2\mu_{22}^{(m)}/RT$	1.000	1.007	1.013	1.032	1.060	1.107	1.142
$(\mu_{12}^{(m)}/\mu_{22}^{(m)})$	0	2.3 ± 0.3	5.1 ± 0.3	9.4 ± 0.3	18.9 ± 0.3	34.6 ± 0.3	53.2 ± 1.2
$(L_{12})_0/(L_{11})_0$	0	−1.9 ± 0.3	−4.3 ± 0.3	−7.3 ± 0.3	−14.6 ± 0.4	−26.0 ± 0.5	−34.9 ± 2.0

in the Rayleigh interferometric optical mode.<sup>25,50,51</sup> The refractive-index profile inside a diffusion cell is measured as described in ref. 25 and references therein. Fifty refractive-index profiles were obtained during the course of each experiment. Experiments were performed by the free-diffusion method in a 10-cm vertical diffusion cell with a 2.5 cm horizontal optical path length and a 0.3 cm width. The temperature was regulated to ±0.001 °C precision and ±0.01 °C accuracy. Initial step-function distributions of solute concentrations were prepared with the boundary located at the center of the cell. Initial imperfections from the ideal sharp boundary are removed as described in ref. 50 and references therein. All experimental data were obtained before detectable concentration changes occurred at the top and bottom ends of the cell, consistent with the free-diffusion boundary condition. A minimum of two experiments is required for determining the four diffusion coefficients at a given set of mean concentrations ( $\bar{C}_1, \bar{C}_2$  in Table 1). These two experiments must have different combinations of solute concentration differences across the diffusion boundary. To verify reproducibility, two other duplicate experiments were performed at each set of mean concentrations. The volume-frame diffusion coefficients  $(D_{ij})_V$  were obtained by applying the method of the non-linear least squares as described ref. 51. Due to PEG polydispersity, a small correction was applied to the fringe positions as described in ref. 52 in details. Experimental details related to individual ternary diffusion experiments and binary experiments on the DEG–water system are available as ESI.†

## Results

In this section, we first describe our experimental diffusion coefficients for the PEG(1)–DEG(2)–water(0) system and then use solvent-frame diffusion coefficients to calculate  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  and  $(L_{12})_0/(L_{11})_0$ .

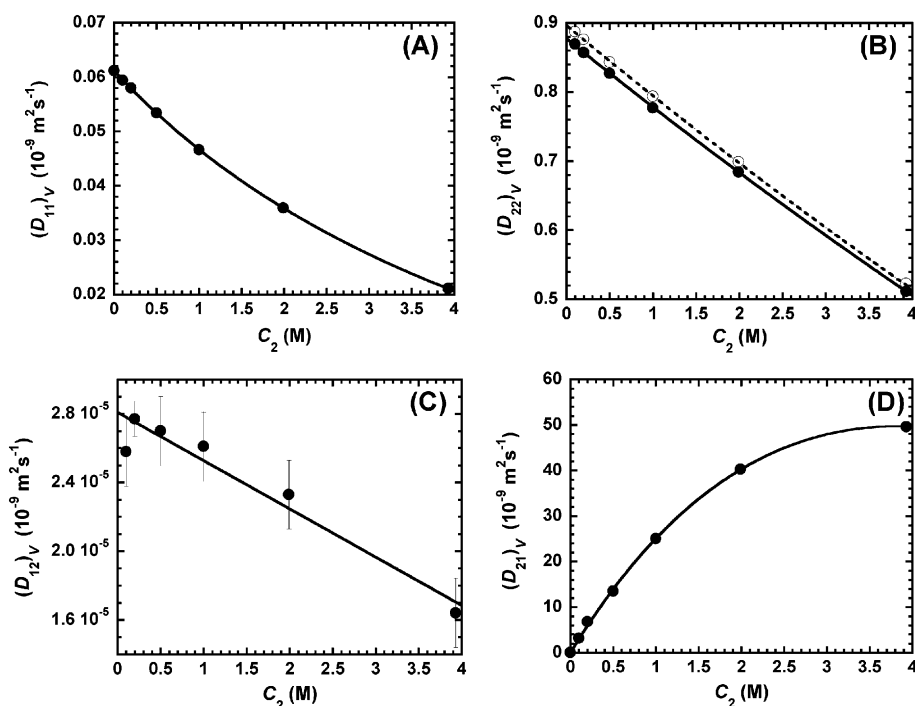
### Diffusion coefficients

In Table 1, we report the experimental volume-frame diffusion coefficients  $(D_{ij})_V$  obtained at 298.15 K for the ternary

PEG(1)–DEG(2)–water(0) system at six mean DEG concentrations  $\bar{C}_2 = 0.10, 0.20, 0.50, 1.00, 1.99$  and  $3.93 \text{ M}$ , and the same mean PEG concentration of  $\bar{C}_1 = 0.25 \text{ mM}$ . We observe that  $\bar{C}_1$  falls inside the dilute-solution regime for PEG with a molecular weight of  $20 \text{ kg mol}^{-1}$ . In Table 1, we also report the mean-composition density,  $\rho$ , and the partial molar volumes:  $\bar{V}_1, \bar{V}_2$  and  $\bar{V}_0$ , obtained from density measurements.<sup>43</sup> The  $\bar{V}_i$  were used to calculate the solvent-frame diffusion coefficients  $(D_{ij})_0$  from the  $(D_{ij})_V$ 's using eqn (2a)–(d).

