Theory of Homopolymer Fractionation and Its Application to Gradient Elution Liquid Chromatography

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ABSTRACT: A theory of the equilibrium distribution of isolated, flexible polymer molecules between a binary solvent mobile phase and a planar stationary phase surface is developed utilizing the Flory–Huggins lattice model for the isolated polymer-associated solvent systems in both chromatographic phases. In the mobile (stationary) phase the polymer molecule is considered to be a spherical (thin, cylindrical) gel of uniform segment density which is penetrated by the solvents. Nearest-neighbor solvent–solvent, solvent–polymer segment, polymer segment–segment, solvent–surface, and polymer segment–surface interactions are included using the Bragg–Williams approximation. When only the less polar, more favorable polymer solvent (e.g., solvent 1) is appreciably adsorbed in the stationary phase, the capacity factor k_3 is given to an excellent approximation by $k_3 = \exp[A_1M(X_{1m} - X_{1mc})]$, where M is the degree of polymerization, X_{1m} is the mobile phase composition of solvent 1, X_{1mc} is a critical mobile phase composition, which for a given temperature depends on M and the various interaction energies, and A_1 ($A_1 < 0$) depends primarily on the solvent–solvent and solvent–polymer segment interchange energies and has only a weak M dependence, which vanishes as $M \to \infty$. The X_{1mc}–M dependence arises from the molecular flexibility of the polymer, which enables it to change its configurational geometry and size in response to its solvent and/or surface environment. For large M, k_3 is either extremely large when $0 < X_{1m} < X_{1mc}$ or extremely small when $1 \geq X_{1m} > X_{1mc}$, and since X_{1mc} increases monotonically with M to an asymptotic limiting value X_{1mc}^{∞} as $M \to \infty$, fractionation of homopolymers of different M is predicted to be feasible by chromatographic methods such as gradient elution HPLC or TLC, which systematically vary the mobile phase composition. Comparison of the retention times, t_R, for HPLC and R_f values for TLC obtained using the theoretical k_3 is made with the experimental fractionation results obtained by Armstrong and Bui for polystyrene homopolymers in a methylene chloride–methanol mixed mobile phase used in conjunction with reversed-phase gradient elution HPLC and TLC. For reasonable selections of the various interaction energy parameters, the theory successfully accounts for the observed trends.

I. Introduction

Recent successful fractionation experiments on high molecular weight homopolymers have been reported which employ either reversed-phase high-pressure liquid chromatography (HPLC) or thin-layer liquid chromatography (TLC).[1] In particular, polystyrene homopolymers in a molecular weight range $105 \leq MW \leq 10^7$ have been fractionated efficiently and rapidly by employing a chemically bonded C-18 stationary phase and a binary solvent mobile phase of methanol and methylene chloride in conjunction with gradient elution HPLC or TLC.[1] The fractionation studies indicate that the transition from complete retention of polymer in the stationary phase to solution in the mobile phase occurs very abruptly, especially for the higher molecular weight homopolymers, when the composition of methylene chloride in the mobile phase is increased slightly beyond a critical composition. The critical composition depends on and in fact increases with the molecular weight of polystyrene and this allows homopolymer fractionation. The HPLC and TLC fractionation experiments[1] also suggest that retention is primarily determined by unfavorable methanol–polystyrene interactions, which promote polymer precipitation into the stationary phase from a mixed methanol–methylene chloride mobile phase deficient in the latter component.

Investigation of the equilibrium distribution of isolated, flexible polymer molecules between a mixed mobile phase and a stationary phase composed of a homogeneous planar surface should provide theoretical criteria for determining the relative preference of a polymer molecule for each chromatographic phase. The phase preference presumably depends on the degree of polymerization, the solvent–polymer segment, polymer segment–segment, and solvent–solvent interactions, the solvent and polymer segment interactions with the surface, and the solvent composition in each chromatographic phase. The HPLC and TLC experiments,[1] however, are not performed under conditions approaching infinite dilution of polymer so that an analysis applied to the phase preference of isolated polymers cannot be expected to provide an entirely meaningful and quantitative interpretation of the chromatographic results. Nevertheless the present analysis ultimately concludes that fractionation by molecular weight for isolated homopolymers can occur by either selective solution into or precipitation out of a mixed mobile phase which is continuously enriched or depleted in the better polymer solvent by gradient elution HPLC or TLC, respectively. Furthermore, the analysis does account for the trends observed in the HPLC and TLC polystyrene fractionations.

An isolated flexible polymer molecule precipitated on a stationary phase surface should assume configurations that minimize contact with an unfavorable mobile phase. Thus the configurational behavior may closely resemble that predicted from the strong-adsorption limit of various theories[2-12] of surface adsorption of isolated, flexible chainlike molecules even though the actual polystyrene C-18 bonded phase surface interactions themselves may be relatively weak. We assume this correspondence in the subsequent analysis.

In section II, we apply the McMillan–Mayer solution theory[13] to an isolated flexible chainlike polymer molecule located in both a binary solvent mobile and stationary phase. We assume a stationary phase consisting of a planar adsorption surface and adsorbed solvents qualitatively simulates the behavior of a chemically bonded C-18 stationary phase. In both chromatographic phases we treat the polymer molecule and the incorporated solvents as a thermodynamic system. We envision the polymer–solvent system to be similar to a spherical (thin, cylindrical) gel having a uniform polymer segment density in the mobile (stationary) phase.[13] Partition functions for the polymer molecule–solvent systems in both phases are constructed from the familiar Flory–Huggins theory[14] applied to a

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singly connected polymer and the incorporated solvents. Nearest-neighbor solvent–solvent, solvent–polymer segment, polymer segment–segment, surface–solvent, and surface–polymer segment interactions are included through the Bragg–Williams approximation.[13,15] From the partition functions, the chemical potentials of a polymer molecule in each phase can be obtained and by equating them, one derives an explicit expression for the capacity factor, which represents the ratio of the number of polymer molecules in the stationary and mobile phases in the limit of infinite dilution of polymer solute. The capacity factor, which is essential for interpreting chromatographic data, depends in general on the degree of polymerization M, the solvent–solvent and solvent–polymer segment interchange energies, the surface–polymer segment and solvent contact interactions, the solvent compositions, and the average dimensions of the polymer molecule in each phase, which, in turn, are dependent upon solvent environment.

When only the less polar and better polymer solvent is appreciably adsorbed in the stationary phase, the general expression for the capacity factor reduces to a result that generates a simple physical interpretation. When M is large, the capacity factor is either extremely large or vanishingly small when the mobile phase composition of the less polar, more favorable solvent is below or above a certain critical composition $X_{1\mathrm{mc}}$. This composition depends on the various interchange and surface contact energies and increases monotonically with M while approaching a limiting value $X_{1\mathrm{mc}}^{\infty}$ as $M \rightarrow \infty$. The $X_{1\mathrm{mc}}$–M dependence arises from the flexibility of the polymer, which enables it to alter its configurational state in response to its solvent and/or surface environment and is absent for completely rigid polymer molecules.

The $X_{1\mathrm{mc}}$–M dependence also suggests chromatographic fractionation of high molecular weight homopolymers can be achieved if a chromatographic method is selected where the mobile phase composition can be systematically varied. The capacity factor also simplifies when only a single solvent is present, and the corresponding retention behavior is briefly discussed in section II. In section III, we apply the theory developed in section II to gradient elution HPLC and TLC and derive explicit expressions for the HPLC retention time t_{R} and the TLC R_f value from the capacity factor obtained in section II. Calculated t_{R} and R_f values using reasonable estimates for the energy parameters that appear in the analysis compare favorably with the results and trends reported by Armstrong and Bui.[1]

II. Derivation of the Capacity Factor for Flexible Homopolymers in Binary Solvent Mixtures

In this section we derive a general expression for the capacity factor for a flexible, chainlike polymer solute of degree of polymerization M distributed between a binary solvent mobile phase and a stationary phase composed of a planar adsorption surface and adsorbed solvent(s). The polymer concentration is assumed to be infinitely dilute so that interaction between different polymer molecules is completely negligible. This assumption permits advantageous application of the McMillan–Mayer theory[13] of dilute solutions in the subsequent analysis. In both mobile and stationary phases, the isolated single polymer molecule and the solvent that penetrates is regarded as a complete thermodynamic system which interacts only weakly with those regions of each phase which are devoid of polymer. The polymer–solvent system in the mobile and stationary phases are, respectively, regarded as gels that have spherical or thin cylindrical disk geometries and uniform polymer segment densities.[13,14] The nearly two-dimensional cylindrical geometry of the retained polymer is consistent with the manifestation of strong adsorption, which can result from unfavorable mobile phase solvent–polymer interaction rather than attractive surface–polymer segment interaction.

Let $\bar{N}_{1\mathrm{m}}$, $\bar{N}_{2\mathrm{m}}$, and $N_{3\mathrm{m}}$ ($\bar{N}_{1\mathrm{s}}$, $\bar{N}_{2\mathrm{s}}$, and $N_{3\mathrm{s}}$) respectively denote the number of solvent molecules of species 1 and 2 and solute polymer molecules (3) in the mobile (stationary) phase and let $\mu_{i\mathrm{a}}$ (i = 1, 2, 3; a = m, s) represent the chemical potentials of each species in each phase; then the grand canonical partition functions are

$$\Xi_{\mathrm{a}}(\{\mu_{i\mathrm{a}}\},V_{\mathrm{a}},\beta) \equiv \exp[\beta(p_{\mathrm{a}} + \pi_{\mathrm{a}})V_{\mathrm{a}}] = \sum_{\bar{N}_{1\mathrm{a}}}\sum_{\bar{N}_{2\mathrm{a}}}\sum_{N_{3\mathrm{a}}} Q_{\mathrm{a}}(\bar{N}_{1\mathrm{a}},\bar{N}_{2\mathrm{a}},N_{3\mathrm{a}},V_{\mathrm{a}},\beta)\prod_{i=1}^{2}\lambda_{i\mathrm{a}}^{\bar{N}_{i\mathrm{a}}}\lambda_{3\mathrm{a}}^{N_{3\mathrm{a}}} = \sum_{N_{3\mathrm{a}}}\psi_{N_{3\mathrm{a}}}(\lambda_{1\mathrm{a}},\lambda_{2\mathrm{a}},V_{\mathrm{a}},\beta)\lambda_{3\mathrm{a}}^{N_{3\mathrm{a}}} \qquad \mathrm{a} \equiv \mathrm{m, s} \quad (1)$$

where V_{a}, p_{a}, and π_{a} are the volume (or area), pressure, and osmotic pressure associated with chromatographic phase a, $\beta \equiv 1/kT$, where T is the absolute temperature and k is Boltzmann's constant, $Q_{\mathrm{a}}(\bar{N}_{i\mathrm{a}},\bar{N}_{2\mathrm{a}},N_{3\mathrm{a}},V_{\mathrm{a}},\beta)$ are the canonical partition functions for each phase, $\lambda_{i\mathrm{a}} \equiv \exp(\beta\mu_{i\mathrm{a}})$ (i = 1, 2, 3; a ≡ m, s) are the corresponding activities, and

$$\psi_{N_{3\mathrm{a}}} \equiv \sum_{\bar{N}_{1\mathrm{a}}}\sum_{\bar{N}_{2\mathrm{a}}} Q_{\mathrm{a}}(\bar{N}_{1\mathrm{a}},\bar{N}_{2\mathrm{a}},N_{3\mathrm{a}},V_{\mathrm{a}},\beta)\prod_{i=1}^{2}\lambda_{i\mathrm{a}}^{\bar{N}_{i\mathrm{a}}} \qquad \mathrm{a} \equiv \mathrm{m, s} \quad (2)$$

The average number of polymer molecules in each phase is

$$\langle N_{3\mathrm{a}}\rangle = \lambda_{3\mathrm{a}}\frac{\partial}{\partial\lambda_{3\mathrm{a}}}[\ln (\sum_{N_{3\mathrm{a}}\geq 0}\psi_{N_{3\mathrm{a}}}\lambda_{3\mathrm{a}}^{N_{3\mathrm{a}}})] = \sum_{N_{3\mathrm{a}}} N_{3\mathrm{a}}\psi_{N_{3\mathrm{a}}}\lambda_{3\mathrm{a}}^{N_{3\mathrm{a}}} \Big/ \sum_{N_{3\mathrm{a}}}\psi_{N_{3\mathrm{a}}}\lambda_{3\mathrm{a}}^{N_{3\mathrm{a}}} \quad (3)$$

