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Generalized derivation of an exact relationship linking different coefficients that characterize thermodynamic effects of preferential interactions

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Abstract

In solutions consisting of solvent water (component '1') and two solute components ('2' and '3'), various thermodynamic effects of differences between solute–solute and solute–solvent interactions are quantitatively characterized by state functions commonly called 'preferential interaction coefficients': $\Gamma_{\mu_1,\mu_3} \equiv (\partial m_3/\partial m_2)_{T,\mu_1,\mu_3}$ and $\Gamma_{\mu_k} \equiv (\partial m_3/\partial m_2)_{T,P,\mu_k}$, where $k = 1,2$ or 3. These different derivatives are not all directly accessible to experimental determination, nor are they entirely equivalent for analyses and interpretations of thermodynamic and molecular effects of preferential interactions. Consequently, various practical and theoretical considerations arise when, for a given system, different kinds of preferential interaction coefficients have significantly different numerical values. Previously we derived the exact relationship linking all three coefficients of the type Γ_{μ_k}, and hence identified the physical origins of the differences between Γ_{μ_1} and Γ_{μ_3} that have been experimentally determined for each of various common biochemical solutes interacting with a protein [J. Phys. Chem. B, 106 (2002) 418–433]. Continuing our investigation of exact thermodynamic linkages among different types of preferential interaction coefficients, we present here a generalized derivation of the relationship linking Γ_{μ_1,μ_3}, Γ_{μ_3} and Γ_{μ_1}, with no restrictions on m_2, m_3 or any physical characteristic of either solute component (such as partial molar volume). Hence, we show that $(\Gamma_{\mu_1,\mu_3} - \Gamma_{\mu_3})$ is related directly to $(\Gamma_{\mu_3} - \Gamma_{\mu_1})$, for which the physical determinants have been considered in detail previously, and to a factor dependent on the ratio of the partial molar volumes $\bar{V}_3/\bar{V}_1$. Our generalized expression also provides a basis for calculating Γ_{μ_1,μ_3}, even in situations where preferential interactions could not be investigated by equilibrium dialysis. To demonstrate this applicability, we analyze isopiestic distillation data for aqueous solutions containing urea and NaCl, two small solute components that cannot be selectively dialyzed.
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1. Introduction

Current understanding of thermodynamic effects due to interactions in solutions containing small and large biochemical solutes has been substantially shaped by the manifold contributions of John Schellman. Among these is the well-known paper [1], published 12 years ago in *Biophysical Chemistry*, in which he introduced 'a simple model for solvation in mixed solvents' with parameters that can be related to 'three measures of selective interaction' (in our notation Γ_{μ_1,μ_3}, Γ_{μ_3} and Γ_{μ_1}). For solutions in which the two solutes consist of a dissociated electrolyte and a highly dilute charged macromolecule with dissociated counterions of the same kind as one of the salt ions, 'usually small' numerical differences between any two of these three selective interaction coefficients were deduced by an analysis based in part on, but more general than, some earlier treatments [2–4].

For solutions in which the two solute components together consist of only three dissociated charged species, we [5] recently showed that a 'common ion' effect on the ideal mixing entropy of component 2 substantially reduces both absolute and relative numerical differences between Γ_{μ_3} and Γ_{μ_1}, but that generally these coefficients can significantly differ for systems in which the solute components do not have a common ion. The present paper has an analogous goal: to identify the thermodynamic variables that govern the exact relationship between Γ_{μ_3} and Γ_{μ_1,μ_3}, and hence to provide a general basis for determining whether (and if so, why) significant differences in the numerical values of these coefficients are to be expected for a given system. The requisite expression is derived without imposing any limit on the concentration of either solute component, or requiring that they have a common ion. This level of generality is mandatory, for example, in rigorous investigations of thermodynamic effects due to macromolecular 'crowding' on preferential interactions in systems where the solute concentrations are comparable to those in biological environments.

The thermodynamic functions Γ_{μ_1,μ_3}, Γ_{μ_3} and Γ_{μ_1} are referred to here as 'preferential interaction coefficients', in conformity with the most common

(though not universally accepted) nomenclature. These coefficients characterize solute concentration-dependent effects of differences between solute–solute and solute–solvent interactions in solutions consisting of three independent components, designated by the conventional numerical labels: '1' for the solvent water; '2' and '3' for the different solutes. (In most systems for which preferential interactions have been investigated, component 2 is much larger and much more dilute than component 3, but in the present development these numerical assignments imply nothing about relative molecular sizes or concentrations.) For any 'three-component' system, the preferential interaction coefficients considered here are defined as different partial derivatives of the two solute molalities: $\Gamma_{\mu_1,\mu_3} \equiv (\partial m_3 / \partial m_2)_{T,\mu_1,\mu_3}$ and $\Gamma_{\mu_k} \equiv (\partial m_3 / \partial m_2)_{T,P,\mu_k}$, for $k = 1,2,3$. Thus, the subscript(s) on Γ indicate(s) explicitly the chemical potential(s) that, when fixed, produce(s) some functional linkage between m_3 and m_2, which otherwise are independently variable. (The derivations presented in this paper do not explicitly take into account any complexation reaction(s) involving the three components that would produce additional linkage(s) between variations in their chemical potentials and hence their molalities. Nevertheless, the derivations presented here can be generalized to include effects due to complexation for applications where sufficient independent information is available.)

The various coefficients that characterize preferential interactions cannot have identical numerical values for all states of any real system, because the differences in the constraints specified on the defining partial derivatives must have some physical consequences. Recent experimental studies [6–8] have revealed that Γ_{μ_3} and Γ_{μ_1} differ significantly (outside experimental uncertainties) for a wide variety of biochemically relevant solutes, including denaturants (such as urea and guanidinium chloride), salts (such as KCl) and osmolytes (such as glycerol and glycine betaine) that interact with the globular protein BSA (bovine serum albumin) over the entire ranges of protein and small solute concentrations investigated (1–5 mmolal in m_2, 10–10^3 mmolal in m_3). As a basis for deducing thermodynamic implications of

numerical differences between Γ_{μ_3} and Γ_{μ_1}, we derived a previously unrecognized exact relationship (Eq. (12) of [5]) that links the three 'constant-pressure' coefficients Γ_{μ_3}, Γ_{μ_2} and Γ_{μ_1}. Hence, we have identified salient general characteristics of the interacting solutes that produce significant numerical differences between Γ_{μ_3} and Γ_{μ_1} (even when one of the solute 2 is highly dilute). Furthermore, we showed why such differences do not imply any conflict with the previous finding [3,4] that $\Gamma_{\mu_3} \cong \Gamma_{\mu_1}$ for certain other types of systems, in particular aqueous solutions containing concentrated (>1.5 M) NaCl and the sodium salt of polyanionic native DNA [9,10].

Whenever different types of preferential interaction coefficients are not approximately equal, relationships that can be used to interconvert them are needed because these thermodynamic derivatives are not equally accessible to accurate experimental evaluation, nor are they simply interchangeable in derivations of the expressions required for rigorous analyses of measurable effects due to preferential interactions. In particular, Γ_{μ_1} can be accurately quantified at low-to-moderate solute concentrations by water-vapor pressure osmometry (VPO), or at moderate-to-high solute concentrations by isopiestic distillation (ID), whereas individual values of Γ_{μ_3} can be *directly* quantified only in exceptional cases. Compared with Γ_{μ_1}, however, coefficients of the type Γ_{μ_3} have substantially more immediate physical significance and interpretability, insofar as they enter directly (as 'stoichiometric combinations') into rigorous analyses of measurable quantities that gauge how a process involving solute 2 as a reactant or product is affected by changing the concentration of a 'perturbing' solute 3 present in excess [11–13]. (Section IIB of [5] and references therein provide more detailed commentary on the crucial role of Γ_{μ_3} in the thermodynamic expression that can be used, in conjunction with a suitably detailed molecular model, to analyze and interpret solute concentration-dependent effects on processes.)

