Biophysical Chemistry 112 (2004) 165–175

Biophysical Chemistry

www.elsevier.com/locate/bpc

Gibbs–Duhem-based relationships among derivatives expressing the concentration dependences of selected chemical potentials for a multicomponent system

Charles F. Anderson[a], M. Thomas Record Jr.[a,b,c,*]

[a]Department of Chemistry, University of Wisconsin-Madison, Madison, WI 53706, United States
[b]John D. Ferry Professor of Chemistry and Biochemistry
[c]Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706, United States

Received 29 June 2004; accepted 12 July 2004

Abstract

For a two-component system, a derivative that specifies the concentration-dependence of one chemical potential can be calculated from the corresponding derivative of the other chemical potential by applying the Gibbs–Duhem Equation. To extend the practical utility of this binary thermodynamic linkage to systems having any number of components, we present a derivation based on a previously unrecognized recursive relationship. Thus, for each independently variable component, κ, any derivative of its chemical potential, μ_κ, with respect to one of the mole ratios $\{m_\kappa \equiv n_\kappa/n_\omega\}$ is related to a characteristic series of progressively higher order derivatives of μ_ω for a single "probe" component, ω, with respect to certain of the $\{m_\kappa\}$. For aqueous solutions in which ω is solvent water and one or more of the solutes (κ) is dilute, under typical conditions each sum of terms expressing a derivative of μ_κ consists of at most a few numerically significant contributions, which can be quantified, or at least estimated, by analyzing osmometric data to determine how the single chemical potential μ_ω depends on the $\{m_\kappa\}$ without neglecting any significant contributions from the other components. Expressions derived here also will provide explicit criteria for testing various approximations built into alternative analytic strategies for quantifying derivatives that specify the $\{m_\kappa\}$ dependences of μ_κ for selected components. Certain quotients of these derivatives are of particular interest in so far as they gauge important thermodynamic effects due to "preferential interactions".

Keywords: Gibbs–Duhem; Preferential interactions; Multicomponent solutions; Thermodynamic nonideality; Osmotic pressure; Vapor pressure osmometry

As one of the founding fathers of modern polymer science, John Ferry should be remembered not only for the large number and widespread impact of his pioneering contributions, but also for the breadth of his interests, the depth of his scientific intuition, and his innovative ability to make significant advances by applying a diverse battery of experimental techniques to well-chosen systems. Surveying his hundreds of papers, published over a span of six decades, one finds some striking instances that show how well he understood the value of thermodynamic information, although it rarely was the primary target of his own research.

Toward the beginning of John Ferry's tenure at the University of Wisconsin, he reported, with G.V. Browning, the first osmotic pressure measurements for moderately concentrated solutions of a polar polymer in two different polar (nonaqueous) solvents [1]. This study was motivated originally by observations that the steady flow viscosity and other mechanical properties of a polymer, such as poly(vinyl acetate), exhibit differences attributable to its interactions with different solvents. Additional physical implications were recognized more than 30 years later. Shortly after DesCloiseaux and DeGennes introduced their "scaling"

---

[*] Corresponding author. Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Dr., Madison, WI 53706, United States. Tel.: +1 608 2623019; fax: +1 608 2623453.

E-mail address: record@biochem.wisc.edu (M.T. Record).

0301-4622/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.bpc.2004.07.016

theory, John Ferry showed that Browning's measurements constituted the first direct experimental confirmation, over an appreciable concentration range, of the 9/4 power law dependence predicted for the osmotic pressure of a "semidilute" polymer solution [2].

During John Ferry's last two decades at this University, we often benefited from his participation in seminars, as well as private consultations, which repeatedly demonstrated his awareness of the power of thermodynamics and his longstanding interest in the wide variety of phenomena that exhibit sensitivity to the interactions of a polymer (including those in biological environments) with "solvent" (including dissolved solutes of similarly small size). Such effects had been a recurrent theme throughout the dozens of classic papers that he published (ca. 1949–1957) on the conversion of fibrinogen to fibrin (e.g., Ref. [3]). Thus, for various reasons, we believe that John Ferry would have appreciated the objectives of this article, which is dedicated to honor his preeminent scientific career.

In this laboratory, we use various thermodynamic and spectroscopic measurements to investigate processes involving proteins, nucleic acids, and smaller biologically relevant solutes in aqueous solution [4]. The results of such studies elucidate the biofunctional roles of structural phenomena, such as changes in solvent-accessible surface area [5,6] or in excluded volume due to "crowding" agents [7]. To characterize these effects systematically, we analyze thermodynamic derivatives (usually) called "preferential interaction coefficients" [8–10]. Their signs and magnitudes, determined for each system by differences among all the various solute–solute and solute–solvent interactions, can be interpreted with regard to molecular factors that govern solute exclusion from, or accumulation in, the domain of solvation that surrounds a macromolecule [11,12].

Most experimental determinations of preferential interaction coefficients so far have been reported for aqueous solutions that contain (or are treated as if containing) only one macromolecular and one smaller solute component. To quantify these thermodynamic functions for systems comprised of more than three components, our laboratory is developing various experimental and analytic strategies for acquiring and processing data accessible by water vapor pressure osmometry [13–15]. Besides providing quantitative information about particular systems, such studies also will serve to establish how a large and steadily growing database can be utilized directly, appropriately adapted, or sufficiently supplemented to arrive at conclusions about preferential interactions under conditions representative of actual biological environments, which typically include a broad diversity of chemically distinct species.

For systems containing biological macromolecules and smaller (also involatile) solutes, typically only one chemical potential, that of solvent water, can be monitored directly (by vapor pressure osmometry) or fixed (by isopiestic distillation), as solute concentrations are varied over a significant range. But information about the concentration dependences of this single chemical potential can suffice, as input for expressions derived for the first time in this paper, to quantify derivatives that specify the concentration dependences of the chemical potential of any other independently variable component of the system. Our derivation also provides a new basis for testing various alternative approximations [9,13–16], whereby osmometric measurements on a multicomponent system, and on simpler subsystems (containing fewer constituents but corresponding in all other respects), are related to quantities that can be interpreted directly in terms of specific model assumptions, or parametric correlations for homologous series of systems, with the ultimate objective of understanding the molecular origins and implications of thermodynamic effects due to preferential interactions [4–6,12–15].