For very dilute polymer solutions

$$\langle N_{3\mathrm{a}}\rangle \simeq \exp[\beta\mu_{3\mathrm{a}}]\psi_{1\mathrm{a}}/\psi_{0\mathrm{a}}$$

or

$$\beta\mu_{3\mathrm{a}} \simeq \ln [\langle N_{3\mathrm{a}}\rangle\psi_{0\mathrm{a}}/\psi_{1\mathrm{a}}] \qquad \mathrm{a} \equiv \mathrm{m, s} \quad (4)$$

where

$$\psi_{0\mathrm{a}} \equiv \sum_{\bar{N}_{1\mathrm{a}}}\sum_{\bar{N}_{2\mathrm{a}}} Q_{\mathrm{a}}(\bar{N}_{1\mathrm{a}},\bar{N}_{2\mathrm{a}},N_{3\mathrm{a}}{=}0,V_{\mathrm{a}},\beta)\prod_{i=1}^{2}\lambda_{i\mathrm{a}}^{\bar{N}_{i\mathrm{a}}} \quad (5\mathrm{a})$$

and

$$\psi_{1\mathrm{a}} \equiv \sum_{\bar{N}_{1\mathrm{a}}}\sum_{\bar{N}_{2\mathrm{a}}} Q_{\mathrm{a}}(\bar{N}_{1\mathrm{a}},\bar{N}_{2\mathrm{a}},N_{3\mathrm{a}}{=}1,V_{\mathrm{a}},\beta)\prod_{i=1}^{2}\lambda_{i\mathrm{a}}^{\bar{N}_{i\mathrm{a}}} \quad (5\mathrm{b})$$

where $Q_{\mathrm{a}}(...N_{3\mathrm{a}}{=}0...)$ and $Q_{\mathrm{a}}(...N_{3\mathrm{a}}{=}1...)$ are the canonical partition functions for a pure binary solvent system and a single polymer–binary solvent system, respectively. In order to simplify eq 4 we approximate $\psi_{0\mathrm{a}}$ and $\psi_{1\mathrm{a}}$ by taking the maximum term in the sums over $\bar{N}_{1\mathrm{a}}$ and $\bar{N}_{2\mathrm{a}}$:

$$\psi_{0\mathrm{a}} \approx Q_{\mathrm{a}}(\bar{N}_{1\mathrm{a}}^{0},\bar{N}_{2\mathrm{a}}^{0},N_{3\mathrm{a}}{=}0,V_{\mathrm{a}},\beta)\prod_{i=1}^{2}\lambda_{i\mathrm{a}}^{\bar{N}_{i\mathrm{a}}^{0}} \quad (6\mathrm{a})$$

and

$$\psi_{1\mathrm{a}} \approx Q_{\mathrm{a}}(\bar{N}_{1\mathrm{a}}^{1},\bar{N}_{2\mathrm{a}}^{1},N_{3\mathrm{a}}{=}1,V_{\mathrm{a}},\beta)\prod_{i=1}^{2}\lambda_{i\mathrm{a}}^{\bar{N}_{i\mathrm{a}}^{1}} \quad (6\mathrm{b})$$

where $\bar{N}_{i\mathrm{a}}^{0}$ and $\bar{N}_{i\mathrm{a}}^{1}$ (i = 1, 2) denote the values of $\bar{N}_{i\mathrm{a}}$ that maximize the generic terms of the sums given in eq 5a,b. In general, $\bar{N}_{i\mathrm{a}}^{0} \neq \bar{N}_{i\mathrm{a}}^{1}$ (i = 1, 2; a ≡ m, s).

An isolated polymer molecule and the associated $N_{1\mathrm{a}} + N_{2\mathrm{a}}$ solvent molecules are assumed to occupy the volumes

$$\bar{V}_{\mathrm{pa}} = \bar{N}_{\mathrm{a}}\nu = (M + N_{1\mathrm{a}} + N_{2\mathrm{a}})\nu \qquad \mathrm{a} \equiv \mathrm{m, s} \quad (7)$$

where ν is the volume of a lattice site that can accommodate either a type 1 or 2 solvent molecule or a monomeric unit of the polymer and $\bar{N}_a$ is the total number of sites occupied by the polymer segments and the solvent molecules. The volumes $\bar{V}_{pa}$ (a ≡ m, s) are considered to be proportional to the volume occupied by either a spherical or a thin cylindrical polymer coil in the mobile or the stationary phase, respectively. We also assume that

$$\frac{Q_a(\bar{N}_{1a}{}^1,\bar{N}_{2a}{}^1,N_{3a}=1,V_a,\beta)}{Q_a(\bar{N}_{1a}{}^0,\bar{N}_{2a}{}^0,N_{3a}=0,V_a,\beta)} = \sum_{\bar{V}_{pa}} P_a(\bar{V}_{pa}) \left[\frac{Q_a(N_{1a},N_{2a},N_{3a}=1,\bar{V}_{pa},\beta)}{Q_a(N_{1a}{}^0,N_{2a}{}^0,N_{3a}=0,\bar{V}_{pa},\beta)} \right] \times \left[\frac{Q_a(\bar{N}_{1a}{}^1-N_{1a},\bar{N}_{2a}{}^1-N_{2a},N_{3a}=0,V_a-\bar{V}_{pa},\beta)}{Q_a(\bar{N}_{1a}{}^0-N_{1a}{}^0,\bar{N}_{2a}{}^0-N_{2a}{}^0,N_{3a}=0,V_a-\bar{V}_{pa},\beta)} \right] \quad a \equiv m, s$$

and when $\bar{N}_{ia}{}^1 - N_{ia} = \bar{N}_{ia}{}^0 - N_{ia}{}^0$ (i = 1, 2), we have

$$\frac{Q_a(\bar{N}_{1a}{}^1,\bar{N}_{2a}{}^1,N_{3a}=1,V_a,\beta)}{Q_a(\bar{N}_{1a}{}^0,\bar{N}_{2a}{}^0,N_{3a}=0,V_a,\beta)} = \sum_{\bar{V}_{pa}} P_a(\bar{V}_{pa}) \left[\frac{Q_a(N_{1a},N_{2a},N_{3a}=1,\bar{V}_{pa},\beta)}{Q_a(N_{1a}{}^0,N_{2a}{}^0,N_{3a}=0,\bar{V}_{pa},\beta)} \right] \equiv \bar{Q}_a(N_{1a},N_{2a},M,\beta) \quad a \equiv m, s \quad (8)$$

where $\bar{V}_{pa}/\nu = \bar{N}_a = M + N_{1a} + N_{2a} = N_{1a}{}^0 + N_{2a}{}^0$ and the $P_a(\bar{V}_{pa})$ represent the probability that the polymer coil and associated solvent molecules occupy a volume $\bar{V}_{pa}$ in their respective chromatographic phase and $\sum_{\bar{V}_{pa}} P_a(\bar{V}_{pa}) = 1$. The separation of the canonical partition functions for a given $\bar{V}_{pa}$ into a product of partition functions, which apply to regions of volume $V_a - \bar{V}_{pa}$ and $\bar{V}_{pa}$, corresponds to decoupling the interaction between the regions and regarding them as independent systems. Provided the intermolecular interactions are short-ranged and $V_a \gg \bar{V}_{pa}$ this approximation should not be too severe.

It is again convenient to employ the maximum term approximation $\bar{Q}_a(N_{1a},N_{2a},M,\beta)$ in eq 8 by finding the value of $\bar{V}_{pa} = V_{pa}$ that maximizes the generic term of the sum over $\bar{V}_{pa}$:

$$\bar{Q}_a(N_{1a},N_{2a},M,\beta) = P_a(V_{pa}) \frac{Q_a(N_{1a},N_{2a},N_{3a}=1,V_{pa},\beta)}{Q_a(N_{1a}{}^0,N_{2a}{}^0,N_{3a}=0,V_{pa},\beta)} = P_a(V_{pa}) \frac{Q_a(N_{1a},N_{2a},N_{3a}=1,V_{pa},\beta)}{Q_a(N_{1a},N_{2a},N_{3a}=0,V_{pa}-M\nu,\beta)} \times \frac{Q_a(N_{1a},N_{2a},N_{3a}=0,V_{pa}-M\nu,\beta)}{Q_a(N_{1a}{}^0,N_{2a}{}^0,N_{3a}=0,V_{pa},\beta)} \quad (9)$$

where the latter form proves convenient in the subsequent analysis. The chemical potentials μ_{3a} (a ≡ m, s) given by eq 4 and 5a,b can now be expressed by using eq 6a,b, 8, and 9 as

$$\beta(\mu_{3a} - M\sum_{i=1}^{2} X_{ia}\mu_{ia}) = \ln N_{3a} - \ln \left[P_a(V_{pa}) \frac{Q_a(N_{1a},N_{2a},N_{3a}=1,V_{pa},\beta)}{Q_a(N_{1a},N_{2a},N_{3a}=0,V_{pa}-M\nu,\beta)} \right] - \ln \left[\frac{Q_a(N_{1a},N_{2a},N_{3a}=0,V_{pa}-M\nu,\beta)}{Q_a(N_{1a}{}^0,N_{2a}{}^0,N_{3a}=0,V_{pa},\beta)} \right] \quad (10)$$

where we have introduced the solvent volume fractions

$$X_{ia} = N_{ia}/(N_a - M) = N_{ia}{}^0/N_a \quad (11)$$

to simplify the term involving the ratio of the activities, $-\ln [\prod_{i=1}^{2} \lambda_{ia}{}^{N_{ia}-N_{ia}{}^0}]$, and we have written $\langle N_{3a} \rangle \equiv N_{3a}$.

Utilization of eq 10 requires explicit expressions for the canonical partition functions and $P_a(V_{pa})$. We shall apply the familiar Flory–Huggins lattice theory of polymer solutions[13,14] to a single isolated polymer molecule and a binary solvent mixture to estimate $Q_a(N_{1a},N_{2a},N_{3a}=1,V_{pa},\beta)$. That is, one determines the number of ways a flexible chainlike polymer molecule consisting of M connected monomeric segments and N_{1a} and N_{2a} solvent molecules can be arranged on a lattice of $N_a = V_{pa}/\nu = M + \sum_{i=1}^{2} N_{ia}$ sites with coordination number C_a (a ≡ m, s) ($C_m = 6$ and $C_s = 5$ for a cubic lattice) such that each lattice site is singly occupied by either a polymer segment or a solvent molecule. The configurational part of Q_a, Q_{ac}, is approximately

$$Q_{ac} \simeq N_a C_a (C_a - 1)^{M-2} N_a{}^{-M} (N_a!/N_{1a}!N_{2a}!) \quad (12)$$

provided $M \ll N_a$, which is satisfied when the polymer molecule resides in a favorable solvent mixture. We also assume that the solvent molecules and polymer segments interact with themselves and each other through nearest-neighbor pair interactions. The Bragg–Williams random-mixing approximation[13] is employed to determine the total interaction energies βE_a (a ≡ m, s)

$$\beta E_a = \beta C_a \left[\sum_{i=1}^{2} N_{ia} \left(\frac{w_{ii}}{2} - \left(\frac{M}{N_a} \right) \delta_{i3} \right) + M \frac{w_{33}}{2} - N_{1a} N_{2a} \delta_{12}/N_a \right] \quad (13)$$

where the w_{ij} (i = 1, 2, 3; j = 1, 2, 3) are the nearest-neighbor pair interaction energies between solvent molecules and/or polymer segments and

$$\delta_{ij} = (w_{ii} + w_{jj})/2 - w_{ij} \qquad i, j = 1, 2, 3 \quad (14)$$

is the interchange energy required to form an i–j nearest-neighbor pair from an i–i and a j–j nearest-neighbor pair. Also we have expressed the polymer segment interaction terms neglecting the contiguous nature of the chain by employing C_a rather than $C_a - 2$ as the coordination number. This simplifies the energetics slightly without altering the physics and is often done in polymer solution theory.[13,14]

In the stationary phase there are also surface–solvent molecule and surface–polymer segment adhesion energies $\epsilon_{is} \equiv \epsilon_{is}(T)$ (i = 1, 2, 3), which are actually temperature-dependent adhesion free energies since they incorporate the differences in the logarithm of single solvent molecule or polymer segment partition functions in the mobile and stationary phases.[15] The total surface–solvent and surface–polymer segment interaction free energy is

$$\beta\epsilon_s(T) = \beta[\sum_{i=1}^{2} N_{is}\epsilon_{is} + M\epsilon_{3s}] \quad (15)$$

Combination of eq 12, 13, and 15 leads to

$$Q_a(N_{1a},N_{2a},N_{3a}=1,N_a,\beta) = N_a C_a (C_a - 1)^{M-2} \times N_a{}^{-M}(N_a!/N_{1a}!N_{2a}!) \exp[-\beta(E_a + \epsilon_a(T))] \quad a \equiv m, s \quad (16)$$

where $\beta\epsilon_m(T) \equiv 0$ and $N_a = V_a/\nu$.

