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Rigorous analysis of static light scattering measurements on buffered protein solutions

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Abstract

Attention is drawn to the thermodynamic invalidity of the current practice of analyzing static light scattering measurements on globular proteins in terms of theory for a single solute because of its disregard of the need to consider small species such as buffer components as additional cosolutes rather than as part of the solvent. This practice continues despite its demonstrated inadequacy in studies of sucrose-supplemented protein solutions, where the aberrant behaviour was recognized to be a consequence of physical protein interaction with the small cosolute. Failure to take into account the consequences of small cosolute effects renders extremely difficult any attempt to obtain a rigorous thermodynamic characterization of protein interactions by this empirical technique.

Keywords:

Thermodynamic nonideality

Protein–cosolute interactions

Static light scattering

1. Introduction

Thermodynamic nonideality of protein solutions can certainly be quantified in terms of the second virial coefficient obtained from light scattering experiments. However, the parameter derived therefrom (A_2) is not identical to the second virial coefficient for protein self-interaction (B_{22}) that emanates from osmotic pressure, sedimentation equilibrium and size-exclusion chromatography measurements on buffered protein solutions [1]. We were first alerted to this problem by reports of negative B_{22} values from light scattering studies of protein solutions supplemented with high salt concentrations [2–4] – values incompatible with the statistical-mechanical concept of the osmotic second virial coefficient for protein self-interaction as an excluded volume [5,6]. The anomaly has been traced to disregard of the role of buffer and supporting electrolyte components, as if these cosolutes have no effect on the overall intensity of light scattering, even though such effects were described more than fifty years ago [7.8]. Reconsideration of how cosolutes affect the way macromolecules scatter light [9–11] has explained the negative light scattering second virial coefficients [2–4], reflecting situations in which the B_{22} contribution is outweighed by an opposing protein–cosolute counterpart at high cosolute concentration.

The popularity of static light scattering for the characterization of protein interactions has been boosted considerably by the development of an automated procedure [12,13] for measuring the concentration dependence of the excess light scattering (R_θ) at set angle θ relative to the incident laser beam. However, results obtained by using this technique, termed composition gradient multi-angle light scattering (CG-MALS), on nonassociating proteins continue to be interpreted in terms of single-solute theory [14] and hence on the assumption that buffer components can be regarded as part of the solvent. The present communication

examines the consequences of this approximation by subjecting reported CG-MALS results [13,15] to closer thermodynamic scrutiny; and a protocol for the correct interpretation of Debye plots is presented.

2. Theoretical considerations

As re-emphasized recently [1], the inclusion of a single nonassociating protein in solvent at constant temperature gives rise to one of two situations: that in which the protein chemical potential is being monitored under the additional constraint of constant solvent chemical potential, and that in which constant pressure is the second constraint – a distinction that is overlooked in standard textbooks and most experimental studies. Studies performed under the former constraint, which applies in osmometry and size-exclusion chromatography, are the simplest to consider because small partitioning solutes (buffer components and electrolytes) can justifiably be regarded as part of the solvent (species 1).

2.1 Solute chemical potential under the constraint of constant solvent chemical potential

For these simpler situations the pertinent measure of the thermodynamic activity of the protein (species 2 with molecular mass M_2) is defined in terms of its weight per unit volume concentration c_2 by the expression [16]

$$\left(\mu_2\right)_{T,\mu_1} = \left(\mu_2^o\right)_{T,\mu_1} + RT\ln z_2 = \left(\mu_2^o\right)_{T,\mu_1} + RT\ln\left(\gamma_2 c_2/M_2\right) \qquad (1)$$

in which the thermodynamic activity (z_2) of the protein is a molar quantity and therefore written as the product of its molar concentration (c_2/M_2) and a corresponding molar activity coefficient (γ_2). By a purely thermodynamic argument it has been shown [6] that

$$\ln\gamma_2 = 2B_{22}c_2/M_2 + \ldots \qquad (2)$$

where B_{22}, the osmotic second virial coefficient for protein self-interaction, is a rigorously defined parameter that can be described on the statistical-mechanical basis of physical interaction between pairs of protein molecules [5,6]. For globular proteins in a buffer medium with moderate ionic strength ($I \geq 0.1$ M) a reasonably reliable estimate of the osmotic second virial coefficient for protein self-interaction can be obtained from the expression [17,18]

