Journal of Chromatography A, 656 (1993) 591–615
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 005

Review

Chemical composition separation of synthetic polymers by reversed-phase liquid chromatography

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ABSTRACT

Synthetic polymers present many unique separations challenges because, unlike small organic molecules, they consist of a distribution of structurally different chains. Each macromolecule can differ in chain length and end groups; stereochemical and structural isomers are possible along with unique branching architectures. Copolymers are further complicated by the combination of these potential variables with varying ratios and sequence distributions of their comonomer units. Liquid chromatography of individual synthetic polymers is limited by the solution properties of macromolecules to a narrow selection of solvents. In particular the thermodynamic considerations of polymer dissolution are strongly influenced by entropic factors. The application of reversed-phase liquid chromatography to the separation of synthetic polymers is characterized by the delicate balance between strong solvation interactions and weak adsorption interactions and is often further complicated by polymer solubility limitations in gradient elution separations.

CONTENTS

1. INTRODUCTION

The scope of this review article is intended to provide a synopsis of the current understanding of the application of reversed-phase high-performance liquid chromatography (RPLC) to the chemical composition separation of synthetic polymers. In that sense no attempt is made to be comprehensive in the review of recent work; instead only specific experimental investigations will be used to highlight polymer RPLC separations and their mechanisms. In a few cases normal-phase HPLC experimental results are cited to emphasize certain synthetic polymer retention behaviors. The reader is referred to recent articles and monographs for either more generalized or in-depth discussions [1–8]. The various aspects of polymer composition, solubility behavior, and chromatographic retention represent a diverse and highly complex area of investigation. In order to present an overview of this field in this brief review, a number of simplified theoretical and empirical relationships will be discussed which provide a basis for the understanding of experimentally observed phenomena. These descriptions are in no way intended to be comprehensive or definitive and the reader is referred to more-in-depth treatises in each subject area.

Prior to the discussion of the potential of RPLC for characterization of synthetic polymers and copolymers and the mechanisms by which these separations may be accomplished, it is important to describe the variability of structure and composition possible in synthetic polymers. The length, configuration, sequence, branching, etc., which are possible in synthetic polymer structures affect the physical properties for potential application and provide means of distinction that may be made amenable to chromatographic separation. By definition liquid chromatographic separation relies on the solution properties of the analytes. It is therefore crucial to consider the solution properties of polymers which differ considerably from those of small molecule solutes and which can provide both help and hindrance to RPLC separation processes.

The number of individual chemical species present in a polymer sample is so large as to make complete resolution a practical impossibility. Low-molecular-mass oligomers may be resolved into chromatograms of individual chemical species, sometimes up to several dozen repeat units. Oligomers, however, represent relatively simple mixtures of components when compared to polymers with molecular masses of several hundred thousands. The most that can be obtained for these materials is a depiction of the distribution profile of a large number of overlapping bands [9]. Careful separation control can in some cases result in a chromatogram representing the distribution in one type of polymer property, *e.g.*, chain length, composition, or end group type. The fact that several types of molecular distributions may jointly describe a single synthetic polymer sample often makes achieving this goal extremely difficult. Thus, in many cases multiple sequential separations by differing separation mechanisms, cross-fractionation, is required to elucidate synthetic polymer molecular distributions [1,10].

Although the mechanism of retention attributed to RPLC with bonded phase packings is the partitioning of solutes by intercalation between the solvated bonded moieties [11], this model is not likely to apply to high-molecular-mass polymers. The size of polymer molecules in solution is not only significantly larger than the solvent and bonded moieties, but also can exceed the size of the porous structure of the column packing material itself. The overlapping size regimes of solvated polymer molecules and porous column packing structure is evidenced by the success of size-exclusion chromatography (SEC). Taking into account the effect of molecular size provides a more appropriate picture of adsorption as the primary process of retention in RPLC. This is often augmented or conflicted with by the solubility properties of the polymer molecules.

2. SYNTHETIC POLYMER STRUCTURES

As will be observed repeatedly in the following discussion, the single most significant aspect of polymer structure contributing to chemical and physical properties is the presence of a large number of covalently linked repeat units. Unlike small organic molecules whose chemical properties are determined by their atomic arrangement and which can be readily isolated as pure compounds, synthetic polymers consist of a distribution of structurally different chains. Each macromolecule is built up of repeat units of a relatively simple chemical structure or monomer unit. The number of monomer units forming a polymer chain is referred to as the degree of polymerization, *P*. If all repeat units are the same, the material is referred to as a homopolymer. The molecular mass of each chain is the degree of polymerization times the molecular mass of the monomer unit plus that of the chain end groups. The variety of chains of different length in a polymer sample is described by the dispersity: the ratio of weight-average molecular mass (M_w) to number-average molecular mass (M_n).

Each polymer chain (excepting cyclics and branched structures) must also contain two terminal groups of different chemical structure from the backbone monomer unit. These terminal groups result either from intentional chemical synthesis, combination, disproportionation, chain transfer termination, or may be present as residuals from polymerization initiators or degradation products. The contribution of most chain terminal groups to polymer chemical properties can often be ignored due to their relatively low molar concentration in polymers consisting of several hundred or more repeat units. However this is not strictly true for low degrees of polymerization and for chains possessing terminal groups differing significantly in chemical polarity from the main chain. In addition to end group effects, at low degrees of polymerization the difference between polymer homologues differing by one repeat monomer unit can still produce differences in chemical behavior and physical properties. These low-molecular-mass polymers are often referred to as oligomers and can provide especially useful polymer properties (*e.g.*, surfactants [12]).

The configurational structure of a polymer can also affect chemical and physical properties and can vary with synthetic conditions. As a result of the reactions of polymer backbone bond formation during some types of polymerization (*e.g.*, free-radical vinyl addition polymerization), the stereochemical orientation of the monomer units can produce polymer isomeric structures [13]. The stereochemical orientation of unsymmetrical atoms of the backbone monomer units can result in a regular (isotactic), alternating (syndiotactic), or random (atactic) arrangement of substituents. Conditional isomers are also common when *cis* or *trans* configurations may result during polymerization providing 1,2 *versus* 1,4 addition, as in polydienes.

In many polymer synthetic reactions chain-transfer reactions chain-transfer reactions can lead to the formation of branched chains [13]. Intermolecular chain transfer can produce long branches, the incidence of which increases with degree of conversion. Short-chain branching can occur through intramolecular chain transfer. Intentional addition of multifunctional monomers can be used to provide controlled levels of branching. In the extreme, branched structures may be intentionally synthesized in a starburst configuration with many arms emanating from a central core. Whether produced by intentional synthesis or random reaction, polymer branching has the effect of decreasing the molecular volume of the polymer chains relative to equal molecular mass linear polymers and of increasing the mole fraction of end groups.

Due in part to the random statistics governing many polymer synthetic reactions, it is also possible for chains to be formed in which the head and tail of the same chain have reacted to produce a cyclic species [13]. These are often of low concentration and of relatively low degrees of polymerization, but can be ubiquitous in some types of polymer synthesis.

3. SYNTHETIC POLYMER COMPOSITION

Having discussed the variables in structure and configuration of synthetic polymer backbone structures, the next consideration is polymers formed from more than one type of monomer unit, *i.e.*, copolymers. In addition to the structural variables, chemical heterogeneity in the form of a distribution in chemical composition both between chains and along a single polymer chain is significant to copolymer properties. For example, random or statistical copolymers synthesized in homogeneous solution can be described by their sequence distribution, instantaneous heterogeneity, and conversion heterogeneity. For the current discussion this type of copolymer is chosen as an example for which simplified mathematical models exist to describe composition heterogeneity. It is emphasized that actual copolymers of this type may be more compositionally complex and that copolymers formed by other synthetic reactions possess their own compositional variation [13].

Sequence distribution for random copolymers is expressed in terms of the monomer reactivity ratios, r_A and r_B, or copolymerization propagation probabilities, p_{AA} and p_{BB}. The following equations apply when the simplistic model for random copolymerization is assumed with a uniform mechanism of irreversible addition in which only the last unit of the growing copolymer chain affects the probability of addition of the next monomer unit.

$$L_A = \frac{1}{1 - p_{AA}} = 1 + r_A \frac{[A]}{[B]} \tag{1}$$

$$L_B = \frac{1}{1 - p_{BB}} = 1 + r_B \frac{[B]}{[A]} \tag{2}$$

The mole ratio of the monomer reaction mixture, [A]/[B], is thus related to the number average length of uninterrupted sequences, L_A and L_B, of monomers A and B along a polymer chain, respectively [14]. The segregation of monomer units is sometimes referred to as the blockiness of the copolymer. In the extreme, copolymers may be prepared with block structures of complete segregation of monomer units on either end of the polymer molecule, so-called A–B diblock copolymers. Other block structures are also possible with sequential preparation of monomer blocks or by controlled monomer addition to the reaction mixture [15].

The instantaneous heterogeneity at any time during polymerization of a copolymer produces a distribution in average chemical composition of chains related to the random statistics of the reaction. A differential weight distribution, $H(P, y)$, of molecules with degree of polymerization P and composition deviation y with variance s^2 was described by Stockmayer [16] for copolymers of very low conversion. The term conversion refers to the percentage reaction of monomers to polymer.

$$H(P, y) = \left[\frac{P}{P_n^2} \cdot \exp\left(-\frac{P}{P_n}\right)\right] \cdot \left[\frac{1}{s(2\pi)^{1/2}} \cdot \exp\left(-\frac{y^2}{2s^2}\right)\right] \tag{3}$$

$$s^2 = \frac{x(1-x)\kappa}{P} \tag{4}$$

$$\kappa = [1 - 4x(1-x)(r_A r_B - 1)]^{1/2} \tag{5}$$

The number-average degree of polymerization is P_n and the mole fraction composition of an individual copolymer chain is x. This mathematical description uses a highly simplified model of a free-radical polymerization with termination by disproportionation and equal molecular mass monomers. An important result in this model, in agreement with experimental observation, is that the composition variance is predicted to be inversely proportional to the degree of polymerization. This implies that the instantaneous compositional heterogeneity will be high in low-molecular-mass fractions and decrease with higher molecular mass.

In the case of copolymers where the monomer reactivity ratios are both equal to unity or in so-called azeotropic copolymers, the composition of the copolymer will be equal to the monomer ratio in the reaction mixture. As stated above, the instantaneous composition usually differs from the reaction mixture monomer ratio resulting in variation of the reaction mixture composition during the course of the polymerization. As one of the monomers is preferentially depleted, this process produces compositional heterogeneity increasing with percentage conversion.

Lastly, copolymer composition can be combined with chain structure to produce both compositional and structural heterogeneity. One example is the preparation of graft copolymers in which side chains of one composition are pendent from a backbone polymer of a different composition [15]. The synthesis of graft copolymers often leads to a heterogeneous mixture containing backbone polymer, sidechain polymer, and a distribution in composition of the desired graft copolymer.

