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Comparison of Resolving Power and Separation Time in Thermal Field-Flow Fractionation, Hydrodynamic Chromatography, and Size-Exclusion Chromatography

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In this study, a general framework is set up for a theoretical comparison of the capabilities of various methods for the separation of polymers according to molecular mass. The resolving power and separation speed of thermal field-flow fractionation (TFFF), packed-column hydrodynamic chromatography (PCHDC), open-tubular hydrodynamic chromatography (OTHDC), and size-exclusion chromatography (SEC) are evaluated, using well-established relations for retention and efficiency. Resolution is analyzed in terms of the underlying molecular mass selectivity and efficiency factors. For each technique, a dimensionless parameter is defined including the basic variables affecting retention. Selectivity, efficiency, and speed of separation are written as universal functions of this parameter, and optimum conditions are examined. The capabilities of the different methods under optimized working conditions are then compared in terms of their ability to produce a desired resolution between two polymer fractions in the shortest possible time.

The most important chromatographic separation methods for characterizing the molecular mass distribution of polymers are thermal field-flow fractionation (TFFF), hydrodynamic chromatography (HDC), and size-exclusion chromatography (SEC). A comparison of the capabilities of these methods is of great value to select the best technique for a particular analytical purpose.

For an objective comparison, the choice of the performance parameters is crucial. This choice is obviously rather arbitrary because the performance of a separation method can be judged from many different viewpoints. As the goal of each method is to obtain a highly accurate molecular mass distribution in the shortest possible time, we regard the resolving power and the analysis speed as the most important performance features just as in nearly all other chromatographic techniques. Investigating these two aspects will therefore be the main focal point in this study. Other important performance criteria that can be put forward are the peak capacity, the dynamic molecular mass range, and the useful separation range. In this respect also convenience and technical feasibility may be mentioned.

Yau and Kirkland compared the capabilities of sedimentation FFF, HDC, and SEC.[1] They evaluated several features including resolving power, dynamic range of macromolecular size, absolute size range, and peak capacity. To obtain a common basis for comparison, they derived performance parameters which were normalized in terms of fractional differences in solute size. These parameters could be applied to methods for size separation in general. Literature data on separations of polystyrene latex standards were used for a quantitative comparison of resolving power. In the selected chromatograms, the separation times and the size range of the solutes were about the same. The latter is of importance since it was recognized that resolving power is strongly dependent on the size of the solute. The comparison of experimental data showed that sedimentation FFF has a much higher resolving power than HDC and SEC, whereas SEC was found to be superior to HDC.

The concept of method-independent resolution parameters provides a good basis for comparison of separation techniques. However, the treatment of Yau and Kirkland has some drawbacks which mainly concern the experimental data used for comparison. One shortcoming is that the separations used were certainly not optimized with respect to maximum resolution and minimum analysis time and therefore certainly do not represent the optimal performance of the various separation methods. Besides, the comparison of separation performance was based on very few experimental data which cover only a very small range of solute sizes. Furthermore, the resolution was not corrected for the effect of sample polydispersity. Finally, their comparison, being executed more than a decade ago, is based on somewhat dated results. It is doubtful therefore if their conclusions about resolving power have a general validity and if they apply to the present situation.

Giddings et al. treated resolution and analysis speed for polymer separation methods in general.[2-4] They examined polymer resolution in terms of the two underlying factors selectivity and efficiency. In their comparison of resolving power in SEC and TFFF, it was demonstrated that selectivity in TFFF is usually much higher than in SEC. Due to this inherent advantage, TFFF was claimed to have higher resolving power than SEC. However, it has become clear from experimental results that the advantage for TFFF in terms of selectivity is largely offset by poorer efficiency compared to SEC.[4] As Giddings' comparison of resolving power focuses

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(1) Yau, W. W.; Kirkland, J. J. *J. Chromatogr.* **1981**, *218*, 217.

(2) Giddings, J. C. *Pure Appl. Chem.* **1979**, *51*, 1459.
(3) Giddings, J. C.; Yoon, Y. H.; Myers, M. N. *Anal. Chem.* **1975**, *47*, 126.
(4) Gunderson, J. J.; Giddings, J. C. *Anal. Chim. Acta* **1986**, *189*, 1.

mainly on selectivity, the role of efficiency is somewhat underexposed. In contrast to the fairly complete theoretical comparison of selectivity, the differences in efficiency between SEC and TFFF have until now not been treated thoroughly.

A more complete treatment of resolving power should be based on adequate models for both selectivity and efficiency. Such models should properly represent current theoretical insight and experimental state of the art. Use of experimental resolution data will not in general provide good insight in resolving power because resolution can be manipulated by too many experimentally adjustable parameters. Even when retention time and polymer sample are fixed (as was attempted in the work of Yau and Kirkland), many variables remain to influence resolution. This complicates an objective comparison of resolving power and may even result in contradictory conclusions. For instance, the separations used by Yau and Kirkland suggest that the resolving power in SEC is much higher than in PCHDC and in OTHDC. This is contradicted by the experimental results of Nagy[5] and Tijssen.[6]

In this study, a new approach is used for comparing resolving power and separation speed in TFFF, PCHDC, OTHDC, and SEC. Instead of using experimental data, we attempt to evaluate separation performance theoretically. This enables us to look beyond the current technical limits of resolving power and separation speed and to explore separation potentials. Universal relations and reduced parameters are used to describe retention, selectivity, and efficiency. The dependence of resolution and analysis speed on retention is investigated, and optimal conditions are derived. Under retention-optimized conditions, a comparison of the analysis time, needed to obtain a desired resolution, is made between the various techniques. The dependence of analysis time on experimentally adjustable parameters is discussed, and possible future developments are treated.

THEORY

In this section we will address the basic theory of resolving power and analysis speed in polymer separations and in particular elaborate this for TFFF, PCHDC, OTHDC, and SEC. For this purpose we will focus on the separation of two polymer fractions which differ in molecular mass by ΔM, and have an average molecular mass M. First, the resolution R_s will be expressed as a function of M, ΔM, and other basic parameters. Then, the analysis time required to reach this resolution will be considered.

The number of plates, N, necessary to separate, at a resolution of R_s, two adjacent peaks, differing in retention volume by ΔV_r is[2]

$$N = 16R_s^2\left[\frac{V_r}{\Delta V_r}\right]^2 \tag{1}$$

where V_r is the average retention volume. For any of the polymer separation techniques considered, the retention volume is a unique function of the molecular mass, M, yielding

$$N = 16R_s^2\left[\frac{M}{\Delta M}\right]^2\left[\frac{d\log M}{d\log V_r}\right]^2 \tag{2}$$

The selectivity, represented by the last term on the right hand

(5) Nagy, D. J.; Silebi, C. A.; McHugh, A. J. *J. Appl. Polym. Sci.* **1981**, *26*, 1567.
(6) Tijssen, R.; Bos, J.; Van Kreveld, M. E. *Anal. Chem.* **1986**, *58*, 3036.

side of eq 2, has been given the symbol S,[2]

$$S = \left|\frac{d\log V_r}{d\log M}\right| \tag{3}$$

The absolute value is used here because the derivative term may be either positive (FFF) or negative (HDC, SEC).

The separation or residence time, t_r, needed to perform the desired separation can be expressed as [7]

$$t_r = Nt_p = 16R_s^2\left[\frac{M}{\Delta M}\right]^2\frac{t_p}{S^2} \tag{4}$$

where t_p is the time equivalent to a theoretical plate (or TETP). We can express t_p as the plate height H for the polymer, divided by the migration velocity of the polymer $R\langle v_0\rangle$

$$t_p = H/R\langle v_0\rangle \tag{5}$$

where R is the retention ratio V_0/V_r with V_0 being the volume of the mobile phase and $\langle v_0\rangle$ is the average velocity of the mobile phase. For packed columns, V_0 is the interparticle volume and $\langle v_0\rangle$ is the average velocity of the interparticle liquid.

Equation 4 includes all of the relevant parameters to evaluate the separation power. It will serve as the basis for the comparison of the different techniques for polymer separation. For a given polymer sample (i.e., fixed $M/\Delta M$), the time needed to achieve a desired resolution R_s appears to depend on the time necessary for one plate (or the rate of generating plates) and on the selectivity. The separation time is then determined by the ratio t_p/S^2, which consequently has a key role in this study. The selectivity appearing squared in eq 4 shows that the inherent differences in selectivity between separation methods are of great importance.[2-4] The role of efficiency should nevertheless not be underestimated because polymer separation methods are rather different in that respect. Unfortunately, the mutual action of selectivity and efficiency has so far not been investigated thoroughly. To treat this problem, it is necessarry to put seemingly very different separation techniques on a common basis. This has been attempted in the present study for four separation techniques (though the analysis can be extended easily to other techniques as well). Retention, selectivity, and efficiency will be treated in an analogous way using universal relations and dimensionless parameters. For each of the techniques, optimum conditions providing the highest resolution in the shortest possible time (or providing minimum values of t_p/S^2) will be established. Under optimum conditions, the performance of polymer separation techniques will be compared.

Universal Approach to Retention and Column Selectivity. In each of the separation techniques, retention (or R) is a function of several variables. It will appear to be very beneficial to gather all of these basic variables in one dimensionless parameter. This parameter will be named λ. A major advantage of introducing λ is that it enables a very similar treatment of retention, selectivity, and efficiency in the different separation techniques. Although λ will be defined differently in the various techniques, its role will appear to be formally the same. In each technique, retention will remain a function of λ only. The relation between R and λ is of fundamental importance because it describes a universal

(7) Giddings, J. C.; Martin, M.; Meyers, M. N. *J. Chromatogr.* **1978**, *158*, 419.

calibration (or retention) curve that is unique within one separation method. It will further be shown that λ plays a key role in efficiency as well.

In every technique, λ can be defined as a ratio of two characteristic sizes, the denominator being a channel size and the numerator being characteristic of the analyte and related to its size. In TFFF, the parameter λ_{TFFF} is[2]

$$\lambda_{TFFF} = \frac{l}{w} = \frac{D_m}{uw} \simeq \frac{D_m}{D_T \Delta T} \qquad (6)$$

where l is the mean thickness of the compressed solute zone, w is the channel thickness, D_m is the solute diffusion coefficient in the mobile phase, u is the mean solute velocity induced by the thermal field perpendicular to the direction of flow, D_T is the thermal diffusion coefficient, and ΔT is the temperature difference between the hot and cold walls. The thermal diffusion coefficient, depends on polymer type and solvent nature, but can be assumed independent of molecular mass. The latter two expressions in eq 6 show the dependence of λ_{TFFF} on polymer size, since D_m is inversely proportional to polymer size.

