

Review

# Determination of chemical composition distributions in synthetic polymers

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## Abstract

A characteristic feature of synthetic polymers is their dispersity in molar mass and, in many cases, chemical composition. Since dispersity is highly relevant in relation to polymer properties, ongoing efforts are being put in the development of appropriate analysis methods. In this respect, size-exclusion chromatography (SEC) is well known for the determination of molar mass distributions. Methods for chemical composition distributions are less mature than SEC and mainly include liquid chromatography and mass spectrometry and the combination of these techniques. The term chemical composition distribution is considered broad in this paper, i.e. for the chemical composition distribution of a (co)polymer backbone, for the functionality type distribution of a polymers' functional end groups, for the block length distribution of a block copolymer, for the branching distribution and for the tacticity distribution. In this paper, analysis methods for all types of chemical composition distributions are reviewed. Special attention is paid to practical requirements and common misconceptions that sometimes arise. Applications within the last 5 years are summarized.

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*Keywords:* Reviews; Chemical composition distributions; Polymers

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*Abbreviations:* ACN, acetonitrile; AP, atmospheric pressure; ATRP, atom-transfer radical polymerization; BD, branching distribution; BLD, block length distribution (of a block copolymer); CCD, chemical composition distribution (of a copolymer chain); DAD, photodiode-array detection; DCM, dichloromethane; DV, differential viscosity detection; EPDM, poly(ethylene-propylene-diene); ESI, electrospray ionization; FFF, field flow fractionation; FTD, functionality type distribution; FT-ICR, Fourier transform ion cyclotron resonance (MS); FT-IR, Fourier transform infrared spectroscopy; GC, gas chromatography; GE-LC, gradient elution liquid chromatography; IR, infrared spectroscopy; LC, liquid chromatography; LCCC, liquid chromatography at critical conditions; LC-LC, two-dimensional LC (off-line or heart cutting method); LC × LC, comprehensive, two-dimensional LC (on-line); LS, light scattering detection; MALDI, matrix assisted laser desorption ionization; MALLS, multi angle laser light scattering detection; MeOH, methanol; MMD, molar mass distribution; NMR, nuclear magnetic resonance spectroscopy; MS, mass spectrometry; PEO, poly(ethylene oxide); PBMA, poly(butyl methacrylate); PDMA, poly(decyl methacrylate); PDMS, poly(dimethylsiloxane); PEMA, poly(ethyl methacrylate); PI, polyisoprene; PL, poly(L-lactide); PMMA, poly(methyl methacrylate); PPO, poly(propylene oxide); PS, polystyrene; PtBMA, poly(*tert*-butyl methacrylate); PTHF, polytetrahydrofuran; PVAc, poly(vinyl acetate); RAFT, reversible addition-fragmentation chain-transfer; RI, refractive index detection; SAN, styrene-acrylonitrile; SD, sequence distribution; SEC, size-exclusion chromatography; THF, tetrahydrofuran; TOF, time-of-flight; TREF, temperature rising elution fractionation; UV, ultraviolet (detection); 2D-LC, two-dimensional LC

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## 1. Introduction

During the past decade there has been a growing interest in the chemical heterogeneity characterization, i.e. all types of chemical composition distributions of synthetic polymers. This is due to increasing demands on polymer properties with respect to for instance mechanical and chemical resistance and biodegradability, necessitating the development of tailor made products such as copolymers and terpolymers, block copolymers and polymer blends. For this purpose, new polymerization processes such as atom-transfer radical polymerization (ATRP) and reversible addition-fragmentation chain-transfer (RAFT) are being developed [1,2].

Chemical composition distributions in complex synthetic polymers unlike for biological macromolecules results from the stochastic character of all polymerization processes [3]. Therefore, all synthetic macromolecules consisting of more than one monomer type are disperse with respect to chain length (molar mass) and chemical composition. In this paper, the term chemical composition is used not only for the composition of the polymer backbone but in a broad sense: for the chemical composition distribution (CCD) of the polymer backbone, composition distribution according to functional (end) groups (functionality type distribution (FTD)), composition distribution with respect to chain topology, e.g. tacticity or linear molecules versus cyclic products, branching distribution (BD), block length distribution (BLD), etc. A schematic overview of the various heterogeneity types is given in Fig. 1.

Since properties of a polymer largely depend on all its distributions, it is important to have access to appropriate analysis methods for the various distribution types. Distribution analysis can only be performed after separation of the chemical inhomogeneous polymer mixture into its consisting components, since any bulk technique (light scattering, NMR, titration) will only provide an average value of a specific compositional feature [4]. In this respect, the analysis of molar mass distributions (MMDs) by size-exclusion chromatography (SEC) is well known since many years [5].

Therefore, chromatographic separation methods (especially LC) and other hydrodynamic methods such as field flow fractionation (FFF) have become popular in this respect [6,7]. This is largely due to developments in interaction based LC techniques like gradient elution LC (GE-LC) and liquid chromatography at critical conditions (LCCC) during the 1990s. However, due to recent developments in mass spectrometry (MS) with respect to soft ionization techniques, i.e. electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI), MS has become amenable to higher molar mass components. Since MS be-

comes itself in this respect as a separation technique of intact polymer molecules it can be used for the characterization of polymer distributions, especially when coupled to LC, for which purpose it is rapidly becoming important [8,9].

For the complete characterization of complex polymer systems being disperse in more than one dimension (for instance both molar mass and chemical composition) a combination of separation methods is needed, each of which preferably responds to only one specific structural feature. Therefore there is increasing interest for the on-line coupling of separation methods for polymer characterization, such as LC  $\times$  LC [10,11] and LC-MS [8,9].