In Fig. 1a–d, we illustrate the behavior of the four  $(D_{ij})_V$  as a function of DEG concentration. In Fig. 1a, we show that  $(D_{11})_V$  decreases as  $C_2$  increases. At  $C_2 = 3.9 \text{ M}$ ,  $(D_{11})_V$  becomes 34% of its binary value. The value of  $(D_{11})_V$  is proportional to the tracer diffusion coefficient of the macromolecules,  $D_1^*$ . We note that changes in DEG–water viscosity,  $\eta$ , and hydrodynamic radius of the macromolecule,  $R_h$ , contribute to the isothermal dependence of the PEG tracer diffusion coefficient on  $C_2$ , according to the Stokes–Einstein equation,  $D_1^* = k_B T / (6\pi\eta R_h)$ , where  $k_B$  is the Boltzmann constant.<sup>47</sup> Using available viscosity data on the DEG–water binary system,<sup>53</sup> we calculate that  $(1/\eta)$  at  $C_2 = 3.9 \text{ M}$  becomes 30% of its value in water. Thus the viscosity change can be regarded as the main cause for the observed decrease in  $(D_{11})_V$ . The residual dependence of  $(D_{11})_V \eta$  on  $C_2$  is consistent with a reduction of  $R_h$  describing a contraction of PEG macromolecules in the presence of DEG.

In Fig. 1b, we show the behavior of the DEG main-diffusion coefficient  $(D_{22})_V$  (solid circles). In the same Figure, we also include the corresponding binary diffusion coefficients,  $(D_2)_V$ , determined by us (open circles) and the fitted literature data<sup>40</sup> (dashed curve). We can see that our binary measurements are in excellent agreement with literature data. Moreover, ternary  $(D_{22})_V$  are slightly lower (2%) than the corresponding binary  $(D_2)_V$ , and share exactly their same dependence on  $C_2$ . We can describe the small difference between  $(D_{22})_V$  and  $(D_2)_V$  by  $(D_{22})_V = (1 - k\phi)(D_2)_V$ , where  $\phi = \bar{C}_1 \bar{V}_1$  is the polymer volume fraction and  $k$  is a constant factor that characterizes a small obstruction effect of the PEG macromolecules on the motion of DEG molecules. For aqueous DEG in the presence



**Fig. 1** Volume-fixed diffusion coefficients as functions of DEG concentration,  $C_2$ , for the PEG–DEG–H<sub>2</sub>O system at  $C_1 = 0.25$  mM and 25 °C: (a)  $(D_{11})_V$ ; (b)  $(D_{22})_V$ ; (c)  $(D_{12})_V$ ; (d)  $(D_{21})_V$ . The solid curves are fits through the ternary experimental points. The dashed curve (data points omitted) was fit through the binary DEG diffusion coefficients taken from ref. 40. The open circles are our measured binary values.

of PEG, we obtain  $k = 5.0 \pm 0.2$  by fitting the experimental  $(D_{22})_V$  using the available expression of  $(D_2)_V$  (dashed curve in Fig. 1b) and our experimental PEG volume fraction of  $\phi = 0.0042$ . For aqueous KCl in the presence of PEG, we have previously obtained  $k = 4.0 \pm 0.2$  at the same PEG volume fraction.<sup>27</sup> We therefore conclude that the obstruction effect of PEG on the motion of KCl ions is smaller than that on DEG molecules. Since the obstruction effect is expected to increase as the size of the additive increases, our experimental results are consistent with the hydrodynamic radius of DEG being larger than that of  $K^+$  and  $Cl^-$  ions.

In Fig. 1(c,d), we show the behavior of the two cross-diffusion coefficients  $(D_{12})_V$  and  $(D_{21})_V$ . We can see that  $(D_{12})_V$  is positive and decreases as  $C_2$  increases. A positive value of  $(D_{12})_V$  implies that, at uniform PEG concentration, the PEG macromolecules diffuse from high to low DEG concentration. Because  $(D_{12})_V$  is also proportional to the PEG tracer diffusion coefficient,<sup>35,36</sup> the observed decrease in  $(D_{12})_V$  can be partially attributed to the corresponding viscosity increase. In Fig. 1d, we show that  $(D_{21})_V$  is positive and increases with  $C_2$ . We note that the value of  $(D_{21})_V$  must approach zero at  $C_2 = 0$ . This occurs because the corresponding flux of DEG becomes zero, independent of PEG concentration gradient. A positive value of  $(D_{21})_V$  implies that DEG diffuses from high to low PEG concentration in the presence of uniform DEG concentration. The positive values of both cross-diffusion coefficients indicate the presence of net repulsive interactions between PEG and DEG in water. Our results are consistent with previous multicomponent diffusion studies on ternary aqueous mixtures of ethylene-glycol oligomers.<sup>36–39</sup> We will further discuss the experimental behavior of our cross-diffusion coefficients after the determination and examination of  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  and  $(L_{12})_0/(L_{11})_0$ .