The earliest applications of VPO to quantify Γ_{μ_3} as well as Γ_{μ_1} were reported in 1996 [6]. Previously the general lack of awareness that these coefficients can differ significantly for systems that are in no respect exceptional may be attributed in part to the scarcity of the experimental information needed to determine Γ_{μ_3} as well as Γ_{μ_1} for various types of solutes over appreciable ranges of their concentrations. The most widely cited (indirect) comparison of Γ_{μ_3} and Γ_{μ_1}, which are numerically indistinguishable for the NaCl–NaDNA solutions mentioned above, is discussed in Chapter 2 of [4] and references therein. The existence of systems and conditions for which Γ_{μ_3} cannot be well approximated by Γ_{μ_1} motivates the present inquiry into whether for those cases (or any others) Γ_{μ_3} also can differ significantly from Γ_{μ_1,μ_3}. To provide a generalized means of addressing this question for any given system, we derive the pertinent exact thermodynamic relationship, valid for any types of solute components at any absolute or relative concentrations. The resulting expression can also be used to calculate Γ_{μ_3} for any systems in which this coefficient differs significantly from the corresponding Γ_{μ_1,μ_3}, which generally, though not in all cases of interest, is much more directly accessible to experimental determination.

Coefficients of the type Γ_{μ_3} can be directly quantified when $\Gamma_{\mu_3} \cong \Gamma_{\mu_1,\mu_3}$ for solutions in which one (and only one) of the solute components can pass through a dialysis membrane. Currently the most widely utilized, albeit indirect, approach entails measuring density as a function of the molality of each solute in a series of simultaneously dialyzed solutions, then matching these results (by suitably precise interpolations) with density measurements on a parallel series of undialyzed solutions. Far more extensively applied than any other, this method has yielded important information about the preferential interactions of a wide variety of small solutes with diverse types of proteins ([14] and references therein). However, for the purpose of quantifying Γ_{μ_1,μ_3} and related coefficients as functions of the concentrations of the two solutes, applications of equilibrium dialysis are not always optimal with regard to experimental accuracy [7,15]. Moreover, dialysis cannot be used to study the preferential interactions of certain pairs of solutes, for example, if they are so similar in molecular size that both, or neither, would diffuse across any (non-hypothetical) membrane permeable to solvent water.

The primary objective of this paper is to establish a more generalized basis than any previously available for assessing the relative importance of the physical factors that, for a given system, determine the range of validity of the approximation $\Gamma_{\mu_3} \cong \Gamma_{\mu_1,\mu_3}$. From the standpoint of practical applications, the model-independent expression derived in this paper also can be used to calculate Γ_{μ_1,μ_3} for systems in which preferential interactions cannot be studied by any method involving equilibrium dialysis. Thus, values of Γ_{μ_1,μ_3} can be obtained from corresponding values of two other preferential interaction coefficients: Γ_{μ_1}, which can be quantified by VPO, or (at higher solute concentrations) ID, with an accuracy limited only by typically small experimental uncertainties; and Γ_{μ_3}, which can be calculated using either of two approximate expressions previously derived [5] to analyze experimental input that also can be acquired by VPO or ID. To demonstrate calculations of Γ_{μ_1,μ_3} as well as Γ_{μ_3} and Γ_{μ_1} for two biochemically significant solutes that cannot be selectively dialyzed, we analyze ID measurements previously reported [16] for NaCl (component 2) and urea (component 3) over extensive ranges of m_2 and m_3. In general, information about the preferential interactions of such relatively small solutes with each other is needed in order to calculate the coefficients that characterize their competitive preferential interactions with a macromolecule, and hence their combined effects on a process involving that macromolecule as a reactant or product.

2. Strategy for derivations of exact thermodynamic relationships linking different preferential interaction coefficients

In Section IIIA of [5], the exact relationship among the three 'constant-pressure' coefficients Γ_{μ_k} ($k = 1$, 2 or 3) was derived using some of the basic mathematical and thermodynamic principles that are also needed for the present objective of deriving the exact linkage of Γ_{μ_1,μ_3}, Γ_{μ_3} and Γ_{μ_1}. Although the two derivations proceed by analogous stages, they differ in one important respect, because Γ_{μ_1,μ_3}, unlike any of the Γ_{μ_k}, characterizes effects of preferential interactions in a solution

where changes in the molalities of the two solutes must be accompanied by changes in the (mechanical) pressure, P, acting on the system. The derivative that expresses this pressure variability is ultimately eliminated, but the concomitant partial molar volumes of components 1 and 3 remain as potentially significant factors in the general expression that relates Γ_{μ_1,μ_3} exactly to derivatives of μ_1 and of μ_3, and hence to Γ_{μ_1} and Γ_{μ_3}. Calculations based on this relationship therefore require knowledge of the partial molar volumes of the (quasi-)diffusible components 1 and 3, in addition to the measurements made by VPO or ID that suffice to quantify Γ_{μ_1}, without approximation, and Γ_{μ_3}, with either of the two alternative approximate expressions (derived in Section IIIC of [5]) that entail measurable derivatives of μ_1 with respect to m_2 or m_3.

The Gibbs–Duhem equation is the ultimate physical origin of the exact thermodynamic correlation of the three Γ_{μ_k}. Consequently, derivation of this relationship requires that each of the three constant-pressure coefficients, defined as a partial derivative of the type $(\partial m_3 / \partial m_2)_{T,P,\mu_k}$ must be alternatively represented as a quotient of first partial derivatives of μ_k with respect to the different solute molalities. Then the five independent derivatives of μ_k are interrelated by two linear Gibbs–Duhem-based linkage expressions, which together yield the exact, non-linear relationship that links Γ_{μ_3}, Γ_{μ_2} and Γ_{μ_1}. Coefficients of the type Γ_{μ_2} generally are no more readily accessible than Γ_{μ_3} to direct experimental determinations. For the special situation of a solution in which the solubility of component 2 is saturated, values of Γ_{μ_2} can be quantified using density measurements to monitor changes in the equilibrium solubility m_2 as a function of m_3, as long as this solute does not coprecipitate. Despite the general experimental inaccessibility of Γ_{μ_2}, we have shown (in Section IIID of [5]) that, by considering the physical determinants of the magnitude of this coefficient for a particular system and range of solute concentrations, inferences can be drawn about the origin(s) of significant differences between Γ_{μ_1} and Γ_{μ_3}. With regard to the factors that cause these coefficients to differ, our previous discussion [5] is directly relevant (so will not be reproduced) in

this paper, because the derivation presented here shows that $(\Gamma_{\mu_1} - \Gamma_{\mu_3})$ is directly related to $(\Gamma_{\mu_{1,3}} - \Gamma_{\mu_3})$.

3. Relating the three constant-pressure coefficients

In an aqueous solution that contains two solute components, a general differential change in any concentration variable at constant temperature produces changes in all three chemical potentials that are linked by the Gibbs–Duhem equation:

$$V_m dP = m_1^{\bullet} d\mu_1 + m_2 d\mu_2 + m_3 d\mu_3 \tag{1}$$

where the molality of each solute has the usual definition (proportional to a mole ratio in which the divisor is the total moles of water in the solution); $m_1^{\bullet}$ is the molality of pure water, which has the constant value 55.5 mol/kg; and V_m is the volume of solution that contains 1 kg of water. At constant pressure, each of the $d\mu_k$ in Eq. (1) can be expressed in terms of the two partial derivatives of μ_k with respect to solute molality. These derivatives are conventionally designated with double numerical subscripts:

$$\text{For } i = 1,2,3; \quad j,k = 2,3$$
$$\mu_{ij} = (\partial\mu_i/\partial m_j)_{T,P,m_{k \neq j}} \tag{2}$$

Of these six derivatives, only five are independent, because by Euler reciprocity: $\mu_{23} = \mu_{32}$. These cross-partial derivatives, expressing the dependence of the chemical potential of either solute on the molality of the other, generally are the most important of the factors that determine the magnitudes of the various preferential interaction coefficients. For each of the derivatives μ_{ij}, the sign and magnitude are determined by two physically distinct contributions: from ideal mixing entropy and from differences between the concentration-dependent effects of solute–solute and solute–solvent interactions. For the different general types of solute components, the Appendix to [5] presents a detailed consideration of the explicit functional forms of the contributions from ideal mixing entropy to each of the three μ_k and to all of their derivatives with respect to m_2 or m_3. These expressions exhibit differences, determined solely by whether the two solute components have a common ion, that have a substantial impact on the extent to which different types of preferential interaction coefficients are numerically distinguishable.

