



Method development in interaction polymer chromatography

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ABSTRACT

Interaction polymer chromatography (IPC) is an umbrella term covering a large variety of primarily enthalpically-dominated macromolecular separation methods. These include temperature-gradient interaction chromatography, interactive gradient polymer elution chromatography (GPEC), barrier methods, etc. Also included are methods such as liquid chromatography at the critical conditions and GPEC in traditional precipitation-redissolution mode. IPC techniques are employed to determine the chemical composition distribution of copolymers, to separate multicomponent polymeric samples according to their chemical constituents, to determine the tacticity and end-group distribution of polymers, and to determine the chemical composition and molar mass distributions of select blocks in block copolymers. These are all properties which greatly affect the processing and end-use behavior of macromolecules. While extremely powerful, IPC methods are rarely employed outside academic and select industrial laboratories. This is generally because most published methods are “bespoke” ones, applicable only to the particular polymer being examined; as such, potential practitioners are faced with a lack of inductive information regarding how to develop IPC separations in non-empirical fashion. The aim of the present review is to distill from the literature and the author's experience the necessary fundamental macromolecular and chromatographic information so that those interested in doing so may develop IPC methods for their particular analytes of interest, regardless of what these analytes may be, with as little trial-and-error as possible. While much remains to be determined in this area, especially, for most techniques, as regards the role of temperature and how to fine-tune this critical parameter, and while a need for IPC columns designed specifically for large-molecule separations remains apparent, it is hoped that the present review will help place IPC methods in the hands of a more general, yet simultaneously more applied audience.

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1. Introduction

A quick look around us should suffice to demonstrate that polymers, both natural and synthetic, form an integral part of our existence. From providing comfort (e.g., polyurethane foam cushions, cotton and nylon clothing) and safety (e.g., poly(vinyl butyral) interlayer for windshields and hurricane-resistant windows) to their roles in life-saving technologies e.g., acrylate copolymers in nonbiodegradable stent coatings and lactic acid copolymers in biodegradable ones, silicone or polyurethane-based shunts), polymers and polymeric-based materials are ubiquitous and our reliance upon them continues to increase.

To characterize the distribution of chain lengths, and accompanying molar mass distribution (MMD), of polymers, size-exclusion chromatography (SEC) has emerged over the last half-

century as the premier method by which to do so [1–4]. Coupled to a variety and, oftentimes, multiplicity of detection methods, SEC has also shown itself capable of providing information related to average changes in branching, chemical composition, sequence length, and conformation as a function of molar mass (M). For polymers not amenable to SEC analysis because of their large size and concomitant chain fragility, other, gentler techniques have emerged to fill the void, such as hydrodynamic chromatography (HDC) and flow field-flow fractionation (FIFFF) [5–7]. All these so-called size-based methods (SEC, HDC, FIFFF) possess the commonality of separating analytes by their size in solution, i.e., by differences in the hydrodynamic or solvodynamic volume of macromolecules at a given set of solvent and temperature conditions. This commonality is also responsible for one of the shortfalls of size-based methods: Within a given sample, analytes which differ from each other in topology (architecture – e.g., branching – or conformation) or in chemical composition (monomeric ratio or

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monomeric sequence) can occupy the same hydrodynamic volume as each other, resulting in their coelution within a given separation slice.

Addressing the aforementioned shortcomings of the size-based methods, in which separation is primarily entropy-controlled, is a group of techniques which fall under the umbrella term Interaction Polymer Chromatography (IPC) and in which separation is primarily driven by enthalpic forces. These techniques, which include methods such as gradient polymer elution chromatography (GPEC, both traditional and interactive), solvent gradient interaction chromatography (SGIC), temperature gradient interaction chromatography (TGIC), and various so-called “barrier” methods, have the ability to separate polymers according to chemical composition, *inter alia*. They can thus provide, on their own, the chemical composition distribution (CCD) of copolymers and, as the first dimension in a two-dimensional liquid chromatography (2D-LC) set-up with SEC as the second dimension, the combined CCD \times MMD of copolymers and blends (henceforth, when SEC is mentioned other size-based methods are implied, unless otherwise noted).

It is these IPC techniques which are the topic of this review. We examine here their mechanisms of separation and their limitations and focus primarily on rational approaches to method development in IPC. It is the latter, especially, that has suffered from a dearth of information. Published methods appear to be mostly “bespoke,” i.e., developed for a particular sample, primarily using empirical approaches with limited, if any, inductive value to those attempting to adapt the methods to, or to design protocols for, polymers other than the specific ones discussed in a particular publication. Those fundamental books which have been published on the topic [8–11], while an invaluable addition to any polymer chromatographer’s library, are both out-of-print and quite dated as regards examples and current developments.

We include here a discussion of SEC “gradient” methods, part of the family of “barrier” techniques and a relatively novel addition to the IPC family. We discuss also liquid chromatography at the critical condition (LCCC), a technique which capitalizes upon the possibility of a balance between entropic and enthalpic forces during a separation, and which yields compositional information akin to that obtained via the enthalpically-dominated IPC methods.

For readers interested in the historical development of the various macromolecular separation techniques, development of SEC is covered in chapter 1 of [1] and in Ref. [4], of HDC in Ref. [6], and of flow and related FFF methods in Refs. [12]. As regards IPC, a nice, recent introduction can be found in Ref. [13]; more details regarding development primacy of the various IPC methods can be found in several of the book chapters by Berek, most notably in Refs. [14].

2. Macromolecular distributions and heterogeneities

All synthetic macromolecules as well as most natural ones (or, at least, the most abundant natural ones) possess a distribution of chain lengths which, as mentioned above, results in a distribution of molar masses. The breadth of this molar mass distribution (MMD) can affect processing and end-use properties such as elongation, tensile strength, and melt viscosity. Likewise, various processing characteristics of polymers have been correlated to different statistical moments of the MMD; for example, the number-average molar mass (M_n) has been correlated to brittleness and flow properties, the weight-average molar mass (M_w) to tensile

strength and hardness, and the z-average molar mass (M_z) to flex life and stiffness [1].

Besides a distribution of chain lengths, macromolecules may also exhibit distributions in a number of other physico-chemical properties, such as branching (long- and short-chain), chemical composition, tacticity, polyelectrolytic charge, and block sequence or sequence length, or even in base-pair sequence for the case of certain biopolymers or, for particulate matter, in a supramolecular property such as particle size. Some examples of how these properties can influence both processing and end-use of materials are given in Table 1, along with representative separation (and a few closely associated) methods employed for determining property distributions and heterogeneities.

At this point, we try to distinguish between the terms “distribution” and “heterogeneity,” as these are often either conflated, confused with one another, or used interchangeably. Here, “heterogeneity” is understood to describe the change in the average of a particular property of the polymer as a function of the polymer’s size in solution, or as a function of its molar mass uncorrected for so-called “local polydispersity” effects (i.e., uncorrected for the fact that, as mentioned in the previous section, a particular separation slice may contain analytes of different molar mass which, because of architectural and/or chemical differences, occupy the same hydrodynamic volume as each other and thereby co-elute in a size-based separation). “Distributions,” on the other hand, may be either “differential” or “cumulative:” A “differential distribution” denotes the proportion or weight fraction of a population to which a certain property value applies. A “cumulative distribution” gives the proportion or weight fraction of the population for which the property does not exceed a certain value [15]. In Fig. 1 are depicted the chemical heterogeneity, differential and cumulative MMDs, and differential and cumulative CCDs of a generic AB random copolymer, where both chemical heterogeneity and CCD are given for the percentage of compositional monomer A (% A) of the copolymer. As can be seen from this figure, while the MMD of a copolymer may be monomodal, the CCD may be bi- or multimodal (the opposite case of a monomodal CCD and a multimodal MMD, though not depicted in the figure, is also possible). Determination of the CCD requires separation by composition, whereas determination of heterogeneity requires separation by size.

The MMD is usually determined via SEC or some other size-based method coupled to a detection method such as multi-angle static light scattering (MALS) along with concentration-sensitive detection, most commonly differential refractometry (DRI). The chemical heterogeneity can also be determined by SEC, by adding a suitable “chemical” detector to the MALS + DRI combo, where the added detector may be a UV/visible spectrophotometer (for polymers that possess a chromophore), or an IR or NMR spectroscope, for example. The CCD, which has been shown to influence the morphology and miscibility of polymers in blends as well as the solubility of polymers, is usually determined by one of the various IPC techniques or by LCCC.

For a multicomponent mixture, a 2D-LC experiment with IPC in the first dimension and SEC in the second can yield more accurate MMDs for the individual components than would an individual SEC experiment. This is because the latter type of experiment suffers from the potential for local polydispersity effects to manifest themselves in the results, due to the possibility of chemically different (different constituent monomer or comonomer, different block lengths or sequence lengths, etc.) chains coeluting, resulting in inaccurately calculated M averages and MMDs. Employing IPC as the first dimension of a two-dimensional experiment allows the

Table 1
Macromolecular distributions: Their measurement and end-use effects.

Macromolecular Property	Representative Processing or End-Use Property Affected	Separation Method Used for Determination
Molar mass	Elongation, tensile strength, adhesion	SEC, HDC, flow FFF, TGIC, CEC, SFC, MALDI-MS
Long-chain branching	Shear strength, tack, peel, crystallinity	SEC-MALS, SEC-VISC, rheology, enzymology
Short-chain branching	Haze, stress-crack resistance, crystallinity	SEC-IR, SEC-NMR, TREF, ^a CRYSTAF, ^a enzymology
Cross-linking	Gelation, vulcanization, surface roughness	SEC-MALS, SEC-VISC, rheology
"Architecture"	Flow modification, diffusion, encapsulation	SEC-MALS-QELS-VISC
Tacticity	Crystallinity, anisotropy, solubility	SEC-NMR, TGIC, LCCC
Chemical composition	Morphology, miscibility, solubility	GPEC, TGIC
Chemical heterogeneity	Toughness, brittleness, biodegradability	SEC-spectroscopy/spectrometry, LCCC
Chemical composition vs. molar mass	Mechanical properties, blending, plasticization	2D-LC (e.g., SEC-GPEC)
Block sequence	Dielectric properties, reactivity, miscibility	SEC-spectroscopy, 2D-LC
Base-pair sequence	Genetic code, heredity, sequencing, mutations	Automated DNA sequencing, MALD/I-MS
Polyelectrolytic charge	Flocculation, transport, binding of metals	SEC-conductivity
Particle size	Packing, drag, friction, mixing	HDC, FFF (flow, sedimentation, gravimetric), DLS, PSDA, sieving, microscopy

Adapted from Ref. [1]. Many techniques require a concentration-sensitive detector, omitted here for simplicity.

^a For crystalline polymers only.

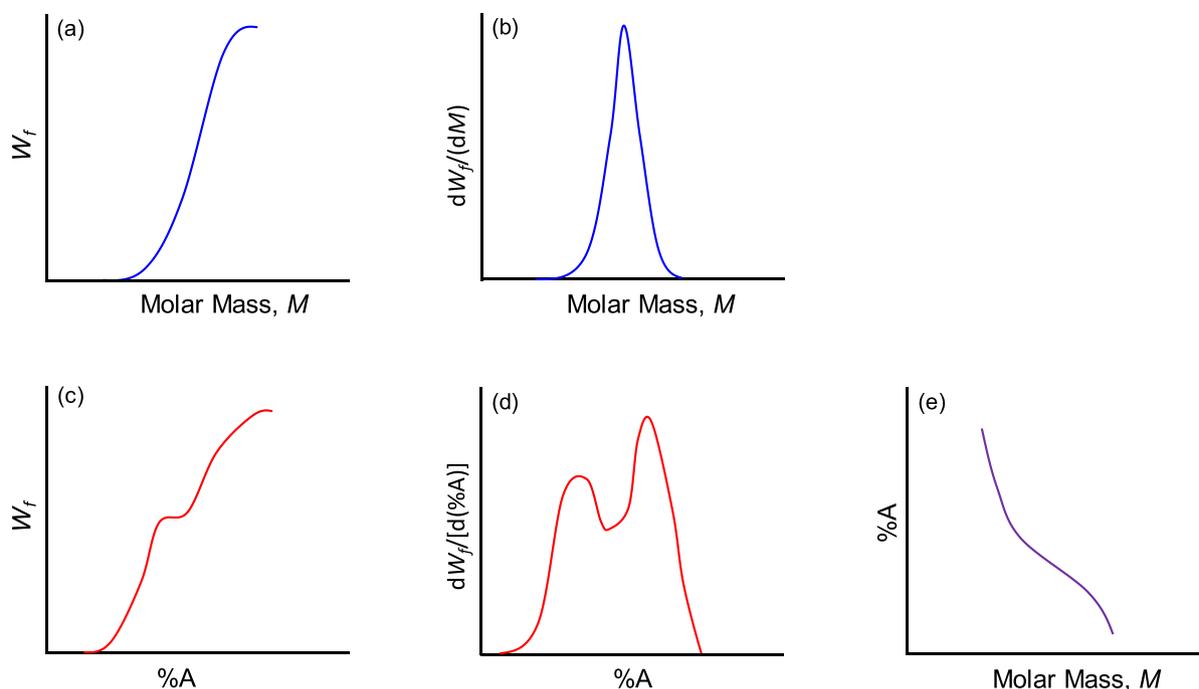


Fig. 1. Generic random AB copolymer with monomodal molar mass distribution (MMD) and bimodal chemical composition distribution (CCD). (a) Cumulative MMD, (b) Differential MMD, (c) Cumulative CCD, (d) Differential CCD, (e) Chemical heterogeneity. W_f : Weight-fraction of species of a given molar mass or chemical composition.

components in the mixture to first be separated by chemistry. Then, in the second dimension, SEC can determine the M averages and MMD of each component (i.e., of each first-dimension peak) with minimized concern regarding the coelution of different components.

Because copolymers are the subject of much academic research and most "real world" materials are comprised of copolymers and blends, the ability to understand the potential and limitations of IPC techniques and the ability to develop methods for particular IPC techniques has become an area of growing importance not only in the field of macromolecular separations, in particular, but also in the more general arena of macromolecular characterization. We begin with method development in gradient polymer elution chromatography (GPEC), as traditionally understood.

3. Gradient polymer elution chromatography (GPEC) – traditional

3.1. Terminology

Given the title of this section it is, perhaps, wise to begin by defining the terms involved. For the separations terms, we rely on the definitions provided by IUPAC [16]:

- **Chromatography:** Physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) and the other (mobile phase) moves in a definite direction.

- *Elution chromatography*: Chromatography in which the mobile phase passes through the chromatographic bed after the introduction of the sample.
- *Gradient elution*: Elution in which the composition of the mobile phase is changed continuously or stepwise.

As regards the term “polymer” and how to distinguish between a polymer and an oligomer, this is discussed rather extensively in chapter 13 of [1], where it is shown that a generally accepted discriminant between polymer and oligomer remains lacking. For the purposes of this review, a polymer will be considered any multimer (i.e., any molecule composed of more than one repeat unit n) wherein end group effects do not change a desirable property when comparing a molecule with n repeat units to one with either $(n - 1)$ or $(n + 1)$ repeat units (e.g., when comparing a 10-mer to either a 9-mer or an 11-mer), or to one wherein the same n repeat units are arranged in an architecturally different manner (e.g., when comparing a linear n -mer to a branched n -mer). Mers in the previous sentence are assumed to be chemically identical. Without wishing to complicate matters further, copolymers will also be assumed to have reached polymeric status (i.e., to no longer be oligomers) once a desirable property becomes constant as a function of n . The most relevant, though of course not the only, “desirable properties” will be the specific refractive index increment ($\partial n/\partial c$), of fundamental importance in refractometry and light scattering, and the absorptivity (a), for its crucial role in UV/visible spectrophotometry.

We use the term “traditional” GPEC to denote a GPEC experiment where the analyte does not, at least in theory, interact chemically with the column packing material. It is referred to as “traditional” because this is the way most GPEC experiments were originally performed, with many still being performed in this manner. This is meant to distinguish from “interactive” GPEC, which will be discussed more fully in Section 5.

3.2. The GPEC experiment and evaporative-type detection

A traditional GPEC experiment relies on the differential solubility of polymers, where solubility differences are due chiefly to differences in the chemical composition of polymer chains. The polymer sample is first dissolved in either a poor solvent or in a solvent mixture that is a poor solvent for the polymer, so that before injection onto the column the sample is already at conditions close to precipitation. The solution is then injected onto the chromatographic column, which should be packed with an inert or non-interacting substrate (packing material) and filled at this time with non-solvent, i.e., filled with a solvent that is unable to dissolve the polymer. Upon reaching the head of the column, the sample then precipitates onto the column packing. The next step is to gradually increase the goodness of the solvent (goodness with respect to chemical functionality of interest; solvent goodness is discussed in Section 5.1). As solvent goodness increases, chains with increasing percentage of the functionality of interest will redissolve and travel through the column to the detector. After all the sample components have eluted, the column is then flushed with non-solvent to prepare it for the next injection. The separation process is shown schematically in Fig. 2.

The experiment employs mixed solvents, where preferential solvation by one solvent over the other generally occurs and can bias results obtained using differential detectors [18]. Use of mixed solvents therefore precludes accurate use of common detection methods such a refractometry, UV/visible absorption, light scattering, or viscometry, among others. The most common type of detector employed, not only in GPEC but in most IPC experiments, is an evaporative-type detector. This class of detectors includes the

so-called evaporative light scattering and evaporative mass detectors, as well as charged aerosol detectors. An in-depth discussion of these, including of the many caveats associated with their use in polymer chromatography, is beyond the scope of the present paper; refs. [19–21] provide excellent reviews. Briefly as regards operation, in evaporative light scattering and evaporative mass detectors upon entering the detector the column eluate is nebulized to form an aerosol, which then enters a heated drift tube (the evaporator) wherein the mobile phase evaporates and leaves behind particles of analyte. These particles then enter the optical cell of the detector, where they interact with a beam from a collimated light source, producing an array of optical phenomena including scattering, reflection, refraction, etc. referring to this instrument as a “light scattering” detector is thus misleading).

The main advantage of evaporative-type detectors in IPC experiments is their ability to handle mixed solvents and, especially, solvent gradients. Main disadvantages are, of course, that these are destructive detectors, precluding the placement of any additional detectors after them or the collection of post-detector fractions; and that the detector operation and response is largely empirically described including, for example, attempts to describe the dependence of droplet size on instrumental parameters.

3.3. GPEC and Hansen solubility parameters

Returning to the description of a traditional GPEC experiment, above, we must now describe how to determine whether or not a particular solvent or solvent mixture will be either a solvent or a non-solvent for a particular polymer. (Caveat: Just because a polymer solution appears clear to the eye does not mean that the polymer is fully dissolved in the solvent, or that it is dissolved in unaggregated form! Light scattering experiments, both dynamic and multi-angle static, can help greatly in this regard; see, e.g., Ref. [22,23] for further information). A common approach to this question is to compare the Hildebrand solubility parameters of analyte and solvent, with closely matched values implying the potential solubility of the former in the latter. Hildebrand solubility parameters are single-value parameters, however. Given that polymer dissolution is generally more difficult than that of small molecules because of the numerous inter- and intramolecular interactions that can occur in macromolecules and the accompanying diversity in molecular forces responsible for these interactions, the three-value Hansen solubility parameters have been found more useful than their Hildebrand counterparts when discussing polymer solubility.

The Hansen solubility parameter δ is comprised of a dispersive term δ_d , a polar term δ_p , and a hydrogen bonding term δ_H , such that:

$$\delta^2 = \delta_d^2 + \delta_p^2 + \delta_H^2 \quad (1)$$

Values for the various terms for numerous solvents and polymers can be found in Ref. [24–26] and in chapter 7 of [1]. Experimental methods by which to determine δ_d , δ_p , and δ_H can be found in Ref. [27–29], respectively.