In both packed-column and open-tubular HDC, the parameter λ_{HDC} is the ratio of the solute size to the size of the flow channels [6,8]

$$\lambda_{HDC} = r_{eff}/R_0 \qquad (7)$$

in which r_{eff} is the effective size of the polymer coil near a solid wall and R_0 is the radius of the capillary tube. In packed-column HDC, R_0 should be substituted by the hydraulic radius of a packed column, $R_H = d_p\epsilon/3(1-\epsilon)$,[8] where d_p is the mean particle diameter and ϵ is the column porosity.

Similarly, for SEC the parameter λ_{SEC} is the ratio of the effective polymer size to the pore radius

$$\lambda_{SEC} = r_{eff}/R_p \qquad (8)$$

where R_p is the average pore diameter. Retention in SEC is not solely a function of λ_{SEC} but is also dependent on the ratio of the interstitial volume V_0 to the pore volume V_i and on the pore size distribution.[9] These factors may change considerably among different types of packing materials. In the coming discussion, these variables will be fixed, leaving R as a function of λ_{SEC} only.

For every separation method, retention depends on the solute size related parameter λ according to a relation specific for that method. Such a simple state of affairs does not exist for the dependence of retention on the molecular mass, the reason being that polymer size (and thus λ) depends on molecular mass in a way that may vary with solvent goodness and polymer type. The selectivity parameter S in eq 3, being based on the relation between retention volume and molecular mass, is thus dependent on the particular polymer–solvent combination. If this dependence were not present, a more universal selectivity parameter would be obtained. Therefore, we split S in two factors, one method-specific and the other

specific for the polymer–solvent combination, by writing[7]

$$S = \left|\frac{d \log V_r}{d \log M}\right| = \left|\frac{d \log R}{d \log M}\right| = \left|\frac{d \log R}{d \log \lambda} \frac{d \log \lambda}{d \log M}\right| \qquad (9)$$

The term d log R/d log λ, being based on the universal calibration relation, is specific for a separation method and will be called a universal selectivity parameter. The derivative d log λ/d log M can be calculated using known relations between polymer size and M. A polymer radius in general, r_p, can be written

$$r_p = aM^b \qquad (10)$$

where both a and b depend on the polymer–solvent combination and the temperature. In contrast to parameter a, exponent b is expected to have the same value irrespective of the particular polymer radius considered (e.g., in good solvents $b \approx 0.6$).[10] It is then obvious that the absolute value for d log λ/d log M is equal to b in each technique. Consequently, we find for the universal selectivity parameter

$$\left|\frac{d \log R}{d \log \lambda}\right| = \frac{S}{b} \qquad (11)$$

For the different separation techniques, we shall now consider selectivity, efficiency, and separation time as a function of λ and examine optimum conditions. Reduced parameters will be used to ensure universal applicability.

Selectivity, Efficiency, and Separation Time in TFFF. *Selectivity.* In TFFF the relation between R and λ_{TFFF} can be approximated by[2]

$$R = 6\lambda_{TFFF}\left[\coth\left[\frac{1}{2\lambda_{TFFF}}\right] - 2\lambda_{TFFF}\right] \qquad (12)$$

From this equation, a universal selectivity plot is derived in Figure 1a. It can be seen that the selectivity is highest when λ is small. In TFFF, high selectivity thus corresponds to high retention. The asymptotic selectivity limit, $S/b = 1$, is reached when λ_{TFFF} approaches zero. Whether this limit can be reached in practice will be discussed later.

For random coil polymers, b in general has a value between 0.5 and 0.6, depending on the goodness of the solvent. It is thus expected in TFFF that the maximum limit for S lies in the same range. This is confirmed by experimental data for various polymer–solvent combinations.[4,11]

Efficiency. Peak broadening in TFFF may stem from longitudinal molecular diffusion, nonequilibrium, polydispersity, and effects related to nonideal circumstances, such as channel irregularities, concentration effects, relaxation processes, and extra-column zone broadening.[12] Sample polydispersity is not considered to be a true broadening factor because it does not affect fractionating power. The contributions from nonideal circumstances can be made negligible in well-designed systems. This leaves only longitudinal molecular diffusion and nonequilibrium in an ideal parallel plate column to be discussed. In that case, we can write for

(8) Stegeman, G.; Oostervink, R.; Kraak, J. C.; Poppe, H.; Unger, K. K. *J. Chromatogr.* **1990**, *506*, 547.

(9) Yau, W. W.; Kirkland, J. J.; Bly, D. D. *Modern Size Exclusion Liquid Chromatography*; Wiley: New York, 1979.

(10) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, N.Y., 1971.

(11) Giddings, J. C.; Myers, M. N.; Janca, J. *J. Chromatogr.* **1979**, *186*, 37.

(12) Smith, L. K.; Myers, M. N.; Giddings, J. C. *Anal. Chem.* **1977**, *49*, 1750.
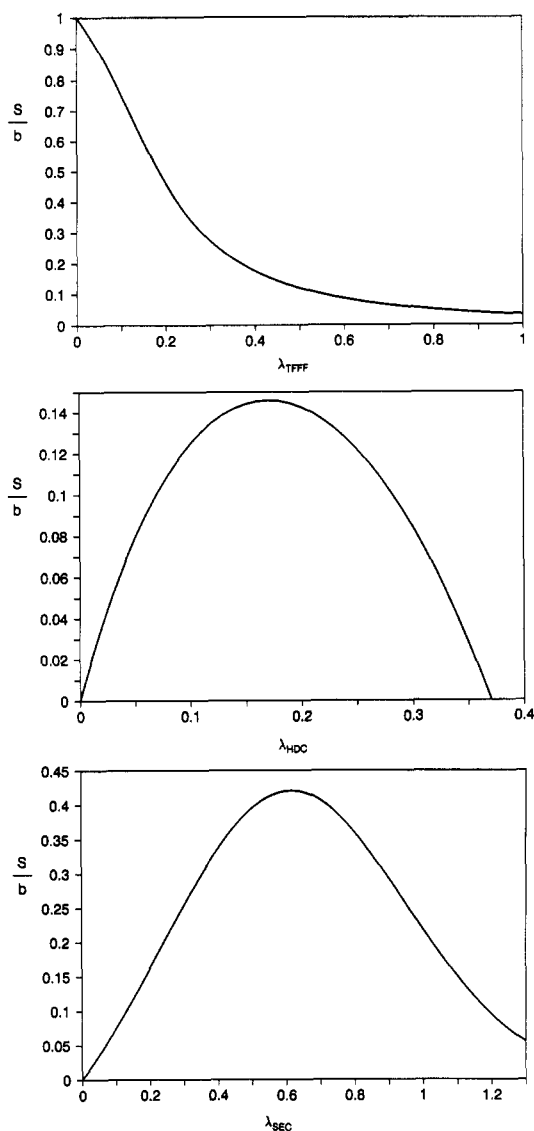
Figure 1. Universal selectivity plot for (a, top) TFFF, (b, middle) HDC and (c, bottom) SEC.

Figure 2. Dependence of χ on λ_{TFFF}.

the plate height in TFFF

$$H_{TFFF} = \frac{2D_m}{R\langle v_0 \rangle} + \frac{\chi w^2 \langle v_0 \rangle}{D_m} \qquad (13)$$

where χ is a complicated function of λ_{TFFF}.[13] In Figure 2, χ is plotted as a function of λ_{TFFF}. As diffusion coefficients for polymers are very small, the longitudinal molecular diffusion term can in practice mostly be neglected compared to the nonequilibrium term.

A more general approach of dispersion in TFFF is possible, when reduced variables are used. Following dispersion in open tubes and in packed columns, we introduce for a parallel plate column the reduced plate height $h_{TFFF} = H/w$ and the reduced velocity $v_{TFFF} = \langle v_0 \rangle w/D_m$. We then find h_{TFFF} to depend solely on v_{TFFF} and λ_{TFFF}, according to

$$h_{TFFF} = \frac{2}{R v_{TFFF}} + \chi v_{TFFF} \qquad (14)$$

Plots of h_{TFFF} against v_{TFFF} are shown in Figure 3a for different values of λ_{TFFF}. It is demonstrated that efficiency in TFFF is strongly dependent on λ_{TFFF}.
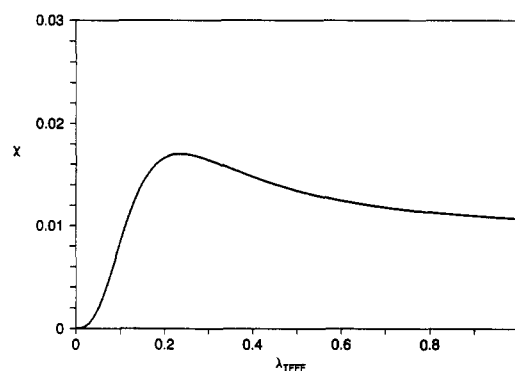
To optimize separation speed, one should aim at the highest rate of generating plates or minimum TETP. This is accomplished in the ascending part of the plate height curve, where plate height is dominated by nonequilibrium. The minimum value for TETP is proportional to $1/R$ and to the slope of the h–v curve at high velocities and is thus a function of λ_{TFFF}. The overall effect of λ_{TFFF} on TETP and on separation time will be discussed next.

Separation Time. The aspiration of obtaining the highest resolution in the shortest possible time requires minimization of the ratio t_p/S^2 with respect to λ_{TFFF}. In the high-speed limit we can write for the TETP

$$t_{p,TFFF} = \chi w^2/RD_m \qquad (15)$$

where χ and R are functions of λ_{TFFF} only. w can be adjusted freely, without altering λ_{TFFF}. Minimum values for $t_{p,TFFF}$ are obtained by reducing λ_{TFFF} as far as possible. We have already found that minimal λ_{TFFF} ensures the highest selectivity and therefore conclude the speed of separation to be highest in the limit of λ_{TFFF} approaching zero (and retention approaching infinity). This is confirmed in Figure 4a where the λ_{TFFF}-dependent factor of t_r (i.e., $(\chi/R)(b/S)^2$) is plotted against λ_{TFFF}.