By applying standard mathematical transformations (explained, for example, in the second chapter of [4]), each of the three constant-pressure coefficients can be represented as a quotient of different derivatives of μ_k:

$$\text{For } k = 1,2,3 \quad \Gamma_{\mu_k} = -\mu_{k2}/\mu_{k3} \tag{3}$$

Derivation of the exact relationship linking all three of these coefficients requires two additional expressions that follow from Eq. (1) with $dP = 0$:

$$\text{For } k = 2,3 \quad -m_1^{\bullet}\mu_{1k} = m_2\mu_{2k} + m_3\mu_{3k} \tag{4}$$

These linear Gibbs–Duhem-based linkage equations relate the three unknown derivatives (μ_{22}, μ_{33} and $\mu_{23} = \mu_{32}$) to μ_{12} and μ_{13}, each of which can be quantified by analyzing either VPO or (more indirectly, by means of interpolations) ID measurements to determine the following two derivatives:

$$\text{For } k = 2,3 \quad \Omega_k \equiv RT(\partial\text{Osm}/\partial m_k)_{T,P,m_{k' \neq k}}$$
$$= -m_1^{\bullet}\mu_{1k}/RT \tag{5}$$

where the osmolality of the solution, Osm, which can be measured directly (for example) by a commercially available vapor pressure osmometer, is related to the activity (a_1) of solvent water, and hence to the difference between μ_1 and the chemical potential of pure liquid water, $\mu_1^{\bullet}$, by:

$$\text{Osm} \equiv -m_1^{\bullet}\ln a_1 = -55.5(\mu_1 - \mu_1^{\bullet})/RT \tag{6}$$

With Eq. (3) and Eq. (5), the isoosmolal coefficient can be expressed in terms of different derivatives of osmolality:

$$\Gamma_{\mu_1} = -\Omega_2/\Omega_3 \tag{7}$$

Combining the five expressions given by Eq. (3) and Eq. (4) yields the following non-linear relationship in which, besides the three Γ_{μ_k}, the only other explicit thermodynamic variable is the mole ratio of the two solute components:

$$\Gamma_{\mu_3} = [\Gamma_{\mu_1} + m_2\Gamma_{\mu_2}\Gamma_{\mu_3}/m_3]/[1 + m_2\Gamma_{\mu_2}/m_3] \tag{8a}$$

This expression can be rearranged in various instructive ways, including the following particularly useful form:

$$(\Gamma_{\mu_1} - \Gamma_{\mu_3})/\Gamma_{\mu_1} = (1 - \Gamma_{\mu_3}/\Gamma_{\mu_2})/(1 - m_3/m_2\Gamma_{\mu_2})$$
$$= \left(1 - (\mu_{23})^2/\mu_{22}\mu_{33}\right)/$$
$$(1 + m_3\mu_{23}/m_2\mu_{22}) \quad (8b)$$

As discussed in Section IIID of [5], Eq. (8b) can be used to examine effects on the relative difference between Γ_{μ_3} and Γ_{μ_1} due to contributions from the ideal mixing entropy of component 2 and from non-ideality due to its interactions with component 3.

Besides providing the exact thermodynamic linkage of all three of the constant-pressure coefficients shown in Eq. (8a) and Eq. (8b), the Gibbs–Duhem linkages expressed by Eq. (4) are also essential for the derivation of two expressions for Γ_{μ_3} that incorporate one of the following alternative approximations:

$$\text{For} \quad k = 2,3: \qquad m_k\mu_{kk} \cong m_k\mu_{kk}^{o(j)} = RT\,\Omega_k^{o(j)} \quad (9)$$

For solute components that do not have a common ion, the approximations in Eq. (9) yield two independent expressions for Γ_{μ_3}, each of which depends only on m_2/m_3 and on derivatives of the osmolality that can be experimentally determined for solutions containing both solutes and for corresponding solutions containing only one solute:

$$\Gamma_{\mu_3}^{(I)} = -(\Omega_2 - \Omega_2^{o(3)})/$$
$$\left[\Omega_3 - (m_2/m_3)(\Omega_2 - \Omega_2^{o(3)})\right] \quad (10a)$$

$$\Gamma_{\mu_3}^{(II)} = -(\Omega_3 - \Omega_3^{o(2)})/(m_2/m_3)\Omega_3^{o(2)} \quad (10b)$$

Steps in the derivation leading to these expressions for Γ_{μ_3} have been presented and discussed in Section IIIC of [5]. When Eq. (10a) and Eq. (10b) yield consistent values of Γ_{μ_3}, together with Eq. (7) they suffice to analyze experimental input accessible by VPO or ID for the purpose of quantifying Γ_{μ_1,μ_3}, because this coefficient is related exactly to Γ_{μ_1} and Γ_{μ_3} by the model-independent derivation presented in the following sections.

4. Representing Γ_{μ_1,μ_3} in terms of derivatives of chemical potentials

In parallel with the derivation leading to Eq. (8a), the first objective is to obtain an expression

analogous to Eq. (3) by relating Γ_{μ_1,μ_3} to the appropriate derivatives of both $_{\mu_1}$ and $_{\mu_3}$. Although Eq. (3) and Eq. (4) are essential for the following derivation, they do not suffice, because, unlike any of the Γ_{μ_k}, the coefficient Γ_{μ_1,μ_3} pertains to a system in which the pressure cannot be held fixed while m_2 and m_3 change. When T, Γ_{μ_1} and Γ_{μ_3} are held constant in a series of solutions containing variable concentrations of two thermodynamically independent solute components, the pressure on each of these solutions must, in general, be different, because the Gibbs phase rule allows a maximum of three constraints on any function that varies with solution composition. Accordingly, three thermodynamic functions must be fixed in defining any partial derivative that can be measured, or related to any other measurable property of a three-component system.

The derivation proceeds by considering isothermal changes in the chemical potentials of the components, each of which, in accordance with the Gibbs phase rule, can be considered as a function of the three independent intensive variables: P, m_2 and m_3. Thus:

$$\text{For} \quad k = 1,2,3:$$
$$d\mu_k = \left(\partial\mu_k/\partial P\right)_{T,m_2,m_3}dP + \mu_{k2}dm_2 + \mu_{k3}dm_3 \quad (11)$$

where the coefficients of the molality differentials are derivatives of the μ_k given by Eq. (2). The coefficients of the pressure differential for each of the three solutes can be expressed in terms of independently measurable quantities on the basis of Eq. (1), in conjunction with the two Gibbs–Duhem linkage equations expressed by Eq. (4) and the additivity of partial molar volumes:

$$V_m = m_1^{\bullet}\bar{V}_1 + m_2\bar{V}_2 + m_3\bar{V}_3 \quad (12)$$

Thus:

$$\text{For} \quad k = 1,2,3:$$
$$\left(\partial\mu_k/\partial P\right)_{T,m_2,m_3} = \bar{V}_k \equiv \left(\partial V/\partial n_k\right)_{T,P,n_{k'\neq k}} \quad (13)$$

In many cases partial molar volumes may be well approximated as having the concentration-invariant values pertaining to an infinitely dilute solution. Otherwise, for systems in which the solute concentrations are not dilute and vary over an extensive

range, $\bar{V}_1$ and $\bar{V}_3$ (also $\bar{V}_2$, though it does not enter into the relationship linking Γ_{μ_1,μ_3} to other preferential interaction coefficients) can be individually quantified *without approximation*, provided that the density, ρ, of the solution can be accurately measured *and* sufficiently well characterized, as a function of the molalities of both solutes, so that the derivatives $\left(\partial\rho/\partial m_2\right)_{m_3}$ and $\left(\partial\rho/\partial m_3\right)_{m_2}$ also can be accurately evaluated.

By imposing the constraints of fixed μ_1,μ_3 on Eq. (11) for $k=1$ and 3, and taking m_2 as the differentiating variable, the following two equations are obtained for the (quasi-)diffusible components, 1 and 3:

$$0=\bar{V}_1\Pi'_2+\mu_{12}+\mu_{13}\Gamma_{\mu_1,\mu_3} \tag{14a}$$

$$0=\bar{V}_3\Pi'_2+\mu_{32}+\mu_{33}\Gamma_{\mu_1,\mu_3} \tag{14b}$$

In these equations the derivative expressing the m_2-dependence of P is:

$$\Pi'_2\equiv\left(\partial\Pi/\partial m_2\right)_{\mu_1,\mu_3}=\left(\partial P/\partial m_2\right)_{\mu_1,\mu_3} \tag{15}$$

The 'osmotic pressure' of the solution containing both solutes is related to water activity and the molar volume of pure water at 25 °C, $\bar{V}_1^{\bullet}$ by the conventional expression: $\Pi=-\left(RT/\bar{V}_1^{\bullet}\right)\ln a_1$. As indicated by Eq. (15), the derivative Π'_2 that appears in Eq. (14a) and Eq. (14b) also expresses the change in P, the mechanical pressure on a solution containing solutes 2 and 3, that must occur when m_2 is changed while μ_1, μ_3 and T are held fixed.