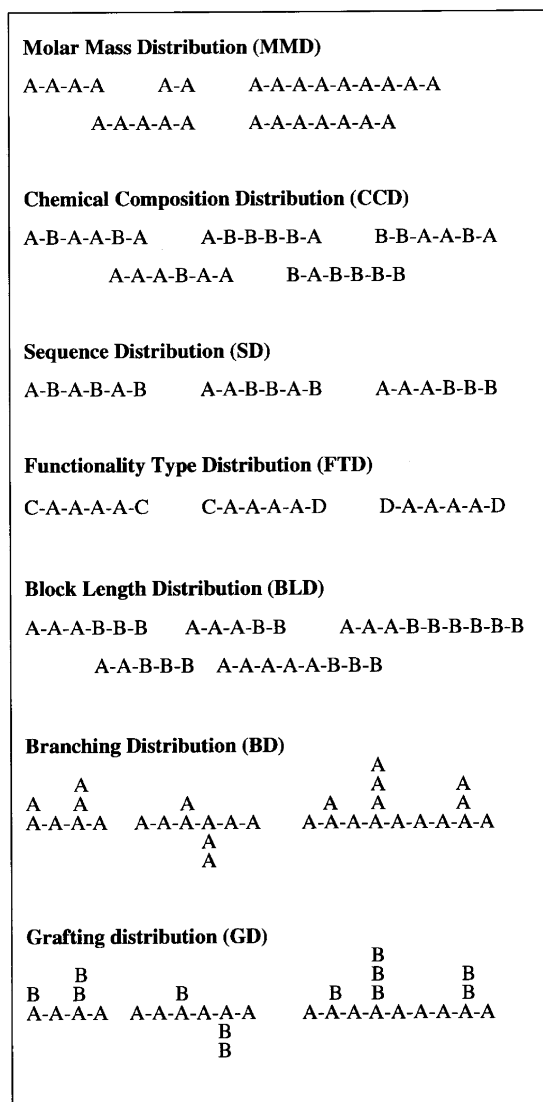


Fig. 1. Schematic representation of chemical inhomogeneities in polymers. A, B: monomeric repeat units; C, D: end groups.

It must be emphasized here that the application of separation methods to intact polymer mixtures can only be used for the determination of the so-called intermolecular microstructure: CCD, FTD, BLD, and BD. The intramolecular microstructure, i.e. the sequence distribution, which refers to the distribution of micro blocks of A and B along the polymer chain (Fig. 1) cannot be obtained in this way. From spectroscopic methods such as NMR and FT-IR [12,13] or pyrolysis followed by GC [14,15] some information with respect to sequence, actually: average block lengths, can be obtained. This however, goes beyond the scope of this review.

In this paper, methods for characterization of the various types of chemical composition distributions of synthetic polymers are reviewed. Since LC techniques are dominant in this respect, first a brief theoretical description of the various chromatographic modes for polymers is given. This is followed by an overview of the most important analysis techniques including mass spectrometry, and their recent developments. To this end literature between 1998 and 2003 was reviewed. Special attention was paid to practical requirements and common misconceptions that sometimes arise.

## 2. General theory of liquid chromatography of polymers

In liquid chromatography, generally porous column packings are used as stationary phase. High molar mass solutes can, depending on their size, partly penetrate into the pores of the column packing and subsequently undergo interactions with the active stationary phase, which is mainly located inside the pores. Therefore, two main processes can be distinguished in LC of polymers: steric exclusion and enthalpic interactions. The latter can either be ‘true’ adsorption or partition but will further be indicated as ‘adsorption’. The retention volume,  $V_r$  can therefore be expressed as [6]:

$$V_r = V_i + K_{\text{sec}} V_p + K_{\text{ads}} V_s \quad (1)$$

where  $V_i$  is the interstitial volume,  $V_p$  the pore volume and  $V_s$  is the stationary phase volume.  $K_{\text{sec}}$  and  $K_{\text{ads}}$  represent the chromatographic distribution coefficients ( $K_D$ ) for steric exclusion and for adsorption, respectively. A chromatographic distribution coefficient is defined as:

$$K_D = \frac{c_s}{c_m} = \exp^{-\Delta\mu_0/RT} \quad (2)$$

where  $c_s$  and  $c_m$  represent the concentration of a solute in the stationary and the mobile phase, respectively and  $\Delta\mu_0$  is the standard chemical potential difference for solute molecules in both phases.

In the case that a thermodynamically good solvent for the polymer which also effectively suppresses enthalpic interactions with the stationary phase (a strong ‘displacer’) is used as the mobile phase,  $K_{\text{ads}} = 0$  and retention is governed by entropic exclusion effects. In thermodynamic terms this means that  $\Delta h = 0$  and  $\Delta\mu = -T\Delta s > 0$

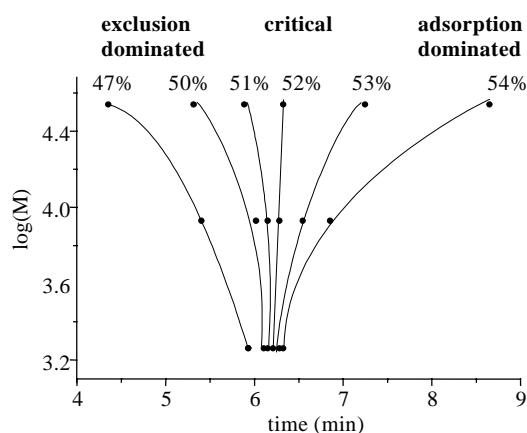


Fig. 2. Separation modes in the chromatography of polymers. Polystyrene standards on a Nucleosil  $C_{18}$  column, in various compositions of acetonitrile–dichloromethane at 25 °C. % (v/v) ACN as indicated in figure.

( $\Delta h$  and  $\Delta s$  are the partial molar enthalpy and entropy change, respectively). This chromatographic mode is known as size-exclusion chromatography (SEC).  $K_{\text{sec}}$  varies between 0 for large molecules, which are totally excluded from the pores (total exclusion) to 1 for small molecules, which can completely enter the pores (total permeation). Retention therefore decreases with increasing molar mass.

When the thermodynamic quality of the solvent is decreased by either the addition of a poor solvent [16] or a temperature change [17],  $K_{\text{ads}}$  may increase as enthalpic, adsorptive interactions start contributing to the total retention. These interactions increase exponentially with the degree of polymerization,  $p$ , due to the fact that more monomeric units are available for interactions with the stationary phase. In practice, a linear dependence between the logarithm of retention factor,  $\log(k)$ , and  $p$  is found (the Martin rule [6]). At a certain point  $K_{\text{ads}}$  is large enough such that  $K_{\text{sec}} V_p + K_{\text{ads}} V_s > V_p$  and retention is dominated by adsorption. In that case  $\Delta\mu = \Delta h - T\Delta s < 0$  and retention increases with increasing molar mass. The two separation modes and the transition from SEC to adsorption are shown in Fig. 2.

From this figure, it can be seen that under certain conditions, entropic exclusion effects and enthalpic adsorption effects are (nearly) balanced, such that  $K_{\text{sec}} V_p + K_{\text{ads}} V_s = V_p$  and  $\Delta\mu = 0$ , and retention is (almost) independent of molar mass. These conditions, commonly referred to as critical conditions, have both been predicted theoretically [18] and found experimentally [16,17] for various polymer systems. Chromatography under these conditions will further be called liquid chromatography at critical conditions (LCCC).