The canonical partition functions in the absence of polymer are assumed to be given in the Bragg–Williams approximation[15] by

$$Q_a(N_{1a}{}^0,N_{2a}{}^0,N_{3a}=0,N_a,\beta) = (N_a!/N_{1a}{}^0!N_{2a}{}^0!) \times \exp[-\beta C_a(N_{1a}{}^0 N_{2a}{}^0(-\delta_{12})/N_a + \sum_{i=1}^{2} N_{ia}{}^0 w_{ii}/2)] \times \exp[-\beta(\sum_{i=1}^{2} N_{ia}{}^0 \epsilon_{ia})] \quad (17a)$$

$$Q_a(N_{1a},N_{2a},N_{3a}{=}0,N_a{-}M,\beta) = ((N_a - M)!/N_{1a}!N_{2a}!) \exp[-\beta C_a[N_{1a}N_{2a}(-\delta_{12})/(N_a - M) + \sum_{i=1}^{2} N_{ia}w_{ii}/2]] \exp[-\beta \sum_{i=1}^{2} N_{ia}\epsilon_{ia}] \quad (17b)$$

where $\epsilon_{1m} = \epsilon_{2m} = 0$ and $N_{ia} = X_{ia}(N_a - M)$ and $N_{ia}^0 = X_{ia}N_a$ (i = 1, 2; a = m, s). Division of eq 17b by eq 17a and evaluation of the logarithm of their quotient yield

$$\ln\left[\frac{Q_a(N_{1a},N_{2a},N_{3a}{=}0,N_a{-}M,\beta)}{Q_a(N_{1a}^0,N_{2a}^0,N_{3a}{=}0,N_a,\beta)}\right] = M[\sum_{i=1}^{2} X_{ia}(\ln X_{ia} + \beta C_a w_{ii}/2 + \beta\epsilon_{ia}) - \beta C_a X_{1a}X_{2a}\delta_{12}] \quad (18)$$

Note this result is independent of N_a and provides an explicit expression for the last term in eq 10.

Evaluation of $\beta(\mu_{3a} - M\sum_{i=1}^{2} X_{ia}\mu_{ia})$ in eq 10 requires $P_a(V_{pa})$ (a ≡ m, s), where the $V_{pa} \equiv N_a\nu$ are the volumes of the isolated polymer–binary solvent systems in each chromatographic phase that minimize the free energy difference between the polymer–solvent system and a pure binary solvent mixture at the same composition. The configurational entropy of the polymer in each chromatographic phase is proportional to $\ln P_a(V_{pa})$ and is taken explicitly into account in the minimization that leads to V_{pa}. That an optimum value of V_{pa} is attained arises from the realization that a favorable (unfavorable) solvent environment will generate an expansion (contraction) of the polymer dimensions relative to its unperturbed dimensions in the absence of solvent or in a Θ solvent.[14] Such expansion or contraction cannot proceed indefinitely, however, because it is opposed by the corresponding decrease in the configurational entropy of the flexible polymer coil.

In the mobile phase, the Gaussian approximation gives

$$P_m(V_{pm}) = (3/2\pi Mb^2)^{3/2} \exp[-3R_m^2/2Mb^2] \quad (19)$$

where $V_{pm} \equiv N_m\nu = \pi R_m^3/6 = N_m\pi b^3/6$. Here, R_m represents the diameter of an equivalent spherical coil of uniform segment density and we have set $\nu \boxminus \pi b^3/6$ to represent the volume of a single lattice site. The mean square end-to-end distance of an unperturbed coil is $\langle R_m^2\rangle_0 = 4\pi\int_0^\infty dR_m\, R_m^4 P_m(V_{pm}) = Mb^2$, and the average volume of the unperturbed polymer molecule is $V_{pm}^0 = N_m^0\pi b^3/6 = \pi\langle R_m^2\rangle_0^{3/2}/6 = \pi M^{3/2}b^3/6$. Hence $N_m^0 = M^{3/2}$ is the number of lattice sites associated with the unperturbed polymer in the absence of solvent (or in a Θ solvent).

In the stationary phase, a polymer molecule is assumed to have the conformational geometry of a thin cylindrical disk, which is promoted by unfavorable mobile phase solvent–polymer interactions and favorable solvent–polymer interactions in the stationary phase. We further assume that the normalized distribution function for an isolated polymer molecule precipitated on the surface is

$$P_s(V_{ps}) = P_s(N_s\nu) = (\pi Mb^2d)^{-1} \exp[-R_s^2/Mb^2]\theta(d - z) \quad (20)$$

where R_s represents the diameter of an equivalent cylindrical disk of uniform segment density which lies flat on the surface. The factor $\theta(d - z)$ denotes the Heaviside unit step function:

$$\theta(d - z) = \begin{bmatrix} 1 & 0 < z < d \\ 0 & d < z < \infty \end{bmatrix}$$

where z denotes the distance normal to the surface and represents the actual thickness of the polymer coil retained on the surface. For a polymer molecule compactly deposited on a stationary phase surface either by unfavorable interaction with the mobile phase or by strong surface adsorption,[12] d should be independent of M and be comparable to the length b. The mean square end-to-end distance of the polymer disk parallel to the surface is $\langle R_s^2\rangle_0 = 2\pi\int_0^\infty dz \int_0^\infty dR_s\, R_s^3 P_s(V_{ps}) = Mb^2$. We expect $V_{ps} \boxminus N_s\nu \equiv \pi R_s^2 d/4$ while the average volume of the unperturbed polymer at the surface is $V_{ps}^0 = \pi\langle R_s^2\rangle_0 d/4 = \pi Mb^2d/4 = N_s^0\nu$. Also $N_s^0 = M$ if $\nu = \pi b^3/6 = \pi b^2 d/4$, which is satisfied if $d = (2/3)b$. Of course, a polymer molecule retained on a C-18-bonded stationary phase rather than an idealized planar surface would probably penetrate into the domain occupied by the C-18 chains and thus have a thickness comparable to the thickness of the bonded phase and perhaps a somewhat larger V_{ps}.

The values of $N_a = V_{pa}/\nu$ that maximize $F_a \equiv P_a(N_a) Q_a(N_{1a},N_{2a},N_{3a}{=}1,N_a,\beta)/Q(N_{1a},N_{2a},N_{3a}{=}0,N_a{-}M,\beta)$, which is proportional to the generic term of eq 8 with the rearrangement introduced in eq 9, are obtained by solving $\partial \ln F_a/\partial N_a = 0$. Employment of eq 16, 17b, 19, and 20 to evaluate $\partial \ln F_a/\partial N_a = 0$ leads to the following equations for the three- and two-dimensional expansion factors $\alpha_m^3 \equiv N_m/N_m^0 = N_m M^{-3/2}$ and $\alpha_s^2 \equiv N_s/N_s^0 = N_s M^{-1}$:

$$\alpha_m^5 - \alpha_m^3 = (\tfrac{1}{2} - \sum_{i=1}^{2} X_{im}\chi_{i3}^m + X_{1m}X_{2m}\chi_{12}^m)M^{1/2} \equiv g_m M^{1/2} \quad (21a)$$

and

$$\alpha_s^4 - \alpha_s^2 = (\tfrac{1}{2} - \sum_{i=1}^{2} X_{is}\chi_{i3}^s + X_{1s}X_{2s}\chi_{12}^s)M \equiv g_s M \quad (21b)$$

where we have introduced

$$g_a \equiv g_a(X_{1a},\{\chi_{ij}^a\}) = \tfrac{1}{2} - X_{1a}\chi_{13}^a - (1 - X_{1a})\chi_{23}^a + X_{1a}(1 - X_{1a})\chi_{12}^a \quad (22)$$

with

$$\chi_{ij}^a = -\beta C_a\delta_{ij} = -\beta C_a[(w_{ii} + w_{jj})/2 - w_{ij}] \quad (23)$$

Clearly, when $X_{1a} = 1$, $g_a \equiv g_a(1) = {}^1/_2 - \chi_{13}^a$ (a ≡ m, s). For a mixed-solvent system the expansion factors α_m and α_s depend on the solvent composition and the pair interchange energies $\{\chi_{ij}^a\} \equiv \chi_{12}^a, \chi_{13}^a, \chi_{23}^a$ (a = m, s). For good polymer solvents $\chi_{i3}^a \leq 0$ (i = 1, 2) and, if $g_a > 0$, the expansion factors exceed unity and the polymer molecule will expand beyond its unperturbed dimensions. The expansion factors for high molecular weight polymers in favorable solvent environments have the following M dependences: $\alpha_m \approx g_m^{1/5}M^{1/10}$ and $\alpha_s \approx g_s^{1/4}M^{1/4}$. Hence

$$N_m = \alpha_m^3 M^{3/2} \simeq g_m^{3/5}M^{1.8} \quad (24a)$$

and

$$N_s = \alpha_s^2 M \simeq g_s^{1/2}M^{1.5} \quad (24b)$$

where $g_a > 0$ (a ≡ m, s) for all compositions; otherwise eq 21a,b must be employed. Whenever a composition X_{1a} exists such that $0 \leq X_{1a} \leq 1$ and

$$X_{1a} = ((\chi_{12}^a + \chi_{23}^a - \chi_{13}^a) \pm ((\chi_{12}^a + \chi_{23}^a - \chi_{13}^a)^2 + 4(\tfrac{1}{2} - \chi_{23}^a)\chi_{12}^a)^{1/2})/2\chi_{12}^a$$

when $\chi_{12}^a \neq 0$ and $X_{1a} = ({}^1/_2 - \chi_{23}^a)/(\chi_{13}^a - \chi_{23}^a)$ when $\chi_{12}^a = 0$, then $\alpha_a = 1$ satisfies either eq 21a or eq 21b and the polymer assumes its unperturbed dimensions and $N_a = N_a^0$ (a = m, s). Thus at a given temperature (the χ's are temperature dependent) a solvent composition (Θ solvent) may exist for a given solvent pair in which the molecular dimensions of the polymer are unperturbed by long-range interferences and the excluded volume (area) effect is nullified.[13,14] In a single solvent, eq 21a,b reduce, respectively, to the familiar results derived by Flory[14] for the expansion factors for a three-dimensional and two-dimensional polymer coil in solution.

Equilibrium transfer at constant temperature of a polymer molecule between the mobile and stationary phases with the concomitant displacement of M solvent molecules from the phase entered by the polymer requires that

$$\mu_{3\mathrm{m}} + M\left(\sum_{i=1}^{2} X_{i\mathrm{s}}\mu_{i\mathrm{s}}\right) = \mu_{3\mathrm{s}} + M\left(\sum_{i=1}^{2} X_{i\mathrm{m}}\mu_{i\mathrm{m}}\right) \tag{25}$$

This condition together with eq 10 generates an expression for the capacity factor of the polymeric solute:

$$k_3 = \lim_{N_{3\mathrm{s}}\to 0, N_{3\mathrm{m}}\to 0} (N_{3\mathrm{s}}/N_{3\mathrm{m}}) = (P_\mathrm{s}(N_\mathrm{s})/P_\mathrm{m}(N_\mathrm{m})) \times \frac{Q_\mathrm{s}(N_{1\mathrm{s}},N_{2\mathrm{s}},N_{3\mathrm{s}}=1,N_\mathrm{s},\beta)}{Q_\mathrm{s}(N_{1\mathrm{s}},N_{2\mathrm{s}},N_{3\mathrm{s}}=0,N_\mathrm{s}-M,\beta)} \times \frac{Q_\mathrm{m}(N_{1\mathrm{m}},N_{2\mathrm{m}},N_{3\mathrm{m}}=0,N_\mathrm{m}-M,\beta)}{Q_\mathrm{m}(N_{1\mathrm{m}},N_{2\mathrm{m}},N_{3\mathrm{m}}=1,N_\mathrm{m},\beta)} \times \exp\left[M\left\{\sum_{i=1}^{2}(X_{i\mathrm{s}} \ln X_{i\mathrm{s}} + \beta C_\mathrm{s}X_{i\mathrm{s}}w_{ii}/2 + \beta X_{i\mathrm{s}}\epsilon_{i\mathrm{s}} - X_{i\mathrm{m}} \ln X_{i\mathrm{m}} - \beta C_\mathrm{m}X_{i\mathrm{m}}w_{ii}/2) - \beta(C_\mathrm{s}X_{1\mathrm{s}}X_{2\mathrm{s}} - C_\mathrm{m}X_{1\mathrm{m}}X_{2\mathrm{m}})\right\}\right] \tag{26}$$

where we have also employed eq 18. Substitution of eq 11, 12, 16, 17b, 19, and 20 into eq 26 yields after some simplification