#### Determination of $\mu_{12}^{(m)}/\mu_{22}^{(m)}$ and $(L_{12})_0/(L_{11})_0$

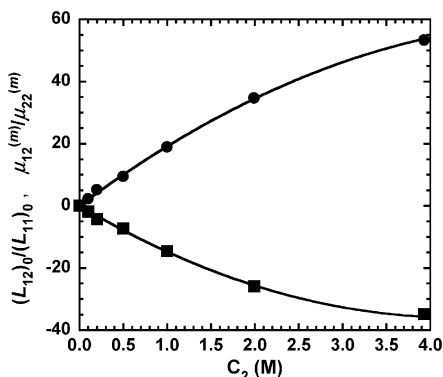
In Table 1, we report the values of  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  as a function of DEG concentration calculated using eqn (5). We can see that  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  is positive at all ternary compositions. Indeed, its value increases as  $C_2$  increases starting from  $\mu_{12}^{(m)}/\mu_{22}^{(m)} = 0$  at  $C_2 = 0$ . In Table 1, we also include the values of  $m_2\mu_{22}^{(m)}/RT$  used in eqn (5), where  $R$  is the ideal-gas constant. The quantity  $\mu_{22}^{(m)}$ , was assumed to be equal to the corresponding chemical-potential derivative of the DEG–water binary system at the same  $m_2$ . The values of  $m_2\mu_{22}^{(m)}/RT$  were calculated using the Van Laar coefficients taken from ref. 40. Technical details are given as ESI.† These estimates of  $\mu_{22}^{(m)}$  can be considered accurate because PEG concentration is low in the ternary system. Indeed the DEG main-diffusion coefficient,  $(D_{22})_V$ , which is essentially directly proportional to  $\mu_{22}^{(m)}$ , is only 2% lower than the corresponding binary value within our experimental concentration ranges. Furthermore, this small difference is nearly independent of DEG concentration and can be entirely attributed to obstruction effects as described above.

Values for  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  were calculated by assuming that  $m_1\mu_{11}^{(m)}/RT = 1.38$ , independent of  $m_2$ . This value was obtained from available light-scattering and isopiestic data on the PEG–water binary system.<sup>54</sup> Technical details are given as ESI.† For comparison, we have also calculated  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  by assuming ideal behavior with respect to PEG, *i.e.*  $m_1\mu_{11}^{(m)}/RT = 1$ . We found that they coincide with those reported in Table 1. Indeed, numerical examination shows that  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  is independent of the chosen value of  $m_1\mu_{11}^{(m)}/RT$  within the experimental error provided that  $0.6 < m_1\mu_{11}^{(m)}/RT < 2.5$ . Thus, large errors on the estimation of  $\mu_{11}^{(m)}$  have a negligible effect on the extracted value of  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$ . This finding is a

consequence of the fact that the term multiplying  $\mu_{11}^{(m)}/\mu_{22}^{(m)}$  in eqn (5) is small.

In Table 1, we finally report the values of  $(L_{12})_0/(L_{11})_0$  as a function of DEG concentration calculated using eqn (6). We can see that  $(L_{12})_0/(L_{11})_0$  is negative at all ternary compositions. Its value decreases as  $C_2$  increases starting from  $(L_{12})_0/(L_{11})_0 = 0$  at  $C_2 = 0$ . We further notice that the values of  $-(L_{12})_0/(L_{11})_0$  are of the same order of magnitude as those of  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$ . It is important to remark that negative values of  $(L_{12})_0/(L_{11})_0$  have been previously obtained for both lysozyme and PEG in aqueous salt solutions.<sup>25,27</sup> Our results on the PEG–DEG–water systems allow us to conclude that large negative values of this transport coefficient can be also obtained for ternary systems in which all components are neutral species. This implies that  $(L_{12})_0$  should not be neglected in multicomponent-diffusion theoretical models on non-associating solutes even in the absence of ionic interactions.

To examine the effect of  $\mu_{11}^{(m)}/\mu_{22}^{(m)}$  on the calculated values of the transport-coefficient ratio, we have also calculated  $(L_{12})_0/(L_{11})_0$  by assuming that  $m_1\mu_{11}^{(m)}/RT = 1$ . We have found that this ideal-solution approximation produces a relative error that is 19% at the highest experimental DEG concentration and reduces to 7% at  $C_2 = 0.1$  M. We further observe that the effect of  $\mu_{11}^{(m)}/\mu_{22}^{(m)}$  on  $(L_{12})_0/(L_{11})_0$  becomes negligible when the magnitude of  $[(D_{12})_0/(D_{11})_0]/C_1$  is small compared to  $\mu_{12}^{(c)}/(C_1\mu_{11}^{(c)})$  in eqn (6). Indeed, in the limit of  $[(D_{12})_0/(D_{11})_0]/C_1 = 0$ , the ratio  $(L_{12})_0/(L_{11})_0$  becomes independent of  $(\mu_{11}^{(c)}/\mu_{22}^{(c)})$  and equal to  $-(\mu_{12}^{(c)}/\mu_{22}^{(c)})$ . Although  $[(D_{12})_0/(D_{11})_0]/C_1$  is small for our system, its contribution to  $(L_{12})_0/(L_{11})_0$  cannot be neglected, especially at the highest experimental  $C_2$  values. Here the accuracy of  $(\mu_{11}^{(c)}/\mu_{22}^{(c)})$  on the calculated value of  $(L_{12})_0/(L_{11})_0$  becomes more important. However, our estimation of  $(\mu_{11}^{(c)}/\mu_{22}^{(c)})$ , which is based on  $\mu_{11}^{(m)}$  of the PEG–water binary system, is less reliable at high DEG concentrations because we are neglecting the dependence of  $\mu_{11}^{(m)}$  on DEG concentration. Thus, our procedure will yield accurate results only for the limiting value of the quotient  $[(L_{12})_0/(L_{11})_0]/C_2$  at  $C_2 = 0$ .



**Fig. 2** Thermodynamic ratio,  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  (circles), and transport-coefficient ratio,  $(L_{12})_0/(L_{11})_0$  (squares), as a function of DEG concentration,  $C_2$ , for the PEG–DEG–H<sub>2</sub>O system. The solid curves are fits through the experimental points performed using  $aC_2(1 - bC_2)$ . We obtain  $a = (21.0 \pm 0.3) \text{ M}^{-1}$  and  $b = (0.089 \pm 0.005) \text{ M}^{-1}$  for the  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  data, and  $a = -(16.7 \pm 0.4) \text{ M}^{-1}$  and  $b = (0.117 \pm 0.009) \text{ M}^{-1}$  for the  $(L_{12})_0/(L_{11})_0$  data.