4. POLYMER SOLUTION BEHAVIOR

During a conventional RPLC separation the polymer molecules are dissolved in the eluent in order to be transported through the column and eluted. However, several important aspects of polymer solution behavior differ from those of small molecules and thereby affect their RPLC separation behavior. Foremost among these properties are the limited solubility of polymers, the size of solvated macromolecules and their associated diminished diffusion coefficients, and the effect of solvated polymer conformation on adsorption interactions [1,2,17].

Dissolution of a solute is associated with a negative free energy change of mixing.

$$\Delta G_{\mathrm{mix}} = \Delta H_{\mathrm{mix}} - T\Delta S_{\mathrm{mix}} < 0 \qquad (6)$$

In simple terms, the enthalpic interactions can be considered as associated with specific chemical interactions between the molecules of solvent and polymer. Due to the relative size of polymer and solvent molecules, these are dominated by the interaction of solvent molecules with individual monomer units along the polymer chain. Entropic contributions result from the change in order of the system on going from separate solvent and polymer to homogeneous solution. It is primarily these entropic contributions that cause polymer solubility behavior to differ significantly from that of small molecules [18]. Due to the high molecular mass of polymers, equal mass percent solutions of polymeric solutes contain drastically fewer solute particles per unit volume than low-molecular-mass solute solutions. In addition, when a polymer molecule is dissolved, the linkage of monomer units in a polymer molecule results in a maintenance of the ordered configuration of attachment in the polymer chain. In fact the long range order of the polymer chain may actually result in an increase in order for the solvent molecules that become associated with the dissolved polymer. The impact of these conditions is to produce a more positive entropy ($-T\,\Delta S_{\mathrm{mix}}$) contribution to polymer dissolution relative to that common for low-molecular-mass compounds. Thus in order to satisfy the requirement of a $\Delta G_{\mathrm{mix}} < 0$, a much more negative enthalpy of mixing ($\Delta H_{\mathrm{mix}} < 0$) is required for most polymer solutions, resulting in the experimentally observed limited number of solvents for a given polymer [19].

Equations for the entropy of ideal mixing employ the assumption that the solute and solvent are particles of similar size. This assumption obviously fails for polymer solutions and an alternative formulation is given by the Flory–Huggins theory in the limit of large difference in size between solvent and solute [13,18]. The change in chemical potential of the solvent on mixing in a polymer solution, $\Delta\mu_{\mathrm{solv}}$, is described as

$$\Delta\mu_{\mathrm{solv}} = RT\left[\ln(1-\varphi_{\mathrm{poly}}) + \left(1 - \frac{\bar{V}_{\mathrm{solv}}}{\bar{V}_{n,\mathrm{poly}}}\right)\varphi_{\mathrm{poly}} + \chi\varphi_{\mathrm{poly}}^{2} + \cdots\right] \qquad (7)$$

where φ_{poly} is the volume fraction of polymer, $\bar{V}_{\mathrm{solv}}/\bar{V}_{n,\mathrm{poly}}$ is the partial molal volume ratio of the solvent to the number-average value for the polymer, and χ is the Flory–Huggins interaction parameter. Additional terms in the polynomial expansion in φ_{poly} are usually considered negligible. The first two terms of this equation account for configurational entropy of mixing. The χ parameters term accounts for the energy resulting from the changes in relative intermolecular contact areas and the change in randomness of each polymer segment relative to the preceding segment. For a given polymer, the χ parameter varies with solvent and temperature. Since the partial molal volume ratio of solvent to polymer decreases with increasing degree of polymerization (P), this equation agrees with the experimentally observed general decrease in solubility with increasing polymer chain length.

The second-order dependence of eqn. 7 on φ_{poly} implies that, for high values of χ, conditions exist for which two values of polymer volume fraction possess equal solvent chemical potential. The solution therefore separates into two phases; a polymer-rich gel phase and a solvent-rich solution phase [2,17,18]. As the quality of the solvent is increased (*e.g.*, by change in T), the value of χ decreases through a critical value below which a single homogeneous solution is formed. For a given polymer–solvent system this critical value is referred to as the θ point or θ temperature. At the θ point the polymer molecules are said to be at their unperturbed dimensions in solution. As the quality of the solvent is further increased, the polymer chains increase in dimension with increasing solvent–polymer interactions. Chain dimensions can of course be further affected by rigid segments and charged groups which cause the polymer molecule to be more fully extended toward the limiting model of a rigid rod.

Uncharged flexible polymer molecules in solution are most simply described as random coils with associated solvent molecules. The unperturbed dimensions referred to above correspond to a minimum size associated with the molecular dimensions of the monomer units and their rotational freedom of movement within the restrictions of the polymer backbone [13]. An estimate of the effective size of polymer molecules in solution is given by their effect on the intrinsic viscosity, $[\eta]$, of the solution. The so-called hydrodynamic radius, r_h, of an equivalent hard sphere is given by

$$r_h = \left(\frac{3[\eta]M_r}{10\pi N_a}\right)^{1/3} \tag{8}$$

where N_a is Avogadro's number. The dramatically larger size of polymer molecules in solution, relative to solvent molecules, is demonstrated by considering data for polystrene in tetrahydrofuran (THF) at $T = 30°C$. At $M_r = 10\,000$, $r_h = 24$ Å, this increases to 90 Å at $M_r = 100\,000$ and to over 330 Å at $M_r = 10^6$ [1]. Considering that many RPLC packing materials have pore diameters in the range of 60 to 300 Å, attention must be paid to avoid overlap between size-exclusion and adsorption phenomena in RPLC separations [4,10,20,21]. Overlap of these effects would essentially result in different size polymer molecules sampling drastically different amounts of adsorbent surface area based on the ability of the solvated polymers to enter the porous structure.

Since RPLC separation is based on the transport processes of the solute molecules in solution, additional effects of polymer solution dimensions must be considered. The diffusion coefficients of conventional solvent molecules are on the order of $D = 10^{-5}$ cm^2/s. Since the rate of diffusion is dominated by viscous drag on the moving solute molecules, D decreases with the cross-sectional area of the solute. Empirical relationships [22,23] indicate that polymer diffusion coefficients are in the range of 10^{-6} to 10^{-7} cm^2/s for molecular masses between 10^4 and 10^6. Chromatographic transport would thus be aided by minimizing the distances over which the polymer solutes must diffuse. The optimum chromatographic packing particle diameter, d_p, based on minimizing band broadening, has been estimated at 0.4 μm for $M_r = 10^5$ [3]. This limit may be impractical due primarily to shear forces developed in the eluent flow. Polymer molecules are not infinitely rugged. If the shear forces accompanying solvent velocity gradients in the packed bed becomes great enough, covalent bonds along the polymer backbone may be broken leading to a reduction in M_r and erroneous separation results. Scaling arguments for the relationship between the critical M_r of polymer solutes (M_c), above which shear degradation occurs, and the parameters of the flow system provide the following relation [24].

$$M_c = I\left(\frac{d_p}{60\Theta\eta V}\right)^m \tag{9}$$

The scaling parameters I and m depend only on the solvent system, Θ is the scaling factor relating extensional forces to shear forces, η is the solution viscosity, and V is the mean linear flow velocity. Estimates of extensional forces in packed beds from SEC and viscometry [24] provide values 1000 times greater than that due to shear forces alone. Additional shear forces may also be developed as a result of the porous structure of the packing particles which may further increase polymer degradation by 25% relative to non-porous packings. This analysis indicates that polymer shear degradation can be reduced by decreasing solution viscosity and solvent velocity and increasing packing particle diameter. A practical balance must therefore be sought between separation efficiency and minimization of shear effects.

To this point the behavior of solutions of polymer molecules in a single solvent has been considered. RPLC, however, involves the use of mixed solvents to provide additional selectivity and control of retention by adjustment of solvent strength and concommitant changes in solute distribution coefficients. Complex relationships based upon the thermodynamics of phase equilibria for multicomponent systems are well established for polymer fractionation by selective precipitation or selective dissolution [25]. These descriptions are well beyond the scope of this review and the reader is referred to ref. 17 for in-depth discussions. True RPLC separation, however, is based on the differential migration imparted by selective retention of polymer solutes by adsorption interactions with the stationary phase. Selective precipitation/redissolution is therefore a competing mechanism to RPLC polymer compositional separation which may be either complementary or in opposition to the desired selectivity [1,26]. Turbidimetric titration [27,28] yields empirical relationships describing the precipitation behavior of polymer solutions upon gradual addition of a nonsolvent. The volume fraction of nonsolvent, Φ^*_{NS}, at the point of incipient phase separation follows an empirical log-linear relationship to polymer concentration, c^*_{poly} [1].

$$\Phi^*_{\mathrm{NS}} = A + B \log c^*_{\mathrm{poly}} \qquad (10)$$

In a similar manner, the volume fraction of nonsolvent at the point of incipient phase separation can be empirically related to polymer molecular mass [29].

$$\Phi^*_{\mathrm{NS}} = \acute{A} + \acute{B} M_r^{-1/2} \qquad (11)$$

The solvation of polymer molecules in mixed solvents does not result in an entirely homogeneous solution. As discussed previously, the enthalpic solvation interactions of solvent molecules with a polymer chain can be considered primarily as interactions with the monomer units of the polymer. For two solvents of differing thermodynamic quality, even if both are solvents for the polymer, the solvent composition in the vicinity of the polymer coil is enriched in the better solvent. This effect has been quantitatively observed by both dialysis experiments and NMR relaxation studies [2]. This selective solvation can have pronounced effects on RPLC elution behavior due primarily to the kinetics of exchange of the preferred solvent from the solvated polymer coil [20,30].

5. POLYMER ADSORPTION

In much the same manner that solvation of a polymer can be considered as being partly constituted from solvent molecule interactions with the individual monomer units along the polymer chain, adsorption processes are similarly affected by the adsorption interactions of each monomer unit. When the three-dimensional solvated polymer coil is adsorbed at a surface, steric restrictions allow only a limited number of monomer units to be immediately adsorbed. If the adsorption interactions are strong enough to overcome the energetics within the solvated polymer coil, then the polymer can unfold with many points of adsorption. For high-molecular-mass polymers with degrees of polymerization in excess of several hundred, it is unlikely that unfolding will be complete, but the polymer will become adsorbed with many loops pendent from the surface into the solvent [2].

At equilibrium, the distribution of a polymer solute between solution and adsorbed state involves two dynamic processes. An equilibrium is reached between the chemical potential of the polymer in solution and the adsorbed state in terms of the polymer as individual solute molecules. This equilibrium is a function of the polymer concentration, the temperature, the net solvation and adsorption interactions, and the adsorbent surface area [31]. Each adsorbed polymer chain also undergoes an intramolecular process in which individual monomer units are adsorbed and desorbed from the surface. The adsorption of each monomer unit along the polymer chain can be described as the balance of enthalpic and entropic interactions. Enthalpic interactions result from contacts with solvent and surface sites, whereas entropic contributions include the restricted movement of individual monomers in the polymer backbone and the three-dimensional solvation of the chain *versus* surface adsorption. These processes, as well as their kinetics, have significant effects on the experimentally observed polymer adsorption phenomena in chromatographic retention [2,21,28,32].

