$$\sum\ldots\sum\langle f_0(1)f_0(2)\ldots f_0(l)f_m\rangle_{\mathrm{c},m} = \frac{(-\upsilon)^m}{V^m}\sum_{\mathbf{q}}\{a_0(\mathbf{q})\}^{m-l}[\{a''(\mathbf{q})\}^l - \sum_{k=1}^{l}{}_lC_k\{\tilde{G}_1(0,n)a_0(\mathbf{q})\}^k\{a''(\mathbf{q}) - \tilde{G}_1(0,n)a_0(\mathbf{q})\}^{l-k}] =$$
$$\frac{(-\upsilon)^m}{V^m}\sum_{\mathbf{q}}\{a_0(\mathbf{q})\}^{m-l}[\{a''(\mathbf{q})\}^l - \sum_{k=1}^{l}\sum_{p=0}^{l-k}{}_lC_{k\,l-k}C_p(-1)^{l-k-p}\{\tilde{G}_1(0,n)a_0(\mathbf{q})\}^{l-p}\{a''(\mathbf{q})\}^p] \quad \text{(B.9)}$$

Changing the order of the summations from $\sum_{k=1}^{l}\sum_{p=0}^{l-k}$ to $\sum_{p=0}^{l-1}\sum_{k=1}^{l-p}$ and making use of the equality

$$\sum_{k=1}^{l-p}{}_lC_{k\,l-k}C_p(-1)^{l-k} = -{}_lC_p(-1)^l \quad \text{(B.10)}$$

we obtain

$$\sum\ldots\sum\langle f_0(1)\ldots f_0(l)f_m\rangle_{\mathrm{c},m} = \frac{(-\upsilon)^m}{V^m}\sum_{\mathbf{q}}\{a_0(\mathbf{q})\}^{m-l}[\{a''(\mathbf{q})\}^l + \sum_{p=0}^{l-1}(-1)^{l-p}{}_lC_p\{\tilde{G}_1(0,n)a_0(\mathbf{q})\}^{l-p}\{a''(\mathbf{q})\}^p] =$$
$$\frac{(-\upsilon)^m}{V^m}\sum_{\mathbf{q}}\{a_0(\mathbf{q})\}^{m-l}\{a''(\mathbf{q}) - \tilde{G}_1(0,n)a_0(\mathbf{q})\}^l \quad \text{(B.11)}$$

Multiplying eq B.11 by the number of ways selecting l molecules out of m molecules, ${}_mC_l$, gives the right-hand side of eq B.1.

With the cases 1 and 2 we have proved the equality B.1.

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Theory of Homopolymer Retention in Semidilute Solutions Using Liquid Chromatography

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ABSTRACT: A previously developed theory of fractionation of flexible homopolymers by gradient elution high-performance liquid chromatography (HPLC) is extended to include intermolecular polymer–polymer interactions and hence be applicable to polymer solutions of finite concentration. The analysis predicts that in favorable mobile-phase solvent environments, the retention time or capacity factor should increase with polymer loading. The theory also suggests the potential utilization of HPLC to obtain osmotic second virial coefficients and hence average molecular volumes of flexible polymer molecules in dilute solution. Also briefly considered is the possibility of verification by HPLC of the osmotic pressure equation of state in semidilute polymer solutions where scaling concepts are applicable. Chromatographic measurements of osmotic second virial coefficients are expected to become more difficult as the degree of polymerization increases, and equation of state measurements in semidilute solutions would require careful operational selection of the mobile-phase composition to achieve low polymer retention at relatively high loading of polymeric solute.

I. Introduction

The transition between infinitely dilute solutions of isolated flexible polymer molecules to semidilute solutions where different polymer molecules begin to interpenetrate substantially occurs in good solvents for a volume fraction of chain monomers given by $\Phi \sim M^{-0.8}$, where M is the degree of polymerization.[1] Thus departures from infinite dilution can be anticipated for small values of Φ when M is large (e.g., when $10^3 \leq M \leq 10^5$, $4 \times 10^{-3} \geq \Phi \geq 1 \times 10^{-4}$). High-performance liquid chromatography (HPLC) has been successfully applied to the reversed-phase fractionation of polystyrene homopolymers using gradient elution with mixed methylene chloride–methanol mobile phases,[2] and a theoretical interpretation of the separation process has been introduced on the basis of assumption of infinite dilution of polymer solute.[3] Preliminary HPLC measurements of the retention time of polystyrene homopolymers distributed between a chemically bonded C-18 stationary phase and a methylene chloride–methanol mixed mobile phase indicate that the retention increases with the amount of polymer loaded into the column.[4] The polystyrene samples had M values in the range $600 \leq M \leq 8600$ and, assuming a molar volume for styrene monomers of 125 cm^3/mol, Φ values in the range $3 \times 10^{-4} < \Phi < 3 \times 10^{-2}$. Thus for the higher polymer loadings $\Phi > M^{-0.8}$, which indicates these samples were semidilute. The purpose of the present analysis is to extend a recent theory of HPLC homopolymer fractionation[3] to include intermolecular polymer–polymer interactions.

In section II we apply the McMillan–Mayer theory[3,5] to dilute (but not infinitely dilute) polymer solutions and scaling theory[1] to semidilute polymer solutions to derive

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an explicit expression for the capacity factor K_3 for flexible, chainlike polymer molecules distributed between a binary solvent mobile phase and a stationary phase consisting of a homogeneous, planar surface. The dependence of K_3 on intermolecular interactions between different polymer molecules appears through temperature and solvent-composition dependent osmotic virial coefficients B_{la} ($l = 2, 3, ...$; a = m, s) associated with interacting clusters of $l = 2, 3, ...$ polymer molecules in each chromatographic phase (m → mobile, s → stationary). The first correction from infinite dilution involves the second osmotic virial coefficients, B_{2m} and B_{2s}, associated with interacting pairs of polymer molecules in each chromatographic phase. The virial coefficients B_{2m} and B_{2s} can be determined from the potentials of mean force $W_m(r)$ and $W_s(r)$, which represent the reversible work required to bring the centers of mass of two isolated polymer molecules in solution together from $r = \infty$ to a distance r in the mobile and stationary phases. An approximate calculation[5] of $W_a(r)$ and hence B_{2a} (a = m, s) is performed by assuming the polymer molecules and associated solvent molecules can be described by spherical or thin cylindrical gels of uniform polymer segment densities in the mobile and stationary phases, respectively.[3]

In good solvent environments B_{2m} and B_{2s} are positive indicating that polymer–polymer interpenetration is repulsive. At sufficiently low polymer concentration, C_3, where only polymer pair intermolecular interactions contribute appreciably, K_3 is greater than (less than) its value at infinite dilution, K_3^∞, whenever the mobile-phase solvent composition, X_{1m}, is sufficiently enriched (depleted) in the better polymer solvent (e.g., solvent 1) so that polymer retention in the stationary phase is small (i.e., $K_3 \ll 1$) (large(i.e., $K_3 \gg 1$)). These results have a simple physical interpretation: when $K_3 \ll 1$ ($K_3 \gg 1$), the polymer solute is predominantly in the mobile (stationary) phase and the repulsive intermolecular pair interactions tend to enhance precipitation (dissolution) into the stationary (mobile) phase relative to the situation prevailing at infinite dilution. These predictions are consistent with the increased retention observed with increased polymer sample loading.[4]

At higher polymer concentrations higher order osmotic virial coefficients, B_{la} ($l = 3, 4, ...$; a = m, s) must be included to obtain K_3. The B_{la} ($l = 3, 4, ...$; a = m, s) are estimated by applying the Carnahan–Starling equations of state for hard spheres and disks[6,7] to obtain the osmotic pressure expansions for polymer molecules in the mobile and stationary phases, respectively, and from these virial expansions the capacity factor K_3. However when the polymer concentration becomes sufficiently large so that $\Phi > M^{-0.8}$, the virial expansions become inaccurate and an analysis based on scaling arguments[1] must be invoked to obtain the osmotic pressure equations of state and ultimately K_3 for polymer solutions in the semidilute concentration region.