Here, we follow the approach first described by Hansen [24,28,29], and later adopted by Staal for GPEC [17], of determining a sphere of solubility for a polymer in a particular solvent. Solubility is expected inside this sphere, insolubility outside of it. As such, the radius of the sphere is referred to as the *radius of interaction of the solvent*, R_A^S . This radius is defined as:

$$R_A^S = \sqrt{4(\delta_d^S - \delta_d^P)^2 + (\delta_p^S - \delta_p^P)^2 + (\delta_H^S - \delta_H^P)^2} \quad (2)$$

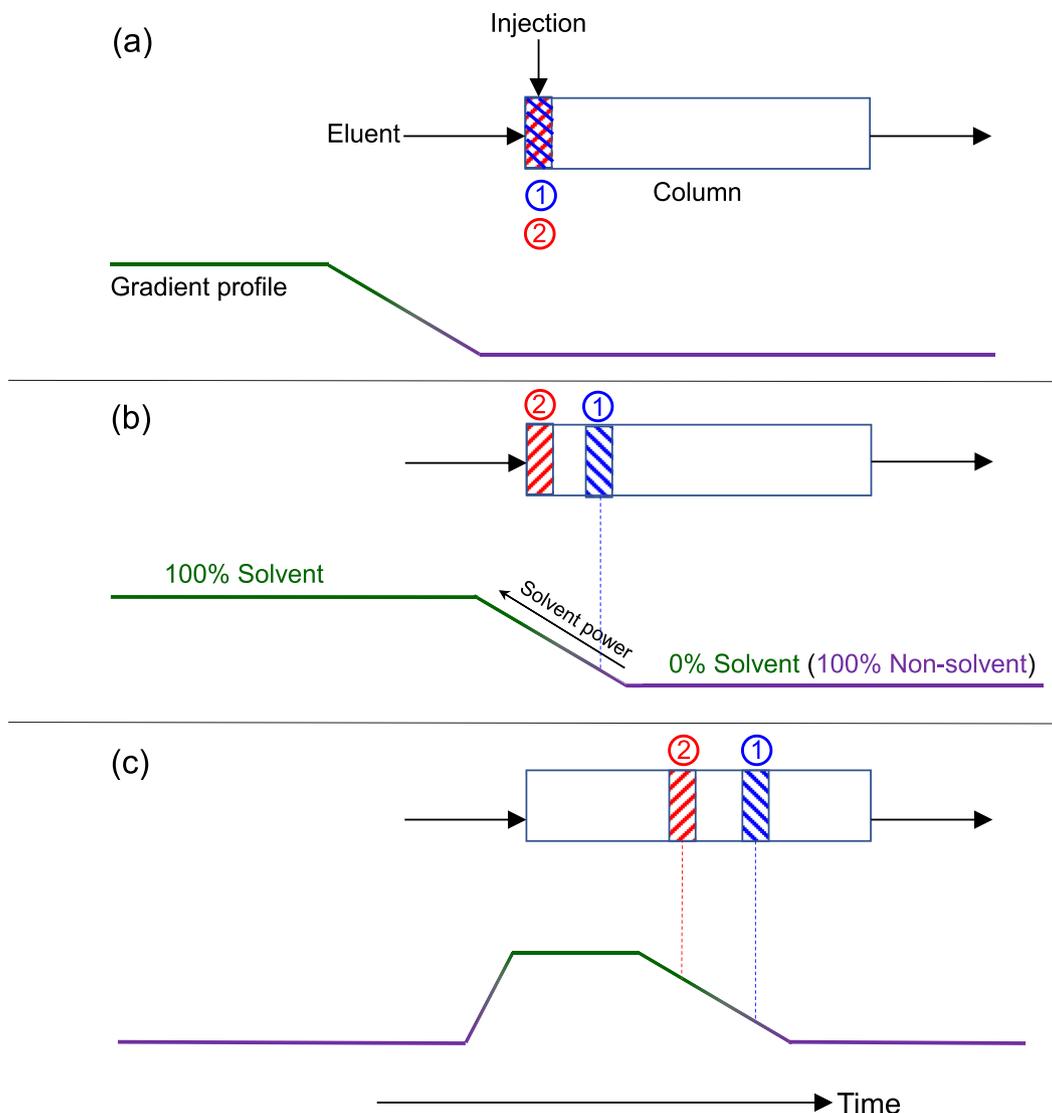


Fig. 2. Precipitation-redissolution process in traditional GPEC. Gradient profiles represent longitudinal solvent composition within the column. (a) Injection of polymer solution into the eluent (non-solvent) and precipitation of solution components 1 and 2 at the head of the column packed in non-solvent. (b) Redissolution and elution of component 1 at early stage of good solvent gradient. (c) Redissolution and elution of component 2 (while component 1 continues to elute) at later stage of solvent gradient, comprising stronger eluent. Adapted from Ref. [17].

where the superscripts *S* and *P* refer to solvent and polymer, respectively, and all other symbols retain their same meaning as above. On a coordinate system defined by the solubility parameters of a polymer (those with superscript *P*) we can then draw a sphere based on the solubility parameters of candidate solvents for said polymer (superscript *S*), as shown in Fig. 3. The radius of this sphere, $R_{A_0}^P$, is known as the *radius of interaction of the polymer*; it is an empirically-determined value based on a best-fit radius in the cloud of observed solvents [29]. Solvents for the polymer will be

located inside this sphere (e.g., pale blue dot a distance R_A^S from the sphere center in Fig. 3, where $R_A^S < R_{A_0}^P$), non-solvents outside the sphere (e.g., green dot a distance R_A^{NS} from the sphere center in Fig. 3, where $R_A^{NS} > R_{A_0}^P$).

For a binary mixture of a solvent and a non-solvent that are 100% miscible in each other, the radius of interaction of the solvent mixture R_A^{mix} (analogous to R_A^S above in the single-solvent case) can be calculated from:

$$R_A^{mix} = \sqrt{4 \left[\left(\Phi^S \delta_d^S + \Phi^{NS} \delta_d^{NS} \right) - \delta_d^P \right]^2 + \left[\left(\Phi^S \delta_p^S + \Phi^{NS} \delta_p^{NS} \right) - \delta_p^P \right]^2 + \left[\left(\Phi^S \delta_h^S + \Phi^{NS} \delta_h^{NS} \right) - \delta_h^P \right]^2} \quad (3)$$

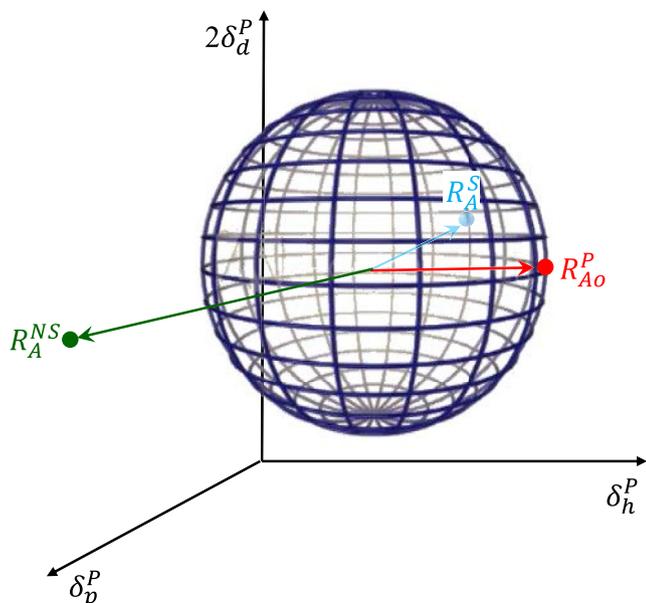


Fig. 3. Schematic representation of the sphere of solubility of a polymer. The radius of interaction of a solvent (R_A^S) is located inside the sphere, that of a nonsolvent (R_A^{NS}) outside the sphere. The radius of interaction of the polymer is $R_{A_0}^P$. The coordinate system is based on the Hansen solubility parameters of the polymer. See text for details.

where Φ^S and Φ^{NS} refer to the volume fractions of the solvent and non-solvent, respectively, and all other terms retain their same meaning as above, with the superscript *S* referring to solvent and *NS* to nonsolvent.

Other approaches to describing solubility parameters can be found in the literature, including four- and five-parameter approaches that seek to account for various dipolar and acid-base interactions [30,31]. It should be noted that all of these approaches, including that by Hansen, fail to adequately address the case of strongly hydrogen-bonding solvents and of water (also, most values for the four- and five-parameter models are not commonly available in the literature for most polymers). For these types of solvents, the Hansen approach provides a first-order approximation, from which corrections need to be made based on observations.

We next describe how to determine the solubility range of a polymer as well as an approximate value for the polymer solubility parameter δ^P . First, one runs two gradient experiments employing the same polymer and a solvent/non-solvent mixture where the solvent is the same in both cases. One case will employ a non-polar non-solvent in the mixture and the other case a polar non-solvent; in each case, the GPEC column will be packed with the non-solvent when the polymer is injected onto it. After precipitation of the analyte onto the column head, the percentage of solvent is increased in each case. Once the polymer elutes, the apex of its peak in the chromatogram for the solvent/polar-non-solvent case represents δ_{po}^{mix} , the apex of the polymer's peak in the chromatogram for the solvent/non-polar-non-solvent case represents δ_{np}^{mix} . The region between these values corresponds to the *range of solubility of the polymer*. The solubility parameter of the polymer δ^P can be approximated as the midpoint between δ_{po}^{mix} and δ_{np}^{mix} , i.e., as:

$$\delta^P \approx \frac{(\delta_{po}^{mix} + \delta_{np}^{mix})}{2} \quad (4)$$

To determine δ_{po}^{mix} and δ_{np}^{mix} , one simply notes the volume fractions of the solvent and the non-solvents at the points corresponding to the apexes of the polymer's peak in the two chromatograms mentioned above. The solubility parameter of each mixture of solvent and non-solvent (polar or non-polar) is calculated using the rule of mixtures [32], as per:

$$\delta^{mix} = \Phi^S \delta^S + \Phi^{NS} \delta^{NS} \quad (5)$$

This same rule of mixtures can be applied to determine the dispersive, polar, and hydrogen bonding terms of δ^{mix} , individually.

3.4. An Object Lesson [33]

As an example of the application of GPEC for the separation of homo- and copolymers based on their chemistry, Staal examined a blend of poly(methyl methacrylate) or PMMA, poly(styrene-co-acrylonitrile) or SAN, polystyrene or PS, styrene-butadiene-styrene rubber or SBS, and polybutadiene or PB [33]. The main aims of the project were to obtain a quick impression of the quality of the separation and to characterize the polymers in terms of solubility parameters. To determine the solubility range of the polymer, as well as to assess the quality of the separations, two gradients were run, with tetrahydrofuran (THF) as the solvent in both cases and water as the polar ($P' = 10.2$) non-solvent in one case and isooctane ($P' = 0.1$) as the non-polar non-solvent in the other.

Cloud points were obtained both chromatographically and titrimetrically. Calculation of cloud points was performed employing Hansen solubility parameters. The shell of the solubility sphere of a polymer was determined by the transition from polymer solubility to insolubility, i.e., by the cloud point area. Addition of non-solvent to a solvent moved the solvent coordinate of the mixture toward the shell of the sphere. Outside this shell, the polymer is insoluble. On the shell, i.e., at the cloud point, the radius of interaction of the solvent/non-solvent mixture (R_A^{mix}) is equivalent to the radius of interaction of the polymer ($R_{A_0}^P$). At the sphere boundary, it is the volume fraction of non-solvent (Φ^{NS}) that is the unknown parameter. At this point, the cloud point composition can be calculated using equation (3) above [17].

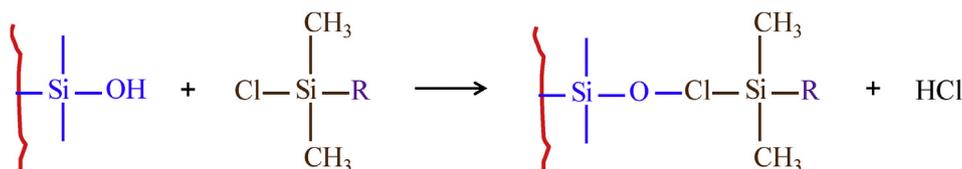
With few exceptions (polycarbonate, or PC, and SBS), correlation between the two sets of cloud points was good. For PC in the isooctane/THF gradient, with the poor solvent (THF) PC tended to adsorb onto the cyanopropyl sorbent. For SBS in this same gradient, PB has strong affinity for polar sorbents when dissolved in isooctane, while PS tends to adsorb when dissolved in non-polar solvents. It therefore appeared likely that SBS would adsorb onto the cyanopropyl sorbent. 19% THF was required to remove the copolymer from the sorbent.

Titrimetrically-obtained cloud points predicted that the elution order in the water/THF gradient would be opposite that in the isooctane/THF gradient, which was also observed experimentally. This supports the contention that elution in GPEC is dominated by a solubility mechanism. Thus, while the polar SAN copolymer was repelled by the non-solvent isooctane and eluted late in the isooctane/THF experiment, this copolymer's attraction to water resulted in its early elution (relative to PS, SBS, or PB) in the water/THF experiment (see Fig. 4). From a practical point of view, by applying cloud points obtained by titration the chromatographic separation could be predicted, and the exceptions explained.

For a more complete description of the traditional GPEC process and of the theoretical background underlying the discussions in this section, including applications, the reader is referred to the work of Staal, in particular to Ref. [17].

4. "Interactive" polymer LC

In interactive polymer LC, macromolecular retention is governed by analyte sorption onto active sites of the chromatographic column's stationary phase, as dictated by the polarity of the solute, mobile phase, and stationary phase. The polymer must want to be in the stationary phase more than it wants to be in the mobile phase; therefore, mobile phase polarity relative to that of the stationary phase is also important in this type of analysis (as we shall soon see, it is this relative relation which dictates whether a sep-



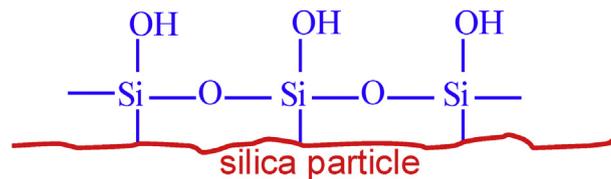
aration proceeds via a normal-phase or a reversed-phase mechanism). Desorption of the analyte from the stationary phase will depend on the "strength" of the mobile phase, as discussed later in this section.

It is very difficult to elute interacting disperse polymers isocratically, i.e., employing a fixed solvent composition. As such, interactive polymer separations usually require long solvent gradients. In this section we will attempt to provide answers for why gradients are needed (i.e., why polymers can't be eluted isocratically), what factors contribute to the length of these gradients, and why different types of gradients are sometimes preferred. The answer to all these questions lies in the very nature of polymers.

4.1. Liquid-liquid partition chromatography

Generally, liquid-liquid partition chromatography employs "bonded-phase supports," wherein molecules comprising the stationary phase are covalently bonded to a silica or silica-based support. The most popular bonded phases are siloxanes, made by

heating silica particles in dilute acid for one or more days to generate reactive silanol groups:



The above is then treated with an organochlorosilane:

These bonded phases are quite stable between pH 2 and 9 and at temperatures up to approximately 80 °C. The surface polarity of the phases is determined largely by the nature of the R group which is, most commonly but certainly not exclusively, C18. Lists of the different phases, including properties and typical applications, can be found in most LC column manufacturers' supplies catalogs.

4.2. Normal- and reversed-phase LC

The most commonly employed modes of polymeric LC remain normal- and reversed-phase chromatography (though hydrophilic interaction liquid chromatography, or HILIC, has seen increased application in recent years). In normal-phase liquid chromatography, or NP-LC, the stationary phase is more polar than is the mobile phase. Generally, highly polar stationary phases and relatively non-polar mobile phases are employed in NP-LC, so that the least polar solute elutes first and the most polar one last. Increasing the polarity of the NP-LC mobile phase generally results in decreased retention times.

In reversed-phase liquid chromatography, or RP-LC, the mobile phase is more polar than is the stationary phase. General practice is to employ a polar mobile phase and a non-polar stationary phase. Polar analytes will thus prefer being in the mobile phase over the stationary phase and will elute first, ahead of their non-polar counterparts. Interestingly, increasing the polarity of the mobile phase usually results in larger retention times. It appears that the driving force for retention in RP-LC is derived less from solute-stationary-phase interactions that it is from the ability of the polar mobile phase to "force" solute into the non-polar stationary phase.

A few of the more commonly used stationary phases for adsorption chromatography are classified according to their polarity in Table 2.

4.3. Solvent "strength" and eluotropic series

"Strength" is a relative term for solvents. "Stronger" mobile phases provide for a smaller value of the retention factor k which, for generic analyte Pol is defined as:

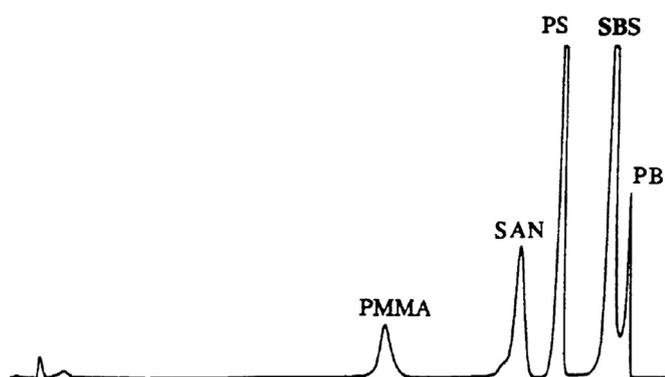


Fig. 4. GPEC chromatogram of blend of PMMA, SAN, PS, SBS, and PB. Concentration: 10 mg mL⁻¹; injection volume: 10 μL; flow rate: 1 mL min⁻¹; linear gradient from 100% H₂O to 100% THF in 15 min; detection: UV, λ₀ = 350 nm; column: Nova-Pak CN, 3.9 mm × 75 mm, 60 Å pore size, 4 μm particle size; temperature: 40 °C. See Refs. [33] for details.

Table 2
Classification of stationary phases in adsorption chromatography.

Non-polar	Medium-polar	Polar
Styrene-divinylbenzene	Cross-linked hydroxyethyl methacrylate	Silica gel
<i>n</i> -Octadecyl (C ₁₈)	Agarose	Aluminum oxide
<i>n</i> -Octyl (C ₈)	Cyanopropyl	Nitrophenyl
Phenyl	Diol	Aminopropyl

Sources: Refs [34,35].

$$k_{Pol} = \frac{K_{D,Pol}V_S}{V_M} \quad (6)$$

where $K_{D,Pol}$ corresponds to the solute distribution coefficient of analyte *Pol* and V_S and V_M to the stationary phase and mobile phase volumes, respectively (noting that V_M is oftentimes referred to as the “dead volume” of a column). In RP-LC, water is therefore considered a “weak” solvent while organic solvents are “strong” solvents. These trends are reversed in NP-LC, where more polar solvents are considered “stronger.”

Solvent strengths can be quantitated as *elutropic values*, ϵ^o , corresponding to the free energy of adsorption per unit surface area of solvent. The order in an elutropic series of solvents depends upon stationary phase polarity. Therefore, the elutropic values of solvents need to be determined experimentally for each stationary phase. Series rankings for alumina, silica, and C18 can be found in the literature, with some values provided below in Table 3.

For a mixture of solvents A and B, the elutropic strength ϵ of the mixture is given by equation (7) [37]:

$$\epsilon = \epsilon_A^o + \frac{\log(N_B 10^{n_B(\epsilon_B^o - \epsilon_A^o)} + 1 - N_B)}{n_B} \quad (7)$$

where ϵ_A^o and ϵ_B^o correspond to the elutropic values of neat solvents A and B, respectively, N_B corresponds to the mole fraction of solvent B in the mobile phase, and n_B corresponds to the relative size (area) of solvent B. As regards this last term, values (given relative to that of benzene as solvent B, for which $n_B = 6$) can be found in the literature for various solvents, with n_B values for common solvents given in Table 3 above.

4.4. Why are gradients needed for interactive polymer separations?

In a multicomponent system, at a given set of experimental conditions, each component will have its own solute distribution coefficient K_D . Given the relation between K_D and the retention factor k , given by equation (6) above, this means that each component in the sample will have its own k . To optimize a separation, one desires a k value, or values, that work for each and every component of a sample.

Let us look at the case of a mixture of benzene ($M = 78 \text{ g mol}^{-1}$), insulin ($M = 9000 \text{ g mol}^{-1}$), cytochrome *c* ($M = 13,000 \text{ g mol}^{-1}$), and a nonapeptide ($M = 1400 \text{ g mol}^{-1}$), where the relation between the retention factor k and the percentage of acetonitrile (% ACN) in the buffer mobile phase is shown in Fig. 5. From the figure one may deduce that any given % ACN in the range of $\approx 22.5\%$ – 30.5% would be sufficient to elute both benzene and the nonapeptide; i.e., separation of both components could be performed isocratically, in reasonable time, at $k \approx 20$ to 25 (actual percentages are not important, only how they relate to one another and to the respective analytes for the purposes of this example). The % ACN range over which both benzene and insulin elute is narrower, from $\approx 24.5\%$ to 31.75% , meaning that we are now more restricted with respect to the

value of % ACN we can employ in an isocratic separation. For the separation of benzene and cytochrome *c*, this range is even narrower, from $\approx 29.75\%$ to 33% . Were one to use a % ACN of between $\approx 32\%$ and 33% , however, only cytochrome *c* would elute, but not insulin. Conversely, a % ACN of between $\approx 25\%$ and 29% would elute only insulin, but not cytochrome *c*.

Fig. 5 shows a number of discrete, monodisperse species, each with a given degree of polymerization and, therefore, a given molar mass M . Macromolecules, as mentioned in the Introduction, are disperse, possessing a molar mass distribution (MMD) comprised of a large number of individual species (chains) each of which differs from each other with respect to molar mass. As seen in Fig. 6, in oligomers and, especially, polymers, there is a systematic change in analyte retention as the size of the solute increases, with the relation between the retention factor and the

Table 3
Elutropic values of solvents, ϵ^o . Relative size of solvent B, n_B , in a solvent mixture.