$$k_3 = \left(\frac{2\pi M}{3}\right)^{1/2} \exp\left[-\left(\langle R_\mathrm{s}^2\rangle - \frac{3}{2}\langle R_\mathrm{m}^2\rangle\right)/Mb^2\right] \times \left(\frac{C_\mathrm{s}(C_\mathrm{s}-1)}{C_\mathrm{m}(C_\mathrm{m}-1)}\right)^{M-2}\left(\frac{N_\mathrm{s}}{N_\mathrm{m}}\left(\frac{N_\mathrm{m}}{N_\mathrm{s}}\right)^{M}\frac{N_\mathrm{s}!}{N_\mathrm{m}!}\,\frac{(N_\mathrm{m}-M)!}{(N_\mathrm{s}-M)!}\right) \times \exp\Big[M\Big\{\chi_{12}{}^{\mathrm{s}}X_{1\mathrm{s}}X_{2\mathrm{s}}(2 - M/N_\mathrm{s}) - \chi_{12}{}^{\mathrm{m}}X_{1\mathrm{m}}X_{2\mathrm{m}}(2 - M/N_\mathrm{m}) - \sum_{i=1}^{2}(\chi_{i3}{}^{\mathrm{s}}X_{i\mathrm{s}}(1 - M/N_\mathrm{s}) - \chi_{i3}{}^{\mathrm{m}}X_{i\mathrm{m}}(1 - M/N_\mathrm{m})) + \beta\sum_{i=1}^{2}(C_\mathrm{s}X_{i\mathrm{s}} - C_\mathrm{m}X_{i\mathrm{m}})w_{ii}/2 - \beta(C_\mathrm{s} - C_\mathrm{m})w_{33}/2 + \sum_{i=1}^{2}(X_{i\mathrm{s}} \ln X_{i\mathrm{s}} - X_{i\mathrm{m}} \ln X_{i\mathrm{m}} + \beta X_{i\mathrm{s}}\epsilon_{i\mathrm{s}}) - \beta\epsilon_{3\mathrm{s}}\Big\}\Big] \tag{27}$$

where we have estimated $\exp[-(R_\mathrm{s}^2 - (3/2)R_\mathrm{m}^2)/Mb^2]$ by $\exp[-(\langle R_\mathrm{s}^2\rangle - (3/2)\langle R_\mathrm{m}^2\rangle)/Mb^2] \equiv \exp[-(\alpha_\mathrm{s}^2\langle R_\mathrm{s}^2\rangle_0 - (3/2)\alpha_\mathrm{m}^2\langle R_\mathrm{m}^2\rangle_0)/Mb^2]$. We shall also employ eq 24a,b to evaluate N_m and N_s in the subsequent analysis since we usually consider large-M polymers in favorable solvent environments. Hence

$$\exp[-(\langle R_\mathrm{s}^2\rangle - \tfrac{3}{2}\langle R_\mathrm{m}^2\rangle)/Mb^2] = \exp[-M(g_\mathrm{s}^{1/2}M^{-0.5} - \tfrac{3}{2}g_\mathrm{m}^{2/5}M^{-0.8})] \tag{28}$$

where we have utilized $\langle R_\mathrm{s}^2\rangle = \alpha_\mathrm{s}^2\langle R_\mathrm{s}^2\rangle_0 = g_\mathrm{s}^{1/2}M^{3/2}b^2$ and $\langle R_\mathrm{m}^2\rangle = \alpha_\mathrm{m}^2\langle R_\mathrm{m}^2\rangle_0 = g_\mathrm{m}^{2/5}M^{1.2}b^2$. If $M/N_\mathrm{a} \ll 1$ (a ≡ m, s), we can also approximate the factor

$$\left(\frac{N_\mathrm{m}}{N_\mathrm{s}}\right)^{M}\frac{N_\mathrm{s}!}{N_\mathrm{m}!}\,\frac{(N_\mathrm{m}-M)!}{(N_\mathrm{s}-M)!} \approx (1 - M/N_\mathrm{m})^{N_\mathrm{m}-M}/(1 - M/N_\mathrm{s})^{N_\mathrm{s}-M} \approx \exp[M^2(N_\mathrm{m}^{-1} - N_\mathrm{s}^{-1})] = \exp[M(g_\mathrm{m}^{-3/5}M^{-0.8} - g_\mathrm{s}^{-1/2}M^{-0.5})] \tag{29}$$

where we have recalled eq 24a,b.

The mobile and stationary phase solvent compositions in the region encompassed by a polymer molecule in each chromatographic phase are dependent and the $X_{1\mathrm{m}}$–$X_{1\mathrm{s}}$ isotherm can be obtained from

$$\beta(\mu_{1\mathrm{m}} - \mu_{2\mathrm{m}})_\mathrm{p} = \beta(\mu_{1\mathrm{s}} - \mu_{2\mathrm{s}})_\mathrm{p}$$

where, in principle, these solvent chemical potential differences should be evaluated in the presence of a polymer solute molecule (hence the subscript p). The solvent compositions $X_{i\mathrm{a}}$ (i = 1, 2; a = m, s) ($\sum_{i=1}^{2}X_{i\mathrm{a}} = 1$) appearing in eq 27 are, in general, different from the bulk mobile and stationary phase compositions $X_{i\mathrm{a}0}$ (i = 1, 2; a ≡ m, s) in the absence of polymeric solute and the relationship between $X_{i\mathrm{a}}$ and $X_{i\mathrm{a}0}$ can be obtained from

$$\beta(\mu_{1\mathrm{m}} - \mu_{2\mathrm{m}})_0 = \beta(\mu_{1\mathrm{m}} - \mu_{2\mathrm{m}})_\mathrm{p} = \beta(\mu_{1\mathrm{s}} - \mu_{2\mathrm{s}})_\mathrm{p} = \beta(\mu_{1\mathrm{s}} - \mu_{2\mathrm{s}})_0$$

When the binary solvent mixture provides a favorable environment for a polymer molecule in both phases, $M/N_\mathrm{a} \ll 1$ (a ≡ m, s) and the compositions $X_{i\mathrm{a}}$ and $X_{i\mathrm{a}0}$ (i = 1, 2) should be approximately identical and the $X_{1\mathrm{m}}$–$X_{1\mathrm{s}}$ isotherms are then determined from

$$\beta(\mu_{1\mathrm{m}} - \mu_{2\mathrm{m}})_0 = -\frac{\partial \ln Q_\mathrm{m}(\bar N_{1\mathrm{m}}{}^0, V_\mathrm{m}-\bar N_{1\mathrm{m}}{}^0, V_\mathrm{m},\beta)}{\partial \bar N_{1\mathrm{m}}{}^0} = \beta(\mu_{1\mathrm{s}} - \mu_{2\mathrm{s}})_0 = -\frac{\partial \ln Q_\mathrm{s}(\bar N_{1\mathrm{s}}{}^0, V_\mathrm{s}-\bar N_{1\mathrm{s}}{}^0, V_\mathrm{s},\beta)}{\partial \bar N_{1\mathrm{s}}{}^0}$$

where

$$Q_\mathrm{a}(\bar N_{1\mathrm{a}}{}^0, V_\mathrm{a}-\bar N_{1\mathrm{a}}{}^0, V_\mathrm{a},\beta) = (V_\mathrm{a}!/\bar N_{1\mathrm{a}}{}^0!(V_\mathrm{a} - \bar N_{1\mathrm{a}}{}^0)!) \times \exp\left[-\left\{\chi_{12}{}^{\mathrm{a}}\bar N_{1\mathrm{a}}{}^0(V_\mathrm{a} - \bar N_{1\mathrm{a}}{}^0)V_\mathrm{a}^{-1} + \frac{\beta}{2}C_\mathrm{a}(\bar N_{1\mathrm{a}}{}^0(w_{11} - w_{22}) + V_\mathrm{a}w_{22}) + \beta(\epsilon_{1\mathrm{a}} - \epsilon_{2\mathrm{a}})\bar N_{1\mathrm{a}}{}^0 + \beta\epsilon_{2\mathrm{a}}V_\mathrm{a}\right\}\right]$$

with $\bar N_{2\mathrm{a}}{}^0 = V_\mathrm{a} - \bar N_{1\mathrm{a}}{}^0$, and $\epsilon_{1\mathrm{m}} = \epsilon_{2\mathrm{m}} = 0$ (see eq 17a and 23). We thus obtain the isotherm

$$\beta(\mu_{1\mathrm{m}} - \mu_{2\mathrm{m}})_0 = \ln[(X_{1\mathrm{m}}/X_{2\mathrm{m}}) \exp[\chi_{12}{}^{\mathrm{m}}(1 - 2X_{1\mathrm{m}}) + \beta C_\mathrm{m}(w_{11} - w_{22})/2]] = \beta(\mu_{1\mathrm{s}} - \mu_{2\mathrm{s}})_0 = \ln[(X_{1\mathrm{s}}/X_{2\mathrm{s}}) \times \exp[\chi_{12}{}^{\mathrm{s}}(1 - 2X_{1\mathrm{s}}) + \beta C_\mathrm{s}(w_{11} - w_{22})/2 + \beta(\epsilon_{1\mathrm{s}} - \epsilon_{2\mathrm{s}})]] \tag{30}$$

Also, in the stationary phase the distribution of adsorbed solvent molecules at equilibrium is obtained from $\partial \ln Q_\mathrm{s}/\partial \bar N_{1\mathrm{s}}{}^0 = 0$:

$$X_{1\mathrm{s}}/(1 - X_{1\mathrm{s}}) = \exp[-\{\chi_{12}{}^{\mathrm{s}}(1 - 2X_{1\mathrm{s}}) + \beta C_\mathrm{s}(w_{11} - w_{22})/2 + \beta(\epsilon_{1\mathrm{s}} - \epsilon_{2\mathrm{s}})\}] \tag{31}$$

If $\beta(\epsilon_{1\mathrm{s}} - \epsilon_{2\mathrm{s}}) \ll 0$, $\chi_{12}{}^{\mathrm{s}} \geq 0$, and $w_{11} \lesssim w_{22}$, then $X_{1\mathrm{s}} \sim 1$, and solvent 1 will be predominantly adsorbed. For C-18-bonded phase surfaces we anticipate that the less polar component solvent will be more strongly adsorbed in the stationary phase.[16] Equations 30 and 31 can be utilized to give

$$\exp\left[M\sum_{i=1}^{2}(X_{i\mathrm{s}} \ln X_{i\mathrm{s}} - X_{i\mathrm{m}} \ln X_{i\mathrm{m}})\right] = \{X_{1\mathrm{s}} \exp[X_{2\mathrm{s}}(\chi_{12}{}^{\mathrm{s}}(1 - 2X_{1\mathrm{s}}) + \beta C_\mathrm{s}(w_{11} - w_{22})/2 + \beta(\epsilon_{1\mathrm{s}} - \epsilon_{2\mathrm{s}})) - X_{2\mathrm{m}}(\chi_{12}{}^{\mathrm{m}}(1 - 2X_{1\mathrm{m}}) + \beta C_\mathrm{m}(w_{11} - w_{22})/2)] + X_{2\mathrm{s}} \exp[X_{1\mathrm{s}}(\chi_{12}{}^{\mathrm{s}}(1 - 2X_{2\mathrm{s}}) + \beta C_\mathrm{s}(w_{22} - w_{11})/2 + \beta(\epsilon_{2\mathrm{s}} - \epsilon_{1\mathrm{s}})) - X_{1\mathrm{m}}(\chi_{12}{}^{\mathrm{m}}(1 - 2X_{2\mathrm{m}}) + \beta C_\mathrm{m}(w_{22} - w_{11})/2)]\}^{M} \tag{32}$$