$$B_{22} = \frac{16\pi N_A R_2^3}{3} + \frac{Z_2^2\left(1+2\kappa R_2\right)}{4I\left(1+\kappa R_2\right)^2} - \frac{Z_2^4\left(1000\kappa^3\right)}{128\pi N_A I^2\left(1+\kappa R_2\right)^4} + \ldots \qquad (3)$$

where the first term is the hard-sphere contribution for a protein with pair-exclusion diameter $2R_2$; and where the subsequent terms account for the exclusion of one solute molecule from further space around another arising from charge–charge repulsion between them, each of which has a symmetrically distributed net charge Z_2. The appearance of the 1000 factor in the last term reflects calculation of the Debye–Hückel inverse screening length κ (in cm^{-1}) as $3.27 \times 10^7 \sqrt{I}$ from the ionic strength I, which also appears as a numerical factor with implicit molar units (M) in the denominator. Avogadro's number (N_A) is included to convert the virial coefficient from a molecular to a molar basis. An alternative procedure for evaluating B_{22} entails an adaptation of scaled-particle theory [19,20] for which the counterpart of Eq. (3) is

$$B_{22} = \frac{16\pi N_A R_{eff}^3}{3} \qquad (4)$$

in which the effective radius of the molecule (R_{eff}) is increased to take into account the excluded volume contributions arising from the charge-charge repulsion terms in Eq. (3) [21]. This effective size is determined as the effective specific volume v_{eff}, from which the second virial coefficient is calculated as $B_{22} = 4M_2 v_{eff}$.

Although not properly applicable, the above theory is also used to interpret static light

scattering measurements in the mistaken belief that the same definition of solute chemical potential [Eq. (1)] applies without further consideration of which thermodynamic variables are chosen to be independent.

2.2 Solute chemical potential under the constraint of constant pressure

In common with most physicochemical situations, the second constraint applying to static light scattering measurements is constant pressure, whereupon the expression for the thermodynamic activity of the protein becomes more complicated. Under the constraints of constant temperature and pressure the thermodynamic activity of a single nonassociating macromolecular solute (a_2) needs to be written as [16]

$$\left(\mu_2\right)_{T,P} = \left(\mu_2^o\right)_{T,P} + RT \ln a_2 = \left(\mu_2^o\right)_{T,P} + RT \ln\left(y_2 w_2 / M_2\right) \tag{5}$$

where a_2 is the molal activity that is most logically expressed in terms of the molal concentration (w_2/M_2) with w_2 defined in terms of g per kg of solvent) and the corresponding molal activity coefficient (y_2). Furthermore, the relevant expression for chemical potential in terms of a virial expansion is now [16]

$$-\frac{\left(\mu_1\right)_{T,P} - \left(\mu_1^0\right)_{T,P}}{RT} = \left(w_2/M_2\right) + C_{22}\left(w_2/M_2\right)^2 + \ldots \tag{6}$$

and the counterpart of Eq. (2) becomes

$$\ln y_2 = 2C_{22}\left(w_2/M_2\right) + \ldots \tag{7}$$

The molal second virial coefficient (C_{22}) is not amenable to simple statistical-mechanical rationalization except for incompressible solutions of solute in a single component solvent. Under those restrictive circumstances the molal and molar second virial coefficients for solute self-interaction are related by the expression

$$C_{22}/\rho_1 = B_{22} - M_2\bar{v}_2 \tag{8}$$

where $\bar{v}_2$ is the partial specific volume of the protein, independent of concentration; and where ρ_1, the solvent density, is required to convert the units of C_{22} (mol per kg solvent) into those of the osmotic second virial coefficient for self-interaction and the molar volume (mol per liter of solution). After replacement of w_2 in Eq. (7) by its more commonly used counterpart c_2 via the relationship $w_2 = c_2/\left[\rho_1\left(1-\bar{v}_2 c_2\right)\right]$, applicable to incompressible solutions, the expression for the molal activity coefficient becomes, correct to linear order in protein concentration,

$$\ln y_2 = \left(2B_{22} - M_2\bar{v}_2\right)\left(c_2/M_2\right) + \ldots \tag{9}$$

which differs only slightly from its counterpart for the molar activity coefficient [Eq. (2)].