Although μ_{12} and μ_{13} can be quantified with input from VPO or ID, four unknown thermodynamic functions remain in Eq. (14a) and Eq. (14b). For direct experimental determinations of Γ_{μ_1,μ_3} according to the definition $\left(\partial m_3/\partial m_2\right)_{T,\mu_1,\mu_3}$, the indicated chemical potentials can be held constant in a series of simultaneously dialyzed isothermal solutions, provided that solute component 3, like water, can diffuse across a membrane that is impermeable to solute component 2. However, regardless of the molecular size of the solutes (or any chemical characteristics that determine whether they can diffuse across some solute-specific dialyzing membrane), Γ_{μ_1,μ_3} is a well-defined state function for any type of three-

component system. Similarly, the osmotic pressure Π has a definite thermodynamic significance, although it cannot be directly measured for a dialyzed solution unless solvent water is the only component that can traverse the dialysis membrane. In contrast, P could, at least in principle, be directly measured using a 'colloid osmometer' even though component 3 diffuses across the membrane.

Although $\Pi\neq P$, the derivatives of these two pressures, as indicated in Eq. (15), *are* always equal in (the usual) situations where the pressure on the dialyzing (two-component) solution is constant, determined by the ambient atmosphere. Because the primary objective of this paper is the exact relationship linking Γ_{μ_1,μ_3} to Γ_{μ_3} and Γ_{μ_1}, the pressure derivative Π'_2 is eliminated from Eq. (14a) and Eq. (14b). Alternatively, Γ_{μ_1,μ_3} could be eliminated, and the resulting expression taken as a basis for calculating Γ_{μ_3} from knowledge of Γ_{μ_1}, μ_{13}, and Π'_2. Because this pressure derivative is directly related to the corresponding derivative of Osm, it could, in principle, be quantified by VPO measurements on a series of simultaneously dialyzed three-component solutions. However, calculations of Γ_{μ_3} via Π'_2, rather than Γ_{μ_1,μ_3}, probably would incur more experimental uncertainty, in part because the magnitude of Π'_2 typically is substantially smaller than that of Γ_{μ_1,μ_3}.

An expression equivalent to Eq. (14b) has been used [4] to derive a relationship between Γ_{μ_1,μ_3} and Γ_{μ_3}, by approximating Π'_2 on the basis of van't Hoff's law for the ideal osmotic pressure. The need to approximate Π'_2 is avoided here by eliminating this derivative in the following derivation of the exact relationship linking Γ_{μ_1,μ_3} to Γ_{μ_3} and Γ_{μ_1}. By introducing into Eq. (14b) the form of Γ_{μ_3} given by Eq. (3), the difference between Γ_{μ_3} and Γ_{μ_1,μ_3} can be expressed as:

$$\Gamma_{\mu_3}-\Gamma_{\mu_1,\mu_3}=\bar{V}_3\Pi'_2/\mu_{33} \tag{16}$$

A parallel expression for the difference between Γ_{μ_1} and Γ_{μ_3} is obtained by eliminating Γ_{μ_1,μ_3} from Eq. (14a) and Eq. (14b), and using Eq. (3) to represent Γ_{μ_1}:

$$\Gamma_{\mu_1}-\Gamma_{\mu_3}=\left[\bar{V}_1\mu_{33}-\bar{V}_3\mu_{13}\right]\Pi'_2/\mu_{13}\mu_{33} \tag{17}$$

The differences between the pairs of preferential interaction coefficients shown in Eq. (16) and Eq. (17) are correlated by eliminating Π'_2:

$$\Gamma_{\mu_3} - \Gamma_{\mu_1,\mu_3} = m_3 \bar{V}_3 (m_1^{\bullet} \mu_{13})(\Gamma_{\mu_1} - \Gamma_{\mu_3})/$$
$$\left[m_1^{\bullet} \bar{V}_1 (m_3 \mu_{33}) - m_3 \bar{V}_3 (m_1^{\bullet} \mu_{13}) \right] \qquad (18)$$

The factors of $m_1^{\bullet}$ and m_3 are introduced in this expression to anticipate the utility of considering the dimensionless quotients $m_3 \bar{V}_3 / m_1^{\bullet} \bar{V}_1$ and $m_1^{\bullet} \mu_{13}/m_3 \mu_{33}$.

5. Deriving the exact linkage of Γ_{μ_1,μ_3} to Γ_{μ_3} and Γ_{μ_1}

In the form given by Eq. (18), the exact relationship linking the three preferential interaction coefficients also explicitly depends on $m_1^{\bullet} \mu_{13}/m_3 \mu_{33}$. Before eliminating this quotient by introducing the appropriate Gibbs–Duhem-based linkage, some conclusions about relationships between various pairs of coefficients can be deduced for situations where simplifying approximations are valid. According to Eq. (18), the difference between Γ_{μ_1} and Γ_{μ_3} is one determinant of the difference between Γ_{μ_3} and Γ_{μ_1,μ_3}, but the latter difference also depends on the quotient $\bar{V}_3 \mu_{13}/\bar{V}_1 \mu_{33}$. Consequently, $\Gamma_{\mu_1,\mu_3} \cong \Gamma_{\mu_3}$ whenever $\Gamma_{\mu_1} \cong \Gamma_{\mu_3}$, but the converse need not hold. If $\bar{V}_3 \mu_{13}/\bar{V}_1 \mu_{33}$ is small enough, then $\Gamma_{\mu_1,\mu_3} \cong \Gamma_{\mu_3}$ even for systems in which Γ_{μ_3} differs significantly from Γ_{μ_1}. If $\bar{V}_3 \mu_{13}/\bar{V}_1 \mu_{33}$ is large enough, then $\Gamma_{\mu_1,\mu_3} \cong \Gamma_{\mu_1}$, regardless of whether these coefficients are similar in magnitude to Γ_{μ_3}. In fact, Γ_{μ_1} has been found to differ significantly from both Γ_{μ_3} and Γ_{μ_1,μ_3} for the preferential interactions with BSA of each of the solutes that have been studied by VPO [7,8].

The partial molar volumes appearing in Eq. (18) originate in Eq. (11) and Eq. (13). The pressure derivative that multiplies both $\bar{V}_1$ and $\bar{V}_3$ cannot in general vanish, because the Gibbs phase rule requires that P must change with m_2 if μ_1, μ_3 and T are fixed. Unequal pressures on solutions separated by a dialysis membrane impermeable to solute 2 would be manifested even by a hypothetical system in which the magnitudes of Γ_{μ_1} and

Γ_{μ_3} are determined entirely by contributions of ideal mixing entropy to derivatives of μ_1 and μ_3, respectively. After an addition of the non-diffusible solute to such 'ideal dilute' solutions at dialysis equilibrium, both water and solute 3 redistribute across the membrane in order to preserve the constraints on μ_1 and μ_3. Thus, to prevent any changes in these chemical potentials, m_2 and m_3 must change in such a way that the resulting changes in the contributions from ideal mixing entropy to the chemical potentials of the diffusible components are balanced by the concomitant changes in their standard-state chemical potentials, which must occur because of the change in pressure on the three-component system. (In typical experimental situations, the two-component dialyzing solution is subject to the ambient atmospheric pressure.)

The remaining quotient of derivatives of chemical potentials in Eq. (18) can be eliminated by combining the appropriate Gibbs–Duhem-based linkage from Eq. (4), with the representation of Γ_{μ_3} given by Eq. (3):

$$-m_1^{\bullet} \mu_{13}/m_3 \mu_{33} = (1 - m_2 \Gamma_{\mu_3}/m_3) \qquad (19)$$

After combining Eq. (18) and Eq. (19), the exact relationship linking Γ_{μ_1,μ_3} to the other two coefficients can be expressed:

$$\Gamma_{\mu_1,\mu_3} = \Gamma_{\mu_3} + Q_v (\Gamma_{\mu_1} - \Gamma_{\mu_3})/(1 + Q_v) \qquad (20)$$

Thus, the pairwise differences $(\Gamma_{\mu_1,\mu_3} - \Gamma_{\mu_3})$ and $(\Gamma_{\mu_1} - \Gamma_{\mu_3})$ are correlated by a factor that depends directly, though not exclusively, on the ratio of the solution volumes occupied by the two (quasi-)diffusible components:

$$Q_v \equiv m_3 \bar{V}_3 (1 - m_2 \Gamma_{\mu_3}/m_3)/m_1^{\bullet} \bar{V}_1 \qquad (21)$$

Like the factor of m_3 subsumed into Q_v, the factor of m_2 multiplying Γ_{μ_3} in Eq. (21) originates in one of the Gibbs–Duhem linkages expressed by Eq. (4). The functional form of Eq. (21) indicates considerable disparity between m_3 and m_2 in their effects on the magnitude of Q_v. This asymmetry arises not because of any assumption about the absolute or relative values of m_2 and m_3, but rather ultimately because no constraint has been placed

on the chemical potential of solute 2, the (quasi-)non-diffusible component. For the same reason, $\bar{V}_2$ does not appear in Eq. (21).