In section III, we apply and discuss the analysis developed in section II to obtain and interpret theoretical $K_3 - X_{1m}$ isotherms for selected values of M and C_3. The transition from high to low retention is predicted to broaden as C_3 is increased, and its implications on chromatographic measurements of the retention time are briefly considered. The broadening of the isotherm reflects the transition from the region of dilute to semidilute polymer solutions. We also investigate the dependence of K_3 on C_3 for fixed mobile-phase compositions and discuss the possibility of employing chromatographic measurements to determine the second osmotic virial coefficient for polymer moelcules in dilute solution by extrapolation to obtain the limiting slope of $\ln K_3$ vs. $C_3/(1 + K_3)$ plots in the limit where the mobile-phase composition is sufficiently enriched in the better polymer solvent to render $K_3 \rightarrow 0$.

II. Derivation of the Capacity Factor for Flexible, Chainlike Polymer Molecules as a Function of Polymer Concentration

Here we derive an expression for the capacity factor K_3 for flexible, chainlike polymer molecules with degree of polymerization M distributed between a binary solvent mobile phase and a stationary phase composed of a homogeneous planar surface and an adsorbed solvent monolayer. Application of the McMillan–Mayer theory[5] to dilute polymer solutions ultimately leads to a virial expansion for the osmotic pressure Π_a (a = m, s) in each chromatographic phase

$$\Pi_a/kT = C_{3a}/M + \sum_{l\geq 2} B_{la}(X_{1a},M,T)(C_{3a}/M)^l \qquad (1)$$

where C_{3a} (a = m, s) is the number density of monomers comprising the polymer chains in each chromatographic phase, C_{3a}/M is the number density of polymer coils, and the $B_{la}(X_{1a},M,T)$ ($l = 2, 3, ...$; a = m, s) are the osmotic virial coefficients which depend on M, T, and the solvent composition on a volume fraction basis X_{1a} (a = m, s) in each chromatographic phase. Even in binary solvent–polymer solute systems the B_{la} will depend on the composition of a single solvent at least to the level of approximation applied in the subsequent analysis. The chemical potential of the polymer solute μ_{3a} (a = m, s) in each chromatographic phase can be obtained by integrating the thermodynamic relationship

$$\left(\frac{\partial(\mu_{3a}/kT)}{\partial(C_{3a}/M)}\right)_{X_{1a},X_{2a},T} = \left(\frac{C_{3a}}{M}\right)^{-1}\left(\frac{\partial(\Pi_a/kT)}{\partial(C_{3a}/M)}\right)_{X_{1a},X_{2a},T} \qquad (2)$$

to obtain

$$\mu_{3a}/kT = \ln N_{3a} + \ln K_a + \sum_{l\geq 1}(l + 1/l)B_{l+1a}(C_{3a}/M)^l \qquad (3)$$

where $N_{3a} = C_{3a}V_a/M$ represents the number of polymer molecules in each chromatographic phase of volume V_a and K_a denotes an integration constant which is determined from the chemical potential in the limit of infinite dilution of solute. The equilibrium distribution of solute between the chromatographic phases is obtained by setting $\mu_{3m} = \mu_{3s}$, and after slight rearrangement

$$K_3 \equiv N_{3s}/N_{3m} = K_3^\infty \exp[\sum_{l\geq 1}(l + 1/l)\{B_{l+1m}(C_{3m}/M)^l - B_{l+1s}(C_{3s}/M)^l\}] \qquad (4)$$

where

$$K_3^\infty \equiv K_s/K_m \equiv \lim_{N_{3s}\rightarrow 0, N_{3m}\rightarrow 0} N_{3s}/N_{3m} \qquad (5)$$

represents the capacity factor in the limit of infinite dilution of solute. An explicit expression for K_3^∞ has been derived from a previous analysis[3] and will be discussed later.

The concentrations C_{3m}/M and C_{3s}/M can be eliminated from eq 4 by using $K_3 = N_{3s}/N_{3m} = C_{3s}V_s/C_{3m}V_m$ and invoking mass conservation of polymer solute, $C_3V_3/M = ((C_{3m}/M)(V_m/(V_m + V_s)) + (C_{3s}/M)(V_s/(V_m + V_s)))V_3$, to express

$$C_{3m} = \frac{1 + \phi}{1 + K_3}C_3 \qquad C_{3s} = \frac{1 + \phi}{1 + K_3}\frac{K_3}{\phi}C_3 \qquad (6)$$

where $\phi = V_s/V_m$ is the chromatographic phase ratio and C_3V_3 represents the total number of polymer solute monomers introduced into the chromatographic system. Hence eq 4 can be expressed as

$$K_3/K_3^{\infty} = \exp\left[\sum_{l\geq 1}\left(\frac{l+1}{l}\right)\left(\frac{1+\phi}{1+K_3}\right)^l (C_3/M)^l \times \{B_{l+1\mathrm{m}} - (K_3/\phi)^l B_{l+1\mathrm{s}}\}\right] \quad (7)$$

Thus corrections to the capacity factor which result from solute–solute interactions can be obtained provided the osmotic virial coefficients $B_{l+1\mathrm{a}}$ (a = m, s) are available or can be estimated. When C_3, the concentration of polymer monomers loaded into the column, is sufficiently dilute so that $[(1 + \phi)/(1 + K_3)](C_3/M) \ll 1$ only the leading term in the virial expansion summation contributes appreciably and

$$K_3/K_3^{\infty} = \exp\left[2\left(\frac{1+\phi}{1+K_3}\right)(C_3/M)\{B_{2\mathrm{m}} - (K_3/\phi)B_{2\mathrm{s}}\}\right] \quad (8)$$

These results for K_3/K_3^{∞} are only valid when the virial expansion for the osmotic pressure holds. If, however, the volume fraction of polymer in either chromatographic phase solution becomes sufficiently large, the osmotic pressure becomes independent of M and scaling arguments must be employed to determine the limiting form of the equation of state[1] and ultimately the capacity factor.

We estimate $B_{2\mathrm{m}}$, $B_{2\mathrm{s}}$, and the higher osmotic virial coefficients by extending a previously developed theory[3] of the equilibrium distribution of isolated, flexible polymer molecules between a binary solvent mobile phase and a stationary phase consisting of a homogeneous planar surface and an adsorbed solvent monolayer. In this model, a polymer molecule located in the mobile (stationary) phase is assumed to be a spherical (thin, cylindrical) gel of uniform polymer segment density which is penetrated by the solvents and has a diameter R_m (diameter R_s and thickness d). The equilibrium values of R_m and R_s are those which minimize the total Helmholtz free energies of an isolated polymer molecule and associated solvents in the mobile and stationary phases, respectively. We assume that the free energy in each chromatographic phase consists of a sum of an entropic, energetic, and elastic contribution associated with the swelling of the polymer from its equilibrium dimensions by solvent uptake in favorable solvent environments. The configurational behavior of the isolated polymer-associated solvent systems in each chromatographic phase was estimated with Flory–Huggins lattice statistics.[5,8] Nearest-neighbor solvent–solvent, solvent–polymer segment, polymer segment–polymer segment, solvent–surface, and polymer segment–surface interactions were included by using the Bragg–Williams random-mixing approximation.[5,8] The molecular volumes of the solvents and the monomer segments were assumed to be comparable, and the transfer of a polymer molecule from one chromatographic phase to the other was assumed to proceed with the concomitant displacement of M solvent molecules from the phase entered by the polymer.

