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Predicting the behaviour of polydisperse polymers in liquid chromatography under isocratic and gradient conditions

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Abstract

In this paper we describe how the existing theories to describe retention and peak width in isocratic and gradient-elution liquid chromatography can be expanded to describe the retention behaviour of natural and synthetic repetitive polymers, which feature distributions of molecules with different masses (and often different structures) rather than unambiguous molecular formulas. For polydisperse samples, it is vital that the model accommodates (isocratic) elution of sample components before the onset of a gradient, elution during the gradient, and elution after the completion of the gradient. The expanded models can readily be implemented in standard spreadsheet software, such as Excel. We have created such spreadsheets based on the conventional model for retention in reversed-phase liquid chromatography (RPLC) and on two different models for retention in normal-phase liquid chromatography. The implementation allows an easy visualization of the theoretical concept. Up to three different polymeric series can be entered, with a total of up to 100 peaks being computed and displayed in isocratic or gradient-elution chromatograms. Also visualized are “retention models” (diagrams of isocratic retention vs. composition) and “calibration curves” (retention or elution composition vs. molecular mass or degree of polymerization). The coefficients in the isocratic retention model may be correlated, as has often been observed in RPLC. It is shown that under certain conditions such a correlation corresponds to the existence of so-called critical (isocratic) conditions, at which all the members of a given polymeric series (same composition and end groups, different number of repeat units) show co-elution.

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1. Introduction

1.1. Theory of gradient elution

The theory of gradient-elution liquid chromatog-

raphy of small molecules is quite well established. Major contributions to the development of this theory have been made by Jandera and Churáček [1] and Snyder [2]. In Snyder's linear-solvent strength (LSS) theory the solvent composition is varied in such a way that the retention factor changes exponentially with time:

$$\ln k = \ln k_a - b(t/t_m) \quad (1)$$

where k_a is the retention factor at the start of the

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gradient (initial solvent), t_m is the residence time (or hold-up time) of the mobile phase in the column and b is the gradient-slope parameter. In the common case of reversed-phase liquid chromatography (RPLC), the retention factor varies approximately linearly with the volume fraction (φ) of strong solvent in the mobile phase (usually the volume fraction of organic modifier in water) [2,3]:

$$\ln k = \ln k_0 - S\varphi \quad (2)$$

where k_0 is the retention factor in the pure weak solvent (usually water) and S is the slope. In the case of a linear gradient, the composition can be described by:

$$\varphi = A + Bt = f(t) \quad (3)$$

where A is the initial mobile-phase composition and B the slope (in volume fraction units per minute; $100 \times B$ is the slope in volume-% per minute). Thus, in RPLC a linear gradient corresponds to LSS conditions. Note that this is not generally true in other forms of liquid chromatography, where Eq. (2) is normally not valid. The concepts presented in this paper are valid if polymer retention is described correctly by an accurate model, irrespective of LSS conditions being met.

If we know how retention varies with composition (Eq. (2)) and how composition varies with time (Eq. (3)), we have, in principle, all the information required to describe the retention behaviour of analytes under gradient conditions. In order to establish the retention time under gradient conditions we need to solve the basic integral for gradient elution [4]:

$$\int_0^{t'_k} \frac{d[f^{-1}(\varphi)]}{k(\varphi)} = t_m - \frac{t_D}{k(A)} \quad (4)$$

where $f^{-1}(\varphi)$ is the inverse gradient function. For a linear gradient we find from Eq. (3) that $f^{-1}(\varphi) = (\varphi - A)/B$. $k(\varphi)$ is the relationship between the retention factor and the composition and $k(A)$ is the particular value of k at the initial mobile-phase composition ($\varphi = A$). t_D is the gradient delay time, or dwell time. This is the time it takes for any programmed change in mobile-phase composition to

reach the top of the column. t'_R is the net retention time (t'_R) corrected for the dwell time:

$$t''_R = t'_R - t_D = t_R - t_m - t_D \quad (5)$$

For the case of linear gradients in RPLC (and for many other cases [1]) the integral of Eq. (4) can be solved analytically and the result can be rearranged to yield an expression for the retention time under gradient conditions:

$$t''_R = \frac{1}{SB} \ln \left\{ 1 + SBk(A) \left[t_m - \frac{t_D}{k(A)} \right] \right\} \quad (6)$$

In many cases $k(A)$ is very large, so that $t_m \gg t_D/k(A)$ and $SBk(A)t_m \gg 1$. This yields a very simple and convenient expression for the retention time of solutes eluting well into the gradient:

$$t''_R \approx \frac{1}{SB} \ln [SBk(A)t_m] \quad (7)$$

One important consequence of Eq. (7) is that it makes it straightforward to estimate the model parameters S and k_0 from the results of two gradient runs (I and II) with different slopes (B), but identical starting compositions (i.e., identical values of $k_i(A)$ for a given analyte i). The value for S_i can easily be derived from:

$$S_i = \frac{\ln(B_{II}/B_I)}{B_{II}t''_{R,i,II} - B_I t''_{R,i,I}} \quad (8)$$

and $k_{0,i}$ then follows from:

$$k_{0,i} = \frac{\exp(t''_{R,i,I} S_i B_I + S_i A)}{S_i B_I t_m} \quad (9)$$

Eqs. (8) and (9) form the basis of many successful simulation and optimization programs for RPLC.

A final important parameter that follows from simple gradient-elution theory is an estimate for the peak-width under gradient conditions. For a chromatographic peak eluting under gradient conditions we may write:

$$\sigma = (1 + k_e) \cdot \frac{t_m}{\sqrt{N}} G \quad (10)$$

where k_e is the retention factor at the moment of elution and G (≤ 1) is a band-compression factor, caused by the fact that under gradient conditions the

leading edge of the peak travels somewhat slower (weaker solvent) than the tailing edge [2]. Under isocratic conditions $G=1$ and k_e corresponds to the conventional retention factor (constant throughout the elution), leaving the conventional expression $\sigma = (1+k)t_m/\sqrt{N} = t_R/\sqrt{N}$. Snyder [2] finds for peaks eluting well into the gradient the following approximate expression for k_e :

$$k_e \approx \frac{1}{SBt_m} \quad (11)$$

1.2. Gradient-elution chromatography of polymers

In our work we are trying to understand, describe and ultimately predict the chromatographic behaviour of large polymers, with molecular masses ranging from thousands to millions. Other authors, including Jandera et al. [5], have studied the behaviour of the smallest members of polymeric series (oligomers, with molecular masses up to, say, 1000 Da) in great detail. There are a number of reasons why the theory of gradient elution cannot simply be applied to describe the LC behaviour of synthetic polymers.