For all the systems and conditions that have been investigated thus far by VPO, the magnitude of $m_2\Gamma_{\mu_3}/m_3$ is significantly less than unity, and it has at most a minor dependence on m_3 [7,8]. Moreover, $\bar{V}_3$, and hence Q_v, are positive. (Negative values of $\bar{V}_3$ have been determined only for some exceptional systems, such as sufficiently dilute aqueous solutions of a strong electrolyte with a relatively small multiply charged cation, like Mg^{2+}.) For all systems where Q_v and $(1 - m_2\Gamma_{\mu_3}/m_3)$ are positive, Eq. (20) and Eq. (21) show that the magnitude of $(\Gamma_{\mu_1,\mu_3} - \Gamma_{\mu_3})$ must be less than that of $(\Gamma_{\mu_3} - \Gamma_{\mu_1})$. As long as Q_v is sufficiently small, the difference between Γ_{μ_1,μ_3} and Γ_{μ_3} may be negligible (within experimental uncertainties), even though Γ_{μ_3} differs significantly from Γ_{μ_1}. The m_3-dependence of Q_v explicitly specified in Eq. (21), and the fact that Γ_{μ_3}/m_3 is always finite [5], ensure that at any value of m_2, Γ_{μ_1,μ_3} and Γ_{μ_3} become exactly equal as $m_3 \rightarrow 0$, even though in this limit the difference between Γ_{μ_3} and Γ_{μ_1} never vanishes and may not be negligible in comparison to the magnitude of Γ_{μ_3}. For systems in which neither m_2 nor m_3 is necessarily small, the physical determinants of $(\Gamma_{\mu_3} - \Gamma_{\mu_1})/\Gamma_{\mu_1}$ have been considered in detail in Section IIID of [5], on the basis of the exact relationship given here as Eq. (8b).

6. Effects of high dilution of component 2 on the exact linkage of $\Gamma_{\mu 1,\mu 3}$ to $\Gamma_{\mu 3}$ and $\Gamma_{\mu 1}$

For solutions in which the concentration of component 2 is sufficiently dilute, the linkage among the three coefficients expressed by Eq. (20) can be expressed more simply as:

$$\Gamma_{\mu_1,\mu_3}^{o(2)} - \Gamma_{\mu_3}^{o(2)} = Q_v^{o(2)}(\Gamma_{\mu_3}^{o(2)} - \Gamma_{\mu_1}^{o(2)})/(1 + Q_v^{o(2)})$$

(22)

where the superscript o(2) indicates a thermodynamic function that has no significant dependence on the concentration of component 2. Thus, $Q_v^{o(2)} = m_3\bar{V}_3^{o(2)}/m_1^{\bullet}\bar{V}_1^{\bullet}$. Comparison of Eq. (22) with Eq. (20) and Eq. (21) shows that the relationship

linking the three coefficients is linear at *any* m_3 and for m_2 in a range that may be well above the 'limit of infinite dilution', as long as $m_2\Gamma_{\mu_3}/m_3$ is small enough compared with unity. This term is invariably less than unity, but not always negligible, in VPO studies of the preferential interactions of various solutes with BSA. The largest value, $m_2\Gamma_{\mu_3}/m_3 = 0.2$, was found for glycine betaine at the highest BSA concentration investigated ($m_2 = 4.15 \times 10^{-3}$ molal) [5,7].

For systems that exhibit significant differences between $\Gamma_{\mu_3}^{o(2)}$ and $\Gamma_{\mu_1}^{o(2)}$, Eq. (22) shows that the difference between $\Gamma_{\mu_3}^{o(2)}$ and $\Gamma_{\mu_1,\mu_3}^{o(2)}$ depends only on the magnitude of $m_3\bar{V}_3^{o(2)}/m_1^{\bullet}\bar{V}_1^{\bullet}$. The maximum value of $m_3\bar{V}_3^{o(2)}$ is generally determined by the concentration at which the solubility limit of component 3 is reached. In the usual units (ml/g), the numerical value of $55.5\bar{V}_1^{\bullet}$ is (by definition) identically one for pure water at 25 °C. In the same units, 0.2 is the maximum value of $m_3\bar{V}_3$ (for trehalose at 0.5 molal) that has been investigated in VPO studies of the preferential interactions of small solutes with BSA [7,8]. More significant differences between Γ_{μ_1,μ_3} and Γ_{μ_3} at a given m_3 can be anticipated for solutes as large as some of the polyethylene glycols that have been investigated (for example) by Bhat and Timasheff [17]. The magnitude of the largest possible difference between $\Gamma_{\mu_3}^{o(2)}$ and $\Gamma_{\mu_1,\mu_3}^{o(2)}$ is determined not only by the maximum value of $m_3\bar{V}_3^{o(2)}$, but also by $|\Gamma_{\mu_3}^{o(2)} - \Gamma_{\mu_1}^{o(2)}|$. This difference is illustrated in Fig. 1 of [5] for the preferential interactions of each of 11 common biochemical solutes with a hypothetical component (2) comprised of an oligoanion and six dissociated univalent counterions. As explained in Section IIID of [5], $|\Gamma_{\mu_3}^{o(2)} - \Gamma_{\mu_1}^{o(2)}|$ is primarily determined by the m_2-dependence of the ideal mixing entropy of component 2 and (generally to a comparatively minor extent) by the m_3-dependence of the non-ideality of component 3, which can be quantified using Eq. (9) to analyze VPO measurements on solutions in which component 3 is the only solute.

As m_3 also approaches infinite dilution, Eq. (20) shows that $\Gamma_{\mu_1,\mu_3}^{o(2)} \rightarrow \Gamma_{\mu_3}^{o(2)}$. However, when $m_3 > 0$, $\Gamma_{\mu_1,\mu_3}^{o(2)}$ and $\Gamma_{\mu_3}^{o(2)}$ can never be exactly equal, because, as we demonstrated by formulas derived
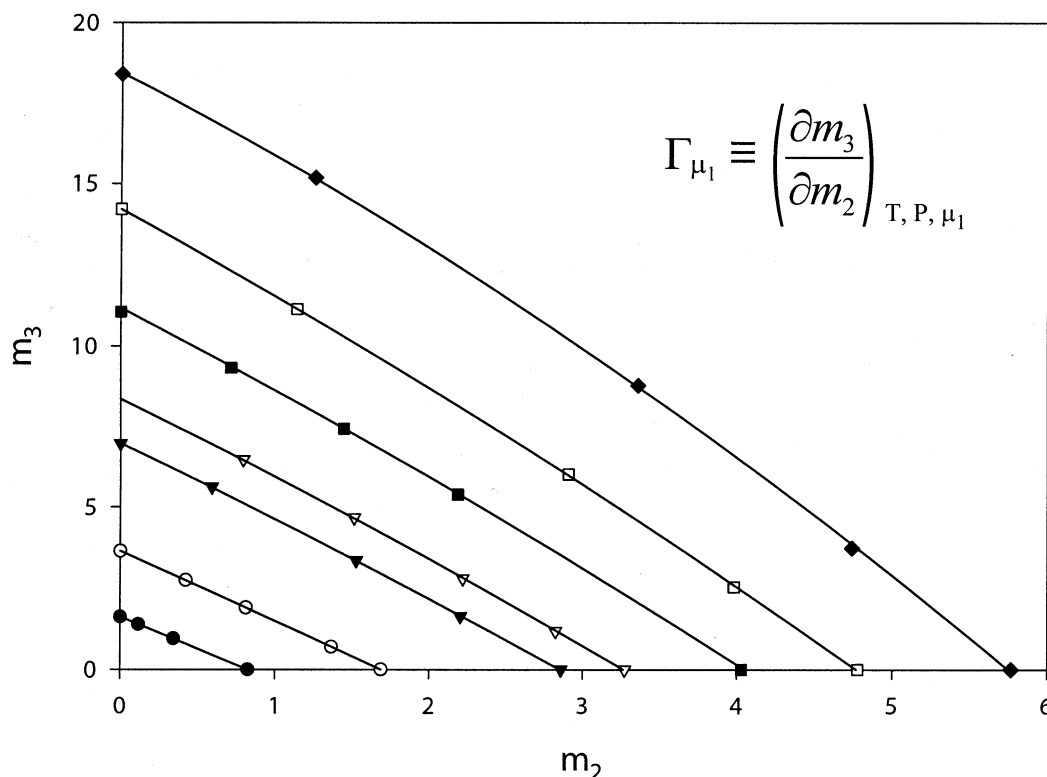
$$\Gamma_{\mu_1} \equiv \left(\frac{\partial m_3}{\partial m_2} \right)_{T, P, \mu_1}$$