The second osmotic virial coefficients $B_{2\mathrm{m}}$ and $B_{2\mathrm{s}}$ are given by[9]

$$B_{2\mathrm{m}} \equiv B_{2\mathrm{m}}(X_{1\mathrm{m}},M,T) = (-1/2)\int_0^{\infty}\{\exp[-W_\mathrm{m}(r,X_{1\mathrm{m}},M,T)/kT] - 1\}4\pi r^2\,\mathrm{d}r \quad (9a)$$

and

$$B_{2\mathrm{s}} \equiv B_{2\mathrm{s}}(X_{1\mathrm{s}},M,T) = (-d/2)\int_0^{\infty}\{\exp[-W_\mathrm{s}(r,X_{1\mathrm{s}},M,T)/kT] - 1\}2\pi r\,\mathrm{d}r \quad (9b)$$

where $W_\mathrm{a}(r,X_{1\mathrm{a}},M,T)$; a = m, s are potentials of mean force which represent the reversible work required to bring two isolated polymer molecules from $r = \infty$ to a distance r between their centers in the corresponding chromatographic phase. The potentials of mean force depend on T, M, and the solvent composition, and they are evaluated from the change in the Helmholtz free energy which occurs when two polymer molecules attain an overlapping configuration where $0 \leq r \leq R_\mathrm{a}$ (a = m, s) starting from an infinite separation. We also assume that the overlap occurs without distortion of the molecular configuration or polymer segment distribution and that the solvent composition for mixed solvents remains equal to the bulk solution value. If $V_{\mathrm{ao}}(r)$ represents the overlap volume of two polymer molecules whose centers are separated by a distance $0 \leq r \leq R_\mathrm{a}$ (a = m, s), then the potential of mean force, which is the free energy change resulting from the overlap, can be estimated from[5]

$$\frac{W_\mathrm{a}(r,X_{1\mathrm{a}},M,T)}{kT} = (\Delta A_\mathrm{a}^{(2)} + \Delta A_\mathrm{a}^{(0)} - 2\Delta A_\mathrm{a}^{(1)})/kT$$

with

$$\Delta A_\mathrm{a}^{(i)}/kT = \sum_{j=1}^{2} N_{j\mathrm{a}}^{(i)} \ln X_{j\mathrm{a}}^{(i)} + (\chi_{12}^{\mathrm{a}}X_{1\mathrm{a}}^{(i)}X_{2\mathrm{a}}^{(i)} + \chi_{13}^{\mathrm{a}}X_{1\mathrm{a}}^{(i)}X_{3\mathrm{a}}^{(i)} + \chi_{23}^{\mathrm{a}}X_{2\mathrm{a}}^{(i)}X_{3\mathrm{a}}^{(i)})V_{\mathrm{ao}}(r)/(\pi b^3/6)$$

where

$$X_{l\mathrm{a}}^{(i)} = N_{l\mathrm{a}}^{(i)}(iM + \sum_{j=1}^{2} N_{j\mathrm{a}}^{(i)})^{-1} \qquad l = 1, 2 \quad (10a)$$

$$X_{3\mathrm{a}}^{(i)} = iM(iM + \sum_{j=1}^{2} N_{j\mathrm{a}}^{(i)})^{-1} \qquad i = 0, 1, 2 \quad (10b)$$

are the volume fractions of solvents 1 and 2 and polymer segments when $N_{1\mathrm{a}}^{(i)}$ and $N_{2\mathrm{a}}^{(i)}$ (i = 0, 1, 2) type 1 and 2 solvent molecules are present in the overlap volume V_{ao} when polymer segments are simultaneously contributed from either i = 0, 1, or 2 polymer molecules, respectively. The quantity $\pi b^3/6$ represents the volume of a monomer unit and is assumed to be comparable to the molecular volumes of both type 1 and 2 solvent molecules. Also the $\chi_{ij}^{\mathrm{a}} = -(C_\mathrm{a}/kT)[(W_{ii} + W_{jj})/2 - W_{ij}]$ (i and j = 1, 2, 3; a = m, s) are reduced pair interchange energies between solvent molecules (1 or 2) and/or polymer segments (3), where W_{ij} represents the nearest-neighbor pair interaction energy between species i and j, and C_a (a = m, s) is the lattice coordination number for each chromatographic phase (C_m = 6 and C_s = 5 for a cubic lattice). We also assume that $X_{3\mathrm{a}}^{(2)} = 2X_{3\mathrm{a}}^{(1)}X_{i\mathrm{a}}^{(2)} = [(1 - 2X_{3\mathrm{a}}^{(1)})/(1 - X_{3\mathrm{a}}^{(1)}]X_{i\mathrm{a}}^{(2)}$, i = 1, 2, and $X_{i\mathrm{a}}^{(1)} = (1 - X_{3\mathrm{a}}^{(1)})X_{i\mathrm{a}}^{(0)}$, i = 1, 2. Substitution into eq 10 gives after some reduction and use of the approximation $\ln(1 - u) \simeq -u - u^2/2$ for $u \equiv X_{3\mathrm{a}}^{(1)}$ and $2X_{3\mathrm{a}}^{(1)}$:

$$\begin{aligned} W_\mathrm{a}(r,X_{1\mathrm{a}},M,T) &= 2V_{\mathrm{ao}}(r)(\pi b^3/6)^{-1}g_\mathrm{a}(X_{1\mathrm{a}})(X_{3\mathrm{a}}^{(1)})^2 \qquad 0 \leq r \leq R_\mathrm{a} \\ &= 0 \qquad r > R_\mathrm{a} \end{aligned} \quad (11a)$$

where

$$g_\mathrm{a}(X_{1\mathrm{a}}) = 1/2 - X_{1\mathrm{a}}\chi_{13}^{\mathrm{a}} - (1 - X_{1\mathrm{a}})\chi_{23}^{\mathrm{a}} + X_{1\mathrm{a}}(1 - X_{1\mathrm{a}})\chi_{12}^{\mathrm{a}} \qquad \mathrm{a = m, s} \quad (11b)$$

and where we have identified $X_{1\mathrm{a}}^{(0)} = X_{1\mathrm{a}}$ and $X_{2\mathrm{a}}^{(0)} = X_{2\mathrm{a}} = 1 - X_{1\mathrm{a}}$ with the volume fractions of the mixed solvents in the absence of polymer. The overlap volume for two interpenetrating spherical polymer coils in the mobile phase is from elementary geometrical considerations[5,8]

$$V_{\mathrm{mo}}(r) = (\pi R_{\mathrm{m}}^3/6)[1 - (3/2)(r/R_{\mathrm{m}}) + (1/2) \times (r/R_{\mathrm{m}})^3] \qquad 0 \le r \le R_{\mathrm{m}} \quad (12)$$

The overlap volume for two interpenetrating pancake-shaped polymer molecules is obtained from the area common to two intersecting circles

$$V_{\mathrm{so}}(r) = (\pi R_{\mathrm{s}}^2 d/4)[1 - 2\pi^{-1}\{(r/R_{\mathrm{s}}) \times (1 - r^2/R_{\mathrm{s}}^2)^{1/2} + \arcsin (r/R_{\mathrm{s}})\}] \qquad 0 \le r \le R_{\mathrm{s}} \quad (13)$$

Substitution of eq 11, 12, and 13 into eq 9a and 9b gives upon introduction of the reduced integration variable $x = r/R_{\mathrm{a}}$ (a = m, s)

$$B_{2\mathrm{m}}(X_{1\mathrm{m}},M,T) = -2\pi R_{\mathrm{m}}^3 \int_0^1 \mathrm{d}x\, x^2\{\exp[-2(b/R_{\mathrm{m}})^3 M^2 g_{\mathrm{m}}(X_{1\mathrm{m}})(1 - (3x - x^2)/2)] - 1\} = 2\pi R_{\mathrm{m}}^3 F(X_{1\mathrm{m}},M,T)/3 \quad (14)$$

and

$$B_{2\mathrm{s}}(X_{1\mathrm{s}},M,T) = -\pi R_{\mathrm{s}}^2 d \int_0^1 \mathrm{d}x\, x\{\exp[-2(2b^3/3R_{\mathrm{s}}^2 d)M^2 g_{\mathrm{s}}(X_{1\mathrm{s}})(1 - 2\pi^{-1}(x(1 - x^2)^{1/2} + \arcsin x))] - 1\} = \pi R_{\mathrm{s}}^2 d H(X_{1\mathrm{s}},M,T)/2 \quad (15)$$

where we have also expressed $X_{3\mathrm{m}}^{(1)} \equiv M(b/R_{\mathrm{m}})^3$ and $X_{3\mathrm{s}}^{(1)} \equiv M(2b/3d)(b/R_{\mathrm{s}})^2$ and have defined

$$F \equiv F(X_{1\mathrm{m}},M,T) = 1 - 3\int_0^1 \mathrm{d}x\, x^2 \exp[-2(b/R_{\mathrm{m}})^3 M^2 g_{\mathrm{m}}(X_{1\mathrm{m}})(1 - (3x - x^2)/2)] \quad (16)$$

and

$$H \equiv H(X_{1\mathrm{s}},M,T) = 1 - 2\int_0^1 \mathrm{d}x\, x \exp[-2(2b^3/3R_{\mathrm{s}}^2 d)M^2 g_{\mathrm{s}}(X_{1\mathrm{s}})(1 - 2\pi^{-1}(x(1 - x^2)^{1/2} + \arcsin x))] \quad (17)$$

