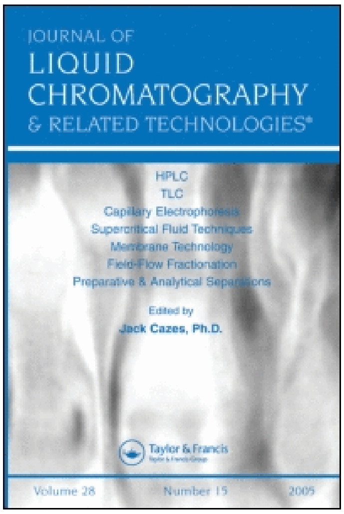

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THE MECHANISM OF COPOLYMER RETENTION IN INTERACTIVE POLYMER CHROMATOGRAPHY. II. GRADIENT SEPARATION

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THE MECHANISM OF COPOLYMER RETENTION IN INTERACTIVE POLYMER CHROMATOGRAPHY.
II. GRADIENT SEPARATION

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ABSTRACT

The molecular-statistical theory of polymer solutions in confined media is applied to the conventional chromatographic theory of gradient elution. This approach leads to the prediction of the special mode of interactive polymer chromatography: gradient elution at critical point of adsorption. We demonstrate theoretically and experimentally that under appropriate conditions elution of each compositionally homogeneous fraction of copolymer occurs at the critical mobile phase composition.

This critical mobile phase composition depends only on the local structure of the copolymer chain and is independent of its molecular weight. As a consequence, gradient elution produces the chemical composition distribution of the copolymer. The theory provides the quantitative conditions for statistical copolymer chains to have a single transition point. Equations describing relationships between the critical eluent composition and the chemical composition and microstructure of macromolecules are developed.

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The experimental verification of the theory was performed by the normal phase isocratic and gradient elution of chlorinated polyethylene with various chemical compositions (chlorine content) and molecular weights. To the best of our knowledge, these experiments, for the first time, demonstrate the existence of the adsorption-desorption transition point for statistical copolymers. The gradient separation at the critical point of adsorption can be applied also to other complex polymers containing various types of molecular heterogeneity.

INTRODUCTION

The fundamental outcome of the molecular-statistical theory of dilute polymer solutions applied to the interactive polymer chromatography is the existence of so-called critical point of adsorption (CPA), viz., a mobile phase composition where retention does not depend on molecular weight of macromolecules. At this point steric and adsorption interactions between macromolecules and the surface of a stationary phase completely compensate each other.

Up to now, the application of the separation at CPA in polymer chromatography was limited by the isocratic elution: separation of functional oligomers and block copolymers.[2] But two inherent features of the chromatographic system performance near the transition point causes numerous practical weaknesses of this technique (see reviews).[3-5] As we discussed in the previous paper,[1] the retention of macromolecules in the vicinity of the CPA is extremely sensitive to the eluent composition, temperature, and other external parameters affecting the segment adsorption energy, especially for high molecular weight macromolecules and narrow pores.

Thus, minute changes in the mobile phase composition can transfer the macromolecules residing inside pores into interstitial space between particles, and vice versa. This feature causes many problems in isocratic separation, such as non-reproducibility of results, excessive peak broadening and skewing, including pronounced peak splitting, poor mass recovery, etc.[3]

Another problem of the isocratic elution at the CPA is that polymer peak elutes with the system peak, i.e., at the total volume $V_T = V_0+V_P$ of the liquid within column (here V_0 and V_P are the interstitial (exclusion) and pore volumes of the column, respectively). This means that macromolecules are not separated from their initial solvent and from non-retained low molecular weight impurities, which may cause peak overlapping difficulties in detection.

In the first part of this work[1] we extended the concept of polymer chromatography at CPA to the cases of statistical copolymers and the stationary phases with heterogeneous surfaces. We showed that any chemically homogeneous random copolymer has a single adsorption-desorption transition point and established conditions when non-random homogeneous statistical copolymers also posses this property.

In the case of copolymers with broad chemical composition distribution (CCD), polymer fractions with different compositions have different transition points. The corresponding critical points of adsorption depend on chemical composition and microstructure but not the molecular weight of individual macromolecules. This feature allows one to perform a gradient separation based solely on the chemical composition and microstructure of polymers. Such gradient elution successfully avoids the aforementioned problems associated with the isocratic separation at CPA. Moreover the high sensitivity of retention to the mobile phase composition has become the most important advantage of the separation at CPA in the gradient mode. The primary goal of this work is developing the theory of gradient separation of homo- and copolymers based on the general molecular-statistical approach to the polymer solutions in porous media, using results obtained in the previous paper.[1]

EXPERIMENTAL

All experiments on chlorinated polymers have been performed by the author several years ago in his former laboratory in Russia (State Research Institute of Chlorine, Moscow, Russia) and part of them have been reported recently.[6,7]

Homogeneous chlorination of four low- and high-density polyethylenes with various molecular weights was performed by continuously passing chlorine gas into a 3%, w/w, polymer solution in carbon tetrachloride (CCl_4) at 77°C with 0.5 wt % AIBN (samples I, II, III, IV) or in chlorobenzene at 130°C without chemical initiator (samples V and VI) (Table 1). Both reactions were performed under moderate pressure. The chlorinated polyethylenes I − V with varying chlorine contents were obtained by sampling from the batch reactor during the course of chlorination and quenching in methanol. The sample VI was obtained in two-stage approach. First, initial polyethylene was chlorinated in batch mode up to 21 wt % chlorine. The subsequent chlorination was carried out in the continuous (flow) reactor by bubbling chlorine gas through the polymer solution. All chlorinated polyethylenes were washed several times with methanol and vacuum dried at 60°C for several days.

The absence of chemical degradation of chlorinated polyethylenes during chlorination (at least up to 40 wt % chlorine) was monitored by measuring the intrinsic viscosity in THF at 30°C (Mark-Houwink coefficients K and alpha 6.92×10^{-4} and 0.6, respectively).[8]

Table 1

Chlorinated Polyethylenes for Chromatographic Studies

Sample	Polymer Type	M x 10^5 (TCB, 135C)	Chlorination Conditions	Chlorine Content, wt%
I.1	LDPE	0.56	CCl_4, 77°C	24.7
I.2			initiator: AIBN	35.3
I.3			batch reactor	41.2
I.4				51.8
I.5				58.0
I.6				60.8
II.1	LDPE	1.78	CCl_4, 77°C	25.5
II.2			initiator:AIBN	35.6
II.3			batch reactor	40.6
II.4				44.7
II.5				52.2
II.6				59.2
II.7				60.9
III.1	HDPE	3.81	CCl_4, 77°C	28.5
III.2			initiator: AIBN	35.6
III.3			batch reactor	41.0
III.4				45.3
III.5				60.7
IV.1	HDPE	5.13	CCl_4, 77°C	30.1
IV.2			initiator: ALBN	35.7
IV.3			batch reactor	40.8
IV.4				47.0
IV.5				55.4
IV.6				59.0
V.1	HDPE	3.81	Chlorobenzene	21.1
V.2			130°C	27.2
V.3			w/o initiator	32.9
V.4			batch reactor	37.8
V.1	HDPE	3.81	Chlorobenzene 130°C w/o initiator continuous reactor	37.6

Gradient and isocratic separations of chlorinated polyethylenes were carried out at room temperature (ca. 25°C) on a Varian Analytical Instruments (San Fernando, CA, USA) Model 5060 ternary gradient chromatograph with a Silasorb 600 5 μm silica 250 x 4.6 mm I.D. column (LabAlliance, CA, USA), average pore diameter 6 nm. A Bruker (Bremen, Germany) variable-wavelength Model LC313-I detector was used at 254 nm.

