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Gradient separation of polymers at critical point of adsorption

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Abstract

Theoretical and experimental analysis of interaction polymer chromatography revealed a new mode of polymer separation: gradient elution at the critical point of adsorption (the eluent composition where size-exclusion and adsorption interactions completely compensate each other). This mode allows for molecular-mass-independent separation by chemical composition and/or other structural differences between macromolecules. The isocratic and gradient elution of narrow polydispersity polystyrenes and poly(methylmethacrylates) on reversed- and normal-phase columns confirmed all basic theoretical assumptions and conclusions. The gradient separation of poly(alkylmethacrylate) and poly(alkylacrylate) blends, as well as styrene–butadiene copolymers provided further experimental verification of the theory.

Keywords: Gradient elution; Critical point of adsorption; Chemical composition distribution; Copolymers; Polymers

1. Introduction

Studies on elucidating retention mechanisms are always popular among HPLC practitioners. Even the most accepted classification of liquid chromatographic methods is based on the mechanism of retention [1]. In polymer chromatography where the polymer characterization is a commonplace goal of separation, the mechanism plays an especially important role. Depending on type of interaction between a macromolecule and a stationary phase, polymer distributions by molecular mass, chemical composition, functional groups, etc., can be measured. Thus, molecular mass distribution (MWD) usually results from the size-exclusion separation. In this case, repulsive polymer–surface steric (excluded volume) forces play a dominant role, while all other types of interaction, typical for conventional HPLC of low-molecular-mass compounds, are undesirable and suppressed. These latter become important in interaction polymer chromatography, where the primary goal of separation is determination of polymer distributions by the structural differences between macromolecules other than molecular mass [2].

From a thermodynamic point of view, steric interaction has an entropic nature and is associated with the loss of conformation entropy due to restriction of fluctuation motion of a flexible chain-like macromolecule. This happens, for example, when a macromolecule penetrates a pore with internal diameter d comparable with the size r_g (root mean square radius, or radius of gyration) of this macromolecule in solution.

Although the concept of entropic origin of sepa-

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ration is commonly accepted in size-exclusion chromatography (SEC) [3], the role of steric interaction in interaction polymer chromatography is still under active consideration. One important example is isocratic separation at critical point of adsorption (CPA) [2, Ch. 6], which is a transition (critical) point between size-exclusion and adsorption modes of separation. In this transition mode, enthalpy-driven attractive forces and steric interaction counterbalance each other as they affect the retention. The complete mutual compensation may occur at a specific mobile phase composition, i.e. CPA, which depends on chemical structure of both polymer and stationary phase, but is independent of molecular mass of macromolecules. As a result, all polymer fractions elute with a solvent band. Note that the CPA does not depend on the pore diameter d, and such compensation occurs even for very wide pores, when $r_g \ll d$ and steric interaction does not exist at all without absorption.

On the other hand, there is a commonly accepted opinion that molecular mass-independent gradient separation in liquid adsorption chromatography [2, Ch. 5] is a direct indication of the absence of steric interaction between macromolecules and a stationary phase [4,5]. This conjecture is believed to be supported by the experimental observation that the best results are usually obtained when molecular mass of polymer exceeds the exclusion limit of the column, so that macromolecules cannot penetrate pores at all [see reviews in 2,4].

However, such explanation has several flaws [6]. First, the adsorption interaction alone always produces strong molecular mass-dependent retention of macromolecules. This fact was demonstrated in many experimental investigations [4], as well as by the theoretical consideration [4,6]. For example, the multiple attachment model of adsorption for isocratic elution of polymer homologous series results in an exponential growth of the capacity factor with molecular mass [4]. Thus, according to this model, even a minute decrease in elution strength of the solvent, which produces just 5% increase in retention of a monomer (from 120 to 126 s), will cause polymer with 100 of such units to be retained on the column for more than 4 h. Only if retention of an isolated unit is exactly zero (i.e. there is no adsorption at all), the whole polymer, irrespective of its size, is also eluted without any retention [4, p. 50].

Secondly, under the conditions of adsorption interaction with the surface, flexible macromolecules can penetrate the pores, even when they are completely excluded in the size-exclusion mode. The probability of penetration depends on the Gibbs free energy of the molecule inside the pore: the lower this energy, the higher the probability [7]. We showed recently [6] that the probability of penetration might even increase when the internal pore diameter d decreases. Moreover, adsorption of macromolecules is always accompanied by steric interaction, even if this adsorption occurs on the external surface of the particles. The conformation entropy of a flexible macromolecule always changes when it is adsorbed by the surface, and the higher molecular mass, the more pronounced is this change [8].

Recently one of us has extended the concept of separation at CPA on the gradient elution [9]. According to this new approach, steric interaction that counterbalances the effect of adsorption on retention is necessary condition for any molecular mass-independent separation in gradient adsorption chromatography of polymers. As a result of this compensation phenomenon, high-molecular-mass polymers leave the chromatographic column with an eluent composition close to the corresponding CPA. The fractions of copolymers or polymer blends with different chemical compositions have different transition points, and molecular-mass-independent gradient separation based solely on chemical composition or other types of structural heterogeneity of macromolecules is achieved.

To support this concept, the molecular–statistical theory of dilute polymer solution in confined media (pores with attractive walls) was applied in combination with conventional chromatographic theory of gradient elution [6,9]. The current publication presents further theoretical and experimental developments of gradient separation of polymers in interaction polymer chromatography. We analyze isocratic and gradient elution of polymers at CPA for normal- and reversed-phase chromatography, discuss the thermodynamic factors affecting the chromatographic separation, and provide quantitative experimental verification of the theory.

2. Experimental

Narrow polydispersity polystyrene and poly(methylmethacrylate) standards were obtained from Waters Technologies (Milford, MA, USA). Broad polydispersity polystyrene standard NBS 706 was purchased from National Institute of Standards and Technologies (Gaithersburg, MD, USA). Acrylate and methacrylate polymer kits of poly(alkylacrylate) and poly(alkylmethacrylate) secondary standards, polybutadiene, polystyrene, butylmethacrylate–isobutyl methacrylate (50:50, by molar ratio) and styrene–butyl methacrylate (50:50) copolymers were purchased from Scientific Polymer Products, (Ontario, NY, USA). The weight average molecular mass of all these polymers varied from 101 000 to 470 000, as determined by gel-permeation chromatography. Five styrene–butadiene statistical copolymers (styrene content 5.2, 16, 23.5, 36 and 50%, mol) prepared by emulsion polymerization at low conversion of monomers (<10%, v/v) were provided by Goodyear Tire and Rubber (Akron, OH, USA).

All HPLC grade solvents [tetrahydrofuran (THF), stabilized or unstabilized, acetonitrile (ACN), *n*-hexane, methanol and toluene] were obtained from J.T Baker (Phillipsburg, NJ, USA) and used without further purification.

Chromatographic separations were performed on Alliance 2690™ Separation Module with column heater at 35 °C (Waters). The module provides a low-pressure quaternary gradient pumping system with lag volume 0.6 ml to the column inlet, online solvent degassing and automatic sample injection from 2-ml vials. Two detectors coupled sequentially were used for HPLC experiments: photodiode array detector PDA model 996™ (Waters) and evaporative light-scattering detector ELSD Model 500™ (Alltech Associates, Deerfield, IL, USA) equipped with LTA module (drift tube at 40 °C, 1.75 l/min nitrogen flow). The mobile phase composition at each elution volume for gradient separation was monitored online using the PDA detector. Data acquisition and manipulation were performed with Millennium^{32} version 3.2 chromatography manager (Waters).