Insertion of this result into eq 27 generates the following alternate general expression for k_3:

$$k_3 = \left(\frac{2\pi M}{3}\right)^{1/2} \exp\left[-\left(\langle R_s^2\rangle - \frac{3}{2}\langle R_m^2\rangle\right)/Mb^2\right] \times \left(\frac{C_s(C_s-1)}{C_m(C_m-1)}\right)^{M-2} \times \left[\frac{N_s}{N_m}\left(\frac{N_m}{N_s}\right)^M \frac{N_s!(N_m-M)!}{N_m!(N_s-M)!}\right] \times \{\sum_{i=1}^{2} X_{is} \exp[-\beta\Delta\epsilon(i,3;s) - X_{is}(1-X_{is}) \times (M/N_s)\chi_{12}{}^{s} + X_{im}(1-X_{im})(M/N_m)\chi_{12}{}^{m} + (M/N_s)\sum_{i=1}^{2} X_{is}\chi_{i3}{}^{s} - (M/N_m)\sum_{i=1}^{2} X_{im}\chi_{i3}{}^{m} + (1-X_{is})(\chi_{12}{}^{s} + \chi_{i3}{}^{s} - \chi_{j\neq i,3}{}^{s}) - (1-X_{im})(\chi_{12}{}^{m} + \chi_{i3}{}^{m} - \chi_{j\neq i,3}{}^{m})]\}^M \tag{33'}$$

where $\chi_{j\neq i,3}{}^{a}$ (a ≡ m, s) means $\chi_{23}{}^{a}$ if $i = 1$ and $\chi_{13}{}^{a}$ if $i = 2$. Also $\beta\Delta\epsilon(i,3;s) \equiv \beta(\epsilon_{3s} - \epsilon_{is} - w_{i3} + w_{ii})$ ($i = 1, 2$) represents the (free) energy change[15] required for the adsorption of a polymer segment on the surface with the concomitant displacement of a type i solvent molecule from the stationary phase to a neat mobile phase of solvent i. While eq 33′ is general within the framework of the approximations already introduced, it is rather cumbersome and we shall specialize it to high molecular weight polymers in favorable solvents. In particular, we employ eq 24a,b, 28, and 29 and approximate $C_s(C_s-1)^{M-2}/C_m(C_m-1)^{M-2}$ by $((C_s-1)/(C_m-1))^M$ for large M to obtain

$$k_3 \simeq \exp[M\{M^{-1} \ln\left[\left(\frac{2\pi}{3}\right)^{1/2} g_s{}^{1/2} g_m{}^{-3/5} M^{1/5}\right] + M^{-4/5}[g_m{}^{-3/5}(5g_m+1)/2] - M^{-1/2}[g_s{}^{-1/2}(2g_s + \tfrac{1}{2})] + \ln\{\sum_{i=1}^{2} X_{is} \exp[-\beta(\Delta\epsilon(i,3;s) + kT\ln((C_m-1)/(C_s-1))) + (1-X_{is})(\chi_{12}{}^{s} + \chi_{i3}{}^{s} - \chi_{j\neq i,3}{}^{s}) - (1-X_{im})(\chi_{12}{}^{m} + \chi_{i3}{}^{m} - \chi_{j\neq i,3}{}^{m})]\}\}] \tag{33}$$

Two important special cases of eq 33 include a (i) single-solvent (e.g., 1) system and (ii) a binary solvent mobile phase but a single adsorbed solvent (e.g., 1) stationary phase. For the neat solvent system, eq 33 reduces to

$$k_3 \equiv k_3{}^{(1)} = \exp\left[M\left\{-\beta(\Delta\epsilon(1,3;s) + kT\ln((C_m-1)/(C_s-1))) - M^{-1/2}[g_s{}^{-1/2}(1)(2g_s(1)+\tfrac{1}{2})] + M^{-4/5}[g_m{}^{-3/5}(1)(5g_m(1)+1)/2] + M^{-1}\ln\left[\left(\frac{2\pi}{3}\right)^{1/2} g_s{}^{1/2}(1) g_m{}^{-3/5}(1) M^{1/5}\right]\right\}\right] \tag{34}$$

where $g_a(1) \equiv {}^1/_2 - \chi_{13}{}^{a}$ (a ≡ m, s). For the binary mobile and neat stationary phase with $X_{1s} = 1$ and $X_{2s} = 0$ (see eq 31 and the subsequent comments)

$$k_3 = \exp\left[M\left\{-\beta(\Delta\epsilon(1,3;s) + kT\ln((C_m-1)/(C_s-1))) - (1-X_{1m})(\chi_{12}{}^{m} + \chi_{13}{}^{m} - \chi_{23}{}^{m}) - M^{-1/2}[g_s{}^{-1/2}(1)[2g_s(1)+\tfrac{1}{2}]] + M^{-4/5}[g_m{}^{-3/5}(5g_m+1)/2] + M^{-1}\ln\left[\left(\frac{2\pi}{3}\right)^{1/2} g_s{}^{1/2}(1) g_m{}^{-3/5} M^{1/5}\right]\right\}\right] = \exp[Mf(X_{1m},\{\chi_{ij}{}^{m}\},\beta\Delta\epsilon(1,3;s),M)] \equiv \exp[Mf(X_{1m})] \tag{35}$$

Clearly, when $X_{1m} \to 1$, eq 35 reduces to eq 34, which is concisely expressed as $k_3{}^{(1)} \equiv \exp[Mf(X_{1m}=1,\chi_{13}{}^{m},\beta\Delta\epsilon(1,3;s),M)] \equiv \exp[Mf(1)]$. The retention behavior for high polymers predicted by eq 34 and 35 depends on the sign of $f(1)$ and $f(X_{1m})$. If $f > 0$ ($f < 0$), $k_3{}^{(1)}$ and k_3 are very large (very small) and a transition from very high to very low solute retention transpires as f passes through zero from positive to negative values. For a neat solvent $f(1)$ can be altered only through temperature variation while for a two-component mobile phase $f(X_{1m})$ varies with composition at fixed T. The critical temperature T_c and critical composition for a given T, $X_{1mc} \equiv X_{1mc}(T)$, which (if they exist) render $f(1) = 0$ and $f(X_{1mc}) = 0$ satisfy

$$\chi_{13c}{}^{m}(\Delta\epsilon(1,3;s))C_m{}^{-1}\delta_{13}{}^{-1} - \ln((C_m-1)/(C_s-1)) - M^{-1/2}[(\tfrac{1}{2} - \chi_{13c}{}^{s})^{-1/2}(\tfrac{3}{2} - 2\chi_{13c}{}^{s})] + M^{-4/5}[(\tfrac{1}{2} - \chi_{13c}{}^{m})^{-3/5}(\tfrac{7}{4} - \tfrac{5}{2}\chi_{13c}{}^{m})] + M^{-1}\left[\ln\left[\left(\frac{2\pi}{3}\right)^{1/2}(\tfrac{1}{2} - \chi_{13c}{}^{s})^{1/2}(\tfrac{1}{2} - \chi_{13c}{}^{m})^{-3/5} M^{1/5}\right]\right] = 0 \tag{36}$$

where $\chi_{13c}{}^{m} \equiv (C_m/C_s)\chi_{13c}{}^{s} \equiv -\beta_c C_m \delta_{13}$, $\chi_{13c}{}^{m} < {}^1/_2$, and

$$f(X_{1mc},\{\chi_{ij}{}^{m}\},\beta\Delta\epsilon(1,3;s),M) \equiv \beta(\Delta\epsilon(1,3;s) + kT\ln((C_m-1)/(C_s-1))) + (1-X_{1mc})(\chi_{12}{}^{m} + \chi_{13}{}^{m} - \chi_{23}{}^{m}) + M^{-1/2}[(\tfrac{1}{2} - \chi_{13}{}^{s})^{-1/2}(\tfrac{3}{2} - 2\chi_{13}{}^{s})] - M^{-4/5}[g_m{}^{-3/5}(X_{1mc})(5g_m(X_{1mc})+1)/2] - M^{-1}\ln\left[\left(\frac{2\pi}{3}\right)^{1/2}(\tfrac{1}{2} - \chi_{13}{}^{s})^{1/2} g_m{}^{-3/5}(X_{1mc}) M^{1/5}\right] = 0 \tag{37}$$

where

$$g_m(X_{1mc}) \equiv \tfrac{1}{2} - X_{1mc}\chi_{13}{}^{m} - (1-X_{1mc})\chi_{23}{}^{m} + X_{1mc}(1-X_{1mc})\chi_{12}{}^{m}$$

In the limit $M \to \infty$, eq 36 and 37 lead to the limiting values $T_c \to T_c{}^{\infty}$ and $X_{1mc} \to X_{1mc}{}^{\infty}$

$$T_c{}^{\infty} = -\Delta\epsilon(1,3;s)/(k\ln((C_m-1)/(C_s-1))) \tag{38}$$

and

$$X_{1mc}{}^{\infty} = 1 + \beta(\Delta\epsilon(1,3;s) + kT\ln((C_m-1)/(C_s-1))) \times (\chi_{12}{}^{m} + \chi_{13}{}^{m} - \chi_{23}{}^{m})^{-1} \tag{39}$$

where $\chi_{12}{}^{m} + \chi_{13}{}^{m} - \chi_{23}{}^{m} \neq 0$. Clearly, $\Delta\epsilon(1,3;s) < 0$ if meaningful values of $T_c{}^{\infty}$ are to result. For a cubic lattice $C_m = 6$, $C_s = 5$, and $\Delta\epsilon(1,3;s)/kT_c{}^{\infty} = \ln {}^4/_5 = -0.2231$ and when $\Delta\epsilon(1,3;s)/kT_c{}^{\infty}$ is less (greater) than $\ln {}^4/_5$ adsorption (no adsorption) of the polymer occurs at the surface. DiMarzio and McCrackin[4] have obtained $\epsilon/kT_c = \ln {}^1/_2$, where ϵ is the polymer segment–surface adhesion energy for the adsorption of a completely isolated, flexible one-dimensional polymer chain in the limit $M \to \infty$. The factor ${}^1/_2$ corresponds to the relative number of ways, 1 or 2, an individual internal polymer segment can be placed on a one-dimensional lattice if the adjacent segment is in contact or not in contact with the surface. The corresponding result for a polymer chain on a three-dimensional cubic lattice is ${}^4/_5$ since a polymer segment has four (five) options if the adjacent segment is adsorbed (not adsorbed) and the site occupied by the adjacent segment is excluded.

Clearly, the last term on the right-hand side of eq 39 must be negative in order to obtain a physically meaningful result for $X_{1mc}{}^{\infty}$, which obeys $0 < X_{1mc}{}^{\infty} < 1$. For favorable (unfavorable) polymer solvents $\chi_{i3}{}^{m} \lesssim 0$ ($\chi_{i3}{}^{m} \gg 0$) ($i = 1, 2$) and if solvents 1 and 2 are respectively favorable and unfavorable, while solvents 1 and 2 are miscible, $\chi_{12}{}^{m} < 2$ ($\chi_{12}{}^{m} = 2$ is the critical value for miscibility of two solvents of similar molecular size in the Bragg–Williams approximation[13]), then we anticipate that $\chi_{12}^{\mathrm{m}} + \chi_{13}^{\mathrm{m}} - \chi_{23}^{\mathrm{m}} < 0$. The numerator $\beta(\Delta\epsilon(1,3;\mathrm{s}) + kT \ln [(C_{\mathrm{m}} - 1)/(C_{\mathrm{s}} - 1)])$ of the last term on the right-hand side of eq 39 is positive provided $-\beta\Delta\epsilon(1,3;\mathrm{s}) < \ln [(C_{\mathrm{m}} - 1)/(C_{\mathrm{s}} - 1)] \equiv 0.223$ for a cubic lattice, and this condition should be satisfied for compatible adsorbed solvent 1–polymer surface layers. It is, of course, also possible to obtain meaningful $X_{1\mathrm{mc}}^{\infty}$ values when $\chi_{12}^{\mathrm{m}} + \chi_{13}^{\mathrm{m}} - \chi_{23}^{\mathrm{m}} > 0$ and $\beta(\Delta\epsilon(1,3;\mathrm{s}) + kT \ln [(C_{\mathrm{m}} - 1)/(C_{\mathrm{s}} - 1)]) < 0$ although this situation does not correspond to the methylene chloride–methanol–polystyrene system to be discussed in section III.