(1) The mechanism of polymer retention in gradient-elution LC is still a matter of dispute [6,7]. Glöckner et al. [8,9] have argued in favour of a precipitation-elution mechanism, in which polymers are either fully retained or rapidly eluted, depending on the mobile-phase composition. Similar mechanisms have been put forward by Regnier [10] and by Martire and co-workers [11,12]. Snyder and Dolan [6] and others [13–15] have suggested that polymers behave similar in kind to smaller analyte molecules, but different in degree, i.e., Eq. (2) applies with very high values of S .

(2) The retention of polymers under isocratic conditions may not follow Eq. (2) [6,15]. Even if the retention mechanism of large (polymeric) molecules would be similar to that of small molecules, various types of stationary phases and mobile phases are applied [7,16]. This includes typical reversed-phase systems with aqueous–organic mobile phases, non-aqueous reversed-phase systems with mixtures of organic solvents as the mobile phase, and typical

normal-phase systems using bare silica or polar-bonded materials as the stationary phase.

(3) Repetitive polymers, including almost all synthetic polymers, as well as some natural ones, are not well-defined molecules. Instead, they are characterized by distributions of molecular mass, functional groups or end groups, chemical composition, etc. Samples of synthetic polymers are always “polydisperse” to some extent.

(4) Different polymeric series and individual series members are often spread across the entire chromatogram, with distinct possibilities of peaks eluting before the onset or after completion of the gradient. As a result, simplified gradient-elution theory (e.g., Eq. (7) and Eq. (11)) is not applicable.

We have overcome the second, third and fourth of these limitations in the work reported here. We have developed models and accompanying software, which now allow us to rigorously test the first item on the list, i.e., the retention mechanism(s) pertaining to the liquid chromatography of synthetic polymers.

Regarding the first point, we follow the Snyder philosophy in this paper. A solution–precipitation (or on–off) mechanism does not allow a discussion of the effect of polydispersity on the behaviour of polymers in liquid chromatography. One of the most intriguing aims of our study into polymer LC is to clarify the phenomenon of critical conditions, at which retention is independent of analyte molecular mass – and the effect of sample polydispersity (M_w/M_n) is negligible. The treatment below encompasses the behaviour of small and large molecules, monodisperse and polydisperse samples, and the occurrence of critical conditions.

1.3. Retention models for normal-phase liquid chromatography (NPLC)

At the start of this paper we have introduced Eq. (2), which is equivalent to assuming a typical RPLC-type behaviour. It is far from evident that this assumption is reasonable for the RPLC of polymers. For very large molecules that show very high apparent S values, the extent to which Eq. (2) is followed may be of little practical relevance, as the predicted retention under gradient conditions will not be greatly affected by slight deviations from an (almost) infinitely steep line. However, we clearly

wish to describe all kinds of polymers (low molecular-mass oligomers as well as large macromolecules) under conditions of low or high retention. This requires other models to be studied, notably those suitable for describing retention in NPLC.

We implemented two models described by Jandera et al. [17], as shown in Table 1. The first model (NPLC-a) corresponds to a linear dependence of the logarithm of the retention factor on the logarithm of the volume fraction of the strong solvent, i.e.:

$$\log k = \log k_1 - m \log \varphi \quad (12)$$

where k_1 is the retention factor at $\varphi=1$ (100% strong solvent) and m is the observed slope. In the special case in which $m=1$, Eq. (12) corresponds to a linear dependence of k on $1/\varphi$, which is sometimes observed in NPLC.

Clearly, Eq. (12) is not valid at $\varphi=0$, where an infinitely large value of k is predicted. This problem is overcome by the second model (NPLC-b) listed in Table 1. In logarithmic form, this model reads:

$$\log k = -m' \log(a + b\varphi) \quad (13)$$

where m' is a slope parameter different from m in Eq. (12) and a and b are two additional model coefficients. As indicated in the table, this model yields finite values both at $\varphi=0$ and at $\varphi=1$. However, it is a three-parameter equation, so that a minimum of three data points are required to estimate the model coefficients from experiments.

1.4. Early-eluting and late eluting peaks

In gradient elution LC of polymers, not all peaks will elute well after the onset, yet before the completion of the gradient. Often some of the sample components (e.g., the smallest members of a poly-

meric series) are not highly retained at the initial conditions. Likewise, a fraction of the sample will often elute late. Problems with early eluting peaks are also likely to occur in the separation of physical mixtures (“blends”) of polymers into the individual homo- or copolymers. Gradient-elution LC is an excellent tool for this purpose. In this case, the starting conditions ideally represent a strong solvent for one of the constituting polymers, causing this fraction to elute before the onset of the gradient. Clearly, when establishing models for the gradient-elution LC of repetitive polymers, we need to be able to deal with both early and late-eluting peaks.

When we know the coefficients for a given solute, any of the models in Table 1 will allow us to rapidly establish whether an analyte will be eluted before it is overtaken by the gradient. If the initial mobile-phase composition is A (see Eq. (3)), the isocratic retention model (Table 1) will yield a value for the initial retention factor [$k(A)$]. The isocratic retention time in the absence of a gradient is:

$$t_{\text{before}} = t_m [1 + k(A)] \quad (14)$$

The onset of a gradient will reach the end of the column at a time:

$$t_{\text{start}} = t_m + t_D + t_{\text{init}} = t_m + t_D^* \quad (15)$$

where t_{init} is an initial hold time, programmed by the analyst. In fact, t_D and t_{init} are equivalent, with the former being an unintended (and possibly undesirable delay) and the latter an intended, desirable delay. t_D^* is the total delay time ($t_D^* = t_D + t_{\text{init}}$), which is the correct parameter in the equations listed in Table 2.

