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Measurement of osmotic second virial coefficients by zonal size-exclusion chromatography

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ABSTRACT

Numerical simulation of protein migration reflecting linear concentration dependence of the partition isotherm has been used to invalidate a published procedure for measuring osmotic second virial coefficients (B_{22}) by zonal exclusion chromatography. Failure of the zonal procedure to emulate its frontal chromatographic counterpart reflects ambiguity about the solute concentration that should be used to replace the applied concentration in the rigorous quantitative expression for frontal migration; the recommended use of the peak concentration in the eluted zone is incorrect on theoretical grounds. Furthermore, the claim for its validation on empirical grounds has been traced to the use of inappropriate B_{22} magnitudes as the standards against which the experimentally derived values were being tested.

The determination of second virial coefficients by size-exclusion chromatography of protein solutions was reported and refined many years ago [1–3]. Those studies employed frontal chromatography [4,5] to allow unequivocal assignment of the protein concentration to which each measured elution volume referred. However, such attention to theoretical detail was deemed unnecessary in a much later investigation [6], which purportedly demonstrated the successful determination of second virial coefficients by zonal size-exclusion chromatography with the effective protein concentration taken as that corresponding to the maximum in the eluted zone.

In view of the continual dilution that occurs during zonal migration [7,8], the validity of that claim certainly warrants further investigation. To that end, calculations based on an analytical solution for concentration-dependent migration with axial dispersion [9] reveal deficiencies in the zonal chromatography approach. Its limitations as an empirical procedure are also exposed by more critical assessment of the extent of agreement between experimentally determined and theoretically predicted second virial coefficients for the two proteins examined. Thus, this communication serves to reemphasize the importance of employing frontal size-exclusion chromatography for the quantitative characterization of protein interactions—a point stressed at the outset of such investigations [4,5].

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Theoretical considerations

The quantitative treatment of the effects of thermodynamic nonideality in size-exclusion chromatography was developed before general recognition of the need to consider the constraints pertaining to the definition of solute chemical potential under the experimental conditions being used [10,11]. Therefore, it is appropriate to consider this aspect first.

Definition of solute chemical potential in size-exclusion chromatography

The equilibrium partitioning of solvent between the mobile and stationary phases of a size-exclusion chromatography column ensures the identity of its chemical potential (μ_1) in the two phases. A similar situation applies to buffer and supporting electrolyte species, which may be regarded as part of the solvent. Under those circumstances, the chemical potential of the protein solute (μ_2) is being monitored on the molar scale and is described by the relationship [10,11].

$$(\mu_2)_{T,\mu_1} = \left(\mu_2^0\right)_{T,\mu_1} + RT\ln z_2 = \left(\mu_2^0\right)_{T,\mu_1} + RT\ln(\gamma_2 c_2/M_2), \quad (1)$$

where z_2 is the molar thermodynamic activity and $((\mu_2^0)_{T,\mu_1}$ is the standard state chemical potential of solute under the constraints of constant temperature and solvent chemical potential. In the second

representation, the thermodynamic activity of solute is replaced by the product of molar activity coefficient γ_2 and the molar concentration, c_2/M_2, where c_2 is expressed in g/L and M_2 is the solute molar mass. Furthermore, a purely thermodynamic argument has established that the activity coefficient is described by the expression [10].

$$\gamma_2 = \exp[2B_{22}c_2/M_2 \; + \; ...], \tag{2}$$

where B_{22} is the osmotic second virial coefficient, a rigorously defined parameter that may be described in terms of physical interaction between pairs of protein molecules [12]. For a spherical solute with radius R_2, the osmotic second virial coefficient can be expressed in terms of $u_{22}(x)$, the potential-of-mean-force between two molecules separated by center-to-center distance x [10–12], and hence calculated as

$$B_{22} = 2\pi N_A \left[(2R_2)^3 \Big/ 3 - \int_{2R_2}^{\infty} f_{22}(x) x^2 dx \right] \tag{3a}$$

in which

$$f_{22} = \exp[-u_{22}(x)/(kT)] - 1, \tag{3b}$$

where k is the Boltzmann constant and where Avogadro's number (N_A) is included to define the virial coefficient on a molar basis. For systems with a moderate ionic strength and relatively low net protein charge, a reasonably accurate estimate of the second virial coefficient can be obtained from the expression [13].

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

The first term is the hard-sphere contribution for a protein with Stokes radius R_2, and subsequent terms account for the additional excluded volume arising from charge–charge repulsion between symmetrically distributed net charge Z_2; the factor of 1000 in the final term of Eq. (4) reflects the 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 measured on the molar scale.