The equilibrium dimensions of an isolated polymer molecule in either chromatographic phase are expanded relative to its unperturbed dimensions in good solvent environments.[8] In particular

$$R_{\mathrm{m}}^3 = g_{\mathrm{m}}^{3/5}(X_{1\mathrm{m}})M^{1.8}b^3 = g_{\mathrm{m}}^{3/5}(X_{1\mathrm{m}})M^{0.3}\langle R_{\mathrm{m}}^2\rangle_0^{3/2} \quad (18)$$

and

$$R_{\mathrm{s}}^2 = g_{\mathrm{s}}^{1/2}(X_{1\mathrm{s}})M^{1.5}b^2 = g_{\mathrm{s}}^{1/2}(X_{1\mathrm{s}})M^{0.5}\langle R_{\mathrm{s}}^2\rangle_0 \quad (19)$$

where $g_{\mathrm{a}}(X_{1\mathrm{a}}) > 0$ (a = m, s) for good solvent environments and $\langle R_{\mathrm{m}}^2\rangle_0 = \langle R_{\mathrm{s}}^2\rangle_0 = Mb^2$ represent the equilibrium average square end-to-end separation of an unperturbed polymer coil in three and two dimensions. The solvent environment is favorable for polymer molecules in chromatographic phase a (a = m, s) whenever

$$X_{1\mathrm{a}} \ge \{\chi_{12}^{\mathrm{a}} + \chi_{23}^{\mathrm{a}} - \chi_{13}^{\mathrm{a}} - ((\chi_{12}^{\mathrm{a}} + \chi_{23}^{\mathrm{a}} - \chi_{13}^{\mathrm{a}})^2 + 4\chi_{12}^{\mathrm{a}}(1/2 - \chi_{23}^{\mathrm{a}}))^{1/2}\}/2\chi_{12}^{\mathrm{a}} \qquad \chi_{12}^{\mathrm{a}} \neq 0$$

$$X_{1\mathrm{a}} \ge (\chi_{23}^{\mathrm{a}} - 1/2)/(\chi_{23}^{\mathrm{a}} - \chi_{13}^{\mathrm{a}}) \qquad \chi_{12}^{\mathrm{a}} = 0 \quad (20)$$

If only a single solvent were present (e.g., 1), then the condition for a favorable solvent environment reduces to $g_{\mathrm{a}}(X_{1\mathrm{a}} = 1) = 1/2 - \chi_{13}^{\mathrm{a}} > 0$, which is the familiar Flory criterion.[8]

Substitution of eq 18 and 19 into eq 14 and 15, respectively, gives

$$B_{2\mathrm{m}} = 2\pi b^3 g_{\mathrm{m}}^{3/5}(X_{1\mathrm{m}})M^{1.8}F/3 \quad (21)$$

with

$$F \equiv F(X_{1\mathrm{m}},M,T) = 1 - 3\int_0^1 \mathrm{d}x\, x^2 \exp[-2g_{\mathrm{m}}^{2/5}(X_{1\mathrm{m}})M^{0.2}(1 - (3x - x^2)/2)]$$

and

$$B_{2\mathrm{s}} = (\pi/2)b^2 d g_{\mathrm{s}}^{1/2}(X_{1\mathrm{s}})M^{1.5}H \quad (22)$$

with

$$H \equiv H(X_{1\mathrm{s}},M,T) = 1 - 2\int_0^1 \mathrm{d}x\, x \exp[-2(2b/3d)g_{\mathrm{s}}^{1/2}(X_{1\mathrm{s}})M^{1/2}(1 - 2\pi^{-1}[x(1 - x^2)^{1/2} + \arcsin x])]$$

The functions $1 - (3/2)x + x^2/2$ and $1 - 2\pi^{-1}[x(1 - x^2)^{1/2} + \arcsin x]$ are nonnegative over the range of integration $0 \le x \le 1$, and for good solvent environments $0 < F(X_{1\mathrm{m}},M,T) \le 1$ and $0 < H(X_{1\mathrm{s}},M,T) \le 1$. Furthermore $F(X_{1\mathrm{m}},M,T) \to 1$ and $H(X_{1\mathrm{s}},M,T) \to 1$ as $M \to \infty$, and in this limit $B_{2\mathrm{m}} \to (2/3)\pi R_{\mathrm{m}}^3$ and $B_{2\mathrm{s}} \to \pi R_{\mathrm{s}}^2 d/2$, which are the second virial coefficients for impenetrable hard spheres and thin disks, respectively.[7] In general, for good solvents $B_{2\mathrm{m}}$ and $B_{2\mathrm{s}}$ are both positive and interpenetration of different polymer molecules in dilute solution is discouraged.

More sophisticated models have been employed for determination of the second osmotic virial coefficient for dilute polymer solutions.[9,10] However, we do not consider them here because they would exceed the scope of the analysis utilized to obtain K_3^{∞}.[3]

For positive $B_{2\mathrm{m}}$ and $B_{2\mathrm{s}}$ we observe from eq 8 that intermolecular polymer–polymer pair interactions decrease (increase) retention relative to K_3^{∞} when high ($K_3 > 1$) (low $K_3 < 1$) retention in the stationary phase prevails. These tendencies suggest a simple physical interpretation. At sufficiently high polymer concentration and when $K_3 > 1$, repulsive intermolecular interactions between different pairs of precipitated polymer molecules tend to promote entry into the mobile phase relative to the situation at infinite dilution. However when $K_3 < 1$, repulsive intermolecular polymer–polymer pair interactions in the mobile phase can promote precipitation into the stationary phase relative to the infinite dilution limit. Clearly at a mobile-phase composition where $B_{2\mathrm{m}} = (K_3/\phi)B_{2\mathrm{s}}$ a balance exists between these two opposing tendencies to retard and promote polymer retention, and the retention behavior corresponds to that predicted at infinite dilution.

Equation 8 applies only when $B_{2\mathrm{a}}C_{3\mathrm{a}}/M \ll 1$ (a = m, s); otherwise, higher order terms in the virial summation of eq 4 must be included and this requires evaluation of the higher order osmotic virial coefficients. If the polymer molecules in the mobile and stationary phase behaved respectively as perfectly impenetrable hard spheres and hard disks, then the third[11] and higher order osmotic virial coefficients, $B_{l+1\mathrm{m}}$ and $B_{l+1\mathrm{s}}$ ($l \ge 2, 3, ...$), might be reasonably approximated by the virial coefficients for a homogeneous hard sphere and hard disk fluid[6,7]

$$B_{3\mathrm{m}} = \tfrac{5}{8}B_{2\mathrm{m}}^2,\ B_{4\mathrm{m}} = \tfrac{9}{32}B_{2\mathrm{m}}^3,\ B_{5\mathrm{m}} = \tfrac{7}{64}B_{2\mathrm{m}}^4,\ B_{6\mathrm{m}} \simeq \tfrac{5}{128}B_{2\mathrm{m}}^5, \text{ etc.}$$

$$B_{2\mathrm{s}} = \tfrac{3}{4}B_{2\mathrm{s}}^2,\ B_{4\mathrm{s}} = \tfrac{1}{2}B_{2\mathrm{s}}^3,\ B_{5\mathrm{s}} = \tfrac{5}{16}B_{2\mathrm{s}}^4,\ B_{6\mathrm{s}} \simeq \tfrac{3}{16}B_{2\mathrm{s}}^5, \text{ etc.}$$