Most HPLC experiments were performed on Waters 300×3.9 mm, 4-μm particle size Nova-Pak® 60-Å pore silica-based columns. Bare silica or bonded C_{18} packings were used for normal- or reversed-phase experiments, respectively. Waters 150×3.9 mm, 5-μm particle size SymmetryShield RP_8 column with median pore diameter $d=90$ Å was also used for reversed-phase gradient separations. Exclusion volume, V_0, and pore volume, V_P, were determined in THF with a set of narrow polydispersity polystyrene standards for the reversed-phase columns and poly(methylmethacrylate) standards for the normal-phase column.

Linear mobile phase gradient with a flow-rate of 1 ml/min was used for all gradient separations; flow-rates 1, 0.5 or 0.25 ml/min were used for isocratic experiments, as indicated at corresponding figures. Sample solutions were prepared in unstabilized THF for all gradient runs, or in the corresponding mobile phase for isocratic separations. The concentrations of narrow polystyrenes and poly(methylmethacrylates) varied from 0.03 to 0.5% (v/v) depending on molecular mass. The concentration of all other polymers was 0.2% (v/v). The injection volume was 10 μl for all samples. At least duplicate injections for each sample were performed and presented at corresponding figures. A 5-min hold time in initial mobile phase (solvent A) was used in all normal-phase gradient experiments to insure full separation of polymers from the initial solvent band.

3. Theory of gradient polymer separation

3.1. Thermodynamics of polymer retention

The basic assumption in any chromatographic theory is that retention is determined by the thermodynamic factors. In such a way, mobile and stationary phases are interpreted as true thermodynamic phases with volumes V_{mob} and V_{stat}, respectively, so that retention volume V_R in isocratic elution depends on the partition (distribution) equilibrium coefficient K of the solute in these two phases:

$$V_R = V_{mob} + KV_{stat} \quad (1)$$

Enthalpic interactions and entropic transformations in solute molecules during chromatographic elution

occur inside the stationary phase. In the case of low-molecular-mass compounds, all such transformations and interactions are localized in a thin adsorption layer at the solid surface. The width of this layer, a, depends on the size of the functional groups at the surface and usually is the order from a few tenths of nanometers to a few nanometers [10]. This layer with volume V_a represents the stationary phase, which constitutes only a small portion of entire pore volume V_P, so that $V_{stat} = V_a \ll V_P$, and practically the entire liquid volume inside the column, $V_L = V_0 + V_P$, comprises the mobile phase: $V_{mob} = V_L$. In the case of a binary mobile phase, the eluent composition (the volume fraction of a chromatographically ''stronger'' solvent B) in the surface layer, Φ_{surf}, may be different from the overall composition Φ and depends on interaction energy between the components of the eluent and the solid surface. Usually, $\Phi_{surf} > \Phi$, as the component B stronger interacts with the functional groups at the pore surface that causes a preferential sorption of solvent B. In HPLC of low-molecular-mass compounds, the accurate determination of the volume V_{stat}, and hence the equilibrium constant K, is problematic, and retention is usually described by the capacity (retention) factor $k = (V_R - V_L)/V_L$ [11].

The situation can be different in the HPLC of polymers. The size of a synthetic macromolecule in solution, r_g, usually significantly exceeds the width a of the monomolecular adsorption layer and can be comparable or even larger than the internal pore diameter d. While the enthalpic interactions are still confined by the volume V_a, the entropic fluctuations of macromolecular conformations occur across the entire width of the pore. What this means is the entire pore volume V_P represents the stationary phase, yet the mobile phase is formed by the interstitial volume V_0 only, i.e. $V_{stat} = V_P$, $V_{mob} = V_0$. Note that eluent composition in the vicinity of the surface of the pores, Φ_{surf}, can still differ from the average composition Φ due to preferential sorption. In this paper we consider separation of synthetic polymers, when $V_{stat} = V_P$, and use the equilibrium constant K as a fundamental parameter describing the retention of macromolecules.

The distribution coefficient, K, decreases from $K > 1$ to $K < 1$ when the elution mode changes from adsorption to size exclusion. Complete entropy–enthalpy mutual compensation occurs at the transition point (CPA), where $K = 1$. At this point retention does not depend on the molecular mass of the polymer, and, in isocratic elution, all polymer fractions move with the solvent band V_L [2].

In gradient elution of macromolecules, the value of K decreases rapidly with the eluent composition Φ, beginning with the extremely strong adsorption ($K \gg 1$) at the injection point, Φ_{inj}. At this initial point, the polymer adsorption is often accompanied by polymer precipitation (phase separation) to give two immiscible liquid phases. This happens when a thermodynamically poor solvent (or nonsolvent) is used as the initial component A of the mobile phase, so that the solubility threshold for the polymer, Φ_{sol}, exceeds the initial mobile phase composition: $\Phi_{sol} > \Phi_{inj}$. Without the solid surface, a polymer-rich liquid thermodynamic phase with high concentration of strong solvent B would form a suspension of small liquid droplets. But in porous media with high adhesion of solvent B to the surface of pores, such a phase covers the internal surface of particles close to the column inlet.

In both cases (with or without phase separation), the injected polymer is separated from the initial band of the sample solution (usually solvent B) and is practically irreversibly retained at the column inlet. According to the concept of gradient elution at CPA [9], macromolecules begin to move through the column with a distinct velocity only in the vicinity of the transition (critical) point, $K = 1$, which is achieved at some composition Φ_{cr} (CPA). All polymer fractions independently of molecular mass leave the column practically simultaneously with the eluent composition close to Φ_{cr}. If polymer precipitated in the beginning of the process, then polymer redissolution into a homogeneous phase should occur before the critical composition Φ_{cr} is achieved, i.e. $\Phi_{sol} < \Phi_{cr}$ [9]. In this case, polymer adsorption follows the redissolution before the gradient elution at the CPA may occur. Otherwise ($\Phi_{sol} > \Phi_{cr}$), the CPA does not exist, and elution of solute occurs by solubility of individual polymer fractions. This last situation has been considered theoretically and experimentally in a number of publications (see review in [12]), and will not be discussed here, except for one example depicted in Figs. 9 and 10, where separation solely by solubility is achieved. The detail comparative

consideration of all possible modes of gradient elution of polymers will be published soon [13].

3.2. Quantitative description of gradient elution

The goal of a quantitative chromatographic theory is to predict or at least explain the results of gradient elution from the dependence of the distribution coefficient K (or free energy change due to solute sorption on the stationary phase) on the parameters describing the structure of macromolecules, such as molecular mass, chemical composition, functional groups, etc., as well as mobile phase composition, temperature, pore geometry and surface properties.

The continuous random-flight model of a flexible homopolymer chain interacting with the attractive surface of the porous material relates the distribution coefficient K to the segment interaction energy ϵ in the vicinity of the transition point [7,9]:

$$K = \exp\left(\frac{2\,r_g^2}{da} \cdot \frac{\epsilon_{cr} - \epsilon}{k_B T}\right) \quad (2)$$

where k_B is the Boltzmann's constant and T is the absolute temperature.