The criterion for a peak eluting before the onset of the gradient is now:

Table 1
Retention models for RPLC and NPLC

Type of LC	Equation for retention factor	Retention factor	
		100% weak solvent ($\varphi=0$)	100% strong solvent ($\varphi=1$)
RPLC	$\ln k = \ln k_0 - S\varphi$	k_0	$k_0 e^{-S}$
NPLC-a	$k = \frac{k_1}{\varphi^m}$	∞	k_1
NPLC-b	$k = \frac{1}{(a + b\varphi)^{m'}}$	$\frac{1}{a^{m'}}$	$\frac{1}{(a + b)^{m'}}$

Table 2
Retention in gradient LC

Type of LC	Corrected net retention time ($t_R'' = t_R - t_m - t_D^*$)	
	During the gradient	After the gradient
RPLC	$\frac{1}{SB} \cdot \ln \left\{ 1 + SBk(A) \left[t_m - \frac{t_D^*}{k(A)} \right] \right\}$	$k(F) \cdot \left[t_m - \frac{t_D^*}{k(A)} \right] + \frac{k(F)}{SB} \ln \left[\frac{k(F)}{k(A)} \right] + \frac{F-A}{B}$
NPLC-a	$\frac{1}{B} \cdot \left\{ Bk_1(m+1) \left[t_m - \frac{t_D^*}{k(A)} \right] + A^{m+1} \right\}^{\frac{1}{m+1}} - \frac{A}{B}$	$\frac{k(F)}{B} \cdot \left\{ B \left(t_m - \frac{t_D^*}{k(A)} \right) - \frac{1}{k_0(m+1)} [F^{m+1} - A^{m+1}] \right\} + \frac{F-A}{B}$
NPLC-b	$\frac{1}{bB} \cdot \left\{ bB(m'+1) \left[t_m - \frac{t_D^*}{k(A)} \right] + (a+bA)^{m'+1} \right\}^{\frac{1}{m'+1}} - \frac{(a+bA)}{bB}$	$\frac{k(F)}{B} \cdot \left\{ B \left(t_m - \frac{t_D^*}{k(A)} \right) \frac{1}{b(m'+1)} [(a+bF)^{m'+1} - (a+bA)^{m'+1}] \right\} + \frac{F-A}{B}$

$$t_{\text{before}} \leq t_{\text{start}} \tag{16}$$

or

$$k(A) \leq \frac{t_D^*}{t_m} \tag{17}$$

The criterion for a component eluting after the completion of the gradient is more complicated. This will depend on the actual gradient program. In the case of a linear gradient (Eq. (3)), we can define the duration of the linear (segment of the) gradient (t_G) between the initial composition A and the final composition F as:

$$t_G = \frac{F - A}{B} \tag{18}$$

where t_G is in minutes if A and F are dimensionless volume fractions and the gradient slope B is expressed in min^{-1} . The end of the linear gradient reaches the column at:

$$t_{\text{end}} = t_m + t_D + t_{\text{init}} + t_G = t_m + t_D^* + t_G \tag{19}$$

Table 2 lists the equations from which the retention times of peaks eluting during the linear segment of the gradient can be calculated. The criteria for these equations to be applicable is that (i) the peak should not elute before the onset of the gradient (Eq. (17)) and (ii):

$$t_{\text{during}} \leq t_{\text{end}} \tag{20}$$

or

$$t_{\text{during}}'' \leq t_G \tag{21}$$

If Eq. (21) is false, then a different integral equation needs to be solved [4]. In the case of a linear gradient this equation reads:

$$\frac{1}{B} \int_A^F \frac{d\varphi}{k(\varphi)} + \frac{1}{B} \int_F^{A+Bt_R''} \frac{d\varphi}{k(F)} = t_m - \frac{t_D^*}{k(A)} \tag{22}$$

The resulting equations for the retention times of components eluting after completion of the gradient are listed in Table 2.

1.5. Calculation of the bandwidth

We have used Eq. (10) for all eluting peaks, as it is universally applicable. Eq. (11) applies only to peaks eluting well into the gradient and it is difficult to indicate a clear distinction between analytes that elute early during the gradient (and do not obey Eq. (11)) and analytes that allow this simplification. One assumption we have made is that $G=1$ in all cases. While theoretically a reduction in σ by 10 or 20% may be expected for analytes eluting during the gradient [2], this is not always observed in practice [6]. Therefore, $G=1$ is a reasonable estimate.

The momentary retention factor at the point of elution (k_e) can be readily estimated as follows.

For components eluting before the onset of the gradient ($t_{\text{before}} \leq t_{\text{start}}$):

$$k_e = k(A) \tag{23a}$$

For components eluting during the gradient ($t_{\text{start}} \leq t_{\text{during}} \leq t_{\text{end}}$):

$$k_e = k(A + Bt_R'') \quad (23b)$$

For components eluting after the completion of the gradient ($t_{\text{during}} \geq t_{\text{end}}$):

$$k_e = k(F) \quad (23c)$$

1.6. Critical conditions

It has often been observed [3,18] that the two coefficients in Eq. (2) ($\ln k_0$ and S) are strongly correlated. This is especially true for a well-defined group of solutes with a similar chemical structure (e.g., a homologous or polymeric series). A straight-line correlation can be represented as:

$$S = p + q \ln k_0 \quad (24)$$

where p and q are empirical coefficients. If we substitute Eq. (14) into Eq. (2) we find:

$$\begin{aligned} \ln k &= \ln k_0 - (p + q \ln k_0)\varphi \\ &= (1 - q\varphi) \ln k_0 - p\varphi \end{aligned} \quad (25)$$

It appears from Eq. (15) that at a given composition ($\varphi_{\text{cr}} = 1/q$) all k values are equal ($\ln k_{\text{cr}} = -p/q$), irrespective of the value of $\ln k_0$. At this so-called critical composition, all members of a homologous or polymeric series co-elute. The existence of a critical composition for polymeric series is well documented [19]. This so-called critical chromatography is of significant practical relevance, because it allows us – in principle – to separate a polymeric mixture into groups with different chemical structures. For example, all molecules with end group X (irrespective of their molecular mass) may be separated from all molecules with end group Y (irrespective of their molecular mass). While definite-

ly worthwhile, critical chromatography is also a highly challenging proposition. It has proven difficult to establish and maintain critical conditions for a given polymer, with the degree of difficulty rapidly increasing with increasing molecular mass of the polymer. It is one of the aims of the present research to gain a better understanding of the critical chromatography of polymers and to devise strategies to rapidly establish the critical conditions.

Correlations between the different coefficients in the retention models for NPLC are not as well documented as those in RPLC. However, it is illustrated in Table 3 that a similar reasoning can be applied to link correlations between the coefficients in the NPLC models to the existence of critical conditions.

Through the equations in Table 3, we have established a possible procedure to rapidly determine whether critical conditions exist for a given group of solutes (polymeric series) on a given system (given stationary phase and mobile phase) and to determine the value of the critical composition and the critical retention factor. The latter is equally as important as the critical composition. If all groups of analytes (e.g., polymers with different end groups) show the same or very low retention factors, then no separation between the different groups can be obtained.