The exclusion volume and total volume of the column were determined with polystyrene standards in tetrahydrofuran: V_0 = 2.11 mL, V_T = 3.48 mL. The gradient pumping system lag volume was 1.6 mL.

Both isocratic and linear gradient separations were performed with a mixture of chloroform (stabilized by 1% ethanol) and n-hexane as a mobile phase. Both solvents were HPLC grade and helium sparged during use. The flow rate was 0.7 mL/min for isocratic elution and varied from 0.3 to 1 mL/min in gradient elution for different runs. Chloroform was used as a solvent for all polymers subjected to the gradient separations (polymer concentration − from 0.1 to 0.6%, w/w). The mobile phase was a polymer solvent for all isocratic separations. A linear gradient slope varied from 0.3 to 1% vol. $CHCl_3$ per minute for different runs. Ten minutes of isocratic elution at initial mobile phase composition immediately after injection always preceded the start of the gradient. The injection volume for isocratic elution was 0.05 mL, for gradient elution − 0.1 or 0.2 mL.

All chlorinated polyethylenes under investigation were completely dissolved in pure chloroform. The solubility (clouds points) of chlorinated polyethylenes in n-hexane/chloroform mixtures at 25°C was visually determined by turbidity titration with n-hexane of 10 mL of 0.2% polymer solution.

THEORY

Gradient Separation of Homopolymers

Consider, first, a polydisperse homopolymer subjected to a gradient elution in a binary mobile phase. The fundamental equation of gradient elution,[9,10]

$$\int_0^{V_g} dV / V = 1 \qquad (1)$$

allows one to calculate the corrected retention volume V_g for any polymer fraction in a gradient run provided that its instantaneous (or "actual") value V at any given time is known. In the case of IPC the volume V is affected by both entropic and enthalpic factors and given by the equation,[1]

$$V = V_0 + K\,V_P \qquad (2)$$

where K is the distribution coefficient. For pores of an internal diameter D, $K = K_D$.

The segment interaction energy between the polymer and stationary phase, $\varepsilon < 0$, depends on the mobile phase composition Φ (the volume fraction of a "strong" solvent, i.e., more polar solvent in the case of normal phase or less polar for reverse phase separation): $\varepsilon = \varepsilon(\Phi)$. Usually (but not necessarily) this solvent is also a better one for the polymer in the thermodynamic sense.

Suppose that the composition at the injection point, $\Phi = \Phi_{inj}$, corresponds to the adsorption state for the polymer: $\varepsilon_{inj} = \varepsilon(\Phi_{inj}) < \varepsilon_{cr}$, where ε_{cr} is the threshold (critical) value for ε.[1] This means that entire polymer adsorbs at the top of the column, assuming it is separated from the initial band of the injected solution. We consider also the situation when the whole polymer or some of its fractions precipitate, i.e., the corresponding solubility limit is not achieved at the initial mobile phase composition. In this case we assume that the solubility improves with an increase of the amount of good solvent B and at some point $\Phi = \Phi_{precip}$ the precipitated polymer redissolves and adsorbs immediately from the solution on the stationary phase. Accordingly, we assign ε_{inj} to this eluent composition, so that $\varepsilon_{inj} = \varepsilon(\Phi_{precip}) < \varepsilon_{cr}$. The opposite situation, when redissolved polymer fractions elute from the column without the succeeding adsorption, constitutes the precipitation-redissolution mechanism of separation[11] and will be reviewed later in this section.

During gradient elution the value of $\varepsilon(\Phi)$ increases (usually linearly) with Φ and at some point $\Phi = \Phi_{cr}$ can reach its critical value ε_{cr}. The corresponding changes in the distribution coefficient K_D for each polymer fraction can be described by equation:[1,12]

$$K_D = \exp U(\varepsilon), \quad U(\varepsilon) = \frac{2\,R_g^2}{D\,a} \times \frac{\varepsilon_{cr} - \varepsilon}{k_B T}, \qquad (3)$$

where a is the width of adsorption layer at the surface of a stationary phase, and parameter U depends on the molecular weight of macromolecules through their size R_g (radius of gyration).

According to this equation, a necessary condition for all polymer fractions to stay in adsorbed state immediately after injection is $K_{D,inj} = K_D(\varepsilon = \varepsilon_{inj}) >> 1$, i.e., $U_{inj} = U(\varepsilon = \varepsilon_{inj}) >> 1$ (cf. equation (8) in previous paper).[1] Substituting expressions (2) and (3) into equation (1) and keeping in mind this condition, we have

$$V_g / V_{cr} = 1 + U_{inj}^{-1} \ln [V_P(\exp Q - 1) / V_0] \tag{4}$$

Here V_{cr} is the retention volume, which corresponds to the critical composition $\Phi = \Phi_{cr}$ and depends on the chemical structure of polymer, and $Q = V_0 U_{inj} / V_{cr}$ is a dimensionless parameter of crucial importance, as will soon become evident. This parameter depends on molecular weight of macromolecules and hence varies for different polymer fractions.

Notice that we have assumed the linearity of gradient shape Φ (V) when deriving equation (4). This means that $V_g / V_{cr} = (\Phi_g - \Phi_{inj}) / (\Phi_{cr} - \Phi_{inj})$, where Φ_g is the final mobile phase composition for a given polymer fraction with gradient retention volume V_g, when the corresponding band is located at the end of the column. The assumption of the gradient linearity has been made for the sake of simplicity and does not affect the following conclusions.

One can see from equation (4) that the polymer retention in the gradient mode generally depends on both polymer chemical structure (mostly through the critical eluent composition Φ_{cr} and hence the critical volume V_{cr}) and the molecular weight (through the molecular size R_g in U_{inj}).