Solvent	ϵ^o			n_B
	On Alumina	On Silica	On Octadecylsilica	
Acetone	0.56	0.53	8.8	4.2
Acetonitrile	0.65	0.52	3.1	3.1
Benzene	0.32	0.25		6.0
<i>n</i> -Butyl alcohol	0.7			
<i>n</i> -Butyl chloride	0.30			
Carbon tetrachloride	0.18	0.11		5.0
Chlorobenzene	0.30			6.8
Chloroform	0.40	0.26	11.7	5.0
Cyclohexane	0.04	0.03		6.0
Cyclopentane	0.05			5.2
<i>n</i> -Decane	0.04			10.3
Dimethyl formamide		0.51	7.6	
Dimethyl sulfoxide	0.62	0.41		4.3
1,4-Dioxane	0.56	0.51	11.7	6.0
Ethyl acetate	0.58	0.48		5.2
Ethyl alcohol	0.88	0.69	3.1	3.8
Ethyl ether	0.38	0.43		4.4
Ethylene dichloride	0.49	0.38		4.8
Formamide		0.55		
Heptane	0.01	0.01		
Hexane	0.01	0.01	0.00	
Iso-octane	0.01	0.01		6.7
Isopropyl alcohol	0.82	0.60	8.3	4.4
Methanol	0.95	0.70	1.0	3.7
2-Methoxyethanol	0.74			
Methyl acetate	0.60			4.8
Methyl- <i>t</i> -butyl ether	0.3–0.4	0.48		4.1
Methyl ethyl ketone	0.51	0.39		4.6
Methyl isobutyl ketone	0.43			5.3
Methylene chloride	0.42	0.30		4.1
Pentane	0.00	0.00		7.6
<i>n</i> -Propyl alcohol	0.82	0.60	10.1	4.4
Pyridine	0.71	0.55		5.8
Tetrahydrofuran	0.45	0.53	3.7	5.0
Toluene	0.29	0.22		6.8
Trichlorotrifluoroethane	–0.25	0.02		
2,2,2-trifluoroethanol		0.62		
Water	Large	0.72	Small	
<i>o</i> -Xylene	0.26			7.6

For n_B , values relative to $n_B = 6$ for benzene as solvent B. Adapted from Ref. [10,36,37].

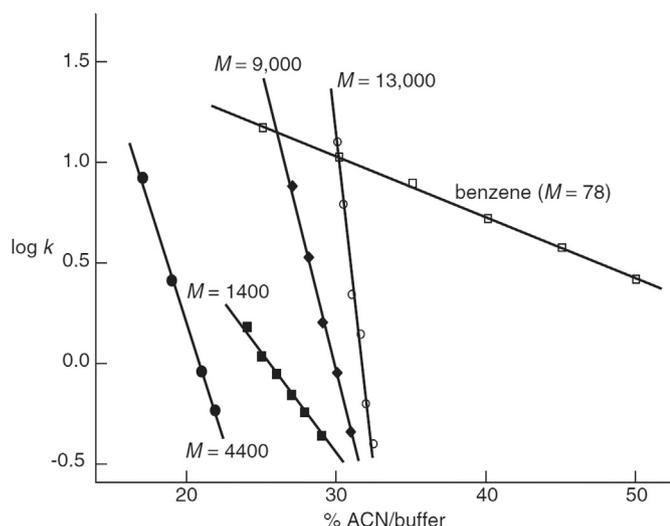


Fig. 5. Change in isocratic retention k with change in % B as a function of analyte molar mass. (Reprinted with permission from Refs. [38]).

percentage of solvent B in the mixture becoming increasingly steep with increasing degree of polymerization (the latter denoted as p in Fig. 6). This means that for large polymers, or for the larger chains of a polymer with a broad MMD, there will be only a very narrow range of mobile phase composition (narrow range of % B) over which the polymer will elute isocratically. To elute the entire MMD of a broadly disperse polymer, one needs to employ a range of % B over the course of a separation, i.e., one needs to employ a solvent gradient.

4.5. What type of gradient to employ?

In small-molecule separations, gradients are employed to (a) optimize retention (optimize K_D or k), (b) improve chromatographic resolution, and (c) shorten run time (t_G). In these types of separations, the gradients employed are usually linear. Polymer LC also employs mostly linear gradients, though occasionally segmented gradients are also used, both in combination with step gradients and/or gradient delays. This is done to effect retention, provide some gain in chromatographic resolution, clean late-eluting compounds from the column, and restore the column to its starting value (to “flush” the column). These and other types of gradients (concave, convex) are shown in Fig. 7. An explanation for their use in IPC follows.

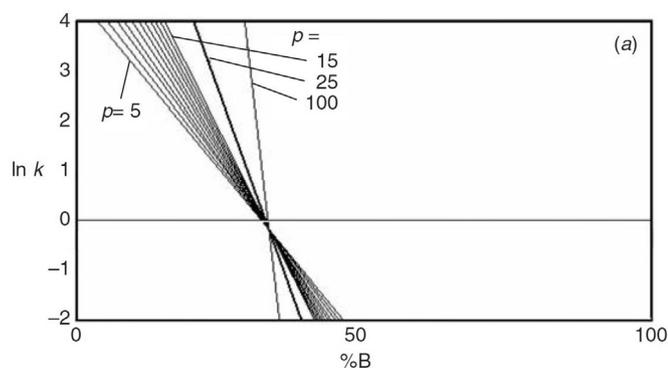


Fig. 6. Macromolecular retention in IPC. Retention behavior of a series of homopolymers, each of degree of polymerization p . (Reprinted with permission from Ref. [37]).

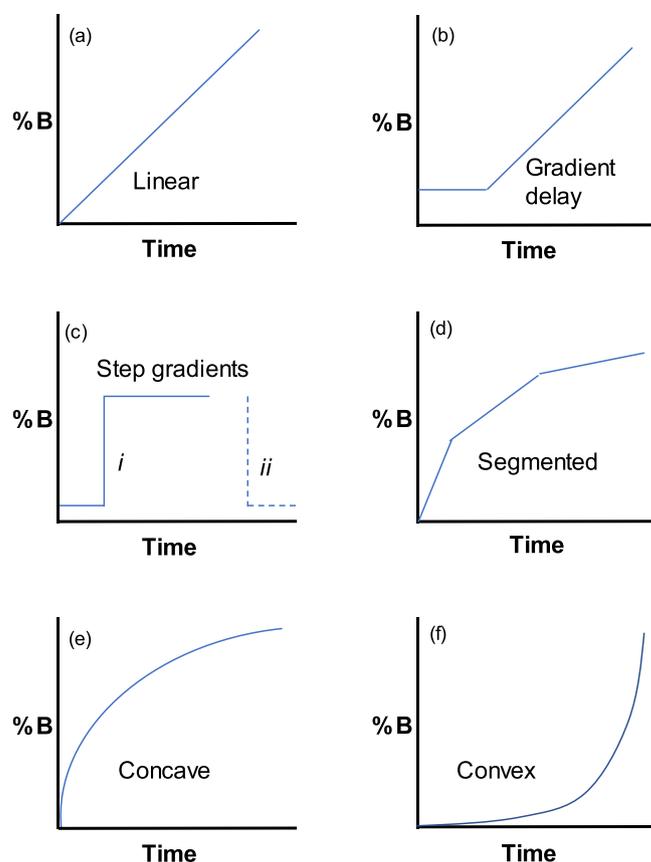


Fig. 7. Different types of solvent gradients (as % B versus time).

While there is some disagreement in the IPC literature as to whether linear or segmented gradients are to be preferred, the use of linear gradients (Fig. 7a) during method development is strongly recommended. In the final method, the case for linear gradients can be seen in Fig. 6: Here, the retention of each species (each chain in a polymer sample) corresponds to an intersection of the various diagonal lines in the figure with a horizontal line corresponding to a given value of k^* (which, as shall be seen in Section 4.6, is equivalent to k in gradient elution). Higher values of k^* (horizontal lines with larger y -intercept) correspond to larger differences in retention time for adjacent peaks, i.e., to better chromatographic resolution, but also to longer gradients, as compared to smaller values of k^* (smaller y -intercept), which result in faster, but lower resolution gradients. A horizontal line in Fig. 6 corresponds to one value of $\ln k$ only, i.e., to only one value of k^* . Thus, there is in this case no separation of adjacent peaks, as both peaks will have identical k^* to each other.

Some have suggested the use of segmented gradients (Fig. 7d) to improve resolution by adjusting chromatographic selectivity for different portions of a separation. Taking the example of Fig. 5, a gradient from $\approx 25\%$ to $\approx 33\%$ ACN, at constant $\Delta(\% \text{ ACN})/\Delta t$ (i.e., at a constant change in percent acetonitrile with change in time), will show favorable separation conditions for benzene throughout all of the separation, for the nonapeptide for most of the separation, for insulin for approximately half of the separation, and for cytochrome c for less than one-quarter of the separation. If, however, $\Delta(\% \text{ ACN})/\Delta t$ is not constant but, rather, it adopts different, smaller values in different, longer-time regions (i.e., the gradient slows down as time progresses), in the given example this is expected to provide for more time in regions of the separation favorable to insulin and cytochrome c . In other words, segmented gradients are expected to

improve the resolution of high-molar-mass solutes. However, given that segmented gradients are usually less reproducible than linear ones when transferred to different hardware, and given the mostly limited advantages of segmented gradients over their linear counterparts (with certain specific exceptions, such as the need to separate a critical pair of peaks in a multicomponent mixture), the use of linear gradients as outlined in the previous paragraph can generally be recommended in IPC, both for method development as well as in the final method.

Steps can be used in a gradient (step gradients, which can come at the beginning and/or end of a linear or other type of gradient) to clean late-eluting compounds from the column, which can be done with a sudden increase in % B (step *i* in Fig. 7c), or to recondition the column back to its original state. The latter can be effected by a sudden decrease in % B (step *ii* in Fig. 7c). This type of sudden decrease is not a concern with silica-based columns, which generally possess excellent mechanical stability, but is not generally recommended (nor is a sudden increase in % B) when using the more fragile polymer-based columns.

The use of curved gradients (concave and convex, Fig. 7e and f, respectively) has fallen into disfavor, as segmented gradients have been found to provide most of the same advantages while being easier to design and to replicate. As such, curved gradients are mentioned here only for the sake of completeness.

4.6. Why can IPC gradients become so (damn) long?

In NP-LC isocratic separations, retention is given as a function of % B by [37]:

$$\log k \approx \log k_B - n \log \phi \quad (8)$$

where ϕ is the volume fraction of solvent B in the mobile phase (and not to be confused with the phase ratio of a column, for which the same symbol is employed in Section 6), k_B is the value of the retention factor k when pure B solvent is used as mobile phase (i.e., 100% B), and n is the number of B solvent molecules displaced by solute (approximately equal to the number of polar substituent groups in a molecule of solute).

In isocratic RP-LC, the relation between retention and % B can be expressed as [37]:

$$\log k = \log k_w - S\phi \quad (9)$$

where, once again, ϕ is the volume fraction of solvent B in the mobile phase, and k_w is the extrapolated value of k for $\phi = 0$ (% B = 0). The parameter S corresponds to the change in $\log k$ for a unit change in ϕ (assuming isocratic elution) and can be considered a constant for a given compound when only ϕ is varied. When $M < 500 \text{ g mol}^{-1}$, $S \approx 4$. In general, $S \approx \sqrt{M}/4$ [37].

When employing gradient separations, retention is given by equation (10) [37]:

$$k^* = \frac{0.87t_G F}{V_m(\Delta\phi)S} \quad (10)$$

where k^* is the retention factor of the gradient (equivalent to k for isocratic separations), t_G is the gradient time, F is the volumetric flow rate, V_m is the column dead-volume, $\Delta\phi$ is the gradient range, and S remains as defined above. Let us focus a bit more on the relation $S \approx \sqrt{M}/4$, however.

In Fig. 5, S is represented by the slopes of the various straight-line fits. As can be seen, these slopes become steeper as the molar mass M of the analytes becomes larger. Fig. 8 shows the SEC

chromatogram of a typical broad-dispersity linear polystyrene (PS), overlaid upon which is the relation between M and retention volume. The so-called “good data” region of molar mass can confidently be found between approximately $4 \times 10^4 \text{ g mol}^{-1}$ and $2 \times 10^6 \text{ g mol}^{-1}$, i.e., this range of M should cover most of the polymer’s MMD.

From equation (10), we see that $t_G \propto k^* S \approx k^* (\sqrt{M}/4)$. When developing a gradient for small-molecule ($S \approx 4$) separations, one generally aims for $k^* \approx 5$. Taking this latter value as our guide, we see that to elute a $2 \times 10^6 \text{ g mol}^{-1}$ analyte at $k^* = 5$, t_G must be increased by a factor of $354/4 \approx 90$. Even for a $4 \times 10^4 \text{ g mol}^{-1}$ PS chain, lying at the lower end of the MMD of the polymer in Fig. 8, t_G must be increased by a factor of ≈ 12 to ensure $k^* \approx 5$. If the gradient for an equivalent small (low M) molecule lasted 10 min, that for the $4 \times 10^4 \text{ g mol}^{-1}$ analyte would last 2 h!

Clearly, IPC would *not* be employed for the separation, by molar mass, of the PS shown in Fig. 8. As stated by Chang, “... isocratic (constant eluent composition) or isothermal [interaction chromatography] elution of a polymer sample having a wide [molar mass distribution] is practically impossible” [13]. It is, however, instructive to see how t_G depends on M in gradient LC and the large influence macromolecular M has on the length of gradients. Other factors in equation (10) can be adjusted to shorten t_G , such as increasing F (though this may well be limited by the potential for on-column, flow-induced degradation of macromolecules [39–43], which is not a concern in small-molecule separations) or decreasing $\Delta\phi$ (if chemical composition permits). Also, the y -intercept of the horizontal line in Fig. 6 may be adjusted to find a suitable value of k^* for the particular separation being attempted.

4.7. “Interactive” polymer LC summary

In “interactive” polymer LC, retention is governed mainly by sorptive-desorptive interactions between the solute and the stationary phase, as mediated by the mobile phase. Consequently, the choice of both mobile and stationary phase is critical, with this type of polymer chromatography showing strong similarity to small-molecule LC.

The polarity of the mobile phase, with respect to that of the stationary phase, is important as it will dictate the “mode” (NP, RP, other) of the separation. Solvent “strength” is equally important, as it dictates the extent of an analyte’s preference for being in the mobile phase vis-à-vis the stationary phase. Not discussed yet is the thermodynamic “quality” of the solvents, i.e., whether a solvent or, more appropriately, a set of solvent and temperature conditions may be considered good, poor, or theta for a particular polymer. This will be discussed in section 5.1 below.

Interactive polymer separations usually require gradients. The gradients themselves are needed to allow the separation of all the analytes (or all the relevant analytes) in a sample, by covering the range of k of a disperse polymer. Interactive separation by molar mass is not usually performed, as this type of separation would require long gradients. These long gradients are the result of needing to provide for acceptable levels of k ; if lower values of k are acceptable in a molar-mass-based separation, then the gradient time can be lowered. It is generally recommended that linear gradients be used for method development and, if at all possible, in the final application of a method. Segmented gradients should be employed sparingly, e.g., for the separation of a critical solute pair in a multicomponent sample. As we shall see in Section 6.1, segmented gradients are used often in TGIC.

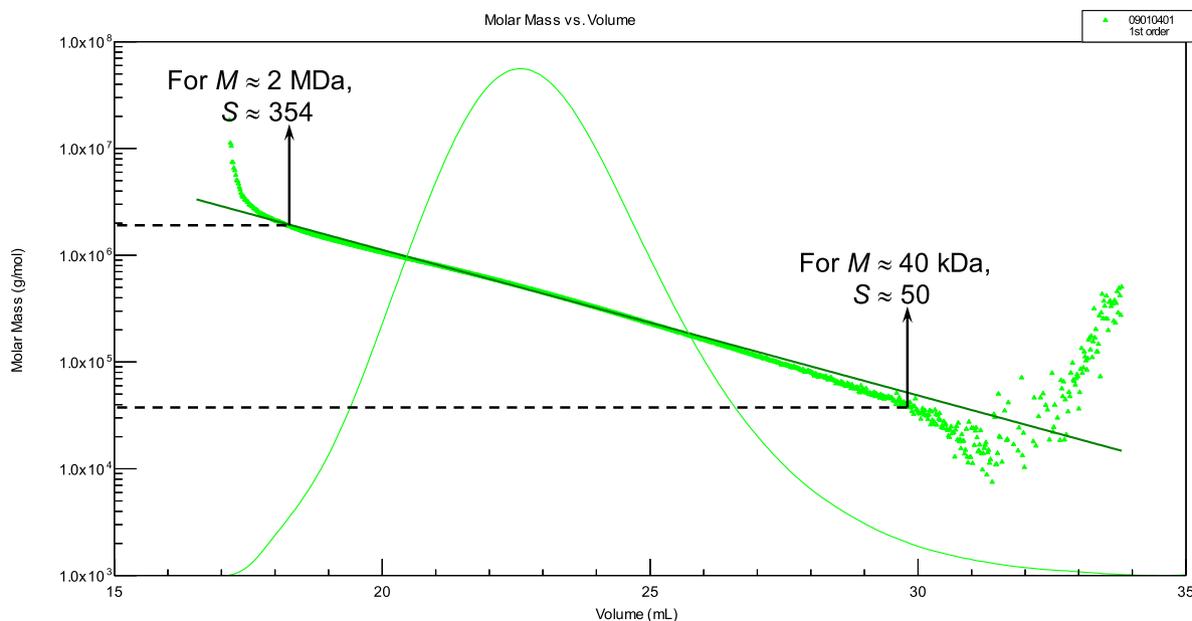


Fig. 8. SEC chromatogram of broad MMD linear PS (solid green curve), with overlaid molar mass versus retention volume relation (filled green triangles; straight line represents linear fit to these data). Columns: Set of three PSS GRALinear 10 μm particle size columns and one PSS GRAL1000 10 μm particle size column, preceded by a guard column; solvent: *N,N*-dimethyl acetamide +0.5% LiCl; temperature: 35 $^{\circ}\text{C}$; flow rate: 1 mL min^{-1} ; detectors: Wyatt DAWN E MALS and Optilab DSP DRI. (Adapted from Ref. [4]).

5. “Interactive” GPEC (a.k.a. solvent gradient interaction chromatography)

As described in Section 3.2, “traditional” GPEC relies on precipitation-redissolution (phase separation or solubility) phenomena to effect separation based on chemical composition. It is generally considered to be a low-resolution chromatographic method. “Interactive” LC relies on sorption-desorption phenomena to effect these (and other types of) separations. In the latter method, long solvent gradients are generally needed.

“Interactive” GPEC (referred to, of late in the polyolefin chromatography literature, as solvent gradient interaction chromatography or SGIC) combines the two types of methods in an attempt to improve the resolution of the former and shorten the analysis time of the latter. Both the strength and thermodynamic quality of the mobile phase are important, as is also the polarity of the stationary phase.

We will attempt to understand the principles of “interactive” GPEC via an object lesson. First, though, a brief discussion of polymer dissolution and solvent “quality.”

5.1. Polymer dissolution and thermodynamic solvent “quality”

Why do certain solvents dissolve certain polymers? This was originally addressed in Section 3.3, where it was mentioned that the Hildebrand and, even, Hansen approaches fail to adequately

explain the case of strongly hydrogen-bonding solvents, *inter alia*. This shortfall also applies regarding the solubility of not only macromolecules with a large degree of intra- and/or intermolecular H-bonding, but also to polymers where “synergistic” inter/intra-molecular non-covalent bonding effects other than H-bonding may occur. An example of the first type is cellulose, which possesses a wide net of both inter- and intramolecular H-bonds, rendering its dissolution quite challenging. While dimethylformamide and dimethyl sulfoxide have solubility parameters in the range calculated for cellulose, neither one of them is known to dissolve this polysaccharide [44].

The length and strength of various types of bonds are given in Table 4. As seen, dipole interactions are weaker than those due to H-bonding. Even so, dissolution of polyacrylonitrile (PAN) is rendered difficult by the fact that dipole interactions occur at the nitrile group of every other carbon in a PAN chain. van der Waals interactions are even weaker than dipole ones. Nevertheless, the fact that the former occur at every methylene group in linear polyethylene, thus binding chains together at every repeat unit, results in a polymer for which a limited number of solvents exist and which necessitates elevated temperature for dissolution and to maintain solubility (the effect of temperature on dissolution is addressed below).