Although the adsorption interactions of a polymer can at least partially be considered in terms of the interactions associated with individual monomer units, chromatographic elution involves the transport of the entire polymer molecule. Any given monomer unit cannot be transported down the column until each monomer in the polymer chain has been desorbed. This requires that the retention interactions associated with each monomer unit be relatively weak, *i.e.*, the elution solvent must be strong, in order for polymer elution to take place in a reasonable time frame [1]. During the adsorption process the structure of the adsorbent becomes important. The multiple adsorption interactions can be enhanced by the curvature of the surface in the porous particles used in RPLC [2,20]. Thus the energetic interactions opposing chain unfolding can be more readily overcome by multiple adsorption interactions in a polymer coil close to the dimensions of an adsorbent pore. In addition, the kinetics of chain unfolding may be encouraged by the increasing number of adsorption contacts provided by penetration of a large polymer coil into a small pore. This process, related to polymer reptation, has been reported experimentally with measurements of the molecular mass dependence of chromatographic efficiency on porous adsorbents [33,34].

6. EXPERIMENTAL

RPLC separations described in Figs. 1, 2 and 3 were performed on a Varian 5060 ternary HPLC. HPLC-grade acetonitrile (MeCN) was obtained from J.T. Baker. Water was purified with a Milli-Q system (Millipore). Both solvents were helium sparged during use.

Poly(ethylene oxide) was obtained from American Polymer Standards Corp., $M_w = 645$, $M_n = 580$. Isocratic separation was performed on a 250 × 4.6 mm Supelco 5 μm LC8 bonded silica column at MeCN–water (18:82, v/v) at 1.0 ml/min. Samples were dissolved in water at 10 mg/ml with 20 μl injected. Detection was performed with an Applied Chromatography Systems Model 750/14 evaporative light scattering (ELS) detector set at temperature = 100 and pressure = 25 p.s.i.g. filtered nitrogen (1 p.s.i.g. $= 6.9 \cdot 10^3$ Pa).

Gradient elution separation at 1.0 ml/min were run from 5 to 100% (v/v) MeCN in water over 30 min with a 10-min hold at 100% MeCN on a 250 × 4.6 mm Brownlee 10 μm RP8-bonded silica column.

Samples of nonylphenoxypolyglycidol were obtained from Olin Corp. Samples were dissolved in MeCN–water (5:95, v/v) at 50 mg/ml with 20 μl injection volume. RPLC detection was performed with two detectors in series. A Kratos Model 757 UV absorbance detector set at 230 nm was followed by an ELS detector set at temperature = 100 and pressure = 35 p.s.i.g. filtered nitrogen.

Samples of perfluoroalkyl-terminated poly(ethylene oxide) were obtained from DuPont. Samples were dissolved in MeCN–water (5:95) at 10 mg/ml with 100 μl injected. Detection was performed with an ELS detector set at temperature = 110 and pressure = 20 p.s.i.g. filtered nitrogen.

RPLC–mass spectrometry was performed under the same RPLC conditions described above on a Hewlett-Packard Model N-50 LC with 0.01 *M* ammonium acetate added to the eluent. Molecular ion masses were used for identification of eluted components as provided by a Hewlett-Packard Model 5989 mass spectrometer with thermospray inlet.

7. LOW-MOLECULAR-MASS POLYMER RPLC SEPARATIONS

7.1. *Isocratic elution*

In order to described the chromatographic separation of synthetic polymers by RPLC, it is useful to begin with consideration of the elution characteristics of low-molecular-mass polymers, *i.e.*, oligomers. A basic relationship, usually referred to as the Martin equation [35], provides a starting point in the description of the elution of compounds related by the incremental increase in the number of repeating molecular subunits. This idealized linear free energy relationship for the retention process may be described by referring to the generalized chemical structure of a member of an oligomer series with n repeat units and two, possibly different, end groups: X–Y_n–Z.

$$\Delta G_{\text{retention}} = \Delta G_{\mathrm{X}} + \Delta G_{\mathrm{Z}} + n\,\Delta G_{\mathrm{Y}} = -RT\ln\left(\frac{a_{\text{stat}}}{a_{\text{mobile}}}\right) \qquad (12)$$

The free energy contribution of each molecular subunit to the retention process, ΔG_i, can thereby be related to the thermodynamic distribution coefficient, K (the ratio of the solute activity in each chromatographic phase, a_{stat} and a_{mobile}). If the retention process may be further described by a single mechanism which is of the same type for every oligomer in a series, then

$$V_{\mathrm{r}} = V_{\mathrm{m}} + KV_{\mathrm{s}} \qquad (13)$$

where the solute retention volume, V_{r}, is given by the sum of the mobile phase volume, V_{m}, and the product of the distribution coefficient and stationary phase volume, V_{s}.

The linear free energy relationship known as the Martin equation is obtained by combining eqns. 12 and 13 to give

$$\ln k' = \ln\left(\frac{V_{\mathrm{r}} - V_{\mathrm{m}}}{V_{\mathrm{m}}}\right) = \left[\frac{-(\Delta G_{\mathrm{X}} + \Delta G_{\mathrm{Z}})}{RT} + \ln\left(\frac{V_{\mathrm{s}}}{V_{\mathrm{m}}}\right)\right] - n\left(\frac{\Delta G_{\mathrm{Y}}}{RT}\right) \qquad (14)$$

where the solute capacity factor, k', is described in terms of the retention contribution of each molecular subunit. To be consistent with the earlier discussion for polymers, the number of repeat units, n, can be replaced with the degree of polymerization, P. It should be noted that in RPLC of small molecules the phase ratio, $V_{\mathrm{s}}/V_{\mathrm{m}}$, is difficult to determine accurately because V_{s} varies with the type of stationary phase, its solvation, and solute access to the stationary phase [11]. The regular logarithmic increase in retention with P has been demonstrated in many experimental investigations. Fig. 1 shows an example of this behavior for an isocratic RPLC separation of poly(ethylene oxide) (PEO) oligomers. For these separation conditions the Martin equation has been observed to continue to apply for oligomers in excess of 60 ethylene oxide repeat units.

The adherance to a linear free energy relationship in isocratic elution is a valuable tool in designing and interpreting separations; however, it must be emphasized that eqn. 14 does not imply a particular mechanism of interaction of solutes with the stationary phase. For example, eqn. 7 which describes polymer solubility behavior can be rearranged into a similar form [1]. The only information available from a separation following eqn. 14 is that the retention mechanism is constant for related solutes and that a linear free energy relationship applies to the distribution coefficient. Separation which follows a linear free energy relationship is representative of the consistency of retention interactions and is not limited to hydrocarbon-based solutes or aqueous-based eluents in RPLC. This is clearly demonstrated by the work of Steudel *et al.* [36] in which the separation of bis-alkoxysulfanes up to $P = 17$ is demonstrated to give the same $\mathrm{d}(\ln k')/\mathrm{d}P$ slope for both methoxy- and n-butoxy-terminated species in pure alcohol eluents on C_{18}-bonded silica.

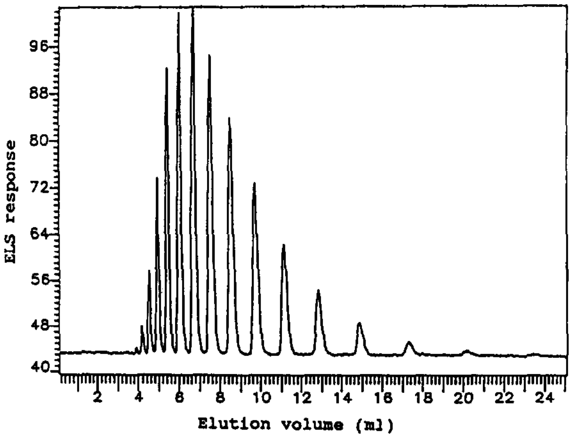

Fig. 1. Chromatogram of isocratic elution of poly(ethylene oxide) oligomers in MeCN–water (18:82, v/v) at 1.0 ml/min on 5 μm Supelco LC8-bonded silica with evaporative light scattering detection.

In the chromatograms of ref. 36, the elution of the cyclic S_8 species is observed early in the sequence of oligomers. Although linear oligomers generally follow a linear free energy relationship in distribution coefficient, caution must be exercised with the separation of cyclic species. The constrained structure of the cyclic species allows for structural variations between subsequent members of a series. For example, the separation of mixtures of linear and cyclic *p-tert.*-butylphenol–formaldehyde resins was described by Ludwig *et al.* [37]. The $P = 5$ cyclic species shows anomolously high retention attributable to the formation of intramolecular hydrogen bonds.

It was noted in the separation of oligomeric sulfanes [36], that the plots of $\ln k'$ *vs.* P provided the same slope for two series of oligomers with different terminal groups. In agreement with eqn. 14, the terminal groups affect retention by changing the intercept of $\ln k'$ *vs.* P. This represents an important practical feature of the adherance of oligomers to eqn. 14. In separations development and compositional characterization of oligomers, plots of $\ln k'$ *vs.* peak number can be used to classify oligomers belonging to different series in a mixture by grouping components which display a constant change in $\ln k'$. A separation of oligomeric Bisphenol-A polycarbonates was described by Bailly *et al.* [38] in which three separate oligomeric series were identifiable based on whether two aromatic, one aromatic and one hydroxyl, or two hydroxyl terminal groups were present.

In the discussion of the effects of terminal groups on the retention behavior of oligomeric series, it is important to clarify the thermodynamic meaning of a terminal group in eqn. 14. The linear free energy relationship essentially places all contributions to retention other than those which provide a constant increase in $\Delta G_{\text{retention}}$ (eqn. 12) into the intercept term of the linear equation. A terminal group for this application may therefore not necessarily correspond to the chemical structure as usually written. The effective terminally group may include several repeat units before linear $\ln k'$ *vs.* P behavior is observed [39]. This is demonstrated in Fig. 1 of ref. 40 for RPLC retention of oligomeric polystyrene. For isocratic elution in 1,4 dioxane–water (83:17) on C_{18}-bonded silica, the first five oligostyrenes show significant nonlinearity. It should also be noted at this point that curvature of $\ln k'$ *vs.* P data can result if an inaccurate V_m is used to calculate k' values. However, this effect produces curvature of the entire data set, not just the low P range. McCormick and Karger [41] have shown that the use of 1% replacement ^2H_2O in aqueous–organic eluents in bonded-phase RPLC provides the most thermodynamically consistent single point measure of V_m.