Preferential interaction coefficients can be represented in terms of certain derivatives of chemical potentials that, although not directly accessible to experimental determination, are rigorously related, via the Gibbs–Duhem Equation, to corresponding functions that can be quantified experimentally [8–10]. Essential background on this thermodynamic linkage is summarized in the next section, which also serves to introduce symbols, nomenclature, and some usages that may not be universally familiar. On this basis, we proceed to delineate more precisely the scope and motivation of this article. In the following sections, we define a "composite" component in order to reduce the number of explicit terms in the Gibbs–Duhem Equation for a multicomponent system to the minimum needed for our general derivation; then, we introduce equalities based on Euler Reciprocity, in order to minimize the number of independent higher order partial derivatives, with respect to appropriately chosen concentration variables, that must be considered to complete the derivation. After introducing a recursive relationship based on the Gibbs–Duhem Equation in order to derive expressions that link derivatives of corresponding partial molal functions, we contrast this analytic strategy with the only previously available alternatives and indicate future applications forthcoming from this laboratory.

1. Gibbs–Duhem linkage of changes in partial molal functions

For a system comprised of σ chemically distinct species (either ions or net neutral molecules), any extensive state function Y (such as volume or the Gibbs Free Energy, G) can be expressed, in accordance with Euler's theorem for first-order homogeneous functions [17], as a mole-number-weighted linear combination of σ "partial molal functions", each defined as a partial derivative of Y with respect to the mole number of one of the constituent species:

$$\bar{Y}_i = (\partial Y/\partial n_i')_{T,P,\{n_{i'\neq i}\}} \tag{1}$$

As a mathematical consequence of fundamental thermodynamic principles, the existence of each of the $\bar{Y}_i$ is guaranteed even for state functions like Y=G that cannot be measured and for a charged solute, although its mole number cannot be varied by a macroscopic increment (in the absence of an external electric field) while all the other $\{n_i\}$ are held fixed.

From the additivity of the complete set of σ functions $\{\bar{Y}_i\}$ and the total differential of Y represented as a linear combination of differentials of the σ mole numbers $\{n_i\}$ at constant T and P, follows the "generalized" Gibbs–Duhem Equation (so-called because Y can be a function other than G). This purely thermodynamic relationship specifies the linkage among changes in all the $\{\bar{Y}_i\}$ that result from a change in any of the $\{n_i\}$:

$$0 = \sum_i n_i \mathrm{d}\bar{Y}_i \tag{2}$$

If Y=G, then $\bar{Y}_i$=μ_i, the chemical potential of each species i, and Eq. (2) becomes the ordinary (isothermal, isobaric) Gibbs–Duhem Equation. For our derivation, expressions are presented in terms of derivatives of $\bar{Y}_i$ rather than μ_i, not only to indicate general applicability to any type of partial molal function, but also because ambiguities or unnecessary complications could be introduced by adopting and extending the conventional asymmetric notation for the first derivative of a chemical potential with respect to a mole ratio.

Each of the $\bar{Y}_i$ is an intensive function of the thermodynamic state of the system, which is determined at a given T and P by a set of $\sigma-1$ intensive concentration variables. For these, we choose the mole ratios, $\{m_i \equiv n_i/n_\omega\}$, rather than (for example) the corresponding molarities, so that equalities based on Euler Reciprocity (as explained in a following section) can be used to simplify profoundly the subsequent derivation. According to the most common (but not obligatory) specification, the mole ratio divisor, n_ω, pertains to the most abundant component—typically solvent water. In quantitative studies of thermodynamic effects due to interactions among solutes, their concentrations usually are expressed as "molalities", with units of moles per kilogram of (pure) water. In this paper, various expressions are simplified by defining m_i as the dimensionless mole ratio of species i, rather than the corresponding molality (which differs merely by a constant factor). Units assigned to the m_i would cancel anyway in the principal relationship derived here.

In many systems, the m_i of some of the σ species cannot be varied individually by any macroscopically significant increment, for example, because they are reactants or products of some unsaturated stoichiometric process, or because overall electroneutrality must be maintained. To allow for this constraint in analyzing thermodynamic effects due to changes in composition, two or more of the charged species are grouped together as a "component". (In general numerous alternative groupings are possible, but typically a single choice is clearly indicated by the experimental situation). Such components consist of an electroneutral combination of dissociated species, in fixed proportion(s) determined by their respective charges [17].

By introducing appropriate definitions of the mole number and partial molal function pertaining to each electroneutral component, κ, the number of terms in Eq. (2) is reduced to include only those for which the n_κ actually could be varied independently (in some feasible series of sample solution preparations). In this paper, we introduce formally analogous definitions for a "composite" component, ζ, again for the purpose of simplifying the multicomponent Gibbs–Duhem Equation. Before considering in detail how and why n_ζ and $\bar{Y}_\zeta$ are defined for the composite component, we outline in the next section the range of applicability of the expressions to be derived in this paper and the primary motivation for such applications.

2. Scope of the Gibbs–Duhem-based analysis

Expressions derived in this paper pertain to systems comprised of $\chi(\geq 3)$ components, which consist, in general, of various molecular species bearing no (net) charge and/or electroneutral combinations of two (or more) dissociated charged species. For each of these individual components, κ, the mole number n_κ is independently variable. To accomplish the objective of deriving relationships among different derivatives of $\bar{Y}_\kappa$, we need to consider variations in the relative proportions of different components, but not in their absolute amounts. Consequently, our derivation does not entail (for example) the powerful matrix methods that have been developed [18] to specify the conservation of molecular material in calculations of the populations of different species present when multiple (unsaturated) reactions are equilibrated. In such "reactive" multicomponent systems, the n_κ can be controlled (fixed or varied) for certain sums of reactants and products, but (generally) not for the individual stoichiometric participants in a process at equilibrium. The resulting reduction in "degrees of freedom" can be readily taken into account in formulating the Gibbs–Duhem Equation for derivatives of $\bar{Y}_i$=μ_i by grouping together, for each process at equilibrium, the reactant and product species as a composite component and specifying its concentration as an independently variable mole ratio. For such "reactive" systems, analogous (but more complicated) Gibbs–Duhem-based expressions can be derived for derivatives of partial molal functions other than the $\{\mu_i\}$.

For applications to (by far) the most common situation, we suppose that one and only one "probe" component, ω, can be monitored directly by some experimental means in order to determine $\bar{Y}_\omega$ accurately as a function of an appropriately chosen set of independently variable mole ratios $\{m_\kappa \equiv n_\kappa/n_\omega\}$. Specifically, with regard to the analyses of osmometric measurements, $\bar{Y}_\omega$ is proportional to the logarithm of the activity of solvent water, which is directly measurable, by commercially available instrumentation, as the “osmolality” of any solution in which the total molality of all solute species is less than ~4 mol/kg [13]. For more concentrated solutions, the $\{m_\kappa\}$ dependences of $\bar{Y}_\omega$ can be determined, less directly, by global fitting of data obtained by quantifying the solute concentrations in a series of solutions equilibrated by isopiestic distillation [15,19].