In Fig. 2, we plot both  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  and  $(L_{12})_0/(L_{11})_0$  as a function of  $C_2$ . We fit both sets of data to the function:  $aC_2(1 - bC_2)$ , where  $a$  and  $b$  are the parameters to which the method of least squares is applied (see least-square results in Fig. 2 caption). At  $C_2 = 0$ , we obtain:  $[\mu_{12}^{(m)}/\mu_{22}^{(m)}]/C_2 = (21.0 \pm 0.3) \text{ M}^{-1}$  and  $[(L_{12})_0/(L_{11})_0]/C_2 = -(16.7 \pm 0.4) \text{ M}^{-1}$ , where the reported errors are standard deviations obtained from the fits. For comparison, we have calculated that a 7.6% higher value of  $|(L_{12})_0/(L_{11})_0|/C_2$  is obtained using the ideal-solution approximation:  $m_1\mu_{11}^{(m)}/RT = 1$ .

## Discussion

In this section, we will first examine our results on  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  and  $(L_{12})_0/(L_{11})_0$  and their relation to macromolecular hydration and preferential hydration. We will then discuss their role on the behavior of the two cross-diffusion coefficients.

### Examination of $\mu_{12}^{(m)}/\mu_{22}^{(m)}$

In the limit of small  $C_1$ , the thermodynamic ratio,  $-\mu_{12}^{(m)}/\mu_{22}^{(m)}$ , is the preferential-interaction coefficient:<sup>55–57</sup>

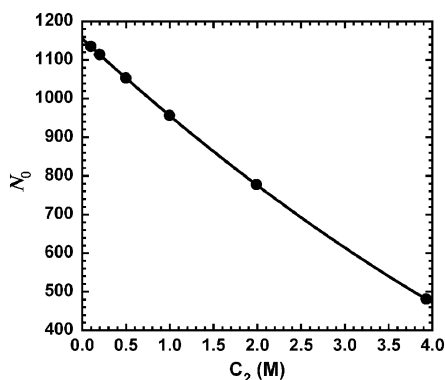
$$\Gamma_{12} \equiv \lim_{C_1 \rightarrow 0} \left( \frac{\partial m_2}{\partial m_1} \right)_{\mu_2} = - \lim_{C_1 \rightarrow 0} \frac{\mu_{12}^{(m)}}{\mu_{22}^{(m)}} \quad (8)$$

The preferential-interaction coefficient characterizes the macromolecule–osmolyte thermodynamic interactions. This coefficient is linked to preferential hydration by a model based on the existence of two domains.<sup>2,48</sup> The first domain is represented by the water–osmolyte layers surrounding the macromolecules. This local domain is in chemical equilibrium with a bulk domain, representing the water–osmolyte remaining solution. Since macromolecules interact with the osmolyte and water molecules in their vicinity, the concentration of osmolyte in the local domain is different from that of the unperturbed bulk domain. If the osmolyte concentration in the local domain is lower than that of the bulk domain, preferential solvation of the macromolecule occurs. In this case, the preferential-interaction coefficient is negative. On the other hand, a positive value of this coefficient is obtained if the macromolecule preferentially interacts with the osmolyte. To describe the chemical equilibrium between the local domain and the bulk unperturbed domain we can introduce the partitioning constant

$$\alpha = \frac{(N_2/N_0)}{(m_2/m_0)} \quad (9)$$

where  $m_0 = 55.51 \text{ mol kg}^{-1}$ ,  $N_2$  and  $N_0$  are respectively the number of osmolyte and water molecules of one macromolecular layer. When  $\alpha < 1$ , the osmolyte is preferentially excluded from the macromolecule domains. On the other hand, when  $\alpha > 1$ , the osmolyte preferentially interacts with the PEG molecules. By considering chemical equilibrium between the two domains and applying Gibbs–Duhem equations,<sup>2</sup> it can be shown that

$$\Gamma_{12} = -N_0^{\text{ex}} \frac{m_2}{m_0} \quad (10)$$



**Fig. 3** Water excess,  $N_0$ , as functions of DEG concentration,  $C_2$ , for the PEG–DEG–H<sub>2</sub>O system. The solid curve is a fit through the data.

where  $N_0^{\text{ex}} \equiv N_0(1 - \alpha)$  is the excess (or deficit) number of solvents in the local domain of the macromolecule. We note that eqn (10) can only be used to extract  $N_0^{\text{ex}}$  and not the individual values of  $N_0$  and  $\alpha$ . In practice, the value of  $N_0$  can be assigned on the basis of assumptions. For preferentially solvated macromolecules, it can be assumed that osmolyte molecules are completely excluded from the local domain by setting  $\alpha = 0$  and  $N_0 = N_0^{\text{ex}}$ . In this way, there is a clear analogy between  $N_0$  and the actual amount of solvent bound to a macromolecule. Nonetheless, it is important to remark that no direct link exists between these two quantities. In general, we should regard  $N_0$  as just a useful model-based property that describes the net thermodynamic effect of the osmolyte on the chemical potential of a macromolecule in solution.<sup>58</sup>