Fig. 1. Thermodynamics of urea–NaCl–H$_2$O (3:2:1) mixtures. Isoosmolal plots of m_3 vs. m_2 (data from [16]) at the following osmolalities: 1.5339 (●), 3.2610 (○), 5.9195 (▼), 6.9645 (▽), 9.0129 (■), 11.2557 (□), and 14.4400 (◆).

in Section III-B of [5], $\Gamma_{\mu_3}^{o(2)}$ and $\Gamma_{\mu_1}^{o(2)}$ remain different as $m_3 \rightarrow 0$. Specifically, if ν_2 is the total number of charged particles that dissociate from the binary component 2, and if the binary components 2 and 3 have no ion in common, then:

$$\Gamma_{\mu_3}^{o(2)} - \Gamma_{\mu_1}^{o(2)} = \nu_2 / \Omega_3^{o(2)} \qquad (23)$$

This expression is inapplicable, however, to solutions in which components 2 and 3 have a common ion. For such systems, as demonstrated in the Appendix of [5], ν_2 has an 'effective' value of unity, as if component 2 were a single species with no net charge. Consequently, in solutions where component 2 is comprised of a highly charged macromolecule and many dissociated counterions, the difference between $\Gamma_{\mu_3}^{o(2)}$ and $\Gamma_{\mu_1}^{o(2)}$ is drastically reduced when these counterions are of the same type as one of the ions dissociated from component 3. This 'entropic dilution' effect

is largely, though not completely, responsible for the approximate equality of $\Gamma_{\mu_3}^{o(2)}$ and $\Gamma_{\mu_1}^{o(2)}$ in solutions containing NaCl and the sodium salt of polymeric native DNA [9,10].

In summary, when m_3 is not negligibly small, the physical origins of any significant numerical differences between $\Gamma_{\mu_1,\mu_3}^{o(2)}$ and $\Gamma_{\mu_3}^{o(2)}$ can be traced to those that determine the corresponding difference between $|\Gamma_{\mu_3}^{o(2)} - \Gamma_{\mu_1}^{o(2)}|$ and those that determine the magnitude of $Q_v^{o(2)}$. This ratio of the solution volumes occupied by components 3 and 1 is determined by the quotient of the partial molar volumes, which originate in Eq. (14a) and Eq. (14b) as factors expressing effects of 'osmotic' pressure variability, and by the ratio m_3/m_1^*, which originates in the molality factors that appear explicitly in the Gibbs–Duhem linkage specified by Eq. (4) for $k=3$. The expressions derived in this section as limiting forms of Eq. (8a) and Eq.

(20) are consistent with Eq. (A10) in [1]. Thus, that equation corresponds to a combination of our Eq. (22), appropriate for solutions where component 2 is highly dilute, and our Eq. (23), with ν_2 assigned the effective value 1 appropriate for two solute components that share a common ion.

7. A three-component system for which all preferential interaction coefficients differ significantly

In this section we show how Eq. (20) for Γ_{μ_1,μ_3}, together with Eq. (10a) for Γ_{μ_3} and Eq. (7) for Γ_{μ_1}, can be applied to analyze ID measurements on systems for which equivalent information about preferential interactions could not be obtained by any method based on equilibrium dialysis. Each of the two small solute components considered here, NaCl and urea, is known to have important, but distinctly different, effects on measurable thermodynamic and kinetic parameters that characterize processes involving at least one biopolymer as a reactant or product. Effects on such processes caused by the combined preferential interactions of 1:1 salt and urea are worth investigating because, as studied both in vitro (e.g. [18,19]) and in vivo [20], environments that contain appreciable concentrations of urea also usually contain non-negligible concentrations of univalent salt ions. Information about the mutual preferential interactions of 1:1 salt and urea in solutions where these are the only solutes will be utilized in a subsequent paper from this laboratory (Hong et al., in preparation), in which we will analyze effects of changing the concentration of urea on a protein–nucleic acid binding process in solutions where the salt molality is fixed.

Fig. 1 presents plots of the molality of urea (component 3) vs. the molality of NaCl (component 2) for each of seven series of isoosmolal solutions. These data, shown as representative of the more extensive results published by Bower and Robinson [16], were taken directly from their Table I (for solutions in which urea is the only solute) or their Table III (for solutions containing both solutes and for 'reference' solutions, in which NaCl is the only solute.) These tabulated values, with at least four significant figures, were determined to this high level of accuracy by gravimetric analysis of the composition of each different equilibrated solution in the various isoosmolal series. The values of m_2 for which data are shown in Fig. 1 cover nearly the entire range over which NaCl is soluble in pure water at 25 °C; the corresponding range of m_3 is more than three-fold larger, consistent with the extraordinarily high solubility of urea in water. The range of fixed osmolalities in the seven series of isoosmolal solutions varies nearly 10-fold from 1.53 to 14.4 molal.

The preferential interaction coefficient that follows most directly from the output of ID experiments, such as those for which data are shown in Fig. 1, is $\Gamma_{\mu_1} \equiv (\partial m_3/\partial m_2)_{\mu_1,P,T}$, which can be quantified as the slope of the line tangent to the curve that characterizes the isoosmolal dependence of m_3 on m_2. The points in each of the seven series represented in Fig. 1 all pertain to solutions in which T is controlled at 25 °C, P is the pressure of pure water vapor in the sealed ID apparatus, and μ_1 corresponds to the osmolality that, for each different isoosmolal series, is quantified by determining the composition of the reference (NaCl) solution and interpolating this value of m_2 onto a plot of Osm vs. m_2 that has been independently determined with high accuracy by measurements on corresponding standard solutions. Each of the solid lines shown in Fig. 1 is a best-fitted quadratic function that exhibits no significant systematic deviations from the data points. Therefore, the slope of each of these isoosmolal lines, in all cases negative, can be expressed as a linear function of m_2 having the form: $\Gamma_{\mu_1} = \Gamma_{\mu_1}^{o(2)} + \left[(\partial \Gamma_{\mu_1}/\partial m_2)_{T,P,\mu_1}^{o(2)} \right] m_2$. For all of the conditions considered here, Γ_{μ_1} is dominated by $\Gamma_{\mu_1}^{o(2)}$, but the term linear in m_2 becomes progressively more significant with increasing osmolality. [The superscript o(2) has the same physical significance here as in Eq. (22), where the superscripted variables also have a generally substantial dependence on m_3.]

According to Eq. (7) the slope of each isoosmolal plot also can be expressed as $-\Omega_2/\Omega_3$, but knowing only the quotient of these derivatives does not suffice to determine corresponding values of Γ_{μ_3}. To quantify this coefficient with Eq. (10a)

or Eq. (10b), individual values of Ω_2, $\Omega_2^{o(3)}$, Ω_3 and/or $\Omega_3^{o(2)}$ must be determined. Whereas VPO measurements could be used to quantify each of these derivatives directly, feasible experiments utilizing ID cannot be designed to hold constant the molality of one solute while the molality of the other is varied at will to produce changes in Osm. As indicated by the non-Cartesian pattern of the data points in Fig. 1, neither m_2 nor m_3 (nor Osm itself) was controlled (fixed at values chosen a priori, or varied in a prescribed way) during the isopiestic equilibration of each solution in an isoosmolal series through evaporation or condensation of the requisite amount of water. Nevertheless, we now proceed to show that these ID measurements can be analyzed to quantify not only Γ_{μ_1}, but also Γ_{μ_3} and Γ_{μ_1,μ_3}, provided that the functional dependence of Osm on m_2 and m_3 can be determined by fitting a sufficiently large, and sufficiently accurate, set of data.