For aqueous solutions containing two or more solutes, or any other such homogeneous multicomponent systems, our primary objective in this paper is to derive expressions that require as input information about the $\{m_\kappa\}$ dependences of $\bar{Y}_\omega$ for just one of the components in order to determine, for any other selected pair (α and β), the three independent first partial derivatives that specify how $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ depend on m_α and m_β. Interest in such derivatives is motivated primarily because for Y=G, certain quotients of such derivatives are “preferential interaction” coefficients [8–10] that express how concentration-driven changes in the chemical potentials μ_α and μ_β are correlated under the standard experimental constraints of constant T and P. These coefficients have significant molecular implications that can be interpreted in terms of particular model assumptions [11,12]; they also play a crucial role (first pointed out by Wyman [20]) in model-independent analyses of thermodynamic measurements that characterize an “unsaturated” process driven toward reactant(s) or product(s) by changing the concentration(s) of some “perturbing” solute(s) not among the direct stoichiometric participants [4,21].

3. Assigning components

To derive tractable expressions for solutions having any number of components, we first reduce the number of terms in Eq. (2) by grouping together, as the “composite” component, ζ, all species other than those comprising the probe, ω, and a selected pair of ordinary “individual” components, α and β. Thus, the constituents of ζ for which the relative proportions can be controlled experimentally consist of $\chi-3$ components. In general, some of these may be electroneutral combinations of charged species, and one or more of the ionic species included in ζ may also be constituents of the components α and/or β. The definitions of the mole number n_ζ and partial molal function $\bar{Y}_\zeta$ for the composite component are analogous to those conventionally adopted for a strong electrolyte with respect to its ionic constituents [17], except that the mole number of each component comprising ζ actually could be varied individually without changing the mole number of any other component.

Our ultimate objectives in this paper are Gibbs–Duhem-based expressions for partial derivatives of $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ subject to constraints that fix the mole ratios of all the components comprising ζ and hence their relative proportions. Consequently, a single mole number, n_ζ, suffices to specify the amount of the composite component, and it can be equated to n_λ for any component, λ, among those comprising ζ. If, for example, the least abundant of these is chosen as λ, then for every other constituent of ζ, $n_{\lambda'}$ is proportional to n_ζ, with a proportionality constant $\nu_{\lambda'} \equiv n_{\lambda'}/n_\lambda$ greater than unity. The numerical value of each of the (quasi-stoichiometric) $\{\nu_{\lambda'}\}$ is determined unambiguously, not by a physical constraint such as electroneutrality but rather by the particular composition of the system under investigation.

Although n_ζ and n_λ are by definition numerically identical, they are not in general functionally equivalent, for example, as differentiating variables for the purpose of defining partial molal functions. Any change in n_ζ would not only change n_λ but also produce a proportionate change in the mole number of every other constituent of ζ. Hence, the partial molal derivative of Y with respect to n_ζ, defined by analogy to Eq. (1), can be expressed as the following linear combination, in which each partial molal function pertaining to a constituent of ζ is weighted by $\nu_{\lambda'}$, its (fixed) mole ratio with respect to n_λ:

$$\bar{Y}_\zeta \equiv \bar{Y}_\lambda + \sum_{\lambda'} \nu_{\lambda'} \bar{Y}_{\lambda'} \tag{3}$$

According to Eq. (3), for any change in composition that preserves the constancy of the $\{\nu_{\lambda'}\}$, the number of terms in Eq. (2) is reduced from σ to four, and the number of independently variable mole ratios is reduced from $\chi-1$ to three:

$$\mathrm{d}\Omega = m_\alpha \mathrm{d}\bar{Y}_\alpha + m_\beta \mathrm{d}\bar{Y}_\beta + m_\zeta \mathrm{d}\bar{Y}_\zeta \tag{4}$$

To emphasize the (supposed) unique experimental accessibility of $\bar{Y}_\omega$, the symbol $\Omega \equiv -\bar{Y}_\omega$ is introduced in Eq. (4). Like the minus sign, a factor of 55.5 mol/kg also would be subsumed into the definition of Ω for aqueous solutions if the solute concentrations were specified as molalities with the conventional units.

For a given choice of α, β, and the $\{\nu_{\lambda'}\}$ in Eq. (3), each derivative specifying how $\bar{Y}_\zeta$ depends on m_κ for κ=α or β is actually a superposition of contributions from the various constituents of ζ. Any of these individual contributions could be evaluated separately, on the basis of Eq. (4), by interchanging one or more of the constituents originally included in ζ for one or both of those initially selected as α and β. This pair of individual components can be chosen in any of $(\chi-1)(\chi-2)/2$ distinct ways for the designated probe, ω. In fact, for many solutions containing biological solutes, the concentration dependences of the $\{\bar{Y}_\kappa\}$ are not all of equivalent physical significance. Moreover, buffers, supporting electrolytes, etc., may be present at low enough concentrations so that they do not affect the magnitudes of derivatives that gauge the preferential interactions of biopolymers and smaller biologically significant solutes.

Even when the concentration dependences of some of the $\bar{Y}_\kappa$ for a multicomponent system do not merit individualized attention, values of derivatives of $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ for the selected pair may in some situations be affected by the presence of the less-interesting components, as a consequence of the linkage required by Eq. (4). Fortunately, any such nonnegligible effects due collectively to some or all of the constituents of ζ are taken rigorously (in effect, automatically) into account in the expressions that we derive by introducing the Gibbs–Duhem recursion. Thus, when this approach is applied to evaluate derivatives of $\bar{Y}_\alpha$ and $\bar{Y}_\beta$, the analysis is streamlined in so far as it does not require separate evaluations of corresponding derivatives specifying the concentration dependences of all the various constituents of ζ.

The differentiation of one function with respect to another, subject to constraints on every other relevant variable, is inherently a binary operation. Consequently, the dependence of either $\bar{Y}_\alpha$ or $\bar{Y}_\beta$ on either m_α or m_β can be analyzed for any pair of individual components by including in Eq. (4) only two explicit individual components in addition to ω. Because the derivatives of $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ could be examined sequentially for every possible designation of the components α and β, the modular character of such analyses ensures that the reduced Gibbs–Duhem Equation from which the requisite expressions are derived need not consist of more than four explicit terms regardless of the total number of independently variable components in the system.

The number of explicit terms in Eq. (4) could be further reduced to three by including β among the constituents of ζ. However, this degree of simplification would restrict the expressions derived to derivatives of $\bar{Y}_\alpha$ or $\bar{Y}_\zeta$ with respect to m_α or m_ζ. In this situation, $\bar{Y}_\zeta$ is a superposition of contributions from at least two individual components, and information about its derivatives (even in conjunction with corresponding information about $\bar{Y}_\alpha$) would not suffice, for example, to determine any isobaric preferential interaction coefficient that can be represented as a quotient of derivatives of the chemical potentials of two individual components with respect to the mole ratio of one of them [8–10].