Our extracted values of  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  are positive thereby implying that PEG is preferentially hydrated as expected. We calculate  $N_0$  as a function of  $C_2$  by applying eqn (12) with  $\alpha = 0$  and  $\Gamma_{12} = -\mu_{12}^{(m)}/\mu_{22}^{(m)}$ . At  $C_2 = 0$ , we use the value of  $[\mu_{12}^{(m)}/\mu_{22}^{(m)}]/C_2$  from the fit to calculate  $N_0 = 1160 \pm 20$ . This corresponds to an excess of 2.6 water molecules per PEG monomeric units. Our results, which are shown in Fig. 3, reveal that  $N_0$  decreases as  $C_2$  increases, becoming 42% of the limiting value at the highest experimental DEG concentration. The observed decrease in  $N_0$  can be explained by considering the osmotic stress<sup>59</sup> on the deformable macromolecule induced by the osmolyte. If DEG molecules are sterically excluded from the PEG local domain, an increase in DEG concentration favors PEG conformers that are relatively more compact and release local water to the bulk domain. This explanation is consistent with a corresponding reduction of PEG hydrodynamic radius.

### Examination of $(L_{12})_0/(L_{11})_0$

The physical meaning of transport-coefficient ratio,  $(L_{12})_0/(L_{11})_0$ , can be examined starting from the frictional-coefficient formulation of diffusion developed by Bearman.<sup>60</sup> Within this approach, a diffusion process is thought to occur in a quasi-stationary regime in which the thermodynamic driving forces equal the opposing frictional forces between component pairs. Based on frictional forces, the following linear laws for diffusion can be written for a ternary system:<sup>35</sup>

$$-C_1 \nabla \mu_1 = \zeta_{10} C_0 (J_1)_0 + \zeta_{12} [C_2 (J_1)_0 - C_1 (J_2)_0] \quad (11a)$$

$$-C_2 \nabla \mu_2 = \zeta_{20} C_0 (J_2)_0 + \zeta_{21} [C_1 (J_2)_0 - C_2 (J_1)_0] \quad (11b)$$

where  $\zeta_{ij} = \zeta_{ji}$  (with  $i, j = 0, 1, 2$  and  $i \neq j$ ) are coefficients describing the frictional force between component  $i$  and component  $j$ . Eqn (11a),(b) are equivalent to resistance based equations previously reported by Onsager<sup>61</sup> and to the Stefan–Maxwell equations.<sup>62</sup> It is important to remark that  $\zeta_{ij}$  are frame independent and have a direct physical interpretation in terms of friction between the diffusing species inside the system.<sup>63</sup>

The ratio  $(L_{12})_0/(L_{11})_0$  is related to the  $\zeta_{ij}$  by the following equation:<sup>23,35</sup>

$$\frac{(L_{12})_0}{(L_{11})_0} = C_2 \frac{\zeta_{12}}{C_0 \zeta_{20} + C_1 \zeta_{12}} \quad (12)$$

Since frictional coefficients are positive quantities, the ratio  $(L_{12})_0/(L_{11})_0$  is also expected to be positive according to eqn (12). This is inconsistent with our experimental findings.

It has been recently shown that negative values of  $(L_{12})_0/(L_{11})_0$  can be observed if the frictional-coefficient formalism is applied to *solvated* solutes, which are the actual diffusing species in solution.<sup>35</sup> Due to solvent binding, the chemical potentials of the solvated solutes are  $\hat{\mu}_1 = \mu_1 + \nu_1 \mu_0$  and  $\hat{\mu}_2 = \mu_2 + \nu_2 \mu_0$ , where  $\nu_i$  is the number of solvent molecules bound to solute  $i$ . We can therefore rewrite eqn (11a),(b) in terms related to the solvated solutes:

$$-C_1 \nabla \hat{\mu}_1 = \hat{\zeta}_{10} \hat{C}_0 (J_1)_0 + \hat{\zeta}_{12} [C_2 (J_1)_0 - C_1 (J_2)_0] \quad (13a)$$

$$-C_2 \nabla \hat{\mu}_2 = \hat{\zeta}_{20} \hat{C}_0 (J_2)_0 + \hat{\zeta}_{21} [C_1 (J_2)_0 - C_2 (J_1)_0] \quad (13b)$$

where  $\hat{\zeta}_{10} = \hat{\zeta}_{01}$  and  $\hat{\zeta}_{20} = \hat{\zeta}_{02}$  describes the frictional force between solvated solutes and free solvent, and  $\hat{\zeta}_{12} = \hat{\zeta}_{21}$  that between the two solvated solutes. The quantity  $\hat{C}_0 = C_0 - \nu_1 C_1 - \nu_2 C_2$  is the concentration of free solvent and the subscript “0” denotes that the fluxes are defined with respect to the free solvent frame of reference. The  $\hat{\zeta}_{ij}$  are related to the  $\zeta_{ij}$  by the following equations:<sup>35</sup>

$$\zeta_{10} = \frac{(C_0 - \nu_2 C_2) \hat{\zeta}_{10} + \nu_1 C_2 \hat{\zeta}_{20}}{C_0 - \nu_1 C_1 - \nu_2 C_2} \quad (14a)$$

$$\zeta_{20} = \frac{(C_0 - \nu_1 C_1) \hat{\zeta}_{20} + \nu_2 C_1 \hat{\zeta}_{10}}{C_0 - \nu_1 C_1 - \nu_2 C_2} \quad (14b)$$

$$\zeta_{12} = \hat{\zeta}_{12} - \frac{\nu_2 (C_0 - \nu_2 C_2) \hat{\zeta}_{10} + \nu_1 (C_0 - \nu_1 C_1) \hat{\zeta}_{20}}{C_0 - \nu_1 C_1 - \nu_2 C_2} \quad (14c)$$