In practice the optimum value for λ_{TFFF} is not attainable. Speed optimization in TFFF is thus basically a matter of trying to reach this limit as closely as possible by increasing the field strength.[7] According to eq 15, separation speed can (for fixed λ_{TFFF}) further be enhanced strongly by reducing w. In this study the possibilities and limitations for reducing w and λ_{TFFF} will be important aspects in the evaluation of the performance of TFFF.

Selectivity, Efficiency, and Separation Time in HDC.
Selectivity. Both in packed-column and in open-tubular HDC, the following relation between R and λ_{HDC} was found to hold for various polymer–solvent combinations[6,14]

$$R = 1 + 2\lambda_{HDC} - 2.7\lambda^2_{HDC} \qquad (16)$$

In Figure 1b, the universal selectivity parameter S/b is plotted as a function of λ_{HDC}. It appears that the maximum value, corresponding to the flattest part of the (universal) calibration curve, is obtained for $\lambda_{HDC} = 0.17$. The maximum value of S/b is 0.146. This is much lower than the maximum value of one achievable in TFFF. This disadvantage can only be compensated for when the efficiency in HDC is much higher

(13) Giddings, J. C.; Yoon, Y. H.; Caldwell, K. D.; Myers, M. N.; Hovingh, M. E. *Sep. Sci.* **1975**, *10*, 447.

(14) Stegeman, G.; Kraak, J. C.; Poppe, H.; Tijssen, R. *J. Chromatogr. A* **1993**, *657*, 283.
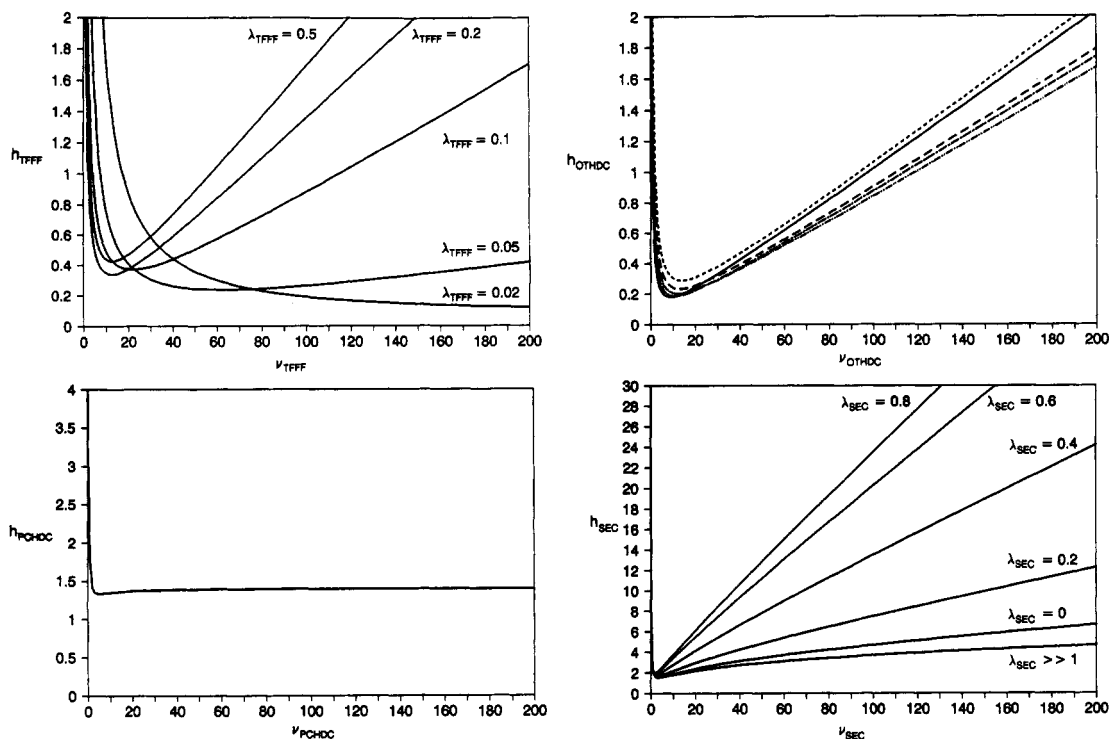
Figure 3. Reduced plate height vs reduced velocity in (a, top left) TFFF; (b, bottom left) PCHDC ($B = 1.2$); (c, top right) OTHDC [λ_{HDC} is equal to 0 (- - -), 0.05 (– – –), 0.10 (–···–), 0.15 (– – –), and 0.20 (—)]; (d, bottom right) SEC ($A = 0.8$ and $B = 1.2$).
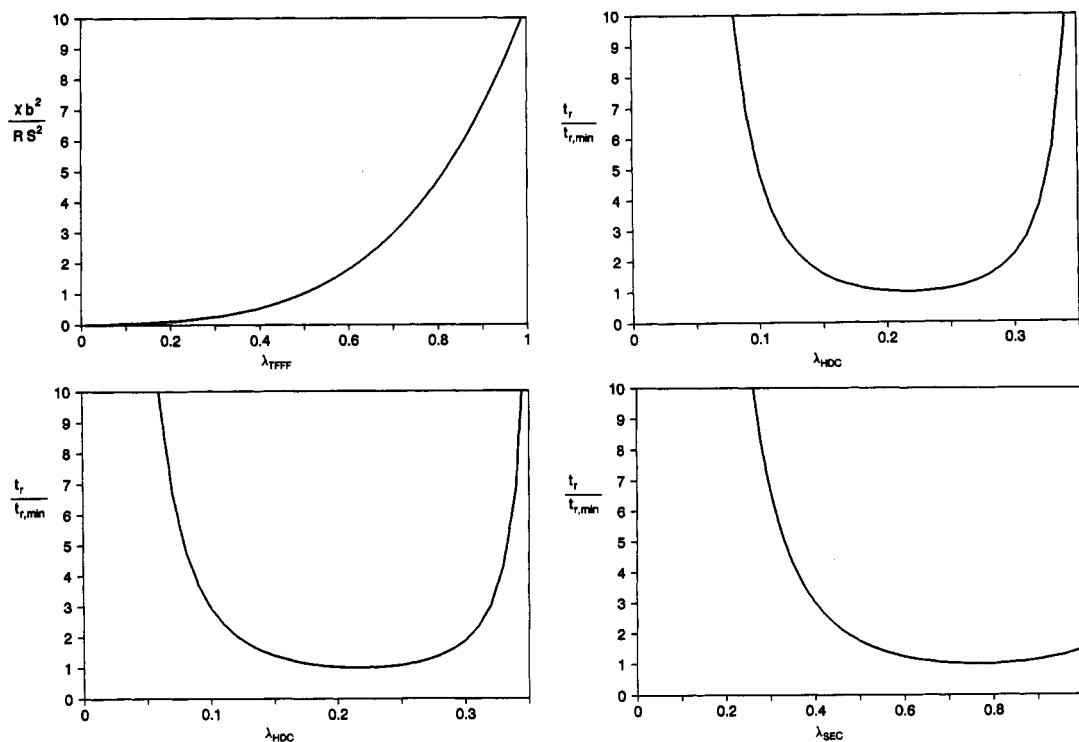
Figure 4. Dependence of the separation time t_r on λ in (a, top left) TFFF, (b, bottom left) PCHDC, (c, top right) OTHDC, and (d, bottom right) SEC, with $d_p/R_p = 32$.

than in TFFF. When both HDC and TFFF are operated under conditions of maximum selectivity, the number of plates in HDC should be about 50 times higher than that in TFFF to obtain the same resolution.

Efficiency. In packed-column HDC, the reduced plate height, $h_{PCHDC} = H/d_p$, was found to be virtually independent of λ_{HDC} and of the reduced velocity, $\nu_{PCHDC} = <v_0>d_p/D_m$, for $\nu_{PCHDC} > 5$.[15] Over a broad range of reduced velocities the reduced plate height was found to be approximately 1.4.

A relation able to accurately match these experimental results is

$$h_{PCHDC} = \frac{B}{R\nu_{PCHDC}} + \cfrac{1}{\left[\cfrac{1}{\nu_{PCHDC}} + \cfrac{1}{1.4}\right]} \qquad (17)$$

where $B/R\nu_{PCHDC}$ represents the longitudinal molecular

(15) Stegeman, G.; Kraak, J. C.; Poppe, H. *J. Chromatogr.* **1993**, *634*, 149.

diffusion contribution with B between 1.2 and 1.4, as in other forms of column liquid chromatography.[16] In practice this plate height term only becomes relevant for very small solutes, which are solutes for which R approaches one. The dependence of plate height on R has therefore not been observed in practice but is introduced here on theoretical grounds and for completeness only. The potential influence of R on h_{PCHDC} is of minor importance for our purposes and will therefore further be neglected. This yields a λ_{HDC}-independent single plate height curve shown in Figure 3b. The plate height being constant at higher velocities implies that the time needed to generate a given number of plates is a function of the velocity. Aiming at the highest rate of generating plates requires that reduced velocities should be chosen as high as possible.

In OTHDC the plate height can be calculated using the Aris–Taylor dispersion theory. Modifications are, however, necessary to account for nonzero values of λ_{HDC}. DiMarzio and Guttman[17] treated the effect of finite solute size for spherical solutes. Their analysis leads to a modified Aris–Taylor dispersion coefficient, which is a function of λ_{HDC}. A more sophisticated analysis was presented by Brenner and Gaydos.[18] Their calculations for homogeneous solid spheres are accurate up to a particle to tube radius ratio of approximately 0.2. Although these results are, in their original form, only applicable to solid spheres, they may provide an estimate for solutes like polymer chains as well. Identifying the solid sphere radius with the effective polymer radius, we obtain for the reduced plate height $h_{\text{OTHDC}} = H/2R_0$, as a function of the reduced velocity $\nu_{\text{OTHDC}} = 2<\nu_0>R_0/D_m$

$$h_{\text{OTHDC}} = \frac{2}{R\nu_{\text{OTHDC}}}f_1(\lambda_{\text{HDC}}) + \frac{\nu_{\text{OTHDC}}}{96R}f_2(\lambda_{\text{HDC}}) \quad (18)$$

where $f_1(\lambda_{\text{HDC}}) = (1 - \lambda_{\text{HDC}})^{-2} (1 - 1.125\lambda_{\text{HDC}} \ln(1/\lambda_{\text{HDC}}) - 1.539\lambda_{\text{HDC}})$ and $f_2(\lambda_{\text{HDC}}) = (1 - \lambda_{\text{HDC}})^{-2} (1 - 3.862\lambda_{\text{HDC}} + 14.40\lambda_{\text{HDC}}^2)$. The two parts of eq 18 represent contributions from longitudinal molecular diffusion and nonequilibrium, respectively. Also in OTHDC, the reduced velocities commonly used are very high owing to the small diffusion coefficients of the solutes. Therefore, in practice, the plate height contribution from longitudinal molecular diffusion can usually be neglected so that only the nonequilibrium plate height has to be considered.