The molecular weight dependence is significant if $Q << 1$. In this case

$$V_g / V_{cr} = 1 + U_{inj}^{-1} \ln (V_P Q / V_0) < 1, \tag{5}$$

and macromolecules leave column well before the eluent reaches its critical composition Φ_{cr}, i.e., $\Phi_g < \Phi_{cr}$. This mode of gradient elution ($Q << 1$) can be named “adsorption gradient chromatography” (AGC), because high molecular weight macromolecules elute later than their low molecular weight counterparts. In this mode $|V_g - V_{cr}| \sim R_g^{-2} \sim M^{-1}$, which means the higher the molecular weight of a given monodisperse polymer fraction, the closer its retention is to the critical volume V_{cr} for this specific polymer. An important feature of AGC is that the dependence of retention on polymer chemical structure and molecular weight is affected by the initial mobile phase composition or solubility limit (through ε_{inj} in U_{inj}) and gradient rate (through the ratio V_0 / V_{cr} in Q). Note also that the width of the polymer peak is affected mostly by its MWD rather than by the bandwidth of the individual compounds in this mode.

The situation dramatically changes with the increase of the parameter Q. Thus, if $\exp Q >> 1$ for all polymer fractions, then

$$V_g = V_{cr} + V_0 \tag{6}$$

What this means is all macromolecules of the polymer stay in adsorbed state until eluent composition Φ reaches the critical value Φ_{cr}, when $\varepsilon = \varepsilon_{cr}$. At this point the entire polymer leaves pores and flows with the liquid out of the column. This mode of gradient elution can be named “gradient chromatography at CPA." In this mode, the polymer retention does not depend on its molecular weight, gradient shape and steepness, flow rate, initial mobile phase composition, and solubility limit (as soon as Φ_{precip} and $\Phi_{sol} < \Phi_{cr}$), and is solely determined by the chemical structure of the polymer and mobile and stationary phases. In most cases $V_0 << V_{cr}$, and the entire polymer band elutes at the critical mobile phase composition $\Phi_g = \Phi_{cr}$ and hence has a narrow bandwidth. The last does not depend on the MWD of the polymer and is controlled exclusively by the mass transfer factors.

Finally, consider the intermediate situation, when Q ~ 1. Let the magnitude of Q exceed 1 for higher molecular weight fractions, and is less than 1 for lower ones. Then the high molecular weight macromolecules elute close to the critical volume V_{cr}, while their lower molecular weight counterparts have less retention and hence broaden the elution peak.

The shape and width of this peak depend mostly on the lower molecular weight portion of the polymer MWD. Thus, equation Q = 1 provides the border between the molecular weight dependent and molecular weight independent gradient elution, respectively.

As we have shown above, the retention behavior of any polymer at gradient elution is determined solely by the magnitude of the parameter Q, which can be presented as a production of three terms:

$$Q = \frac{2R_g^{\,2}}{Da} \times \frac{\left(\varepsilon_{cr} - \varepsilon_{inj}\right)/k_BT}{\Phi_{cr} - \Phi_{inj}} \times \frac{\Phi_{cr} - \Phi_{inj}}{V_{cr}/V_0} \approx \frac{2R_g^{\,2}}{Da} \times \frac{d\left(\varepsilon/k_BT\right)}{d\Phi} \times \frac{d\Phi}{d\left(V/V_0\right)} \tag{7}$$

The first term reflects the relation between the size of macromolecules and pores, the second one – the effect of eluent composition on the segment interaction energy, and the last one – the gradient rate.

It is evident from equation (7) that the decrease of internal diameter of individual pores, the increase of segment interaction energy in the presence of a strong solvent, and the increase of gradient steepness constitute the factors favorable to the transition to the gradient elution at CPA.

Recall[1] that there is a thermodynamic preference for a macromolecule in adsorbed state to penetrate most narrow pores and stay there until the segment interaction energy achieves its critical value due to varying eluent composition. This means that the diameter D in equation (7) represents the narrowest of the available pores, providing the volume of these pores is large enough to accommodate the entire polymer.

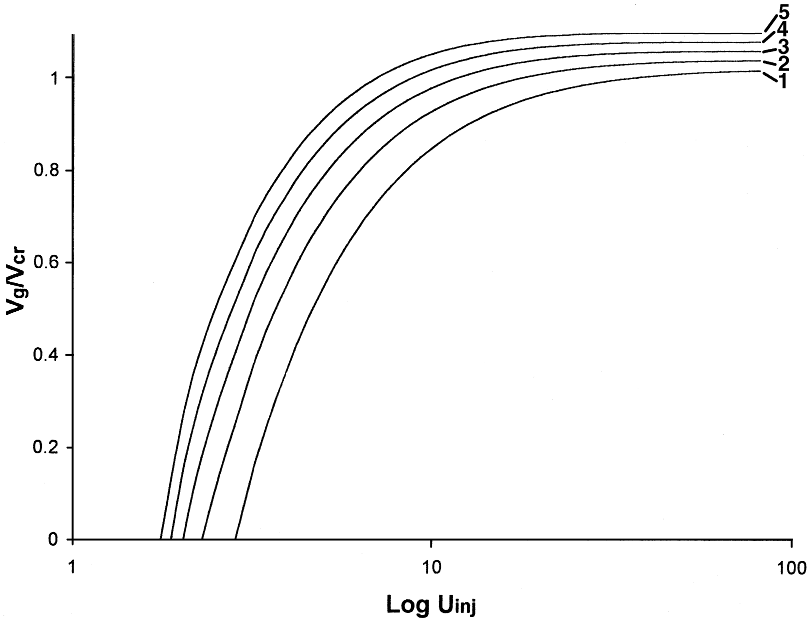

Figure 1. Theoretical dependence of the ratio V_g/V_{cr} on log U_{inj} calculated from equation (4) for the linear gradient slopes V_0/V_{cr}: 0.02 (1), 0.04 (2), 0.06 (3), 0.08 (4), 0.1 (5).

The gradient elution represents probably the simplest way to measure the adsorption-desorption threshold. Practically, a single chromatographic run is enough to obtain the critical composition Φ_{cr} for a given polymer. Thus, the calibration curve log M versus Φ_g obtained in gradient mode for a mixture of several low polydispersity samples with known molecular weights (narrow standards) of this polymer should have the vertical asymptote at high molecular weights. The position of this asymptote indicates the critical composition Φ_{cr}, which does not depend on the gradient rate or the initial eluent composition Φ_{inj}. This finding is demonstrated at Figure 1, where the ratio V_g/V_{cr} from equation (5) as a function of log U_{inj} (proportional to log M) is plotted for various gradient slopes V_{cr}/V_0. Note that the asymptote $1 + V_0/V_{cr}$ for each curve on this graph slightly depends on the gradient steepness.