From the above it can be concluded that terms like ‘SEC’ and ‘adsorption chromatography’ refer to a thermodynamic condition of a separation system, rather than to the use of a specific column. The latter is a common misconception. It should be emphasized that for instance SEC can in principle be carried out on any porous column packing, provided that thermodynamic conditions (eluent, temperature) are chosen

properly. The main reason for the existence of dedicated SEC columns is that for realizing enough separation capacity more column packing (pore volume!) is needed than for adsorption chromatography. Furthermore, the range of pore sizes is more critical than in adsorption chromatography.

Both SEC and LCCC are isocratic methods. Chromatography under adsorption conditions for polymers however, generally requires the use of gradient elution. This is due to the rapid increase of retention with polymerization degree in combination with the fact that synthetic polymers are highly disperse with respect to molar mass. Caused by the limited solubility of polymers, which is predicted from the Flory Huggins theory [19], the starting eluent in gradient elution is often a non-solvent for at least part of the injected polymer. Thus, retention in gradient elution mode is governed by three parameters: exclusion, solubility effects and adsorption [6].

Enthalpic interactions playing a role in the critical and the adsorption mode are affected by both molar mass and chemical composition of a polymer. Therefore, LCCC and gradient elution techniques are suited to study various forms of chemical composition distributions of synthetic polymers as will become clear in the next sections.

### 3. Chemical composition determination by size-exclusion chromatography

As was pointed out in the previous section, SEC is an entropy governed separation technique. It mainly responds to differences in molecular size, which is dominated by chain length/molar mass making the technique especially suited for the study of a polymers' MMD. However, molecular size in solution is also influenced by molecular architecture such as branching and, to a somewhat lesser extent, by chemical composition via affinity towards the used solvent.

SEC coupled to more than one detector can provide information on the average chemical composition as function of molar mass (or to be more precise: hydrodynamic volume) via the comparison of the respective detector signals as function of elution volume. To be successful, the number of detectors preferably at least equals the number of different chemical components of a chemical heterogeneous polymer, each of which should respond differently to those components. It should be emphasized that although in this way very useful information on chemical heterogeneity of synthetic polymers can be obtained, the method does not reveal a chemical composition distribution! Furthermore, it is impossible to distinguish between polymer blends and copolymers, especially when, in the case of a blend, molar masses of the polymer components are pretty much the same. Finally, the translation of elution volume to molar mass for copolymers, in order to obtain the chemical composition as function of molar mass is not straightforward [20]. The accurate molar mass determination of copolymers goes beyond the scope of this review but the interested reader is referred to [7,10,21].

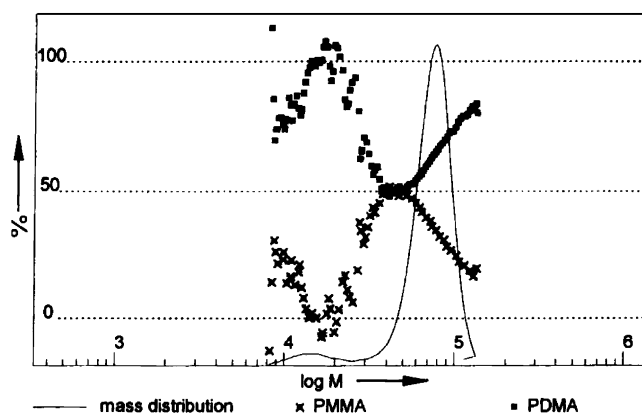


Fig. 3. MMD and chemical composition determination of a PMMA–PDMA block copolymer by SEC with combined refractive index and density detection. From [7] with permission.

The most well known and easy to handle multi detector setup is the combination of ultraviolet (UV) and refractive index (RI) detection, which, however, is only suited for copolymers containing a chromophore. Starting with the analysis of the corresponding homopolymers, the response factors and their ratios are determined for each detector. Subsequently for the copolymer the average composition as function of elution volume can be determined by solving a set of two equations [10]. Recent examples were shown by Medrano et al. who described the chemical heterogeneity determination of poly(styrene-*co*-methyl methacrylate) (PS-*co*-MMA) [22] and by Chiantore et al. for poly(ethylene-propylene-diene-graft-styrene-acrylonitrile) (EPDM-graft-SAN) [23]. This method, however, relies on the assumption that  $dn/dc$  is proportional to composition, which unfortunately is not always true.

An alternative for copolymers that lack a chromophore is the combination of RI and density detection [21], although examples are rare due to the restricted availability of commercial instruments. In Fig. 3, the simultaneous determination of MMD and average chemical composition as function of molar mass for the non-UV-absorbing poly(methyl methacrylate)-poly(decyl methacrylate) (PMMA-PDMA) block copolymer by SEC with combined RI and density detection is shown [7].

A critical feature in each multi detector approach is the matching of elution volumes, which are slightly different for the respective detectors due to the interdetector delay. Furthermore, it may be necessary to correct for resulting band broadening differences between the detectors. Incorrect correction leads to erroneous quantitative results, i.e. to high chemical composition differences [7].

Mourey and Balke presented their so called 'local polydispersity' method, relying on the combination of a RI, a differential viscosity (DV) and a light scattering (LS) detector [24,25]. In this method, an apparent number-average molar mass ( $M_n$ ) is calculated using the DV and RI detectors, assuming that no differences in refraction index



increment,  $dn/dc$ , due to chemical inhomogeneities as function of molar mass occur. Inhomogeneities cause so-called local polydispersity, as molecules with equal hydrodynamic volumes may differ in molar mass due to differences in chemical composition. Comparably, an apparent  $M_w$  is calculated from the LS and RI detectors. From the combination of both results a theoretical RI chromatogram with the assumption of ‘no local polydispersity’ is reconstructed. This result is compared with the experimentally obtained RI chromatogram and differences (as function of molar mass) represent chemical inhomogeneity. Also for this method,  $dn/dc$  is assumed to be proportional to composition (see before).

The combination of two SEC systems both with RI and LS detection, with different eluents each being isorefractive to either one of the constituting components of a PMMA-graft-poly(dimethylsiloxane) (PMMA-graft-PDMS) copolymer was described by Mrkivickova [26]. By combining the results of both systems the variation in chemical composition and molar mass of individual copolymer blocks as function of hydrodynamic volume could be determined. Results were in good agreement with those of other fractionation methods. Nevertheless, the method is of restricted importance since the requirement of two isorefractive solvents both of which must also be suited for SEC, will rarely be met.