Quantitative treatment of nonideality in frontal size-exclusion chromatography

Fortunately, the correct choice of concentration scale was made unwittingly in the original treatment of thermodynamic nonideality, and hence those expressions [1–3] retain validity [11]. Partition of the protein between mobile (α) and stationary (β) phases of a size-exclusion chromatography column ensures the identity of its chemical potentials in the two phases; consequently, it follows from Eq. (1) that

$$z_2^{\beta} \Big/ z_2^{\alpha} = \exp\left[\left\{ \left(\mu_2^0\right)^{\alpha} - \left(\mu_2^0\right)^{\beta} \right\} \Big/ (RT) \right]. \tag{5}$$

On the other hand, the partition coefficient (σ) obtained experimentally from the elution volume (V_e) of a solute on a column (volume V_{tot}) with V_o and V_s as the respective mobile and stationary phase volumes via the expression

$$\sigma = \; (V_e - V_o)/V_s \; = \; (V_e - V_o)/(V_{tot} - V_o) = c_2^{\beta} \Big/ c_2^{\alpha}, \tag{6}$$

is the corresponding ratio of protein concentrations in the two phases. Inasmuch as the ratios of activities, z_2^{β}/z_2^{α}, and concentrations, c_2^{β}/c_2^{α}, are identical under thermodynamically ideal conditions, the experimental partition coefficient in the limit of zero protein concentration (σ^0) also defines the exponential of the difference in standard state chemical potentials (Eq. (5)). Incorporation of Eq. (2) for the activity coefficient γ_2 then gives [1,2].

$$\sigma = \; \sigma^0 \exp\left[(2B_{22}/M_2)\left(c_2^{\alpha} - c_2^{\beta}\right) = \sigma^0 \exp\left[(2B_{22}/M_2) c_2^{\alpha}(1-\sigma) \right] \right. \tag{7}$$

or, in logarithmic format [3,11],

$$\ln \sigma \; = \; \ln \sigma^0 + (2B_{22}/M_2) \; c_2^{\alpha} \; (1-\sigma) \tag{8}$$

which allows determination of the second virial coefficient from the slope of a linear dependence of $\ln \sigma$ on $c_2^{\alpha}(1-\sigma)$. Further support for the use of Eqs. (7) and (8) has been provided by a subsequent demonstration of their validity for effects of thermodynamic nonideality reflecting nearest neighbor interactions [14].

Quantitative treatment of zonal migration in chromatography

An obvious problem with the application of Eq. (8) to zonal size-exclusion chromatography data is the need for specification of the concentration to which V_e (or its partition coefficient counterpart σ) refers. In the reported use of zonal size-exclusion chromatography [6], that concentration was calculated by combining the amount of protein applied with the width of the eluted zone at half-maximal height (concentration). For a symmetrical elution profile, that approach merely provides an alternative estimate of the concentration at the peak of the zone (c_p). The validity of choosing this substitute for c_2^{α} has been examined by employing an analytical expression for concentration-dependent zonal migration [9] to simulate the zonal chromatographic behavior of a solute for which partitioning is described by the isotherm

$$\sigma = \sigma^0 + Kc_2. \tag{9a}$$

By expressing the exponential term in Eq. (7) as a series expansion, it becomes

$$\begin{aligned} \sigma \; &= \; \sigma^0 \left[1 \; + \; (2B_{22}/M_2) \; c_2^{\alpha} \; (1-\sigma) + \; ... \right] \\ &= \; \sigma^0 + (2B_{22}/M_2)\sigma^0\left(1-\sigma^0\right)c_2^{\alpha} \\ &\quad - \left[(2B_{22}/M_2)\sigma^0\left(1-\sigma^0\right) \right]^2 (c_2^{\alpha})^2 + ... \end{aligned} \tag{9b}$$

Consequently, the consideration of partition in terms of linear concentration dependence suffices in situations where $2(B_{22}/M_2)c_2^{\alpha}\,\sigma^0(1-\sigma^0) << 1$ to allow truncation of Eq. (9b) at the linear concentration term. Indeed, the same proviso ($Kc_2 << 1$) is incorporated into the theoretical treatment of concentration-dependent zonal chromatographic migration.

The analytical solution [9] refers to migration on a chromatography column with length L, through which solvent flows with linear velocity u. Zonal spreading is defined in terms of an origin migrating with the linear velocity of a solute for which partition is governed by σ^0. In those terms, that limiting solute velocity (U) and the extent of spreading from the median position (ξ) are given by the expressions [9].

$$U = u \Big/ \left(1 + \sigma^0 \Big/ \varepsilon\right) \tag{10a}$$

$$\xi \; = \; l - Ut, \tag{10b}$$