Similar to TFFF, the reduced plate height in OTHDC is a function only of the reduced velocity and the parameter λ_{HDC}. For several values of λ_{HDC} a plot of h_{OTHDC} against ν_{OTHDC} is presented in Figure 3c. This figure demonstrates that the plate height does not strongly depend on λ_{HDC}. In OTHDC, the analysis speed is greatest in the ascending part of the plate height curve, where longitudinal molecular diffusion is negligible. In our effort to maximize separation speed we will therefore focus on the nonequilibrium plate height contribution.

Separation Time. At higher reduced velocities, eq 17 reduces to $h_{\text{PCHDC}} = 1.4$. Combining this with eq 5, we obtain

a simple formula for the TETP in packed-column HDC

$$t_{\text{p,PCHDC}} = \frac{1.4d_p}{R<\nu_0>} = \frac{4.2r_{\text{eff}}(1 - \epsilon)}{R<\nu_0>\lambda_{\text{HDC}}\epsilon} \quad (19)$$

The first form of this equation is the more general expression. However, when optimizing $t_{\text{p,PCHDC}}$ with respect to λ_{HDC}, we find this to be a somewhat misleading form since altering d_p does, for a given solute size, also change λ_{HDC}. Therefore, in the second form of eq 19, d_p has been rewritten as a function of λ_{HDC} (according to eq 7, with $R_0 = R_H$). For a fixed value of λ_{HDC}, the only adjustable variables in the expression for $t_{\text{p,PCHDC}}$ are the eluent velocity and the solute size (ϵ is assumed to be a constant of value 0.4). If the solute size is fixed as well (i.e., for a given sample), the TETP can only be influenced by the eluent velocity.

When the λ_{HDC} value providing the highest speed of separation is searched for, a compromise has to be found between the minimum TETP, obtained for $\lambda_{\text{HDC}} = 0.68$, and maximum selectivity, reached at $\lambda_{\text{HDC}} = 0.17$. The overall effect of λ_{HDC} on separation time is shown in Figure 4b. From this figure it can be concluded that the highest speed of separation occurs at $\lambda_{\text{HDC}} = 0.22$.

In OTHDC we can write for the TETP

$$t_{\text{p,OTHDC}} = \frac{f_2(\lambda_{\text{HDC}})R_0^2}{24R^2D_m} = \frac{f_2(\lambda_{\text{HDC}})r_{\text{eff}}^2}{24R^2\lambda_{\text{HDC}}^2D_m} \quad (20)$$

When minimizing $t_{\text{p,OTHDC}}$ with respect to λ_{HDC}, we have to consider that R_0 cannot be varied freely, without altering λ_{HDC}. This is depicted in the second form of eq 20, where R_0 is substituted by $r_{\text{eff}}/\lambda_{\text{HDC}}$. Subsequent differentiation of $t_{\text{p,OTHDC}}$ with respect to λ_{HDC} reveals that the highest rate of evolving plates is obtained for $\lambda_{\text{HDC}} = 0.30$.

In Figure 4c the separation time is given as a function of λ_{HDC}. For the highest speed of separation, one should work at $\lambda_{\text{HDC}} = 0.22$. If OTHDC separations are performed under these maximum speed conditions, $t_{\text{p,OTHDC}}$ depends only on the size of the solute (r_{eff} and D_m). Once the solutes and solvent are chosen, the minimum time needed for a given resolution is fully determined.

Selectivity, Efficiency and Separation Time in SEC. *Selectivity.* Appropriate relations between R and λ_{SEC} can be derived from migration models in which it is assumed that size separations in SEC can be explained on a purely steric basis. A convenient model, able to predict the calibration curves with high precision, is that based on the random spheres model (RSM) as developed by Van Kreveld and Van den Hoed.[19] In this model, the pore structure is generated by randomly placed, freely overlapping, uniform solid spheres. The RSM model is applicable to a rigid porous material with a fairly uniform pore size distribution. The exclusion coefficient K_{SEC} in the RSM model is given by

$$K_{\text{SEC}} = \psi^{((R_{rs} + r_{\text{eff}})/R_{rs})^3 - 1} \quad (21)$$

where ψ is the pore fraction of the porous material (i.e., the porous particles) and R_{rs} is the radius of the randomly placed uniform spheres. An approximate relation between the average pore radius R_p and R_{rs}, which holds well for $0.5 <$

(16) Guiochon, G.; Martin, M. *J. Chromatogr.* **1985**, *326*, 3.
(17) DiMarzio E. A.; Guttman, C. M. *Macromolecules* **1970**, *3*, 131.
(18) Brenner, H.; Gaydos, L. J. *J. Colloid Interface Sci.* **1977**, *58*, 312.

(19) Van Kreveld, M. E.; Van den Hoed, N. *J. Chromatogr.* **1973**, *83*, 111.

$\psi < 0.7$, is[20]

$$R_p = \alpha R_{rs} \qquad (22)$$

with $\alpha = -2(1.32 - \psi)/(3 \ln \psi)$. K_{SEC} can then be expressed as a function of λ_{SEC} according to

$$K_{SEC} = \psi^{(1+\alpha\lambda_{SEC})^3 - 1} \qquad (23)$$

Furthermore, K_{SEC} is related to R through

$$R = \frac{V_0}{V_0 + K_{SEC}V_i} \qquad (24)$$

It can then be seen that retention in SEC is a function of λ_{SEC}, V_0/V_i, and ψ. To let λ_{SEC} be the only variable affecting retention, V_0/V_i and ψ should be fixed. In this study we choose $V_0 = V_i$ and $\psi = {}^2/_3$, which are realistic values for packing materials in SEC. Because the RSM model simulates a pore size distribution and λ_{SEC} is based on the average pore size, the exclusion limit (corresponding to $R = 1$) is not yet reached at $\lambda_{SEC} = 1$ but is approached when $\lambda_{SEC} \gg 1$.

The selectivity in SEC is plotted as a function of λ_{SEC} in Figure 1c. In our retention model, the maximal value for S/b is 0.42. This optimum selectivity is reached for $\lambda_{SEC} = 0.62$. Giddings derived, from a much simpler representation of a SEC calibration curve, that the limiting value for S is 0.22 in SEC.[2] For random coil polymers with $b = 0.5$–0.6, this is in good agreement with our results.

Efficiency. In SEC, a general expression, based on the Knox equation, has been proposed for the reduced plate height h_{SEC} as a function of the reduced interparticle velocity ν_{SEC}[21]

$$h_{SEC} = (B/\nu_{SEC}) + A\nu_{SEC}^{1/3} + C\nu_{SEC} \qquad (25)$$

where $h_{SEC} = H/d_p$, $\nu_{SEC} = \langle v_0 \rangle d_p/D_m$, A and B are constants, and C is the mass-transfer coefficient. A is between 0.8 and 2 for a well-packed column, B is between 1.2 and 1.4 (as in PCHDC), and C is between 0.01 and 0.02 for very small solutes[16] but usually larger in SEC. The three terms in eq 25 account for peak broadening from, respectively, axial molecular diffusion, the complex flow pattern in the interparticle space, and nonequilibrium arising from the slow mass transfer within the pores. Similar to the previously mentioned techniques, the reduced velocities in SEC are usually very high, because of the small diffusion coefficients for polymers. Therefore, in practice, the third term is the dominant plate height term in SEC. This term is also of major importance when separation time is optimized. Greatest separation speed is obtained at higher velocities, where the terms B/ν_{SEC} and $A\nu_{SEC}^{1/3}$ are very small compared with $C\nu_{SEC}$. We will therefore focus on the mass-transfer coefficient, which can be written as[22]

$$C = R(1 - R)/30\gamma_s \qquad (26)$$

where γ_s is an obstructive factor for diffusion in the pores. Unfortunately, this factor is not well established. In both theoretical and experimental studies, there is no agreement as to the exact value of γ_s or as to its dependence on λ_{SEC}.[22] According to the best accepted theories, γ_s is composed of a λ_{SEC}-dependent constriction factor K_r and a tortuosity factor

τ, which is constant for a given porous matrix [9]

$$\gamma_s = D_s/D_m = K_r/\tau \qquad (27)$$

where D_s is the effective diffusion coefficient in the pores. K_r accounts for the reduction in diffusion occurring when the size of the solute is relatively large compared with the pore size. Hindered diffusion in the pore network within packing particles is rather difficult to describe theoretically. Therefore, we have to apply results from studies involving simpler pore geometries like cylindrical or slit-like pores. For hindered diffusion of solid spheres in monosized cylindrical pores, approximate and exact theoretical expressions have been derived, which conform well to experimental results for rigid molecules and particles.[23] The approximate expressions (the so-called centerline approximations) have been used frequently in the literature on SEC. However, a drawback of these models is that their predictions were not very successful when applied to random coil polymers. This is probably connected to the difficulty of assigning a correct equivalent sphere size to polymer chains. We therefore prefer an empirical relation describing hindered diffusion for (long-chain) linear polystyrenes in track-etched membranes[24]

$$K_r = \left[1 - 1.83\frac{r_h}{R_p} + 4.18\frac{r_h^2}{R_p^2} \right] e^{-6.52(r_h/R_p)} \qquad (28)$$

where r_h is the hydrodynamic or Stokes radius, which is close to but not equal to our preferred size measure r_{eff}. This equation can be rewritten as a function of λ_{SEC} when we use the approximate relation $r_h/r_{eff} = 0.88$.[25,26]

The tortuosity factor τ depends on the pore structure and on the porosity of the porous material, and its exact value may vary considerably among different types of porous chromatographic particles. Tortuosity values cited frequently in the literature on SEC are those for a randomly packed bed of nonporous particles with bed porosity of about 0.4 ($\tau = 1.7$)[22] and those measured for porous silica–alumina beads with porosity of approximately 0.45 ($\tau = 2.1$–2.4).[9] These values, however, do not in general apply to SEC because they concern porous structures which are less porous than commonly employed SEC particles. For a pore structure according to the RSM model Ho and Strieder[27] calculated the tortuosity as a function of porosity. For $\psi = {}^2/_3$, which is the assumed particle porosity in this study, they found $\tau = 1.20$.