An alternative for the multidetector approach is the coupling of more informative, spectroscopic techniques either on-line or off-line, to SEC. From the full spectroscopic data compositional information as function of elution volume can be obtained, provided that absorption characteristics of the constituting chemical components in a heterogeneous polymer are sufficiently different.

Although the most well-known spectroscopic detection method in LC is UV-photodiode-array detection (DAD), its application in polymer composition analysis is rare. This is due to the low specificity of UV spectra in combination with the fact that the condition of all components being UV active is rarely met for synthetic polymers.

Therefore, the combination of LC and FT-IR is more successful. In most cases a semi on-line setup using the commercially available solvent elimination interface is used thus bypassing the problem of too much IR background absorption of the used solvents. Recent applications include determination of functional group content as function of molar mass [27], backbone compositional heterogeneities of polyalkenes [28] and short chain branching in polyalkenes [29]. Recently, Schoonover et al. described the use of factor analysis of large data sets obtained by SEC-IR to obtain insights in polymer degradation mechanisms, which were not readily apparent from visual inspection of raw data files [30]. Furthermore, there is growing attention for difficulties related to quantitative aspects of SEC-FT-IR coupling which are affected by factors like film formation during evaporation of the solvent [31,32], eluent flow [33] and molar mass of the investigated polymer [33].

Until now, the coupling of SEC to NMR is less popular than LC-FT-IR, which is mainly due to the need of expen-

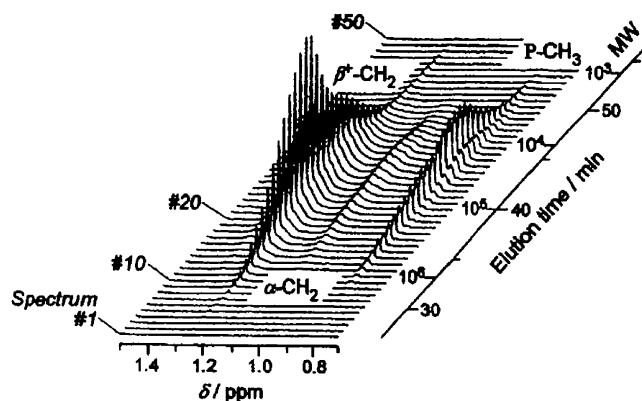


Fig. 4. Example of on-line SEC-NMR for the determination of backbone inhomogeneity as function of molar mass in an EPDM sample. Stacked trace plot of a series of 50 750 MHz SEC-NMR spectra plotted against elution time. Eluent:  $[^2\text{H}]\text{chloroform}$ , data acquisition per spectrum: 90 °C pulse, 4.5 s interval, eight scans. From [37] with permission.

sive high field instruments and the inherent lack of sensitivity of NMR. Nevertheless, due to its specificity and improved quantification aspects in comparison with IR and MS, SEC-NMR is very powerful in the compositional characterization of copolymers and functional polymers. An extensive review on the practical aspects of coupling LC in general, to NMR has been presented by Albert [34]. Recent applications include the distribution of end groups in several thermoplasts by off-line SEC-NMR [35], the off-line determination of chemical composition as function of molar mass for acrylate copolymers [36] and the on-line application to backbone heterogeneity of ethylene-propylene-diene (EPDM) prerubbers (Fig. 4 [37]).

Undoubtedly, the most important development in polymer characterization of recent years is the coupling of LC to MS using soft ionization techniques. Although MS is a separation technique itself it is nowadays generally accepted that for polymer analysis the coupling to another technique, mostly LC, is necessary for obtaining results that are representative for the entire polymer sample. Otherwise, essential information will be missed due to severe discrimination of high molar mass species as will be further pointed out in Section 7.

SEC-MS, especially off-line SEC-MALDI-MS, is nowadays frequently used for the absolute molar mass calibration of SEC [38,39]. But even more interesting in the present context is its use for chemical heterogeneity characterization. Especially during the latest years, examples have been shown for end group characterization [40–42], detection of cyclic species [40–42] and evaluation of the chemical composition of the polymer backbone as function of molar mass [36,38,44,45].

A unique feature of SEC coupled to DV and LS detection is the ability of obtaining information on branching. Branching seriously affects mechanical and rheological properties of synthetic polymers [46], but the chemical characterization is not always easy. Spectroscopic techniques like

NMR and FT-IR are rather insensitive to branching, i.e. the number of branches needed for affecting polymer properties is often much lower than the detection limits of these techniques. Since adsorption phenomena are hardly influenced by branching, interaction based forms of chromatography are also inadequate in this respect.

Branching, however, significantly reduces a molecules' hydrodynamic volume and therefore also influences retention in SEC. Furthermore, molecular density increases and thus the intrinsic viscosity,  $[\eta]$ , which in fact is the reciprocal density, decreases.  $[\eta]$  is defined as the limiting value of the ratio of specific viscosity and concentration, given by Eq. (3) [33]:

$$[\eta] = \lim_{c \rightarrow 0} \frac{\eta - \eta_0}{\eta_0 c} = \lim_{c \rightarrow 0} \frac{\eta_{sp}}{c}; \quad \text{for } c \downarrow 0 \quad (3)$$

where  $\eta_{sp} = (\eta - \eta_0)/\eta_0$ .  $[\eta]$  can be determined from the combination of DV and RI detection coupled to SEC. By comparing  $[\eta]$  of a linear sample with that of a branched sample, a quantitative impression of the average degree of branching as function of molar mass can be obtained from the branching parameter  $g'$  where  $g'$  is defined as  $[\eta]_{\text{branched}}/[\eta]_{\text{linear}}$  at equivalent molar mass [47].