The energy change associated with the adsorption of macromolecules is related to the fact that a certain amount of monomer units of a polymer chain replace the solvent molecules near the surface and create reversible bonds with the adsorption (active) sites on this surface at the solvent composition Φ_{surf}. The energy gain in polymer adsorption is proportional to molecular mass of the macromolecule. The corresponding specific value ϵ describes this energy change per one such bond, and is molecular mass independent. As the adsorption is caused by attractive forces, ϵ is always negative. The critical value, $\epsilon = \epsilon_{cr} < 0$, corresponds to the transition point, $K = 1$, and is independent of the nature of polymer.

Notice the strong exponential molecular-mass-dependence of distribution constant K (through the size r_g) in the vicinity of the critical point, $\epsilon = \epsilon_{cr}$. This makes retention of high-molecular-mass molecules extremely sensitive to the segment interaction energy ϵ, and the mode of elution can change dramatically from practically irreversible adsorption to size exclusion within an extremely narrow interval of the energy ϵ surrounding ϵ_{cr}.

The interaction energy depends on chemical nature of macromolecules, and different polymers have different values of ϵ at the same eluent composition. This is also true for individual fractions of statistical copolymers, and ϵ can be calculated as a function of macromolecular chemical composition and structure [6]. On the other hand, ϵ depends on the eluent composition on the surface, Φ_{surf}, and, consequently, its overall composition Φ. As a result, macromolecules with different chemical composition and structure have different values of Φ_{cr}, and hence different retention volumes in gradient elution. In the case of linear gradient with slope $d\Phi/dV$, the volume of liquid needed for the mobile phase to reach the critical composition Φ_{cr}, is

$$V_{cr} = (\Phi_{cr} - \Phi_{inj})/d\Phi/dV \quad (3)$$

As mentioned above, Eq. (2) is valid in the vicinity of the critical point, $\epsilon_{cr} = \epsilon(\Phi_{cr})$. In this region, the segment interaction energy ϵ can be expressed as a linear function of Φ [9]:

$$\epsilon - \epsilon_{cr} = (d\epsilon/d\Phi) \cdot (\Phi - \Phi_{cr}) \quad (4)$$

where the derivative is taken at the point $\epsilon = \epsilon_{cr}$. This assumption of linearity is probably a good approximation even outside the immediate proximity of the critical point, as it also leads to the log-linear Φ-dependence of the distribution constant (2), which is typical for various mechanisms of HPLC of polymers. Thus, the well-known linear solvent strength gradient model also leads to this approximation in the case of a linear gradient [11].

The retention volume in gradient elution, V_g, is defined as the volume of liquid needed for the solute band to pass through the entire column during the mobile phase gradient. It can be calculated from the mass-balance equation [11]:

$$\int_0^{V_g} dV/V_R = 1 \quad (5)$$

where dV is the incremental volume of mobile phase that passes through the band center during its migration along the column, and V_R is an instantaneous value from Eq. (1) with $V_{mob} = V_0$, $V_{stat} = V_P$, calculated for isocratic elution at instant mobile phase composition Φ. Substituting Eqs. (1)–(4) into

Eq. (5) and taking into account that adsorption is very strong at the injection point, i.e. $K \gg 1$ at $\Phi = \Phi_{inj}$, we have [9]:

$$V_g - V_{cr} = V_0 \ln[(e^Q - 1) V_P / V_0]/Q$$
$$Q = \frac{2r_g^2}{Da} \cdot \frac{d(\epsilon/k_B T)}{d\Phi} \cdot \frac{d\Phi}{d(V/V_0)} \quad (6)$$

Here the derivatives as well as the size of macromolecules are calculated at the critical composition Φ_{cr}. A similar equation can be obtained for the eluent composition Φ_g, which accompanies the polymer band leaving the column:

$$\Phi_g - \Phi_{cr} = d\Phi/d(V/V_0) \ln[(e^Q - 1) V_P / V_0]/Q \quad (7)$$

As we see from Eqs. (6) and (7), the retention of macromolecules in gradient elution is governed by two crucial parameters: the critical point of adsorption, Φ_{cr}, and the dimensionless parameter Q. The latter consists of three factors. The first term reflects the relation between the dimensions of macromolecule and pore, the second reflects the effect of eluent composition on the segment interaction energy, and the last one is the gradient rate.

The molecular mass dependence of retention is determined solely by the magnitude of Q. If $Q \gg 1$, Eqs. (6) and (7) turn into an asymptotic form:

$$V_g - V_{cr} = V_0, \quad \Phi_g - \Phi_{cr} = d\Phi/d(V/V_0) \quad (8)$$

and retention becomes completely molecular mass independent, as well as independent of gradient conditions, initial eluent composition, Φ_{inj}, etc. Usually, $V_0 \ll V_{cr}$ and $d\Phi/d(V/V_0) \ll 1$, so that the polymer band reaches the column outlet at an eluent composition close to Φ_{cr}. As can be seen from Eq. (8), the slower the gradient the closer the asymptotic value of Φ_g to the critical composition. But the analysis of Eq. (7) shows (see also Fig. 7) that for shallow gradients this asymptotic value (8) is achieved at higher values of molecular mass of the polymer, compared with the elution at higher gradient rates.

Previously [9], we suggested to call such a mode the ''gradient elution at CPA''.

The molecular mass dependence can be significant in the opposite case, $Q \ll 1$:

$$V_g - V_{cr} = V_0 \ln(Q V_P / V_0)/Q < 0,$$
$$\Phi_g - \Phi_{cr} = d\Phi/d(V/V_0) \ln(Q V_P / V_0)/Q < 0 \quad (9)$$

In this mode, the macromolecules leave the column well before the eluent reaches its critical composition, Φ_{cr}, but the difference between Φ_g and Φ_{cr} diminishes with the increase of gradient rate. We suggested [9] naming this mode ''adsorption gradient chromatography'', because high-molecular-mass macromolecules elute later than their low-molecular-mass counterparts. In this mode, the dependence of retention on polymer chemical structure and molecular mass is affected by the chromatographic conditions such as initial eluent composition and the gradient slope.

Note that parameter Q is proportional to the mean square radius r_g^2 and hence strongly increases with molecular mass. This means that when the polymer homologous series is subjected to gradient elution, lower molecular mass fractions can be effectively separated ($Q < 1$), whereas the macromolecules with higher molecular masses ($Q > 1$) will have practically the same retention.

3.3. Gradient separation of polymer blends and copolymers by chemical composition

If chromatographic conditions for gradient separation of a polymer blend are chosen the way that the parameter Q for each component of the mixture significantly exceeds unity, then separation by chemical composition and/or microstructure of macromolecules occurs exclusively due to the difference in their critical mobile phase compositions. Thus, for the mixture of two homopolymers A and B with critical mobile phase composition $\Phi_{A,cr}$ and $\Phi_{B,cr}$, respectively, the difference in retention volumes $V_{A,g}$ and $V_{B,g}$ of corresponding chromatographic peaks is proportional to the difference between the transition points:

$$V_{B,g} - V_{A,g} = V_{B,cr} - V_{A,cr}$$
$$= (\Phi_{B,cr} - \Phi_{A,cr})/(d\Phi/dV) \quad (10)$$

The same concepts can be applied also to gradient separation of statistical copolymers [6,9]. We have shown recently [6] that copolymers with narrow (Gaussian) chemical composition distribution (we

called them ergodic copolymers [6,9,14]) have a single CPA similar to homopolymers. The effective segment interaction energy ϵ_{eff} for such copolymers depends on average chemical composition and microstructure of macromolecules, as well as the interaction energies of corresponding comonomer units. For example, for a random copolymer comprised of comonomers A_i with segment interaction energies ϵ_i, the chemical composition dependence of ϵ_{eff} is described by Eq. [6]:

$$X_{eff} = \sum_1 [X_i P(A_i)] \quad (11)$$

where $X_{eff} = \exp(-\epsilon_{eff}/k_B T)$, $X_i = \exp(-\epsilon_i/k_B T)$, $P(A_i)$ is the probability (molar fraction) of the comonomer A_i in the copolymer, and summation is over all types of monomer units.