2. Experimental

The spreadsheet workbooks were written in various versions of the same software, i.e., Excel 5.0 (Windows 95), Excel 97 and Excel 2000 from Microsoft (Seattle, WA, USA). Current versions run in all three versions on various personal computers (with Intel 486, Pentium and Pentium II processors)

Table 3
Parameter correlations

Type of LC	Correlation	Critical point	
		Composition (φ_{cr})	Retention factor (k_{cr})
RPLC	$S = p + q \ln k_0$	$1/q$	$e^{-p/q}$
NPLC-a	$\ln k_1 = p + qm$	e^q	e^p
NPLC-b	$\ln\left(a + \frac{b}{p}\right) = \frac{q}{m'}$	$1/p$	e^{-q}

without significant problems. Table 4 provides a summary of all the inputs required by and results obtained from the software.

2.1. Column, instrument and eluent properties

A few simple constants have to be provided, such as the column plate count (N), the column hold-up time (t_m) and the instrument dwell time (t_D). A trivial change in the software would allow the user to specify the column hold-up volume (V_m) and the instrument dwell volume (V_D), together with the volumetric flow-rate (F_v). As stated before, if there is any initial time programmed in the gradient, then this is added (by the software) to the dwell time prior to performing the calculations.

Other gradient parameters are the initial and final compositions and the duration of the (linear segment of the) gradient. It is assumed that the final com-

position is held indefinitely. Incomplete elution of the polymer is usually evident from the predicted chromatogram (see below). Recovery can easily be calculated in the program.

The program computes the gradient slope – an essential parameter in the calculations. Isocratic conditions can be accommodated, by entering a very long initial time or by choosing equal values of A and F . There is a provision in the program not to apply the equations of Table 2 if the gradient slope (B) is equal to zero. In that case all peaks elute before the onset of the gradient and Eq. (14) applies.

2.2. Sample properties

The way in which the retention parameters for a polymeric series are entered requires a bit more explanation. Up to three polymeric series with a total

Table 4
Input and output of Excel spreadsheet for simulating the gradient-elution LC of polymers

Input data	Output data
<ul style="list-style-type: none"> ■ Column hold-up time (t_m) ■ Instrument dwell time (t_D) ■ Column plate count (N) 	
Gradient parameters <ul style="list-style-type: none"> ■ Initial time (t_{init}) ■ Initial composition (A) ■ Final composition (F) ■ Gradient duration (t_G) or Isocratic composition 	<ul style="list-style-type: none"> ■ Gradient slope (B) ■ Total dwell time ($t_D^* = t_D + t_{init}$)
<ul style="list-style-type: none"> ■ Number of peaks in a polymeric series ■ Parameter value (e.g., $\ln k_0$) for first peak in series and incremental increase (e.g., $\Delta \ln k_0$) or Peak molecular mass (M_p) and polydispersity of polymeric standard 	<ul style="list-style-type: none"> ■ For each peak <ul style="list-style-type: none"> • Area • Isocratic model parameters (e.g., S, $\ln k_0$) • Indication of elution before (B), during (G) or after (A) the gradient • Retention time (t_R) • Elution composition (φ_c) • Retention factor at the point of elution (k_c) • Width (σ) • Height (h) • (Gaussian) profile ■ Isocratic retention vs. composition curves for all solutes
<ul style="list-style-type: none"> ■ Location of the critical point or Coefficients of the inter-parameter correlation (see Table 3) 	
<ul style="list-style-type: none"> ■ Total concentration of a group of peaks (polymeric series) 	
	<ul style="list-style-type: none"> ■ Calibration curves φ_c vs. MM (or degree of polymerization, n); t_R vs. MM (or n)
	<ul style="list-style-type: none"> ■ Predicted chromatogram

of up to 100 members can be entered in the current implementation.

In our original implementation, based on the RPLC model, the program required the $\ln k_0$ value of the smallest (first eluting member) of the series. It was then assumed that the Martin rule was applicable, i.e.:

$$\ln k_0 = x + yn \quad (26)$$

where n is the degree of polymerization (i.e., the number of monomeric units and not, as in the conventional Martin rule, the number of carbon units). The combination of Eq. (24) and Eq. (26) implies that the Martin rule is applicable at any composition ($0 < \varphi < 1$):

$$\ln k = x(\varphi) + y(\varphi)n \quad (27)$$

with Eq. (26) being a special case of Eq. (27) for $\varphi = 0$. According to Eq. (27) the Martin rule applies at any composition, where the functions $x(\varphi)$ and $y(\varphi)$ take on constant values. The models proposed by Jandera et al. [5] for RPLC and NPLC are also special cases of Eq. (27), with x and y being linear ($x = x_0 + x_1\varphi$; $y = y_0 + y_1\varphi$) or logarithmic functions ($x = x_0 + x_1 \log \varphi$; $y = y_0 + y_1 \log \varphi$), respectively.

By providing the $\ln k_0$ value of the first peak and the step increment [$\Delta \ln k_0 = y(0)$], the $\ln k_0$ values for the specified number of members of the series can be calculated. S values then follow either from known (user-supplied) values of the correlation parameters p and q (see Eq. (24)), or, equivalently, from (user-supplied) values of the critical point (see Table 3).

We often find it more convenient to establish a relationship between each individual parameter (either $\ln k_0$ and S , k_1 and m , or a , b , and m' ; see Table 1) and the molecular mass of series members. In this case, we need to provide the appropriate relationships, but the properties of a polymeric series are more easily entered in terms of the number of members, the peak molecular mass and the polydispersity. We do not make any assumptions about the existence of a critical composition, either real ($0 < \varphi_{cr} < 1$) or imaginary ($\varphi_{cr} < 0$ or $\varphi_{cr} > 1$). A disadvantage of this second approach is that the individual peaks do not necessarily represent members of the series. In general, the distance between the imaginary peaks is a non-integer number of mono-

meric units. In case the individual members are not resolved (e.g., when modelling the behaviour of polymeric standards), this is not a problem.

2.3. Program output

After providing data to the program as outlined above, the analyst is provided with a complete, interactive prediction of the chromatographic behaviour of the sample. All information about the individual peaks is available from the spreadsheet, but the graphs provided are infinitely more useful. A plot of isocratic retention vs. composition illustrates the behaviour of the series. Calibration curves indicate how the retention (or the elution composition) varies along one (or more) series.

Chromatographers, however, assign most value to the predicted chromatograms. The user may provide the length of the displayed chromatogram (opting to focus on one particular part if so desired). He or she may immediately observe the predicted effects of changes in the gradient parameters (A , t_G , and F) or in the isocratic composition, changes in the properties of the sample, etc.

One noticeable limitation is the number of data points that can be displayed in the current implementation. In order to obtain a proper representation of the predicted chromatogram, we must keep the value of N rather low (up to about 5000).