7.2. Gradient elution

For a given solvent composition, the logarithmic relationship between solute P and retention, given by eqn. 14, places a practical limit on isocratic RPLC separations. Solvent strength can be increased to elute more strongly retained solutes. According to eqns. 12, 13 and 14, early elution corresponds to a smaller distribution coefficient, a less negative $\Delta G_{\text{retention}}$, and a decrease in resolution as a function of P. For polymeric solutes of broad molecular mass distribution and especially for compositionally heterogeneous copolymers, gradient elution is required to achieve separation within a reasonable time frame, while maintaining as much resolution as possible. The effect of eluent solvent strength on retention in RPLC has been described on a theoretical basis by Schoenmakers *et al.* [42,43], as a second order polynomial in volume fraction strong solvent, φ_s, or more simply as a linear function in experimental studies by Larmann *et al.* [32].

$$\ln k' = A\varphi_S^2 + B\varphi_S + C \approx \ln k'_w - S\varphi_S \qquad (15)$$

The coefficients of the linear solvent strength (LSS) formulation are characteristic of each solute. The extrapolated solute capacity factor in pure weak solvent (water in aqueous–organic RPLC) is k'_w and the slope $S = -\text{d}(\ln k')/\text{d}\varphi_S$. The retention of solutes under gradient elution, k'_g, can be described in the LSS model by [32]

$$k'_g = (V_D/V_m) + \left(\frac{t_G}{\Delta\varphi_S}\right)\cdot\left(\frac{1}{V_m S}\right) \cdot \log[2.303(\Delta\varphi_S/t_G)SV_m k_0 + 1] \qquad (16)$$

where $\Delta\varphi_S/t_G$ is the gradient strong solvent slope, k'_0 is the solute k' in the starting solvent composition, and V_D is the lag volume of the HPLC system for the gradient solvent change to reach the column inlet. The general utility of these and other models for gradient elution RPLC will be discussed further in terms of high-molecular-mass polymer retention behavior.

7.3. Chemical composition separation

Fig. 2 shows a gradient elution separation of nonylphenoxy-terminated polyglycidol in MeCN–water on C_8-bonded silica. Although eqns. 12–14 are strictly written for isocratic conditions, the concept of ΔG per molecular subunit is still useful in interpreting this chromatogram. A fraction of the oligomer chains are not nonylphenoxy terminated and elute early in the gradient at 9 min, as shown by the comparison of UV absorbance and evaporative light scattering detector responses. As the gradient solvent strength is increased the retention per repeat unit decreases, however in this separation oligomer retention is dominated by the non-polar terminal group. The envelope of nonylphenoxy-terminated oligomers is seen to display increasing resolution with increasing retention. Unlike the elution profile shown in Fig. 1, increasing *P* corresponds to decreasing retention. Consistent with eqn. 14, this would imply that in the range of eluent composition required to elute nonylphenoxy-terminated oligomers, ΔG per glycidol monomer [$-CH_2-CH(CH_2OH)-O-$] is positive. Referring to eqn. 12, a positive ΔG corresponds to a capacity factor less than unity. However, since this discussion considers the ΔG contribution per molecular subunit, this demonstrates that there is no *a priori* reason why ΔG per molecular subunit must be negative, only that the total $\Delta G_{\text{retention}} < 0$. Neither is this RPLC oligomer elution behavior unique. Jandera observed a positive ΔG per ethylene oxide repeat unit for nonylphenoxy-terminated PEO in 2-propanol–water on C_{18}-bonded silica (Fig. 3 in ref. 40). It should also be noted that Jandera's log k' *vs.* φ_S data show a distinct curvature more in agreement with Schoenmaker's solvent strength model, eqn. 15.

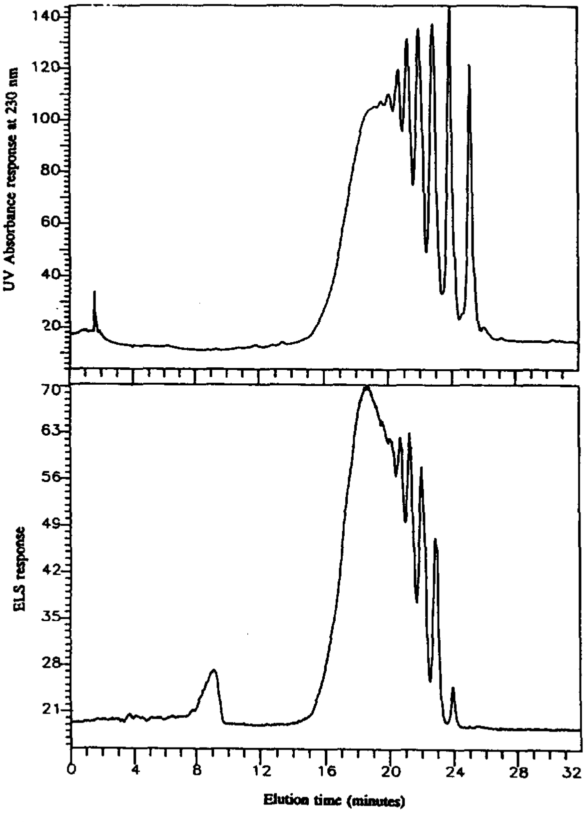

Fig. 2. Comparison of UV absorbance at 230 nm and ELS detector response gradient elution chromatograms of nonylphenoxypolyglycidol eluted from Brownlee 10 μm RP8-bonded silica with 5 to 100% MeCN in water over 30 min.

When the terminal group of an oligomer distribution is composed of an homologous series, the two distributions are convolved as in the following example of a perfluoroalkyl-terminated PEO surfactant, $F_3C-(CF_2-CF_2)_n-CH_2CH_2O-(CH_2CH_2O)_m-CH_2CH_2OH$.

The PEO distribution of $m = 3$–18 is present for each perfluoralkyl chain length from $n = 2$–6. A gradient elution separation of this oligomeric surfactant is shown in Fig. 3. Hydroxyl-terminated PEO oligomers are eluted at 16 min with partial resolution of individual oligomers ($\Delta G_{EO} < 0$). Perfluoroalkyl-terminated PEO chains are resolved in groups of oligomers differing by the length of the perfluoroalkyl terminal group. The elution profile of this separation is interpreted in terms of eqn. 14 as a large negative ΔG per ($-CF_2-CF_2-$) unit relative to a small negative ΔG per EO unit at the φ_S necessary to

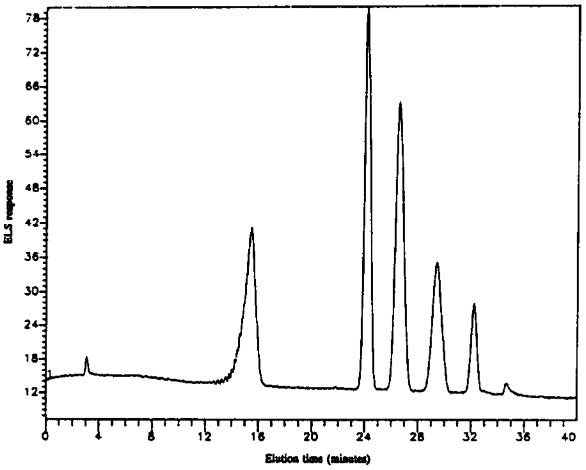

Fig. 3. Gradient elution ELS response chromatogram of perfluoroalkyl-terminated poly(ethylene oxide) eluted from Brownlee 10 μm RP8-bonded silica with 5 to 100% MeCN in water over 30 min.

elute the perfluoroalkyl-terminated chains. The composition of components in this separation was verified by determining the variation of molecular ion masses by thermospray RPLC–mass spectrometry.

The observation of altering ΔG per monomer repeat unit while maintaining a more negative ΔG of the terminal groups leads to a type of chromatographic separation referred to as elution under "critical conditions" [21,44–46]. The following description of the chromatographic conditions corresponding to this type of separation is presented in an oversimplified manner for ease of explanation. The reader is referred to refs. 21 and 44–46 for a more complete derivation. Eqn. 17 is obtained by writing eqn. 13 to take into account both exclusion phenomena (V_pK_{SEC}) associated with the porous structure of the adsorbent and adsorption interactions (V_sK_{ads}). This formula, however, ignores the importance of the overlap between exclusion and adsorption phenomena which results from the restricted access of solutes to the porous adsorbent. A more comprehensive version of eqn. 17 is given in ref. 10.

$$V_r = V_i + V_pK_{SEC} + V_sK_{ads} \tag{17}$$

The mobile phase volume of eqn. 13 is subdivided into the interstitial volume V_i and the pore volume V_p. When $K_{ads} = 0$ and $K_{SEC} = 0$, the polymer solute is excluded from the pore volume and elutes at V_i. In the range $0 < K_{SEC} < 1$, SEC separation is observed in a good solvent for the polymer ($K_{ads} \approx 0$). If the quality of the solvent is decreased by either addition of a poor solvent [21] or temperature change [47], then K_{ads} becomes non-zero and adsorption interactions overlap exclusion. If K_{ads} becomes large enough such that

$$V_pK_{SEC} + V_sK_{ads} > V_p$$

the solute then elutes under adsorption conditions ($V_r > V_m$) with possible contribution from exclusion phenomena depending upon polymer access to the pore volume. These various elution regimes are demonstrated in Fig. 4 for PEO as a function of eluent composition [21]. At the point where exclusion and adsorption contributions are balanced such that

$$V_pK_{SEC} + V_sK_{ads} = V_p \tag{18}$$

all components elute at V_m. When the polymeric solute consists of a functionally terminated chain

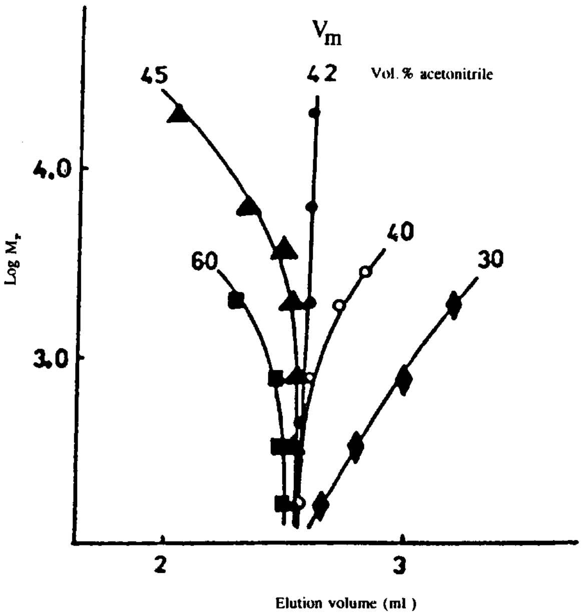

Fig. 4. Near critical condition elution for poly(ethylene oxide) homopolymers. Numbers indicate isocratic MeCN volume % in MeCN–water eluents on Chrompak RP-18 at 25°C and 1.0 ml/min. From ref. 21.

and the separation conditions provide adsorption contribution of the terminal groups greater than that of the repeat unit, separation can occur over a limited range of P in which the polymer is essentially "invisible" [44]. Obviously separation under "critical conditions" must be carried out under isocratic conditions.

Theoretical arguments have been presented [41,44] which suggest that elution under "critical conditions" may be applicable to the separation of block and graft copolymers. Random copolymers and short-chain branched copolymers are theoretically limited to small pore adsorbents such that $K_{SEC} = 0$ for each block of uniform composition. The comparison of the elution profile of PEO–poly(propylene oxide) (PPO) block and random copolymers is shown in Fig. 5 under RPLC "critical conditions" for PEO homopolymers [21]. The effect of copolymer structure (PPO sequence) is apparent in the broad profile of the random copolymer. Since application of "critical conditions" to oligomers is restricted to isocratic conditions, it is limited by the possibility of finding solvent and adsorbent conditions for which eqn. 18 can be obtained for one molecular subunit while K_{ads} for the other subunits (terminal groups or comonomer) are small enough to permit elution in a reasonable time frame [21].