Comparably, branching influences the radius of gyration,  $R_g$ . For large molecules with  $R_g$  exceeding 10 nm (for  $q$  of a  $\approx 680$  nm incident source), this parameter can be determined from combined multi angle light scattering (MALLS) and RI detection. Without going into detail in light scattering theory, the angular dependence of the Rayleigh scattering,  $P(\theta)$ , of a macromolecule in solution is related to its  $R_g$  according to Eq. (4),

$$\frac{1}{P(\theta)_i} = 1 + \frac{q^2 (R_g^2)_i}{3}; \quad q = \left\langle \frac{4\pi}{\lambda_0} \right\rangle \sin \frac{P(\theta)}{2} \quad (4)$$

where  $\lambda_0$  is the wavelength of the scattered light in vacuum. Analogous to  $g'$  a branching factor  $g$  is defined as  $\langle R_g^2 \rangle_{\text{branched}} / \langle R_g^2 \rangle_{\text{linear}}$ . Finally,  $g$  and  $g'$  are interrelated according to  $g' = g^\epsilon$ .  $\epsilon$  depends on the conformation of the macromolecule in solution and varies approximately between 0.5 and 2. A more detailed description of light scattering in combination with SEC can be found in [48].

It must be emphasized that both SEC–DV and SEC–LS do not provide a true branching distribution. The only method by which mainly a separation according to branching is obtained is temperature rising elution fractionation (TREF). The application of TREF, however, is restricted to (semi-)crystalline polymers such as polyalkenes (see Section 8) making its applicability very limited. Therefore, SEC coupled to DV and LS is nowadays the most sensitive and general applicable method for determining chemical heterogeneity caused by branching, as has been shown in numerous publications during the past 15 years [49,50]. Recent papers describe its application to macromolecules with complex architecture such as hyperbranched polymers [38,39] and starblocks [51]. Tackx and Tacx described the determination of both  $g$  and  $g'$  for low density polyethyl-

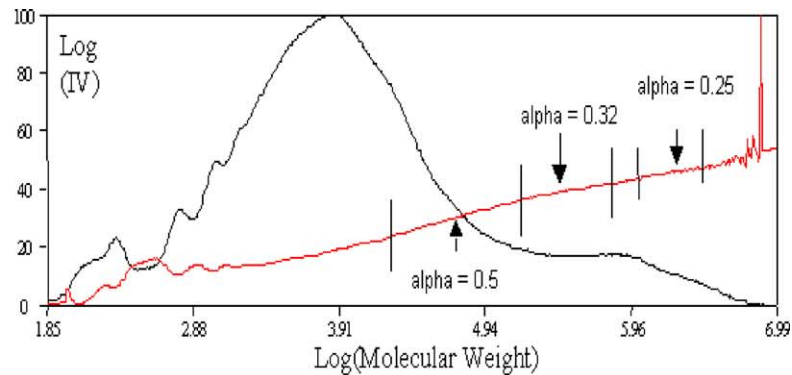
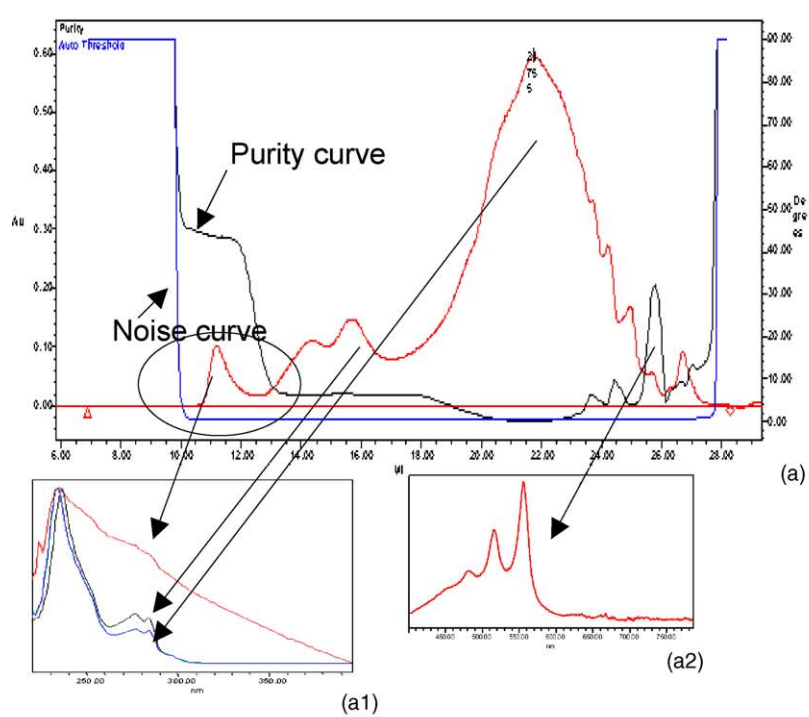
ene using SEC–MALLS in combination with universal calibration [52]. They showed that a reliable estimate for  $\epsilon$  could only be obtained at high molar masses, due to the inability to measure  $R_g$  below 10 nm. Podzimek and Kastanek showed that SEC–LS can also be applied for rather low molar mass polymers such as epoxy resins [53]. The sensitivity of SEC–DV to differences in architecture other than branching was recently demonstrated for copolyesters in work from our own lab [45].

Finally, the author wants to emphasize the practical importance of SEC for the characterization of complex, chemical inhomogeneous polymer systems. Due to its isocratic character, SEC is a relatively simple method that can easily be coupled to a large variety of detectors. The combination of DAD/RI/DV/LS is practically easy to handle and provides a wealth of information in one single analysis, despite the fact that no true chemical composition distribution is obtained. This is especially interesting for 'unknown' products for which an overall impression or a comparison with related products is needed, rather than a quantitatively exact characterization of polymer distributions. This situation often occurs in industry.

An example from our own lab is given in Fig. 5 where the analysis of an 'unknown' sample is shown. MMD information immediately shows a relatively low molar mass resin together with high molar mass fractions (Fig. 5a and b). Both comparison of UV and RI and impurity information from DAD indicates no significant chemical inhomogeneity as function of molar mass, except for the very high molar mass part (Fig. 5a). The UV–Vis spectra enable the classification of both the polymer type and the dye that is observed in the low molar mass part (Fig. 5a). The Mark–Houwink  $\alpha$  value obtained from DV detection indicates highly branched structures in the high molar mass fraction (Fig. 5b). And finally a comparison of DV and LS proves the presence of micro gels in the highest fraction (Fig. 5c), explaining the impurity indication from DAD. The excessive response of the LS detector at the low retention side is caused by these micro gels.

#### 4. Gradient elution liquid chromatography

The potential of GE-LC for polymer analysis was already recognized in the late 1970s. van der Maeden et al. were the first to separate oligomer series according to molar mass [54] and in 1979 Teramachi et al. showed the first example of a chemical composition separation of a copolymer [55]. Nevertheless it took nearly another 15 years before the technique became more widely applicable. This was mainly due to a lack of understanding of the separation mechanisms. Much pioneerings work in this respect was done by Glöckner [6]. In 1987, a standard work appeared from his hand dealing with fundamental aspects of polymer properties and polymer chromatography, which is still highly recommended for studying purposes [56].