The theory of gradient polymer elution developed in this paper extends the conventional chromatographic theory[9,10] to the case of IPC, viz., polymer chromatography accompanied by the common retention process: adsorption (or sorption) of isolated solute molecules on the surface of the column packing. Our approach uses molecular-statistical theory of flexible macromolecules in confined space with attractive walls (pore space) and constitutes a logistical extension of the formal (phenomenological) chromatographic theory in the case when polymer adsorption is mainly responsible for retention.

The opposite situation of precipitation-redissolution gradient elution has been considered by Armstrong and co-workers (see[11] for review). In this case the main role of the stationary phase is to preferentially adsorb the thermodynamically strong solvent B of the mobile phase (i.e., the good solvent). Saturation of the stationary phase with this solvent occurs rapidly and the concentration of B near the surface remains essentially constant as the composition of the mobile phase changes.

Once this state is reached, the separation is predominantly controlled by the mobile phase regulation and occurs through the multiple successive precipitation-dissolution steps. This assumption provided the basis for the derivation of the quantitative chromatographic theory[11] based on the Flory's theory of phase separation in dilute and semi-dilute polymer solutions.

The major conclusion from the theory[11] is the existence of so-called critical mobile phase composition $\Phi = \Phi_{precip}$ (here Φ is the concentration of solvent B as before). This composition can be considered roughly as the composition of a binary solvent system that will just dissolve an immobilized homopolymer or just precipitate a dissolved polymer in the presence of a stationary phase. Consequently, polymers are either infinitely retained or tend to elute rapidly, depending on whether the solvent composition is below or above the critical composition Φ_{precip}, respectively. Polymer adsorption does not play any role in retention in this case.

The fundamental difference between Φ_{precip} and Φ_{cr}, introduced in this paper for the polymer gradient elution controlled by the adsorption mechanism of retention, is that Φ_{precip} depends on molecular weight and concentration (i.e., injection volume) of the polymer.[11] This difference renders the precipitation-redissolution retention mechanism impractical for CCD separation of copolymers, and the condition

$$\Phi_{cr} > \Phi_{precip} \tag{8}$$

can be considered as a prerequisite to the molecular weight independent separation.

The dependence of retention on polymer concentration and injection volume makes the precipitation-redissolution mechanism questionable even for the gradient separation of homopolymers, however quantitative results on the gradient molecular weight separation of polystyrenes have been reported recently.[13]

Note that the increase of sample concentration can transform the regular sorption mechanism into the precipitation-redissolution retention[14] with adverse impact on separation. Further discussion on this subject, supported by both published and original experimental data, is to be published.[15]

Gradient Separation of Statistical Copolymers and Blends According to Chemical Composition and Microstructure

The gradient elution at CPA opens a new exciting opportunity for polymer chemists: the gradient separation of statistical copolymers and blends according to chemical composition and microstructure. For example, if two polymers A and B have distinct critical eluent compositions $\Phi_{A,cr}$ and $\Phi_{B,cr} > \Phi_{A,cr}$, respectively, and parameters Q_A and Q_B given by equation (7) significantly exceed unity for both polymers, the difference in retention volumes for these polymers is proportional to the corresponding difference in critical mobile phase compositions:

$$V_{B,g} - V_{A,g} = V_{B,cr} - V_{A,cr} = (\Phi_{B,cr} - \Phi_{A,cr}) / (d\Phi/dV) \qquad (9)$$

This separation is determined by segment interaction energies ε_A and ε_B, i.e., polymer structure, rather than by MWD.

The existence of a single CPA for a homogeneous ergodic statistical copolymer established in previous paper[1] implies that its retention in gradient mode is also described by equations (4) – (6), where the segment interaction energy ε should be replaced with corresponding effective energy ε_{eff}. If exp Q >> 1, the entire copolymer elutes practically at a single point (6). This point does not depend on molecular weight and is determined exclusively by the corresponding critical mobile phase composition Φ_{cr}, for which $\varepsilon_{eff} = \varepsilon_{cr}$. The peak width and shape also do not depend on MWD of the copolymer. Thus, the gradient elution at CPA does not provide any fractionation for the ergodic copolymer as soon as exp Q >> 1. On the other hand, the separation by both molecular weight and chemical composition takes place when $Q \le 1$. In this last case the molecular weight dependence can be further enhanced if the precipitation precedes the polymer adsorption.

The opposite situation occurs in the case of heterogeneous non-ergodic statistical copolymers. Each ergodic class of such copolymer has a specific retention volume corresponding to its critical eluent composition, which reflects the chemical composition and microstructure, but not molecular weight or MWD of the corresponding fraction. Thus, the gradient elution at CPA provides the separation of non-ergodic copolymer into individual ergodic fractions according to their chemical composition and microstructure.

In other words, the gradient elution of the non-ergodic copolymer at CPA allows one to obtain its CCD in the same manner as SEC provides the way to measure the MWD of homopolymers. If a single concentration detector is used, the set of compositionally "narrow" standards with known chemical compositions is necessary for quantitation. For example, free-radical copolymerization at low conversion of monomers usually produces an ergodic copolymer with narrow CCD. The chemical composition of this copolymer $\xi \approx \langle\xi\rangle = P(A)$ corresponds to a certain critical eluent composition $\Phi = \Phi_{cr}$ for a selected chromatographic system (recall[1] that P(A) is the probability of units A in the copolymer). Several such samples covering broad range of chemical compositions may serve as a set of "narrow" standards for constructing the calibration curve ξ vs. Φ, which does not depend on flow rate or gradient shape and steepness.

The areas of these peaks allow one to calculate the dependence of detector response on the copolymer composition. The non-ergodic statistical copolymer synthesized at high conversion of these monomers can be quantitated against the calibration curve to obtain the CCD. Obviously, the use of compositional detectors (IR, UV at two or more different wavelength, MS, etc.) enables one to measure CCD directly, i.e., without the calibration curve. Nevertheless, the detectors should be calibrated appropriately to take into account the difference in the sensitivity due to the change in mobile phase composition during the gradient run.

Note that there is no necessity of establishing the critical conditions at gradient elution. Thus, the independence of eluent composition at elution point Φ_g from the flow rate, gradient steepness, and initial mobile phase composition is an unambiguous verification of the separation mechanism.