Polymer dissolution in a given solvent requires a negative Gibbs free energy of solution, $\Delta G_{\text{solution}}$. Main factors considered in dissolution are the contributions from polymer crystallinity, derivatizing and/or complexing, and mixing [44]:

Table 4
Bond parameters.

Bond Type	Dissociation Energy (kcal mol^{-1})	Interatomic distance (nm)
Primary covalent	50 to 200	0.1 to 0.2
Hydrogen bond	3 to 7	0.2 to 0.3
Dipole interaction	1.5 to 3	0.2 to 0.3
van der Waals bond	0.5 to 2	0.3 to 0.5
Ionic bond	10 to 20	0.2 to 0.3

Source: Ref [45].

$$\Delta G_{\text{solution}} = \Delta G_{\text{fusion}} + \Delta G_{\text{reaction}} + \Delta G_{\text{mixing}} \quad (11)$$

For a polymer to dissolve in a given solvent, it is only necessary for the sum of these three factors to be negative, not for each individual one to be so (this is a theme that will be revisited several times during the course of this paper). Here, we will ignore the effects of complexation or derivatization, as this usually creates a different moiety with which to deal and which can exert its own effect on other parts of the dissolution equation. For many nonpolar polymers (e.g., polypropylene), crystalline forces are overcome by input of heat, resulting in a molten polymer more amenable to dissolution once the crystalline structure has been destroyed. (It should be noted that dissolution of certain high- M crystalline polymers may not follow Fick's second law of diffusion. The reasons behind this non-Fickian behavior, also termed Type II transport, are discussed more fully in section 7.2.1 of ref [1]). Polymers such as cellulose, however, as well as many proteins possess such high intermolecular forces that, when heated, they will char or otherwise decompose rather than melt. For these macromolecules, either a sufficiently negative ΔG_{mixing} is needed to overcome the positive ΔG_{fusion} , or physical work input or chemical changes are needed. Physical work may involve vigorous shaking or stirring of solutions, while chemical changes may involve derivatization or employing solvent additives (e.g., salts) to create soluble non-covalent polymer-solvent-ion complexes. In the case of cellulose (CellOH), which has been found to dissolve in the complex solvent DMAc/LiCl (DMAc: N,N -dimethyl acetamide), dissolution requires heat, vigorous stirring/shaking, and the formation of the complex $[\text{CellOH} \cdot \text{Cl}]^- [\text{DMAc} \cdot \text{Li}]^+$ in solution [46–48].

As regards mixing, we have

$$\Delta G_{\text{mixing}} = \Delta H_{\text{mixing}} - T\Delta S_{\text{mixing}} \quad (12)$$

The entropy of mixing, ΔS_{mixing} , is normally positive for polymer solutions. If there is a net positive attraction favoring the formation of solute-solvent pairs, then the enthalpy of mixing, ΔH_{mixing} , is negative and dissolution will occur at any temperature. If ΔH_{mixing} is positive, however, then an increase in temperature may be needed so that $-T\Delta S_{\text{mixing}}$ becomes the dominant term on the right-hand side of equation (12) (this assumes that, as mentioned in the previous paragraph, the polymer will not char or decompose at elevated temperature).

When only dispersion forces are involved, the ΔH_{mixing} can be calculated using the solubility parameters discussed in Section 3.3, as per:

$$\Delta H_{\text{mixing}} = \Phi^S \Phi^P (\delta^S - \delta^P)^2 \quad (13)$$

where Φ^S and Φ^P correspond to the volume fractions of solvent and polymer in solution, respectively, and δ^S and δ^P to their respective solubility parameters.

Thermodynamic solvent quality can be assessed quantitatively via the second virial coefficient of dilute polymer solutions, A_2 . If, for a particular polymer dissolved in a particular solvent at a particular temperature, the formation of solute-solvent pairs is favored, then $A_2 > 0$ and these solvent-temperature conditions are referred to as “good.” At “poor” conditions, $A_2 < 0$ and dissolution is difficult, with the possibility of precipitation occurring with only a small change in temperature.

At so-called “theta” conditions, the excess Gibbs free energy of mixing (excess chemical potential, which itself cannot be measured directly) is zero, and so is A_2 ; i.e., at theta conditions $A_2 = 0$. It is important to note (as shall be seen when discussing LCCC in Section 8) that this does not imply that both ΔH_{mixing} and ΔS_{mixing} are zero,

which would be the case in a true ideal solution. At theta conditions, the terms on the right-hand side of equation (12) compensate each other, so that:

$$\Delta H_{\text{mixing}} \equiv \theta \Delta S_{\text{mixing}} \quad (\text{at } T = \theta) \quad (14)$$

meaning that the theta state is not a thermodynamically ideal state but, rather, a thermodynamically pseudoideal one [49]. For certain polymers, e.g., cellulose and polyethylene, poor or theta conditions do not exist. A sufficiently high temperature is needed for dissolution that those solvent-temperature conditions which have been found for these polymers are, by definition, “good” ones.

The second virial coefficient of a solution can be measured using static light scattering (see Refs. [3,22,23] and chapter 9 of ref [1]). For mixed solvents, measurement accuracy is often compromised by the difference in refractive indices of the solvents and the tendency for preferential solvation to occur, i.e., for the hydrodynamic volume occupied by the polymer in solution to be enriched by one solvent over the other, as compared to the solvent mix outside this volume. The use of an isorefractive solvent pair can overcome this limitation, provided solvent miscibility and other considerations are met. This case is discussed in detail in Ref. [18].

An interesting case is provided by the dissolution of a polymer in a mix of two non-solvents. About a dozen examples of this phenomenon can be found in Table 7.5 of reference [1]. While somewhat beyond the topic of the present paper, dissolution in these cases can be explained entirely from considerations of intermolecular forces.

To summarize, when discussing dilute solution thermodynamics one should not speak of “good,” “poor,” or “theta” solvents so much as of the respective solvent-temperature conditions: A particular solvent might be a thermodynamically good solvent for a particular polymer at a particular temperature, but be a poor, theta, or even a non-solvent for the same polymer at a different temperature. Solvent and temperature are inextricably linked to each other in dilute solution polymer science.

5.2. An object lesson [50]

Given that interactive GPEC relies on the combined principles of traditional GPEC and interactive polymer LC, both of which were explained in detail previously, how these combine to provide for improved resolution and speed is best explained by example. Here, we examine the interactive GPEC analysis of poly(vinyl butyral) or PVB, to determine the chemical composition distribution (CCD) of the vinyl alcohol (VOH) content of this polymer.

As seen in Fig. 9, what is normally called “PVB” is actually a random terpolymer more accurately referred to as poly(vinyl butyral-co-vinyl alcohol-co-vinyl acetate) [50,51]. The principal application of this polymer is in automotive and architectural safety glass, though a large number of niche applications for it also exist [52]. Determining the vinyl alcohol content of PVB is of interest, as this datum has been found to control adhesion to surfaces, to improve the properties of thermoset resins, to affect the miscibility and morphology of blends, to influence crosslinking behavior, and to affect polymer solubility. ASTM wet chemistry methods exist to determine the percentage of VOH (VOH %) in PVB [53], which can also be done employing either near-IR or NMR. All these methods provide only a single value, however, giving no insight into the shape and/or breadth of the VOH % distribution in a PVB sample.

Fig. 10 shows the Hansen solubility spheres of six PVB samples (each color sphere corresponds to two samples) [52], akin to the generic sphere depicted in Fig. 3. The relative energy difference (RED) between polymer and solvent (or solvent mixture) is

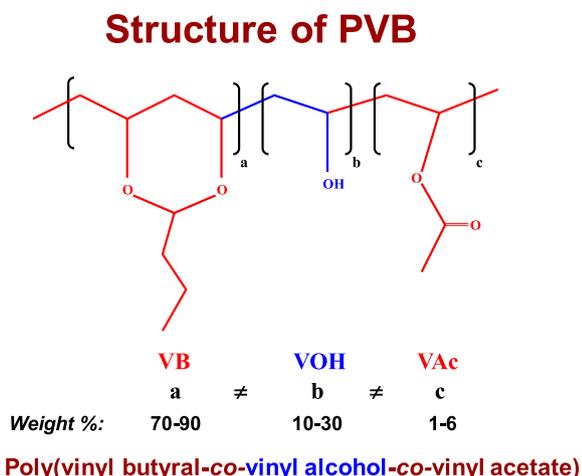


Fig. 9. Structure of the polymer commonly referred to as poly(vinyl butyral) or PVB.

defined as the right side of equation (2) divided by the left side. Solvents or solvent mixtures with $RED < 1$, i.e., with coordinates within the polymer sphere, will dissolve the polymer; those with $RED \geq 1$ are nonsolvents. It has been determined [50] that PVB with $VOH \% \leq 13$ to 15 (all percentages refer to weight percentages in this discussion) is soluble in ethyl acetate, EtAc. Above this value, PVB swells in EtAc but is insoluble in it. Conversely, PVB with $VOH \% \leq 13$ to 15 is insoluble in methanol (MeOH); above this value, it is soluble. It was also determined that acetonitrile, ACN, is a non-swelling non-solvent for PVB, regardless of $VOH \%$. As such, the polymer was dissolved in a 7:7:6 mixture of EtAc:MeOH:ACN so that, regardless of $VOH \%$, samples would be in solution and injected onto the column at conditions close to precipitation (i.e., the solvent mixture was thermodynamically poor; all separations were conducted at room temperature), which was shown to decrease band broadening, peak asymmetry, and tailing, an admittedly empirical result.

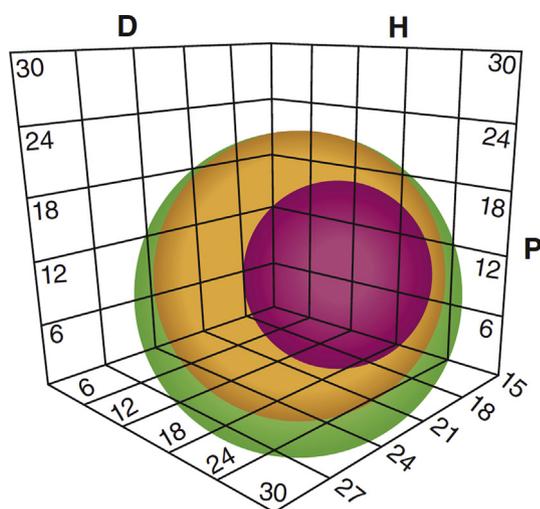


Fig. 10. Hansen solubility sphere for PVB. Different color spheres denote three different pairs of PVB samples, with samples differing from each other in both molar mass and comonomer ratios. D, P, and H correspond to the dispersive, polar, and H-bonding terms of the Hansen solubility parameter of the polymer, respectively. Axis units are $MPa^{1/2}$ in all cases. (Reprinted with permission from Ref. [52].)

Separation was performed employing a Diol column, which is a moderately-polar stationary phase (see Table 2), filled with EtAc at the start of the analyses. Upon injection, PVB chains with $VOH \% > 15$ precipitate. Chains with $VOH \% < 15$ continue to travel through the column, separating by adsorption. These latter chains are dissolved in EtAc, a low eluotropic strength eluent which promotes analyte transfer from the mobile to the stationary phase.

As the MeOH content of the gradient gradually increases (see Fig. 11), PVB chains with $VOH \% > 15$ redissolve as a function of increasing $VOH \%$ and travel through the column. Sorption of these chains onto the Diol packing is largely prevented by the eluotropic solvent strength of MeOH in the gradient, which displaces the analyte from active sites on the column packing material.

After all the analyte has eluted, the column is flushed and refilled with EtAc for the next run. As can be seen from Fig. 11, initially a gradient hold is employed for EtAc, followed by a linear gradient (linear increase in MeOH content), followed by another hold to ensure elution of all chains, followed by a sudden decrease to return the column to its starting EtAc value and a hold at this value to flush and recondition the column. An evaporative mass detector (evaporative light scattering detector, ELS) was employed for the analysis, given this detector's ability to handle solvent gradients (see Section 3.2). Separation proceeded by a normal-phase mechanism, i.e., by NP-GPEC.

A series of well-characterized samples, the $VOH \%$ of which had been determined by both NIR and titration analysis, was employed to create a first-order calibration curve. This served to allow for a more quantitative determination of the CCD and associated averages than is generally given in literature. An interactive GPEC chromatogram for a typical PVB sample analyzed is shown in Fig. 12a. Applying the calibration curve in Fig. 11 to these data, the CCD of the polymer, presented as both cumulative and differential distributions of the $VOH \%$, is obtained. These are shown in Fig. 12b, along with statistical averages (number- and weight-average $VOH \%$) and dispersity ($PDI_{VOH\%} \equiv (VOH \%)_n / (VOH \%)_w$) of the distribution.

6. Temperature gradient interaction chromatography (TGIC)

The simplest interpretation of TGIC is that it employs thermal gradients, rather than solvent gradients, to effect separation either by chemical composition, molar mass, tacticity, or some other macromolecular property. To understand how this occurs, to follow solute migration within a chromatographic column, and to assist in designing experiments, we shall follow Chang's approach to the subject [54].

6.1. Retention in TGIC

To determine the temperature-dependent migration rate $v(T)$ of an analyte in a TGIC experiment, we first define the retention factor k (earlier defined in eq. (6) based on the solute distribution coefficient and the volumes of the stationary and mobile phases) as:

$$k = \frac{t_R - t_0}{t_0} \quad (15)$$

where t_R is the retention time of the analyte and t_0 is the elution time of the injection solvent (noting that the void volume of the column is equal to the product of t_0 and flow rate). The retention factor is related to the thermodynamic components of the Gibbs free energy of transfer between phases as per:

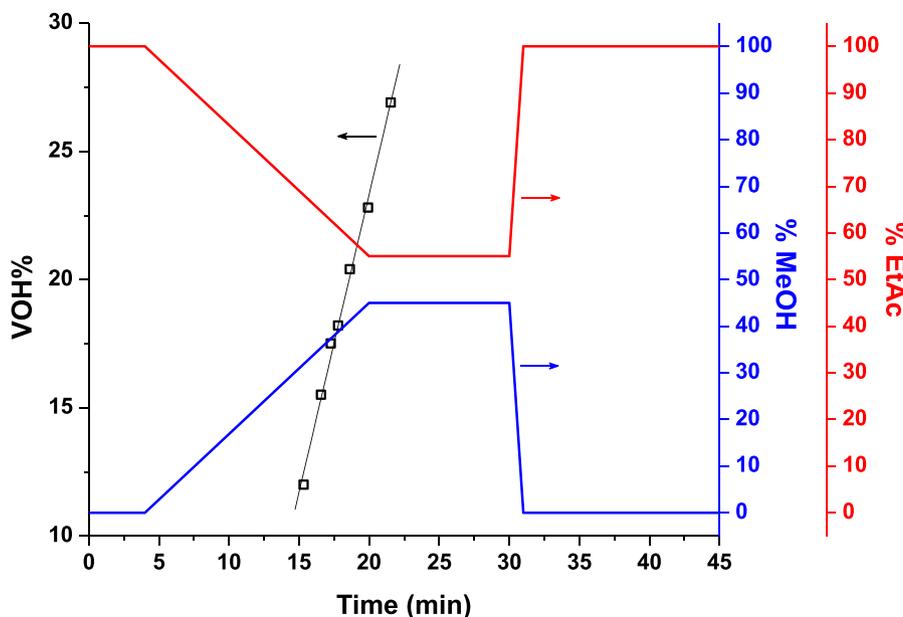


Fig. 11. MeOH:EtAc gradient profile for interactive GPEC analysis of PVB, to determine the VOH % distribution. Square markers represent elution times of peak maxima of calibration samples (each marker representing the average of three determinations, with error bars substantially smaller than marker size and, therefore, not shown) with VOH % determined by both NIR and titration analysis. Straight line represents first-order fit to the calibration data, with $r^2 = 0.995$. Column: Diol 10 μm particle size, 4.6 mm \times 250 mm; flow rate: 1 mL min^{-1} ; room temperature; detector: PL-EMD 960 ELSD. See Ref. [50] for details.

$$\ln k = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \phi \quad (16)$$

where, in this case, ϕ is the phase ratio of the column, i.e., $\phi \equiv V_S/V_M$ (this ϕ should not be confused with the volume fraction of solvent B in a solvent gradient, as the term was employed earlier in Section 4). Equation (16) is also known as the van't Hoff equation.

By plotting $\ln k$ versus $1/T$ in a so-called van't Hoff plot, the thermodynamic parameters of a separation are obtained. These will be employed in discussions below. To keep with Chang's nomenclature in these discussions, we define the slope and intercept of this plot as:

$$a \equiv \frac{-\Delta H^0}{R} \quad (= \text{slope of van't Hoff plot}) \quad (17a)$$

$$b \equiv \frac{\Delta S^0}{R} + \ln \phi \quad (= \text{intercept of van't Hoff plot}) \quad (17b)$$

van't Hoff plots are expected to be linear as long as there is zero change in heat capacity for transfer, i.e., that ΔH^0 , ΔS^0 , and ϕ are temperature-invariant. Non-linearity in this type of plot can correspond to the presence of mixed retention mechanisms and/or to sorption onto the stationary phase being influenced by changes in solute or stationary phase conformation [55]. It should be noted that accurate determination of the intercept is complicated by the non-trivial nature of determining the phase ratio of columns for most LC techniques, SEC being a notable exception in this regard.

At a fixed temperature T (i.e., in an isothermal elution), the dependence of retention time on thermodynamic parameters is given by:

$$t_R = t_0 \left[\exp\left(\frac{a}{T} + b\right) + 1 \right] \quad (18)$$

and the relation between solute migration rate and temperature by:

$$v(T) = \frac{L}{t_R} = \frac{L}{t_0 \left[\exp\left(\frac{a}{T} + b\right) + 1 \right]} \quad (19)$$

Neglecting extracolumn volume (for which a constant correction term can be added, if needed), a solute migrating through a column of length L , at temperature T , is expected to elute at retention time t_R as per the relation:

$$\int_0^{t_R} v(T) dt = L \quad (20)$$

Because in a TGIC experiment retention is controlled by varying the column temperature, equation (20) can be employed to predict retention time in this type of experiment. To do so, time t is substituted for temperature T as the integration variable, as the integrand is a function of the latter (see equation (19)).

For a linear temperature gradient $T = T(0) + \gamma t$, where γ is the column heating rate (usually given in units of K min^{-1}), one can write:

$$dt = \left(\frac{1}{\gamma}\right) dT \quad (21)$$

substituting equation (19) and (21) into equation (20) yields:

$$\int_{T(0)}^{T(0)+\gamma t_R} \frac{1}{\gamma \left[\exp\left(\frac{a}{T} + b\right) + 1 \right]} dT = t_0 \quad (22)$$

Employing equation (22), one can obtain t_R numerically given knowledge of a and b , which depend on molar mass M and can be obtained from the slopes and intercepts of van't Hoff plots as described above.

It is often the case in TGIC experiments that a multistep linear temperature gradient (i.e., a segmented gradient; see Fig. 7d) is needed to effect the type of separation desired when dealing with a multicomponent blend. In such cases, provided that a and b are constant over the temperature variation range, equation (22) is easily extended to:

$$\frac{t_1}{\exp\left(\frac{a}{T(0)+b}\right) + 1} + \int_{T(t_1)}^{T(t_2)} \frac{dT}{\gamma_1 \left[\exp\left(\frac{a}{T} + b\right) + 1 \right]} + \int_{T(t_2)}^{T(t_3)} \frac{dT}{\gamma_2 \left[\exp\left(\frac{a}{T} + b\right) + 1 \right]} = t_0 \quad (23)$$

where $T(t_1) = T(0)$, $T(t_2) = T(0) + \gamma_1(t_2 - t_1)$, and $T(t_3) = T(0) + \gamma_1(t_2 - t_1) + \gamma_2(t_3 - t_2)$. This scenario is shown graphically, for a generic three-step gradient, in Fig. 13.