Mw:	Mn:	Mz:	MwD:	IW:	alfa:	logK
191,900	1,780	4,018,000	107.81	0.281	0.353	-2.139

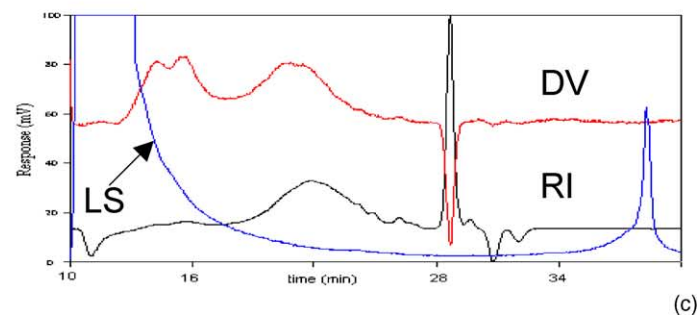


Fig. 5. SEC analysis with combined DAD/VIS, RI, DV and LS detection of an 'unknown' product. (a) UV at 277 nm, including DAD purity plot (220–400 nm). (a1) UV spectra (220–400 nm) at three different elution times. (a2) VIS spectrum (400–790 nm) of a dye. (b) Molar mass results and Mark-Houwink plot from DV/RI detection. (c) Combined RI/DV/LS detection. SEC conditions—columns: 2 × PC mixed C, eluent: THF + acetic acid (1% (v/v)),  $T = 35^{\circ}\text{C}$ . Explanation: see text.

GE-LC is more complex than SEC since retention and separation are governed by more than one mechanism (Section 2). This also explains why different terms were proposed for the same technique: liquid adsorption chromatography (LAC), high performance precipitation liquid chromatography (HPPLC) and gradient polymer elution chromatography (GPEC).

Very recently, Brun presented in an excellent paper a combination of the molecular-statistical theory of polymer solutions in porous media and the conventional theory of GE-LC resulting in a comprehensive description of gradient separation of polymers and all its relevant aspects [57]. To the authors' opinion, this paper can be regarded as state of the art knowledge on GE-LC of polymers.

In short, at the start of the analysis a polymer is injected in a weak eluent. Due to limited solubility of polymers [19] this is often a non-solvent causing precipitation of the polymer. By increasing eluent and solvent strength, the polymer gradually dissolves and desorbs and thus starts eluting. Elution depends on both molar mass and chemical composition. At certain eluent strength, the critical conditions are reached (Section 2). At this point two situations are possible. Either the polymer is completely dissolved and at critical conditions the high molar mass fraction elutes independent of molar mass due to the compensation of adsorption and exclusion effects, i.e. ( $\Phi_{cr} > \Phi_{sol}$ ), where  $\Phi_{cr}$  is the fraction strong solvent at the critical point and  $\Phi_{sol}$  is the fraction at the point of complete solubility. Or, if no complete dissolution already occurred due to insufficient affinity of the eluent towards the polymer, the remaining high molar fractions are eluted later at higher solvent strength, due to redissolution effects ( $\Phi_{cr} < \Phi_{sol}$ ). In the former case molar mass independent elution is observed above a certain molar mass (Fig. 6a), in the latter case no such situation exists, as is shown in Fig. 6b [57]. The difference between these two situations often led to misconceptions in the past.

The practical meaning of the situation where ( $\Phi_{cr} > \Phi_{sol}$ ) is that after reaching critical conditions elution only depends on the chemical composition of the polymer backbone, functional groups, etc. A chemical heterogeneous polymer can be regarded as mixture of fractions with different composition, each having its own, specific, critical conditions. Each separate fraction will elute, independent of molar mass, when its specific critical point is reached.

From the above it is clear that eluent system and pore size of the column are important parameters that have to be chosen carefully. The eluent system must be chosen such that ( $\Phi_{cr} > \Phi_{sol}$ ) which can only be found out experimentally from trial and error. In this respect the recent work of Schoenmakers et al. is of help who showed that with use of existing theories and a few experiments, critical conditions of homopolymers can easily be predicted [58]. Furthermore, pore size of the column must be chosen such that no polymer fractions are totally excluded from the pores, which can easily be checked from an experiment under exclusion conditions, i.e. at high eluent strength.

Unlike what is often thought, the occurrence of solubility effects is generally unwanted since it leads to an increased molar mass dependence in GE-LC thus complicating chemical composition determinations. The belief, a priori, that solubility dominates polymer separations in some cases even leads to wrong choices in practical parameters. For instance, (separation) results presented in [59] could have been much better if the authors had realized that sorption dominates their separations and therefore had chosen a proper analytical column instead of a pre-column.

Like for small molecules GE-LC can be performed in the reversed-phase (RP) as well as in the normal-phase (NP) mode. However, it should be realized that in NP sorptive interactions are generally much stronger than in RP and hence the favorable condition  $\Phi_{cr} > \Phi_{sol}$  is more easily met. Therefore, NP is often used for chemical composition