Unfortunately, physicochemical studies of aqueous protein solutions require supplementation of the solvent (water) with low molecular mass buffer and supporting electrolyte components. Whereas these small species could be regarded as part of the solvent in osmometry and size-exclusion chromatography, the experimental constraint of constant pressure (rather than constant solvent chemical potential) necessitates their consideration as additional cosolutes [7,8]. To simplify nomenclature we shall regard them as a single "buffer" component (species 3) present at molar concentration c_3/M_3. The counterpart of Eq. (6) must now be written as

$$\begin{aligned} -\frac{\left(\mu_1\right)_{T,P} - \left(\mu_1^0\right)_{T,P}}{RT} &= \left(w_2/M_2\right) + \left(w_3/M_3\right) \\ &+ C_{22}\left(w_2/M_2\right)^2 + C_{23}\left(w_2/M_2\right)\left(w_3/M_3\right) + C_{223}\left(w_2/M_2\right)^2\left(w_3/M_3\right) + \ldots \end{aligned} \tag{10}$$

whereupon the counterpart of Eq. (5) becomes [10]

$$\ln y_2 = 2C_{22}\left(w_2/M_2\right) + C_{23}\left(w_3/M_3\right) + 2C_{223}\left(w_2/M_2\right)\left(w_3/M_3\right) + \ldots \tag{11}$$

in which C_{23} is the second molal virial coefficient for protein interaction with a "buffer" molecule; and where C_{223} , the third virial coefficient for physical interaction between two protein molecules and one "buffer" molecule, is included to retain rigor of the expression to terms linear in both w_2/M_2 and w_3/M_3.

The important point to emerge from Eqs. (10) and (11) is that the activity coefficient for protein (species 2) now contains contributions from protein–cosolute interactions, and that the second virial coefficient derived from light scattering measurements is certainly not B_{22}, the osmotic second virial coefficient for protein self-interaction. Nor is it the corresponding molal parameter C_{22}. We shall therefore revert to the original light scattering nomenclature that designated the experimental second virial coefficient as A_2.

2.3 Expressions for the concentration dependence of light scattering measurements

Concentration dependence of the excess light scattering (Rayleigh ratio) for a single nonassociating solute has traditionally expressed in Debye format as

$$Kc_2/R_\theta = \left(n_1/n\right)^2\left[1 + c_2\left(d\ln\gamma_2/dc_2\right)\right]/M_2 = 1/M_2 + 2A_2c_2 + \ldots \qquad (12)$$

where $K = 4\pi^2 n_1^2\left(dn/dc_2\right)^2/\left(N_A\lambda\right)^4$ is an optical constant defined in terms of the solvent refractive index (n_1), the specific refractive index increment for solute ($\chi_2 = dn/dc_2$) and the wavelength of the incident laser beam(λ); and where $A_2 = B_{22}/M_2^2$ in conformity with the experimental convention that it has the dimensions mL mol g^{-2}. However Eq. (12) is based on the presumption that molar thermodynamic activity is being monitored, rather than the molal activity appropriate to the constraints of constant temperature and pressure relevant to the refractive index fluctuations that produce light scattering. Accommodation of this change in concentration scale requires the replacement of Eq. (2) by Eq. (7) for the definition of the

activity coefficient as well as expression of the refractive index increment as $(\partial n/\partial w_2)_{T,P}$: we thus need a revised version of the product $(n_1/n)^2(\partial n/\partial w_2)^{-2}$ that is consistent with the definition of the solution turbidity upon which a rigorous version of Eq. (12) is based [7,8].