It should be pointed out that the composition of polymer solution injected (Φ_{sample}) may not coincide with the initial eluent composition Φ_{inj} for gradient elution at CPA, as opposed to isocratic separation at critical conditions. It is obvious that Φ_{sample} should exceed the solubility threshold Φ_{precip}, while the injection volume should be kept as small as possible to avoid the polymer breakthrough with the initial band of the injected solution. Selecting Φ_{inj} less than Φ_{precip} can facilitate the problem of polymer separation from this band. More detailed discussion of this and other practical aspects of gradient elution at CPA emerging from the theory and the corresponding experimental data will be published soon.[15]

EXPERIMENTAL VERIFICATION OF THEORY

In Introduction section of the previous paper[1] we have mentioned already a well-known experimental observation that gradient separation of statistical copolymers becomes molecular weight independent with a decrease in a pore diameter and/or an increase in a polymer molecular weight. It occurs when the retention is governed by the adsorption-desorption mechanism, rather than the precipitation-dissolution one. According to the theory developed in the previous section, this fact indicates the transition from the AGC to the gradient elution at CPA.

The combination of isocratic and gradient elution for the same chromatographic system and polymers would be a more convincing proof of the theory. In series of publications Mori et al.[16-21] carried out both isocratic and gradient separations of several statistical copolymers with different chemical composition on silica with the intent of elucidating the mechanism of separation. Very narrow pores (30 Å) provided conditions when macromolecules were completely excluded from the pore volume without sufficient adsorption interactions with free silanol groups on the surface of silica gel.

It has been shown[17] that styrene-methyl methacrylate copolymers of a given composition elute from the column with the chloroform (or 1,2-dichloroethane) /ethanol mobile phase above a certain amount of ethanol and are retained practically infinitely below that critical value. This critical content of ethanol in the mobile phase depends on temperature and the copolymer composition. In other words, a critical content of ethanol in the mobile phase exists for a copolymer of a certain composition at the specified temperature, and a critical composition of the copolymer also exists for the mobile phase of a certain composition.[17] The peak retention volume at isocratic elution was unchanged with column temperature and coincided with the interstitial volume V_0 for copolymers of every composition as soon as ethanol content exceeded the corresponding critical value.[17] At the same conditions, the linear gradient allowed separating copolymers according to chemical composition exclusively, i.e., independently of molecular weight.[17]

These experimental observations, reported also for other statistical copolymers of acrylates and methacrylates (methyl-, ethyl- and n-butyl-) with styrene,[18-20] are consistent with the theory developed in this article. The only question remains is the observed value (0.5 or 0.6 mL)[17,19] of retention volume in isocratic elution, which was reported as the interstitial volume V_0 of the system. Note that for a very short column used for the separations[16-20] (50 x 4.6 mm I.D.), with the total internal volume just 0.83 mL, the pore volume V_P can not exceed 0.2 – 0.3 mL, which corresponds approximately to the width of the polymer peaks presented in the papers.[17-20] For this reason it was probably difficult to detect any differences in retention volume from V_0 to $V_T = V_0 + V_P$, which could occur with the increase of amount of ethanol in the eluent.

In a more recent publication[21] Mori and Naito used a larger column (250 × 4.6 mm I.D., ODS silica) with V_0 = 2.0 mL and V_T = 3.0 mL to separate styrene-acrylonitrile statistical copolymers with mixtures of chloroform and n-hexane as mobile phases. In isocratic mode the retention volume for all copolymers was 2.0 mL (interstitial volume) with up to a certain amount of poor solvent (n-hexane) in the mobile phase, which depended on the copolymer composition. On further increasing the n-hexane content, the second peak at 3.0 mL (total volume) appeared and partially replaced the first one at V_0. Eventually, the second peak (at 3.0 mL) replaced the first one completely at a certain amount of poor solvent, and this amount again depended on the copolymer composition. On further increase of n-hexane, second peak also disappeared for some of copolymers, when they precipitated on the column. Separation according to the copolymer composition was possible by gradient elution from n-hexane to chloroform. No molecular weight dependence was observed.[21]

Note that the copolymer was dissolved in pure chloroform as sample solution in all instances, including isocratic separations.[21] Taking into account a relatively high injection volume (0.1 mL), the real composition of the solution surrounding the macromolecules could differ significantly from the mobile phase composition. In this situation the peak splitting between interstitial and total volumes of the system is typical for isocratic separation of homopolymers near CPA.[3-5,22] This phenomenon can be explained as a manifestation of "macroscopic" fluctuations (between equilibrium and metastable states) which accompany the first order phase transition in a thermodynamic system.[1] In the case of isocratic elution of large macromolecules in narrow pores near CPA these two states of the chromatographic system are represented by elution at interstitial volume and total volume, respectively. Thus, the peak splitting in isocratic elution of statistical copolymers, when the sample solution composition does not coincide with the eluent composition, could be additional evidence in favor of the separation at CPA.

Unfortunately, the separations in the paper[21] were accompanied by precipitation phenomena. Thus, Mori and Naito associated the second peak with dramatic change of the macromolecule size during initial stage of precipitation process, which enabled the macromolecules to penetrate even small pores. Accordingly, they described the transition region of eluent compositions where two polymer peaks (at V_0 and V_T) coexisted in the isocratic elution as a pre-precipitation state of the polymer solution. However valid this explanation may be, it is obvious that the proximity of the cloud point significantly complicates the issue and the experimental verification of the theory based on the adsorption–desorption equilibrium has to be carried out well outside the precipitation limit.

We performed the chromatographic experiments on the set of chlorinated polyethylenes listed in Table 1(see Experimental section). Chlorination of polyethylene is a substitution reaction proceeded by a free-radical mechanism.[23] The products of this reaction, chlorinated polyethylenes, can be considered as statistical copolymers comprised of three types of monomer units: CH_2-, CHCl- and CCl_2-groups. n When performed in homogeneous conditions (in solution), the chlorination usually does not accompany chain degradation, dehydrochlorination, crosslinking, or any other undesirable side reactions.[24]

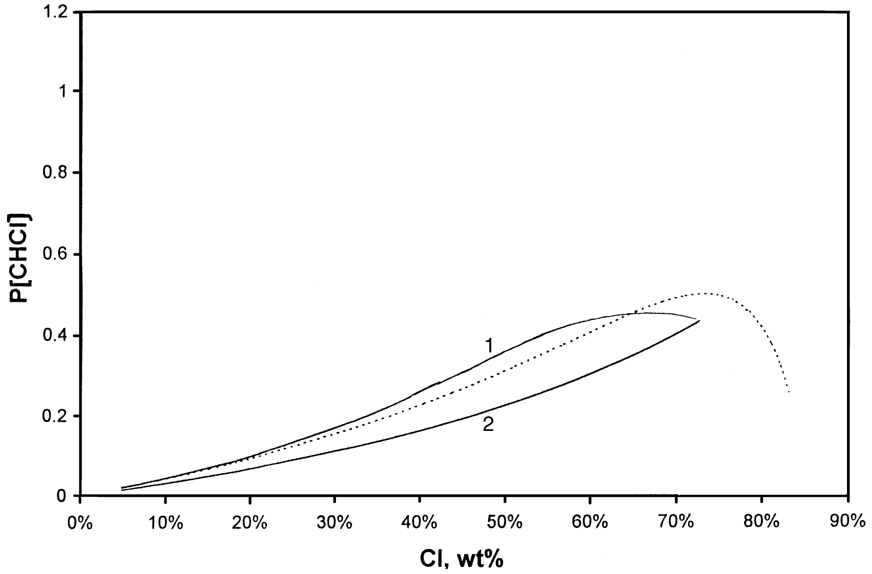

Figure 2. Probability of CHCl-groups in chlorinated polyethylenes as a function of chlorine content calculated for the products of: homogeneous chlorination in chlorobenzene (1), partly heterogeneous chlorination in carbon tetrachloride (2), and for the random model of statistical copolymer comprised of three types of units, i.e.,CH_2-, CHCl- and CCl_2-groups (dashed curve).