The chemical composition heterogeneity of ergodic copolymers (such as many condensation copolymers or products of free-radical copolymerization at low conversion of comonomers) becomes negligible with increase of molecular mass, and their chromatographic behavior is similar to that of homopolymers with segment interaction energy $\epsilon = \epsilon_{eff}$. Recently, we demonstrated this chromatographic behavior for isocratic elution of chlorinated polyethylenes with different molecular masses on silica at several hexane–chloroform compositions [9].

The copolymers with high chemical composition heterogeneity, such as products of free-radical copolymerization at high conversion of monomers, are comprized of polymer fractions with widely different compositions, and hence different critical points of adsorption. The situation is similar to that of polymer blends. At gradient separation, each such fraction elutes at corresponding CPA, so that separation by chemical composition is achieved.

4. Results and discussion

4.1. Reversed-phase separations

To establish the gradient elution at CPA, we first performed a series of isocratic separations of narrow polydispersity polystyrene standards (molecular masses from 474 to 710 000) on the silica-based C_{18} reversed-phase column using ACN–THF mixtures at various compositions (Figs. 1–4). The elution of such polymers in THF ($\Phi = 1$) is depicted in Fig. 1. A typical size-exclusion mode of elution is observed with higher-molecular-mass polymers eluting earlier. This can be explained by the fact that the relatively nonpolar THF suppresses reversed-phase interaction between polystyrene and aliphatic groups at the surface of the bonded silica. The opposite situation

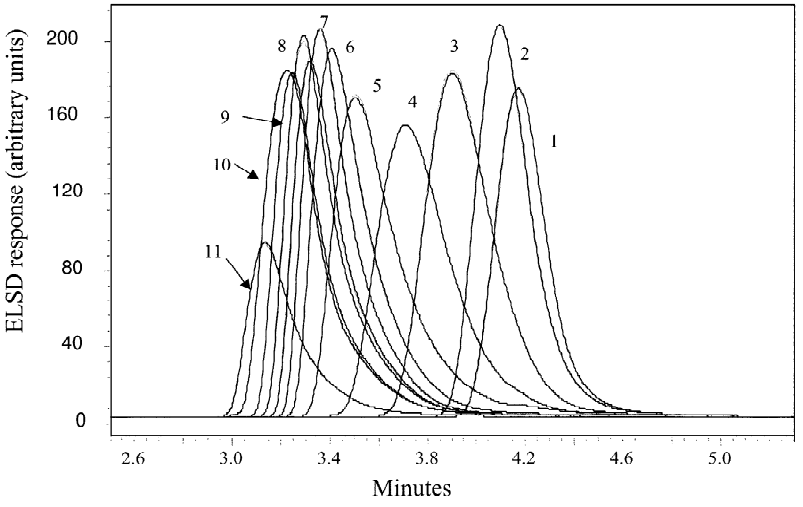

Fig. 1. Isocratic elution of narrow polystyrene standards (two injections of each) with peak molecular masses (as determined by GPC): 474 (1), 890 (2), 2630 (3), 5570 (4), 9100 (5), 18 100 (6), 37 900 (7), 96 400 (8), 186 000 (9), 355 000 (10), 710 000 (11). Detector: evaporative light-scattering, column: Nova-Pak C_{18}, temperature: 35 °C, mobile phase: unstabilized THF, flow-rate: 0.5 ml/min.

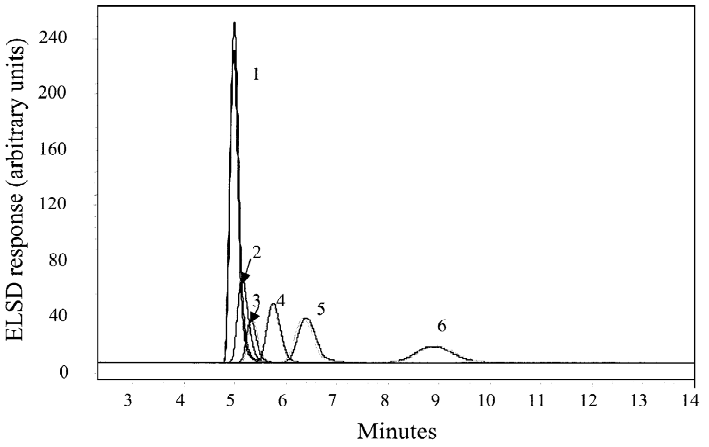

Fig. 2. As Fig. 1, except the mobile phase composition: ACN–THF (55:45, v/v), and highest molecular mass polystyrene: 18 000 (6).

has come to light when the amount of the more polar component ACN in the mobile phase exceeds 52% (v/v) ($\Phi < 0.48$). In this case depicted in Fig. 2, the overall polarity of the eluent is high enough to avoid suppression of nonpolar interaction, and higher-molecular-mass fractions elute later on, which is inherent in the adsorption mode of elution. Finally, the molecular mass independent isocratic elution of polystyrenes was found at 48% (v/v), THF ($\Phi_{cr} = 0.48$) (Fig. 3), which represents the critical point of adsorption for this system. Different modes of isocratic elution of polymer homologous series are usually shown as a set of molecular mass calibration curve [2]. Such a fan-like diagram for polystyrenes in ACN–THF mixtures summarizes the results of current investigation and is presented in Fig. 4.

Note that polystyrenes with molecular masses 100 000 or higher precipitated at room temperature in ACN–THF mixtures with THF content less than 47% (v/v) ($\Phi_{sol} = 0.47$), and could not be subjected to isocratic elution. (Only columns and detectors were kept at 35 °C during the experiments, while all polymer samples for isocratic runs were dissolved in mobile phase and injected at room temperature). Another factor affecting the isocratic elution is the strong dynamic effect, which is usually observed in

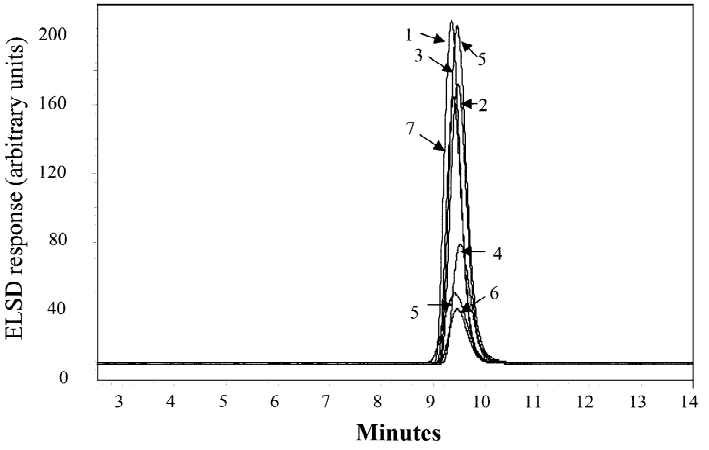

Fig. 3. As Fig. 1, except mobile phase composition: ACN–THF (52:48, v/v), flow-rate: 0.25 ml/min, and highest-molecular-mass polystyrene: 37 900 (7).