Furthermore in terms of the dimensionless parameters $Y_{\mathrm{m}} = B_{2\mathrm{m}}C_{3\mathrm{m}}/4M$ and $Y_{\mathrm{s}} = B_{2\mathrm{s}}C_{3\mathrm{s}}/2M$

$$\Pi_{\mathrm{m}}/kT \simeq (C_{3\mathrm{m}}/M)[1 + 4Y_{\mathrm{m}} + 10Y_{\mathrm{m}}^2 + 18Y_{\mathrm{m}}^3 + 28Y_{\mathrm{m}}^4 + 40Y_{\mathrm{m}}^5 + ...] \simeq (C_{3\mathrm{m}}/M)[1 + \sum_{l=1}^{\infty}(l^2 + 3l)Y_{\mathrm{m}}^l] \quad (23)$$

and

$$\Pi_{\mathrm{s}}/kT \simeq (C_{3\mathrm{s}}/M)[1 + 2Y_{\mathrm{s}} + 3Y_{\mathrm{s}}^2 + 4Y_{\mathrm{s}}^3 + 5Y_{\mathrm{s}}^4 + 6Y_{\mathrm{s}}^5 + ...] \simeq (C_{3\mathrm{s}}/M)[1 + \sum_{l=1}^{\infty}(l + 1)Y_{\mathrm{s}}^l] \quad (24)$$

Comparison of eq 23 and 24 with eq 1 indicates that the higher order osmotic virial coefficients can be related to $B_{2\mathrm{m}}$ and $B_{2\mathrm{s}}$ by

$$B_{l+1\mathrm{m}}(X_{1\mathrm{m}},M,T) = (l^2 + 3l)\left[\frac{B_{2\mathrm{m}}(X_{1\mathrm{m}},M,T)}{4}\right]^l \qquad l \geq 1$$

and

$$B_{l+1\mathrm{s}}(X_{1\mathrm{s}},M,T) = (l + 1)\left[\frac{B_{2\mathrm{s}}(X_{1\mathrm{s}},M,T)}{2}\right]^l \qquad l \geq 1$$

can be performed to give the Carnahan–Starling equations of state for hard spheres[6] and disks[7,12]

$$\Pi_{\mathrm{m}}/kT = (C_{3\mathrm{m}}/M) \times [1 + Y_{\mathrm{m}} + Y_{\mathrm{m}}^2 - Y_{\mathrm{m}}^3]/(1 - Y_{\mathrm{m}})^3 \qquad 0 \leq Y_{\mathrm{m}} < 1 \quad (25)$$

and

$$\Pi_{\mathrm{s}}/kT = (C_{3\mathrm{s}}/M)[1 - Y_{\mathrm{s}}]^{-2} \qquad 0 \leq Y_{\mathrm{s}} < 1 \quad (26)$$

The dimensionless parameters

$$Y_{\mathrm{m}} = B_{2\mathrm{m}}C_{3\mathrm{m}}/4M = (\pi/6)b^3C_3g_{\mathrm{m}}^{3/5}(X_{1\mathrm{m}})M^{0.8}(1 + \phi)F(1 + K_3)^{-1} = B_{2\mathrm{m}}C_3(1 + \phi)/4M(1 + K_3) \quad (27)$$

and

$$Y_{\mathrm{s}} = B_{2\mathrm{s}}C_{3\mathrm{s}}/2M = (\pi/6)b^3(3d/2b)C_3g_{\mathrm{s}}^{1/2}(X_{1\mathrm{s}})M^{0.5}(1 + \phi)H(K_3/\phi)(1 + K_3)^{-1} = B_{2\mathrm{s}}C_3(1 + \phi)K_3/(2M\phi(1 + K_3)) \quad (28)$$

increase with M for a given C_3, ϕ, and K_3, indicating that higher order virial corrections to the osmotic pressure become more important for solutions and precipitated monolayers of higher molecular weight homopolymers. Clearly $(B_{2\mathrm{m}}/4M)(1 + \phi)C_3$ and $(B_{2\mathrm{s}}/2M)[(1 + \phi)/\phi]C_3$ respectively represent the maximum values of Y_{m} and Y_{s}.

When $Y_{\mathrm{m}} \geq 1$ or $Y_{\mathrm{s}} \geq 1$ or when Y_{m} or Y_{s} becomes sufficiently large so that the virial expansion of the osmotic pressure becomes either inapplicable or inaccurate, it is necessary to modify the osmotic pressure so that it becomes independent of M. The correct limiting equations of state can be deduced from a scaling analysis[1] by setting

$$\Pi_{\mathrm{m}}/kT = \frac{C_{3\mathrm{m}}}{M}f(Y_{\mathrm{m}}/F(X_{1\mathrm{m}},M,T)) \quad (29)$$

or

$$\Pi_{\mathrm{s}}/kT = \frac{C_{3\mathrm{s}}}{M}h(Y_{\mathrm{s}}/H(X_{1\mathrm{s}},M,T)) \quad (30)$$

where $f(Y_{\mathrm{m}}/F(X_{1\mathrm{m}},M,T))$ and $h(Y_{\mathrm{s}}/H(X_{1\mathrm{s}},M,T))$ are functions whose asymptotic forms for large Y_{m}/F and Y_{s}/H are $f(Y_{\mathrm{m}}/F) = A(Y_{\mathrm{m}}/F)^{\lambda}$ and $h(Y_{\mathrm{s}}/H) = B(Y_{\mathrm{s}}/H)^{\delta}$, respectively, where λ and δ are selected to render the osmotic pressures in each chromatographic phase indepenent of M and A and B are constants independent of M. Thus $\Pi_{\mathrm{m}}/kT \rightarrow A(C_{3\mathrm{m}}/M)[(\pi/6)b^3C_{3\mathrm{m}}g_{\mathrm{m}}^{3/5}M^{0.8}]^{\lambda}$ and $\Pi_{\mathrm{s}}/kT \rightarrow B(C_{3\mathrm{s}}/M)[(\pi/6)b^3((d/2b)/3)C_{3\mathrm{s}}g_{\mathrm{s}}^{1/2}M^{0.5}]^{\delta}$ and the selections $\lambda = 5/4$ and $\delta = 2$ will respectively ensure that Π_{m}/kT and Π_{s}/kT are independent of M. Furthermore

$$(\Pi_{\mathrm{m}}/kT)[(\pi/6)b^3] = Ag_{\mathrm{m}}^{3/4}\Phi_{\mathrm{m}}^{9/4} \quad (31)$$

and

$$(\Pi_{\mathrm{s}}/kT)[(\pi/6)b^3] = Bg_{\mathrm{s}}^2(3d/2b)^2\Phi_{\mathrm{s}}^3 \quad (32)$$

where $\Phi_{\mathrm{a}} = (\pi/6)b^3C_{3\mathrm{a}}$ (a = m, s) represents the volume fraction of monomers in each chromatographic phase.

We assume that Π_{m}/kT is given by eq 25 for $0 \leq Y_{\mathrm{m}} \leq Y_{\mathrm{m}}^*$ where $0 < Y_{\mathrm{m}}^* < 1$ and by eq 31 for $Y_{\mathrm{m}} \geq Y_{\mathrm{m}}^*$ and similarly that Π_{s}/kT is given by eq 26 for $0 \leq Y_{\mathrm{s}} \leq Y_{\mathrm{s}}^*$ where $0 < Y_{\mathrm{s}}^* < 1$ and by eq 32 for $Y_{\mathrm{s}} \geq Y_{\mathrm{s}}^*$. The constants A and B are chosen so that the osmotic pressures given by eq 25 and 31 and eq 26 and 32 are continuous at $Y_{\mathrm{m}} = Y_{\mathrm{m}}^*$ and $Y_{\mathrm{s}} = Y_{\mathrm{s}}^*$, respectively. Hence $A \equiv (1 - Y_{\mathrm{m}}^*)^{-3}[1 + Y_{\mathrm{m}}^* + Y_{\mathrm{m}}^{*2} - Y_{\mathrm{m}}^{*3}]\ (Y_{\mathrm{m}}^*/F)^{-5/4}$ and $B \equiv [1 - Y_{\mathrm{s}}^*]^{-2}(H/Y_{\mathrm{s}}^*)^2$, and