Substituting the appropriate expression for K_r in eq 27 and taking $\tau = 1.20$, a relation is obtained between γ_s and λ_{SEC}. In Figure 5, the factors γ_s and $R(1 - R)$ as well as the overall C term are plotted as a function of λ_{SEC}. A similar shape of the C term curve has been derived before, both from experimental data and from theoretical models.[28] However, whether the current model provides accurate estimates for the magnitude of the C term is not certain because the C term in SEC is not well established. In our model, C can assume values up to 0.2. Values calculated from experimental data are (although varying considerably) mostly found in a range up

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Figure 5. Dependence of γ_s (–·–), $R(1-R)$ (– – –), and C (—) on λ_{SEC}.

to 0.1.[21,29,30] This difference indicates that the actual diffusion reduction for relatively large solutes (i.e., high λ_{SEC} values) may be less pronounced than predicted by eq 28. For totally excluded solutes, the C term is predicted to drop to zero. Note that this limit is not included in Figure 5, as full exclusion requires $\lambda_{SEC} \gg 1$. In our model, both extremes of λ_{SEC} ($\lambda_{SEC} > 1$ and $\lambda_{SEC} = 0$) are advantageous as far as plate height concerns, but at these extremes selectivity becomes zero. The least favorable conditions with respect to dispersion occur near $\lambda_{SEC} = 0.8$, which unfortunately is rather close to the point where selectivity is highest.

In analogy with TFFF and HDC, the reduced plate height in SEC can be considered as a function of the reduced velocity and λ_{SEC} only. The influence of both variables on h_{SEC} is made clear in Figure 3d. The figure demonstrates that plate height is strongly dependent on λ_{SEC}, especially at higher reduced velocities.

Separation Time. In SEC, the minimum TETP is obtained when plate height is dominated by nonequilibrium (i.e., the C term). The TETP can then be expressed as

$$t_{p,SEC} = (1 - R)d_p^2/30\gamma_s D_m \qquad (29)$$

In this equation, the terms that are directly related to λ_{SEC} are $(1 - R)$ and γ_s. The particle diameter is in principle freely adjustable, without changing λ_{SEC}. However, when considering the lower limit of d_p, one should realize that the particle diameter must always be much larger than the pore diameter. Therefore, in the limit of maximum separation speed d_p is a function of the pore size and can thus be expressed as a function of solute size.

In a theoretical study, Giddings treated the limits of reducing the ratio d_p/R_p in SEC.[31] He stated that high selectivity demands a high velocity contrast between pore space and interstitial regions. Substantial pore flow should therefore be avoided, and the pore liquid should preferably remain stagnant. According to Giddings, a reasonable suppression of flow in pores requires that the particle diameter should at least be 32 times larger than the pore radius. On the other hand, the occurrence of pore flow may facilitate the mass transfer between pores and the interparticle space and can thus increase efficiency.[32] It is not clear whether this gain in efficiency can fully compensate the loss in selectivity. Accounting only for maintenance of high selectivity, and using

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Table 1. Parameters for Maximum Separation Speed

	λ	R	S/b
TFFF	0	0	1
PCHDC	0.22	1.31	0.14
OTHDC	0.22	1.31	0.14
SEC	0.77	0.89	0.38

Table 2. Parameters and Relations Used in the Comparison of Separation Performance[a]

			ref
b	=	0.588 (in HDC and SEC) or 0.564 (in TFFF)	6, 33
D_m	=	$3.45 \times 10^{-8} M^{-0.564}$ m^2/s	33
D_T	=	1.00×10^{-11} m^2 s^{-1} K^{-1}	34
$M/\Delta M$	=	5	
M		variable	
R_s	=	1	
r_{eff}	=	$1.23 \times 10^{-11} M^{0.588}$ m	6

[a] The data concern linear polystyrenes in THF at 298 K.

the restriction on d_p as derived by Giddings, we obtain for $t_{p,SEC}$ under conditions of highest separation speed

$$t_{p,SEC} = 34(1 - R)r_{eff}^2/\gamma_s\lambda^2_{SEC}D_m \qquad (30)$$

This equation demonstrates that the TETP decreases continuously with increasing λ_{SEC}. The optimum λ_{SEC} for separation speed again is a compromise between minimum TETP and maximum selectivity. In Figure 4d, the dependence of the separation time on λ_{SEC} is plotted. As shown, the maximum speed of separation is obtained for $\lambda_{SEC} = 0.77$.

RESULTS AND DISCUSSION

Writing the speed of separation as a function of a dimensionless retention parameter λ enabled finding optimum conditions in the different techniques, expressed in terms of λ. The parameters corresponding to maximum separation speed have been summarized in Table 1. We will now proceed with a comparison of separation performance. In each technique, optimum λ is assumed, except in TFFF, where near optimum conditions are chosen.

If λ is fixed (at the best value), only a few adjustable parameters remain to alter the analysis time. Most of these adjustable parameters, namely r_{eff}, D_m, D_T, and b, are fixed when a particular combination of polymer type and solvent, temperature, and solute molecular mass are chosen. In the coming comparison of separation performance, we will confine ourselves to the problem of separating, at room temperature, linear polystyrenes dissolved in THF. The polymer fractions to be used are assumed to have such narrow molecular mass distribution that their polydispersities need by no means be considered in our analysis. All parameters and relations used in the comparison are summarized in Table 2. Note that the empirical relations for D_m and r_{eff} yield a b value in TFFF which slightly differs from that in HDC and SEC.

Minimum Separation Time in TFFF. In the theoretical section it was shown already that separation speed is highest when either or both w and λ_{TFFF} are made as small as possible. The limit of infinite separation speed, reached when these parameters approach zero, is not attainable in practice. It is therefore of importance to study how close this limit can be

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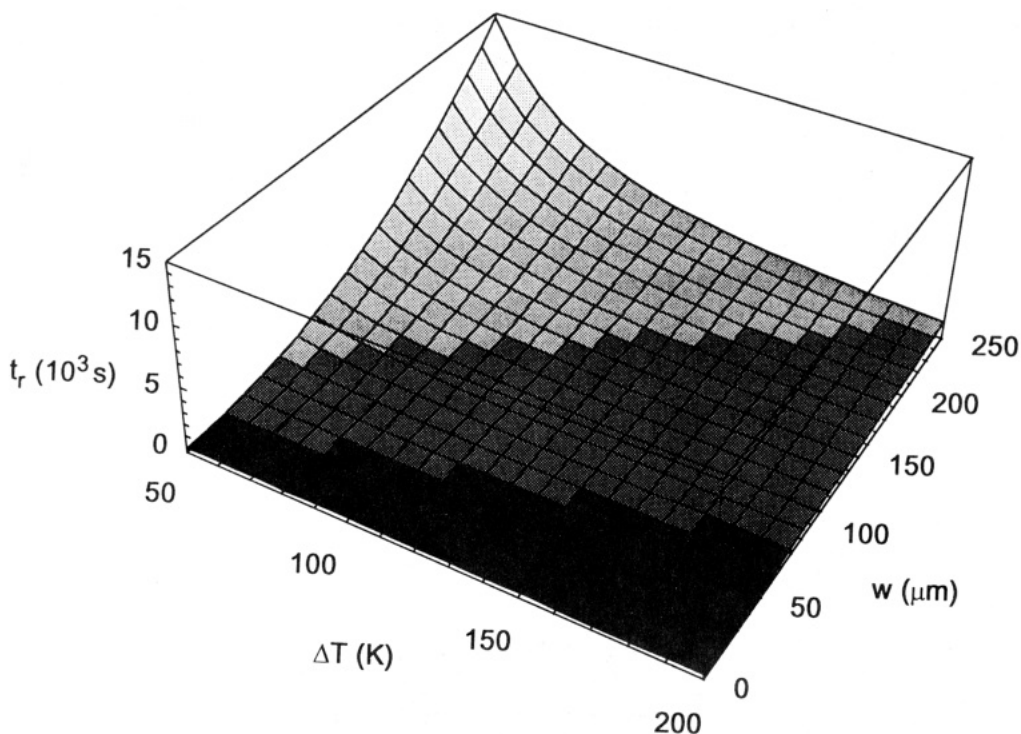(34) Schimpf, M. E.; Giddings, J. C. *J. Polym. Sci. Part B* **1989**, *27*, 1317.

Figure 6. Separation time in TFFF plotted as a function of ΔT and w for two polystyrene fractions in THF with $M = 10^6$, $M/\Delta M = 5$, and $R_s = 1$.

approached and what role is played in this by the different variables. Considering the fixed polymer–solvent system, only D_m, ΔT, and w are left as variables for the speed of separation. In a three-dimensional graph in Figure 6, the dependence of the analysis time on ΔT and w is plotted for a fixed molecular mass (or fixed diffusion coefficient). This figure shows how in general analysis speed can be improved by increasing the temperature difference across the channel and reducing the channel thickness. Both actions, meant to increase the field strength, have technical limitations and in practice appear to exclude each other to some extent: a combination of both would lead to an excessively great heat flux. A maximum temperature difference of 158 K has been realized across a 245-μm channel,[35] whereas in a 51-μm channel the attainable temperature difference was found to be at most 56 K.[7] The latter combination of w and ΔT, which is the more favorable one with respect to separation time, marks the limit of the present technology.

The role of the diffusion coefficient is twofold because D_m directly affects both λ_{TFFF} (eq 6) and H_{TFFF} (eq 13). The net result at high retention is that the separation time is proportional to D_m. In contrast to Giddings et al.,[7] we accordingly find small diffusion coefficients to be advantageous for rapid separations. This is illustrated in Figure 7a, where the separation time (for fixed w and ΔT) is shown to decrease with increasing molecular mass.

In the limit of λ_{TFFF} approaching zero, it has been shown that the separation time is simply proportional to $(w/\Delta T)^2$.[7] This would indicate that one single curve in Figure 7a represents a series of w and ΔT combinations, having an equal ratio $w/\Delta T$. Coinciding lines indeed appear in the limit of high retention, but the curves diverge when their corresponding λ_{TFFF} values differ too much from zero.