This means that only the types of monomer units are changing during chlorination, i.e., CH_2-groups turn into CHCl-groups, or these latter – into CCl_2-groups, while the total number of them (*L*) in each individual macromolecule stay unchanged.

The kinetic of this reaction and the microstructure of its products are well established,[24-30] and the chlorinated polyethylene with chlorine content 20 wt % or higher are soluble in chloroform (and in some chloroform-hexane mixtures) at room temperature. The CCD of chlorinated polyethylenes is the distribution of macromolecule with the chlorine amount. The chlorine content heterogeneity of chlorinated polyethylenes described by the CCD is the major factor affecting their properties.[24,29] These combined features make chlorinated polyethylenes amenable to the chromatography study.

Due to the negative induction effect of the chlorine atom incorporated into polymer chain on the activity of neighboring CH-groups, the reaction slows down with an increase of chlorine amount in the polymer. Thus, the CH_2-group next to CCl_2-group is practically non-active, and the relative activities of central

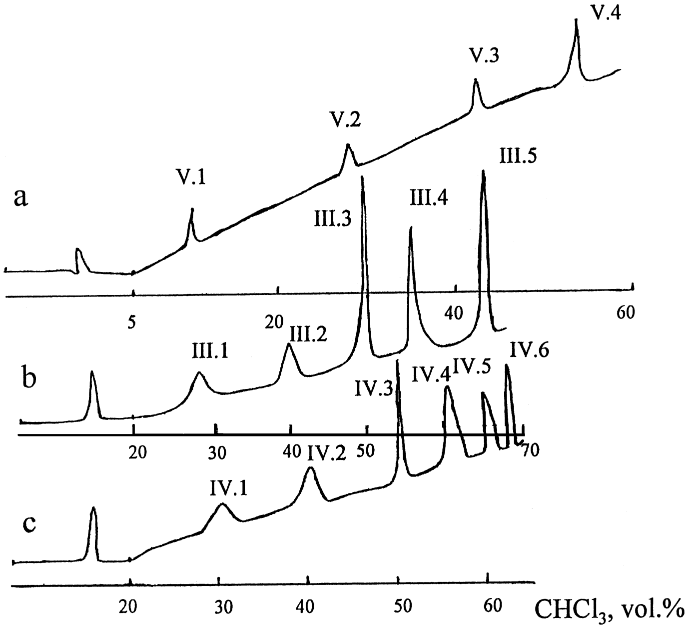

Figure 3. Gradient separations of chlorinated polyethylenes from Table 1: V (a), III (b) and IV (c), in n-hexane/chloroform 0.8 vol.% of $CHCl_3$ per minute linear gradient at flow rate 0.7 mL/min, injection volume 0.1 mL.

groups in the triads $-CH_2-CH_2-CH_2-$, $-CHCl-CH_2-CH_2-$, $-CHCl-CH_2-CHC-$ are related as 1 : 0.35 : 0.08.[24] The CCl_2-group can be generated only from the CHCl-group surrounded by two CH_2-groups, i.e., inside the triad $-CH_2-CHCl-CH_2-$, and the corresponding kinetic constant is three times less than the reactivity of the CH_2-group inside the triad $-CHCl-CH_2-CHCl-$.[25]

This homogeneous kinetics describes the chlorination of polyethylene in chlorobenzene in a batch reactor (samples V in Table 1). The mathematical model has been developed,[24,29] which allows one to calculate the relative amounts (probabilities) of different groups in chlorinated polyethylenes as functions of average chlorine content, based on foregoing kinetic parameters (Figure 2, curve 1). The distribution of these groups is close to a random one with a slight tendency to alternating.[24] In this case of homogeneous chlorination the polymer is ergodic (chemical correlation segment[1] $n^* \sim 1$) and its CCD is very narrow (especially for high molecular weight polymers) and is described by the normal law (see equation (12) in previous paper[1]). The gradient separation of these copolymers with different amount of chlorine is presented at Figure 3a.

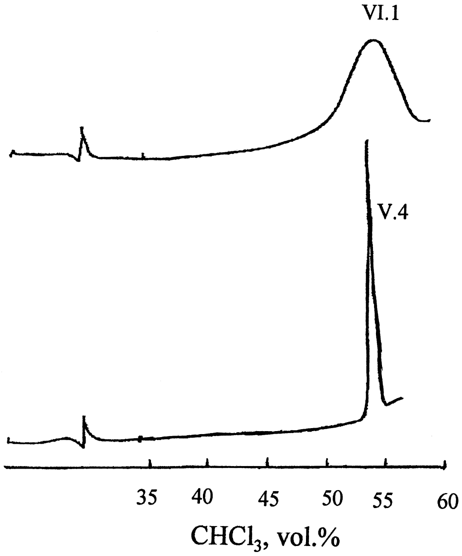

Figure 4. Effect of chlorination conditions (continuous vs. batch reactors) on peak widths of two gradient chromatograms, obtained at the same chromatographic conditions as of Figure 3. Chlorinated polyethylenes VI.1 and V.4 have similar average chlorine content but diverge considerably in the width of the chemical composition distributions.

By contrast, the chlorinated polyethylene VI.1 (Table 1) is substantially non-ergodic and is characterized by very broad CCD, even though the chlorination has been carried out in homogeneous conditions (chlorobenzene at 130°C). The reason for that is the second stage of the reaction performed in the continuous (flow) reactor, when the chlorine content in the copolymer has been changed from 21.0 to 37.6 wt %. During this stage various macromolecules were exposed to chlorine for different time intervals due to broad residence time distribution in the reaction mixture. The difference in peak width of two chromatograms depicted in Figure 4 demonstrates the significant difference in chlorine content heterogeneity (CCD) for the products of batch and continuous processes, respectively.