$$\Pi_{\mathrm{m}}/kT = (C_{3\mathrm{m}}/M)[(1 + Y_{\mathrm{m}} + Y_{\mathrm{m}}^2 - Y_{\mathrm{m}}^3) \times (1 - Y_{\mathrm{m}})^{-3}\Theta(Y_{\mathrm{m}}^* - Y_{\mathrm{m}}) + (1 + Y_{\mathrm{m}}^* + Y_{\mathrm{m}}^{*2} - Y_{\mathrm{m}}^{*3})(1 - Y_{\mathrm{m}}^*)^{-3}(Y_{\mathrm{m}}/Y_{\mathrm{m}}^*)^{5/4}\Theta(Y_{\mathrm{m}} - Y_{\mathrm{m}}^*)] \quad (33)$$

and

$$\Pi_{\mathrm{s}}/kT = \left(\frac{C_{3\mathrm{s}}}{M}\right)[(1 - Y_{\mathrm{s}})^{-2}\Theta(Y_{\mathrm{s}}^* - Y_{\mathrm{s}}) + (1 - Y_{\mathrm{s}}^*)^{-2}(Y_{\mathrm{s}}/Y_{\mathrm{s}}^*)^2\Theta(Y_{\mathrm{s}} - Y_{\mathrm{s}}^*)] \quad (34)$$

where $\Theta(x) = 1$, $x > 0$, and 0, $x < 0$, is the Heaviside unit step function. The selections of Y_{a}^* (a = m, s) are arbitrary within the range $0 < Y_{\mathrm{a}}^* < 1$ and reflect the uncertainty about the osmotic pressure equation of state for polymer solutions as the transition from dilute to semidilute solutions in both three and two dimensions occurs.[1]

The chemical potentials $\mu_{3\mathrm{a}}$ (a = m, s) are obtained by integrating eq 2 by using eq 33 and 34, respectively, and are given by

$$\mu_{3\mathrm{m}}/kT = \ln K_{\mathrm{m}} + \ln N_{3\mathrm{m}} + P(Y_{\mathrm{m}},Y_{\mathrm{m}}^*) \quad (35)$$

where

$$P(Y_{\mathrm{m}},Y_{\mathrm{m}}^*) \equiv Y_{\mathrm{m}}(8 - 9Y_{\mathrm{m}} + 3Y_{\mathrm{m}}^2) \times (1 - Y_{\mathrm{m}})^{-3}\Theta(Y_{\mathrm{m}}^* - Y_{\mathrm{m}}) + [Y_{\mathrm{m}}^*(8 - 9Y_{\mathrm{m}}^* + 3Y_{\mathrm{m}}^{*2})(1 - Y_{\mathrm{m}}^*)^{-3} + (9/5)(1 + Y_{\mathrm{m}}^* + Y_{\mathrm{m}}^{*2} - Y_{\mathrm{m}}^{*3}) \times (1 - Y_{\mathrm{m}}^*)^{-3}((Y_{\mathrm{m}}/Y_{\mathrm{m}}^*)^{5/4} - 1)]\Theta(Y_{\mathrm{m}} - Y_{\mathrm{m}}^*)$$

and

$$\mu_{3\mathrm{s}}/kT = \ln K_{\mathrm{s}} + \ln N_{3\mathrm{s}} + R(Y_{\mathrm{s}},Y_{\mathrm{s}}^*) \quad (36)$$

where

$$R(Y_{\mathrm{s}},Y_{\mathrm{s}}^*) = [(3Y_{\mathrm{s}} - 2Y_{\mathrm{s}}^2)(1 - Y_{\mathrm{s}})^{-2} - \ln (1 - Y_{\mathrm{s}})]\Theta(Y_{\mathrm{s}}^* - Y_{\mathrm{s}}) + [(3Y_{\mathrm{s}}^* - 2Y_{\mathrm{s}}^{*2})(1 - Y_{\mathrm{s}}^*)^{-2} - \ln (1 - Y_{\mathrm{s}}^*) + (3/2)(1 - Y_{\mathrm{s}}^*)^{-2}((Y_{\mathrm{s}}/Y_{\mathrm{s}}^*)^2 - 1)]\Theta(Y_{\mathrm{s}} - Y_{\mathrm{s}}^*)$$

and K_{a} (a = m, s) has been introduced in eq 3. At equilibrium $\mu_{3\mathrm{m}} = \mu_{3\mathrm{s}}$, and equating eq 35 and 36 gives the capacity factor

$$K_3 = \frac{N_{3\mathrm{s}}}{N_{3\mathrm{m}}} = K_3^{\infty} \exp[P(Y_{\mathrm{m}},Y_{\mathrm{m}}^*) - R(Y_{\mathrm{s}},Y_{\mathrm{s}}^*)] = K_3^{\infty} \exp[P(B_{2\mathrm{m}}C_3(1 + \phi)/4M(1 + K_3),Y_{\mathrm{m}}^*) - R(B_{2\mathrm{s}}C_3(1 + \phi)K_3/2M\phi(1 + K_3),Y_{\mathrm{s}}^*)] \quad (37)$$

where the latter form of eq 37 follows from eq 27 and 28 and demonstrates that K_3 can be determined implicitly from this equation as a function of M, C_3, solvent composition, and K_3^{∞}.

A previous analysis provides an explicit expression for the capacity factor K_3^{∞} at infinite dilution of polymer solute. When only the less polar, more favorable polymer solvent (e.g., 1, which is methylene chloride in the methylene chloride–methanol–polystyrene system) is appreciably retained in the stationary phase

$$K_3^{\infty} = \exp[|A|M(X_{1\mathrm{mc}} - X_{1\mathrm{m}})] \qquad (38)$$

where $X_{1\mathrm{m}}$ is the volume fraction of solvent 1 in the mobile phase, $X_{1\mathrm{mc}}$ is a critical mobile-phase composition, which for a given temperature depends on M and the various interaction energies, and $|A|$ depends primarily on the solvent–solvent and solvent–polymer segment pair interchange energies and has only a weak M dependence which vanishes as $M \to \infty$.

Specifically for a cubic lattice

$$X_{1\mathrm{mc}} = X_{1\mathrm{mc}}^{\infty} + aM^{-1/2} + bM^{-4/5} + cM^{-1} \qquad (39)$$

where

$$X_{1\mathrm{mc}}^{\infty} = 1 + (\Delta\epsilon(1,3;\mathrm{s})/kT + \ln(5/4))(\chi_{12}^{\mathrm{m}} + \chi_{13}^{\mathrm{m}} - \chi_{23}^{\mathrm{m}})^{-1} \qquad (40)$$

and

$$|A| = \chi_{12}^{\mathrm{m}} + \chi_{13}^{\mathrm{m}} - \chi_{23}^{\mathrm{m}} + dM^{-4/5} + eM^{-1} \qquad (41)$$

Here χ_{12}^{m}, χ_{13}^{m}, and χ_{23}^{m} respectively represent reduced pair interchange energies in the mobile phase between type 1 and 2 solvent molecules themselves and with polymer segments (3). Also $\Delta\epsilon(1,3;\mathrm{s})$ is the free energy for adhesion of a polymer segment onto the stationary-phase surface with the concomitant displacement of a type 1 solvent molecule. The coefficients a, b, c, d, and e depend on the interchange energies, and coefficients b, c, d, and e also depend weakly on the mobile-phase solvent composition. Explicit results for these coefficients are given by eq 37 and 44 of ref 3.

The $X_{1\mathrm{mc}}$–M dependence results from the molecular flexibility of the polymer molecule which permits it to alter its configurational geometry and size in response to its solvent and/or surface environments. For large M, K_3^{∞} is either extremely large when $0 < X_{1\mathrm{m}} < X_{1\mathrm{mc}}$ or extremely small when $X_{1\mathrm{mc}} < X_{1\mathrm{m}} \leq 1$, and since $X_{1\mathrm{mc}}$ increases monotonically with M to an asymptotic limiting value of $X_{1\mathrm{mc}}^{\infty}$ as $M \to \infty$, fractionation of homopolymers of different M is possible by chromatographic methods such as gradient elution HPLC or TLC in which the mobile-phase composition is systematically varied.