3. Results and discussion

The models developed and implemented in this study have since been applied to real data and extensively validated. This is the subject of several other papers [20,21]. Here we suffice with a graphical illustration of the presently proposed retention model and with examples of the chromatograms and various other plots provided by the software.

Most experience has been obtained with chromatographic systems in which the retention of polymers is most closely described by the RPLC-type model. If Eqs. (2), (24), and (26) are all valid, a retention vs. composition plot for a homopolymeric series (one kind of monomer, same end groups for all members) can be depicted by a series of lines as shown in Fig. 1. Each line in this figure represents the variation in

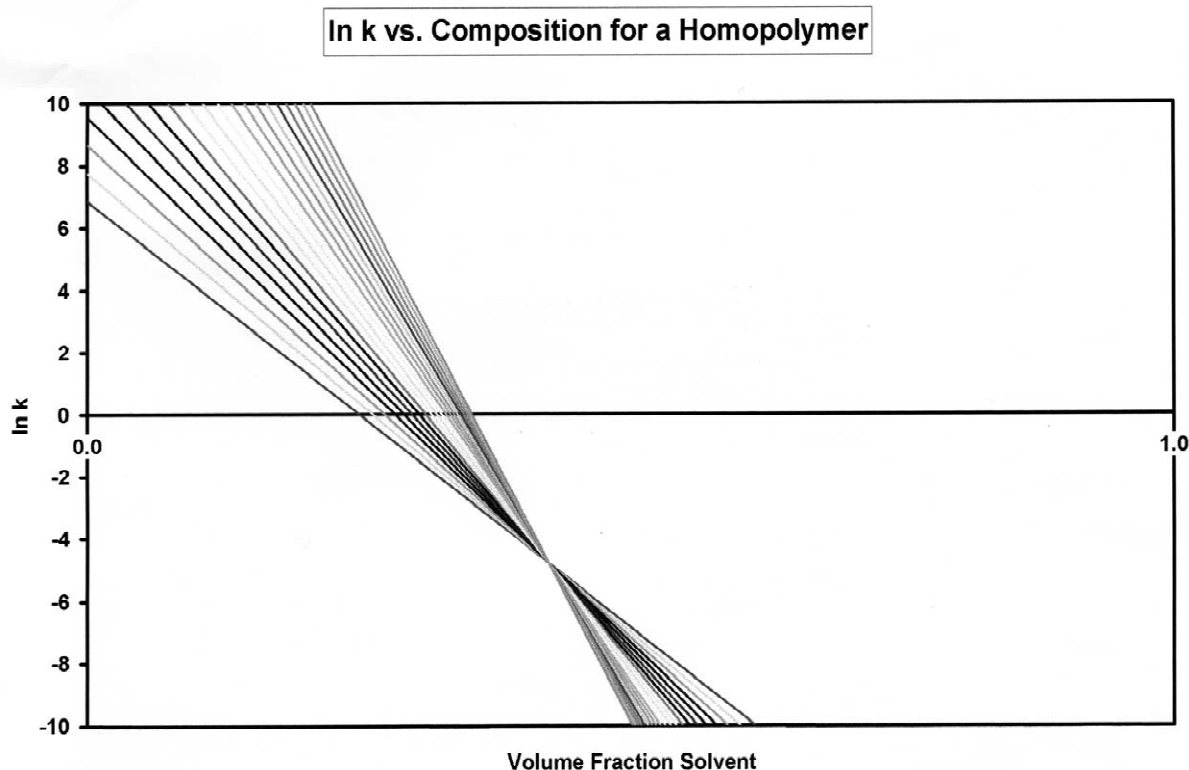


Fig. 1. Retention vs. composition plot of a homopolymeric series. Parameter estimates based on experiments reported elsewhere [21]. Critical mobile-phase composition: 43% THF (strong solvent) in *n*-hexane (weak solvent); critical retention factor: 0.008.

retention with composition for a specific member of the series (specific number of monomeric units). The validity of Eq. (2) implies that these lines are straight. The validity of Eq. (24) corresponds to the occurrence of a common intersection point for all lines. This is equivalent to the occurrence of critical elution conditions, at which retention within a polymeric series is independent of molecular mass [19]. The critical composition at which the lines intersect may represent a real mobile-phase mixture ($0 < \varphi < 1$) or a “virtual” mixture when φ is outside this range. The validity of Eq. (26) implies that along any vertical line in Fig. 1 the lines are equidistant. Note that Eq. (26) does not need to be obeyed in order for critical conditions to occur.

Once the retention behaviour of a polymeric series has been established experimentally [20,21], the theory outlined in this paper allows the prediction of the chromatograms obtained for that series under any kind of isocratic conditions or linear gradients for a

given system. Although we have so far only implemented single-step gradients (defined by the initial composition, initial-hold period, final composition, and duration of the gradient segment, assuming an indefinite hold at the final composition in the calculations), extension of the current treatment to include multi-step linear gradients is trivial.

Fig. 2 shows two gradient-elution chromatograms, predicted using the retention model of Fig. 1. The two chromatograms are indicative of commonly observed chromatograms. In the first case (Fig. 2a), good separation is attained for a number of members of a polymeric series. Retention is strongly affected by the molecular mass (or degree of polymerization). In the second chromatogram (Fig. 2b), all members of this series elute in a compact group, which (depending on the chromatographic efficiency or plate count) may appear as a single, broad, fronting peak. In this case retention is rather independent of molecular mass and the term pseudo-critical chroma-