The compositionally heterogeneous non-ergodic chlorinated polyethylenes are usually generated by solid phase chlorination, e.g., by suspension chlorination.[30] The products of this reaction have some crystallinity even at high content of chlorine. This reflects the blockiness of the chlorine distribution, which leads to the presence of unchlorinated segments sufficiently long to crystallize, and of high amount of CCl_2-groups.[27-30] An intermediate situation happens when chlorination is carried out in relatively poor solvents such as tetrachloroethane[30] or carbon tetrachloride.[29]

For example, in the latter case, the initial polyethylene is not soluble in CCl_4 at 77°C. Because of this, early in the chlorination the reaction proceeds in a heterogeneous matter, and becomes homogeneous only after introducing ca. 5 wt % chlorine. The resulting statistical copolymers have more blocky microstructure, broader CCD (up to ca. 40 wt % chlorine), less fraction of CHCl-groups, and higher portion of CCl_2-groups, than corresponding products of a completely homogeneous reaction (see Fig.2, curve 2, which was calculated based on the mathematical model developed previously).[24,29]

The corresponding difference in CCDs between chlorinated polyethylenes obtained in homogenous and heterogeneous conditions respectively is clearly seen at the gradient chromatograms presented in Figure 3. The products of chlorination in CCl_4 have broader peaks at low chlorine content. Due to the reaction slow down with an increase of the chlorine content, the compositional heterogeneity decreases and achieves almost the level of ergodic copolymers at 40 – 42 wt % chlorine. On further chlorination, the solubility of chlorinated polyethylenes in CCl_4 decreases, which may cause some heterogeneity of chlorination with broadening the CCD of associated products (Figure 3b,c).

Figure 3a-c represent the examples of gradient elution of chlorinated polyethylenes with linear gradient of n-hexane/chloroform. We performed gradient separation of each set of chlorinated polyethylenes (from I to V, Table 1) under various gradient conditions. In particular, the gradient rate was varied from 0.3 to 1 vol.% of $CHCL_3$ per minute in different runs at flow rate 0.7 mL/min. It turned out that for all polymers eluent composition at peak elution point Φ_g did not depend on the gradient rate beginning with 0.8 vol. % of $CHCl_3$ per minute. At these conditions the elution did not depend, also, on polymer molecular weight for chlorinated polymers with the same amount of chlorine (Figure 5a). It was shown also that all polymers were completely soluble in mobile phases of related compositions Φ_g. This eliminates the possibility that the precipitation-redissolution mechanism affects the elution volume V_g.

As can be seen from Figure 5a, the chlorinated polyethylenes obtained in chlorobenzene demonstrated slightly higher retention comparisons with the corresponding products of chlorination in CCl_4. To account for this observation, we suggested that the retention of chlorinated polyethylenes on silica gel occurs due to the adsorption interaction between silanol groups and polarized CHCl-groups on polymer chains. Accordingly, the retention should depend on the probability of these groups rather than on the total amount of chlorine in a polymer chain.

To prove this assertion, we used the calculated curves in Figure 2 to present the experimental data for Φ_g as a function of the concentration of CHCl-groups (Figure 5b). One can see a one-to-one correspondence between Φ_g and the concentration of CHCl-groups for all polymers in full conformity with the proposed mechanism of adsorption.

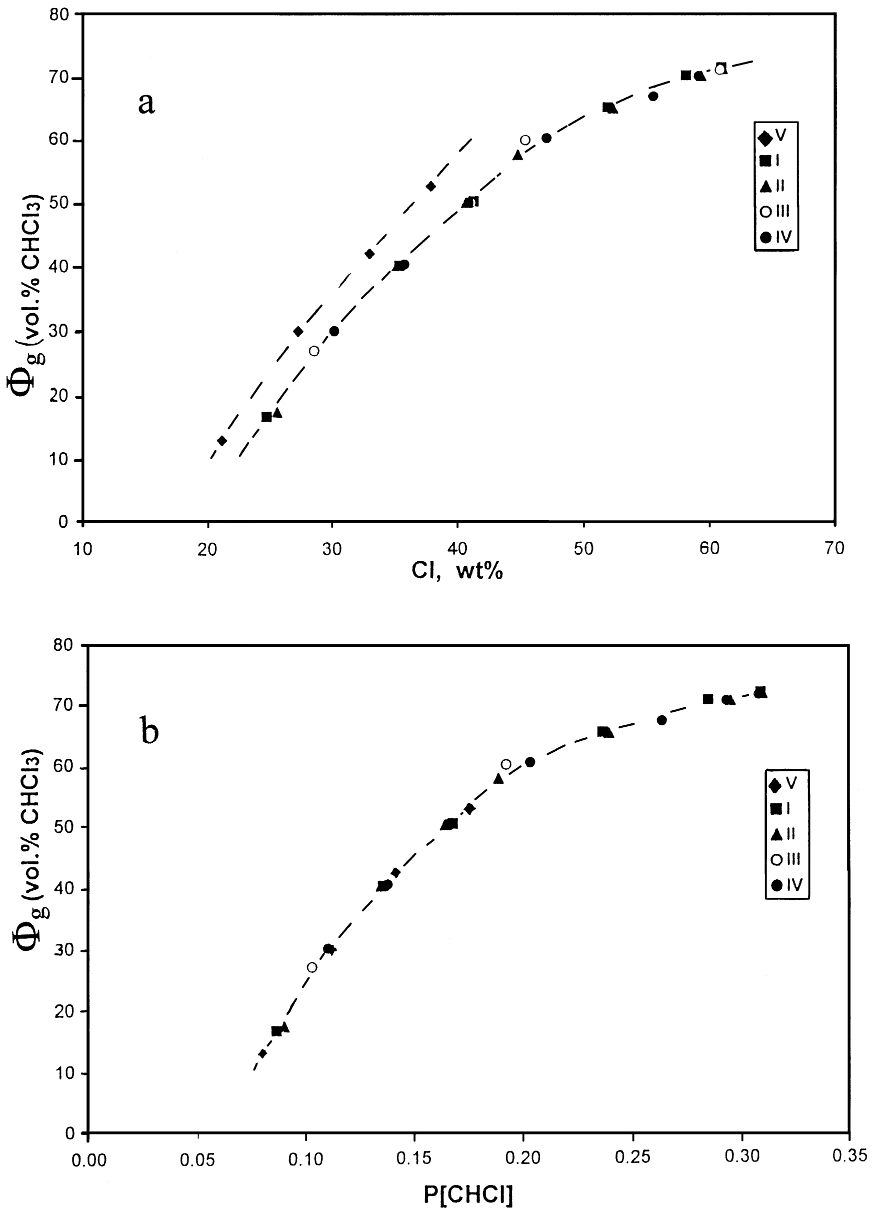

Figure 5. Chloroform content Φ_g in the mobile phase for gradient elution of chlorinated polyethylenes from Table 1 plotted as functions of the average chlorine content (a) or the concentration of CHCl-groups (b) in the polymers. Gradient conditions are the same as at Figure 3.