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Minimum Separation Time in PCHDC. Specifying the polymer–solvent system and fixing λ_{HDC} at its optimal value leaves only r_{eff} and $<v>$ as adjustable variables for the analysis time in packed-column HDC. In contrast to TFFF, the analysis time is found to increase when the solute size is increased. The proportionality between $t_{r,min}$ and r_{eff} makes PCHDC a potentially fast separation technique for smaller polymers. This becomes also evident from Figure 7b, which displays the dependence of $t_{r,min}$ on molecular mass for various eluent velocities. As shown in this figure, separation time can in general be reduced to a large extent by increasing the eluent velocity. It needs, however, no discussion that high velocities are ultimately limited by the pressure drop across the column. This and other restraints to high separation speed will be discussed shortly.

Minimum Separation Time in OTHDC. In open-tubular HDC, the minimum separation time is dependent only on r_{eff}. When a solute size is chosen, the minimum separation time achievable is fully established and can by no means be further reduced. Theory predicts that $t_{r,min}$ increases with the third power of polymer size. This strong dependence on size is translated in terms of molecular mass in Figure 7c. Not only the slope of the curve but also the magnitude of $t_{r,min}$ indicate that OTHDC has impressive potentials for high-speed separations in the region of lower molecular masses. For the smaller solutes OTHDC can be much faster than TFFF and PCHDC (see Figure 7a,b), but the oposite is found for higher molecular masses. The practical consequences for applying OTHDC to smaller molecules remain to be discussed.

Minimum Separation Time in SEC. Similar to OTHDC, the minimum separation time in SEC is also dependent on the third power of the polymer size only. The plot of $t_{r,min}$ against M in Figure 7d is therefore very similar to Figure 7c. Again it is seen that the fastest separations are possible for the lower
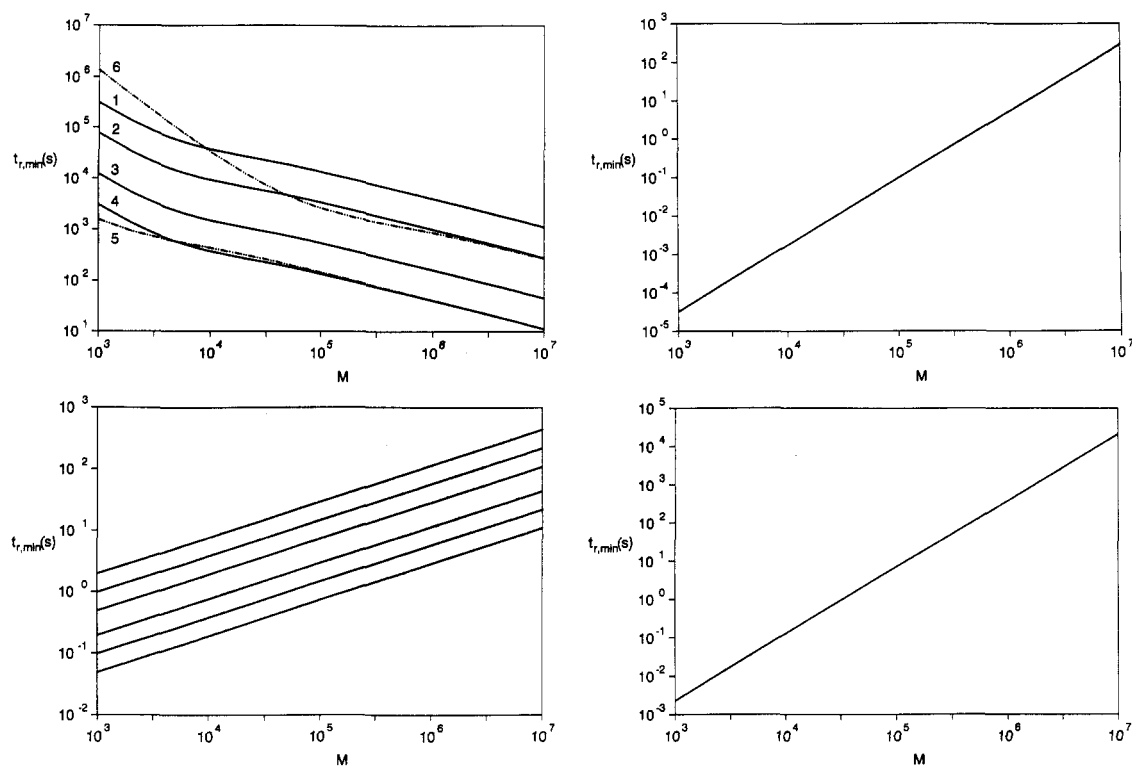
Figure 7. Minimum separation time as a function of molecular mass for polystyrenes in THF with $M/\Delta M = 5$ and $R_s = 1$. (a, top left) TFFF with combinations of ΔT and w as follows: (1) $\Delta T = 100$ K, $w = 250$ μm; (2) $\Delta T = 100$ K, $w = 125$ μm; (3) $\Delta T = 100$ K, $w = 50$ μm; (4) $\Delta T = 100$ K, $w = 25$ μm; (5) $\Delta T = 200$ K, $w = 50$ μm; and (6) $\Delta T = 20$ K, $w = 25$ μm. (b, bottom left) PCHDC with linear eluent velocities $<v_0>$ from top to bottom, 0.5, 1, 2, 5, 10, and 20 mm/s. (c, top right) OTHDC. (d, bottom right) SEC with $d_p/R_p = 32$.

molecular masses. However, the minimum attainable separation time is considerably larger than in OTHDC. In the present model, OTHDC is faster by almost 2 orders of magnitude for every molecular mass.

Examples and Practical Aspects. Now that separation time has been calculated as a function of all key variables, it is of importance to consider the practical feasibility of the theoretically optimized separations. The practical aspects, including current technical limitations and possible future improvements, will be treated on the basis of several time-optimized computer-simulated separations of polystyrenes dissolved in THF. The parameters and relations used in the simulations are again those from Table 2. In HDC and SEC, optimum λ, values are within the usual range of working conditions and thus feasible in practice. For this reason optimum values are assumed in the simulated separations. The practical consequences of this choice will be discussed. In TFFF, the optimum λ value cannot be reached. In the simulations, therefore, values according to current technical standards are chosen and some possible future improvements are explored.

In TFFF, OTHDC, and SEC, highest separation speed was shown to occur when efficiency is dominated by nonequilibrium. This requires that the eluent velocity should be much higher than the velocity corresponding to the minimum in the plate height curve. When this condition is fulfilled, the separation time is independent of the actual eluent velocity. The actual velocity is nonetheless of great practical importance because it determines what column length and pressure drop will be required. From the practical point of view, velocity should not be too high to prevent excessively high pressure drops and unnecessarily long columns. The velocities used in

the simulations are those at which the plate height, in the ascending part of the plate height curve, has risen to twice the value in the minimum of the curve. At these velocities, it is assumed that plate height is dominated by nonequilibrium and that other plate height contributions are negligibly small so that the expressions for TETP, derived in this work, apply. The column length L, required to perform the separation, is then equal to $2L_{min}$, where L_{min} is the minimum column length needed to generate the required plate number or the column length required to obtain the desired resolution if one works at the minimum of the plate height–velocity curve. The reported values for column length and pressure drop can be seen as a rough estimate of the minimum practical demands put on the separation. These estimates are appropriate for TFFF and OTHDC but may not be very accurate for SEC because the assumption of a negligibly small plate height term $Av_{SEC}^{1/3}$ is not entirely correct (see also Figure 3a,c,d). In SEC, the actual column length and eluent velocity needed to closely approach the reported minimum separation time are therefore somewhat larger than calculated in the simulations.

The minimum separation time in PCHDC is, in contrast to the other techniques, a function of the eluent velocity. In the simulations, widely differing velocities are chosen, but they are all sufficiently high to ensure that the assumption $h_{PCHDC} = 1.4$ holds. The required column length L is then equal to $1.4d_p N$. Since at higher velocities plate height is almost equal to its minimum value, $L \approx L_{min}$.