4. Applying Euler Reciprocity

Our ultimate objective is to utilize Eq. (4) as a basis for deriving expressions whereby any of the derivatives of $\bar{Y}_\kappa$ for $\kappa=\alpha$, β, or ζ can be related to higher order derivatives of the directly observable Ω. Although $\bar{Y}_\zeta$ may lack any particular physical significance, it must be considered as an intermediate variable en route to the objective of deriving expressions for derivatives of $\bar{Y}_\alpha$ and $\bar{Y}_\beta$. The number of independent terms entering into the derivation of these expressions can be minimized by considering some purely mathematical characteristics of derivatives of partial molal functions.

Each of the $\{\bar{Y}_\kappa\}$ is defined as a derivative (according to Eq. (1)) or a combination of derivatives (according to Eq. (3)) with respect to a mole number. The resulting state functions however are intensive, and therefore their dependences on the composition of the system are analyzed most economically in terms of intensive variables. For this purpose, each of the partial molal functions that appear explicitly in Eq. (4), except Ω, is represented alternatively as a partial derivative, with respect to $m_\kappa \equiv n_\kappa/n_\omega$, of the intensive function $I \equiv Y/n_\omega$:

$$\bar{Y}_\kappa = \left(\partial I/\partial m_\kappa\right)_{T,P,\{m_{\kappa \neq \kappa'}\}} \tag{5}$$

Conversion of Eq. (1) (or Eq. (3)) to Eq. (5) follows from the same general mathematical characteristics of thermodynamic state functions for an isobaric system that lead to Eq. (2). (Derivation of the total differential representation of I as a linear combination of differentials of the $\{m_\kappa\}$ does not require that n_ω be fixed).

Because $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ each can be represented as a derivative of I in accordance with Eq. (5), the cross partial derivatives of I with respect to m_β and m_α are equal as a consequence of Euler Reciprocity :

$$\left(\partial \bar{Y}_\alpha/\partial m_\beta\right)_{T,P,\{m_{i\neq\beta}\}} = \left(\partial \bar{Y}_\beta/\partial m_\alpha\right)_{T,P,\{m_{i\neq\alpha}\}} \tag{6}$$

Expressions analogous to Eq. (6) pertain to derivatives of $\bar{Y}_\zeta$ for the composite component. Thus, for example, the partial derivative that expresses how all of the $\bar{Y}_\iota$ comprising $\bar{Y}_\zeta$ (in Eq. (3)) change as a result of changing the mole ratio of the single component, α, must be equal, by Euler Reciprocity, to the partial derivative that expresses the change in $\bar{Y}_\alpha$ for the single component, α, caused by simultaneous changes, in fixed proportions, of the mole ratios of all the constituents of ζ.

The principle of Euler Reciprocity also is readily extended to show that the value of any higher order mixed partial derivative of I does not depend on the order in which the differentiations are performed with respect to m_α, m_β, and/or m_ζ. Consequently, the number of such derivatives can be decreased to an irreducible minimum by introducing equalities based on Euler Reciprocity, which therefore substantially simplifies the derivation that follows from Eq. (4). Before proceeding to derive the resulting expressions, we indicate in the next section more precisely how the applicability of Euler Reciprocity determines the optimal choice of concentration variables for the purpose of analyzing systems subject to the most common thermodynamic constraints.

5. Choosing concentration variables

Eq. (6) is valid for the partial molal functions of every pair of the χ independently variable components that does not include ω. No partial derivative of $\bar{Y}_\omega$ with respect to a mole ratio of the type $m_\kappa \equiv n_\kappa/n_\omega$ can be equated on the basis of Euler Reciprocity to a corresponding single derivative of any of the $\bar{Y}_\kappa$, because no relationship analogous to Eq. (5) can be derived to express $\bar{Y}_\omega$ alternatively as a single derivative of $I=Y/n_\omega$. Only for mole ratios specified with some divisor other than n_ω can simple equalities analogous to Eqs. (5) and (6) be derived in order to relate a derivative of $\bar{Y}_\alpha$ or $\bar{Y}_\beta$ to a corresponding single "cross" partial derivative of the directly observable$\bar{Y}_\omega$ (such as Eq. (5) in Ref. [22]). However, the resulting expressions are equivalent to particular combinations of derivatives that express how $\bar{Y}_\alpha$ or $\bar{Y}_\beta$ depends on a mole ratio of the type $m_\kappa \equiv n_\kappa/n_\omega$. These concentration variables are of primary interest because they are proportional to the conventional molalities that almost always are specified when the concentration dependences of thermodynamic properties of solutions are investigated. To evaluate separately individual derivatives of any of the $\bar{Y}_\kappa$ with respect to a mole ratio of the type $m_\kappa \equiv n_\kappa/n_\omega$, given only corresponding information about the concentration dependences of $\bar{Y}_\omega$, we introduce the Gibbs–Duhem-based approach in the following section.

Eq. (6) and its generalized analogs minimize the number of independent higher order mixed partial derivatives that enter explicitly into our derivation of expressions for derivatives of partial molal functions with respect to mole ratios of the type $m_\kappa \equiv n_\kappa/n_\omega$. These simplifications based on Euler Reciprocity strongly motivate the use of mole ratios (or the equivalent molalities) as concentration variables, although from an experimental standpoint, molarities may be more readily quantifiable because of the volumetric (micropipetting) procedures routinely used for the preparation of sample solutions, especially those containing biopolymers. Moreover, particle densities, expressed per unit volume, are the natural concentration variables for any analytic theory of thermodynamic nonideality based on interaction potentials expressed as functions of spatial coordinates.

Unfortunately, in view of the experimental and theoretical advantages of molarities, substituting n_κ/V for each of the corresponding n_κ/n_ω for the purpose of analyzing the concentration dependences of partial molal functions does not produce relationships of simplicity comparable to Eqs. (5) and (6). No intensive state function analogous to I has a differential that can be represented as a simple linear combination of differentials of the molarities at constant pressure. Therefore, isobaric partial derivatives $(\partial \bar{Y}_\alpha/\partial C_\beta)_{T,P,\{C_{i\neq\beta}\}}$ and $(\partial \bar{Y}_\beta/\partial C_\alpha)_{T,P,\{C_{i\neq\alpha}\}}$ are not equal, for any two solutes having significantly different partial molal volumes, even as both of their concentrations approach infinite dilution. In this limit, molalities and molarities become numerically identical (albeit dimensionally distinct) because by definition one gram of pure water occupies one cubic centimeter at standard temperature and pressure. Nevertheless, even when none of the C_κ differs significantly in numerical magnitude from the corresponding m_κ, molalities and molarities still are not functionally equivalent as variables or constraints for the partial derivatives that are the objectives of our derivation.