It proves convenient in the subsequent analysis to rewrite eq 34 and 35 as

$$k_3^{(1)} = \exp[M\sum_{j=1}^{\infty} A_j^{(1)}(\chi_{13}^{\mathrm{m}} - \chi_{13\mathrm{c}}^{\mathrm{m}})^j] \tag{40}$$

and

$$k_3 = \exp[M\sum_{j=1}^{\infty} A_j(X_{1\mathrm{m}} - X_{1\mathrm{mc}})^j] \tag{41}$$

where $f(1,\chi_{13\mathrm{c}}^{\mathrm{m}}) = 0$, $f(X_{1\mathrm{mc}}) = 0$, and

$$A_j^{(1)} = \frac{1}{j!}\,\frac{\partial^j f(1)}{\partial \chi_{13\mathrm{c}}^{\mathrm{m}j}}, \qquad A_j = \frac{1}{j!}\,\frac{\partial^j f}{\partial X_{1\mathrm{mc}}^{j}} \qquad j = 1, 2, ..., \infty \tag{42}$$

with

$$\frac{\partial f(1)}{\partial \chi_{13\mathrm{c}}^{\mathrm{m}}} = A_1^{(1)} = \Delta\epsilon(1,3;\mathrm{s})/C_{\mathrm{m}}\delta_{13} + M^{-1/2}[g_{\mathrm{sc}}(1) - \tfrac{1}{2}]5g_{\mathrm{sc}}^{-3/2}(1)/6 + M^{-4/5}[0.3 - g_{\mathrm{mc}}(1)]g_{\mathrm{mc}}^{-8/5}(1) + M^{-1}[\tfrac{3}{5}g_{\mathrm{mc}}(1) - \tfrac{1}{2}g_{\mathrm{sc}}(1)]$$

and

$$\frac{\partial^j f(1)}{\partial \chi_{13\mathrm{c}}^{\mathrm{m}j}} = M^{-1/2}\frac{\partial^{j-1}}{\partial \chi_{13\mathrm{c}}^{\mathrm{m}j-1}}[5g_{\mathrm{sc}}^{-3/2}(1)[g_{\mathrm{sc}}(1) - \tfrac{1}{2}]/6] + M^{-4/5}\frac{\partial^{j-1}}{\partial \chi_{13\mathrm{c}}^{\mathrm{m}j-1}}\{[0.3 - g_{\mathrm{mc}}(1)]g_{\mathrm{mc}}^{-8/5}(1)\} + M^{-1}\frac{\partial^{j-1}}{\partial \chi_{13\mathrm{c}}^{\mathrm{m}j-1}}[\tfrac{3}{5}g_{\mathrm{mc}}(1) - \tfrac{1}{2}g_{\mathrm{sc}}(1)] \qquad j \geq 2 \tag{43}$$

where $g_{\mathrm{mc}}(1) \equiv g_{\mathrm{m}}(1)|_{\chi_{13}^{\mathrm{m}}=\chi_{13\mathrm{c}}^{\mathrm{m}}} = {}^1/_2 - \chi_{13\mathrm{c}}^{\mathrm{m}}$ and $g_{\mathrm{sc}}(1) = g_{\mathrm{s}}(1)|_{\chi_{13}^{\mathrm{s}}=(5/6)\chi_{13\mathrm{c}}^{\mathrm{m}}} = {}^1/_2 - 5\chi_{13\mathrm{c}}^{\mathrm{m}}/6$. Also

$$\frac{\partial f}{\partial X_{1\mathrm{mc}}} = A_1 = \chi_{12}^{\mathrm{m}} + \chi_{13}^{\mathrm{m}} - \chi_{23}^{\mathrm{m}} + [M^{-4/5}g_{\mathrm{m}}^{-8/5}(X_{1\mathrm{mc}})(g_{\mathrm{m}}(X_{1\mathrm{mc}}) - 0.3) - \tfrac{3}{5}M^{-1}g_{\mathrm{m}}^{-1}(X_{1\mathrm{mc}})]\{\chi_{12}^{\mathrm{m}} + \chi_{23}^{\mathrm{m}} - \chi_{13}^{\mathrm{m}} - 2X_{1\mathrm{mc}}\chi_{12}^{\mathrm{m}}\}$$

and

$$\frac{\partial^j f}{\partial X_{1\mathrm{mc}}^{j}} = \frac{\partial^{j-1}}{\partial X_{1\mathrm{mc}}^{j-1}}\left[\left(M^{-4/5}g_{\mathrm{m}}^{-8/5}(X_{1\mathrm{mc}}) \times (g_{\mathrm{m}}(X_{1\mathrm{mc}}) - 0.3) - \frac{3}{5}M^{-1}g_{\mathrm{m}}^{-1}(X_{1\mathrm{mc}})\right)\right]\{\chi_{12}^{\mathrm{m}} + \chi_{23}^{\mathrm{m}} - \chi_{13}^{\mathrm{m}} - 2X_{1\mathrm{mc}}\chi_{12}^{\mathrm{m}}\} \qquad j \geq 2 \tag{44}$$

The higher order coefficients $A_j^{(1)}$ ($j \geq 2$) and A_j ($j \geq 2$) vary as inverse powers of M and $|A_j^{(1)}| < |A_1^{(1)}|$ and $|A_j| < |A_1|$ for $j \geq 2$ and sufficiently large M provided $\Delta\epsilon(1,3;\mathrm{s}) \neq 0$ and $\chi_{12}^{\mathrm{m}} + \chi_{13}^{\mathrm{m}} - \chi_{23}^{\mathrm{m}} \neq 0$. Also if $\chi_{13}^{\mathrm{m}} \sim \chi_{13\mathrm{c}}^{\mathrm{m}}$ and $X_{1\mathrm{m}} \sim X_{1\mathrm{mc}}$ and when M is large, only the leading terms of the expansions $\sum_{j=1}^{\infty}A_j^{(1)}(\chi_{13}^{\mathrm{m}} - \chi_{13\mathrm{c}}^{\mathrm{m}})^j$ and $\sum_{j=1}^{\infty}A_j(X_{1\mathrm{m}} - X_{1\mathrm{mc}})^j$ contribute significantly and

$$k_3^{(1)} \simeq \exp[MA_1^{(1)}(\chi_{13}^{\mathrm{m}} - \chi_{13\mathrm{c}}^{\mathrm{m}})] = \exp[M(\beta_{\mathrm{c}}\Delta\epsilon(1,3;\mathrm{s}))(1 - T_{\mathrm{c}}/T)\{1 + (C_{\mathrm{m}}\delta_{13}A_1^{(1)}(\Delta\epsilon(1,3;\mathrm{s}))^{-1} - 1)\}] \tag{45}$$

where $T_{\mathrm{c}} \equiv -C_{\mathrm{m}}\delta_{13}/k\chi_{13\mathrm{c}}^{\mathrm{m}}$ and $T \equiv -C_{\mathrm{m}}\delta_{13}/k\chi_{13}^{\mathrm{m}}$; and

$$k_3 \simeq \exp[MA_1(X_{1\mathrm{m}} - X_{1\mathrm{mc}})] = \exp[M(\chi_{12}^{\mathrm{m}} + \chi_{13}^{\mathrm{m}} - \chi_{23}^{\mathrm{m}})(X_{1\mathrm{m}} - X_{1\mathrm{mc}})\{1 + (A_1(\chi_{12}^{\mathrm{m}} + \chi_{13}^{\mathrm{m}} - \chi_{23}^{\mathrm{m}})^{-1} - 1)\}] \tag{46}$$

In the limit $M \to \infty$, eq 45 and 46, respectively, reduce to

$$k_{3\infty}^{(1)} \equiv \lim_{M\to\infty} k_3^{(1)} = \exp[M(\ln((C_{\mathrm{m}} - 1)/(C_{\mathrm{s}} - 1))) \times (T_{\mathrm{c}}^{\infty}/T - 1)] = \exp[0.223M(T_{\mathrm{c}}^{\infty}/T - 1)] \tag{47}$$

and

$$k_{3\infty} \equiv \lim_{M\to\infty} k_3 = \exp[M(\chi_{12}^{\mathrm{m}} + \chi_{13}^{\mathrm{m}} - \chi_{23}^{\mathrm{m}})(X_{1\mathrm{m}} - X_{1\mathrm{mc}}^{\infty})] \tag{48}$$

where the latter form of eq 47 applies to a cubic lattice and T_{c}^{∞} and $X_{1\mathrm{mc}}^{\infty}$ are given by eq 38 and 39, respectively. Clearly, if $\chi_{12}^{\mathrm{m}} + \chi_{13}^{\mathrm{m}} - \chi_{23}^{\mathrm{m}} = 0$ and $\chi_{12}^{\mathrm{m}} = \chi_{13}^{\mathrm{m}} = \chi_{23}^{\mathrm{m}} = 0$, which represents an ideal ternary mixture, the expansion of $f(X_{1\mathrm{m}})$ defined by eq 41 and 42 does not exist and eq 46 and 48 do not apply since $f(X_{1\mathrm{m}},\{\chi_{ij}^{\mathrm{m}}=0\},\beta\Delta\epsilon(1,3;\mathrm{s}),M)$ is independent of $X_{1\mathrm{m}}$ (see eq 37).

Equations 45 and 46 and/or eq 47 and 48 reveal that $k_3^{(1)}$ and k_3 and/or their limiting forms $k_{3\infty}^{(1)}$ and $k_{3\infty}$ change abruptly from very large to very small values as T and $X_{1\mathrm{m}}$ approach and pass through T_{c} and $X_{1\mathrm{mc}}$ and/or T_{c}^{∞} and $X_{1\mathrm{mc}}^{\infty}$. The transition from retained to unretained polymer becomes increasingly abrupt as M increases but becomes discontinuous only in the limit $M \to \infty$ at $T = T_{\mathrm{c}}^{\infty}$ and/or $X_{1\mathrm{mc}}^{\infty}$. Since we anticipate $\beta_{\mathrm{c}}\Delta\epsilon(1,3;\mathrm{s}) < 0$ and $\chi_{12}^{\mathrm{m}} + \chi_{13}^{\mathrm{m}} - \chi_{23}^{\mathrm{m}} < 0$, $k_3^{(1)}$ and k_3 should be very large (small) if $T < T_{\mathrm{c}}$ and $X_{1\mathrm{m}} < X_{1\mathrm{mc}}$ ($T > T_{\mathrm{c}}$, $X_{1\mathrm{m}} > X_{1\mathrm{mc}}$). Since both T_{c} and $X_{1\mathrm{mc}}$ depend on M (recall eq 36 and 37), the transition from large to small values of $k_3^{(1)}$ and k_3, respectively, occurs at different temperatures and mobile phase compositions (at constant temperature) and this is the essential feature that, in principle, permits homopolymer separation by molecular weight with temperature or composition gradient HPLC and/or TLC. The molecular-weight dependence of T_{c} and $X_{1\mathrm{mc}}$ ultimately arises from the molecular flexibility of a chainlike polymer molecule, which enables it to alter its configurational geometry and dimensions in response to its environment.

Comparison of eq 36 and 37 and eq 45 and 46 indicates an important advantage of employing a mixed solvent rather than a neat mobile phase since the transition from high to low solute retention and ultimately homopolymer fractionation can be monitored by systematically adjusting the mobile phase composition rather than the temperature, which is of considerable practical importance in HPLC and TLC. Separation of polystyrene homopolymers in methylene chloride–methanol mixed solvents by gradient elution HPLC and TLC is discussed in the next section. Separation of homopolymers by molecular weight in single-solvent HPLC or TLC by temperature variation is probably not feasible because a large operational temperature range would be necessary to cover a broad molecular weight range. In Table I, $\chi_{13\mathrm{c}}^{\mathrm{m}\infty}/\chi_{13\mathrm{c}}^{\mathrm{m}} \equiv T_{\mathrm{c}}/T_{\mathrm{c}}^{\infty}$–$M$ results determined from solving eq 36 with $\Delta\epsilon(1,3;\mathrm{s})/\delta_{13} = -1$ are presented. We have assumed the temperature dependence of $\Delta\epsilon(1,3;\mathrm{s})$ is negligible in performing the calculations. The results suggest that temperature variation methods used in conjunction with HPLC might have limited applicability for homopolymers within a narrow molecular weight range and might be suitable for con-

Table I

$\chi_{13c}^{m\infty}/\chi_{13c}^{m} = T_c/T_c^{\infty}$ Values as a Function of M Calculated from Eq 36 Using $-\Delta\epsilon(1,3;s)/\delta_{13} = 1$

M	T_c/T_c^{∞}	M	T_c/T_c^{∞}
22.38	0.329	3714.3	0.832
166.67	0.531	35238	0.937
333.33	0.610	95238	0.960
1047.6	0.729	∞	1.000

venient separation of high molecular weight homopolymers in a single solvent, which provides a convenient operational value of T_c^{∞}.

In Table II, X_{1mc}–M results determined from eq 37 are presented for a few selections of $\{\chi_{ij}^{m}\}$ and $\beta\Delta\epsilon(1,3;s)$ consistent with $\chi_{12}^{m} + \chi_{13}^{m} - \chi_{23}^{m} < 0$ and $\beta(\Delta\epsilon(1,3;s) + kT \ln [(C_m - 1)/(C_s - 1)]) > 0$. The values of X_{1mc} increase monotonically with increasing M and asymptotically approach X_{1mc}^{∞}. The difference of successive X_{1mc} values decreases with increasing M and $X_{1mc}^{\infty} - X_{1mc} \sim M^{-1/2}$ for large M. This suggests that homopolymer fractionation might become more difficult as M increases.