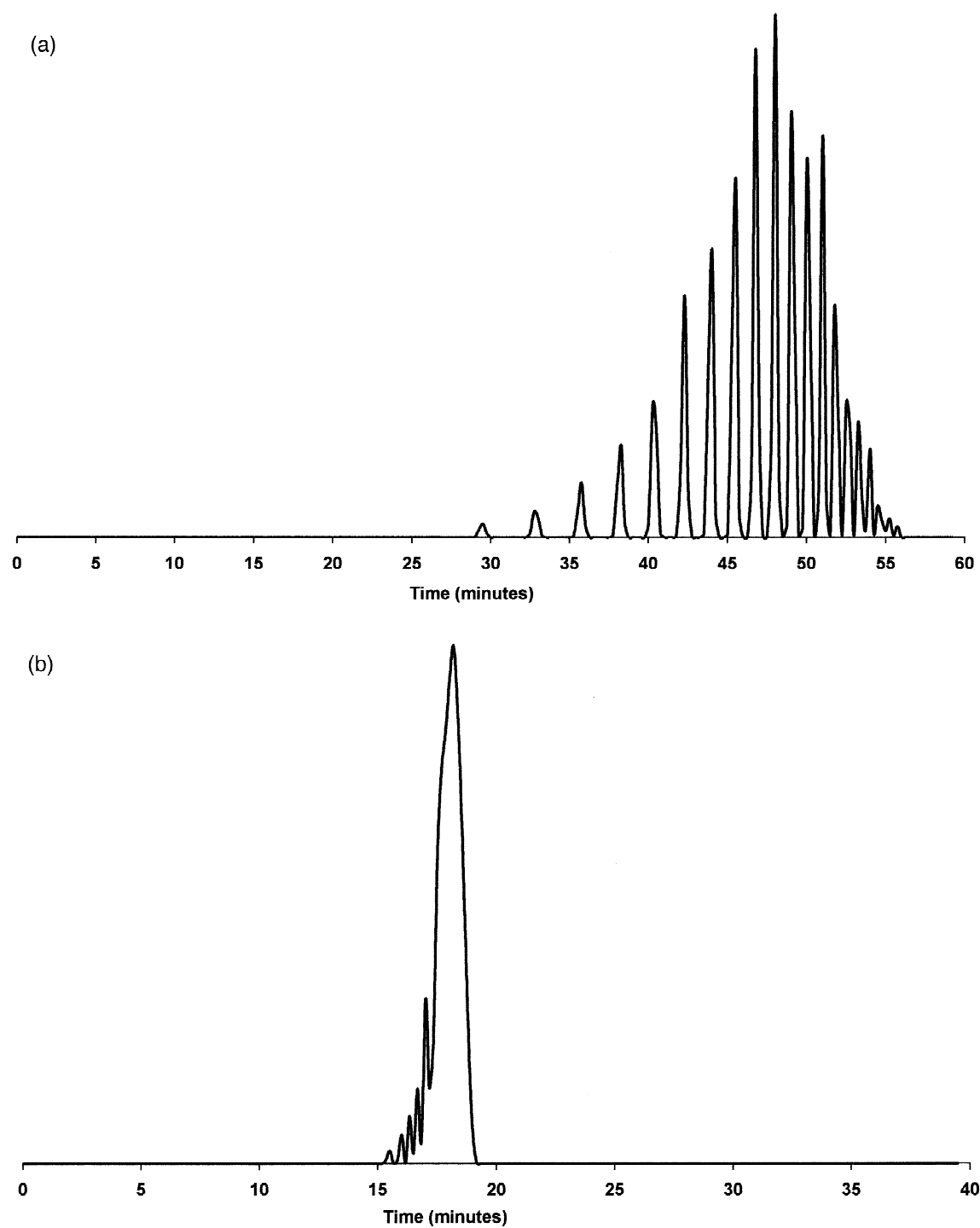


Fig. 2. (a) Gradient-elution chromatogram predicted for the homopolymeric series of Fig. 1. Polymer molecular mass: 20 000 Da; polydispersity 1.1; gradient: 10 to 60% THF in *n*-hexane in 90 min; $t_m = 108$ s; $t_D = 556$ s; $N = 2000$. (b) Gradient: 10 to 60% THF in 15 min; all other conditions as in (a).

tography has been used for such gradient separations [16].

Genuine critical chromatography occurs under isocratic conditions, as is illustrated by the calibration curves in Fig. 3. As is common in size-exclusion chromatography, we refer to a relationship between retention and molecular mass as a calibration curve. At the critical point (bottom curve) retention is clearly independent of molecular mass. However, as soon as the mobile-phase is only slightly weaker than the critical mixture, retention is seen to be a strong function of the molecular mass. Although less dramatically, a mobile phase stronger than the critical mixture also results in retention varying with composition. It is clear that critical chromatography can only be achieved at a very specific composition. This is in agreement with the common observation that critical conditions are very hard to achieve and maintain in practice [22]. Gradient-elution LC allows high-resolution separa-

tions of polymers. A calibration curve can be constructed to relate retention to molecular mass and to convert a chromatogram to a molecular-mass distribution (MMD), as is commonly done in size-exclusion chromatography (SEC). An interesting and potentially beneficial aspect is that the calibration curve can be changed almost at will by varying the conditions of the gradient. Fig. 4 shows examples of calibration curves corresponding to the chromatograms of Fig. 2. Alternatively, we may plot the elution composition as a function of the molecular mass. In this case, the calibration curves are less affected by the gradient conditions.

Isocratic chromatography of a single homopolymeric series is not very relevant. Isocratic LC alleviates some of the detection problems induced by gradient-elution LC. For example, refractive-index detection may be used only under isocratic conditions. However, isocratic chromatography of polymers can be achieved much more easily in the