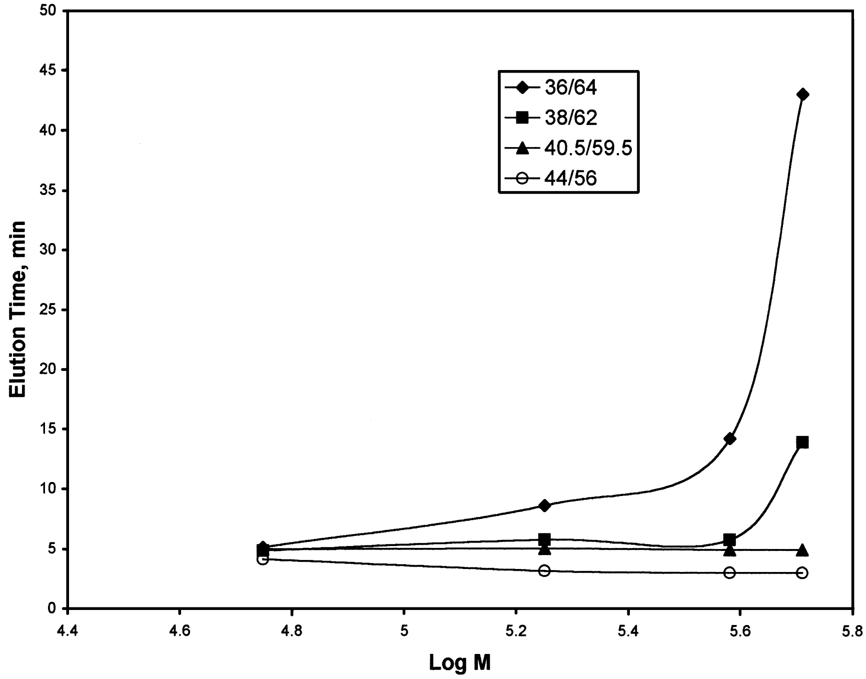

Figure 6. Critical diagram for isocratic elution of four chlorinated polyethylenes: I.2, II.2, III.2 and IV.2 with chlorine content 35.3, 35.6, 35.6 and 35.7 wt%, respectively. All these polymers elute at critical eluent composition Φ_g = 40.5 vol.% of $CHCl_3$ in gradient mode, which corresponds to the critical point of adsorption.

The foregoing results of gradient studies on chlorinated polyethylenes validate the gradient separation at CPA according to the theory developed in this paper. To prove that the chlorinated polyethylenes do have a critical point of adsorption at the mobile phase composition Φ_g corresponding to the elution of the same polymer in the gradient mode, we analyzed the isocratic elution of several chlorinated polyethylenes with closely related chemical compositions but different molecular weights. The critical diagram at Figure 6 unambiguously demonstrates the existence of CPA (40.5 vol % of $CHCl_3$) for chlorinated polyethylenes with ca. 35 wt % of chlorine (or 21 mol % CHCl-groups): higher molecular weight copolymers have stronger retention for eluent compositions with less than 40.5 % of chloroform (the adsorption mode), while the size-exclusion order of elution is observed for the chloroform content higher than 40.5 vol %. The retention volume at 40.5 % of $CHCl_3$ corresponds to the total volume of the system V_T = 3.48 mL for polymers with broad range of molecular weights.

Critical diagrams are always presented in literature as sets of log M versus retention volume V graphs, corresponding to different eluent compositions at isocratic elution. These plots are called relative calibration curves in SEC, because they depend on chemical structure of polymer chains.

More general presentation can be achieved by plotting a universal calibration curve: log H versus V, where H is the hydrodynamic volume of macromolecules. In ideal SEC this curve has universal nature, i.e., it does not depend on polymer, because the separation occurs according to the hydrodynamic volume and not to the molecular weight of a polymer chain. The same way as the dependence log H vs. V is universal for the ideal SEC, any deviation from this curve caused by non-steric interactions with the stationary phase also have universal nature in the sense that the deviation is determined only by the magnitude of the energy ΔE of these interactions.[31] Thus, the critical diagram consisted of the set of log H vs. V curves for different eluent compositions can be considered as a universal critical diagram in IPC, which is independent on polymer, stationary, and mobile phases.

We showed recently[31] that the hydrodynamic volume H for each fraction of the polymer subjected to a chromatographic separation can be measured on-line using signals from three coupled detectors: refractometer, capillary viscometer, and static light scattering photometer. In this way, the entire calibration curves can be obtained in a single chromatographic run as soon as the polymer has broad size distribution.[31] Thus, this triple detector combination provides a simple way to generate the universal critical diagram in IPC.

CONCLUSIONS

The interactive chromatography of polymers is an important technique, complementary to SEC, which makes available information about the chemical structure and composition distribution of complex polymers. The existence of the critical point of adsorption for statistical copolymers established in this work, furnishes a new mechanism of gradient separation of statistical copolymers: gradient elution at CPA.

We have shown that only this mode of elution is associated with a completely molecular weight independent separation. The theory of gradient elution of polymers developed above allows one to determine the necessary conditions for this mode.

Although many particular conclusions from the theory are still needed to be verified experimentally, we hope that the approach developed in this article can provide fresh insight into the gradient chromatography of polymers. One important field of application is the liquid chromatography separation of biopolymers, including reverse phase separation of proteins. Notwithstanding the fact that the real mechanism of retention of biological polymers can be much more complex than the one considered in this article (as much as the chemical structure of proteins is more complex than the structure of synthetic statistical copolymers), the gradient separation of proteins and other biological polymers at CPA might be not too far from the reality.

For example, we have analyzed the published data[32] on the isocratic and linear gradient separations of eight commercial proteins with molecular weights from 12,400 (cytochrome *c*) to 67,000 Dalton (bovine serum albumin) on the silica-based column material modified by butyl and phenyl groups. The recovery of all proteins was almost quantitative when 0.1 *M* phosphate buffer (pH 6.0) was used with varying ammonium sulphate concentration (from 2 *M* to 0).[32] We correlated capacity factors for isocratic elution at various salt concentrations with appropriate gradient compositions Φ_g. It turned out that four proteins with highest molecular weights had almost identical isocratic capacity factors (close to V_P/V_0) at corresponding ammonium sulphate concentrations Φ_g. What this means is gradient elution of these proteins occurs at their respective critical points of adsorption, i.e., $\Phi_g = \Phi_{cr}$. This example provides additional grounds to assume that gradient elution at CPA is an effective way of separation of complex polymers with various types of molecular heterogeneity, including biopolymers.

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