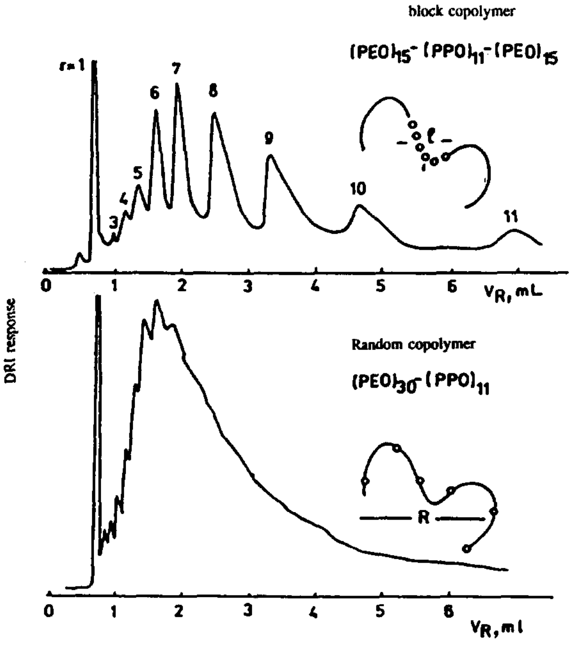

Fig. 5. Isocratic elution differential refractive index (DRI) detection chromatograms of a block and a random copolymer of similar average composition under critical conditions for PEO homopolymer as shown in Fig. 4. From ref. 21.

7.4. Stationary phase influences

For general information on stationary phase influences on RPLC separations of small molecules the reader is referred to the recent review literature [11,48,49], as well as other articles in the current issue of this journal. A few specific comments relevant to RPLC separations of oligomers are made here. Polar-bonded phases on silica (aminopropyl, propyldiol, cyanopropyl) have been found to be more suited to normal-phase separation [50] to enhance selectivity for polar monomer units [12]. Secondary interactions can strongly influence the choice of bonded-phase materials to either minimize or accentuate polar groups selectivity. Hydrocarbon-bonded phase interactions have been studied for the separation of oligostyrenes on phenyl-bonded silica [51]. Experimental studies on the effect of bonded hydrocarbon chain length on the RPLC retention of homologous series is relevant to oligomers of low P [52]. It has been noted that a change in ln k' *vs.* P is observed when the length of the solute exceeds that of the bonded hydrocarbon moieties [53,54].

The discussion of RPLC retention presented is sufficiently general that even separations on novel chromatographic adsorbents are found to be in agreement with the performance of conventional RPLC supports. For example, Rheinländer *et al.* [55] described a thermodynamic study of the adsorption behavior of PEO oligomers on graphite with aqueous methanol or acetonitrile eluents. Although unlike conventional hydrocarbon-bonded silica RPLC materials, this combination of a carbon surface with polar eluents fits the definition of a reversed-phase chromatographic system. The retention mechanism is truly adsorption and the results agree well with the Martin equation (eqn. 14) description of oligomer separation.

8. HIGH-MOLECULAR-MASS POLYMER RPLC RETENTION PROCESS PARAMETERS

No distinct or discontinuous difference exists between the chromatographic retention behavior of oligomers and high-molecular-mass synthetic polymers in RPLC. There are, however, many experimentally observable behaviors of synthetic polymers in RPLC elution which result in apparent inconsistencies with conventional RPLC experience. These will be discussed in terms of chromatographic experimental data and the properties of synthetic polymers which have been described in the introduction of this review. As stated in the discussion of polymer solution and adsorption behavior, the most significant aspects of polymer properties which differ from small molecule solutes are solvated polymer conformation, the size of solvated polymers, and their limited solubility.

First, consider the effect of increasing degree of polymerization beyond the oligomer range. In order to obtain elution, stronger eluents are required (and possibly weaker adsorbents) which produce a concomitant decrease in selectivity per monomer unit. The Martin equation is based upon equal retention interactions for each monomer unit in the polymer. However, as the energetics of the three-dimensional structure of the solvated polymer chain become significant with respect to the retention interactions, it is no longer possible for every monomer unit to be adsorbed. Consistent with this effect, as shown in Fig. 6, a monotonic deviation from the oligomeric linear response has been observed for polystyrene (PS) elution with THF–water from 1000 Å pore C_{18}-bonded silica [32]. In this plot, isocratic elution at higher P values was estimated from gradient elution data using eqns. 15 and 16.

For high degrees of polymerization, the size of the solvated polymer reaches the same regime as the diameter of the pores of conventional RPLC adsorbents. Shalliker *et al.* [20] demonstrated that the contribution of adsorption decreases with decreasing pore diameter for RPLC elution of PS in dichloromethane–methanol (DCM–MeOH) gradients on three different pore size C_{18}-bonded silica materials (Fig. 7). For small pore adsorbents, when the size of the solvated polymer molecules exceeded the pore diameter, the significant drop in available surface area caused the polymer to elute at the solubility limit. When large pore (4000 Å) adsorbent was used, the polymer continued to have significant retention beyond the solubility limit even for polymer chains of 10 000 monomer units. Similar observations were presented by Schultz and Engelhardt [26] for the same RPLC system. In comparison, elution of PS at the solubility limit independent of P was observed when DCM–MeOH was used as the eluent on the same pore size bare silica. This was an unconventional combination of a decreasing polarity solvent gradient on a polar adsorbent. The strong adsorption of methanol effectively eliminated PS adsorption contributions. No significant effect of

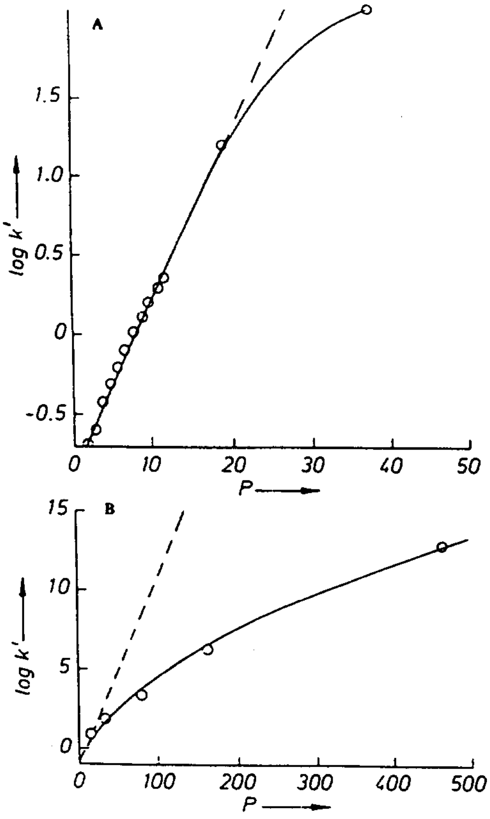

Fig. 6. Deviation of PS elution from the Martin equation (eqn. 14) with increasing P; (A) $2 \leqslant P \leqslant 40$, (B) $19 \leqslant P \leqslant 470$. Elution from 1000 Å pore 30 μm Zipax C_{18}-bonded silica with THF–water (60:40, v/v) for $P \leqslant 11$. Log k' for $P \geqslant 19$ calculated from gradient elution data. From ref. 32.

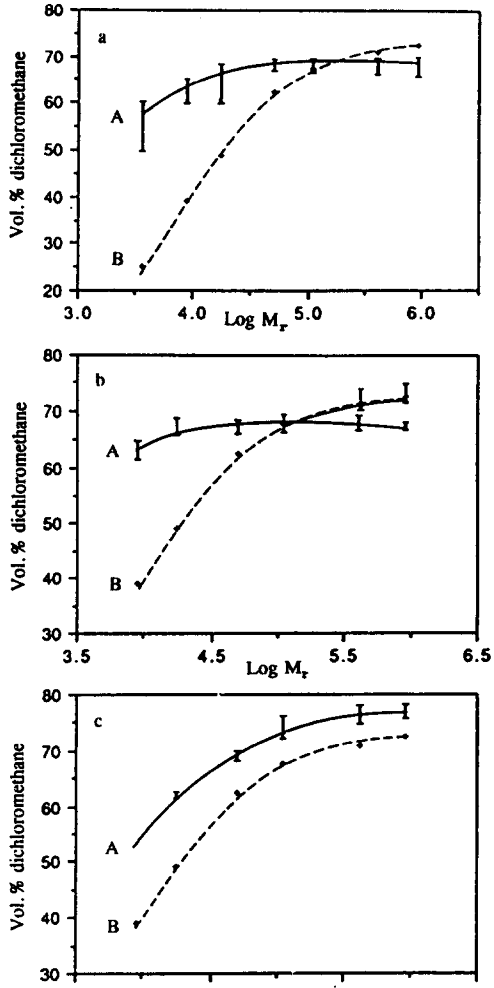

Fig. 7. Relationship between (A) φ_c and log M_r and (B) solubility limit data at 25°C for PS in DCM–MeOH. Chromatographic gradient elution data from (a) 120 Å pore 10 μm μBondapak C_{18}-bonded silica, (b) 300 Å pore 5 μm Serva C_{18}-bonded silica, and (c) C_{18}-derivatized 10 μm LiChrospher SI 4000 Å pore silica. From ref. 20.

adsorbent pore diameter was noted by Larmann *et al.* [32] for PS elution from C_{18}-bonded silica with THF–water.

The effect of adsorbent pore diameter was investigated by Sato *et al.* [56] for RPLC compositional separation of poly(styrene–methyl methacrylate) (SMMA) copolymers. Identical separations were performed for a mixture of SMMA copolymers with molecular masses ranging from 119 000 to 243 000 with DCM–MeCN gradients on two cross-linked PS columns. As shown in Fig. 8, resolution loss due to band broadening was observed for the smaller pore diameter column associated with the overlap of adsorption and size-exclusion phenomena. The compositional selectivity, however, remained very similar between the two columns.