For an incompressible solution the weight concentration c_2 is related to its molal counterpart (w_2) by

$$c_2 = w_2\rho_1/\left(1+\overline{v}_2 c_2\right) = w_2\rho_1\left(1-\overline{v}_2 c_2+\ldots\right) = w_2\rho_1\left(1-\overline{v}_2 w_2\rho_1+\ldots\right) \tag{13}$$

whereupon the expression for the solution refractive index (n) in terms of the refractive index increment (χ_2) becomes

$$n = n_1 + \chi_2 c_2 + \ldots = n_1 + \chi_2\rho_1 w_2\left(1-\rho_1\overline{v}_2 w_2+\ldots\right) \tag{14}$$

Differentiation of Eq. (14) with respect to w_2 then gives

$$\left(\partial n/\partial w_2\right)_{T,P} = \chi_2\rho_1 - 2\chi_2\rho_1^2\overline{v}_2 w_2 + \ldots = \chi_2\rho_1\left(1-2\overline{v}_2 c_2\right)+\ldots \tag{15a}$$

Furthermore, the first expression in Eq. (14) for the solution refractive index may be rearranged as

$$n_1/n = 1\Big/\left[1+\left(\chi_2/n_1\right)c_2+\ldots\right] = 1-\left(\chi_2/n_1\right)c_2+\ldots$$

whereupon the required refractive index term becomes, correct to first order in protein concentration,

$$\begin{aligned}\left(n_1/n\right)^2\left(\partial n/\partial w_2\right)_{T,P}^{-2} &\approx \left[1-\left(\chi_2/n_1\right)c_2\right]^2\Big/\left[\chi_2\rho_1\left(1-2\overline{v}_2 c_2\right)\right]^2 \\ &= \left[1-2\left(\chi_2/n_1\right)c_2\right]\left[\left(1+4\overline{v}_2 c_2\right)\Big/\left(\chi_2\rho_1\right)^2\right] \\ &= \left[1-2\left(\chi_2/n_1\right)c_2+4\overline{v}_2 c_2\right]\Big/\left(\chi_2\rho_1\right)^2\end{aligned} \tag{16}$$

whereupon the revised form of Eq. (12) becomes

$$\frac{Kc_2}{R_\theta} = \frac{1}{M_2} + \frac{\left[2B_{22} - 2\left(\chi_2/n_1\right)\right]c_2}{M_2^2} + \ldots = \frac{1}{M_2} + 2A_2c_2 + \ldots \tag{17}$$

The experimental value of A_2 should thus be only a slight underestimate of B_{22}/M_2^2. Indeed, the contribution of the (χ_2/n_1) term, calculated to be 2.5% of the numerator for isoelectric ovalbumin, would be within the experimental uncertainty limits of an A_2 measurement. However, this situation only applies to a single macromolecular solute (protein) in an unsupplemented solvent.

The presence of buffer and supporting electrolytes in the solvent (water) necessitates their consideration as additional nonscattering cosolutes [7,8] – a task already undertaken elsewhere [10]. The expression for the solute activity coefficient is then given by Eq. (11), but the expression for interchange between concentration scales [Eq. (13)] needs modification to include the cosolute contribution. This is now effected by the relationship (for $i = 2,3$)

$$\begin{aligned} c_i &= \frac{\rho_1 w_i}{1 + \rho_1\overline{v}_2 w_2 + \rho_1\overline{v}_3 w_3} \\ &= \rho_1 w_i\left(1 - \rho_1\overline{v}_2 w_2 - \rho_1\overline{v}_3 w_3 + \rho_1^2\overline{v}_2^2 w_2^2 + 2\rho_1^2\overline{v}_2\overline{v}_3 w_2 w_3 + \ldots\right) \end{aligned} \tag{18}$$

in order to obtain $(\partial n/\partial w_2)_{T,P}$ correct to linear order in both weight molalities. The refractive index of the solution needs to include the buffer contribution, and hence becomes

$$n = n_1 + \chi_3 c_3 + \chi_2 c_2 = n_s + \chi_2 c_2 \tag{19}$$

where n_s, the refractive index of the buffer, replaces n_1 in a revised optical constant K_s. After these modifications the counterpart of Eq. (17) becomes [10]

$$\frac{K_s c_2}{R_\theta} = \frac{A_1}{M_2} + 2A_2c_2 + \ldots \tag{20}$$

where

$$A_1 = 1 + 2(\chi_3/\chi_2)\left[(B_{23} - M_3\overline{v}_3)/M_2\right]c_3 \tag{21}$$