To determine Osm as a function of m_2 and m_3 for aqueous solutions containing both NaCl and urea, and for solutions containing only one of these solutes, a non-linear global fitting procedure (to be described in complete detail elsewhere) has been applied to analyze data from Table III of [16], together with data reported for aqueous urea solutions [21] and for aqueous salt solutions [22]. The appropriate derivatives of the best fitted functional form of Osm(m_2,m_3) provide the input used to quantify Γ_{μ_1} by applying Eq. (7), and corresponding values of Γ_{μ_3} by applying Eq. (10a). [Alternative determinations of Γ_{μ_3} based on Eq. (10b) gave values consistent with calculations based on Eq. (10a), but generally with significantly larger uncertainties.] For each value of Γ_{μ_1} and Γ_{μ_3} at given m_2 and m_3, the corresponding value of Γ_{μ_1,μ_3} was calculated using Eq. (20) and Eq. (21), with $\bar{V}_3$ fixed at $\bar{V}_3^{o(2)} = 0.044$ 1/mol [8]. (The partial molar volume of urea in aqueous solutions where it is the only solute varies by less than 6% over the range of m_3 considered here, so this effect is secondary compared with the dominant effect on $|\Gamma_{\mu_1,\mu_3} - \Gamma_{\mu_3}|$ produced by the variation of m_3 itself.)

Evaluated using derivatives calculated from the best fitted functional form of Osm(m_2,m_3), Γ_{μ_1}, Γ_{μ_3} and Γ_{μ_1,μ_3} are plotted in Fig. 2 as functions of m_3, with m_2 fixed at 2 molal. This value was chosen as representative in part because it subdivides the total number of ID data points used for the global fitting into two approximately equal sets. The size of the points shown in Fig. 2 generally exceeds the minor random scatter due to the uncertainties in the fitting procedure, as indicated by discrepancies between actual data points and the corresponding predictions of the best-fitted function for Osm(m_2,m_3). With regard to the primary theme and scope of the present paper, the most significant information conveyed by Fig. 2 is the extent to which the three preferential coefficients differ. These differences at the median salt concentration $m_2 = 2$ molal are qualitatively representative of analogous plots calculated at different fixed salt concentrations within the range of the ID measurements analyzed. At $m_2 = 2$ molal the magnitude of Γ_{μ_1} always substantially exceeds those of both Γ_{μ_3} and Γ_{μ_1,μ_3}. As noted above, Eq. (20) requires that $|\Gamma_{\mu_3} - \Gamma_{\mu_1,\mu_3}| = 0$ when $m_3 = 0$, but when m_3 exceeds ~ 1.8 molal, the difference between Γ_{μ_3} and Γ_{μ_1,μ_3} exceeds the uncertainties in these coefficients and continues to increase with increasing m_3. Implications of the finding that for urea and NaCl in aqueous solution Γ_{μ_3} is approximately zero over broad ranges of m_2 and m_3 will be explored in detail in a subsequent paper (Anderson et al., in preparation), where we will also introduce a new method of testing and, where necessary, improving upon the approximations incorporated into the alternative expressions for Γ_{μ_3} given by Eq. (10a) and Eq. (10b).

8. Concluding discussion

Different approximate theoretical rationales [4], [23] have been proposed to justify the apparent equivalence of P and μ_1 as constraints on the partial derivatives of m_3 with respect to m_2 that define, respectively, Γ_{μ_3} and Γ_{μ_1,μ_3}. Prior to the derivation presented here, the most comprehensive treatment of the relationship between these two coefficients was offered by John Schellman [1]. His Eq. (A10) shows that for two solute components having a common ion, in the limit $m_2 \to 0$ the difference between Γ_{μ_1,μ_3} and Γ_{μ_3} is determined by the 'volume fraction' (denoted ϕ) of
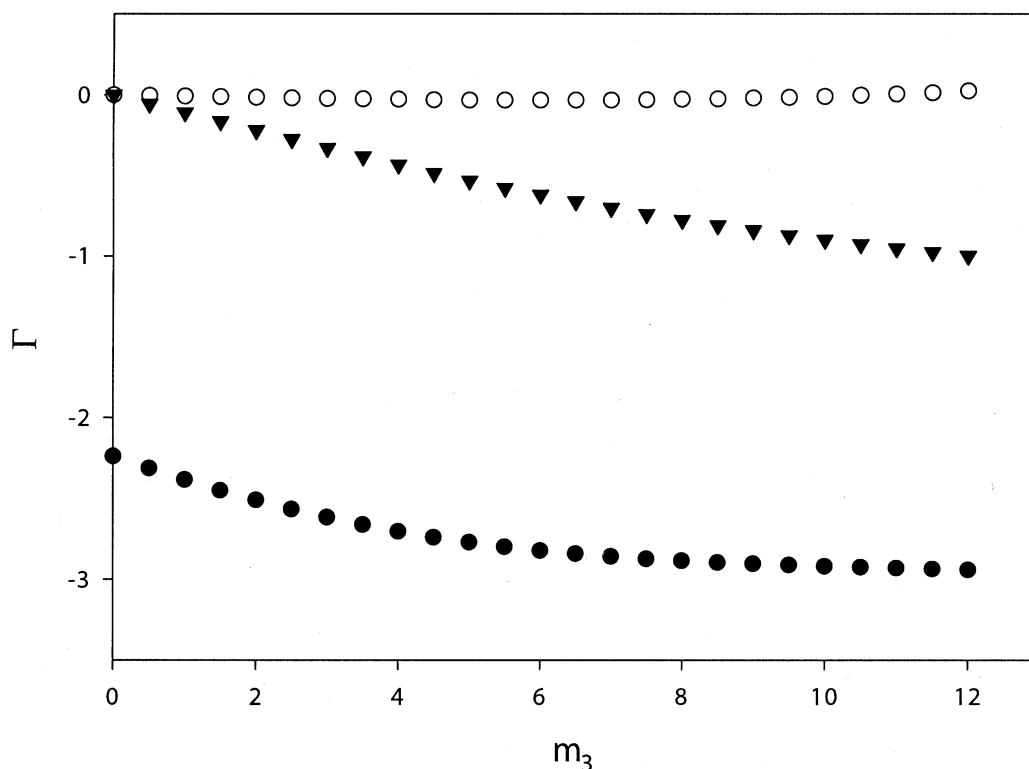
Fig. 2. The three preferential interaction coefficients Γ_{μ_1}, Γ_{μ_3} and Γ_{μ_1,μ_3} for NaCl–urea interactions in aqueous solution expressed as functions of urea (component 3) molality at 2 molal NaCl (component 2): Γ_{μ_1} (●), Γ_{μ_3} (○) and Γ_{μ_1,μ_3} (▼).

the solution occupied by component 3, multiplied by the factor $1/\Omega_3^{o(2)}$ [in our notation, as in Eq. (9)], which does not differ much from unity for most of the solutes that have been investigated by VPO [7,8]. In solutions where solute 2 is not at high dilution and does not have an ion in common with component 3, our Eq. (20), Eq. (21) and Eq. (23) show that an additional factor of ν_2 considerably amplifies the magnitude of $|\Gamma_{\mu_1,\mu_3} - \Gamma_{\mu_3}|$ in systems where component 2 is a highly charged polyelectrolyte. According to Eq. (21), $|\Gamma_{\mu_1,\mu_3} - \Gamma_{\mu_3}|$ also depends on a term that can be viewed as a modified 'volume fraction', in which $\bar{V}_3$ is multiplied by $(1 - m_2\Gamma_{\mu_3}/m_3)$, a factor generally not much different from unity. Moreover, this volume fraction does not depend upon any contribution from component 2, regardless of the magnitude of $m_2\bar{V}_2$ relative to $m_3\bar{V}_3 + m_1^\bullet\bar{V}_1^\bullet$. For a particular pair of solute components and a given

specification of their concentrations, the numerical significance of $|\Gamma_{\mu_1,\mu_3} - \Gamma_{\mu_3}|$ compared with $|\Gamma_{\mu_3}|$ also depends, as indicated by Eq. (3), on the magnitude of μ_{23}, generally the primary thermodynamic gauge of solute specific effects due to preferential interactions.

The model-independent expressions presented in this paper, exact or justifiably approximate, can be used to interconvert Γ_{μ_1,μ_3} and Γ_{μ_3} for two solute components of any type, over any concentration ranges. Regardless of any general or specific physical characteristics of the solute species in a three-component system that may preclude equilibrium dialysis as a means of quantifying Γ_{μ_1,μ_3}, this derivative is a well-defined thermodynamic function. At the molecular level, this coefficient gauges the accumulation or exclusion, relative to solvent water, of one solute in the vicinity of another, on the basis of the local-bulk domain model [13].