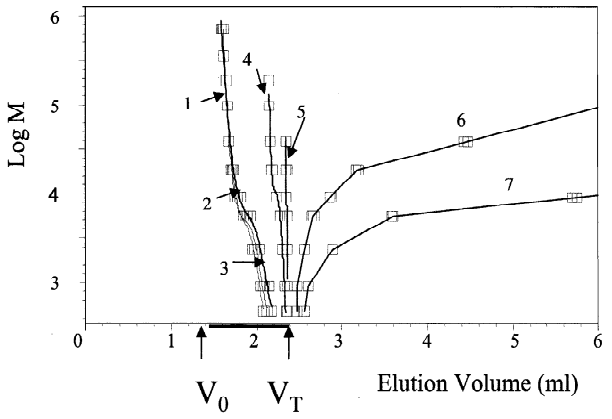

Fig. 4. Molecular weight calibration curves for isocratic elution of narrow polystyrene standards (duplicate injections) with mobile phase ACN–THF of composition Φ: 1 (1), 0.8 (2), 0.7 (3), 0.5 (4), 0.48 (5), 0.45 (6), 0.4 (7). Other conditions as in Fig. 1. The exclusion and total volumes of the column are indicated on the *x* axis.

the vicinity of CPA [2]. We will present experimental evidence and some insight into these phenomena elsewhere [13].

The same polystyrenes were subjected to gradient elution in ACN–THF mixtures beginning with pure ACN ($\Phi_{inj}=0$). The results of such elution at two different gradient rates are presented in Figs. 5 and 6. In qualitative agreement with the theory, the molecular mass-dependence is observed for lower molecular mass samples only ($Q<1$), while larger molecules elute close to Φ_{cr} ($Q>1$). Note the excellent resolution of polystyrene oligomers achieved at gradient elution, which far exceeds the resolution of size-exclusion separation of the same polymers (compare chromatograms 1, 2 and 3 in Figs. 1 and 6, respectively). One can see multiple bimodal peaks

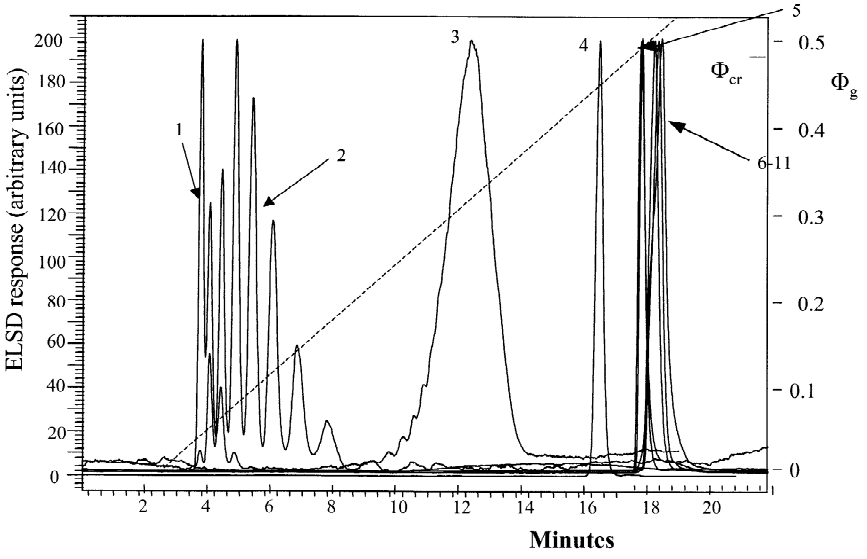

Fig. 5. Gradient elution of polystyrenes from Fig. 1 from 0 to 100% (v/v) THF in ACN linear over 30 min [$d\Phi/d(V/V_0)=0.027$]. Right *y* axis shows the eluent composition at the column outlet, Φ_g (broken line), with indicated $\Phi_{cr}=0.48$. Detector: evaporative light-scattering, column: Nova-Pak C_{18}.

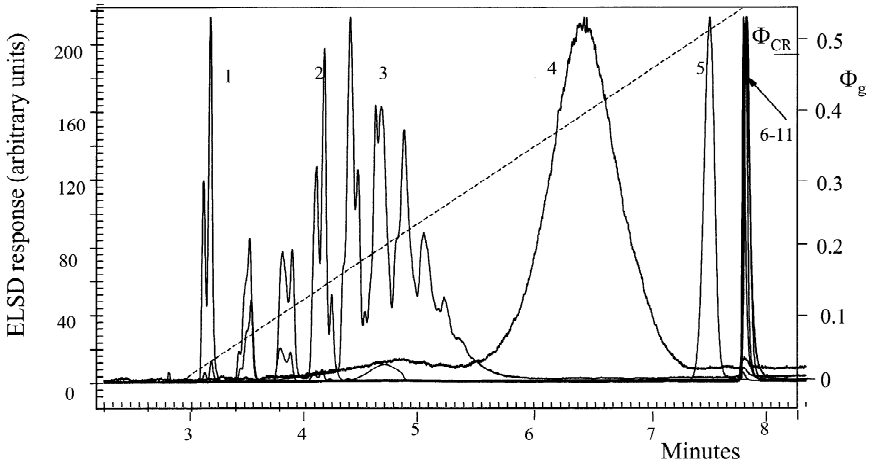

Fig. 6. As Fig. 1, except linear gradient over 10 min [$d\Phi/d(V/V_0) = 0.081$].

from polystyrenes with molecular masses below 2000 in Figs. 5 and 6. We can assume these are individual isomers or molecules with different end groups separated in fast gradient.

To correlate our measurements with theoretical predictions quantitatively, we need to calculate the values of parameter Q in Eqs. (6) or (7) for polymer fractions with different molecular masses.

It is well known [15] that the molecular-mass-dependence of the radius of gyration for flexible linear macromolecules is described by a power function $r_g \sim M^{\alpha}$, were the value of the exponent α changes from 0.6 to 0.5 depending on the thermodynamic quality of the solvent. Thus, polystyrene has $\alpha = 0.57$ in THF at 40 °C [16] (good solvent), and $\alpha = 0.5$ in cyclohexane at 34 °C (Θ-solvent) [15]. The ACN–THF (52:48, v/v) mixture, corresponding to CPA ($\Phi_{cr} = 0.48$), is a rather poor solvent for polystyrene as $\Phi_{sol} = 0.47$ at room temperature and just slightly lower (0.44) at 35 °C (operating temperature). Consequently, the reasonable assumption in this case is $\alpha = 0.5$, so that Q can be expressed as a linear function of M:

$$Q = \gamma M \, d\Phi/d(V/V_0) \qquad (10)$$

where γ is a molecular-mass-independent constant for given polymer and chromatographic system. Substituting relation (10) and the measured values $V_0 = 0.81$ ml, $V_P = 1.1$ ml, $\Phi_{cr} = 0.48$ into Eq. (7), we have:

$$\Phi_g = 0.48 + 0.306/(\gamma M) + \ln\{\exp[\gamma M \, d\Phi/d(V/V_0)] - 1\}/(\gamma M) \qquad (11)$$

The results of calculations by Eq. (11) for three different gradient rates are presented in Fig. 7 along with corresponding experimental data. Excellent agreement between theory and experiment is achieved with the only adjustable parameter $\gamma = 0.0015$, which has been used to fit all three sets of data.