The expression for  $(L_{12})_0/(L_{11})_0$  can be obtained by inserting eqn 14(a–c) into eqn 12:

$$\begin{aligned} & \frac{(L_{12})_0}{(L_{11})_0} \\ &= C_2 \frac{(C_0 - \nu_1 C_1 - \nu_2 C_2) \hat{\zeta}_{12} - \nu_2 (C_0 - \nu_2 C_2) \hat{\zeta}_{10} - \nu_1 (C_0 - \nu_1 C_1) \hat{\zeta}_{20}}{C_1 (C_0 - \nu_1 C_1 - \nu_2 C_2) \hat{\zeta}_{12} + \nu_2^2 C_1 C_2 \hat{\zeta}_{10} + (C_0 - \nu_1 C_1)^2 \hat{\zeta}_{20}} \end{aligned} \quad (15)$$

If we then take the limit of small  $C_1$  and  $C_2$ , eqn (15) finally becomes:

$$\frac{(L_{12})_0}{C_2 V_0^* (L_{11})_0} = -\nu_1 \left( 1 + \frac{\nu_2 \hat{\zeta}_{10}}{\nu_1 \hat{\zeta}_{20}} \right) + \frac{\hat{\zeta}_{12}}{\hat{\zeta}_{20}} \quad (16)$$

where  $V_0^*$  is the molar volume of pure solvent. Thus, a negative term, proportional to  $\nu_1$ , contributes to the value of  $[(L_{12})_0/(L_{11})_0]/C_2$  for solvated solutes. For dilute solutions,  $D_1^* = RTV_0^*/\zeta_{10}^*$  and  $D_2^* = RTV_0^*/\zeta_{20}^*$  are the tracer diffusion coefficients of the two solutes.<sup>23</sup> We will apply eqn (16) to the PEG–DEG–water system in order to calculate  $\nu_1$ . This parameter characterizes PEG hydration. It is important to note that the application of eqn (15) with  $C_1 = 0.25$  mM and  $C_2 = 0$  instead of the limiting expression given by eqn (16), produces the negligible error of 1% into the calculated value of  $\nu_1$ .

We will now estimate  $(\nu_2/\nu_1)(\zeta_{10}/\zeta_{20})$  and  $(\zeta_{12}/\zeta_{20})$ . We shall see that their contribution in eqn (16) is expected to be small. The value of  $\nu_2/\nu_1$  is approximately equal to the molecular-weight ratio between PEG and DEG,  $M_2/M_1$ . For dilute solutions, the ratio  $\zeta_{10}/\zeta_{20}$  is equal to  $D_2^*/D_1^*$ . Using  $D_1^* = 0.061 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$  and  $D_2^* = 0.897 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ ,<sup>40</sup> we obtain  $(\nu_2/\nu_1)(\zeta_{10}/\zeta_{20}) = 0.078$ . Hence, osmolyte solvation introduces the small correction of 7.8% to the factor multiplying  $\nu_1$  in eqn (16).

For an order-of-magnitude estimate of  $\zeta_{12}/\zeta_{20}$ , we can assume that  $\zeta_{ij}/\zeta_{kj} = \alpha_i/\alpha_k$ , where the  $\alpha_i$  are parameters used to scale the ratios of the frictional coefficients.<sup>17</sup> In other words, it is assumed that the ratio  $\zeta_{ij}/\zeta_{kj}$  depends on the chemical nature of  $i$  and  $k$  only. This assumption is consistent with the multicomponent extension of the well-known Darken equation for binary systems.<sup>17,23</sup> Hence, we derive:  $\zeta_{12}/\zeta_{02} = \zeta_{10}/\zeta_{00} = D_0^*/D_1^*$ , where  $\zeta_{00}$  is the water–water frictional coefficient and is related to the tracer-diffusion coefficient of water,  $D_0^*$ , by  $D_0^* = RTV_0^*/\zeta_{00}$ . Using  $D_1^* = 0.061 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$  and  $D_0^* = 2.30 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ ,<sup>64</sup> we obtain:  $\zeta_{12}/\zeta_{20} = 38$ .

We are now in position to calculate  $\nu_1$  from eqn (16). Using the reported value of  $[(L_{12})_0/(L_{11})_0]/C_2$  and  $V_0^* = 0.018 \text{ dm}^3 \text{ mol}^{-1}$ , we obtain  $\nu_1 = 900$ . This corresponds to 2.0 water molecules bound to each PEG monomeric units. We can see that the  $\zeta_{12}/\zeta_{20}$  is only a small fraction (4.2%) of  $\nu_1$ , thereby showing that the accuracy for the estimation of this frictional-coefficient ratio needs not be high. If we assume that our estimates have introduced a systematic error as high as the values of  $\zeta_{12}/\zeta_{20}$  and  $\nu_2/\nu_1$ , we conclude that the calculated value of  $\nu_1$  has an error of the order of 10%. We also note that  $\zeta_{12}/\zeta_{20}$  and  $\nu_2\zeta_{10}/\zeta_{20}$  contribute in eqn (16) with opposite sign. This implies that their net effect on the value of  $[(L_{12})_0/(L_{11})_0]/(C_2V_0^*)$  is small and  $[(L_{12})_0/(L_{11})_0]/(C_2V_0^*) \approx -\nu_1$  can be regarded as a good approximation.