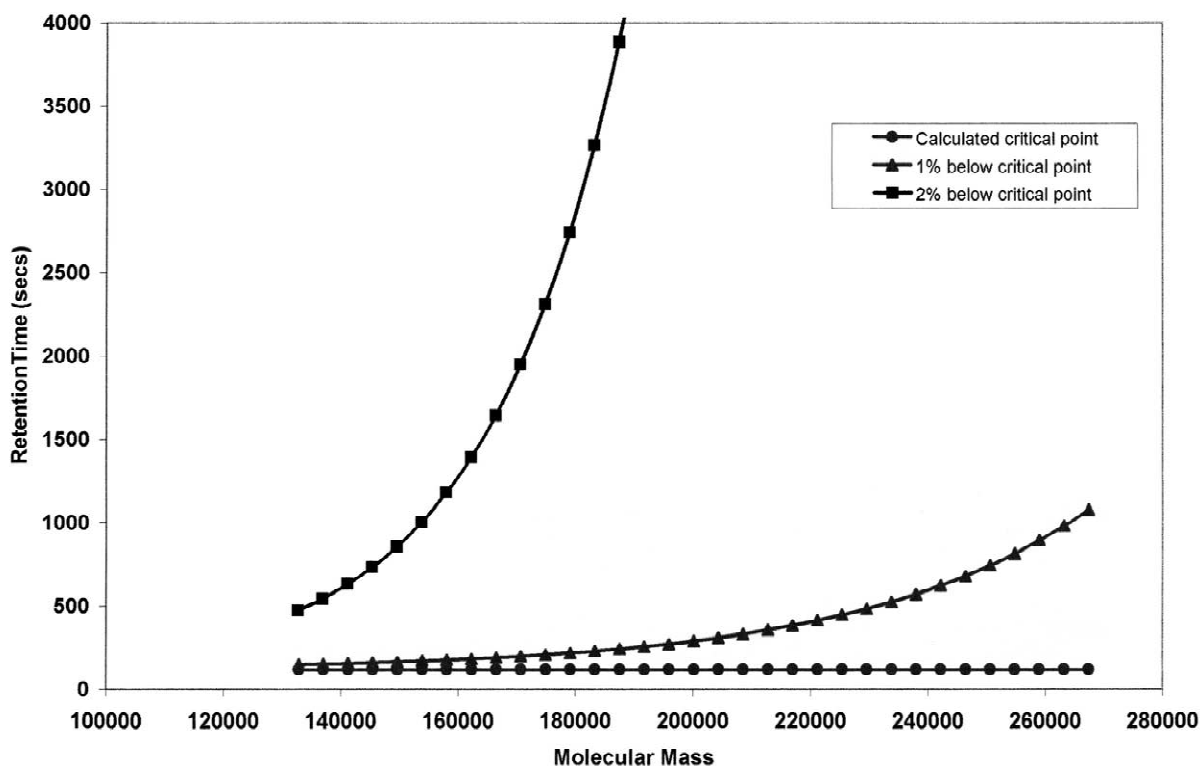


Fig. 3. Retention time vs. molecular mass calibration curve predicted for the homopolymeric series of Fig. 1 at near-critical conditions; isocratic mobile phase, all other conditions as in Fig. 2a.

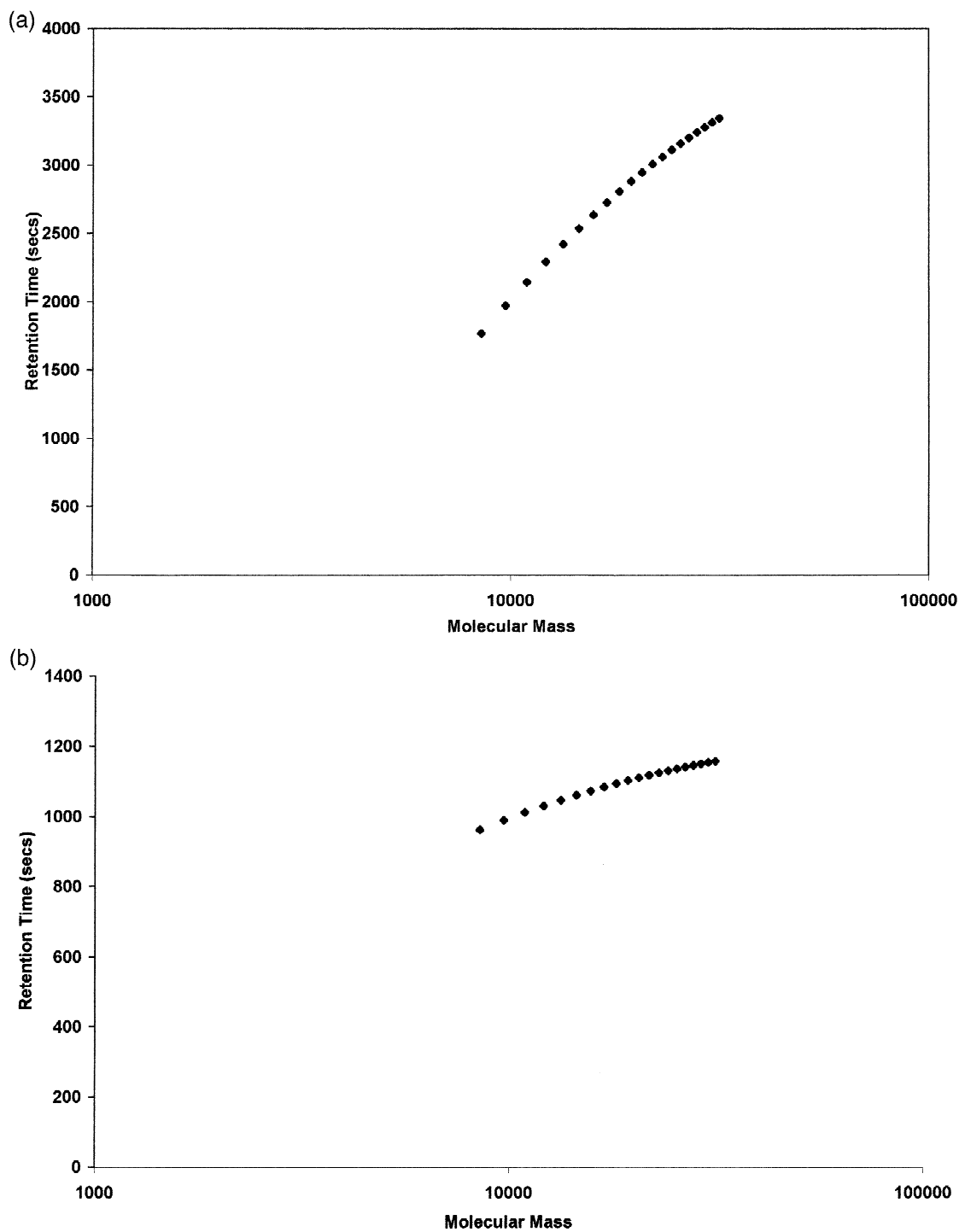


Fig. 4. Calibration curves corresponding to the chromatograms of Fig. 2.

size-exclusion mode. The real interest of (pseudo-)critical chromatography becomes obvious when different polymeric series (different monomeric units and/or different end groups) are considered. We will just present a single example here. Fig. 5 illustrates the retention models of two different homopolymers, such as encountered in a polymeric blend. Two different series, based on different monomeric segments, usually show quite different critical compositions, allowing many blends to be readily separated into the individual homopolymers [16]. A simulated example (“pseudo-critical” conditions) is shown in Fig. 6. Once the individual polymers have been characterized in terms of model coefficients, it becomes easy to simulate and optimize the separation of polymeric blends.

We are presently investigating the use of this kind of retention modelling to other, more-demanding challenges in polymer separations. At the very least, the model is an excellent tool to help us understand the behaviour of repetitive polymers in liquid chromatography.