Consistent with the Flory–Huggins theory (eqn. 7) [18], polymer solubility is generally observed to decrease with increasing degree of polymerization. For a given polymer composition and temperature, its solubility varies as a function of *P*, concentration, and solvent quality (φ_{NS}). In solutions of a mixed solvent and non-solvent, this is empirically described by eqn. 11 as a function of polymer molecular mass and eqn. 10 as a function of polymer concentration. Glöckner has been a proponent of the use of turbidimetric titration for comparison with chromatographic elution data for the elucidation of retention mechanisms [1,57]. Linear plots con-

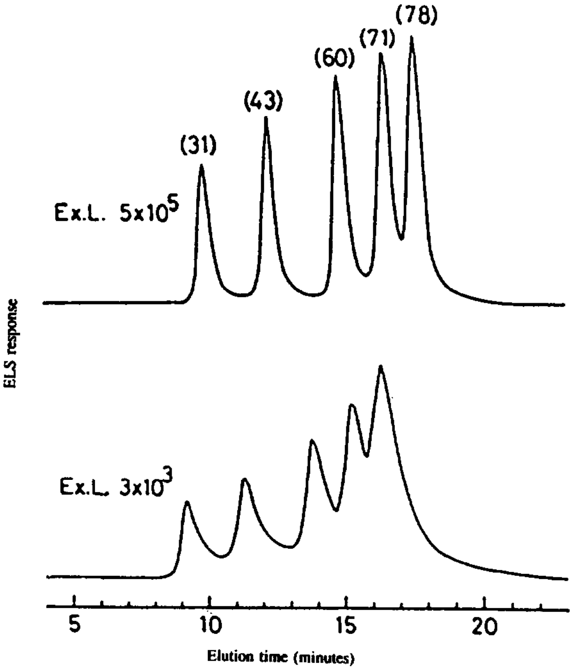

Fig. 8. Exclusion limit (Ex.L.) pore size effect on the separation of SMMA copolymer mixture by linear gradient elution in DCM–MeCN (0 to 60% DCM in 25 min) on cross-linked PS gel columns. Peak numbers indicate the mol% styrene incorporation of the random copolymers. From ref. 56.

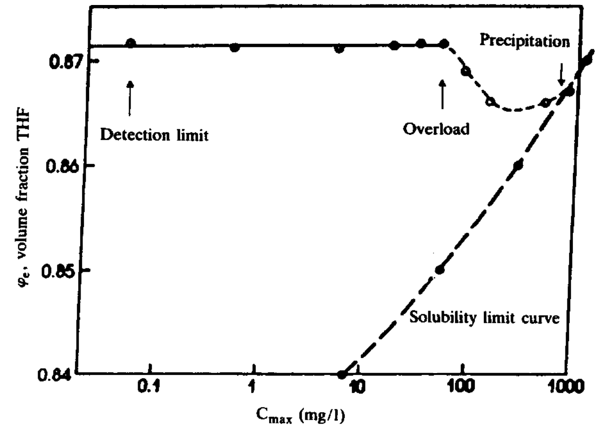

Fig. 9. Gradient elution retention data for M_r 50 000 PS from 150 Å pore Zorbax C_{18}-bonded silica at 35°C with linear gradients from 75 to 95% (v/v) THF in water over 30 min and comparison to solubility data, C_{max}. From ref. 58.

sistent with eqn. 11 (when plotted as φ_{NS} *vs.* $M_r^{-1/2}$) are obtained under precipitation conditions [1,26,57]. Caution must be used however with direct comparisons due to the strong dependence of the solubility limit on polymer concentration [1,58] (eqn. 10). The adherence of RPLC elution to eqn. 10 under precipitation conditions for high-molecular-mass PS was clearly demonstrated by Schultz and Engelhardt [26] in DCM–MeOH on C_{18}-bonded silica over an injected polymer concentration range varying by a factor of 300.

An alternative application of eqn. 10 for interpretation of RPLC retention was proposed by Quarry *et al.* [58]. As shown in Fig. 9, φ_{NS} is plotted as a function of C_{max}, the solubility limit concentration of the polymer in the solvent–non-solvent mixture. Comparison of elution solvent composition of C_{18}-bonded silica with the determined solubility curve for an M_r 50 000 PS in THF–water clearly indicates adsorption polymer retention far in excess of the solubility limit except at very high injected sample concentration [58]. In practical application this approach is limited to very monodisperse polymers so as not to obtain polymer molecular mass fractionation (or composition for copolymers) in the determination of C_{max}.

8.1. *Isocratic elution*

It has been shown that, although high-molecular-mass polymers do not follow the logarithmic increase in k' with P characteristic of oligomers, a monotonic increase is still observed (Fig. 6). This makes isocratic separation impractical at high P due to the range of components present in even narrow dispersity synthetic polymers. The value of both S and k'_w (eqn. 15) is observed to increase with degree of polymerization. Data reported by Stadalius *et al.* [9] for PS on C_{18}-bonded silica in THF–water determined $S = 0.22\ M_r^{0.5}$. According to eqn. 15 for $S = 50$ a change in isocratic k' from 1 to 10 would require a change in φ_s of only 2%. This is experimentally observed in Fig. 10 for an M_r 50 000 PS. This observation is sometimes interpreted as a discontinuous retention behavior of high-molecular-

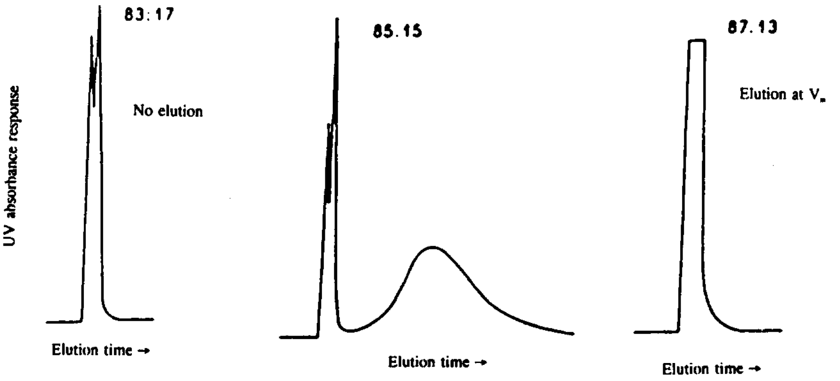

Fig. 10. Isocratic elution of M_r 50 000 PS from 300 Å pore C_{18}-derivatized Zorbax SIL silica. THF–water eluent composition indicated as volume percent on each chromatogram. From ref. 32.

mass polymers in which the polymer is either irreversibly adsorbed or completely unretained. An alternative explanation is that the range of suitable isocratic elution solvent strength becomes increasingly narrow for high *P*, thus eliminating the need to hypothesize discontinuous behavior.

Given these difficulties in performing isocratic elution of high-molecular-mass polymers, the method of elution under "critical conditions", which depends on isocratic conditions, must be reevaluated. Theoretically, elution under "critical conditions" is not limited by the degree of polymerization of the polymer solutes. However, the observations just stated regarding the difficulty of performing isocratic elution at high *P* still applies to the portion of the polymer undergoing adsorption retention. The dispersity present in the adsorbed portion of the polymer would make elution difficult in a reasonable time frame. In addition, the theoretical model of elution under "critical conditions" makes the implicit assumption that the adsorption interactions per molecular subunit are constant regardless of the polymer structure. The data of Fig. 6 shows this not to be the case for high-molecular-mass polymers. Thus obtaining "critical conditions" for one comonomer would be limited to a narrow *P* range.

8.2. *Gradient elution*

The LSS model described for gradient elution (eqn. 16) can be used to investigate some of the experimentally observed differences between small molecule RPLC and that of high-molecular-mass polymers. The LSS gradient elution model can be formulated in terms of the k' of a solute at the column midpoint during a gradient elution separation. Expressed in terms of the strong solvent volume fraction at solute elution, φ_e, the following relationship to chromatographic parameters is obtained [58].

$$\varphi_e = (1/S)\log(2k_0) - (1/S)\log\left[\frac{0.87 t_G F}{V_m \Delta\varphi S}\right] + \varphi_0 \tag{19}$$

The eluent flow-rate is given by *F*, the initial volume fraction of strong solvent in the gradient is φ_0, and other variables are as defined in previous discussions. For high *P* polymers with corresponding large *S* and k'_0 values, the variation of the elution conditions with readily changed variables in gradient RPLC results in very small changes in φ_e. These effects are discussed in refs. 9 and 58. Two observations differing from small molecule RPLC experience will be discussed here. It is observed that polymer elution (φ_e) is not significantly altered for high *P* by changing φ_0 [20] (or injection time after the gradient start [4]) and that little effect on polymer peak width is observed as a function of column length [59,60]. At high *S* the value of k' at the beginning of the gradient (eqn. 15) is very large. In addition, large *S* implies that significant polymer migration takes place over only a narrow range in φ_S (Fig. 9). Therefore, in agreement with eqn. 19, changing the injection time or φ_0 has only a small effect on φ_e, which may be less than the experimental precision of φ_e for high *P*. Since polymer elution band width is more related to polymer dispersity [9] than to conventional band broadening processes [31], changing column length will have the primary effect of changing V_m in eqn. 19. When *S* is large this will have only a small effect on φ_e.

In addition, it is often observed that the peak width of polymers eluting near the end of a gradient are more narrow than those eluting early. This is different from small molecule experience in which peak width is nearly constant or slightly increasing across a gradient elution profile. It must be remembered, however, that polymer samples are not individual species but a distribution of chains [9]. The increase in *S* with *P* therefore yields a more narrow φ_e range for higher-molecular-mass (more retained) peaks, and a resultant band narrowing. It would be interesting to investigate the RPLC separation of a series of high *P* polymers which possess a well retained terminal group and a positive ΔG per repeat unit (PEO in ref. 40). Gradient elution of these polymers would be expected to show an increase in band width with increasing retention. Of course this would be associated with an increasing retention with decreasing *P*.

A statistical thermodynamic theory of homopolymer separation by liquid chromatography has been developed by Boehm and co-workers [4–7]. The distribution of an isolated flexible homopolymer molecule is modeled between a binary mobile phase and a planar adsorbent surface with an adsorbed solvent layer. The equilibrium distribution of polymer depends on φ_S, the degree of polymerization, all available nearest neighbor interactions, and the configurational entropy of the polymer in each chromatographic phase. In simplified form

$$\ln k' = S(\varphi_c - \varphi_S) = |A_1|P(\varphi_c - \varphi_S) \tag{20}$$

$${}_1 = \chi_{12} + \chi_{13} - \chi_{23} + dP^{-4/5} + eP^{-1} \tag{21}$$

$$\varphi_c = \varphi_c^{\infty} - |a|P^{-1/2} + bP^{-4/5} + c_1P^{-1} + c_2P^{-1}(\ln P) \tag{22}$$

These equations employ a different reference point than the LSS model described previously. Whereas the LSS model depends on linear ln k' *vs.* φ_S data to determine the slope S and extrapolation to ln k'_w (or k'_0), this model uses the point at which ln $k' = 0$ as its reference. The isocratic eluent composition at which ln $k' = 0$ is defined as φ_c with slope $S \equiv -\partial(\ln k')/\partial\varphi_c$, for any ln k' *vs.* φ_S data set which need not be linear. Statistically this should provide values with smaller variance by interpolation rather than extrapolation, but nonetheless very precise isocratic k' data are required at high P. In the limit as P approaches infinity, φ_c is predicted to asymptotically approach φ_c^{∞}. The terms χ_{ij} represent the reduced interchange energy to form i–j interactions, where 1 and 2 refer to good and poor solvent, respectively, and 3 refers to the monomeric unit of the homopolymer. The parameters a, b, c_1, c_2, d and e depend on the interchange energies and weakly on the eluent composition [5,7].