The extracted value of  $\nu_1$  is about 20% smaller than that of  $N_0 = 1160$  calculated above. That  $N_0$  is larger than  $\nu_1$  can be explained by invoking molecular crowding. Due to excluded-volume interactions between the two solvated solutes, the center of mass of a DEG molecule will not be able to access the volume occupied by both the solvated PEG macromolecules and their adjacent solvent layers with thickness proportional to the size of DEG molecules. We can make a rough estimate of  $N_0$  by approximating the solvated solutes as spherical particles. In this case, the total volume excluded to DEG molecules is  $V_{\text{ex}} = (\bar{V}_1 + \nu_1V_0^*)(1 + R_2/R_1)^3$ , where  $R_1$  and  $R_2$  are the radii of solvated PEG and DEG molecules respectively.<sup>35,65,66</sup> We note that  $\bar{V}_1 + \nu_1V_0^*$  can be regarded

as the hydrodynamic volume of the macromolecule.<sup>47</sup> If we assume that the ratio  $R_1/R_2$  is approximately equal to the diffusion ratio,  $D_1^*/D_2^*$ , we obtain  $V_{\text{ex}} \approx 40 \text{ dm}^3 \text{ mol}^{-1}$ . The value of  $N_0 \approx 1300$  can be then calculated from  $(V_{\text{ex}} - \bar{V}_1)/V_0^*$ . Considering all approximations involved, we believe that this estimate of  $N_0$  is in good agreement with its actual determined value. Our analysis has shown that multi-component diffusion studies can be used to provide information on both hydration and preferential hydration of macromolecules in ternary aqueous solutions. For our investigated system, the water excess characterizing preferential hydration has been found to be slightly higher than the actual number of water molecules bound to the PEG macromolecules.

## Examination of cross-diffusion coefficients

We will now discuss the role of  $N_0$  and  $\nu_1$  on the cross-diffusion coefficients,  $(D_{ij})_0$ . Because  $(D_{ij})_0$  becomes directly proportional to both  $C_i$  and  $(D_{ii})_0 = D_i^*$  in the limit of dilute solutions, it is convenient to consider the normalized quotient  $[(D_{ij})_0/(D_{ii})_0]/C_i$ .<sup>35</sup> In this way, the explicit dependence of the cross-diffusion coefficient on amount and tracer diffusion of solute can be removed. In Fig. 4, we plot our experimental values of  $[(D_{ij})_0/(D_{ii})_0]/C_i$  as a function of  $C_2$ . We can see that the macromolecule quotient,  $[(D_{12})_0/(D_{11})_0]/C_1$ , is significantly smaller than the osmolyte quotient,  $[(D_{21})_0/(D_{22})_0]/C_2$ . To examine the difference in magnitude between these two diffusion quotients, we employ eqn (3a)–(d) to derive their dilute-solution expressions in terms of thermodynamic ratios,  $\mu_{12}^{(c)}/\mu_{22}^{(c)}$  and  $\mu_{21}^{(c)}/\mu_{22}^{(c)}$ , and transport-coefficient ratio,  $(L_{12})_0/(L_{11})_0$ :

$$\frac{(D_{12})_0}{C_1(D_{11})_0} = \frac{\mu_{12}^{(c)}}{C_2\mu_{22}^{(c)}} + \frac{(L_{12})_0}{C_2(L_{11})_0} \quad (17a)$$

$$\frac{(D_{21})_0}{C_2(D_{22})_0} = \frac{\mu_{21}^{(c)}}{C_2\mu_{22}^{(c)}} + \frac{(L_{12})_0}{C_2(L_{11})_0} \frac{D_1^*}{D_2^*} \quad (17b)$$

where we have also used:  $C_i\mu_{ii}^{(c)}/RT = 1$  and  $(D_{ii})_0 = RT(L_{ii})_0/C_i = D_i^*$ . Eqn (17a),(b) show that the two diffusion quotients are given by the sum of a thermodynamic and a transport term. We also note that  $D_1^*/D_2^*$  is small and the

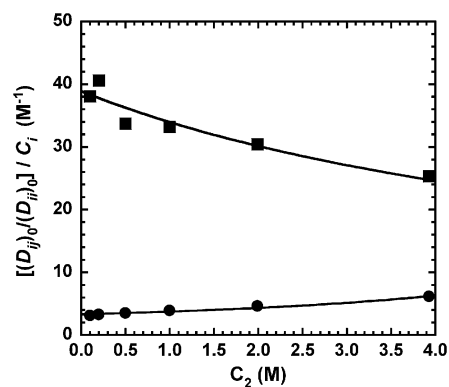


Fig. 4 Diffusion-coefficient ratios:  $[(D_{12})_0/(D_{11})_0]/C_1$  (circles) and  $[(D_{21})_0/(D_{22})_0]/C_2$  (squares) as a function of DEG concentration,  $C_2$ , for the PEG–DEG–H<sub>2</sub>O. The solid curves are fits through the data.



transport term in eqn (17b) can be neglected being only about 3% of  $[\mu_{21}^{(c)}/\mu_{22}^{(c)}]/C_2$ . Thus, the  $[(D_{21})_0/(D_{22})_0]/C_2$  quotient is essentially a thermodynamic quantity.