The molecular-mass-independence of gradient elution at the CPA makes this mode of polymer chromatography a powerful tool for characterization of copolymers, polymer blends, polymers with func-

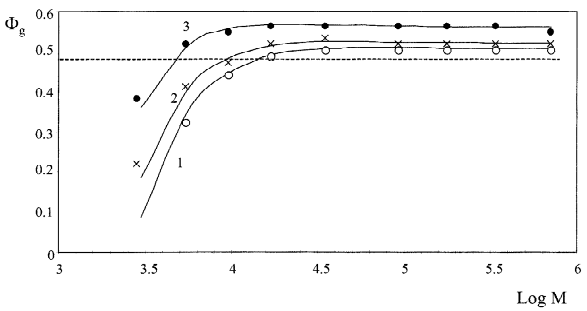

Fig. 7. Comparison of theoretical curves calculated from Eq. (11) with $\gamma = 0.0015$ and experimental data (points) for gradient elution of polystyrenes in ACN–THF at linear gradient rates $d\Phi/d(V/V_0)$: 0.027 (1, ○), 0.041 (2, ×) and 0.081 (3, ●). Other conditions as in Figs. 5 and 6.

tional groups and other complex polymeric samples. The technique is complimentary to SEC, which usually produces MWD of polymers.

As the critical point of adsorption is usually very sensitive to chemical structure of a polymer chain, the gradient elution at CPA has very high resolution even for separation of polymers with close chemical structures. This is demonstrated in Fig. 8 for gradient elution of mixture of five poly(alkylmethacrylates) with weight average molecular masses above 200 000. For such high-molecular-mass polymers, the value of parameter Q far exceeds unity, and the elution of each individual polymer occurs close to the corresponding CPA.

Another feature of gradient elution of polymers at the CPA, which favorably sets off it from other modes of polymer chromatography, is high efficiency of separation, associated with very narrow peaks for individual polymers, even with high degree of polydispersity. This is apparent from Fig. 8 and is a result of the molecular mass independence of elution as well as reduction in the effect of dynamic factors on separation [13]. High selectivity and efficiency of gradient elution at CPA provide excellent resolution for polymer separation based on the chemical composition and microstructure of macromolecules.

Polymers injected in thermodynamically poor solvent can precipitate at the column inlet to give two or more immiscible liquid phases. For example, such a phase separation occurred in our experiments when polystyrenes with molecular mass 100 000 or higher were injected in pure ACN (Figs. 5 and 6). As in this case $\Phi_{cr} > \Phi_{sol}$, these high-molecular-mass polystyrenes redissolved when mobile phase composition reached the solubility threshold $\Phi_{sol} = 0.44$ at 35 °C, and eluted at composition Φ_g slightly higher than the critical point of adsorption, $\Phi_{cr} = 0.48$.

A completely different situation occurred when a stronger nonsolvent was selected as component A of the mobile phase. Thus, we replaced ACN with methanol for gradient reversed-phase separation of the polystyrene standards (Fig. 9) at otherwise identical conditions to those described at Fig. 5. In this new case, critical point of adsorption for polystyrene does not exist at all ($\Phi_{cr} < \Phi_{sol}$), and elution of individual polystyrenes occurs solely by solubility. As can be seen from Fig. 9, a strong molecular mass dependence of elution is observed in this case for the entire range of molecular masses in full agreement with the theoretical prediction [12]. The difference in elution profiles for the broad polystyrene sample measured in ACN–THF and methanol–THF gradients, respectively (Fig. 10) also demonstrates the remarkable difference between the two modes of interaction polymer chromatography.

We analyzed the reversed-phase gradient separation of statistical copolymers at CPA using styrene–butadiene copolymers with close molecular

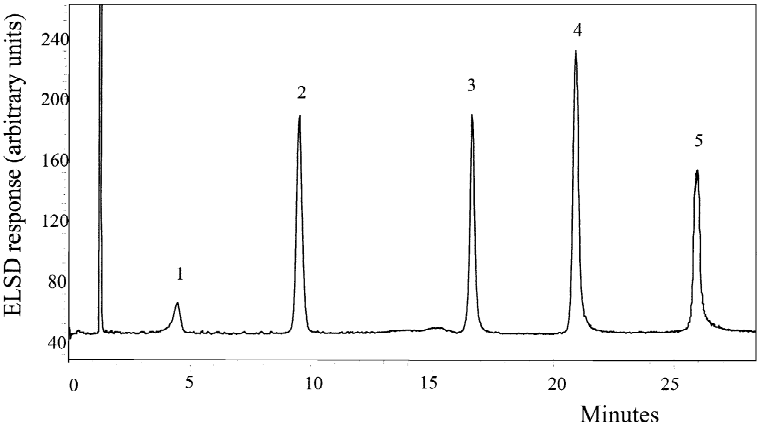

Fig. 8. Gradient separation of mixture of poly(methylmethacrylate) (1), poly(ethylmethacrylate) (2), poly(*n*-butylmethacrylate) (3), poly(*n*-hexylmethacrylate) (4), poly(laurylmethacrylate) (5). Detector: evaporative light-scattering, column SymmetryShield RP_8, gradient: 0–100% (v/v) THF in ACN linear over 30 min.

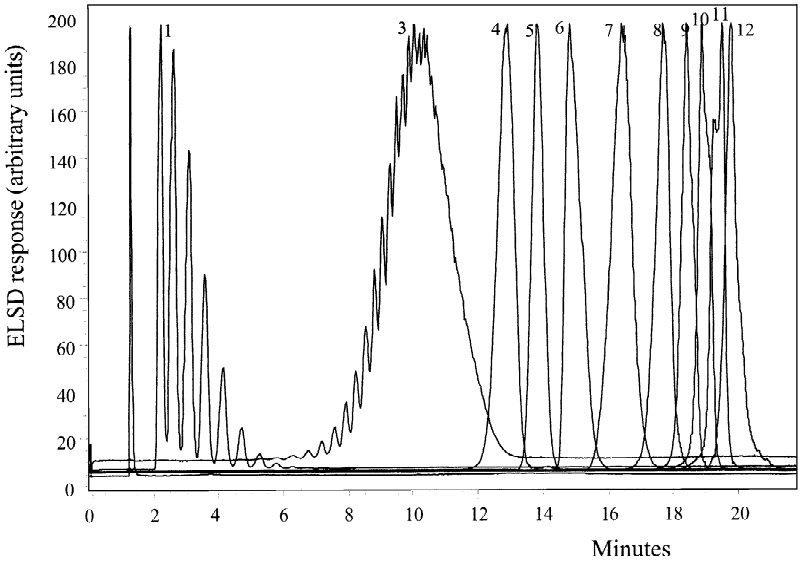

Fig. 9. Gradient elution of polystyrenes from Fig. 1 [additional narrow polystyrene (12) has molecular mass 2890 000] from 0 to 100% (v/v) THF in methanol linear over 30 min. Detector: evaporative light-scattering, column: SymmetryShield RP_8.

masses but different chemical compositions (Fig. 11). All copolymers tested were synthesized at low conversion of monomers, and had narrow chemical composition distribution. Phase separation occurred at the injection point, because all samples were insoluble in pure ACN. But the same way as for polystyrenes, the solubility threshold for each copolymer was lower than corresponding critical point of adsorption, i.e. $\Phi_{sol} < \Phi_{cr}$, so that the elution at CPA independent of molecular mass has been achieved for each copolymer. The related chemical composition calibration curve depicted in Fig. 12, describes how the copolymer chemical composition depends on its CPA, and can be used to calculate the chemical composition distribution of copolymers with high composition heterogeneity.