III. Potential Chromatographic Applications

Utilization of eq 38 for K_3^{∞} permits evaluation of K_3 or $\ln K_3$ from eq 37 for a homopolymer (e.g., polystyrene) of fixed M in a mixed solvent environment (e.g., methylene chloride–methanol) either as a function of $X_{1\mathrm{m}}$ for constant C_3 or as a function of C_3 for constant $X_{1\mathrm{m}}$. Figures 1 and 2 depict $\ln K_3$–$X_{1\mathrm{m}}$ isotherms calculated for (polystyrene) homopolymers with $M = 952$ (molecular weight = 1.0×10^4) and $M = 8570$ (molecular weight = 9.0×10^5) for various values of C_3 (i.e., polymer sample loadings) and for the reduced energies $\chi_{12}^{\mathrm{m}} = 0.5$, $\chi_{13}^{\mathrm{m}} = 0.0$, $\chi_{23}^{\mathrm{m}} = 1.0$, and $\Delta\epsilon(1,3;\mathrm{s})/kT = -0.1$. These energy parameter selections are consistent with methylene chloride–methanol being completely miscible and for pure methylene chloride (methanol) being a good (poor) solvent for polystyrene and with methylene chloride and polystyrene having similar affinities for the surface. We have also set $d = 2b/3$, $\phi = 0.53$, and $\pi b^3/6 = (125\ \mathrm{cm^3/mol})/N_0$, where N_0 is Avogadro's number, to represent the volume of a (styrene) monomer. The corresponding values of F, H, $X_{1\mathrm{mc}}$, and $|A|$ calculated from eq 21, 22, 39, and 41 with the above selections of M and the energy parameters are listed in the associated figure captions. The parameters g, F, and H were evaluated at the critical composition $X_{1\mathrm{mc}}$ for a given M and their variation with $X_{1\mathrm{m}}$ was neglected over the narrow composition range where the polymer solute undergoes the transition from very high to very low retention.

It is apparent from Figures 1 and 2 that the transition from high to low retention gradually broadens with increasing C_3. When C_3 is sufficiently dilute the isotherms broaden relative to the linear isotherm at infinite dilution because of repulsive polymer–polymer intermolecular interactions which promote enhanced entry into the mobile (stationary) phase when the solute retention is high (low). At higher values of C_3 the polymer solutions enter the semidilute region, considerable interpenetration of polymer

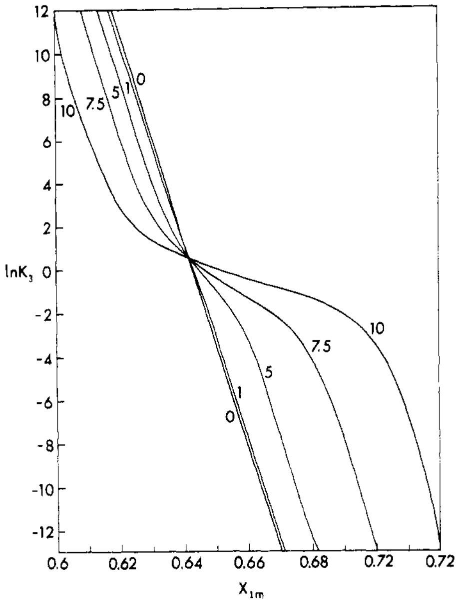

Figure 1. $\ln K_3$–$X_{1\mathrm{m}}$ isotherms calculated from eq 37 for polystyrene homopolymers with $M = 952$ and $\chi_{12}^{\mathrm{m}} = 0.5$, $\chi_{13}^{\mathrm{m}} = 0.0$, $\chi_{23}^{\mathrm{m}} = 1.0$, and $\Delta\epsilon(1,3;\mathrm{s})/kT = -0.1$ for the polymer loadings $C_3 = 0$, 1.0, 5.0, 7.5, and 10 g/cm^3. The isotherms were calculated by using $d = 2b/3$, $\phi = 0.53$, $(\pi/6)b^3 = (125\ \mathrm{cm^3/mol})/N_0$, where N_0 = Avogadro's number and $F = 0.3262$, $H = 0.8760$, $X_{1\mathrm{mc}} = 0.6428$, and $|A| = 0.5022$. The cutoff values $Y_{\mathrm{m}}^* = 0.5$ and $Y_{\mathrm{s}}^* = 0.5$ were also adopted.

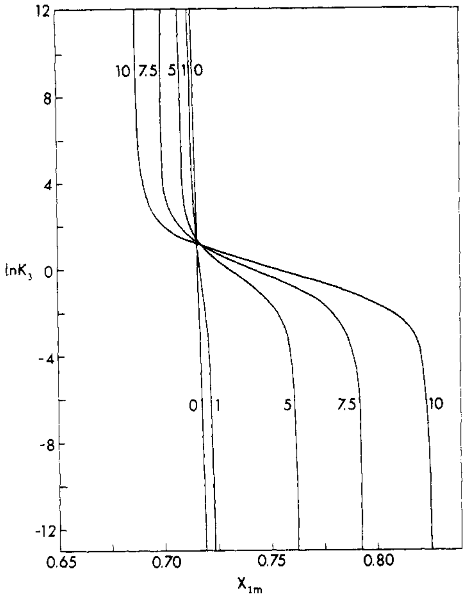

Figure 2. Same as Figure 1 except for the following: $M = 8570$, $F = 0.4255$, $H = 0.9392$, $X_{1\mathrm{mc}} = 0.7158$, and $|A| = 0.5002$.

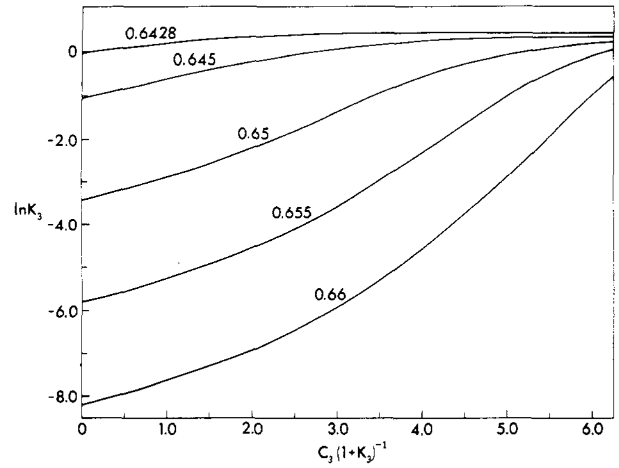

Figure 3. ln K_3–$C_3(1 + K_3)^{-1}$ isotherms evaluated from eq 37 for $M = 952$ and the mobile-phase compositions $X_{1m} = 0.645$, 0.650, 0.655, 0.660, and $X_{1m} = X_{1mc} = 0.6428$. The limiting slopes of the isotherms as $C_3 \rightarrow 0$ are directly proportional to the second osmotic virial coefficient by eq 42.

molecules occurs, and the enhanced number of polymer–polymer segment interactions continues to further mollify the transition from high to low retention.

Preliminary measurements of the retention time as a function of polymer loading have been conducted for polystyrene homopolymers by employing gradient elution HPLC with a methylene chloride–methanol mixed mobile phase and a chemically bonded C-18 stationary phase.[4] A general increase in retention time occurs with increasing C_3, and these observations are qualitatively consistent with the trends predicted from eq 37 and depicted in Figures 1 and 2. However, a quantitative comparison between experimental and theoretical capacity factors is not attempted here because the experimental gradient elution chromatographic results average the retention behavior over a range of X_{1m}, and theoretical comparisons would require a rather formidable integration of K_3^{-1} (see eq 37) over mobile-phase composition.[3,13]

When C_3 is sufficiently small $Y_m < Y_m^*$, $Y_s < Y_s^*$, and eq 37 becomes $\ln K_3 = \ln K_3^\infty + Y_m(8 - 9Y_m + 3Y_m^2)(1 - Y_m)^{-3} - (3Y_s - 2Y_s^2)(1 - Y_s)^{-2} + \ln (1 - Y_s)$, and as $C_3 \rightarrow 0$

$$\ln K_3 \simeq \ln K_3^\infty + (2B_{2m}/M)[1 - (B_{2s}K_3/B_{2m}\phi)]C_3(1 + \phi)(1 + K_3)^{-1} + \ldots \tag{42}$$

Thus the limiting slope as $C_3 \rightarrow 0$ of a plot of ln K_3 vs. $C_3(1 + K_3)^{-1}$ at a fixed value of X_{1m} in principle should provide an estimate of the second osmotic virial coefficient B_{2m}. Furthermore if ln K_3 vs. $C_3(1 + K_3)^{-1}$ plots are generated at several mobile-phase compositions that exceed X_{1mc}, then the limiting slope as $C_3 \rightarrow 0$ for the largest X_{1m} that can be employed to obtain a chromatographically measurable retention time or capacity factor should provide a reasonably direct estimate of $B_{2m}(X_{1m},M,T)$ since the limiting slope approaches $2B_{2m}(1 + \phi)/M$ when $(B_{2s}/B_{2m})K_3/\phi \ll 1$. A theoretical, graphical example of this method is depicted in Figure 3.