Numerical examination of eqn (7b),(c) shows that we can approximately write  $\mu_{12}^{(c)}/\mu_{22}^{(c)} \approx \mu_{12}^{(m)}/\mu_{22}^{(m)}$  and  $\mu_{21}^{(c)}/\mu_{22}^{(c)} \approx \mu_{12}^{(m)}/\mu_{22}^{(m)} + C_2\bar{V}_1$  in dilute solutions. We note that the small partial molar volume of the osmolyte,  $\bar{V}_2 = 0.092 \text{ dm}^3 \text{ mol}^{-1}$ , can be neglected with respect to  $[\mu_{12}^{(m)}/\mu_{22}^{(m)}]/C_2$  ( $> 10 \text{ dm}^3 \text{ mol}^{-1}$ ) in eqn (7b). It is therefore a very good approximation to write the following expressions for the molarity-based thermodynamic ratios in eqn (17a),(b):

$$\frac{1}{C_2} \frac{\mu_{12}^{(c)}}{\mu_{22}^{(c)}} \approx N_0 V_0^* \quad (18a)$$

$$\frac{1}{C_2} \frac{\mu_{21}^{(c)}}{\mu_{22}^{(c)}} \approx N_0 V_0^* + \bar{V}_1 \quad (18b)$$

where we have set  $N_0 = N_0^{\text{ex}}$  and replaced  $m_2/m_0$  with  $V_0^*C_2$ . If we insert eqn (18a),(b) into eqn (17a),(b) and approximate  $[(L_{12})_0/(L_{11})_0]/C_2$  by  $-\nu_1 V_0^*$ , we obtain the following expressions for the two diffusion quotients:

$$\frac{(D_{12})_0}{C_1(D_{11})_0} \approx (N_0 - \nu_1)V_0^* \quad (19a)$$

$$\frac{(D_{21})_0}{C_2(D_{22})_0} \approx \bar{V}_1 + N_0 V_0^* \quad (19b)$$

Eqn (19a),(b) directly relate the diffusion quotients to  $N_0$  and  $\nu_1$ , the hydration parameters discussed above. We can see that  $[(D_{21})_0/(D_{22})_0]/C_2$  is approximately equal to the excluded volume,  $V_{\text{ex}} = \bar{V}_1 + N_0 V_0^*$ , whereas  $[(D_{12})_0/(D_{11})_0]/C_1$  is significantly smaller because it is approximately equal to the difference between  $V_{\text{ex}}$  and the hydrodynamic volume of the macromolecule,  $\bar{V}_1 + \nu_1 V_0^*$ . To appreciate the accuracy of approximate eqn (19a),(b), we insert the determined values of  $N_0$  and  $\nu_1$  in these expressions. We obtain:  $[(D_{21})_0/(D_{22})_0]/C_2 \approx 38 \text{ dm}^3 \text{ mol}^{-1}$  and  $[(D_{12})_0/(D_{11})_0]/C_1 \approx 5 \text{ dm}^3 \text{ mol}^{-1}$  consistent with our results shown in Fig. 4. We believe that eqn (19a),(b) can be used to model multicomponent-diffusion processes in aqueous solution containing solvated solutes of very different size. However, it is also important to observe that estimating the value of  $[(D_{12})_0/(D_{11})_0]/C_1$  seems very difficult because accurate values of  $N_0 - \nu_1$  would be needed.

## Summary and conclusions

We have experimentally characterized coupled diffusion for the PEG–DEG–water ternary system. This is a macromolecule–osmolyte–solvent system in which all three components are neutral species. Ternary diffusion coefficients were used to characterize the thermodynamic factor  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  and the transport-coefficient ratio  $(L_{12})_0/(L_{11})_0$  as a function of DEG concentration. The extracted values of  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$  show that PEG is preferentially hydrated in the presence of DEG. Using the two-domain model, we have characterized PEG preferential hydration by calculating the excess of water,  $N_0^{\text{ex}}$ , in the local domain of a PEG macromolecule. We have found that  $N_0^{\text{ex}}$  decreases as  $C_2$  increases,

consistent with a contraction of PEG macromolecules due to the osmotic stress of DEG molecules.

We have found that  $(L_{12})_0/(L_{11})_0$  is negative with magnitude comparable with that of  $\mu_{12}^{(m)}/\mu_{22}^{(m)}$ . Our results on the PEG–DEG–water systems allow us to conclude that large negative values of  $(L_{12})_0/(L_{11})_0$  can be obtained even for ternary systems in which all components are neutral species. This implies that the Onsager cross-transport coefficient should not be neglected in multicomponent-diffusion theoretical models even when ionic interactions or chemical association between the solute species are absent.

We have extracted the transport quotient  $[(L_{12})_0/(L_{11})_0]/C_2$  in the limit of  $C_2 = 0$ . By including solute solvation into the frictional-coefficient formulation of diffusion,  $(L_{12})_0/(L_{11})_0$  is indeed predicted to be negative for hydrated PEG macromolecules. Specifically,  $[(L_{12})_0/(L_{11})_0]/C_2$  is approximately equal to  $-\nu_1 V_0^*$ , where  $\nu_1$  is the number of water molecules bound to the PEG macromolecules. We have found that  $N_0^{\text{ex}}$  is slightly larger than  $\nu_1$ , consistent with the presence of PEG–DEG excluded-volume interactions. This result contributes to the understanding of macromolecular hydration compared with preferential hydration.

Both experimental cross-diffusion coefficients were found to be positive. The value of  $[(D_{21})_0/(D_{22})_0]/C_2$  was significantly larger than  $[(D_{12})_0/(D_{11})_0]/C_1$  because the first quotient is approximately equal to the excluded volume,  $(\bar{V}_1 + N_0)V_0^*$ , whereas the second quotient is approximately equal to the difference in volume,  $(N_0 - \nu_1)V_0^*$ . This work significantly contributes to the fundamental understanding of coupled diffusion in neutral macromolecule–additive–water ternary systems because it provides the basis for understanding coupled diffusion in more complex aqueous systems such as those containing charged proteins or nucleic acids in the presence of salts or osmolytes.

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