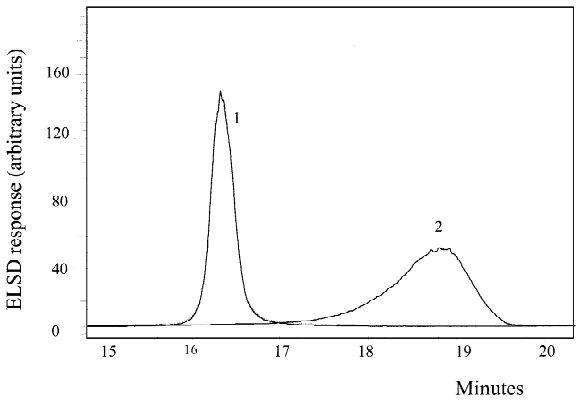

Fig. 10. Gradient elution of broad polystyrene NBS 706 from 0 to 100% (v/v) THF in ACN (1) or methanol (2) linear over 30 min. Detector: evaporative light-scattering, column: Nova-Pak C_{18}.

4.2. Normal-phase separation

Normal-phase columns can serve as a more effective separation material for polar polymers. All of the preceding is completely applicable to normal-phase separation. The results reported above for reversed-phase separations were substantiated by similar experiments with silica columns and toluene–THF and *n*-hexane–THF mixtures as mobile phases (Figs. 13–16). A series of isocratic experiments with narrow polydispersity poly(methylmethacrylates) (molecular masses from 1020 to 1 100 000) in toluene–THF mixtures allowed for determination of CPA, $\Phi_{cr} = 0.41$. The gradient separations of the same polymers confirmed that all polymers with molecular masses 50 000 and higher eluted close to this critical composition. Fig. 13 demonstrates such

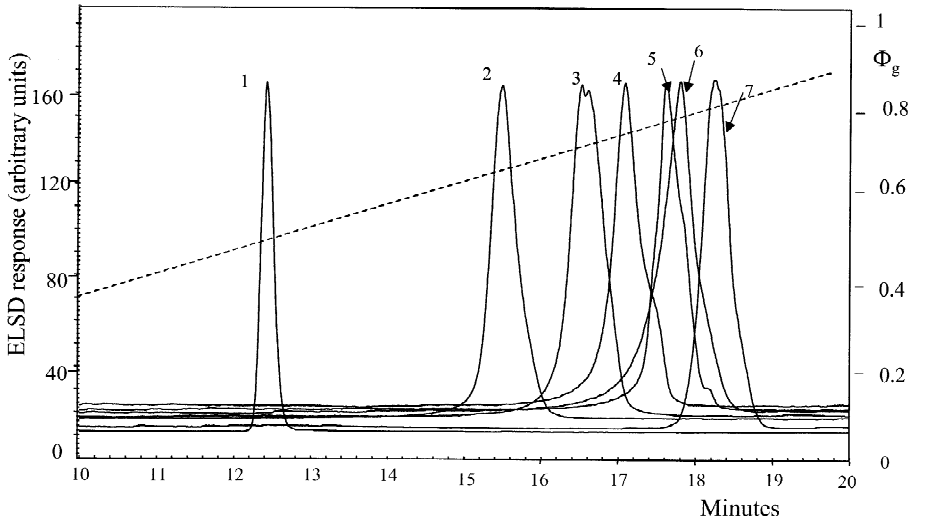

Fig. 11. Gradient elution of polystyrene (1), polybutadiene (7) and five styrene–butadiene statistical copolymers with styrene content, mol%: 50 (2), 36 (3), 23.5 (4), 16 (5), 5.2 (6). Right *y* axis shows the eluent composition at the column outlet, Φ_g (broken line). Detector: evaporative light-scattering, column: SymmetryShield RP_8, gradient: 0–100% (v/v) THF in ACN linear over 20 min.

gradient elution for several poly(methylmethacrylates) including one (7) with broader MWD (M_n = 48 300, M_w = 102 600). As most of the fractions of this high polydispersity polymer eluted close to the critical composition, the width of its peak did not exceed the width of polymers with much lower polydispersity.

The examples of normal-phase gradient elution at CPA of different polymers with close molecular masses are depicted in Figs. 14–16. The order of elution of poly(alkylmethacrylates) in toluene–THF gradient depicted in Fig. 14, is the opposite to that in Fig. 8, in full agreement with the general rule of HPLC: more polar molecules are retained stronger on normal-phase columns, and weaker on reversed-phase columns. In our case, the order of elution reflects the effect of polarity of the solutes on the critical composition of the mobile phase.

The use of less polar *n*-hexane instead of toluene in gradient elution of poly(alkylmethacrylates) and poly(alkylacrylates) can further improve the selectivity of separation, which is demonstrated in Figs. 15 and 16. Thus, Fig. 16 shows that, in spite of similarity in chemical structure of three structural isomers of poly(butylmethacrylate), the difference between the corresponding CPAs allows for a noticeable difference in retention times in their elution in a hexane–THF gradient.

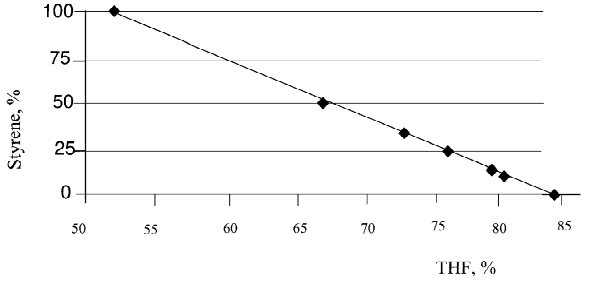

Fig. 12. Chemical composition of styrene–butadiene copolymers as a function of eluent composition for the gradient elution depicted in Fig. 11.