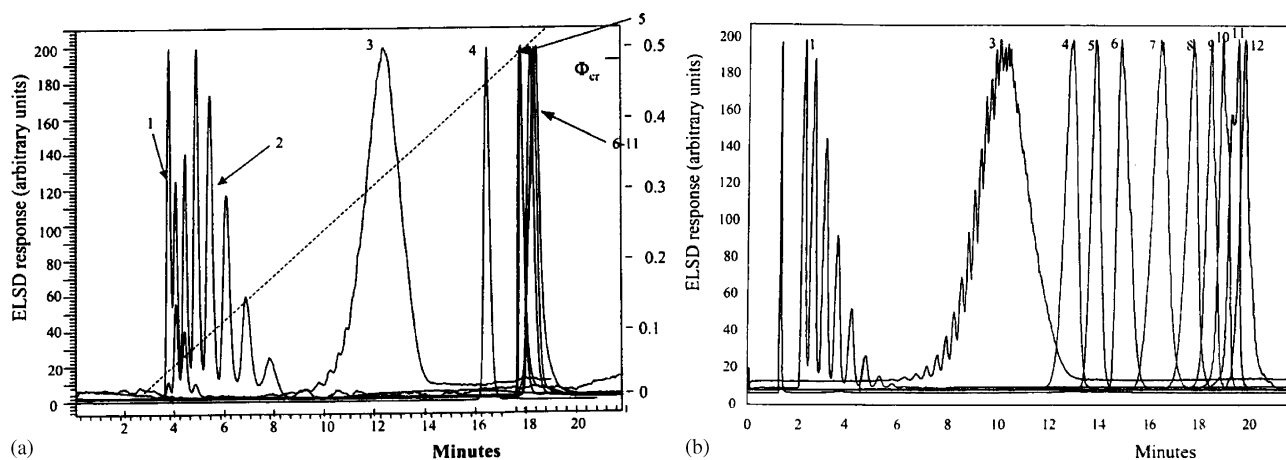


Fig. 6. GE-RPLC of polystyrene standards with peak molar masses (as determined from SEC): 474 (1), 890 (2), 2630 (3), 5570 (4), 9100 (5), 18 100 (6), 37 900 (7), 96 400 (8), 186 000 (9), 355 000 (10), 710 000 (11), 2 890 000 (12). (a) From 0 to 100% THF in ACN linear over 30 min on a Nova-Pak C<sub>18</sub> column. (b) From 0 to 100% THF in MeOH linear over 30 min on a symmetry shield RP<sub>8</sub> column. Right y-axis in (a) shows the eluent composition at the column outlet,  $\Phi_g$  (broken line), with indicated  $\Phi_{cr} = 0.48$ . Detector: ELSD. From [57] with permission.



separations of copolymers and functional polymers despite its more complex practical character. Recent examples can be found in [60–69].

Precipitation of the polymer directly after injection is often unavoidable [6,56]. In many cases, this is not a problem but due to demixing effects so-called breakthrough may occur causing parts of the polymer to elute unretained. This effect should be avoided since breakthrough depends on molar mass and chemical composition, causing the retained part of the polymer to be non-representative for the original product. Recently, the group of Schoenmakers extensively investigated and explained this effect [70]. They concluded that (1) the injection solvent should be as weak as possible, (2) the polymer should be injected at relatively high concentration in a small injection volume and (3) the initial solvent strength should be as weak as possible. In Fig. 7, breakthrough is explained schematically [70].

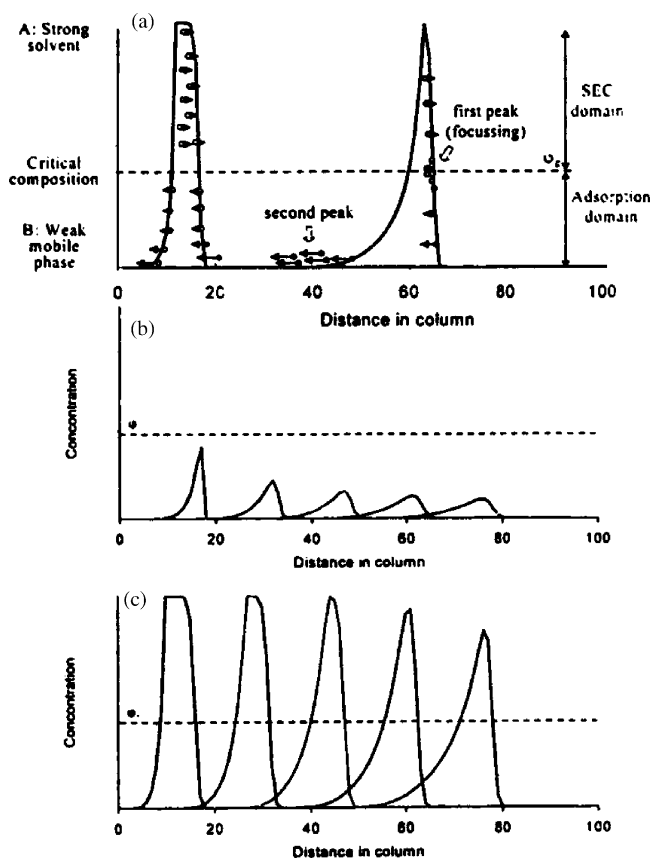


Fig. 7. Simulated representations of the solvent zone in the column. The horizontal dashed line represents the critical composition ( $\Phi_{cr}$ ). (a) A focusing of the polymer molecules within the solvent zone at the exact location of the critical point is expected and polymers in the tail of the solvent plug will be retained properly, giving rise to the second (“real retention”) peak. (b) When both the sample solvent and the mobile phase are weaker than the critical composition, we are in the adsorption mode and the polymer will be retained on the column, producing only one peak. (c) The percentage of solvent A in the solvent zone will decrease and the width of the solvent zone will increase along the column. From [70] with permission.

In the case of (semi)-crystalline polymers a solid crystalline phase can be formed after injection [71]. The redissolution of this phase highly depends on practical conditions and disturbs proper chromatography. To avoid this effect, it was found that the system temperature should be higher than the depressed melting point at initial conditions of the gradient.

Detection in GE-LC is restricted to only a few detector types, since bulk property detectors such as RI cannot be used. As many synthetic polymers are non-UV absorbing, evaporative light scattering detection (ELSD) has become popular in this respect. Although very easy to use, quantification is more complicated than with UV due to its non-linear response characteristics [72,73]. Especially when a comparison of obtained distributions with theory is aimed at, a signal correction is required afterwards to avoid underestimation of polydispersity. Furthermore, ELSD response depends on eluent composition [74,75] and polymer type [61,75]. A correction for differences in peak width, which, however, mainly occurs in isocratic elution, was described by Mengerink et al. [76,77].

Hyphenation of GE-LC with spectroscopic techniques is nowadays very well possible. Like in SEC such coupling directly provides chemical composition information as function of retention time. With respect to quantitative aspects, situation is more complicated than in isocratic (SEC) systems. Coupling with FT-IR via the solvent elimination interface suffers from a changing film formation process due to altered solvent properties during the analysis, which affects quantification [33]. For NMR, solvent suppression becomes more difficult due to (slight) changes in chemical shift with eluent composition [34]. Only few examples have been shown in literature until now [78,79]. In MS, ionization efficiency alters during the analysis. In most cases, however, MS is used only qualitatively. The use of GE-LC in combination with MS to study chemical compositions distributions has been described for a limited number of relatively low molar mass polymer types, e.g. copolyesters [45,80,81], polyethylene–polypropylene block copolymers [63] and hyperbranched polymers [82]. Absolute quantitative analysis of the CCDs, however, is rarely required since in most cases a relative comparison between different samples is sufficient.