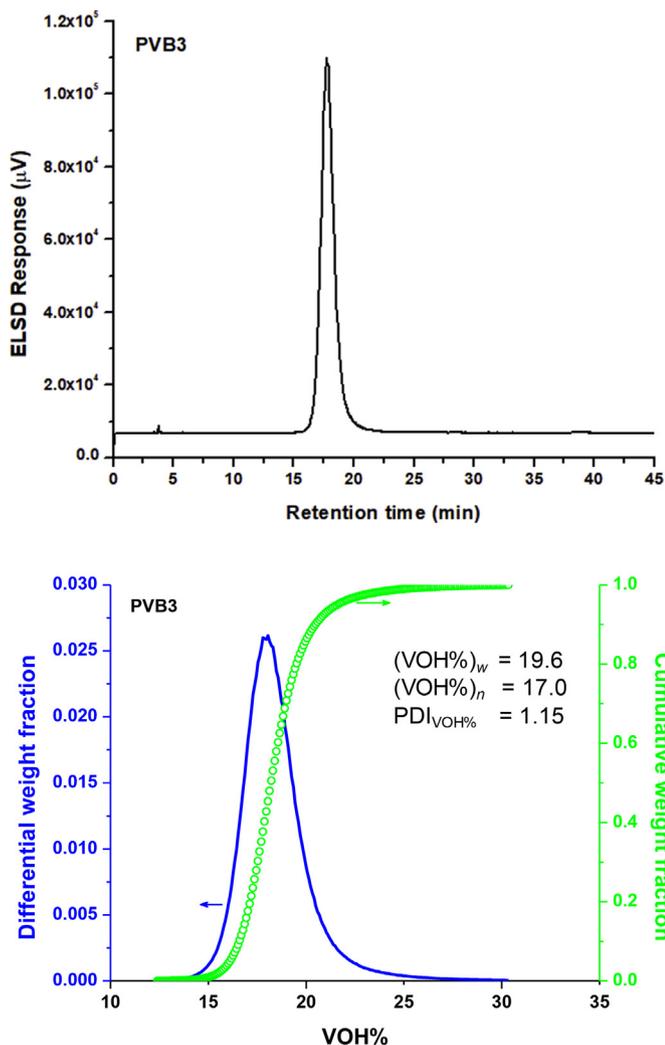


Fig. 12. (a) Interactive, NP-GPEC chromatogram of typical PVB sample (b) Chemical composition distribution of the VOH % of the same PVB sample, given as both differential (blue, left ordinate) and cumulative (green, right ordinate) distributions. Experimental conditions same as in Fig. 11. See Ref. [50] for details.

Finally, it should be noted that the above model of TGIC separation applies only to elution under adsorbing conditions. A molecular statistical model developed by Radke and coworkers predicts both this and elution under either SEC-like or “critical” (see Section 8) conditions. Because the above model should be suitable in most experimental designs, as well as to discussions within the context of this paper, we shall not delve into details of the molecular statistical model, which can be found in Ref. [56].

6.2. Solute migration in TGIC

By changing the value to t_0 in equation (23), it is possible to follow solute migration during a TGIC experiment. For example, to calculate the time it takes for an analyte to migrate halfway through the column, t_0 is replaced by $t_0/2$ in equation (23); to calculate the time it takes to migrate one-quarter way through the column, t_0 is replaced with $t_0/4$; and so on. This is shown in Fig. 14 for the injection solvent (same as the mobile phase) and seven polystyrene (PS) samples of different molar masses on a 50 mm column [54]. The three horizontal lines in the figure show the positions of 1 mm, 10 mm, and 50 mm in the column. The time when the analytes reach the 50-mm line thus represents the retention time t_R of each compound.

As expected, the injection solvent migrates at a constant rate proportional to the mobile phase flow rate. The 16 k PS sample also migrates at a constant rate, albeit at a slower one than the injection solvent. The migration rate of the 31 k PS shows some departure from linearity, but its retention time is still seen to increase steadily as a function of column length. For separation of lower- M analytes, it is clearly beneficial to use a longer column to improve resolution between peaks.

Above 90 kg mol⁻¹ (90 k), the analytes in Fig. 14 hardly move until a column threshold temperature has been reached. Beyond this threshold, movement accelerates until, for each analyte, it reaches a steady rate nearly equal to that of the injection solvent. As evident from the figure, for $M > 55$ kg mol⁻¹ the migration rates through the column display negligible difference beyond the 10-mm position. From this observation, one can interpret that a column as short as 10 mm would suffice to provide a separation of the high- M polymers comparable to that obtained using the 50-mm column. Ryu and Chang were able to confirm this hypothesis by performing the same experiment employing a shorter column and the same temperature gradient program and, separately, by also using a delayed temperature gradient program at the same column length. Details of these experiments can be found in Ref. [54].

6.3. Designing a TGIC experiment

The useful range of temperatures is bracketed by the freezing point of the solvent or solvent mix, at the low end, and by the boiling point at the upper end. Because of this, temperature alone will not provide as effective a control variable (i.e., will not provide a large enough variation in interaction strength) as will solvent composition (which can be varied widely) in the separation of broadly disperse or multicomponent samples. For temperature to play an efficient role with respect to controlling polymer elution, it is therefore necessary to select an appropriate solvent system for each individual polymer sample of interest.

To enable analysis of a broad range of molar masses, or a broad variety of compositions, in a single TGIC experiment, it is necessary that interaction of the analytes with the stationary phase be weak. Otherwise, high- M samples would not elute within a reasonable run time, due to their being retained too strongly and to the aforementioned limitation in the useful temperature range of a solvent. As such, most elutions are started at solvent-temperature

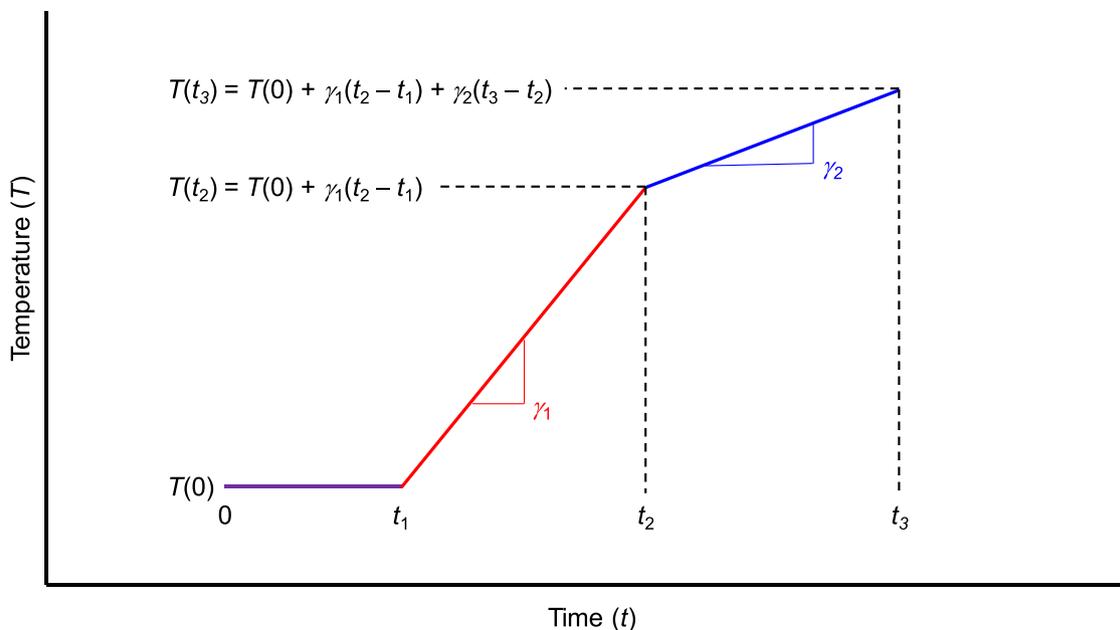


Fig. 13. Generic example of three-step linear temperature gradient for a TGIC separation.

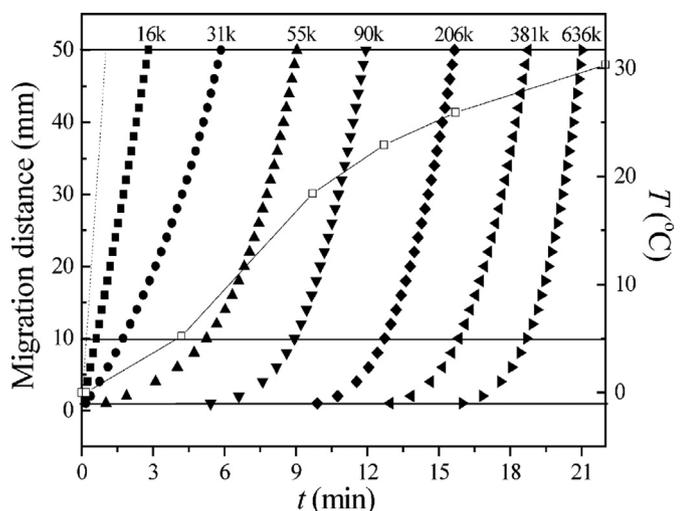


Fig. 14. Simulated analyte migration in TGIC. Filled symbols represent migration of different PS samples (each of molar mass M given at top of plot); dotted line represents injection solvent. Horizontal lines show 1 mm, 10 mm, and 50 mm positions in column. Solid line connecting open squares represents temperature gradient. Separation conditions are as follows: Column: Nucleosil C18, 3 μm particle size, 100 \AA pore size, 50 mm \times 4.6 mm i.d.; eluent: CH_2Cl_2 :ACN 57:43; flow rate: 0.7 mL min^{-1} . (Reproduced with permission from Refs. [54]. Copyright 2005 American Chemical Society).

conditions at or near the critical adsorption point of dilute polymer solutions (see Section 8). From here, temperature is usually raised to promote analyte desorption, because solvent strength usually increases with increasing temperature. This is not always the case, however; in certain cases, solvent strength will increase with decreasing temperature and the latter will need to be decreased for elution to occur.

To date, most TGIC separations have been conducted employing stationary phases composed of aliphatic hydrocarbon bonded silica, predominantly C18, though bare silica and even polymeric stationary phases have also been used. An example of normal-phase TGIC is shown in Fig. 15, where two sets ($1 \times 10^4 \text{ g mol}^{-1}$ and

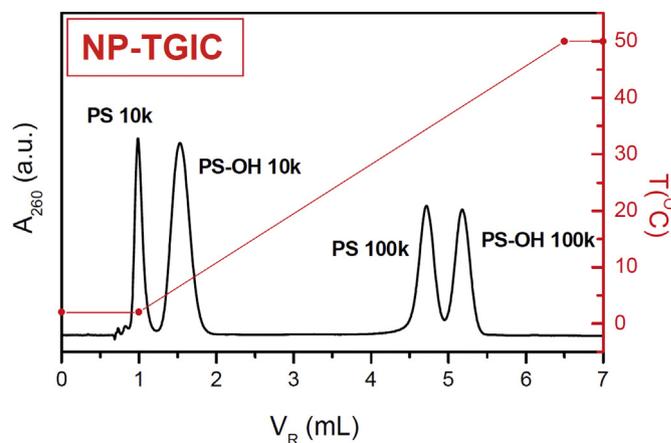


Fig. 15. NP-TGIC analysis of PS samples with different end groups, H-terminated versus OH-terminated. Column: Nucleosil bare silica, 100 \AA pore size, 250 mm \times 2.1 mm i.d.; mobile phase: isooctane:THF 55:45; flow rate: 0.1 mL min^{-1} . (Reproduced with permission from Ref. [57]).

$1 \times 10^5 \text{ g mol}^{-1}$) of PS samples (one H-terminated and one OH-terminated) were analyzed on a bare silica column [57]. The sensitivity of NP-TGIC to polar groups (expected from that same sensitivity in traditional NP-LC) is showcased by the fact that, not only are the four components in the sample clearly resolved from each other but, more impressively, both components of each molar mass set were resolved even though they differed from each other by a single hydroxyl group.

Method development is complicated by the need to find analyte “critical conditions” (see Section 8). Because TGIC experiments are usually begun at conditions near the critical adsorption point of polymers, for each individual analyte a proper combination of solvent(s), temperature, and stationary phase chemistry must be found that provides near-critical conditions for the solution and M -independent elution with the chosen stationary phase. Currently, this is an empirical endeavor. An extensive, though somewhat

dated, tabulation of critical conditions can be found in Ref. [58]. Once appropriate column and solvent conditions for polymer elution have been determined, the separation can be fine-tuned by careful variation of temperature and solvent composition, individually.

Experimentally, it has been found that the most important parameter for controlling retention time in TGIC is the heating rate of the column, more so than either column length or eluent flow rate [59]. The effect of column length was most pronounced with respect to its influence on void volume and chromatographic resolution but, in the latter case only as regards low- M polymers (see Section 6.2). While flow rate appears to be least significant among chromatographic parameters examined, its optimization in conjunction with heating rate and column length should be considered during experimental design.

6.4. High-temperature TGIC (HT-TGIC)

While currently employed only for analysis of polyolefins, high-temperature TGIC or HT-TGIC merits mention here due to the fact that method development in this mode of the technique has become more straightforward through the work of Cong and co-workers [60].

To determine comonomer distribution in polyolefins, HT-TGIC is performed using a Hypercarb porous graphitic column. Separation is effected via the interaction of the polyolefin chains with the graphite surface upon temperature change in an isocratic solvent. The ability to withstand higher sample loads (in common with its non-HT counterpart) means that the technique can also be employed for preparative fractionation.

The schematic of an HT-TGIC experimental set-up is shown in Fig. 16, with variables defined in Table 5. Experiments were performed in *o*-dichlorobenzene (ODCB). An example of separation based on comonomer content is shown in Fig. 17.

The flow rate during the cooling process, F_C , can be either zero (static cooling) or nonzero (dynamic cooling). Advantages of the latter over the former include: (1) Reduced potential for column plugging; the spreading of polymer solution along the entirety of the column leads to a reduction in sample concentration buildup; (2) Unretained polymer chains are flushed further down the

column, thus separating them from retained chains; (3) Minimized potential multilayer adsorption effects by polymer chains retained at same location on the column.

Because of the constant solvent composition, differential detectors such as light scattering and viscometry can be employed with HT-TGIC. These can provide absolute molar masses as well as architectural information about the analytes, in addition to the chemical information provided by the infrared detector that is usually employed in high-temperature LC of polyolefins [1–3]. Also, substantially different behavior was observed for polyolefins depending on whether ODCB or 1,2,4-trichlorobenzene (TCB) was employed as eluent. This suggests that adjusting solvent strength (e.g., by addition of poor solvent to either ODCB or TCB, with separation carried out isocratically) may afford a handle by which to further increase resolution between component peaks. The source of the substantial band broadening observed in HT-TGIC is still not well understood, especially given that its non-HT counterpart generally affords lower band broadening as compared to, e.g., SEC.

6.5. Advantages and limitations of TGIC

First, we state the obvious: TGIC can separate analytes (as can most other IPC methods) by chemical composition, tacticity, or other properties which are not necessarily directly related to the hydrodynamic volume of macromolecules in solution. As such, and reiterating what was mentioned in the Introduction, IPC methods including TGIC provide a view of polymers different from that afforded by SEC and other size-based techniques.

Further advantages of TGIC over SEC include lower band broadening for M -dependent separations (though not in the case of HT-TGIC; see previous section), thus providing for higher M -resolution in TGIC; and a higher column loading capacity, making TGIC an attractive preparative fractionation method.

As compared to solvent gradient elution methods, less signal drift is encountered when employing temperature gradients for separation. Also, because temperature can be readjusted at the detector, differential detection methods (e.g., refractometry, light scattering, viscometry) can be employed in TGIC, but not in most solvent gradient separations due to issues related to the preferential solvation of polymers in mixed solvents [18].

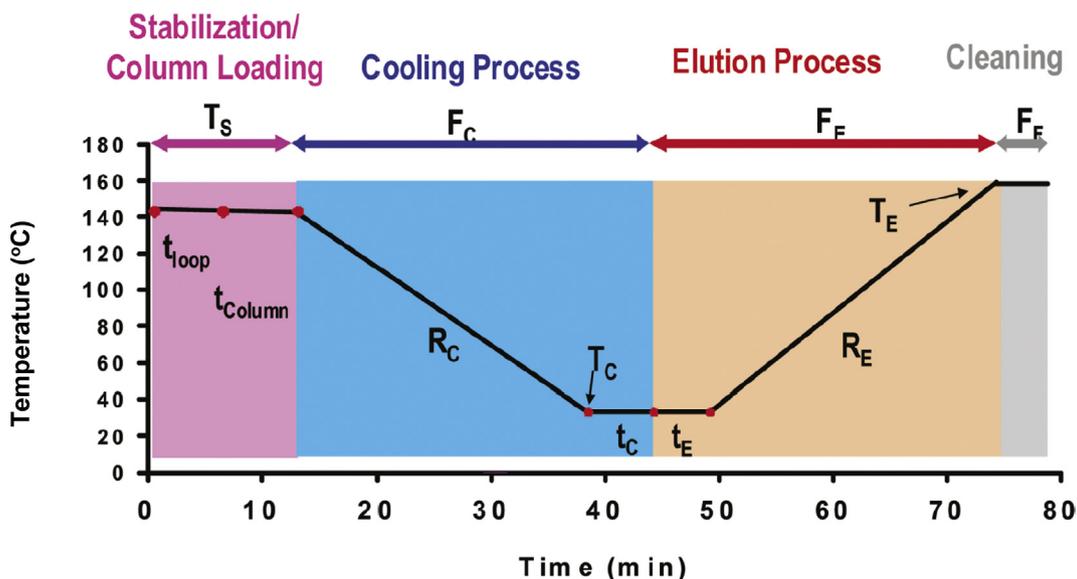


Fig. 16. Schematic of HT-TGIC experimental set-up. See Table 5 for definition of variables. (Reproduced with permission from Ref. [60]. Copyright 2011 American Chemical Society.).

Table 5
Experimental variables in an HT-TGIC experiment, in relation to Fig. 16.

Variable	Units	Symbol	Description
<i>Stabilization and Sample Loading Process</i>			
Stabilization temperature	°C	T_s	Temperature during stabilization and at start of cooling process
Stabilization time	min	t_{loop}	Amount of time sample stays in injection loop in top oven before loading onto column
Precooling time	min	t_{column}	Amount of time sample stays in the front of the column before cooling process begins
<i>Cooling Process</i>			
Cooling rate	°C min ⁻¹	R_C	Thermal rate of main oven during cooling process
Final temperature of cooling process	°C	T_C	Final temperature at end of cooling process
Postcooling time	min	t_C	Time sample stays in column at final temperature of cooling process. (Pump flow rate of cooling process continues, but data are not collected during this time)
Flow rate of pump during cooling process	mL min ⁻¹	F_C	Flow rate during cooling process. Can be zero (static cooling) or nonzero (dynamic cooling)
<i>Elution Process</i>			
Elution rate	°C min ⁻¹	R_E	Thermal rate of main oven during elution process
Final temperature of elution process	°C	T_E	Final temperature at end of elution
Soluble fraction time	min	t_E	Amount of time main oven stays at final temperature during cooling process, with pump at flow rate of elution process (F_E), before increasing temperature. Data collection begins here
Flow rate of pump during elution process	mL min ⁻¹	F_E	Flow rate during elution process

Adapted from Ref. [60].

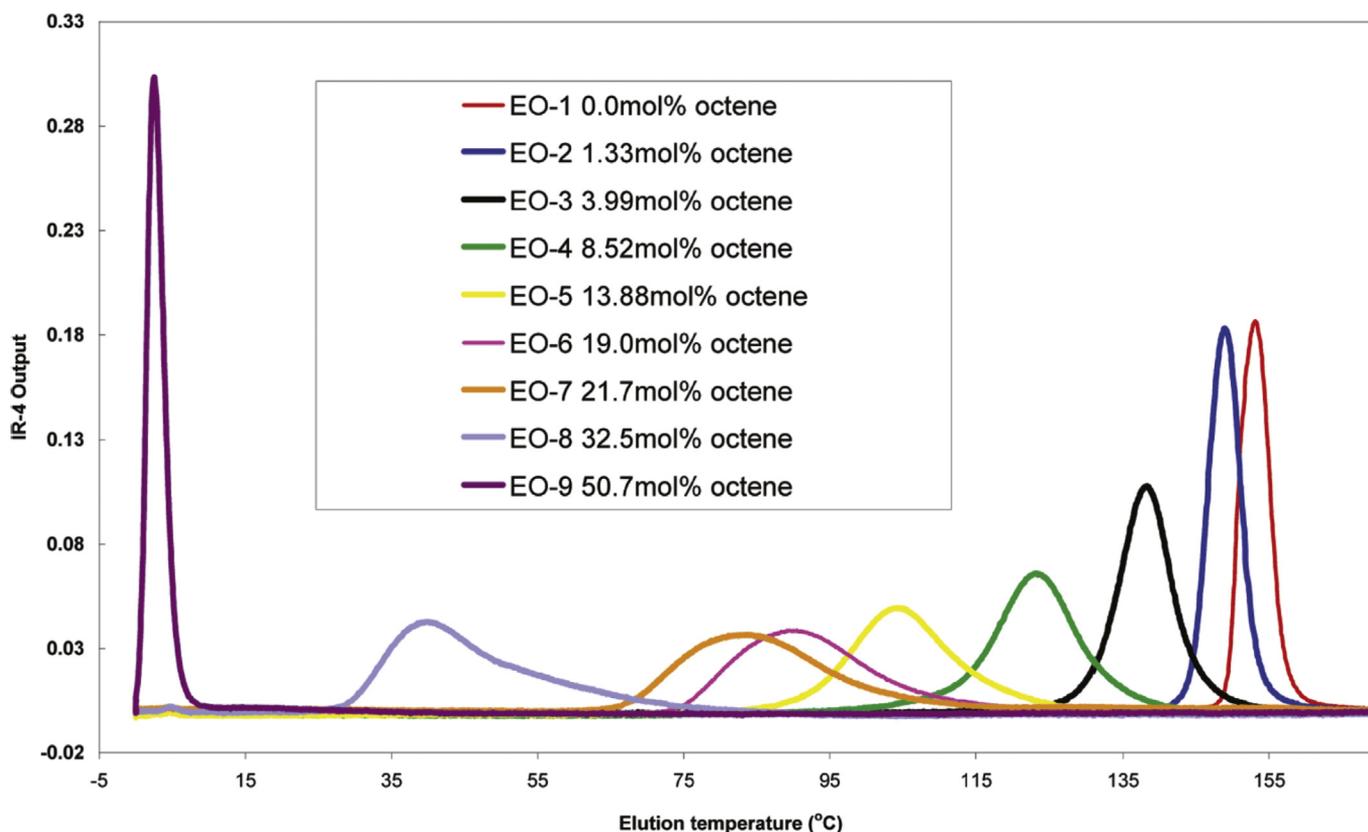


Fig. 17. HT-TGIC separation of ethylene-octene (EO) copolymers based on octene comonomer content. Column: Hypercarb, 7 μ m particle size, 100 mm \times 4.6 mm i.d.; mobile phase: *o*-dichlorobenzene containing 600 ppm butylated hydroxytoluene; detector: Polymer Char IR-4 two-channel infrared detector; T_s : 140 °C; T_C : 0 °C; T_E : 175 °C; R_C : 6 °C min⁻¹; R_E : 3 °C min⁻¹; F_C : 0.03 mL min⁻¹; F_E : 0.5 mL min⁻¹ (Reproduced with permission from Refs. [60]. Copyright 2011 American Chemical Society.).