The preceding comments indicate why our Gibbs–Duhem-based derivation would become intractably complicated if the partial molal functions were analyzed as functions of solute molarities for isobaric systems. However, with molarities as concentration variables simple equalities analogous to Eqs. (5) and (6) can be derived for a multicomponent liquid solution equilibrated by dialysis. Elsewhere we will show how to modify Eqs. (4)–(6), and hence the derivation presented in the following section to arrive at expressions that can be used to analyze experimental measurements or theoretical predictions of derivatives that specify the concentration dependences of the osmotic pressure acting on a dialyzed solution.

6. Introducing Gibbs–Duhem recursion

On the basis of the simplifications considered in preceding sections, we are now in a position to present the most efficient derivation of expressions that relate experimentally accessible derivatives of $\bar{Y}_\omega$ (or Ω in Eq. (4)) to individual derivatives specifying the concentration dependences of $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ for any pair of the other independently variable components. Together with the cross-partial derivative for which Eq. (6) shows equivalent forms, two independent "diagonal" partial derivatives, $(\partial \bar{Y}_\alpha/\partial m_\alpha)_{T,P,\{m_{i\neq\alpha}\}}$ and $(\partial \bar{Y}_\beta/\partial m_\beta)_{T,P,\{m_{i\neq\beta}\}}$, suffice to characterize the variations in $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ caused by changing m_α or m_β while m_ζ is fixed. Appropriate quotients of these derivatives express correlations between changes in $\bar{Y}_\alpha$ and $\bar{Y}_\beta$. For example, the coupling of these partial molal functions produced by a change in (only) m_α can be expressed by $(\partial \bar{Y}_\alpha/\partial \bar{Y}_\beta)_{T,P,\{m_{i\neq\alpha}\}}$. If $Y=G$, this derivative is a generalized type of isobaric preferential interaction coefficient, which could be used, for example, to analyze solute-concentration-dependent effects on a process at equilibrium in a system at constant T and P containing any number of components [21].

Our Gibbs–Duhem-based derivation necessarily considers various higher order mixed partial derivatives of Ω and of I with respect to the three independently variable mole ratios $\{m_\kappa\}$ in Eq. (4). As a more compact notation for such derivatives, ∂_{abz} is placed before the function differentiated. The subscripts a, b, and z on ∂ are nonnegative integers that specify, in the order given, the number of partial differentiations with respect to m_α, m_β, and m_ζ at constant T and P. For any mixed partial derivative designated by ∂_{abz}, the overall order is $(a+b+z)\equiv q$. As noted above, a straightforward generalization of Euler Reciprocity guarantees that multiple differentiations with respect to the three independently variable m_κ can be performed in any sequence without affecting the value of the resulting derivative. Nevertheless, the order of the subscripts on ∂ cannot be permuted without changing the physical meaning of the symbol, and hence, in general, the value of the derivative specified. For example, $\bar{Y}_\alpha \equiv \partial_{100} I \neq \bar{Y}_\beta \equiv \partial_{010} I \neq \bar{Y}_\zeta \equiv \partial_{001} I$.

From the Gibbs–Duhem linkage of differentials given by Eq. (4) follow three equations that relate the three first partial derivatives of Ω, which can be evaluated directly by analyzing experimental input, to the six independent second partial derivatives of I, none of which (presumably) can be evaluated by direct measurement:

$$\partial_{100}\Omega = m_\alpha \partial_{200} I + m_\beta \partial_{110} I + m_\zeta \partial_{101} I \tag{7a}$$

$$\partial_{010}\Omega = m_\alpha \partial_{110} I + m_\beta \partial_{020} I + m_\zeta \partial_{011} I \tag{7b}$$

$$\partial_{001}\Omega = m_\alpha \partial_{101} I + m_\beta \partial_{011} I + m_\zeta \partial_{002} I \tag{7c}$$

For a system comprised of only three components, these equations are decoupled by setting m_ζ=0 and deleting the third subscript on ∂. In the resulting simplified forms of Eqs. (7a) and (7b), only three of the six unknown functions remain. But even for systems comprised of more than three components, the corresponding derivatives ($\partial_{200} I$, $\partial_{110} I$, and $\partial_{020} I$) suffice to specify completely the dependences of $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ on m_α and m_β at any particular fixed value of m_ζ, regardless of the total number of independently variable components included in ζ.

We proceed to show how each first partial derivative of $\bar{Y}_\alpha$ or $\bar{Y}_\beta$ can be expressed in terms of derivatives of Ω, without concomitantly solving for (or approximating) any of the three remaining unknowns in Eqs. (7a), (7b), and (7c) ($\partial_{101} I$, $\partial_{011} I$, and $\partial_{002} I$), each of which is a different first derivative of the composite partial molal function $\bar{Y}_\zeta$. Such derivatives are superpositions of contributions from different components and as such lack, in general, any simply interpretable physical significance. Nevertheless, derivatives of $\bar{Y}_\zeta$ cannot simply be ignored a priori, because of the linkage, via Eq. (4), to the derivatives of $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ that are the objectives of the derivation. This analysis is focused on derivatives of the chosen pair of components, α and β, by grouping all the rest (other than ω) together as constituents of the composite component ζ, as explained in reference to Eq. (3). Successive differentiations of Eqs. (7a), (7b), and (7c) with respect to each of the three independent mole ratios reveal a pattern of linkages among certain families of progressively higher order partial derivatives of Ω and I that conform to a simple recursive relationship. Thus, for any specification of a, b, and z, such that $q \geq 1$:

$$\begin{aligned}\partial_{abz}\Omega = (q-1)\partial_{abz} I + m_\alpha \partial_{(a+1)bz} I + m_\beta \partial_{a(b+1)z} I \\ + m_\zeta \partial_{ab(z+1)} I\end{aligned} \tag{8}$$

Multiplication of Eq. (8) by $m_\alpha^a\ m_\beta^b\ m_\zeta^z$ weights each of the five terms by factors of the three mole ratios, each raised to the power that corresponds exactly to the number of differentiations specified by the respective subscript on ∂. Recognizing this exact correspondence, the notation $[abz]$ is introduced to denote $(m_\alpha^a\ m_\beta^b\ m_\zeta^z)\ \partial_{\mathrm{abz}}$, as applied to either I or Ω in Eq. (8).Then rearrangement of the resulting terms shows that any partial derivative of I of order $q \geq 2$ is proportional to the same partial derivative of Ω, minus the simple sum of all (three) of the next higher (q+1) order partial derivatives of I:

$$\begin{aligned}[abz]I = (q-1)^{-1}([abz]\Omega - [(a+1)bz]I - [a(b+1)z]I \\ - [ab(z+1)]I)\end{aligned} \tag{9}$$