$$A_2 = \left[(B_{22} - M_2\chi_2/n_s + \tfrac{1}{2}\Omega c_3/M_3)\right]/M_2^2 \tag{22}$$

$$\begin{aligned} M_2\Omega = {} & \frac{1}{M_2 M_3 \rho_1^2}\left[C_{223} - C_{23}^2 + 2(C_{22} - C_{23})\overline{v}_3 M_3\rho_1\right] \\ & + \frac{2(\chi_3/\chi_2)}{M_2^2\rho_1^2}\left\{\begin{array}{l} C_{223} + 2C_{22}C_{23} + 2M_2C_{22}\overline{v}_2\rho_1 \\ + 2M_2C_{23}\rho_1\left[2\overline{v}_2 - 2(\chi_2/n_s)\right] \end{array}\right\} \\ & + 6(\chi_3/\chi_2)\overline{v}_2^2 - 4(\chi_3/n_s)\overline{v}_2 \end{aligned} \tag{23}$$

The reason for leaving Eq. (23) in molal virial coefficient format is that little is known about C_{223}, a parameter with the dimensions of a third virial coefficient reflecting the potential-of-mean-force interaction of a single cosolute molecule, which may be an electrolyte, with a pair of protein molecules, and about which little is known [7,10]. Although a fully quantitative interpretation of the effect of the omega term on the magnitude of the light scattering second virial coefficient (A_2) is therefore precluded, the experimental observation of values considerably smaller than B_{22} (even to the extent of negative values) seemingly implicates a dominant effect of the negative $(C_{23}/\rho_1)^2$ term in Ω over a broad range of common experimental conditions [10]. Another consequence of the need to regard buffer components as additional cosolute species is the prediction [Eqs. (20 and 21)] that the ordinate intercept of the Debye plot is no longer the reciprocal of solute molecular mass.

Therefore, any protocol for using light scattering to determine the nature and extent of protein self-interactions, including self-association that may lead to crystallization, must contend with effects of cosolutes, especially electrolytes. This will necessarily involve making measurements at different concentrations of cosolute to make an empirical determination of the magnitude of the Ω term in Eq. (23), being careful, in the case of

electrolytes, to estimate the changing contribution of B_{22} to A_2 [Eq. (22)] through Eq. (3), but also paying attention to the variations in the intercept of the Debye plot [Eq. (21)] or its inverse [see Eq. (24) below]. An independent determination of B_{23}, using a chromatographic technique for example [9,22], would also be advantageous. However, because of these complications light scattering is not the method of choice for investigating protein self-interactions.

3. Appraisal of recent light scattering measurements on globular proteins

The previous section has established that for a solution of a single nonassociating protein in solvent (water) the magnitude of the light scattering second virial coefficient (A_2) differs only slightly from that of B_{22}, the osmotic second virial coefficient [Eq. (17)] despite differences in the nature of the thermodynamic activity and constraints to which the nonideality coefficient refers: *molal* $\left[a_2 = y_2 w_2 / M_2\right]_{T,P}$ in Eqs.(5-7) as opposed to *molar* $\left[z_2 = \gamma_2 c_2\right]_{T,\mu_1}$ in Eqs. (1-2). However, consideration of the magnitude of A_2 to be a reasonable estimate of B_{22} does not extend to a buffered protein solution because of the need to regard buffer and supporting electrolyte components as additional nonscattering cosolutes [Eq. (20)]. That quantitative expression clearly predicts a potential for molecular mass underestimation (M_2/A_1) as well as a second virial coefficient (A_2) that reflects a complicated mixture of protein–protein and protein–cosolute interactions [Eqs. (22) and (23)]. We therefore need to examine the experimental light scattering data that are purported to conform with Eq. (17) rather than Eq. (20).