Disadvantages of TGIC vis-à-vis SEC mainly revolve around the fact that the former is not as universal as is the latter. Each time one wishes to analyze a different polymer by TGIC, experimental conditions need to be found and optimized for that particular analyte, including finding the correct solvent composition and initial temperature, stationary phase, and temperature gradient, none of

which are trivial endeavors, especially if tabulated values for critical conditions for the particular polymer being analyzed cannot be found in the literature.

As regards its comparison to solvent gradient techniques, a main advantage of the latter is the large variety of solvents available, to be employed either neat or in binary or ternary

mixtures with near-infinite possibilities for combinations. This allows chromatographic selectivity to be tuned through a variety of molecular properties though, as mentioned earlier in this manuscript, the principles behind this “tuning” are still being developed.

7. Barrier and SEC-Gradient methods

7.1. Barrier methods

7.1.1. Principles of the method

In barrier methods, multicomponent polymer samples are introduced onto a porous LC column along with various sequentially-injected plugs of “barrier” solvents. In the absence of such plugs and at conditions favoring the desorption of analyte from the stationary phase (i.e., employing a strong solvent as mobile phase), a polymer elutes in SEC mode and ahead of the solvent peak because, due to preferential exclusion from the column pores, the polymer peak travels faster through the column than does the solvent peak (this has also been termed liquid chromatography at limiting conditions of desorption, or LC-LCD). When a plug (barrier) of a different solvent is injected onto the column prior to the polymer injection then, eventually, the polymer peak will catch up with the barrier. If the barrier solvent is a strong solvent for the polymer, then the latter will migrate through the barrier and continue its travel through the column. If, however, the barrier is a weak or a non-solvent for the polymer, then the polymer will either be absorbed by or will precipitate onto the column packing material

(termed liquid chromatography at limiting conditions of insolubility, or LC-LCI). The polymer cannot, however, fall behind the barrier because behind is the strong solvent that promotes analyte desorption from the stationary phase and elution ahead of this strong solvent. The result is that polymer molecules now “pile up” at the back edge of the poor/non-solvent barrier, eluting in an almost M -independent fashion immediately behind this barrier [61]. At its simplest, a bicomponent sample can be separated into its two individual fractions employing a single barrier, where the barrier is a strong solvent for one of the components and a weak solvent for the other. For an n -component sample, $n - 1$ barriers will be needed (assuming one component is completely non-adsorptive at the experimental conditions) [62].

The terms “*adsorli*” and “*desorli*” are commonly employed in the literature of barrier methods, but their meaning should be interpreted with caution, as it will depend on the type of barrier method being applied. For example, in the LC-LCD case described above where the eluent (mobile phase) promotes analyte desorption, this eluent is the *desorli*. The *adsorli* in LC-LCD is the barrier solvent (which can be introduced either as a plug injection before sample injection or can be introduced mixed in with the eluent). On the other hand, with liquid chromatography at limiting conditions of adsorption (LC-LCA), it is the eluent which is the absorptive barrier and, thus, the *adsorli*, with the solvent (or solvents) that promotes desorption being the *desorli* [63].

As a generic example of a barrier method separation, let us take a three-component blend of an A-B block copolymer, homopolymer A, and homopolymer B. Being a 3-component system, $(3 - 1) = 2$

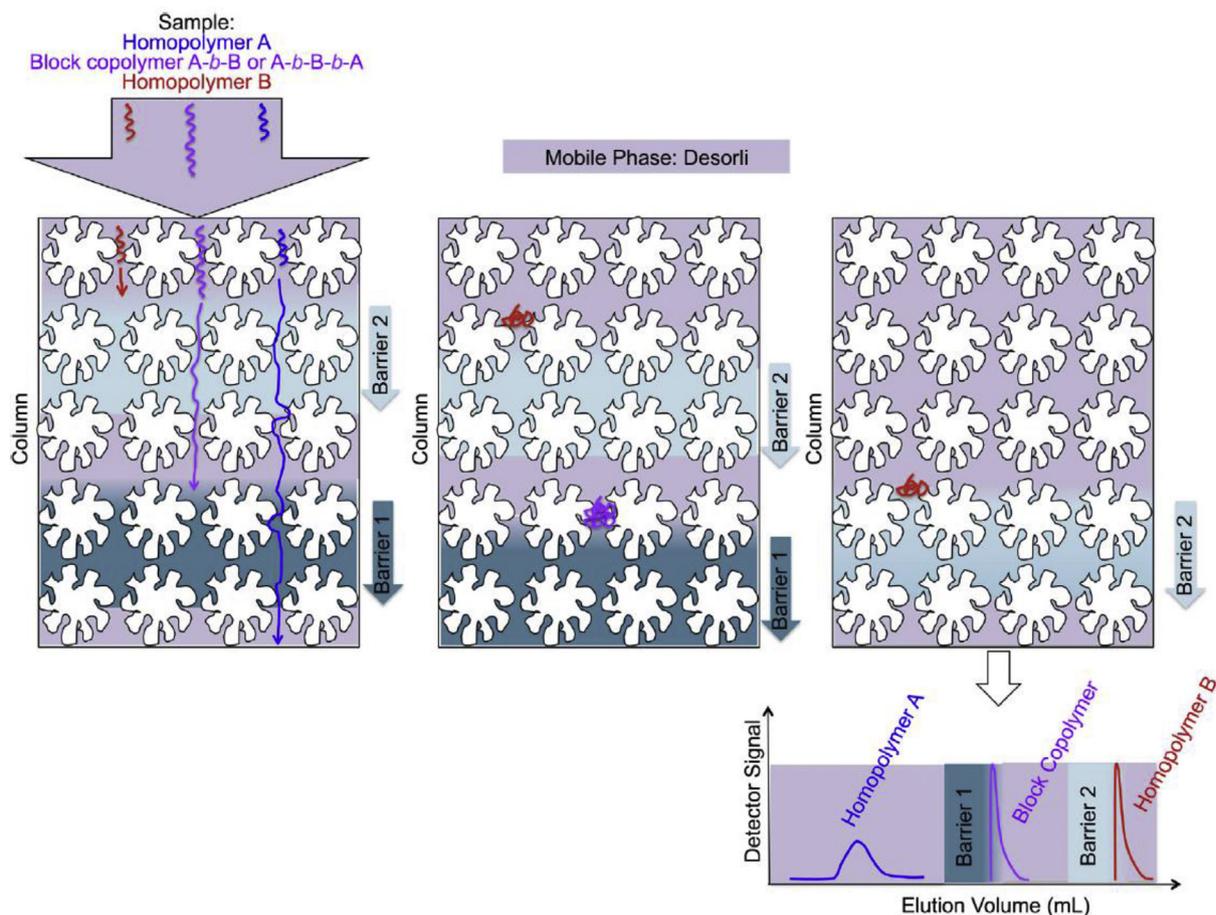


Fig. 18. Barrier method, as applied to a generic three-component blend of a block copolymer and its constituent homopolymers. See text for explanation. (Reprinted with permission from Ref. [64]).

barriers will be needed. This case is illustrated in Fig. 18 [64]. If homopolymer A elutes in LC-LCD mode, i.e., does not adsorb onto the stationary phase at the chromatographic conditions employed, it will not be retarded by either barrier (Barrier 1 or Barrier 2), eluting in SEC mode. Homopolymer B is adsorptive and retarded by the second barrier (Barrier 2). This second barrier is not efficient enough to slow down the elution of the block copolymer, however, which breaks through Barrier 2 and continues to elute in SEC mode until reaching Barrier 1. Barrier 1 being more efficient than Barrier 2 with respect to the block copolymer means that the latter cannot break through Barrier 1 and accumulates at this barrier's trailing edge. Elution order is shown in the figure; as can be seen, the peak for homopolymer A will be relatively broad, as it eluted in SEC mode without barrier retardation. The peaks for homopolymer B and for the block copolymer will be narrower, due to the focusing effect of the individual barrier through which these components cannot travel (Barrier 1 for the block copolymer, Barrier 2 for homopolymer B). The peak tailing observed for homopolymer B and for the block copolymer is due to the individual analytes' solvation by the strong solvent which follows behind the barriers.

7.1.2. Experimental design

Designing a barrier method experiment requires choosing a column, a mobile phase, and barriers (duh!). The column should be porous, ideally packed with particles possessing narrow-diameter pores but a large pore volume. This combination, however, is difficult to attain in commercially-available columns. Because of their large pore volume, small pore size SEC columns are often used. Bare silica as a packing material generally provides appropriate adsorptivity; for highly polar polymers a less adsorptive packing such as surface-modified silica is recommended. Polymers with low adsorptivity may require retention by phase separation instead and the use of C18 or polymeric columns [62]. The reader is referred to Table 2 for a classification of different types of column packing materials.

We distinguish again between solvent goodness and solvent strength. The latter governs interactions between eluent and column packing material, and the adsorption of macromolecules onto the stationary phase, and is measured by the solvent's elutropic strength (ϵ^0) as discussed in Section 4.3. The former is a measure of how a solvent interacts with a polymer at a given temperature and determines the extent of polymer adsorption and enthalpic partition onto the stationary phase. Qualitatively, solvents are classified as good, poor, and theta (and as non-solvents), keeping in mind that temperature forms an integral part of this classification. A quantitative measure of solvent goodness is given by the second virial coefficient of a dilute solution. This topic is discussed in Section 5.1.

In LC-LCD, the mobile phase is generally chosen as in a typical SEC experiment, i.e., it should be a good solvent (at the experimental temperature) for all sample components and one which will be an eluotropically strong solvent or good *desorli*. The sample is dissolved in this same solvent. By contrast, in LC-LCA the mobile phase should promote polymer adsorption onto the column packing material (this eluent is the *adsorli*); the polymer is dissolved and injected onto the column in a solvent (or solvent mix) which promotes desorption from the column packing (the dissolving solvent is thus the *desorli*) [65].

The choice of barriers is complicated by the nature of the components being separated, of the retention mechanisms that each barrier should effect, by the need to tune barrier duration and delay time [66], and by the fact that a barrier may be composed of a single solvent or of a mix of two (or more) solvents. In the latter case, the appropriate solvent ratio also needs to be found. Barriers have usually been chosen to promote one of the following retention mechanisms:

- 1) Adsorption onto the column packing material.
- 2) Enthalpic partition into the stationary phase (absorption), most commonly into alkyl-bonded phases.
- 3) Phase separation or precipitation onto the column packing.

In designing barriers, it should be kept in mind that adsorption will be promoted by eluotropically weak solvents, absorption by thermodynamically poor solvents, and precipitation by non-solvents. It is often the case that, with mixed solvents, the same component solvents can be applied across several barriers, only in different ratios to promote different retention mechanisms.

7.1.3. Advantages and limitations of barrier methods

Barrier methods are generally considered robust and highly repeatable, with separation fairly independent of eluent composition (at least in the case of LC-LCD [67]). Sample capacity is high, enabling characterization of minor components, preparative fractionation, and segmented 2D-LC. Sample recovery is also fairly high. Sample components that elute behind a barrier do so in a relatively *M*-independent fashion (not so for the component(s) eluting ahead of the barrier). In comparison to other IPC techniques, barrier methods are fairly fast, with elution usually accomplished in 15 min or less.

On the other hand, sample components need to be soluble in solvents of different polarities (to identify appropriate *desorli* and *adsorli*) and components should exhibit different adsorptivities with respect to one another. Only a limited number of components can usually be separated. Also, careful choice of barrier solvent or solvents is required, as is the ratio of solvents within each barrier, the width of the barriers, and the delay between barrier and sample.

How these methods may handle copolymers with a broad CCD is not yet clear.

Because of the mixed solvents employed in barrier methods, evaporative-type detectors are normally used. Some benefits and limitations of these types of detectors were discussed above (see Section 3.2).

7.1.4. An object lesson [62]

We examine here the design of an LC-LCD experiment for the separation of a blend containing polystyrene (PS), poly(methyl methacrylate) (PMMA), poly(ethylene oxide) (PEO), and poly(2-vinyl pyridine) (P2VP) [62]. As mentioned above, bare silica as a column packing material has generally been found to provide appropriate adsorptivity for many barrier methods, especially LC-LCD ones, and that was the type of column which was ultimately employed in this study.

All polymer components dissolved in both tetrahydrofuran (THF) and dimethyl formamide (DMF), both of which are polar solvents, so polymer precipitation onto the column packing material could safely be excluded. The solvents themselves tend to interact strongly with bare silica, adsorbing onto it. PEO and P2VP show high adsorptivity on bare silica gel. While tetrahydrofuran (THF) acts as a *desorli* for many medium-polarity polymers on silica gel, in this solvent both PEO and P2VP are fully retained on silica. Because of this DMF, a chromatographically strong solvent, was added to the eluent.

Barriers were needed that would promote adsorption of all but the least adsorptive component, that being PS. Toluene was chosen because, on silica gel, it is a chromatographically weak solvent that acts as an *adsorli* for PMMA, PEO, and P2VP, but as a *desorli* for PS. Toluene is, however, a thermodynamically poor solvent for PEO and a non-solvent for P2VP. Therefore, the solubility of these two polymers in toluene-containing eluent or barriers needed to be

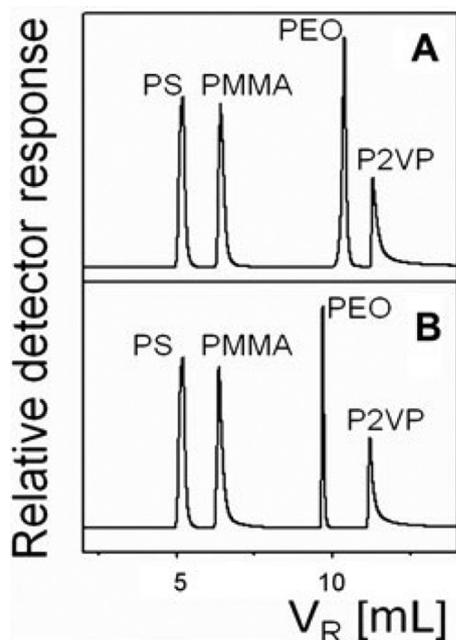


Fig. 19. LC-LCD separation of a 4-component blend of PS, PMMA, PEO, and P2VP. All components present in equal concentrations (approximately 6 mg mL^{-1} each). Column: Kromasil $10 \mu\text{m}$ particle size, 60 \AA pore size; flow rate: 1 mL min^{-1} ; temperature: $22 \text{ }^\circ\text{C}$ – $24 \text{ }^\circ\text{C}$; detector: ELSD model 100. Eluent: DMF/THF/toluene 30:50:20. Barrier 1: Neat toluene; Barrier 2: Neat THF; Barrier 3: 15:55:30 DMF/THF/toluene. Time delays (in minutes): (A) 0-2-4-5, (B) 0-1.5-4-5. (Reproduced with permission from Ref. [62]).

optimized. This was done off-line, employing mobile phases containing 20%–30% DMF, 40%–50% THF, and 20%–30% toluene. Ultimately, an eluent composition of DMF/THF/toluene 30:50:20 was chosen, because it promoted high sample recovery.

Barrier design followed a trial-and-error approach, though the authors note that this stage of the experiment was simple and fast. At constant eluent composition, stock solutions of the individual polymers were dissolved in eluent and injected behind barriers of various compositions. The goal was to design barriers that would decelerate the more polar sample constituents while letting the other constituents pass through. With the bare silica gel stationary phase and barriers of distinct composition, absorptivity increased in the order $\text{PS} < \text{PMMA} < \text{PEO} < \text{P2VP}$. PS was unretained with either THF, DMF, or toluene. PMMA was only slightly more adsorptive than was PS; it was decelerated using a barrier of neat toluene (Barrier 1). Elution of PEO was slowed down by neat THF (Barrier 2), which did not affect the elution of either PS or PMMA. The highly adsorptive P2VP was decelerated with a 15:55:30 mix of DMF/THF/toluene (Barrier 3), which did not influence the elution of the other three polymeric components in the blend. Results of the separation are shown in Fig. 19.

As seen in Fig. 19, good baseline-level separation was obtained between all components in the blend. P2VP showed a lower ELSD detector response as compared to the other three polymers in the blend; P2VP peak skew is due to a low- M tail present in this polymer. Fig. 19B shows the effect of time-delay between barrier and sample injection. The delay between Barrier 1 and Barrier 2 was shortened, meaning that the delay between Barrier 2 and sample injection increased. This resulted in PEO being shifted to a lower retention volume, away from P2VP and toward PMMA.

Not shown are the results of experiments with different ratios of the blend components. These additional studies demonstrated that components could be detected at relative concentrations as low as 1%.

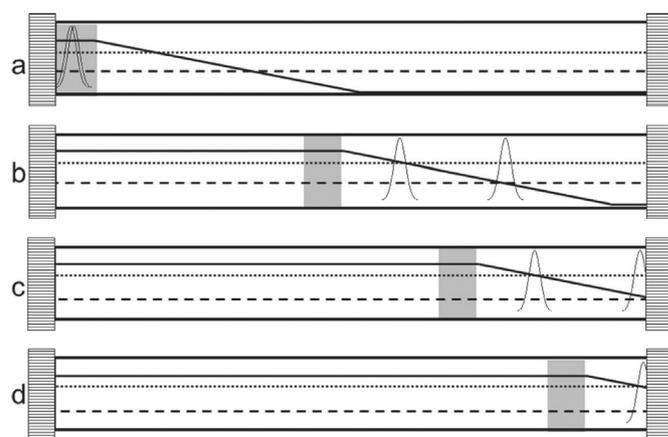


Fig. 20. The SEC-Gradient process. (a) At time of injection. (b) When polymers have reached their individual adsorption thresholds. (c) When first polymer elutes from the column. (d) When second polymer elutes from the column. Dark band corresponds to solvent band, solid line to gradient, intersection of dashed line with gradient to adsorption threshold of first polymer, intersection of dotted line with gradient to adsorption threshold of second polymer. (Reprinted with permission from Ref. [70]).

7.2. SEC-gradient methods

Related to barrier techniques, but improving upon the limited peak capacity of these, are so-called SEC-Gradient methods [68–71]. In these, before sample injection a solvent gradient is created within the column. Samples are dissolved in a chromatographically strong solvent and injected at the end of the gradient (Fig. 20a). At injection, analytes experience strong solvent conditions (similar to LC-LCD) and, as a result of steric exclusion, travel through the column faster than do eluent molecules, until the analytes reach their adsorption threshold in the gradient (Fig. 20b). At this point, analyte molecules with a given chemical composition accumulate (again, similar to the LC-LCD case). The SEC-gradient can thus be thought of as a large number of consecutive barriers of different, continuously decreasing eluotropic strength wherein the polymers will automatically locate their respective adsorption thresholds. Solvent/non-solvent gradients have also been applied. In these cases, polymers automatically locate their precipitation threshold within the gradient.

As with LC-LCD, column packings with a small pore size but a large pore volume are preferred, though hard to come by commercially. Commercially available SEC columns are employed as a compromise. The separation range in SEC-gradient methods is therefore restricted by the pore volume of the column [61].