Eq. (9) is valid for every specification of a, b, and z such that $q \geq 2$, given that the functional dependence of Ω on m_α, m_β, and m_ζ is physically realistic (nondivergent) over the ranges of these concentration variables where measurements actually can be made. Consequently, any selected second (or higher)-order partial derivative of I can be related to second and higher order partial derivatives of Ω for the single component, ω, by successive applications of Eq. (9). The resulting sum of terms for a derivative of the type $[abz]I$ begins with $[abz]\Omega$ and continues with groups of progressively higher order partial derivatives of Ω, each weighted by the corresponding product of powers of m_α, m_β, and/or m_ζ.

Table 1 presents the lowest order terms for each of the three derivatives that specify the dependences of $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ on m_α and m_β at any fixed m_ζ. These expressions are obtained by applying Eq. (9) successively to generate derivatives of Ω through the fourth order (q=4). In each case, the lowest order (q=2) derivative of Ω has the same specification of differentiating mole ratios as does the derivative of I related by Eq. (9) to the entire series. Thus, $[110]I$ is approximated to first order by $[110]\Omega$, which differs, in general, from the derivative [11] Ω for the corresponding three-component subsystem in which m_ζ =0. With regard to the higher order derivatives of Ω, as q increases ($\geq$3) the expressions for $[200]I$ and $[020]I$ begin to have more terms in common, but at each q the expressions for both of these derivatives have more terms in common with $[110]I$ than with each other.

The patterns exhibited by the truncated series appearing in Table 1 conform to the following general prescription. For each order of differentiation $q \geq 2$, the terms consist of a trinomial collection of $q(q-1)/2$ partial derivatives of Ω, all divided by $(q-1)!$. Each of these derivatives, weighted by the numerical coefficient appropriate for a trinomial expansion, also is multiplied by the product $(m_\alpha^a\ m_\beta^b\ m_\zeta^z)$, where the exponents on the mole ratios correspond to the subscripts specified on ∂_{abz}. Analogous statements pertain

Table 1

Expressions for the three independent first partial derivatives of partial molal functions $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ in terms of derivatives of Ω through fourth-order in the series generated by Gibbs–Duhem recursion

$[110]I$=$[110]\Omega-([210]\Omega+[120]\Omega+[111]\Omega)/2$ $+([220]\Omega+[211]\Omega+[121]\Omega)/3+([310]\Omega+[130]\Omega+[112]\Omega)/6-\ldots$
$[200]I$=$[200]\Omega-([300]\Omega+[210]\Omega+[201]\Omega)/2$ $+([310]\Omega+[301]\Omega+[211]\Omega)/3+([400]\Omega+[220]\Omega+[202]\Omega)/6-\ldots$
$[020]I$=$[020]\Omega-([120]\Omega+[030]\Omega+[021]\Omega)/2$ $+([130]\Omega+[031]\Omega+[121]\Omega)/3+([220]\Omega+[040]\Omega+[022]\Omega)/6-\ldots$

to a system containing just the three components α, β, and ω, except that in this case, the binomial expansion dictates, for a given order q, the total number $(q-1)$ of distinct derivatives of Ω and specifies the numerical coefficients that weight each of these derivatives.

7. Theoretical and practical determinants of applicability

Only the most general mathematical characteristics of extensive thermodynamic state functions are inherent in our derivation of Eq. (9). The resulting expressions for derivatives of $\bar{Y}_\alpha$ or $\bar{Y}_\beta$, such as those shown in Table 1, are applicable to systems for which all derivatives of $\Omega(\{m_\kappa\})$ are sufficiently well-behaved over the range of solute concentrations accessible to measurements so that the trinomial collection of qth-order derivatives, being divided by $(q-1)!$ becomes negligibly small as q goes to infinity. This criterion generally is satisfied if the functional form of $\Omega(\{m_\kappa\})$ can be expressed as a finite sum of products of integral powers of the mole ratios pertaining to the various independently variable components.

For a liquid solution containing two or more uncharged solutes, osmotic pressure (Π), hence osmolality (after applying the requisite thermodynamic relationship) can be represented, on the basis of the MacMillan–Mayer Theory [23], as a "virial" power series of products of mole ratios $\{m_\kappa\}$ of the various solute components [24]. This representation is valid over the entire range of homogeneous phase compositions extending to infinite dilution. In contrast, for charged solutes, whether small or macromolecular, the thermodynamic consequences of coulombic interactions cause Π, hence all related thermodynamic variables, to have a transcendental dependence on a universal function of the $\{m_\kappa\}$ ("ionic strength"), which predominates in the limit of infinite dilution.

According to results reported [5,6,13–15] for systems containing a biological macromolecule together with any one of a wide variety of solutes, nonelectrolytes as well as dissociated electrolytes, the functional form of the concentration dependence of osmolality, $\Omega(\{m_\kappa\})$, can be fitted by linear, quadratic, or at most cubic functions of the solute/solvent mole ratios over the entire concentration ranges where measurements with a vapor pressure osmometer are feasible. Higher order polynomials may be required to fit data obtained by analyzing isopiestic distillation experiments on solutions containing multimolar concentrations of highly soluble small molecular species, such as urea, in solution with another small solute [15,19]. Thus far, no nonpolynomial (transcendental) functional expression for $\Omega(\{m_\kappa\})$ has been found necessary to fit the osmometric data.

As indicated by the extant osmometric database for aqueous solutions containing two ([5,6,13–15]; or in a few cases three (J. Hong et al., in press)] solute components, the low-order polynomials that suffice to describe the functional form of Ω $(\{m_\kappa\})$ have fitted coefficients such that $\partial_{abz}\Omega$ is indistinguishable from zero when $q \geq 4$. The negligibility of higher order terms generated via successive applications of Eq. (9) is further promoted because the mole ratio factors $(m_\alpha^a\ m_\beta^b\ m_\zeta^z)$ are integral powers of numbers significantly less than unity, especially for macromolecular solutes. Hence, at least for systems with characteristics similar to those that have been investigated thus far by osmometry, Gibbs–Duhem-based expressions, such as those shown in Table 1, are expected to be analytically useful, because at most a small number of terms make significant contributions to the derivatives of $\bar{Y}_\alpha$ and $\bar{Y}_\beta$ being sought.