For comparison, application of the preceding analysis to rigid-rodlike solute molecules in binary monomeric solvents on a cubic lattice produces the capacity factor

$$k_{3R} = (\eta_s/\eta_m)[X_{1s} \exp[-\beta\{\Delta\epsilon(1,3;s) + (5X_{2s} - 6X_{2m})(w_{11} - w_{12}) + [(4 + 2M^{-1})X_{2m} - (3 + 2M^{-1})X_{2s}](w_{13} - w_{23})\}] + X_{2s} \exp[-\beta\{\Delta\epsilon(2,3;s) + (5X_{1s} - 6X_{1m})(w_{22} - w_{12}) + [(4 + 2M^{-1})X_{1m} - (3 + 2M^{-1})X_{1s}](w_{23} - w_{13})\}]]^{M} \quad (49)$$

where η_s/η_m represents the ratio of the total number of allowed rigid-rod orientations in the stationary and mobile phases. In the derivation we have explicitly considered the connectivity of the internal segments comprising a solute molecule in determining the solute–solvent interactions. Also we have assumed that $M/V_a \rightarrow 0$ ($a \equiv$ m, s), where V_m and V_s are the volumes of the mobile and stationary phases, which is consistent with the limit of infinite solute dilution. In a neat solvent (e.g., 1), $X_{1m} = X_{1s} = 1$, $X_{2m} = X_{2s} = 0$, and

$$k_{3R}^{(1)} = \exp[-M\beta(\Delta\epsilon(1,3;s) + kTM^{-1} \ln (\eta_m/\eta_s))] \quad (50)$$

High (low) retention occurs for large M when $\beta(\Delta\epsilon(1,3;s) + kTM^{-1} \ln (\eta_m/\eta_s))$ is negative (positive). When there is a binary solvent mobile phase but only a single adsorbed solvent (e.g., 1), then $X_{1s} = 1$, $X_{2s} = 0$, and

$$k_{3R} = \exp[A_R M(X_{1mc}^{R} - X_{1m})] \quad (51)$$

where

$$X_{1mc}^{R} = 1 + \beta(\Delta\epsilon(1,3;s) + kTM^{-1} \ln (\eta_m/\eta_s))\{\chi_{12}^{m} + \chi_{13}^{m} - \chi_{23}^{m} + (1 - M^{-1})(\tfrac{1}{3}(\chi_{23}^{m} - \chi_{13}^{m}) + \beta(w_{22} - w_{11}))\}^{-1} \quad (52)$$

and

$$A_R = \chi_{23}^{m} - \chi_{12}^{m} - \chi_{13}^{m} + (1 - M^{-1})(\tfrac{1}{3}(\chi_{13}^{m} - \chi_{23}^{m}) + \beta(w_{11} - w_{22})) \quad (53)$$

If $0 < X_{1mc}^{R} < 1$, then a transition from high to low retention occurs abruptly for large M as the mobile phase composition of solvent 1 is increased beyond X_{1mc}^{R}. The critical composition X_{1mc}^{R} depends only weakly on M for rigid-rod solutes of large M and the M dependence arises from the contiguous nature of the molecular segments comprising a solute molecule, which allows only $4M + 2$ ($3M + 2$) rather than $6M$ ($5M$) nearest-neighbor interactions with the solvents in the mobile (stationary) phase. This dependence, in fact, was suppressed in the analysis of flexible homopolymer solutes. The M dependence associated with the term involving $\ln (\eta_m/\eta_s)$ appears because we have chosen to set $k_{3R} = 1$ rather than $k_{3R} = \eta_s/\eta_m$ when $X_{1mc} \boxminus X_{1mc}^{R}$ and for large M contributes insignificantly to the value of X_{1mc}^{R}. The weak X_{1mc}^{R}–M dependence predicted for rigid-rodlike polymeric solutes suggests that fractionation by molecular weight should be very difficult by gradient elution HPLC or TLC methods. For example, if the polystyrene homopolymer solutes were regarded as rigid-rodlike polymers (same M values), then for the selections $\beta\Delta\epsilon(1,3;s) = 0.1$, $\chi_{12}^{m} = \chi_{13}^{m} = 0$, $\chi_{23}^{m} = {}^{1}/_{2}$, $\beta(w_{11} - w_{22}) = 0$, and $\eta_m/\eta_s = {}^{3}/_{2}$, X_{1mc}^{R} ranges from 0.694 to 0.700 as M increases from 167 to 95000, and for $M = 22.4$, $X_{1mc}^{R} = 0.653$. Thus apparently only the lowest molecular weight solute would be separable from the other "rigid-rodlike" homopolymers. The selection $\eta_m/\eta_s = {}^{3}/_{2}$ corresponds to assuming that the rigid-rod solutes on a simple cubic lattice have three (two) allowed mutually orthogonal orientations in the mobile (stationary) phase.[15]

III. Possible Extensions of the Analysis and Comparison between Theory and Experiment

In this section we briefly consider whether eq 37 and 46 can qualitatively account for the trends observed by Armstrong and Bui[1] in their recent successful polystyrene homopolymer fractionation experiments performed with a methylene chloride–methanol mobile phase in conjunction with reversed-phase HPLC and TLC using a chemically bonded C-18 stationary phase. Separation over a molecular weight range of 105 (polystyrene monomer) to 10^7 daltons was achieved over a narrow range of mobile phase composition enriched in methylene chloride. Achievement of fractionation required gradient elution HPLC or a naturally occurring mobile phase composition gradient, which is inherent in most TLC plate developments with binary solvents. The TLC gradient was verified experimentally[1] starting from a 50:50 methylene chloride–methanol mobile phase and revealed a depletion of methylene chloride in the mobile phase with increasing solvent-front migration distance even though a constant mobile phase composition was maintained in the solvent reservoir. This suggests selective stationary phase ad-

Table II

X_{1mc} and A_1 Values as a Function of M Calculated Respectively from Eq 37 and 44 for the Following Reduced Energy Parameter Sets (χ_{12}^{m}, χ_{13}^{m}, χ_{23}^{m}, $\beta\Delta\epsilon(1,3;s)$): (1) (0, 0, 0.5, −0.102); (2) (0.05, 0, 0.5, −0.115); (3) (0, −0.1, 0.5, −0.075); (4) (0.1, 0, 0.5, −0.1275)

M	X_{1mc}	$-A_1$	X_{1mc}	$-A_1$	X_{1mc}	$-A_1$	X_{1mc}	$-A_1$
22.38	0.2740	0.5998	0.2275	0.5674	0.3303	0.6830	0.1761	0.5498
166.67	0.5181	0.5071	0.4934	0.4571	0.5476	0.6064	0.4612	0.4073
333.33	0.5773	0.5031	0.5593	0.4531	0.5984	0.6028	0.5355	0.4030
1047.6	0.6478	0.5009	0.6376	0.4508	0.6588	0.6007	0.6236	0.4008
3714.3	0.6958	0.5002	0.6910	0.4502	0.7000	0.6002	0.6836	0.4002
35238	0.7364	0.5000	0.7360	0.4500	0.7348	0.6000	0.7342	0.4000
95238	0.7445	0.5000	0.7450	0.4500	0.7418	0.6000	0.7444	0.4000
∞	0.7577	0.5000	0.7597	0.4500	0.7531	0.6000	0.7609	0.4000

sorption of the less polar component—methylene chloride—occurs.

Equations 37 and 46 can only be expected to apply to an idealized liquid chromatographic situation involving isolated polymer molecules, which may not be achieved especially in the stationary phase under any experimentally accessible conditions. Hence an analysis that includes interactions between different polymer molecules may ultimately be required to obtain a meaningful quantitative interpretation of the liquid chromatographic results.[17-19] The analysis would also benefit from a more sophisticated description of the configurational behavior of a polymer molecule retained in the stationary phase. A two-dimensional distribution of polymer configurations may become inaccurate (especially for the lower molecular weight polymers) near the critical composition X_{1mc}. In this regime a precipitated polymer molecule may possess loops that extend well into the mobile phase and a substantial polymer segment concentration profile may develop.[17-19]

The stationary phase has been approximated by an impenetrable planar surface, which is a considerable oversimplification to the actual structure of a chemically bonded C-18 phase, which depends on the extent of surface coverage and the solvent environment.[20,21] For high coverages and a favorable solvent environment (i.e., nonpolar solvents) the bonded phase should be densely packed with the chains in a rather extended configuration in order to facilitate solvent uptake. On the other hand, highly polar solvents probably do not penetrate into the bonded phase appreciably and the chains may assume overlapping configurations, which tend to exclude such solvents. The actual configurations of an isolated polymer chain in a chemically bonded stationary phase may also be less restricted than those confined to the monolayer directly above a planar surface and a possible extension of the present analysis would be a more detailed treatment of the statistical behavior of an adsorbed chain in a bonded phase.

The assumption of uniform solvent composition within the domain of the polymer coil and the bulk solvent is subject to improvement since one anticipates that the solvent composition within the coil should be enriched in the better solvent. Thus a solvent composition gradient and an associated surface free energy at the boundary between regions occupied by the polymer and the bulk solvent devoid of polymer should be included in the analysis. The present analysis was applied to a system where only the less polar solvent was adsorbed appreciably in the stationary phase; hence neglect of this surface energy may not be too severe, especially when the mobile phase is also relatively rich in the less polar solvent.

Despite these shortcomings eq 37 and 46, which at best should only apply to idealized HPLC or TLC situations involving an isolated polymer molecule, can nevertheless qualitatively account for the experimentally observed trends provided reasonable estimates of $\chi_{12}{}^{m}$, $\chi_{13}{}^{m}$, $\chi_{23}{}^{m}$, and $\beta\Delta\epsilon(1,3;s)$ at constant temperature which conform to the experimental system are introduced. The TLC experiments of Armstrong and Bui revealed that polystyrene migrated with the advancing solvent front with neat methylene chloride while migration did not occur in neat methanol. Thus methylene chloride (methanol) is a good (poor) solvent and if we label methylene chloride (methanol) as solvent 1 (2), then we anticipate that $\chi_{13}{}^{m} \ll {}^{1}/_{2}$ and $\chi_{13}{}^{m} \ll \chi_{23}{}^{m} \leq {}^{1}/_{2}$. The upper limit, $\chi_{23}{}^{m} = {}^{1}/_{2}$, guarantees that the methanol–polystyrene system is completely miscible even if $M \to \infty$, at least within the framework of the Flory–Huggins lattice theory of polymer solutions.[13,14] The entries in Table II are for $\chi_{23}{}^{m} = {}^{1}/_{2}$, $\chi_{13}{}^{m} = 0$ or $\chi_{13}{}^{m} = 0.1$, and $\chi_{12}{}^{m} = 0, 0.05$, or 0.1, and these selections are consistent with $\chi_{12}{}^{m} + \chi_{13}{}^{m} - \chi_{23}{}^{m} < 0$ and that solvents 1 and 2 are completely miscible.[13] The requirement that $\beta\Delta\epsilon(1,3;s) > \ln {}^{4}/_{5} = -0.223$ is also satisfied for the entries in Table II in order that physically meaningful solutions of eq 37 result. The lower bound imposed on $\beta\Delta\epsilon(1,3;s)$ is also consistent with a polymer segment and a solvent 1 molecule possessing comparable affinities for the C-18-bonded stationary phase surface and being compatible in solution. No attempt is made here to select values of the energy parameters which correlate with experimental solution and adsorption data. Rather we are content to demonstrate that reasonable selections of these parameters in eq 37 and 46 can predict the experimental trends.