Experimental evaluation of this statistical thermodynamic approach was presented in ref. 4 for both isocratic and gradient elution of PS on C_{18}-bonded silica of two different pore sizes. This experimental evaluation differs from that described by Snyder and co-workers [9,32,58] in that the DCM–MeOH gradient provides a mixed separation mechanism dominated by precipitation phenomena at high P [20,26], thus making comparison difficult. It is clear that adsorption separation is dominant for PS 50 000 in THF–water on C_{18}-bonded silica [58]; however, the dominant mechanism of retention can change with increasing P [20,26]. The separation mechanism sensitivity to choice of solvents is further emphasized by Glöckner's observations in THF–MeOH eluent [57]. Precipitation was shown to be the dominant retention process for PS on C_{18}-bonded silica with THF–MeOH by comparison to turbidimetric titration over the M_r range 36 000 to 2 000 000. Regardless, more concrete comparison of the dependence of separation parameters in both models requires high-precision chromatographic data [4,30,58].

8.3. *Chemical composition separation*

The objective of applying RPLC to the separation of synthetic copolymers is to provide resolution based upon chemical composition or structure rather than molecular mass distribution. Depending upon the mechanism of retention and separation, the polymer degree of polymerization may be convolved with the composition distribution information in the elution profile. Optimum separation conditions are obtained when all compositions of the polymeric sample are soluble in all proportions of the eluent gradient and the adsorption interactions with the column packing the strong [1]. Primarily due to the limited solvent range available for polymers, this case is the exception rather than the rule, particularly in RPLC. The strongest solvent used in the gradient must be capable of effectively displacing the adsorbed polymer, while the weakest solvent should allow adsorption while still being capable of dissolving the polymer. With the relatively weak adsorption interactions available to RPLC stationary phases, the former situation is usually not difficult to obtain. The weak solvent constraint is usually not possible with RPLC of synthetic polymers. Instead, in most situations the weak solvent is a nonsolvent for most if not all of the polymer compositions of interest. The polymer is thus precipitated at the column inlet and is later dissolved by the gradient. This solubility problem is one of the primary reasons why normal-phase chromatography has been much more widely applied to copolymer compositional separations [1].

In situations where the weak solvent is a nonsolvent and adsorption interactions are very weak, all components elute at their solubility limit [26,57] dependent upon both their molecular mass and chemical composition. If the adsorption interactions are sufficiently strong to allow observable retention beyond the point of dissolution in the gradient, then the adsorption interactions may dominate the separation. That is, the magnitude of adsorption dependence on composition may be sufficiently larger than the molecular mass dependence (eqn. 11) to provide supression of molecular mass effects on polymer elution [56]. Once again due to the relatively weak adsorption interactions of RPLC stationary phases, this situation is unlikely, but is more commonly found in normal-phase HPLC [1].

As a result of these potentially overlapping retention phenomena, considerable attention has been paid to differentiating retention mechanisms in copolymer RPLC. Several workers have used the comparison of turbidimetric titration solubility limit results with polymer elution φ_e. Sato *et al.* [56] showed that SMMA elution from cross-linked PS required a significantly higher volume fraction of dichloromethane than was indicated by the solubility data (Fig. 11). The difference was attributed to adsorption interactions. The slope of φ_e *vs.* mol% styrene was not parallel to the solubility limit indicating a decrease in adsorption contribution with increasing styrene content. Similar results were obtained by Glöckner and co-workers [61–64] for the compositional separation of poly(styrene-co-ethyl methacrylate) (SEMA) on C_{18}-bonded silica. A mixture of SEMA copolymers was separated by RPLC with a THF–MeOH gradient after SEC fractionation. For these RPLC conditions the spacing between the φ_e data and the solubility limit was quite small (Fig. 12B). Adsorption retention contribution again decreased with increasing styrene incorporation. However, the φ_e data and solubility limit now overlap at high styrene content indicating the transition to a precipitation mechanism. The data of Fig. 12A and B are consistent with the expected composition and molecular mass variation of low conversion (<5%) copolymers [62] discussed earlier (eqn. 3).

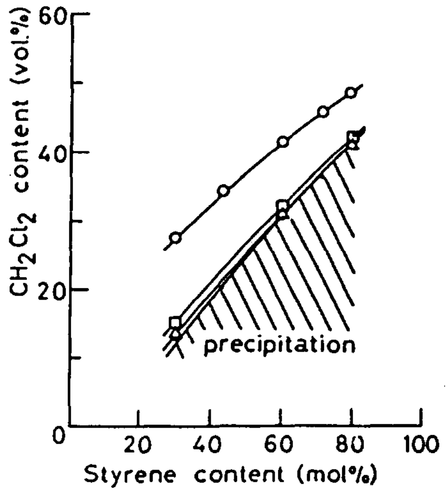

Fig. 11. Gradient elution φ_e data for SMMA copolymers on three cross-linked polymer gel adsorbents with DCM–MeCN eluent with comparison to turbidimetric titration data at 25°C. △ = Polyacrylonitrile; □ = poly(methyl methacrylate); ○ = polystyrene. From ref. 56.

In many cases it is difficult to differentiate precipitation from adsorption retention when the difference between φ_e and turbidimetric titration solubility curves is small. This is true for the high styrene content copolymers in Fig. 12B. The data presented by both Schultz and Engelhardt [26] and Shalliker *et al.* [20] showed a convergence of φ_e and solubility curves for PS eluted from C_{18}-bonded silica with DCM–MeOH. The φ_e due to adsorption contributions may be only very slightly different from the solubility limit when polymer molecules are excluded from the adsorbent pore structure. The dominant retention mechanism may change with *P* or composition when separations are conducted across a broad range. Additional data are then required to investigate the retention mechanism.

The concentration or mass of injected polymer may be used to investigate the retention mechanism for polymer RPLC based on both peak position and peak profile. Recalling the discussion of polymer adsorption processes, as the

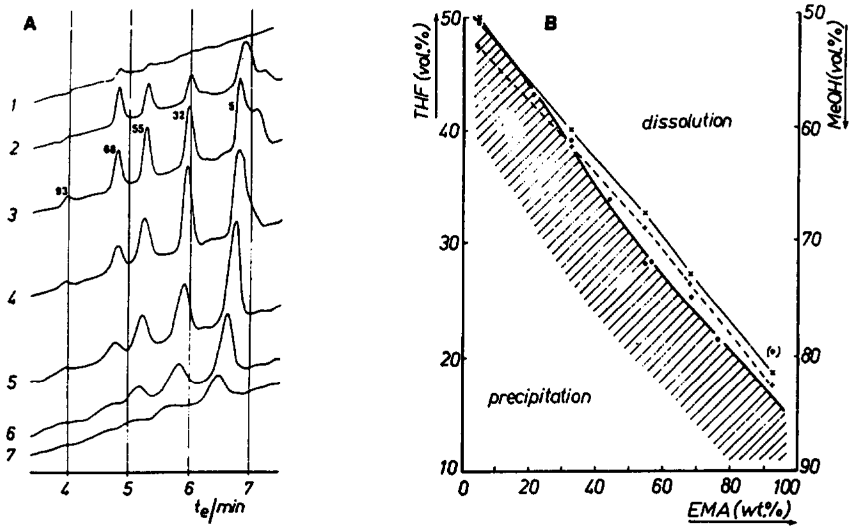

Fig. 12. (A) RPLC gradient elution of SEC fractions from a mixture of SEMA copolymers on 50 Å pore 5 μm Nucleosil C_{18}-bonded silica with linear THF–MeOH gradients (0 to 100% THF in 10 min) at 50°C. Mass percent EMA incorporation for each copolymer is indicated on fraction 3. (B) Elution φ_e of SEMA copolymers of SEC fraction 2 ($M_r = 132\,000$) (×) and fraction 5 ($M_r = 34\,400$) (+). Solubility data were obtained from turbidimetric titration. From ref. 64.

concentration of polymer reaches a limiting value for saturation of accessible adsorption sites, additional polymer must be carried down the column by the eluent until available surface is found. During subsequent elution, this reduces the effective length of the column and thereby produces a tailing peak eluting at earlier time [1,9]. This is consistent with column overload phenomena observed with small molecule separations and the PS data of Fig. 9. If, however, the retention process is dominated by precipitation, then the RPLC packing acts merely as a support for holding the precipitated polymer. As the injected concentration of polymer is increased, multiple layers of polymer are deposited on the support. During elution, polymer is gradually redissolved and the result is a fronting peak at increased elution time [1,20,65]. This type of polymer concentration effect is demonstrated in Fig. 13 for M_r 110 000 PS [26].

At high P the solvent association with the three-dimensional polymer chain also plays a role in distinguishing retention phenomena. Rather than observing polymer elution coincident with the solubility limit, Shalliker *et al.* [20] observed polymer elution prior to the solubility limit (Fig. 7). They proposed a mechanism for "pre-elution" of PS in DCM–MeOH associated with slow exchange of good solvent from the redissolved polymer relative to the gradient slope. It is difficult, however, to make quantitative comparisons of φ_e and solubility data due to the concentration dependence of polymer solubility (eqn. 10). The slow exchange of solvent from a solvated polymer chain has other observable effects on polymer RPLC. Due to the fact that the initial gradient conditions represent

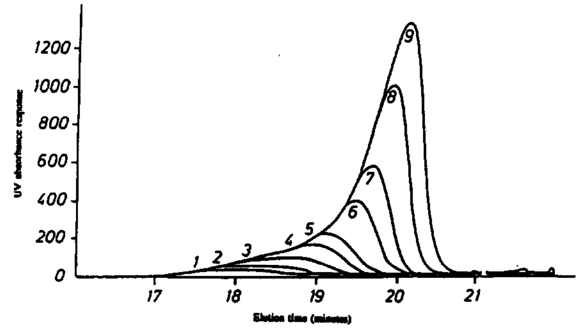

Fig. 13. Sample size effect on elution of PS 110 000 from C_{18}-derivatized 90 Å pore 10 μm silica with DCM–MeOH gradients (20 to 50% in 3 min, then to 80% DCM at 27 min) at 35°C. Sample mass: (1) 12.5 μg; (2) 25 μg; (3) 50 μg; (4) 75 μg; (5) 100 μg; (6) 150 μg; (7) 200 μg; (8) 300 μg; (9) 400 μg. From ref. 26.

a non-solvent for the polymer in many synthetic polymer separations, the polymer sample is injected in a solvent much stronger than the gradient starting composition. This often results in elution of all or part of the polymer as an unretained peak. This type of behavior was reported by Glöckner [66] for RPLC separation of SEMA in which both excluded polymer and a fraction eluting immediately after the solvent front were observed. Lochmüller and McGranaghan [30] studied the use of a low-dispersion mixer on PS RPLC. They succeeded in eliminating the unretained polymer peak while maintaining the same φ_e and polymer peak shape, clearly demonstrating the effect of strong solvent held within the polymer coil.

The effect of temperature has also been suggested to help differentiate polymer retention phenomena [1]. The effect of temperature on adsorption depends on the sign of the entropy of adsorption. For polymer chromatographic retention by adsorption, elution volume is generally expected to increase with increasing temperature [67]. Increasing temperature conformationally favors polymer adsorption since the entropy increase of displacing adsorbed solvent is greater than the entropy loss of adsorbing the conformationally ordered monomer units in the polymer chain [1]. The effect of temperature on solubility is given by eqn. 7 at fixed polymer composition, P, and solvent composition. In general polymer solubility increases with temperature. Precipitation retention would therefore be expected to decrease with increasing temperature. Caution must be used, however, that the polymer and solvent system are not near a lower critical solution temperature (LCST). For LCST systems, solubility increases with decreasing temperature [17].