8. Comparison with alternative analytic strategies

Gibbs–Duhem recursion provides a previously unrecognized model-independent basis for determining the number and kind of measurements that can suffice to characterize completely the concentration dependences of all partial molal functions of a given type for a system comprised of more than two components. From the most general perspective, expressions derived here show how, in principle, measurements (or theoretical calculations) that characterize the concentration dependences of a given type of partial molal function pertaining to a single component, at a single specification of the $\{m_\kappa\}$, can be used to evaluate derivatives of the corresponding partial molal function for any other component. From the standpoint of practical applicability, the comments in the preceding section indicate that expressions such as those shown in Table 1—either by themselves or in conjunction with some type(s) of matching approximation(s) [15]—will prove useful to calculate derivatives that specify the concentration dependences of $\bar{Y}_\kappa$ for any solute component, provided that the consequences of its interactions with other components have been adequately sampled by acquiring sufficiently detailed information about the functional form of Ω $(\{m_\kappa\})$.

In contrast to any other extant approach having similar objectives [22,25], expressions derived here do not require as input measurements (or theoretical predictions) pertaining to conditions far removed from those where the derivatives of interest are to be evaluated. Nor does our derivation require the matching of functions that must be determined under different thermodynamic constraints for systems having identical compositions (evaluated, most commonly, by analyzing parallel series of density measurements on dialyzed and isobaric systems ([26,27]). The Gibbs–Duhem-based expressions derived here have significant implications (to be explored in a subsequent paper) concerning the generality of the derivation, originally presented by Robinson and Stokes [16], of an expression in which the osmolality of a three-component system is matched with corresponding osmolalities for each of two corresponding "subsystems" containing two components.

Various authorities [22,25,28] have pointed out, more or less explicitly, that complete information about the concentration dependences of a single chemical potential for a given multicomponent system suffices, in principle, to evaluate any of the other chemical potentials and hence derivatives that specify their concentration dependences. However, prior to the expressions derived here for this purpose, we know of only two explicit alternatives that have been proposed [22,25]. Any application of either of these would necessitate integration of a chemical potential derivative evaluated by acquiring (or extrapolating) data over the entire range of compositions extending (sufficiently close) to infinite dilution of a given component, α, and then differentiation of the resulting function with respect to a solute/solvent mole ratio m_κ, in order to evaluate such a derivative for $\kappa=\alpha$ or for some other component, and thereby obtain information equivalent to that accessible by applying a Gibbs–Duhem-based expression of the type shown in Table 1. Probably because mathematical operations required for applications of Eq. (6) of Ref. [22] or Eq. (6) of Ref. [25] would place such extreme demands on the quality and quantity of the input data, these analytic expressions apparently never have been used, for example, to quantify solute activity coefficients or their derivatives for a solution containing more than one solute. At least no such applications have been cited, for example, in the book edited by Pitzer [29] or in any other standard monograph on solution thermodynamics.

Neither the derivation of Eq. (9) nor the application of any resulting expression for a derivative of $\bar{Y}_\kappa$, such as those given in Table 1, requires integration (or extrapolation) of any kind nor measurements (or estimates) over concentration ranges significantly outside those where the derivative(s) of interest are to be evaluated. Interpolations using empirically fitted functions also are unnecessary, because the need to match as closely as possible data points, or slopes of curves fitted thereto, determined for (putatively) corresponding systems, does not arise when a derivative of $\bar{Y}_\kappa$ can be represented, as in Table 1, by a small number of terms. Each of these can be quantified by analyzing the functional form of $\Omega(\{m_\kappa\})$ as determined, or estimated within bounds set by experimental considerations, solely by "global fitting" of measurements on a given multicomponent system for the conditions of interest. The feasibility of this approach is implied by results reported in studies that utilize water vapor pressure osmometry [15] or isopiestic distillation [19] to investigate preferential interactions in aqueous solutions containing two or three (J. Hong et al., in press) solute components.

9. Broader implications and future directions

As methods of investigating concentration-dependent effects of preferential interactions in solutions containing at least two solute components, water vapor pressure osmometry and isopiestic distillation to date have been applied less extensively than density measurements on dialyzed solutions [26,27] but are much more common at present than direct determinations of the ("colloid") osmotic pressure, Π, such as those published in the pioneering study of nonaqueous polymer solutions by Browning and Ferry [1]. Despite the dearth of studies reporting direct in vitro measurements of Π for biological systems, "turgor" pressure, the osmotic pressure difference across a cellular membrane can be quantified, at least indirectly, and interpreted with regard to processes in living organisms [30]. More generally, "osmotic pressure" and related phenomena, such as "osmotic stress" [14,31], often are considered in discussions of effects exhibited by—or related to—the activity of solvent water in biological systems, regardless of the thermodynamic function(s) actually measured.

The view that osmotic pressure is the most fundamental measure of thermodynamic nonideality may be traceable to the statistical mechanical treatment originated by MacMillan and Mayer [23], and further developed (for example) by Hill [24]. According to this "cluster" theory, the (hydrostatic) osmotic pressure acting on a multicomponent liquid solution in dialysis equilibrium with "solvent" (defined to include all species diffusible across the dialyzing membrane) is rigorously analogous to the (mechanical) pressure of an imperfect gas mixture, with regard to thermodynamic effects due to interactions, via "solvent-averaged" potentials, among those solutes that cannot pass through the membrane. The osmotic pressure that can be expressed theoretically in terms of integrals over functions of "solvent-averaged" interaction potentials [24] is exactly identifiable with the Π that could be directly measured by dialysis for a real solution, provided that none of the "explicit" solute particles (whose interactions, via "solvent-averaged" potentials, are included explicitly in the theoretical formulation) can diffuse across the dialyzing membrane.

In fact, most biological systems contain, along with macromolecular species, appreciable concentrations of solutes small enough so that they, like water, also would be equilibrated across any size-selective dialyzing membrane. For such systems, the Gibbs–Duhem-based derivation presented in this paper can be modified to accept as input theoretical predictions of derivatives expressing changes in the osmotic pressure Π, subject to constraints on the chemical potentials of a "solvent" that includes, besides water, any (quasi-diffusible) solute components treated implicitly in formulating the "solvent-averaged" potentials. The resulting expressions (to be presented elsewhere) relate derivatives of $\bar{Y}_\kappa$ for any selected explicit (quasi-nondiffusible) solute component(s) to corresponding derivatives of Π, rather than $\bar{Y}_\omega$, with respect to the appropriate molarities, which turn out to be more directly useful than mole ratios for the derivation of expressions that specify the concentration dependences of Π.