Provided that the molalities and partial molar volumes of the solute components are known, Eq. (20) and Eq. (21), in conjunction with Eq. (7) and Eq. (10a) or Eq. (10b), suffice for quantitative analyses of the requisite experimental input from VPO or ID in order to quantify Γ_{μ_1,μ_3}, as well as the other preferential interaction coefficients, for systems where equilibrium dialysis is comparatively less accurate, with regard to experimental uncertainties [15], or even strictly inapplicable.

Methods involving equilibrium dialysis are unsuitable to quantify coefficients of the type Γ_{μ_1,μ_3} not only for solute components of equivalent membrane permeability (similar molecular size) but also for aqueous solutions prepared by dissolving two binary solute components that dissociate completely into a total of four charged species, of which three would diffuse across a dialysis membrane. If such a system were subjected to dialysis, selective diffusion of two different small ions having the same charge sign would produce, at dialysis equilibrium, a solution containing *three* solute components (two diffusible, one non-diffusible) rather than the original two. In this situation, only coefficients of the types $(\partial m_3/\partial m_2)_{T,\mu_1,\mu_3,\mu_4}$ and $(\partial m_4/\partial m_2)_{T,\mu_1,\mu_3,\mu_4}$ could be directly quantified by dialysis. (The number of constraints on these derivatives is dictated by the Gibbs Phase Rule for a system that has, besides water, one non-diffusible and two diffusible binary components, labeled '3' and '4'.) Undialyzed solutions that consist of the same four solute *species*, but only *two* independent solute components, can be prepared by dissolving various amounts of either of two different sets of pairwise electroneutral binary components. In principle, VPO or ID measurements on these solutions could be analyzed with the expression derived in this paper in order to quantify the various coefficients determined by preferential interactions of the pairs of charged species for each choice of the two independent binary solute components.

The results presented in Fig. 2 for the preferential interactions of urea and NaCl show that at $m_2=2$ molal, Γ_{μ_3} and Γ_{μ_1,μ_3} differ significantly over most of the broad range of m_3 for which ID measurements are available. Analogous results calculated at other fixed salt molalities show the same qualitative pattern of substantial differences between the three different types of coefficients. In contrast to these characteristics of the preferential interactions of two small solutes, preliminary applications of the equations derived in this paper to analyze the preferential interactions of each of a wide variety of small solutes with a biomacromolecule (BSA), at concentrations in the range 1–5 molal, indicate that any differences between Γ_{μ_3} and Γ_{μ_1,μ_3} lie close to, or within, the experimental uncertainties with which their magnitudes have been determined, although both of these coefficients differ significantly from Γ_{μ_1}. Thus, for these systems exact analyses based on Eq. (20) and Eq. (21) demonstrate that the reliability of Schellman's Eq. (A10) [1] extends well beyond the limit of infinite dilution of the macromolecular component, for example, into the mmolal range of m_2 required for accurate measurements by VPO. As the thermodynamic and kinetic consequences of preferential interactions continue to be more systematically investigated for an increasingly wider variety of solutes, the generalized model-independent framework developed in this paper will provide a rigorous basis for quantifying the different types of coefficients, even when they differ significantly, and for understanding the physical origins of such differences.

Acknowledgments

References

[1] J.A. Schellman, A simple model for solvation in mixed-solvent. Applications to the stabilization and destabilization of macromolecular structures, Biophys. Chem. 37 (1–3) (1990) 121–140.

[2] E.F. Cassassa, H. Eisenberg, Thermodynamic analysis of multicomponent solutions, Adv. Protein Chem. 19 (1964) 287–395.

[3] E.P.K. Hade, C. Tanford, Isopiestic compositions as a measure of preferential interactions of macromolecules in two-component solvents. Application to proteins and concentrated aqueous cesium chloride and guanidine

hydrochloride, J. Am. Chem. Soc. 89 (1967) 5034–5044.

[4] H. Eisenberg, Biological Macromolecules and Polyelectrolytes in Solution, Clarendon, Oxford, 1976.

[5] C.F. Anderson, E.S. Courtenay, M.T. Record, Thermodynamic expressions relating different types of preferential interaction coefficients in solutions containing two solute components, J. Phys. Chem. B 106 (2002) 418–433.

[6] W. Zhang, M.W. Capp, J.P. Bond, C.F. Anderson, M.T. Record, Thermodynamic characterization of interactions of native bovine serum albumin with highly excluded (glycine betaine) and moderately accumulated (urea) solutes by a novel application of vapor pressure osmometry, Biochemistry 35 (1996) 10506–10516.

[7] E.S. Courtenay, M.W. Capp, C.F. Anderson, M.T. Record, Vapor pressure osmometry studies of osmolyte–protein interactions: implications for the action of osmoprotectants in vivo and for the interpretation of 'osmotic stress' experiments in vitro, Biochemistry 39 (2000) 4455–4471.

[8] E.S. Courtenay, M.W. Capp, M.T. Record, Thermodynamics of interactions of urea and guanidinium salts with protein surface: relationship between solute effects on protein processes and changes in water-accessible surface area, Protein Sci. 10 (2001) 2485–2497.

[9] J.E. Hearst, Determination of the dominant factors which influence the net hydration of native sodium deoxyribonucleate, Biopolymers 3 (1965) 57–62.

[10] G. Cohen, H. Eisenberg, Deoxyribonucleate solutions: sedimentation in a density gradient, partial specific volumes, density and refractive index increments, and preferential interactions, Biopolymers 6 (1968) 1077–1100.

[11] J. Wyman, Linked functions and reciprocal effects in hemoglobin: a second look, Adv. Protein Chem. 19 (1964) 223–286.

[12] C.F. Anderson, M.T. Record, Salt dependence of oligoion–polyion binding: a thermodynamic description based on preferential interaction coefficients, J. Phys. Chem. 97 (1993) 7116–7126.

[13] M.T. Record, W. Zhang, C.F. Anderson, Analysis of effects of salts and uncharged solutes on protein and nucleic acid equilibria and processes: a practical guide to recognizing and interpreting polyelectrolyte effects, Hofmeister effects, and osmotic effects of salts, Adv. Protein Chem. 51 (1998) 281–353.

[14] S.N. Timasheff, Control of protein stability and reactions by weakly interacting cosolvents: the simplicity of the complicated, Adv. Protein Chem. 51 (1998) 355–433.

[15] H. Eisenberg, Protein and nucleic acid hydration and cosolvent interactions: establishment of reliable baseline values at high cosolvent concentrations, Biophys. Chem. 53 (1994) 57–68.

[16] V.E. Bower, R.A. Robinson, The thermodynamics of the ternary system: urea–sodium chloride–water at 25 degrees, J. Phys. Chem. 67 (1963) 1524–1527.

[17] R. Bhat, S.N. Timasheff, Steric exclusion is the principal source of the preferential hydration of proteins in the presence of polyethylene glycols, Protein Sci. 1 (1992) 1133–1143.

[18] J.K Myers, C.N. Pace, J.M. Scholtz, Denaturant m-values and heat capacity changes: relation to changes in accessible surface areas of protein unfolding, Protein Sci. 4 (1995) 2138–2148.

[19] E.S. Courtenay, M.W. Capp, R.M. Saecker, M.T. Record, Thermodynamic analysis of interactions between denaturants and protein surface exposed on unfolding: interpretation of urea and guanidinium chloride m-values and their correlation with changes in accessible surface area (ASA) using preferential interaction coefficients and the local-bulk domain model, PROTEINS, Struct. Funct. Genet. 41 (S4) (2001) 72–85.

[20] S. Ghaemmaghami, T.G. Oas, Quantitative protein stability measurement in vivo, Nat. Struct. Biol. 8 (2001) 879–882.

[21] G. Scatchard, W.J. Hamer, S.E. Wood, Isotonic solutions. I. The chemical potential of water in aqueous solutions of sodium chloride, potassium chloride, sulfuric acid, sucrose, urea, and glycerol at 25 °C, J. Am. Chem. Soc. 60 (1938) 3061–3070.

[22] R.A. Robinson, R.H. Stokes, Electrolyte Solutions, Butterworths Scientific Publications, London, 1959.

[23] D. Stigter, Interactions in aqueous solutions. IV. Light scattering of colloidal electrolytes, J. Phys. Chem. 64 (1960) 842–846.