7.2.1. An object lesson [69]

An SEC-gradient approach was applied to the separation of poly(methyl methacrylate-*stat*-methacrylic acid) samples based on their methacrylic acid (MA) content, for polymers with up to 50% MA. Previous work by the authors had shown that, when dissolved in CHCl_3 , poly(methyl methacrylate) adsorbed onto the packing material of a Proteoma modified silica column and THF could be used to desorb the polymers. However, also in THF, copolymer samples with a high MA content were found to completely adsorb onto the same column material. A stronger solvent than THF was obviously needed for copolymer analysis. *N,N*-dimethyl acetamide (DMAc) was employed and, in isocratic runs, all copolymer samples eluted at the exclusion limit of the column ($\approx 6.1 \text{ mL}$), regardless of molar mass. This indicated that the pore size of the column was too small for effective separation by size, a good thing because in SEC-gradients (as in barrier methods) a small pore size is needed to allow for good separation by chemical composition while

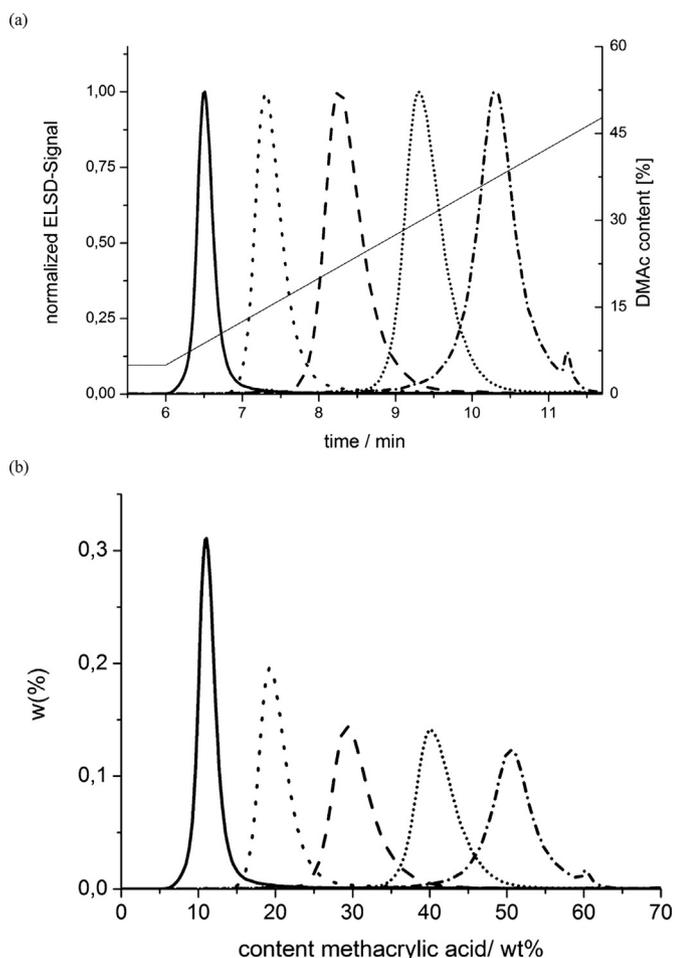


Fig. 21. SEC-gradient separation of poly(methyl methacrylate-*stat*-methacrylic acid) copolymers based on methacrylic acid content. (a) Normalized chromatograms. Gradient: Linear, ranging from 5% to 50% DMAC in CHCl_3 . Straight line represents eluent composition at detector. Gradient: 0 min–3 min: 50% DMAC; 3 min–8 min: 100% DMAC; 8 min–23 min: 100% CHCl_3 ; 23 min–26 min: 5% DMAC; 26 min–32 min: Linear increase from 5% to 50% DMAC. Injection time: 32 min. (b) Differential chemical composition distribution of the percent methacrylic acid in copolymers. (Reprinted with permission from Ref. [69]. Copyright 2013 American Chemical Society.)

minimizing molar mass effect. (As a reminder, in a porous column where the pores are too small to allow for penetration by the polymers, the latter will travel through the column faster than will the injection solvent, because this injection solvent will be able to explore the internal pore volume as well as the interstitial volume of the column, whereas the polymers, being excluded from the pores, will only be able to sample the interstitial volume).

Because the sample with the highest MA content didn't dissolve in CHCl_3 , all samples were instead dissolved in DMAC. Optimizing the separation involved trying various gradients (0%–100% DMAC, 0%–50% DMAC, and 5%–50% DMAC), as well as reducing the final DMAC content in the gradient from 100% to 50%. The lower (50%) DMAC content at time of injection allowed for stronger retardation of late-eluting peaks with higher (40%–50%) MA content, by adjusting adsorption thresholds closer to the injection solvent. This also allowed for slightly stronger retardation of early-eluting peaks with lower (10%–20%) MA content. To further optimize the separation of these latter peaks, the gradient was started at a slightly higher (5%) DMAC content. Results of the optimized analysis are shown in Fig. 21a, where the straight line represents the gradient composition at the detector.

Given that $\approx 12\%$ DMAC in CHCl_3 was required to dissolve the sample with the highest MA content, and that only the sample with the lowest MA content eluted below this value (see Fig. 21a), it was concluded that separation must have occurred based on adsorption, not precipitation.

Applying a calibration curve of MA content (as determined by ^1H NMR) versus elution volume, the authors determined the chemical composition distribution of the samples, based on % MA and shown in Fig. 21b, in a manner akin to that shown earlier for the interactive GPEC measurement of VOH % in PVB (see Section 5.2 and Figs. 11 and 12, as well as refs [50,72]).

8. Liquid chromatography at the critical condition (LCCC)

At its simplest, LCCC involves finding the appropriate combination of solvent(s) and temperature such that, for a particular polymeric chemistry (i.e., homopolymers, or copolymer blocks, composed of a particular monomeric repeat unit), elution employing a particular stationary phase is molar-mass-independent. This allows for determining, among other things, the end group distribution (distribution of end group chemistries) in homopolymers and either the molar mass or length of the “non-critical” block in block copolymers. Separation involves an exact compensation, at a given temperature, of the entropy and enthalpy of analyte transfer between the mobile and stationary phases of a column, resulting in a Gibbs free energy of transfer between phases of zero ($\Delta H^{\text{transfer}} = T\Delta S^{\text{transfer}} \therefore \Delta G^{\text{transfer}} = 0$). Contrary to some claims, the enthalpic and entropic terms do not both have to be zero and, indeed, this will rarely if ever be the situation. Rather, it is only necessary for the two terms to be equal to each other. As such, the critical condition is a thermodynamically pseudoideal condition (rather than an ideal one, which would be the case if both thermodynamic terms were zero), revisiting a theme seen earlier in our discussions of both polymer dissolution and of the theta condition for polymer solutions (see Section 5.1 for both accounts).

The simplicity of the principle underlying LCCC belies the difficulty in finding critical conditions and the limitations of the technique. As such, it has found a home mostly, though certainly not exclusively, in academia, where the study of model (co)polymers predominates, as compared to industry where complex polymers form the basis of numerous plastic products, machine components, etc.

8.1. Determining the critical condition: choosing solvent(s) and column

While some of the caveats associated with LCCC are examined in more detail in Section 8.4, we mention at the outset that there is no way to determine *a priori* whether or not limiting conditions exist for the separation of a particular polymer. As stated recently by Brun and Rasmussen when referring to the central role the critical point of adsorption and, specifically, critical conditions play in many IPC techniques, “a lack of methodology for a systematic approach to find such conditions for novel polymers often hampers the application of IPC techniques for the characterization of complex polymers” [73].

For a particular polymer, critical conditions involve a combination of column stationary phase chemistry, solvent(s), and temperature. The role of temperature is discussed in Section 8.3. Here, we focus on the choice of column and solvents in LCCC.

For an LCCC separation, one usually seeks a system where there will be weak adsorptive interactions between the analyte and the stationary phase [74]. As a popular application of the technique is for the analysis of block copolymers, let us take the case of one such copolymer composed of polar and non-polar segments (blocks).

Table 6
Elution scenarios in LCCC of block copolymers employing RP and NP columns.

Column	Mobile phase polarity	Polar segment	Non-polar segment
RP	High	LCCC	IPC
RP	Low	SEC	LCCC
NP	High	LCCC	SEC
NP	Low	IPC	LCCC

Source: Ref [75].

One question is: If, given a particular type of column (RP or NP) and a particular mobile phase polarity (high or low), critical conditions can be established for one segment of the copolymer (the “critical” block), in what chromatographic mode (SEC or IPC) will the other segment (the “non-critical” block) elute?

If critical conditions can be established for the polar block employing an NP column and a high polarity mobile phase, then the non-polar blocks will be excluded from the pores of the column packing material and the block copolymer will elute ahead of the column void volume according to the length of the non-critical block. Conversely, if critical conditions can be established for the non-polar block on an NP column employing a low-polarity mobile phase, elution of the copolymer will be dictated by the interactive elution of the polar block. On an RP column, if critical conditions can be found for the polar block using a high-polarity mobile phase, then the non-polar block will display stronger interaction with the stationary phase and the copolymer will elute subsequent to the column void volume. If, again using an RP column, critical conditions can be found for the non-polar block using a low-polarity mobile phase, then the polar segments of the copolymer will be excluded from the pores and the copolymer will elute ahead of the void volume according to the segment length of the excluded block. These possibilities are summarized in Table 6.

The pore diameter of the column packing material is also an important consideration, in that it can affect the temperature at which critical conditions occur (see Section 8.3).

Determining the critical condition is oftentimes done by mixing a solvent and a non-solvent for the analyte, where the non-solvent may comprise from as little as less than 1% to as much as 70% of the solvent mixture. Increasing the amount of non-solvent in the mixture will decrease the thermodynamic quality of the latter and, while this doesn't necessarily affect critical conditions *per se* [58], it has been connected to problems related to poor solubility of high-*M* polymers and, even, to these polymers' inability to reach the critical condition due to their precipitation from solution.

A better approach than the solvent/non-solvent approach is to employ a pair of thermodynamically good solvents (these would take on the roles of *adsorli* and *desorli*, as defined in Section 7.1.1), which results in good polymer solubility. In this case, one solvent (the *adsorli*) should support adsorption of polymer onto the column packing material, while the other solvent in the pair (the *desorli*) should support desorption. This type of solvent combination can enable critical behavior up to very high molar masses. It should be noted that this will not guarantee full analyte recovery from the column, however. For example, when PMMA is dissolved in a mix of THF and toluene, both of which are thermodynamically good solvents for this polymer, and analyzed on a silica-based column, either little or no sample recovery was observed for samples with molar mass above $\approx 2 \times 10^5 \text{ g mol}^{-1}$ (though *M*-independent elution was observed below this value), presumably due to strong adsorption of the higher-*M* PMMAs on silica [76].

When employing mixed solvents for LCCC, whether these be a solvent/non-solvent pair or a pair of thermodynamically good solvents, in either case it is possible for very small changes in solvent composition to affect the critical condition [77]. These changes may

be as small as a 0.1% change in the relative ratio of the two solvents in the mixture. As seen in the left-hand side of Fig. 22, a mere 0.5% increase in the amount of methylene chloride in a $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ mixture changes the elution mode of PS from LCCC to SEC at the given experimental conditions, while a decrease of 2% in the amount of methylene chloride leads to a switch from LCCC to IPC at the same conditions.

In aqueous systems, the pH of the mobile phase needs to be considered, as well, when establishing critical conditions.

Critical conditions can also be achieved employing a single eluent. This eliminates problems regarding preferential solvation in mixed solvents (see Section 8.4 and ref [18]), solvent peaks, and the preferential sorption of one mobile phase component versus the other onto the column packing material. Use of single eluents also expands the variety of detection methods that can be accurately used in LCCC. Fig. 23 shows the separation of PMMAs based on tacticity employing a single eluent.

Critical conditions for various polymers have been found to occur near the critical solubility parameter of the analyte, i.e., when the solubility parameters of the polymer and solvent are close to each other (generally interpreted as corresponding to a difference of $\leq 1 \text{ cal}^{1/2} \text{ cm}^{-3/2}$) [58]. However, even for a given type of stationary phase chemistry (e.g., silica gel, styrene/divinyl benzene, etc.), the critical solubility parameter for a given polymer may vary by as much as $\pm 3 \text{ cal}^{1/2} \text{ cm}^{-3/2}$. This variability is possibly due to differences between supposedly identical column packing materials (e.g., differences between silica from different manufacturers, or between silica lots from the same manufacturer, in amount of unreacted silanol groups on the surface of derivatized silica), and/or to differences in experimental temperature. Tables of critical solubility parameters for various polymers on a number of different stationary phases can be found in Ref. [58]. We note that, to date, no clear correlation has been found between the critical solubility parameter and either the eluotropic strength ϵ^0 or the polarity P' of either single or binary eluents.

Brun and Alden elegantly described how to determine if a critical condition can be established in a particular system (combination of solvents, temperature, and column stationary phase) [80]. Their method requires the initial precipitation of analyte onto the column. Redissolution of the sample will occur at its solubility threshold Φ_{sol} . This redissolution will happen at a solvent composition either above or below the critical composition Φ_{cr} . If above ($\Phi_{\text{sol}} > \Phi_{\text{cr}}$), subsequent to redissolution the sample will no longer experience adsorbing interactions with the stationary phase, migrating instead through the column with the same velocity as the surrounding solvent and eluting at $\Phi = \Phi_{\text{sol}}$. In this case, it will be impossible to establish a critical condition in that particular system, because any possible solvent composition able to dissolve the sample will also prevent its interaction with the stationary phase and the sample will elute in SEC mode. If, however, $\Phi_{\text{sol}} < \Phi_{\text{cr}}$ then the dissolved sample will remain adsorbed onto the column packing. As the eluotropic strength of the mobile phase increases, the sample desorbs and elutes near Φ_{cr} . Because, in this latter case, the sample is soluble in the critical eluent, it will be possible to establish critical conditions in the particular system.

8.2. Determining critical eluent composition

The traditional method of determining critical eluent composition involves analyzing, in a particular solvent mixture, a series of narrow dispersity standards with the same monomeric repeat unit as the sample under consideration and covering a wide range in *M*. One performs a series of experiments in which the solvent ratio in the mixture is slowly varied, until all the standards are observed to elute at the same retention volume, i.e., at this solvent ratio analyte

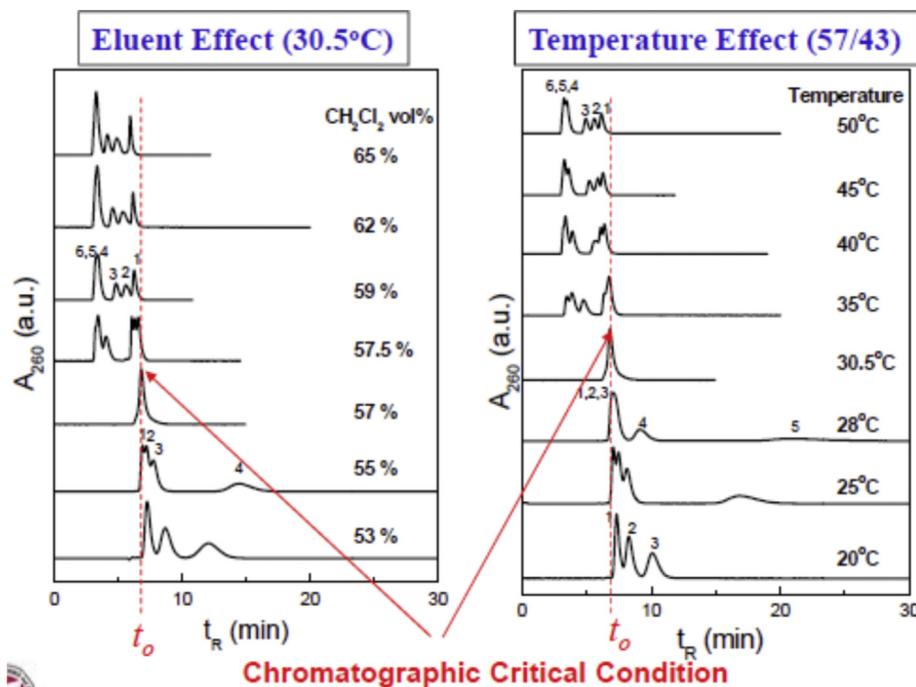


Fig. 22. Temperature and solvent effects on retention in LCCC. Analytes are narrow dispersity linear PS, with molar mass, in g mol^{-1} of 2.5×10^3 (1), 1.2×10^4 (2), 2.9×10^4 (3), 1.65×10^5 (4), 5.02×10^5 (5), and 1.8×10^6 (6). Column: Nucleosil C18, 5 μm particle size, 100 \AA pore size, 4.5 mm \times 250 mm; eluent: $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ mixture; flow rate: 0.5 mL min^{-1} . Critical conditions: 57/43 $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$, 30.5°C . (Reprinted with permission from Refs. [78]).

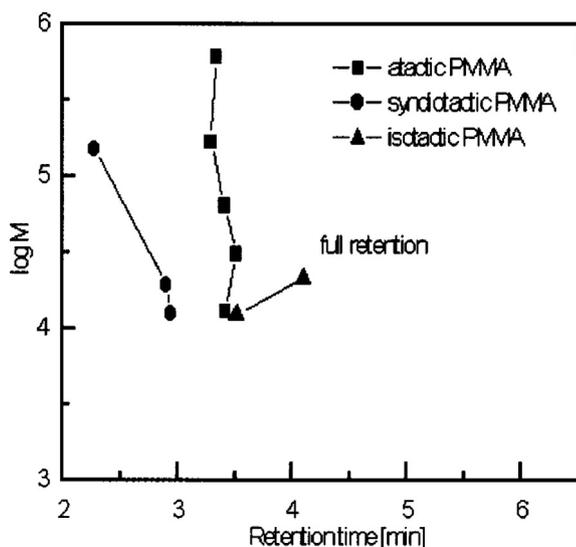


Fig. 23. Retention behavior of PMMA based on tacticity, employing a single eluent. At given conditions, syndiotactic PMMA (filled circles) elutes in SEC-like mode, isotactic PMMA (filled triangles) in adsorptive mode, and atactic PMMA (filled squares) in LCCC mode. Solvent: Acetonitrile; column: Nucleosil C18 5 μm particle size, 300 \AA pore size, 6 mm \times 250 mm; temperature: 68°C . (Reprinted with permission from Ref. [79]. Copyright 2002 American Chemical Society.)

retention is M -independent. This is, to put it mildly, quite tedious – and can be quite frustrating if critical conditions do not exist for the particular analyte which, as mentioned earlier, cannot presently be determined *a priori*.

A simpler approach is that proposed by Radke and coworkers [81], building upon earlier work by German and colleagues [82] and by Brun and Alden [80]. Their proposed strategy for finding the

critical eluent composition goes as follows, employing the separation of poly(ethylene glycol), PEG, as an illustrative example:

- 1) Once a solvent pair has been decided upon (see previous section for some selection guidelines in this respect, as well as the extensive tabulations of critical conditions in Ref. [58]), perform between one and three linear gradient runs with different slopes (e.g., 0%–100% strong solvent in 10 min, 20 min, and 40 min) for a single, high- M sample. For each run, calculate to solvent composition at elution. For simplicity, the maximum of the analyte chromatogram peak provides a convenient point for which to perform this calculation, which can be done employing equation (24):

$$\%B_g = (V_g - V_v - V_d) \frac{\Delta\%B_g}{Ft_G} + \%B_0 \quad (24)$$

where $\%B_g$ is the eluent composition at peak maximum (in terms of the strong solvent), V_g is the elution volume at peak maximum, V_v is the column void volume, V_d the system dwell volume, $\Delta\%B_g$ the total change in composition (of strong eluent) during the gradient, t_G is the gradient time, F the flow rate, and $\%B_0$ the initial composition. Results from this step for the separation of PEG in a mixture of methanol (MeOH) and water are shown in Fig. 24a.