Results of osmometric studies currently in progress in this laboratory will be analyzed and interpreted in subsequent papers, where we will take advantage of expressions based on the Gibbs–Duhem-based derivation presented here to devise optimal strategies for quantitative investigations of thermodynamic effects due to preferential interactions in solutions containing two or more biological solutes. Among the various alternative expressions that have been [5,6,9,13–15] or now can be applied for this purpose, the more rigorous (incorporating fewer thermodynamic approximations) generally consist of more terms and therefore are subject to larger levels of propagated experimental uncertainties. Probably no single formula will invariably prove most satisfactory for every type of system, under all conditions, as a basis for minimizing the inaccuracies that can arise from these two inherently countervailing sources of uncertainty: approximations that simplify the thermodynamic expressions applied; cumulative error due to random scatter in the data acquired. Nevertheless, expressions derived on the basis of Gibbs–Duhem recursion will provide useful guidance toward the design of experiments sufficient to characterize a given system, and toward the most reliable expression(s) for analyzing a given set of measurements.

Acknowledgments

Motivation for the present article was considerably strengthened by numerous conversations with Jonathan Cannon, Michael Capp, Scott Cayley, Daniel Felitsky, and Jiang Hong on a wide variety of topics related to vapor pressure osmometry. We also are grateful to Professor John Schrag for some additional insights about John Ferry's career at the University of Wisconsin, Madison, and for access to a complete collection of reprints, which facilitated placing this article more specifically in the context of work by our distinguished colleague, to whose memory this Festschrift is dedicated. This research was supported by NIH Grant GM 47022.

References

[1] G.V. Browning, J.D. Ferry, Thermodynamic studies of polyvinyl acetate solutions in the dilute and moderately concentrated range, J. Chem. Phys. 17 (1949) 1107–1112.
[2] J.D. Ferry, Concentration dependence of osmotic pressure in the semidilute range, Macromolecules 13 (1980) 1719.
[3] S. Shulman, S. Katz, J.D. Ferry, The conversion of fibrinogen to fibrin XIII: dissolution of fibrin and inhibition of clotting by various neutral salts, J. Gen. Physiol. 36 (1953) 759–766.
[4] M.T. Record Jr., W.T. 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.
[5] E.S. Courtenay, M.W. Capp, R.M. Saecker, M.T. Record Jr., 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) (2000) 72–85.
[6] E.S. Courtenay, M.W. Capp, M.T. Record Jr., 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.
[7] D. Hall, A.P. Minton, Macromolecular crowding: qualitative and semiquantitative successes, quantitative challenges, Biochim. Biophys. Acta 1649 (2003) 127–139.
[8] H. Eisenberg, Biological Macromolecules and Polyelectrolytes in Solution, Clarendon, Oxford, 1976, Chap. 2.
[9] C.F. Anderson, E.S. Courtenay, M.T. Record Jr., Thermodynamic expressions relating different types of preferential interaction coefficients in solutions containing two solute components, J. Phys. Chem., B 106 (2002) 418–433.
[10] C.F. Anderson, D.J. Felitsky, J. Hong, M.T. Record Jr., Generalized derivation of an exact relationship linking different coefficients that characterize thermodynamic effects of preferential interactions, Biophys. Chemist. 102 (2002) 497–511.
[11] M.T. Record Jr., C.F. Anderson, Interpretation of preferential interaction coefficients of nonelectrolytes and of electrolyte ions in terms of a two-domain model, Biophys. J. 68 (1995) 786–794.
[12] D.J. Felitsky, M.T. Record Jr., Application of the local-bulk partitioning and competitive binding models to interpret preferential interactions of glycine betaine and urea with protein surface, Biochemistry 43 (2004) 9276–9288.
[13] W.T. Zhang, M.W. Capp, J.P. Bond, C.F. Anderson, M.T. Record Jr., 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.
[14] E.S. Courtenay, M.W. Capp, C.F. Anderson, M.T. Record Jr., 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.
[15] J. Hong, M.W. Capp, C.F. Anderson, M.T. Record Jr., Preferential interactions in aqueous solutions of urea and KCl, Biophys. Chemist. 105 (2003) 517–532.
[16] R.A. Robinson, R.H. Stokes, Activity coefficients in aqueous solutions of sucrose, mannitol, and their mixtures at 25 degrees, J. Phys. Chem. 65 (1961) 1954–1958.
[17] I.N. Levine, Physical Chemistry, McGraw-Hill, New York, 1988, Chaps. 9 and 10.
[18] R.A. Alberty, Thermodynamics of Biochemical Reactions, Wiley-Interscience, New Jersey, 2003, Chap. 5.
[19] 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.
[20] J. Wyman, Linked functions and reciprocal effects in hemoglobin: a second look, Adv. Protein Chem. 19 (1964) 223–286.
[21] C.F. Anderson, M.T. Record Jr., Salt dependence of oligoion–polyion binding: a thermodynamic description based on preferential interaction coefficients, J. Phys. Chem. 97 (1993) 7116–7126.
[22] H.A.C. McKay, Thermodynamics of three component systems, Nature 169 (1952) 464–465.
[23] W.G. MacMillan, J.E. Mayer, The statistical thermodynamics of multicomponent systems, J. Chem. Phys. 13 (1945) 276–305.
[24] T.L. Hill, Statistical Mechanics, McGraw-Hill, New York, 1956.
[25] L.S. Darken, Application of the Gibbs–Duhem equation to ternary and multicomponent systems, J. Am. Chem. Soc. 72 (1950) 2909–2914.
[26] H. Eisenberg, Protein and nucleic acid hydration and cosolvent interactions—establishment of reliable base-line values at high cosolvent concentrations, Biophys. Chemist. 53 (1994) 57–68.
[27] S.N. Timasheff, Control of protein stability and reactions by weakly interacting cosolvents: the simplicity of the complicated, Adv. Protein Chem. 51 (1998) 355–432.

[28] R.H. Stokes, in: K.S. Pitzer (Ed.), Activity Coefficients in Electrolyte Solutions, Chemical Rubber, Boca Raton, FL, 1991, Chap. 1.

[29] K.S. Pitzer (Ed.), Activity Coefficients in Electrolyte Solutions, Chemical Rubber, Boca Raton, FL, 1991.

[30] D.S. Cayley, H.J. Guttman, M.T. Record Jr., Biophysical characterization of changes in amounts and activity of *Escherichia coli* cell and compartment water and turgor pressure in response to osmotic stress, Biophys. J. 78 (2000) 1748–1764.

[31] V.A. Parsegian, R.P. Rand, D.C. Rau, Osmotic stress, crowding, preferential hydration and binding: a comparison of perspectives, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 3792–3987.