5. Conclusion

A symbiosis of molecular–statistical theory of polymer solutions in confined (porous) media and conventional theory of gradient elution produces an elegant method for the description of peculiarities of gradient separation of polymer blends and copolymers by chemical composition distribution and/or

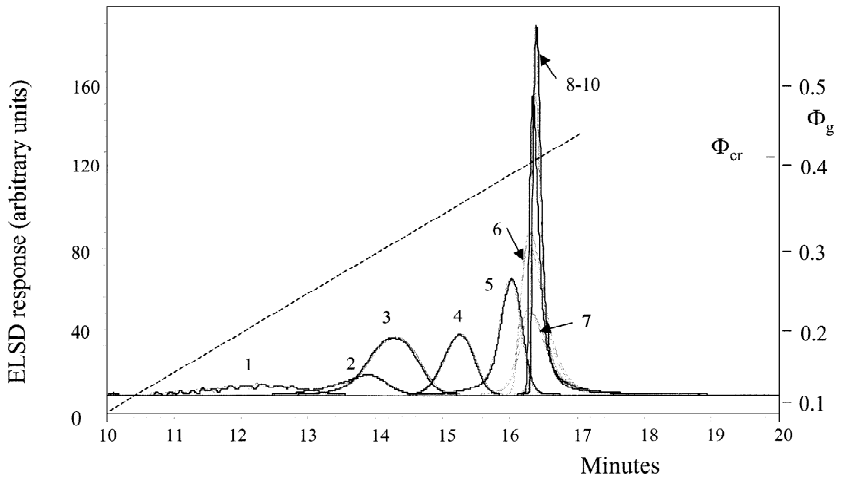

Fig. 13. Gradient elution of narrow poly(methylmethacrylate) standards (two injections of each) with peak molecular masses (as determined by GPC): 1020 (1), 1960 (2), 2500 (3), 5090 (4), 11 300 (5), 105 000 (6), broad (7), 230 000 (8), 530 000 (9), 1 100 000 (10). Right *y* axis shows the eluent composition at the column outlet, Φ_g (broken line), with indicated $\Phi_{cr}=0.41$. Detector: evaporative light-scattering, column: Nova-Pak silica, gradient: 0–100% (v/v) THF in toluene linear over 20 min.

other structural differences in macromolecules. The thermodynamics of chromatographic elution predicts conditions when retention does not depend on molecular mass of macromolecules in both isocratic and gradient modes: separation at critical point of adsorption. The thermodynamic theory explains the reason for high selectivity of polymer chromatography, which allows for the successful gradient separation of macromolecules of close chemical structures and composition.

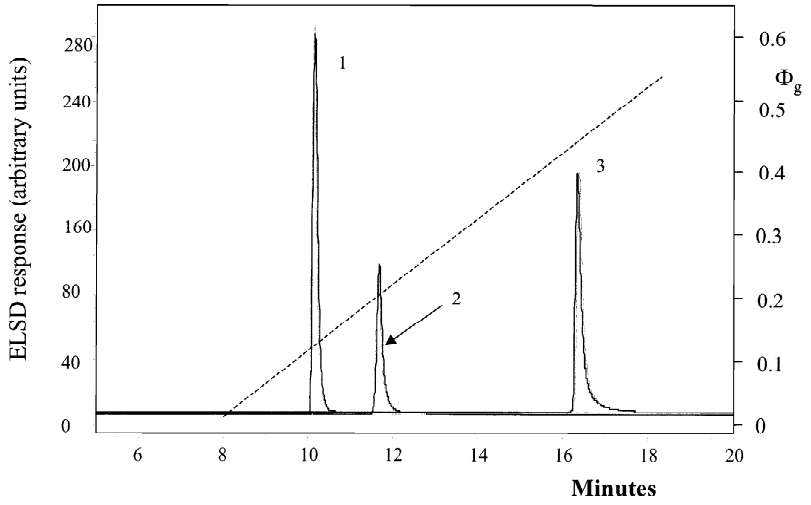

Fig. 14. Gradient elution of poly(*n*-butylmethacrylate) (1), poly(ethylmethacrylate) (2), poly(methylmethacrylate) (3). Right *y* axis shows the eluent composition at the column outlet, Φ_g (broken line). Detector: evaporative light-scattering, column: Nova-Pak silica, gradient: 0–100% (v/v) THF in toluene linear over 20 min.

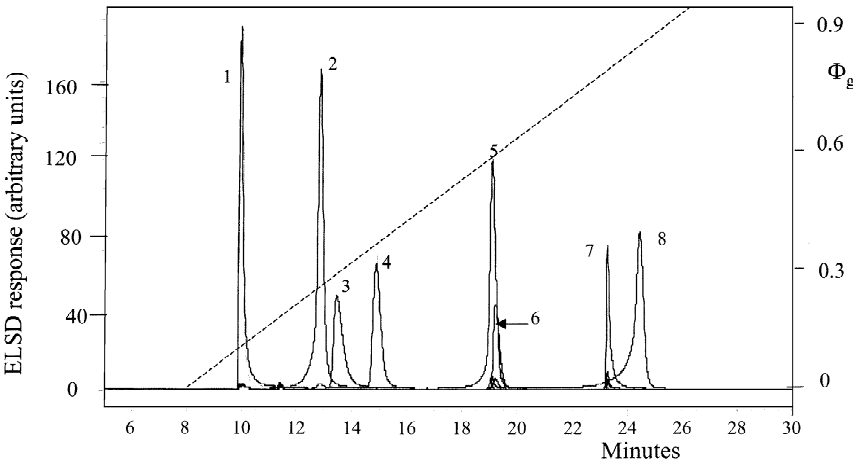

Fig. 15. Gradient elution of poly(laurylmethacrylate) (1), poly(*n*-butylacrylate) (2), poly(*n*-butylmethacrylate) (3), styrene–butylmethacrylate copolymer (4), poly(ethylacrylate) (5), poly(ethylmethacrylate) (6), poly(methylmethacrylate) (7), poly(methylacrylate) (8). Right *y* axis shows the eluent composition at the column outlet, Φ_g (broken line). Detector: evaporative light-scattering, column: Nova-Pak silica, gradient: 0–100% (v/v) THF in *n*-hexane linear over 20 min.

The quantitative description of resolution in chromatographic separation should also include analysis of dynamic effects, which may play an especially important role when the solutes are polymers. Another complication can come from the possible phase separation caused by poor solubility of polymers at the initial stages of separation. All these effects can significantly change the output of the chromatographic process and are the subject of our current theoretical and experimental investigations.

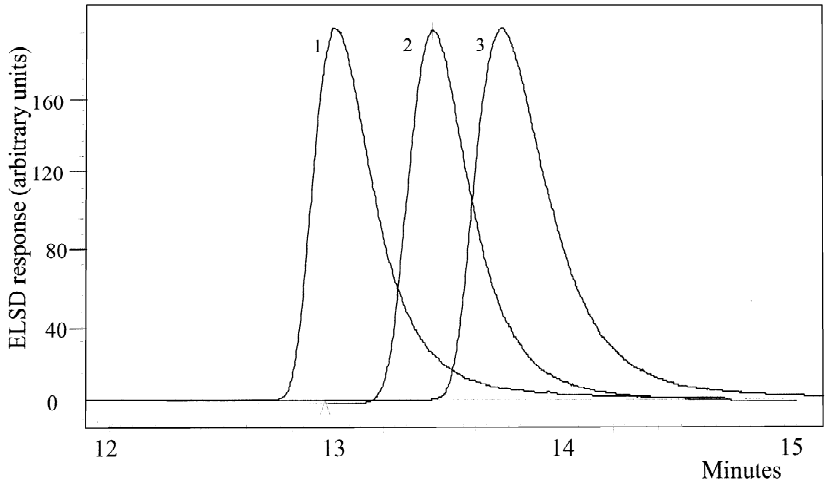

Fig. 16. Normalized chromatograms of poly(isobutylmethacrylate) (1), copolymer of isobutylmethacrylate and *n*-butyl methacrylate (2), poly(*n*-butylmethacrylate) at gradient elution from 0 to 100% (v/v) THF in *n*-hexane linear over 20 min. Detector: evaporative light-scattering, column: Nova-Pak silica.

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