Simulated Separations for $M = 10^6$. First we will consider the separation of two polymer fractions with average molecular mass of 10^6. The results obtained in the four separation techniques are shown in Figure 8. The elution order in TFFF is reversed compared to that in the other methods. Further-
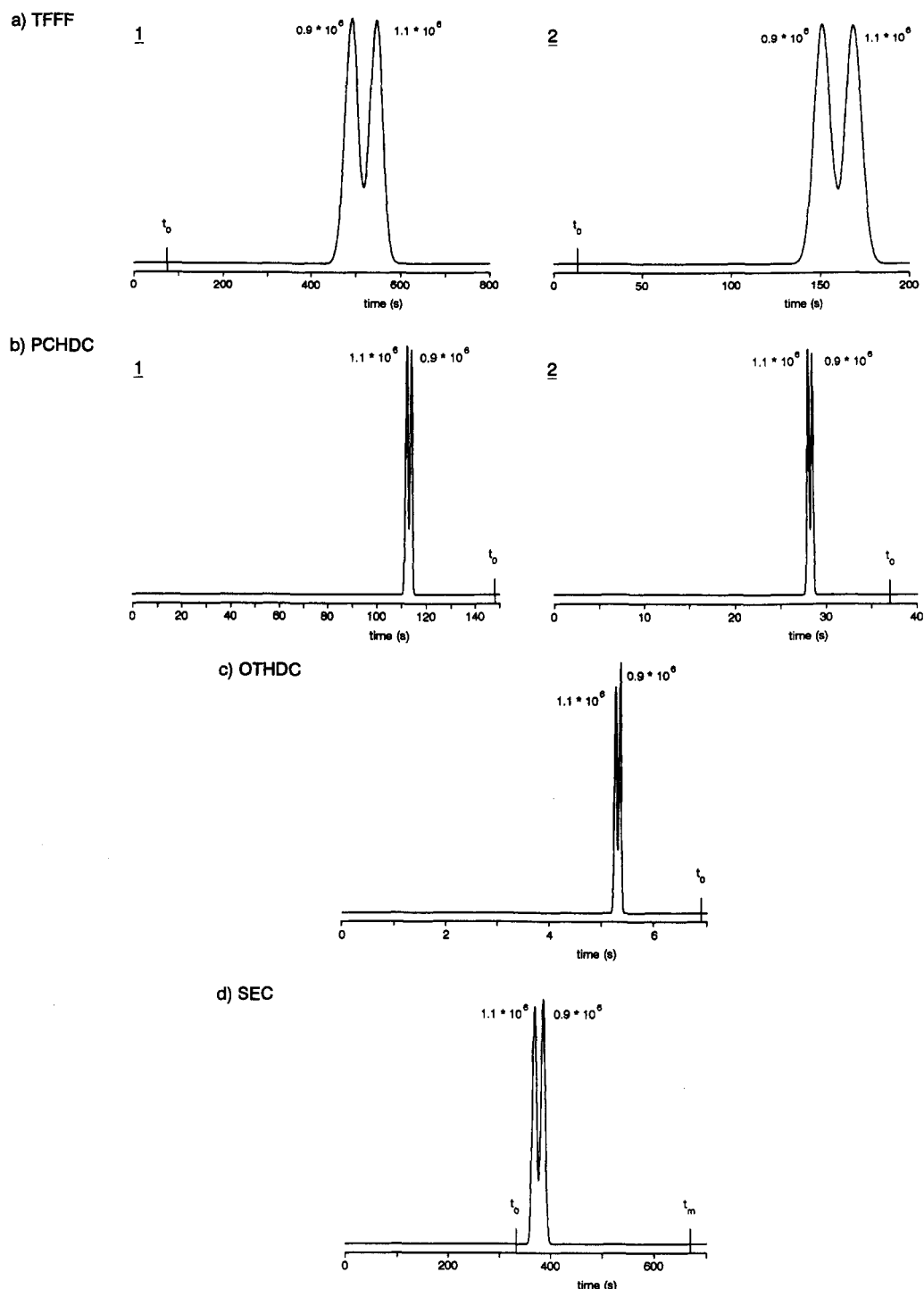
Figure 8. Simulated separations of two polystyrene fractions in THF with $M = 10^6$, $M/\Delta M = 5$ and $R_s = 1$. (a) TFFF separations with (1) $\Delta T = 56$ K, $w = 51$ (μm), $L = 2L_{min} = 17.4$ mm, $\langle v_0 \rangle = 0.233$ mm/s; (2) $\Delta T = 100$ K, $w = 50$ μm, $L = 2L_{min} = 9.47$ mm, $\langle v_0 \rangle = 0.712$ mm/s. (b) PCHDC separations with $d_p = 0.849$ μm for optimal λ_{HDC}, $L = 73.9$ mm and (1) $\langle v_0 \rangle = 0.5$ mm/s, $\Delta P = 93.6$ bar; (2) $\langle v_0 \rangle = 2.0$ mm/s, $\Delta P = 374$ (bar). (c) OTHDC separation with $R_0 = 0.189$ μm for optimal λ_{HDC}, $L = 2L_{min} = 8.37$ mm, $\langle v_0 \rangle = 1.22$ mm/s, $\Delta P = 10.5$ (bar). (d) SEC separation with $R_p = 53.9$ nm for optimal λ_{SEC}, $d_p = 32R_p = 1.72$ μm, $L = 2L_{min} = 56.0$ mm, $\langle v_0 \rangle = 0.167$ mm/s, $\Delta P = 5.74$ (bar).

more, the peaks in TFFF are retained compared to the solvent, eluting at t_0, whereas in HDC and SEC they are accelerated. It must be noted in this regard that t_0 is the elution time of the mobile phase, which in the case of SEC corresponds to the interparticle volume, whereas t_m is the solvent elution time (in HDC and TFFF, $t_0 = t_m$). It is evident from Figure 8 that the separation mechanism of TFFF produces high selectivity but poor efficiency compared to the other methods. In HDC on the other hand, selectivity is rather low but efficiency is relatively high. SEC takes an intermediate position between

TFFF and HDC. The trends in selectivity and efficiency are inherently related to the differences in separation mechanism. Both selectivity and efficiency directly depend on the distribution of solutes over the different velocity domains in the column.

Having fixed M, the minimum analysis time in TFFF is dependent on w and ΔT. In the first separation of Figure 8a the best values of w and ΔT achieved in practice so far are used. If future technical developments enable either smaller w, larger ΔT, or both, analysis time can be much further

reduced as illustrated in the second separation of Figure 8a. At present it is not clear to what extent separation power can be enhanced by technical improvements. In TFFF the pressure drop is not a severe limitation, enabling a large L/L_{min} ratio. This can be useful to prevent possible injection and detection volume limitations.

In PCHDC, working under optimal conditions with respect to λ_{HDC} requires the use of 0.85-μm particles. Filling columns with particles smaller than 1 μm has not been attempted yet, but there seem to be no insurmountable technical problems to this. The two separations shown can thus be assumed viable with current technology. In the examples it is illustrated how the analysis time can be affected by the eluent velocity $<v_0>$. Reducing the analysis time is ultimately limited by the pressure drop across the column, but since this is a technical limitation, there is room for further future improvements.

The minimum separation time in OTHDC is fully determined when M is chosen. The capillary radius of 0.2 μm, which is required for optimal λ_{HDC}, is about one-third of the smallest capillaries used so far.[6] Although the use of such capillaries may be possible with current technology, it poses severe problems in terms of injection and detection volumes. Such problems can be alleviated to some extent by using longer columns to increase the volume scale of separation. If tube lengths and eluent velocity are both scaled up to the same extent, the separation time and resolution are not affected, but the required pressure drop increases rapidly. Although the analysis speed is higher than in PCHDC and much higher than in TFFF, the experimental feasibility of this separation is questionable for current technical standards. Moreover, the analysis speed in TFFF and PCHDC can be increased when technical developments proceed, whereas for OTHDC an absolute and inherent speed limit is reached.

In SEC, the optimal separation conditions require the use of 54-nm pores in 1.7-μm particles. The synthesis and use of such particles seem possible with current technology, and we therefore assume this separation to be practicable. Compared to HDC, the minimum separation time for this sample is rather long, but the separation speed is somewhat higher than in a state-of-the-art TFFF system.

Simulated Separations for $M = 10^5$ and $M = 10^7$. In Figure 9, separations have been simulated for $M = 10^5$ and 10^7. With the current restrictions on w and ΔT, the highest separation speed in TFFF is obtained for the highest molecular masses. This is because a larger molecular mass corresponds to smaller λ_{TFFF}. The current inability to reach small λ_{TFFF} values for lower M causes TFFF to be best suitable to analyze high molecular mass polymers. If another polymer–solvent system had been chosen in the simulations, the same trends in separation speed would have been obtained, although the actual separation time for a given M might be different. Separation speed will in most instances be lower, because for most other polymer–solvent combinations used so far, the thermal diffusion coefficient is lower than for polystyrenes in THF. An interesting field of application (although outside the scope of this study) is the separation of different polymer types, since TFFF offers the unique possibility to separate polymers of different chemical composition but equal size.[36]

Working at the optimal λ_{HDC} in PCHDC is experimentally well possible for $M = 10^7$. For $M = 10^5$ this becomes

troublesome because of the very small particles required. If for the separation of $M = 10^7$ the same eluent velocities had been used as in Figure 9a, the separation speed would have been rather low (this could also be concluded from Figure 7b). As in that case, the pressure drop would also be very low, there is room for speeding up the separation. The eluent velocity being freely adjustable enables us to increase the separation speed until again the pressure limitation is reached. For the separation of $M = 10^5$, consequently, a much lower eluent velocity was chosen to avoid an impractically high pressure drop. We here observe that the pressure drop becomes the dominant criterion for the analysis speed. When we keep the pressure drop constant, we obtain the same analysis time irrespective of M (unless longitudinal molecular diffusion becomes significant). This is because the effects on ΔP of changing d_p, L, and $<v_0>$ exactly compensate each other. This is also evident from Figure 8a and 9a. At present the use of PCHDC is limited to polymers with rather high molecular masses, because only for these solutes can suitable (or optimal) particle diameters be employed.

In OTHDC, the separation speed is seen to drop dramatically when M is increased. For $M = 10^7$, the minimum separation time is longer than in PCHDC and TFFF. For $M = 10^5$, however, an extremely fast separation is predicted. Unfortunately, the optimum conditions to do this require an impractically small tube radius of 50 nm. Although the theoretical prospects for separations in the lower molecular mass range are impressive, the practical consequences restrict the use of OTHDC to higher molecular masses.

In SEC the trends in the molecular mass dependence of t_r are similar to those in OTHDC. Even if suitable packing materials can be developed for the ultrahigh molecular masses, analysis speed will always remain low. For these solutes, better prospects are offered by TFFF and PCHDC. For the lower molecular masses, on the other hand, analysis speed is relatively high in SEC. Pore diameters needed to cover the lower molecular masses are already employed in conventional SEC. The commonly used particle diameters need, however, to be further reduced for optimum performance. The synthesis and use of, for instance, 0.5-μm particles for the separation of $M = 10^5$ have not been attempted yet, but there seems to be no serious hindrance to do this in the near future. In OTHDC the separation speed for the lower molecular masses is potentially higher than in SEC, but the experimental problems are probably more restrictive. We therefore assume SEC to be the best appropriate method for the separation of lower molecular mass compounds and to remain this in the near future.

Final Remarks. In this study, the comparison of separation techniques mainly involves resolving power and separation time under optimized conditions. Many aspects which are of great importance when one has to select a particular separation technique are left aside. The conclusions drawn in this study should therefore be applied with caution.

The modeling of SEC suffers from many uncertainties. Retention and efficiency in SEC strongly vary between different packing materials. The relations used in this work apply under specific conditions and can certainly not be claimed to hold for SEC in general. This probably has caused substantial inaccuracy in the final calculations of separation time, although the trends still hold.