8.4. *Stationary phase influences*

The effect of silica bonded-phase on RPLC retention of PS was studied by Bui *et al.* [60]. They found relatively little difference between C_2-, C_8- and C_{18}-bonded phases. This is reasonable for high-molecular-mass polymers relative to the model presented in eqn. 19 [58]. The retention of PS was also studied by Glöckner on both C_{18}- and phenyl-bonded silica phases [57] in THF–MeOH gradients. Precipitation retention apparently dominated PS elution as a function of degree of polymerization on C_{18}-bonded silica, but an additional adsorption contribution was found on the phenyl-bonded phase at lower P. Compositional separation of SMMA copolymers was compared on phenyl- and C_{18}-bonded silica in RPLC THF–MeCN gradients by Teramachi *et al.* [68]. RPLC elution of SMMA from C_{18}-bonded silica resulted in carryover artifacts [69]. Significant amounts of copolymer were observed to elute on four subsequent blank gradients after sample injection. Strong adsorption interactions were hypothesized as the cause, but the effect was not definitely explained.

A comparison of cross-linked polymer adsorbents was made by Sato *et al.* [56]. As shown in Fig. 11, the adsorption contribution to retention for RPLC separation of SMMA copolymers changes with adsorbent polarity on cross-linked polymer gels of either acrylonitrile, methyl methacrylate, or styrene. The best separation was obtained on PS gel in RPLC with DCM–MeCN eluent. As the polarity of the cross-linked gel increased, the spacing of φ_e from the solubility limit decreased, indicative of decreasing adsorption contributions. In addition, the peak shapes became increasingly broadened with increasing gel polarity [56]. No RPLC compositional selectivity was obtained for poly(styrene–butyl methacrylate) (both *n*-butyl and *tert.*-butyl) on cross-linked octadecyl methacrylate gel.

9. CONTROL OF POLYMER COMPOSITION SEPARATION BASED ON SPECIFIC PROPERTIES

9.1. *Random copolymers*

The compositional separation of random copolymers by RPLC is based upon the relative mole percent incorporation of the comonomer units, as well as their relative polarity. Sato *et al.* [56] compared the RPLC elution of random and alternating SMMA copolymers on a PS gel column with DCM–MeCN gradients. The elution φ_e was the same for equal molar composition copolymers, indicating no discernable effect from comonomer run length (eqns. 1 and 2). A narrow peak was obtained for the alternating copolymer despite a high dispersity (M_w/M_n = 3.7) demonstrating a much higher selectivity for composition relative to *P*. A comparison of the compositional separation of poly(styrene–*n*-butyl methacrylate) (SnBMA) and poly(styrene–*tert.*-butyl methacrylate) (StBMA) showed only slight difference in retention. Increasing selectivity for StBMA relative to SnBMA was observed with increasing styrene content, consistent with the lower polarity of the *tert.*-butyl ester group.

The compositional separation of random copolymers of SEMA has been discussed above and in Fig. 12 [64]. A strong overlap of compositional and P resolution was noted. The adsorption contribution was less on C_{18}-bonded silica than was observed for similar styrene-alkyl acrylate copolymers on PS gel adsorbent [56]. The stronger the non-polar interactions, the more effectively the separation can be conducted under adsorption rather than precipitation conditions.

9.2. Block copolymers

RPLC separations of random and block copolymers of StBMA on phenyl-bonded silica with THF–MeOH gradients [70] yielded copolymer retention increasing with increasing styrene content. Equal composition StBMA block copolymers were observed to be more retained than random copolymers. That is, the block copolymers were retained as though they contained a higher percent incorporation of styrene. These data are presented graphically in Fig. 14 [71]. Since the three-dimensional solvated structure of a high *P* polymer makes it entropically unfavorable for every monomer subunit to be adsorbed, the adsorption process represents a random sampling of the polymer chain. Therefore, if a copolymer contains long sequence of each comonomer, the adsorption process is energetically more similar to a copolymer with a higher mole percent of the more strongly adsorbed comonomer.

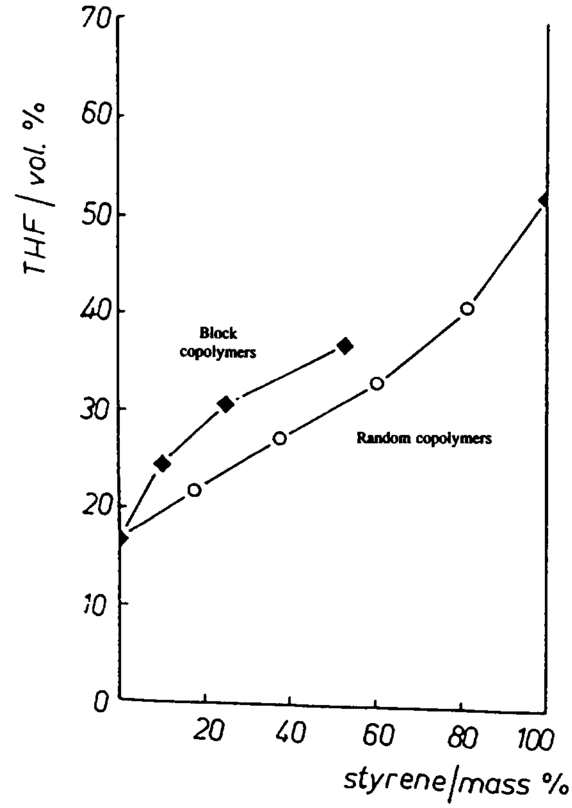

Fig. 14. Elution φ_e of random (○) and block (◆) copolymers of StBMA on 50 Å pore phenyl-bonded 7 μm silica with THF–MeOH gradients (10 to 70% THF in 12 min) at 50°C. From ref. 71.

An important observation associated with this separation of StBMA block copolymers was noted by Glöckner and Wolf [72]. StBMA block copolymers separated by RPLC on C_{18}- or phenyl-bonded silica phases in THF–MeOH eluents showed apparent association of PS with the block copolymer. Separate injection of PS and StBMA block copolymer showed good resolution. Upon mixing PS of similar P (M_r = 130 000) to that of the PS block in the copolymer with the block copolymer sample (M_r = 240 000, 55% tBMA), the PS could not be resolved from the block copolymer by RPLC. Mixing PS 2 000 000 with the same block copolymer resulted in PS elution at the expected retention φ_e. These workers concluded that PS homopolymer became associated with the block copolymer and could not be resolved by RPLC.

9.3. *Graft copolymers*

Graft copolymers represent a class containing both chemical and structural heterogeneity. Polymer side chains of one comonomer are pendant from a backbone polymer of a second comonomer. In most cases these copolymers represent mixtures of backbone and sidechain homopolymers with broad composition distribution graft copolymer [15]. A study of PMMA-graft-PS copolymer composition separation was performed by Teramachi *et al.* [73]. Chemical composition separation could be obtained under RPLC conditions on C_{18}-bonded silica with a THF–MeCN gradient. Composition distributions broader than random copolymers of similar average composition were obtained. Their results indicated increasing chemical heterogeneity with increasing incorporation and decreasing P of the PS side chain. The breadth of the RPLC-determined composition distributions were in general agreement with theoretical predictions, but were somewhat broader than predicted.

9.4. *Configurational isomers*

Successful RPLC composition separations have also been reported for configurational isomers of synthetic polymers. Sato *et al.* [74] separated PMMA configurational isomers based upon differences in tacticity on a cross-linked PS column with a gradient of DCM–nitromethane. Syndiotactic PMMA was observed to be less retained than isotactic PMMA. The retention mechanism was definitively through adsorption interactions as PMMA was soluble in all proportions of the eluent. The resolution of the two isomeric polymers was determined to be greater than that associated with P selectivity by comparison of two different P atactic PMMA samples.

Cis- and *trans*-polybutadienes were resolved by RPLC on a cross-linked PS column with a DCM–MeCN gradient by the same workers [75]. A mixture of polybutadienes varying in their ratios of *cis*-, *trans*- and 1,2-isomeric units were also resolvable, but their elution range overlapped that of the *cis*- and *trans*-isomers. Samples eluted in order of decreasing 1,2-unit incorporation. Comparison with turbidimetric titration results indicated that the polymers eluted very close to their solubility limits, consistent with a precipitation separation mechanism.

10. CONCLUSIONS

A synopsis has been presented of the current understanding of RPLC retention processes controlling the separation of synthetic polymers. Separation may be accomplished based upon the variation of chain length (P), terminal groups, average composition, comonomer sequence, graft composition, or configurational isomerization. The single most significant aspect of polymer structure affecting physical and chemical properties has been shown to be the presence of a large number of covalently linked repeat units constituting the polymer backbone. For such materials, the number of individual chemical species present in even a narrow dispersity synthetic polymer sample is so large as to make complete resolution of its components a practical impossibility.

Oligomer resolution based on chain length or end group functionality is attainable at low degrees of polymerization due to the uniform retention interactions per monomer unit. This behavior is effectively described by the linear free energy relationship of the Martin equation. A monotonic deviation from the Martin equation is observed, however, with increasing P for high-molecular-mass polymers.

Regardless, there is no discontinuous difference in the RPLC retention behavior of oligomers and high P polymers. The solution and adsorption properties of high P polymers become dominated by their limited solubility range, their solvated molecular size, and their three-dimensional solvated conformation. These properties result in many experimentally observed differences between polymer and small-molecule RPLC retention behavior.

The solvated dimensions of high-molecular-mass polymers result in the potential overlap between adsorption and size-exclusion phenomena for conventional porous RPLC adsorbents. Due in part to both the limited solubility of high-molecular-mass polymers and their three-dimensional structure on adsorption, high $d(\ln k')/d\varphi_S$ values for high P polymers result in narrow isocratic elution windows. This results in the variation of polymer elution with readily changed RPLC operating conditions which is very small relative to that observed for small molecules.

Due to the limited solubility of high-molecular-mass polymers, in most situations, polymer RPLC retention is a combination of precipitation and adsorption phenomena. In many cases it is difficult to differentiate which process is controlling retention because of the small adsorption contribution in excess of the solubility borderline. The dominant retention mechanism may change with P or composition when separations are conducted across a broad range. However, the stronger the non-polar interactions, the more effectively RPLC separation can be conducted under adsorption conditions.

For mixed solvents, the solvent composition in the vicinity of the polymer is enriched in the better solvent and the kinetics of exchange can be slow relative to RPLC processes. Similar association behavior between polymer chains can also be observed between block copolymers and similar P homopolymers.

Entropic limitations to the complete unfolding of high P polymers upon adsorption results in multisite adsorption with many loops pendent from the surface. This effect becomes particularly obvious in the differences between random and block copolymer RPLC elution behavior. The adsorption of a block copolymer is observed to be energetically more similar to a random copolymer with a higher mole percent incorporation of the more strongly adsorbed comonomer.

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