These considerations suggest the possibility of chromatographic measurement of the second osmotic virial coefficient and hence average molecular volume (recall eq 14, 18, and 21) of flexible polymer molecules in dilute solution. Ideally measurements of K_3 as a function of C_3 should be performed for mobile-phase composition(s) where retention is as small as possible while still permitting feasible experimental determination of the retention. Such experiments probably would become more difficult with increasing M because the transition from completely retained to unretained polymer becomes increasingly abrupt[2-4] (see Figures 1 and 2) and selection of an operational mobile-phase composition range becomes more difficult.

In the domain of semidilute solutions where C_3 is large enough to render $B_{2m}(1 + \phi)C_3/4M(1 + K_3) > Y_m^*$, eq 37 becomes $\ln K_3 = \ln K_3^\infty + Y_m^*(8 - 9Y_m^* + 3Y_m^{*2})(1 - Y_m^*)^{-3} + (9/5)(1 + Y_m^* + Y_m^{*2} - Y_m^{*3})(1 - Y_m^*)^{-3}[(Y_m/Y_m^*)^{5/4} - 1] - (3Y_s - 2Y_s^2)(1 - Y_s)^{-2} + \ln (1 - Y_s)$. This result predicts that experimental ln K_3 vs. $[C_3/(1 + K_3)]^{5/4}$ plots at a fixed value of X_{1m} should become linear as C_3 increases provided the mobile phase is sufficiently enriched in solvent 1 (the better polymer solvent) so that $K_3 \ll 1$ and $Y_s \rightarrow 0$. In principle, chromatographic determination of the validity of the osmotic pressure scaling law[1] for semidilute polymer solutions is possible. In practice, experimental detection of the rather subtle scaling correction to the dependence of K_3 on C_3 would probably be extremely difficult because the measurements ideally should be performed under conditions of very low retention and high values of C_3 and this would again entail careful operational selection of the mobile-phase composition (see Figures 1 and 2).

Measurement of the retention time at higher polymer loadings may become complicated in the mobile-phase composition domain where the ln K_3–C_3 isotherms broaden into a plateau-like region. In this region $0.1 < K_3 < 10$ (see Figures 1 and 2) and the polymeric solutes will spend an appreciable fraction of time in each chromatographic phase, and this will serve to broaden and perhaps distort the solute sample peak in the chromatogram and cause ambiguity in the determination of the retention time.

Finally the preceding analysis can be applied to a single solvent–polymer solute system. For a single-solvent system temperature variation must be utilized to monitor the transition from low to high solute retention. Since the operational temperature range of HPLC is somewhat limited, the solvent–polymer system must be judiciously selected to provide a transition temperature within the allowed range. The expression for the capacity factor is obtained from eq 37 by setting $X_{1m} = X_{1s} = 1$ and by using[3]

$$K_3^\infty \simeq \exp[M(\Delta\epsilon(1,3;s)/kT_c)(1 - T_c/T) \times \{1 + (6A_1^{(1)}\delta_{13}(\Delta\epsilon(1,3;s))^{-1} - 1)\}]$$

where for a cubic lattice

$$T_c^{-1} = T_c^{\infty-1} + a'M^{-1/2} + b'M^{-4/5} + c'M^{-1}$$

with

$$T_c^\infty = -\Delta\epsilon(1,3;s)/(k \ln (5/4)) \qquad \Delta\epsilon(1,3;s) < 0$$

and

$$A_1^{(1)} = \Delta\epsilon(1,3;s)/6\delta_{13} + d'M^{-1/2} + e'M^{-4/5} + f'M^{-1}$$

where the coefficients a', b', c', d', e', and f' are functions of $\chi_{13c}^m = -6\delta_{13}/kT_c$ and c' also has a weak M dependence. Explicit results for these coefficients are given by eq 36 and 43 of ref 3.

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Changes in Unperturbed Dimensions upon Formation of Regular β Meanders

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ABSTRACT: Configuration partition functions have been formulated for the formation of regular β meanders by homopolypeptides. A regular β meander is a segment of pleated sheet containing three antiparallel strands that are of the same length and in register. The strands are assumed to be connected by tight bends. Generator matrix formalism is used to assess the mean square unperturbed radii of gyration. Radii of gyration of short chains decrease upon formation of β meanders. The extent of the reduction is determined primarily by the number of the residues in the β meanders; it is only slightly dependent upon the cooperativity of the ordering process. For long chains, however, the dimensions are strongly dependent on the cooperativity. At any degree of polymerization, the lowest dimensions are obtained at high β meander content and low transition cooperativity. Unperturbed mean square radii of gyration are examined for chains containing the same number of residues as chicken and T4 lysozymes. Squared radii of gyration decrease by about 10% when each chain contains average β meanders similar to those found in the conformations adopted by these proteins in the cystalline state.

Helices[1] and pleated sheets[2] are two types of ordered structures commonly found in proteins and synthetic polypeptides. While both structures have been subjects of numerous investigations, the process of helix formation is much better understood than is the origin of the pleated sheet.[3] The helix–coil transition in homopolypeptides can be studied in dilute solution by classic techniques used to provide a physical characterization of macromolecules.[4,5] Comparable studies of homopolypeptides undergoing pleated sheet formation proceed with much greater difficulty because aggregation usually accompanies formation of this ordered structure.[6,7]

Backbone hydrogen bonding in the classic α helix[1] always occurs between amino acid residues i and $i + 4$. However, if amino acid residue i is in a pleated sheet, it might be hydrogen bonded to virtually any other amino acid residue in the polypeptide chain. Consequently a theoretical description of the helix–coil transition can focus on interactions of comparatively short range, but a comprehensive treatment of pleated sheet formation must address potentially important interactions of much longer range. For this reason the theory of pleated sheet formation remains in a rather rudimentary form while helix–coil transition theory has become quite sophisticated.[3]

The situation just described is brought well into focus by consideration of a specific example. Poly(L-glutamic acid) undergoes a pH-induced helix–coil transition in aqueous media. The intrinsic viscosity at the midpoint of the transition is significantly lower than that of either the helical or completely disordered form.[5] This observation implies that the mean square radius of gyration must pass through a minimum as the conformational transition occurs. Theoretical support has been provided for this conclusion. Using helix–coil transition theory,[8] the measured unperturbed dimensions of disordered homopolypeptides containing CH_2R side chains[9,10] and generator matrix formalism,[11] Miller and Flory[12] were able to show that a minimum in the unperturbed dimensions would occur if the cooperativity parameter, σ, was not too small. The change in unperturbed dimensions has been analyzed in terms of the principal moments of the gyration tensor.[13] Poly(L-glutamic acid) can also form a β structure in aqueous solution,[14] but detailed studies are not available. On the one hand, experimental characterization is rendered difficult by aggregation. On the other hand, theoretical computation of the unperturbed dimensions is made difficult by the important role played by long-range interactions.

The objective here is characterization of changes in the mean square radius of gyration upon formation of a specific type of pleated sheet in homopolypeptides. Restriction to a special class of pleated sheet is required so that consequences of important long-range interactions can be handled in a satisfactory manner. The pleated sheet dealt with here is among those described by Schultz[15] as a β meander. A β meander is a sheet comprised of three antiparallel strands. The β meander has been identified in crystal structures of several proteins, including both chicken[16] and T4[17] lysozyme. A further restriction that the three strands be in register and connected by tight turns will be imposed here.

Given these restrictions, one can evaluate the manner in which the mean square unperturbed radius of gyration depends on pleated sheet content for homopolypeptides of varying degree of polymerization. Several aspects of the calculations are of particular interest. The first objective

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