4. Conclusions

- (1) Gradient-elution theory can be expanded and applied to describe the chromatographic behaviour of polydisperse macromolecular samples.
- (2) The expanded theory can readily be implemented in standard spreadsheet software.
- (3) Critical chromatography can be incorporated

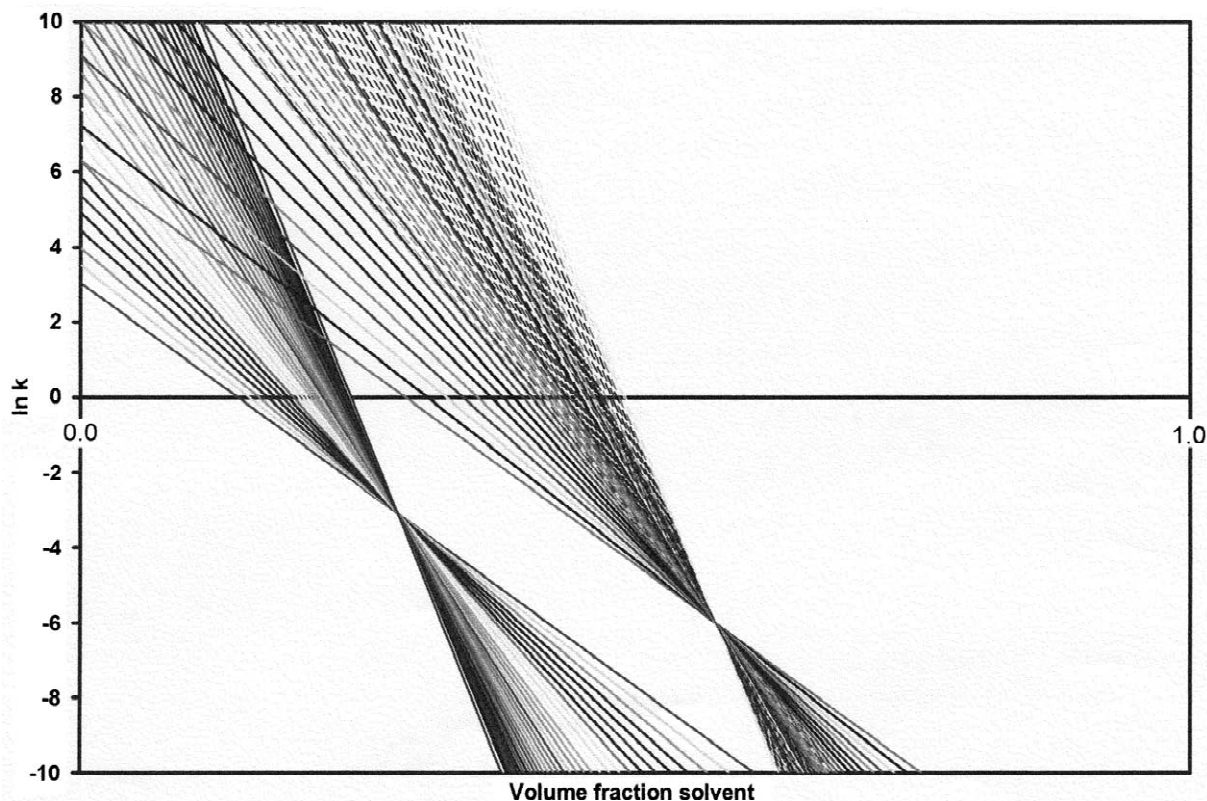


Fig. 5. Retention vs. composition plot of a two-component polymeric blend. Both components: molecular mass 20 000 Da; polydispersity 1.2. Component 1: critical mobile-phase composition: 29% strong solvent; critical retention factor: 0.045. Component 2: critical mobile-phase composition: 57% strong solvent; critical retention factor: 0.0024. All other conditions as in Fig. 2a.

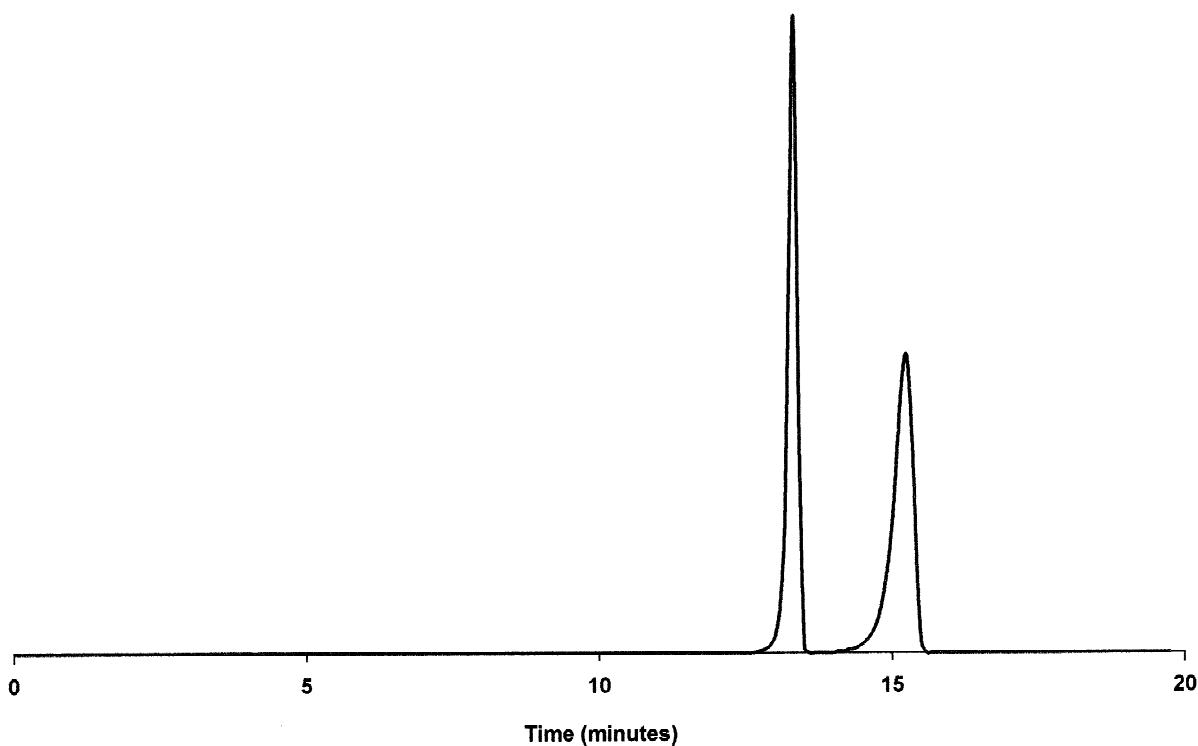


Fig. 6. Gradient-elution chromatogram illustrating the separation of the two-component polymeric blend of Fig. 5; gradient: 0 to 60% in 5 min. All other conditions as in Fig. 2a.

as a logical extension of conventional LC theory and observations.

(4) Computer simulation allows the gradient-elution liquid chromatography of (polydisperse) macromolecules to be efficiently studied and increasingly understood.

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