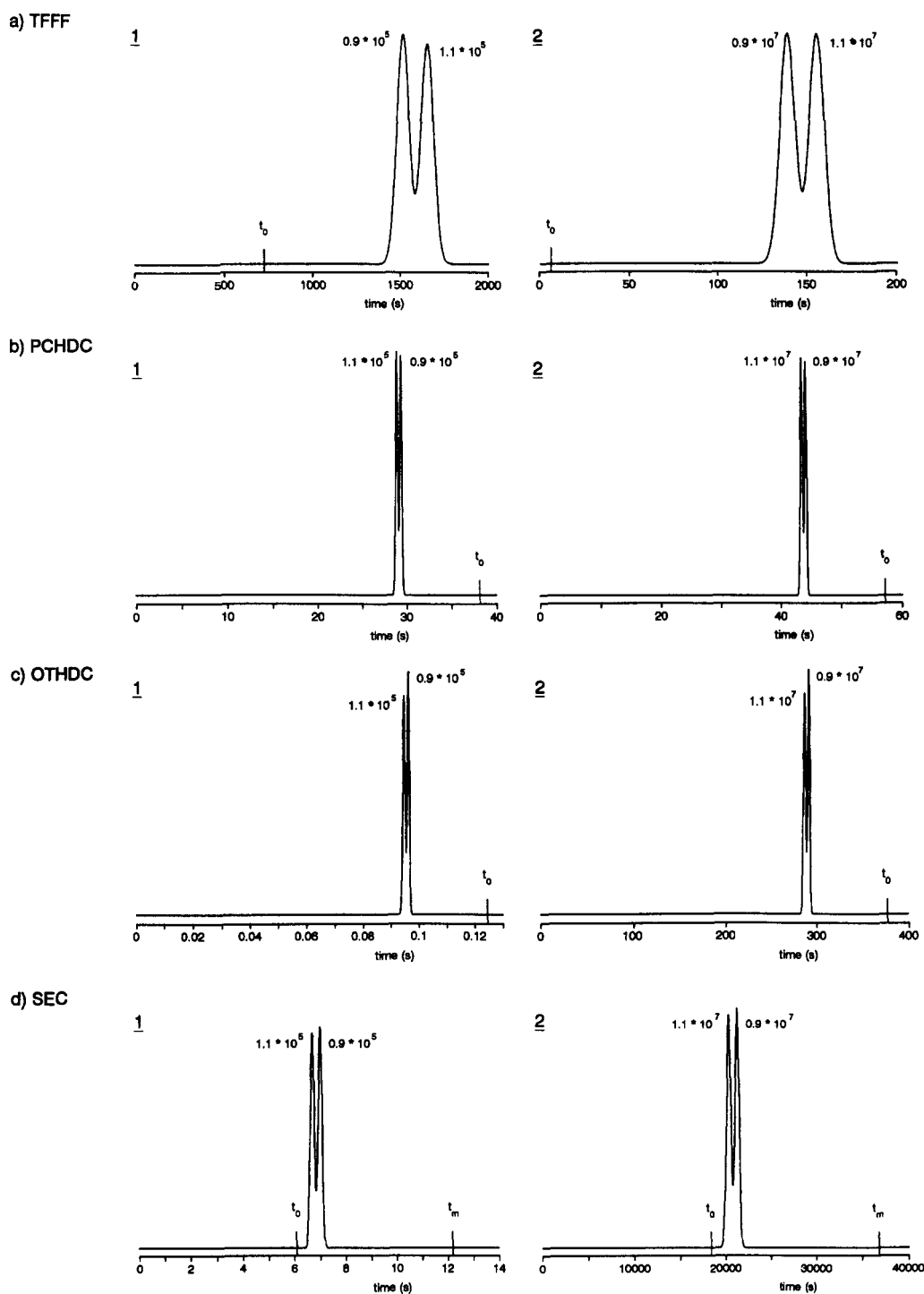(36) Gunderson, J. J.; Giddings, J. C. *Macromolecules* **1986,** *19,* 2618.

Figure 9. Simulated separations of two polystyrene fractions in THF with (1) $M = 10^5$ and (2) $M = 10^7$. $M/\Delta M = 5$ and $R_s = 1$. (a) TFFF separations with $\Delta T = 56$ K, $w = 51$ μm and (1) $L = 2\ L_{min} = 71.7$ mm, $<v_0> = 0.0995$ mm/s; (2) $L = 2L_{min} = 4.67$ mm, $<v_0> = 0.772$ mm/s. (b) PCHDC separations with (1) $d_p = 0.219$ μm, $L = 19.1$ mm, $<v_0> = 0.5$ mm/s, $\Delta P = 362$ (bar); (2) $d_p = 3.29$ μm, $L = 286$ mm, $<v_0> = 5.0$ mm/s, $\Delta P = 242$ bar. (c) OTHDC separations with (1) $R_0 = 0.0487$ μm, $L = 2\ L_{min} = 2.16$ mm, $<v_0> = 17.3$ mm/s, $\Delta P = 575$ bar ; (2) $R_0 = 0.730$ μm, $L = 2L_{min} = 32.4$ mm, $<v_0> = 0.0858$ (mm/s), $\Delta P = 0.190$ bar . (d) SEC separations with (1) $R_p = 13.9$ nm, $d_p = 32R_p = 0.445$ μm, $L = 2L_{min} = 14.4$ mm, $<v_0> = 2.37$ mm/s, $\Delta P = 316$ bar; (2) $R_p = 209$ nm, $d_p = 32R_p = 6.68$ μm, $L = 2\ L_{min} = 217$ mm, $<v_0> = 0.0118$ mm/s, $\Delta P = 0.105$ bar.

Furthermore, the equations presented to describe retention and efficiency were assumed to hold under all circumstances. In all polymer separation methods, however, deviating behavior has been observed when high linear velocities are used to analyze high molecular masses.[14,26,31,37] This can be attributed to a number of phenomena including lateral migration, polymer deformation, and degradation. These phenomena have not

been included in our analysis, but in practice they may have a significant influence. Frequently, preventing undesired flow phenomena will have higher priority than high separation speed.

To obtain a true molecular mass distribution, it is of major concern to choose experimental conditions such that shear degradation of polymers is avoided. These conditions are not known accurately in every separation technique, because the mechanism of shear degradation is not fully understood. In

(37) Giddings, J. C.; Li, S.; Williams, P. S.; Schimpf, M. E. *Makromol. Chem., Rapid Commun.* **1988**, 9, 817.

uniform flow systems, such as in TFFF and OTHDC, polymers are submitted only to tangential shear and the shear stress is thus the critical parameter controlling degradation. In such systems degradation becomes more severe when smaller flow channels and higher flow velocities are used. In PCHDC and SEC the flow field is more complicated and other strain components, in particular elongational flow, can produce chain breakage as well. Smaller particle diameters and higher flow velocities produce higher elongational strain rates and shear stress and thus promote degradation. Note that shear degradation thus limits the use of very high eluent velocities in PCHDC, which were shown to be beneficial for chromatographic performance. To evaluate the significance of shear degradation in each technique, comparable data on flow velocity, and particle and channel size are required. Such data may be derived from this study, but further work is needed to clarify this subject.

CONCLUSIONS

A general scheme has been developed for analyzing and comparing the resolving power and analysis speed of polymer separation methods. The procedures used are universally valid and also apply to other than polymer solutes. Optimal conditions with respect to retention can be derived for different separation techniques when the key chromatographic terms retention, selectivity, and efficiency are expressed as universal functions of a dimensionless parameter λ.

Optimum retention conditions (leading to the highest separation speed) have been derived for TFFF, HDC, and SEC. These provide guidelines for choosing several experimental parameters and may possibly indicate a route for future improvements.

TFFF in theory has the highest separation potentials, but these cannot be fully exploited because of the technical requirements. In comparison to other methods, TFFF performs best for the separation of high molecular mass compounds. HDC is a separation method with low selectivity, but efficiency can be very high. In PCHDC, high analysis speed is possible over a wide range of molecular masses. Currently employed particle diameters limit the use of PCHDC to $M > 10^5$. In OTHDC, very high analysis speed is predicted theoretically for lower M, but this range of solutes cannot yet be covered because of technical problems connected with the use of very small tube diameters. SEC can reach intermediate selectivity between TFFF and HDC. The high separation speed predicted for the lower molecular masses and the practicality of these separations make SEC the most suitable method for $M < 10^5$.

ACKNOWLEDGMENT

SYMBOLS

A,B	constants in the plate height equation for packed columns
a,b	constants depending on polymer–solvent system
C	mass-transfer coefficient in SEC
D_m	diffusion coefficient in free solution (m^2/s)
D_s	effective diffusion coefficient in pores (m^2/s)
D_T	thermal diffusion coefficient ($m^2\ s^{-1}\ K^{-1}$)
d_p	diameter of packing particles (m)
H	plate height (m)
h	reduced plate height
h_X	reduced plate height in technique X, defined as H/x, where $x = w$ for X = TFFF, $x = d_p$ for X = PCHDC and X = SEC, and $x = 2R_0$ for X = OTHDC
K_r	constriction factor
K_{SEC}	exclusion coefficient
L	column length (m)
L_{min}	minimum column length needed to perform a separation (if one works at the minimum of the plate height-velocity curve) (m)
l	mean thickness of the solute zone (m)
M	(average) molecular mass
ΔM	difference in molecular mass
N	number of theoretical plates
R	retention ratio, V_0/V_r
R_0	radius of a tube (m)
R_H	hydraulic radius of a packed bed (m)
R_p	pore radius (m)
R_{rs}	radius of randomly placed spheres (m)
R_s	resolution
r_{eff}	effective radius of a polymer near a wall (m)
r_h	hydrodynamic or Stokes radius (m)
r_p	polymer radius in general (m)
S	selectivity
ΔT	temperature difference (K)
t_0	mobile phase elution time (s)
t_m	solvent elution time (s)
t_r	separation or polymer residence time (s)
$t_{r,min}$	minimum separation or polymer residence time (at optimal λ) (s)
t_p	time equivalent to one theoretical plate or TETP (s)
$t_{p,X}$	time equivalent to one theoretical plate in technique X, where X is TFFF, PCHDC, OTHDC or SEC (s)
u	field-induced solute velocity perpendicular to the direction of flow (m/s)
V_i	pore volume (m^3)
V_r	retention (or elution) volume (m^3)
V_0	interparticle volume (m^3)
$<v_0>$	average (interparticle) mobile phase velocity (m/s)
w	channel thickness (m)
α	function of ψ
γ_s	obstructive factor for diffusion in pores
λ	parameter that includes all variables affecting retention
λ_X	parameter that includes all variables affecting retention in technique X, defined as l/w for X = TFFF, r_{eff}/R_H for X = PCHDC, r_{eff}/R_0 for X = OTHDC, and r_{eff}/R_p for X = SEC
ν	reduced velocity
ν_X	reduced velocity in technique X, defined as $<v_0>x/D_m$, where $x = w$ for X = TFFF, $x = d_p$ for X = PCHDC and X = SEC, and $x = 2R_0$ for X = OTHDC
τ	tortuosity
ψ	pore fraction of porous particles
χ	function of λ_{TFFF}