Although the ordinate intercept of a Debye plot for protein solutions is predicted to underestimate M_2, this potential deficiency of static light scattering for the measurement of protein molecular mass in aqueous solution has only resurfaced recently [10,11,23]. Ordinate intercepts signifying A_1 values greater than unity have been reported for buffered aqueous

solutions of ovalbumin [10], chymotrypsinogen A [11], and IgG1 [23,24]. However, results conflicting with the current prediction of protein molecular mass underestimation by static light scattering have also been reported recently for several proteins [13,15,25,26] as evidence for the validity of considering light scattering by a nonassociating protein in buffer to be amenable to interpretation in terms of single-solute theory [14]. Although the consistency of those light scattering measurements with the molecular masses of these well-characterized proteins by static light scattering seemingly signifies a value of unity for A_1 and hence of zero for $B_{23} - M_3\overline{v}_3$ [see Eq. (21)], they have not been obtained from the ordinate intercept of a Debye plot. Instead, nonideality has been assessed by nonlinear regression analysis of the dependence of R_θ/K_s upon c_2, an analysis that not only avoids the use of a transformed variable but also achieves the desired separation of variables. Reciprocation of Eq. (20) and the incorporation of Eq. (21) for A_1 show that

$$\frac{R_\theta}{K_s} = \frac{M_2 c_2}{1 + 2(\chi_3/\chi_2)\left[(B_{23} - M_3\overline{v}_3)/M_2\right]c_3 + 2A_2 M_2 c_2 + \ldots} \tag{24}$$

which, in principle, predicts a limiting slope of $M_2 \Big/ \left\{1 + 2(\chi_3/\chi_2)\left[(B_{23} - M_3\overline{v}_3)/M_2\right]c_3\right\}$ as $c_2 \rightarrow 0$ for the dependence of R_θ/K_s upon protein concentration. However, interest in nonideality at extremely high protein concentrations in those studies [12,13,25,26] led to the accumulation of much of the data in the concentration region where the middle term in the denominator of Eq.24 could be neglected; and hence to a curve-fitting analysis with minimal input from data in the low concentration region where the consequences of the A_1 factor predominate – see Fig. 1 for results reported for ovalbumin at neutral pH [13]. The limiting slope of the dependence of R_θ/K_s upon protein concentration therefore becomes relatively insensitive to the magnitude of A_1, and hence provides a reasonably accurate estimate of M_2. That rationale is reinforced by results from a subsequent study [15] in which a much lower

ovalbumin concentration range (< 1.5 g/L) was used to examine the effect of sucrose supplementation of the buffer on the limiting slope, $(M_2)_{app}$, of the dependence of R_θ/K_s upon c_2 (Fig. 2A). In accordance with the behavior predicted by Eq. (24), the limiting slope decreases progressively as the concentration of sucrose is increased in 50 g/L steps from zero to 200 g/L. Linearity of the consequent dependence of $(M_2)_{app}$ upon sucrose concentration (c_3) is illustrated in Fig. 2B, where the broken line (the best-fit linear dependence based on $(M_2)_{app}$ for the four finite sucrose concentrations) seemingly favors an ordinate intercept of 42,600 Da rather than the reported experimental estimate of 44,000 Da for the apparent molecular mass of ovalbumin in buffer [26]; and would thus be consistent with a value of 1.03 for A_1 [see Eq. (20)]. Such a difference between $(M_2)_{app}$ estimates would, of course, be well within the limits of experimental uncertainty. However, irrespective of the relevance of that seeming disparity, the important point to emerge from Fig. 2 is that sucrose must be regarded as a nonscattering cosolute [15]; and that other small species such as buffer components must also be regarded in similar vein [1,9,10].

Further evidence for the unacceptability of the single-solute treatment of light scattering measurements on buffered protein solutions [12,13,25,26] comes from a comparison of the magnitudes of A_2 thereby determined with those of B_{22} predicted [Eq. (3)] on the statistical-mechanical basis of excluded volume (Table 1). For both ovalbumin and bovine serum albumin the estimates of A_2 consistently underestimate the B_{22} values predicted by the McMillan and Mayer treatment [5] of thermodynamic nonideality for a single-solute system. Recent CG-MALS measurements on bovine serum albumin in the vicinity of its isoelectric point [33] have also yielded an A_2 value (0.32×10^{-4} mL mol g^{-2}) that is well below that (0.99×10^{-4} mL mol g^{-2}) calculated for the osmotic second virial coefficient [$B_{22} = 4\pi N_A R_2^3/3$]. Clearly, the effective particle volume that emanates from scaled particle theory is not an accurate estimate of $B_{22}/4$ and cannot be relied upon for the evaluation of activity

coefficients [12,13,15,25,26].