- 2) Perform isocratic runs with at least three standards (if these exist for a particular analyte; if not, homopolymeric samples with as narrow a molar mass distribution as possible will have to do) at the solvent composition calculated in point 1 above, and at a composition a few percent higher in the strong solvent. If, in point 1 above, the composition at elution was found to be strongly dependent on gradient slope, then the difference between the composition at elution and the composition calculated from the second isocratic run (that performed with a few higher percent strong solvent) is expected to be larger than if a

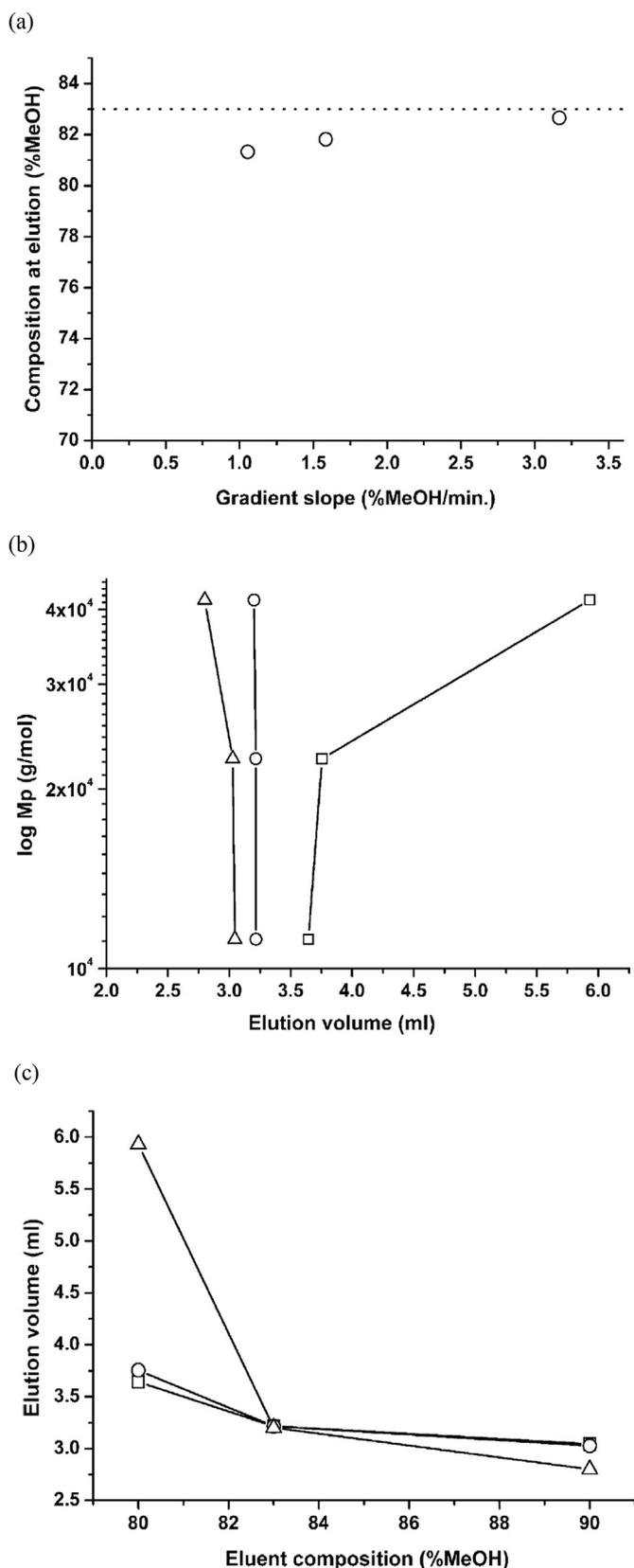


Fig. 24. Fast determination of critical eluent composition for polymers by gradient chromatography. (a) Composition at elution (% MeOH in water) for a PEG with peak-average molar mass M_p of 4×10^4 g mol⁻¹ as a function of gradient slope. Dotted line shows critical composition as determined from isocratic runs. (b) Dependence of elution volume on molar mass of PEGs at different eluent compositions. Open squares: 80% MeOH; open circles: 83% MeOH; open triangles: 90% MeOH. (c) Dependence of

weak dependence had been found for composition at elution on gradient slope. The reason for this is that strong dependences on gradient slope have been found for lower- M polymers which elute at lower compositions. Results from this step are shown in Fig. 24b.

- Plot either the elution volume (most conveniently), capacity factor k , or distribution coefficient K_D versus the isocratic eluent composition for the different M . The eluent composition at the intersection point corresponds to the critical eluent composition. Results from this step are shown in Fig. 24c. It is recommended that one additional isocratic experiment be performed at this composition to verify the results.

8.3. Determining the critical condition: finding the right temperature

It appears that, originally, many researchers would try to locate the critical condition temperature by starting their experiments near the theta temperature of the polymer solution, if such a value was already known. This was due to the fact that, in the vicinity of the theta temperature, polymer coils rapidly change their structure, and below this temperature the thermodynamic quality of solvents generally deteriorates. While one certainly has to begin *someplace* when tuning temperature for an LCCC separation, as Macko et al. concluded from their investigations in this regard, "It is evident that any transition in the structure of macromolecules produced by temperature variation cannot bring the system to critical conditions if the interaction between macromolecules and column packing is either too strong or too weak" [79]. As mentioned earlier in Section 6.3, temperature alone does not provide as large a variation in interaction strength between analyte and stationary phase as does solvent composition.

The above, from nearly two decades ago, virtually summarizes the state-of-the-art as regards published rationale concerning the critical condition temperature. However, as can be seen in the right-hand side of Fig. 22, even a small change of a few degrees (and oftentimes a mere fraction of a degree) in either direction can drive a separation away from critical conditions and into either adsorptive or size-exclusion mode. Experimentalists have thus resorted to varying the system temperature stepwise from low to high or vice-versa via a series of experiments each conducted at an individual temperature and at an individual solvent composition.

A more systematic approach to the problem, though not a particularly enticing one, would involve performing, for temperature, something akin to the 3-step approach outlined in Section 8.2 for determining the critical eluent composition, but doing so in tandem with the 3-step eluent approach (one can think of the problem as similar to trying to optimize a ternary solvent gradient, which is hardly a trivial effort). Data interpretation would likely benefit in this case from a surface response methodology approach [83]. In reality and for all practical purposes, this does not seem easily implementable (without wishing to discourage anyone from attempting it).

It has also been known for some time that the pore size of the column packing material can influence the temperature at which critical conditions occur. For example, employing a series of C18 columns of the same dimensions, particle size, and manufacturer,

elution volume on eluent composition for different molar masses of PEGs (values denote M_p in g mol⁻¹): Open squares: 1.2×10^4 ; open circles: 2.3×10^4 ; open triangles: 4.0×10^4 . In all cases, column: Nucleosil C18, 5 μ m particle size, 300 Å pore diameter, 4.6 mm \times 250 mm; flow rate: 1 mL min⁻¹; temperature: 35 °C; detector: ELSD. (Reprinted with permission from Ref. [81]).

which only differed from each other with respect to pore size, Abdulahad and Ryu noted that, as the pore size decreased from 300 Å to 120 Å to 100 Å to 50 Å, the critical temperature decreased from 29.9 °C to 26.9 °C to 26.4 °C to 24.7 °C, respectively [84]. The authors postulated that the fact that the critical condition occurred at a higher temperature with larger column pore size was due to a weaker surface interaction energy between analytes and column packing material in larger pore size columns. These results appear to have been confirmed by Lattice Monte Carlo simulations of the dependence of critical conditions in LC on the pore size of the column packing material [85].

Some guidance with respect to determining the critical condition temperature can be gleaned from known relations between retention and temperature. For example, recent work has shown that, for non-polar solvents on a C30 stationary phase, the retention factor k decreases by approximately a factor of two for every 10 °C increase in temperature [86]. At any rate, beginning at slightly above room temperature (to nullify the effects of small, and not-so-small, temperature variations in the physical environment within which the instrument is located) for low-viscosity solvents is probably a good idea while, for higher-viscosity solvents beginning at either 50 °C or even 80 °C might be necessary. If, for these latter solvents, lower temperatures are needed, it might then be necessary to reduce the volumetric flow rate of the experiment so as to not damage the column by overpressurizing the packing material.

Fig. 25 shows how, even in a single eluent, a change in temperature can drive the mode of separation from size-exclusion-like to critical to adsorptive.

8.4. Caveats associated with LCCC

Berek has written repeatedly and at length about potential shortcomings and caveats associated with the LCCC analysis of macromolecules [14,87,88]. A summary of these follows:

1) *Demanding identification of critical conditions.* Given that this has been addressed extensively above, we shall not discuss it further here.

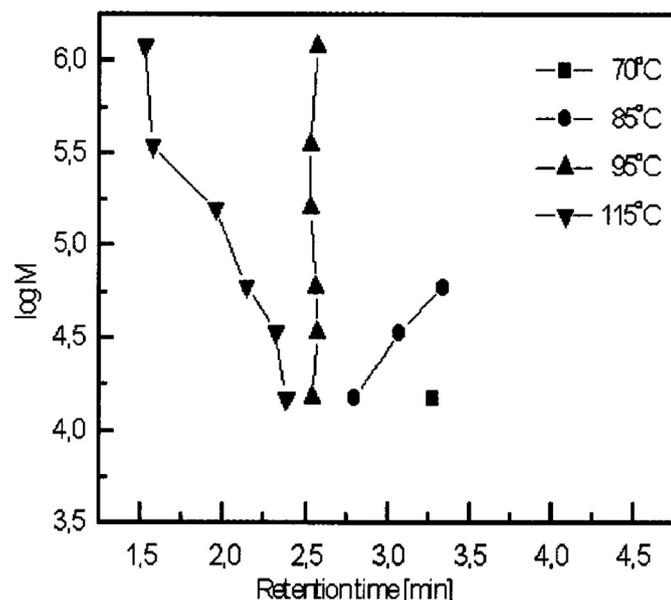


Fig. 25. Effect of temperature on critical conditions. Analytes: Narrow M -distribution linear PS; eluent: Dimethyl formamide; column: Nucleosil C18 5 μm particle size, 300 Å pore size, 6 mm \times 250 mm. (Reprinted with permission from Refs. [79]. Copyright 2002 American Chemical Society.)

- 2) *Extremely high sensitivity of critical conditions to eluent composition and to continuous variation in column interactivity* (due to, e.g., irreversible retention of some sample components). Higher- M chains are more sensitive to critical conditions than lower- M ones. Even if eluents can be prepared repeatedly at the same exact composition, problems can result from preferential evaporation or preferential absorption of atmospheric moisture.
- 3) Problems associated with *frictional heating within the column*, such as the creation of axial and/or radial temperature gradients therein. This may lead to differences in column interactivity within a given column due to the creation of regions where preferential polymer sorption may occur, thus leading to a gradual departure from critical conditions.
- 4) *Excessive peak broadening.* Given that there should be no evidence of molar mass dispersity in LCCC for a critically-eluting polymer (or for the critically-eluting block of a block copolymer), any peak width observed should be the result of intra- and extra-column mixing and diffusion processes. This broadening appears to become more severe with increasing molar mass and with decreasing column packing pore size, and to be less severe in single eluents as compared to mixed mobile phases. Few studies have been performed in this regard, however.
- 5) *Detection problems.* Polymers dissolved in mixed eluents will experience preferential solvation by one solvent over another. This means that, when using differential detectors such as refractometers, light scattering photometers, or viscometers, the solvent baseline will not accurately reflect the solvents' contribution to the analyte peak, thereby compromising the quantitative accuracy of the determinations. As such, evaporative-type detectors (see Section 3.2) are commonly employed for LCCC. These detectors suffer from a limited linearity of response and from the fact that this response can be related only empirically to a variety of instrument and solvent parameters. Issues regarding preferential solvation will generally be absent in special cases, such as when employing an isorefractive solvent pair [18], and are obviated by the use of single eluents.
- 6) *Limited sample recovery.* Part of a sample can become irreversibly adsorbed onto the column packing, leading to incomplete recovery and to a bias in calculated results. This phenomenon becomes increasingly severe at higher molar masses and as the column packing pore size decreases, though the underlying phenomenology remains unknown. Methods to determine column recovery are described in Ref. [87]. This is a problem that plagues much of IPC, not just LCCC and which, unfortunately, is most often ignored.

9. Conclusions and future outlook

Presented here were a set of self-contained guidelines for developing various interaction polymer chromatography methods. These included both traditional and interactive GPEC, general IPC methods, TGIC (room- and high-temperature), barrier and SEC-gradient methods, and LCCC. The aim has been to distill from the literature and the author's experience what relevant information exists regarding the interactions of macromolecules, solvent(s), temperature, and column stationary phase chemistry so that, applying some of the fundamental LC concepts included herein, readers can attempt a more informed design of their own IPC methods for analytes that might not match those exact ones for which a particular, "bespoke" method has been published. The focus has been a predictive and method development approach to the subject matter and, as such, most of the descriptive literature aimed at explaining how a particular method works has not been

covered. For more insights into the latter, the reader is referred to Ref. [61,73].

Much remains to be discovered in this area, especially as regards the fundamentals of the particular separations. For example, the role of temperature in most methods, TGIC notwithstanding, has generally not been explored in sufficiently significant detail. Likewise, while the role of eluotropic strength is understood fairly well in most IPC separations, this is not equally so as regards thermodynamic solvent quality as well as with respect to our understanding of what fundamental parameter governs the strength of interactions between analyte and chromatographic stationary phase. An iterative approach involving experiment, theory, and modeling and simulations appears to be the most promising way of making significant inroads into these problems.

It is worth noting that, to date, most IPC separations are conducted employing columns designed for small-molecule LC, i.e., except for the use of SEC columns for some IPC separations, there are no commercially-available columns designed specifically for the latter. This presents an issue: For small-molecule separations, surface inhomogeneities lead primarily to a loss in efficiency and resolution. For large molecules, these same inhomogeneities may lead to analyte recoveries that are highly reduced or biased with respect to molar mass or chain length or, in a worst-case scenario, to the irreversible adsorption and concomitant non-recovery of analyte from a column. Given the immense variability in results, or even in experimental feasibility, encountered by users of small-molecule LC columns for IPC analyses, a wider choice of commercially-available IPC columns is thus of paramount importance to the general macromolecular separations community.

IPC methods are integral to determining the chemical composition distribution of macromolecules and to separating multi-component polymeric samples according to their chemical constituents, to determining the tacticity and end-group distribution of polymers, and to determining the length and molar mass distributions of select blocks in block copolymers, all properties which greatly affect the processing and end-use behavior of macromolecules. Combined with size-based separation methods as part of a 2D-LC set-up, IPC methods can provide, among other things, the combined CCD \times MMD of complex polymers and blends. For all this to be so, the application of IPC methods will need to become more widespread than is currently the case, especially as regards industrial users. This begins with a better, more fundamental, and thus more versatile understanding of how to develop IPC methods. To this effect, it is the author's hope that the information included herein and in the accompanying references will provide the reader with sufficient guidance to attempt and successfully carry out non-empirical method development in this area.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations¹

ACN:	Acetonitrile
ASTM:	American Society of Testing Materials
C ₈ :	<i>n</i> -Octyl
C ₁₈ :	<i>n</i> -Octadecyl
C30:	<i>n</i> -Triacontyl
CCD:	Chemical composition distribution
CEC:	Capillary electrochromatography
CellOH:	Cellulose
CN:	Cyano
CRYSTAF:	Crystallization analysis fractionation
DLS:	Dynamic light scattering
DMAc:	<i>N,N</i> -Dimethyl acetamide
DMF:	Dimethyl formamide
DNA:	Deoxyribonucleic acid

¹ For list of symbols related to HT-TGIC and their definition, see Table 5.

DRI:	Differential refractometry	k_w:	Value of <i>k</i> at $\phi = 0$ (i.e., at % B = 0)
ELSD:	Evaporative light scattering detection	k*:	Retention factor of gradient
EtAC:	Ethyl acetate	K_D:	Solute distribution coefficient
FFF:	Field-flow fractionation	K_{D,Pol}:	Solute distribution coefficient of polymer
FFFF:	Flow field-flow fractionation	L:	Column length
GPEC:	Gradient polymer elution chromatography	n_B:	Relative size of solvent B in solvent mixture
HDC:	Hydrodynamic chromatography	R_A^S:	Radius of interaction of the solvent
HILIC:	Hydrophilic interaction liquid chromatography	N_B:	Mole fraction of solvent B in mobile phase
HT:	High-temperature	R:	Gas constant
IPC:	Interaction polymer chromatography	R_{AO}^P:	Radius of interaction of the polymer
IR:	Infrared spectroscopy	R_A^{mix}:	Radius of interaction of solvent mixture
IUPAC:	International Union of Pure and Applied Chemistry	S:	Change in log <i>k</i> for unit change in ϕ . $S \approx M^{0.5}/4$
LC:	Liquid chromatography	t:	Time
LCCC:	Liquid chromatography at the critical condition	t_G:	Gradient time
LC-LCA:	Liquid chromatography at limiting conditions of adsorption	t_R:	Retention time of analyte
LC-LCD:	Liquid chromatography at limiting conditions of desorption	t₀:	Retention time of injection solvent
LC-LCI:	Liquid chromatography at limiting conditions of insolubility	T:	Temperature
M:	Molar mass	V_d:	System dwell volume
MA:	Methacrylic acid	V_g:	Eluent volume at peak maximum
MALDI-MS:	Matrix assisted laser desorption/ionization mass spectrometry	V_m:	Column void volume
MALS:	Multi-angle static light scattering	v(T):	Temperature-dependent migration rate
MeOH:	Methanol	V_S:	Volume of stationary phase in column
M_n:	Number-average molar mass	V_M, V_m:	Volume of mobile phase in column ("dead volume")
M_p:	Peak-average molar mass	γ:	Column heating rate
M_w:	Weight-average molar mass	δ:	Hansen solubility parameter
M_z:	z-average molar mass	δ_d:	Hansen solubility parameter dispersive term
MMD:	Molar mass distribution	δ_d^{NS}:	Hansen solubility parameter dispersive term of nonsolvent
NIR:	Near-infrared spectroscopy	δ_d^P:	Hansen solubility parameter dispersive term of polymer
NMR:	Nuclear magnetic resonance spectroscopy	δ_d^S:	Hansen solubility parameter dispersive term of solvent
NP:	Normal-phase	δ_H:	Hansen solubility parameter H-bonding term
ODCB:	o-Dichlorobenzene	δ_H^{NS}:	Hansen solubility parameter H-bonding term of nonsolvent
p:	Degree of polymerization	δ_H^P:	Hansen solubility parameter H-bonding term of polymer
P:	Solvent polarity index	δ_H^S:	Hansen solubility parameter H-bonding term of solvent
PAN:	Polyacrylonitrile	δ_p:	Hansen solubility parameter polar term
PB:	Polybutadiene	δ_p^{mix}:	Hansen solubility parameter of solvent/nonsolvent mixture
PDI:	Dispersity index (ratio of number-average to weight-average)	δ_{np}^{mix}:	Hansen solubility parameter of polymer in mix of solvent and non-polar nonsolvent
PEG:	Poly(ethylene glycol)	δ_p^{NS}:	Hansen solubility parameter polar term of nonsolvent
PEO:	Poly(ethylene oxide)	δ_p^P:	Hansen solubility parameter polar term of polymer
PSDA:	Particle size distribution analyzer	δ_p^S:	Hansen solubility parameter polar term of solvent
PMMA:	Poly(methyl methacrylate)	δ_{po}^{mix}:	Hansen solubility parameter of polymer in mix of solvent and polar nonsolvent
PS:	Polystyrene	δ_p^P:	Hansen solubility parameter of polymer
PVB:	Pol(vinyl butyral)	δ_{NS}^S:	Hansen solubility parameter of nonsolvent
P2VP:	Poly(2-vinyl pyridine)	δ^S:	Hansen solubility parameter of solvent
QELS:	Quasi-elastic light scattering	ΔG_{fusion}:	Gibbs free energy of fusion
RED:	Relative energy difference	ΔG_{mixing}:	Gibbs free energy of mixing
RP:	Reversed-phase	ΔG_{reaction}:	Gibbs free energy of reaction
SAN:	Poly(styrene-co-acrylonitrile)	ΔG_{solution}:	Gibbs free energy of solution
SBS:	Styrene-butadiene-styrene rubber	ΔG_{transfer}:	Gibbs free energy of transfer between phases
SEC:	Size-exclusion chromatography	ΔH_{mixing}:	Enthalpy of mixing
SFC:	Supercritical fluid chromatography	ΔH_{transfer}:	Enthalpy of transfer between phases
SGIC:	Solvent gradient interaction chromatography	ΔS_{mixing}:	Entropy of mixing
TCB:	1,2,4-Trichlorobenzene	ΔS_{transfer}:	Entropy of transfer between phases
TGIC:	Temperature gradient interaction chromatography	Δφ:	Gradient range
THF:	Tetrahydrofuran	ε⁰:	Eluotropic value of solvent
TREF:	Temperature-rising elution fractionation	φ:	Volume fraction of solvent B in mobile phase
UV:	Ultraviolet absorption spectroscopy	φ:	Phase ratio of column ($\phi \equiv V_S/V_M$)
VISC:	Viscometry	φ^{NS}:	Volume fraction of nonsolvent
VOH:	Vinyl alcohol	φ^P:	Volume fraction of polymer in solution
W_f:	Weight-fraction of a particular species	φ^S:	Volume fraction of solvent
2D-LC:	Two-dimensional liquid chromatography	φ_{cr}:	Critical solvent composition
		φ_{sol}:	Solubility threshold of analyte
		θ:	Theta temperature
		%B_g:	Eluent composition at peak maximum, in terms of strong solvent
Symbols			
a:	Slope of van't Hoff plot		
b:	Intercept of van't Hoff plot		
A₂:	Second-virial coefficient		
F:	Volumetric flow rate		
k:	Retention factor		
k_{Pol}:	Retention factor of polymer		