The migration velocity of a solute at a given position Z and time t in a HPLC column is given by[22]

$$v(Z,t) = u/(1 + k_3(X_{1m}(Z,t))) = \mathrm{d}Z/\mathrm{d}t \qquad (54)$$

where u is the flow velocity of the mobile phase and unretained solute. If the mobile phase solvent mole fraction composition varies linearly with time

$$X_{1m}(Z,t) = X_{1m}(0,0) + B(t - Z/u) \qquad (55)$$

where B is a positive constant and $X_{1m}(0,0)$ is the initial composition at the column entrance at $Z = 0$, then eq 54 may be integrated using eq 55 to eliminate the time t to obtain

$$B^{-1}\int_{X_{1m}(0,0)}^{X_{1m}(0,0)+B(t_{R}-t_{m})} \mathrm{d}X_{1m}\, k_3{}^{-1}(X_{1m}) = \int_0^L \mathrm{d}Z/u = L/u \equiv t_{m} \qquad (56)$$

where t_{R} is the solute retention time and $t_{m} \equiv L/u$ is the time required for unretained solute to flow through a column of length L. When eq 46 is employed for k_3, then eq 56 may be easily integrated and rearranged to

$$t_{R} = t_{m} + (|A_1|MB)^{-1} \times \ln\,[1 + |A_1|MBt_{m} \exp[|A_1|M(X_{1mc} - X_{1m}(0,0))]] \qquad (57')$$

and this reduces to

$$t_{R} \simeq t_{m} + B^{-1}(X_{1mc} - X_{1m}(0,0)) + (|A_1|MB)^{-1} \ln\,[|A_1|MBt_{m}] \qquad (57)$$

whenever $|A_1|MBt_{m} \exp[|A_1|M(X_{1mc} - X_{1m}(0,0))] \gg 1$. Clearly, when $M \to \infty$ and $X_{1m}(0,0) < X_{1mc}$, $t_{R} \to t_{m} + (X_{1mc} - X_{1m}(0,0))/B = t_{m} + t_{c}$, where t_{c} is the time required for the mobile phase composition at the inlet ($Z = 0$) to develop to X_{1mc} when a linear composition gradient is maintained. Equations 57 and 37 provide the relationship between t_{R} and M and predict that a very dilute homopolymer mixture can be separated by gradient elution HPLC. Operationally the composition gradient should progressively enrich the mobile phase with the better polymer solvent and $X_{1m}(0,0)$ should be less than the critical composition for the lowest molecular weight homopolymer present. The order of elution then proceeds successively from the lowest to the highest molecular weight constituent. If $X_{1m}(0,0) > X_{1mc}(M_{I})$, where $X_{1mc}(M_{I})$ is the critical composition obtained from eq 37 for some intermediate $M = M_{I}$, then eq 57′ reduces to

$$t_{R} \simeq t_{m}(1 + \exp[-|A_1|M(X_{1m}(0,0) - X_{1mc})]) \simeq t_{m}$$

for all polymer components with $M < M_{I}$. Thus separation for all components with $M < M_{I}$ should be difficult, if not impossible, since they proceed essentially unretained through the column provided their molecular weights are sufficiently high to render $\exp[-|A_1|M(X_{1m}(0,0) - X_{1mc})]$ very small. If $X_{1m}(0,0) \geq X_{1mc}{}^{\infty}$, then $t_{R} \lesssim t_{m}(1 + \exp(-|A_1|M(X_{1mc}{}^{\infty} - X_{1mc})))$ and $t_{R} \approx t_{m}$ for all but the lowest

Table III
Retention Times t_R (min) Calculated from Eq 57 Using the X_{1mc} and $-A_1$ Values Given in Table II for the Listed Sets of Energy Parameters[a]

M	$t_R(1)$	$t_R(2)$	$t_R(3)$	$t_R(4)$	t_R-(exptl)
22.38	3.1	3.0	3.3	2.9	3.2
166.67	6.8	6.0	7.6	5.0	8.3
333.33	8.5	8.0	9.0	7.2	8.8
1047.6	10.4	10.1	10.8	9.7	10.1
3714.3	11.8	11.7	12.0	11.5	11.6
35238	13.1	13.0	13.0	13.0	12.7
95238	13.3	13.3	13.2	13.3	13.3
∞	13.7	13.8	13.5	13.8	

[a] The experimental t_R values obtained by Armstrong and Bui are included for comparison. The values of $\beta\Delta\epsilon(1,3;s)$ in each energy parameter set were selected so that the t_R calculated from eq 57 for M = 95 238 matched the experimental value, 13.3 min. The temporal variation of the composition was chosen to be $X_{1m}(t) = 0.4163 + 0.031t$, where t is in minutes and t_m = 2.72 min.

molecular weight polymers. Thus under these operating conditions the higher molecular weight homopolymers will elute first, probably without separation. The lowest molecular weight component will elute last and may be resolved from the higher molecular weight components.

Retention times, t_R, calculated from eq 57 appear in Table III for the $(\chi_{12}{}^{m},\chi_{13}{}^{m},\chi_{23}{}^{m},\beta\Delta\epsilon(1,3;s))$ sets given in Table II with $X_{1m}(0,0) = 0.4163$, $B = 0.031$ (min^{-1}), t_m = 2.72 min, and M values corresponding to the polystyrene homopolymers investigated by Armstrong and Bui. Their experimentally measured t_R values obtained from gradient elution HPLC are included for comparison. The composition gradient $X_{1m}(Z,t) = 0.4163 + 0.031(t - Z/u)$, where X_{1m} refers to methylene chloride, and t_m = 2.72 min were selected to duplicate the experimental conditions as closely as possible. Armstrong and Bui actually employed an initial 53:47 methylene chloride–methanol mixture by volume and increased the volume percentage of methylene chloride linearly so that after 18.8 min the mobile phase was neat in that solvent. The linear composition gradient employed here reproduces the experimental gradient provided there is no volume change upon mixing. The comparison reveals that for entirely reasonable selections of the energy parameters, the experimental trends can be reproduced and the experimental results semiquantitatively predicted, especially for the large-M homopolymers (especially with sets (1) and (2)). In particular, the trend that the resolution should diminish with increasing M as $X_{1mc} \to X_{1mc}{}^{\infty}$ appears to be observed experimentally.

Isocratic HPLC was observed to be unsuccessful for polystyrene fractionation.[1] This is consistent with the present analysis since for a fixed mobile phase composition, eq 54 indicates that for large M either $v(Z,t) \simeq u$ or $v(Z,t) \simeq 0$, depending upon whether $X_{1m} > X_{1mc}$ or $X_{1m} < X_{1mc}$. Thus the polymeric solutes either move along with the mobile phase or are very strongly retained and separation only between unretained and retained components develops. When $X_{1m} > X_{1mc}{}^{\infty}$ and isocratic HPLC prevails, the higher molecular weight solutes elute together with $t_R \simeq t_m$ and precede the lowest molecular weight component(s), which are weakly retained and have $t_R > t_m$. This prediction is consistent with the experimental isocratic HPLC results in a 77.5:22.5 methylene chloride–methanol mobile phase (by volume), where the high molecular weight polymers with M ranging from 167 to 95 000 appeared together unresolved in the chromatogram while the lowest molecular weight polymer with M = 22.4 possessed the longest t_R and was separable from the other components.

Table IV
TLC R_f Values Calculated from Eq 53 Using the X_{1mc} and $-A_1$ Values Given in Table II for the Listed Sets of Energy Values and $X_{1m}(0) = 0.829$ and κ = 0.455[a]

M	$R_f(1)$	$R_f(2)$	$R_f(3)$	$R_f(4)$	R_f-(exptl)
22.38	0.965	0.976	0.948	0.986	0.715
166.67	0.742	0.793	0.676	0.856	0.568
333.33	0.597	0.639	0.547	0.692	0.507
1047.6	0.418	0.442	0.391	0.475	0.429
3714.3	0.300	0.311	0.289	0.328	0.358
35238	0.204	0.205	0.207	0.209	0.244
95238	0.185	0.184	0.191	0.185	0.185

[a] The experimental R_f values obtained by Armstrong and Bui are included for comparison. The composition $X_{1m}(0) = 0.829$ corresponds to that obtained from $X_{1m}(t) = 0.416 + 0.031t$ for the longest retention time observed, t_R = 13.3 min, in the HPLC experiments. The value of κ was chosen to match the experimental R_f value for the highest molecular weight polymer.

In TLC whenever the composition of the better solvent $X_{1m}(Z,t)$ drops below X_{1mc}, the polymeric solute will precipitate onto the stationary phase. If $X_{1m}(Z,t)$ is available as a function of Z along the TLC plate at time t, then eq 54 may be integrated using eq 46 for k_3 to obtain the ratio of the solute migration distance $d(\tau)$ at time τ to the distance $L = u\tau$ advanced by the solvent front. The ratio $d(\tau)/L \equiv R_f$ is the relevant measure of retention in TLC. If we assume $X_{1m}(Z,t) = X_{1m}(0) - \kappa_1 Z/u$ $(t > Z/u)$, then

$$R_f = (|A_1|M\kappa)^{-1} \ln [1 + (|A_1|M\kappa) \exp[|A_1|M(X_{1m}(0) - X_{1mc})](1 - R_f)]$$

where $\kappa \equiv \kappa_1 L/u$. For selected values of $X_{1m}(0)$ and κ, R_f can be determined as a function of M and the values of X_{1mc} and $|A_1|$, which depend on the energy parameters and M. Results for R_f–M calculated from eq 58 when $X_{1m}(0) = 0.829$ and κ = 0.455 are listed in Table IV. The composition $X_{1m}(0) = 0.829$ is obtained from $X_{1m}(t) = 0.416 + 0.031t$ for the longest retention time observed, t_R = 13.3 min, in the HPLC experiments and κ was selected to fit the experimental R_f value for the highest molecular weight polymer. The monotonic decrease of R_f with M is consistent with experiment but the calculated R_f values only qualitatively correlate with observation. The discrepancy may partially reflect uncertainty about the precise nature of the composition gradient that actually prevails in the TLC experiments.

If $X_{1mc}{}^{\infty} \leq X_{1m}(0) < 1$ and $\kappa \sim 0$ (1), fractionation is predicted from eq 58. However, if $X_{1m}(0) < X_{1mc}(M_I)$, then only partial separation of the homopolymer solutes with $M < M_I$ is achieved and these predictions are consistent with observation. When $X_{1m}(0) \to 1$, the composition gradient must vanish, $\kappa \to 0$, and eq 58 predicts that $R_f \to 1$ irrespective of M in this limit and all the homopolymer solutes elute with the mobile phase without retention and fractionation, which is consistent with observation.[1]

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Empirical Conformational Energy Study of the Diastereomeric Dipeptides MeCO-L-Ala-L-Ala-NHMe and MeCO-L-Ala-D-Ala-NHMe: An Approach to the Conformational Analysis of Stereocopolypeptides

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ABSTRACT: A conformational energy study has been carried out with the program ECEPP on the two blocked diastereomeric dipeptides MeCO-L-Ala-L-Ala-NHMe and MeCO-L-Ala-D-Ala-NHMe as models of stereocopolypeptide dyads (CHR–CO–NH–CHR). The results are reported in the form of maps of the space defined by the two internal torsion angles ψ_1 and ϕ_2, whereby only conformers whose energies have been minimized with respect to the other pair of torsion angles (ϕ_1 and ψ_2) are taken into account. A comparison with the low-energy local minima, obtained using all torsion angles as independent variables, indicates the validity of these maps as an approximate representation of the conformational space allowed to the dipeptides. These maps are used to illustrate the conformational preferences of the relevant dyads. An analysis of the periodic conformations so far proposed on the basis of theoretical or experimental evidence for regular stereocopolypeptides shows that the ψ and ϕ values of the component dyads do indeed fall into the low-energy regions of these maps.

Introduction

In recent years various copolypeptides with specific repeating sequences of enantiomeric D and L residues (regular stereocopolypeptides) have been the object of experimental conformational investigations in different laboratories.[1-9] For stereocopolypeptides with the repeating configurational sequences –LD–,[10-14] –LLDD–,[15] and –LDLL–,[2] theoretical studies have also been carried out, whereby the feasibility of specific periodic conformations has been examined by stereochemical or energetic criteria.

In conformity with the procedure used by other authors[16] for vinyl polymers, a different and more general approach to the conformational analysis of regular stereocopolypeptides appears to be a systematic conformational energy study of appropriate models for the configurationally different dyads[17] CHR–CO–NH–CHR (R = substituent) present in these polymers. Since interactions—especially hydrogen bonding—between the dyad peptide group and one or both of its adjacent peptide groups, as well as interactions of the adjacent peptide groups with one another, are important in determining the conformational preferences of a dyad, models that can take into account such interactions are desirable. Blocked diastereomeric dipeptides of the type MeCO-L-X-L-X-NHMe (or MeCO-D-X-D-X-NHMe) and MeCO-L-X-D-X-NHMe (or MeCO-D-X-L-X-NHMe) (X = amino acid residue) fulfill these requirements.

In view of our own interest in the conformational properties of regular stereooligo-[18-20] and -copolypeptides we have decided to apply this approach, and we have examined two diastereomeric dipeptides of the simplest α-substituted amino acid, namely, MeCO-L-Ala-L-Ala-NHMe and MeCO-L-Ala-D-Ala-NHMe, by using a currently available program[21] to compute conformational energy. In light of the aim of the study, we focused our attention on the dependence of the conformational energy of a dipeptide on the torsion angles ψ_1 and ϕ_2, which describe rotations around the bonds directly connecting the C_α atoms to the central peptide group (the indexes 1 and 2 refer to the first and second residue, respectively, of the dipeptides) (Figure 1). Our results are presented in two-dimensional diagrams where the energy and geometry of those conformers that have locally minimal energy are depicted as functions of these angles. In order to obtain these diagrams we minimized the molecular conformational energy with respect to the two torsion angles ϕ_1 and ψ_2 at fixed, sufficiently close values of ψ_1 and ϕ_2. The validity of these diagrams as an approximative representation of the low-energy region of the four-dimensional conformational energy surface is assessed in a comparison with sets of unconstrained, local low-energy minima for MeCO-L-Ala-L-Ala-NHMe, as obtained by other au-