The first doubts about the thermodynamic status of A_2 arose from light scattering studies reporting negative values for the osmotic second virial coefficient for protein self-interaction [1–3,34]. Those reports prompted sedimentation equilibrium studies on lysozyme [35] and equine serum albumin [9] to show the ionic strength dependent decrease in B_{22} to a positive, asymptotic value defined by the hard particle contribution. This theoretically predicted [5] approach to the limiting value (– – –) is illustrated for lysozyme solutions (pH 4.5) by the solid line in Fig. 3, where the experimental data denote the ionic strength dependence of A_2 reported by Rosenbaum and Zukoski [4]. In that sense the present investigation is reemphasizing the need to regard buffer components as additional nonscattering cosolutes in the interpretation of static light scattering measurements – an important consideration that renders extremely difficult any attempt to obtain a quantitative thermodynamic characterization of protein interactions by this empirical technique.

4. Concluding remarks

This investigation has drawn attention to theoretical shortcomings of the current procedure for analysing static light scattering measurements on globular proteins in terms of expressions for a single solute – an interpretation that disregards the need to regard buffer components as additional cosolutes rather than part of the solvent. Although the value of A_2 thereby obtained does, of course, provide a phenomenological description of nonideality in a nonassociating protein solution, the objective of those light scattering studies [13–15,25,26] was to employ the second virial coefficients for the prediction of protein activity coefficients on the statistical mechanical basis of the potential-of-mean-force (excluded volume) between molecules [5]. For that mechanistic purpose the practice of substituting an incorrect parameter (A_2) for B_{22}, the osmotic second virial coefficient to which McMillan–Mayer

theory [5] applies, has no theoretical justification. This invalidation of a basic tenet of the approach to allowance for the effects of thermodynamic nonideality clearly detracts from the recommended use of static light scattering for the rapid quantitative characterization of weak association equilibria requiring study at high protein concentrations [13–15,25,36].

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Table 1

Comparison of the magnitudes of osmotic second virial coefficients for protein self-interaction with recent estimates of the second virial coefficient arising from single-solute analysis of static light scattering measurements

Protein	pH	I (M)	Second virial coeff. (mL mol g^{-2})		Ref.
			$10^4 B_{22}$	$10^4 A_2$	
Ovalbumin	7.2	0.27	1.57[a]	1.49	[1,9]
	7.4	0.16	1.81	1.37	[10]
Serum albumin	7.2	0.27	1.23[b]	1.12	[1,9]
	7.4	0.16	1.32	1.13	[10]

[a]Calculated from Eq. (3) with, M_2 = 44 kDa [27], R_2 = 2.9 nm [28], and a net charge (Z_2) of –16 in phosphate buffers at neutral pH [29]

[b]Calculated from Eq. (3) with M_2 = 66 kDa [30], R_2 = 3.5 nm [31] and a net charge of –24 at neutral pH [32]

LEGENDS TO FIGURES

Fig. 1. Concentration dependence of the Rayleigh excess ratio (R_θ) for ovalbumin in 0.05 M phosphate buffer (pH 7.2) containing 0.15 M NaCl. (Data taken from Fig. 3 of Fernández and Minton [13].)

Fig. 2. Effect of sucrose supplementation of phosphate buffered saline (pH 7.4) on the thermodynamic nonideality of ovalbumin solutions. A, Concentration dependence of the Rayleigh excess ratio in buffer supplemented with increasing concentrations of sucrose. B, Dependence of the apparent molecular mass (deduced from limiting slopes) upon the concentration of sucrose (c_3) included in the phosphate-buffered saline. (Data in A and B taken from Figs.1B and 2B respectively of Wu and Minton [15]).

Fig. 3. Comparison of the calculated ionic strength dependence (———) of the osmotic second virial coefficient (B_{22}) for lysozyme (pH 4.5) with experimental values (●) of its light scattering counterpart (A_2). The broken line signifies the limiting value of B_{22}. (Experimental data taken from Fig. 4 of Rosenbaum and Zukoski [4].)