GE-LC nowadays is regularly applied for the determination of compositional distribution of the polymer backbone (CCD), in most cases for addition polymers [60–65,67,68,75,83–85], and functional end groups [62,66,69,86]. In the latter case nearly always NPLC is used. Few papers describe the characterization of other heterogeneity types like grafting distribution [75,87] or block length distribution [83,84]. As an example, the chemical composition separation of poly(styrene-*co*-ethylacrylate) by GE-RPLC is shown in Fig. 8 [79].

Like in SEC, the retention axis must be calibrated with standards of known composition in order to obtain the final composition distribution. For the CCD this is often performed by using low conversion copolymers with a

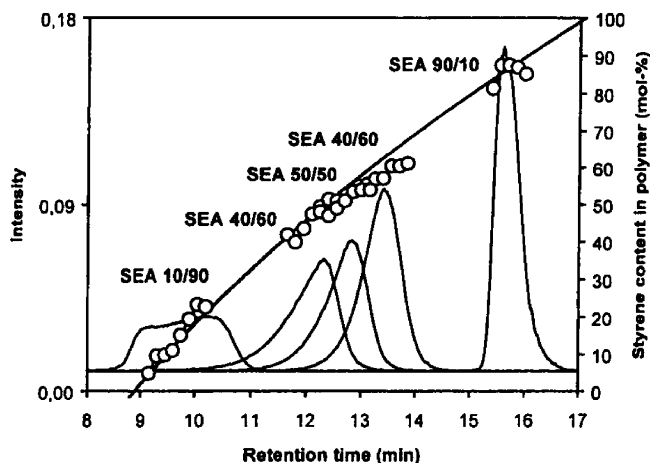


Fig. 8. GE-RPLC chromatograms of (styrene)-*co*-(ethyl acrylate) copolymers (SEA copolymers) of different composition with high conversion. Styrene content obtained by HPLC calibration (line) and on-line HPLC- $^1\text{H}$  NMR (circles) experiments. Column: Nucleosil  $\text{C}_{18}$ , eluent: THF-ACN (10:90 (v/v)) linear to (100:0) in 25 min. Detection: ELSD. From [79] with permission.

subsequent narrow distribution. The average chemical composition of these products is determined by NMR or FT-IR. However, care must be taken to directly translate these results into retention time, since the peak maximum does not necessarily represent the average composition [75]. For those cases where an exact CCD is needed for comparison with polymerization models, a mathematical procedure to overcome this problem was proposed by Teramachi and co-workers [75,88]. Another possibility is the on-line coupling to, e.g.  $^1\text{H}$  NMR to calibrate the retention axis, as was demonstrated by Pasch and co-workers [78,79] (see also Fig. 8).

In those cases where separation is affected by more than one structural feature, translation into distribution of one heterogeneity type becomes more complex. Most well known are molar mass influences next to chemical composition, especially for low molar mass polymers. This must be dealt with by the coupling (off-line or on-line) of (at least) two separation methods as will be discussed in Section 6. The off-line coupling of SEC and GE-LC for the simultaneous determination of MMD and CCD was already extensively described more than a decade ago by Glöckner [6]. Recently Kawai et al. were the first to determine the two-dimensional chemical composition distribution of a terpolymer by the combination of RP and GE-methods [65]. The present author showed the first example of the CCD determination of a low molar mass condensation polymer, a copolyester, independent of its MMD and FTD [68].

## 5. Liquid chromatography at critical conditions and barrier methods

The history of LCCC goes back to the seventies when Russian workers proposed a theory predicting chromato-

graphic conditions for macromolecules on porous media where entropic exclusion effects are exactly compensated by enthalpic interactions [18,89]. At these so-called 'critical conditions' retention for homopolymers is independent of molar mass (Fig. 2) and is solely governed by chemical differences. This makes LCCC in principal perfectly suited for studying chemical composition distributions. It was predicted that LCCC could be used for end group separations of telechelic polymers and for separation of block copolymers according to block length.

Theoretical modeling and comparison with practical findings, mainly performed by Gorbunov and Skvortsov, still goes on until now [90,91]. This work is very relevant since understanding critical conditions provides insight in the entire 'spectrum' of polymer chromatography. It is reminded here that critical conditions also influence elution behavior in gradient elution (see previous chapter [57]).

Nowadays LCCC is becoming increasingly popular and during the latest years the number of papers regarding chemical composition separations using LCCC even exceeded that of GE-LC.

The earliest practical examples of LCCC were those, dealing with end group separations [92,93]. In this case, conditions are chosen such that under critical conditions chains with functional end groups are retained more than non-functionals. Since most functional end groups are more polar than the polymer backbone, end group separations in the majority of cases are performed in the NP mode. Examples were described for polyethers (majority of cases), polyesters, polybutadienes, oligocarbonates and epoxide resins. See [17,94] for early references. Recent examples deal with functionalized PS (see Fig. 9) [94], functionalized PMMA [95,96], and poly(propylene oxide) (PPO) [97]. The separation of fatty polyethers according to the length of the alkyl chain, under RP conditions was shown by Trathnigg [98].

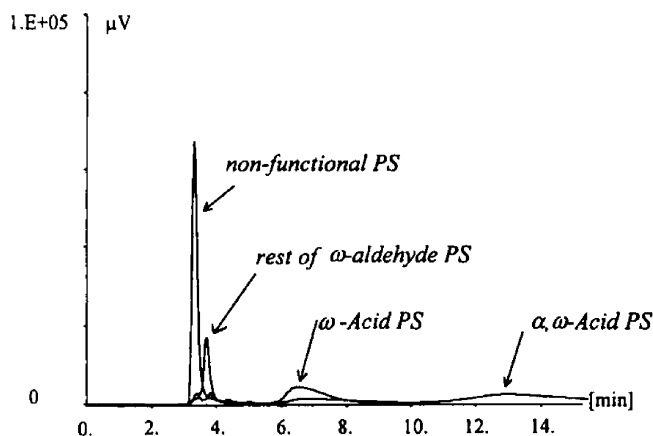


Fig. 9. LCCC chromatograms of non-functional PS standards (average  $M_w = 13700$ ),  $\omega$ -acidic-PS (average  $M_w = 4000$ ) and  $\alpha,\omega$ -acidic-PS (average  $M_w = 2000$ ) in ethyl acetate-hexane (38:62 (v/v)). Column: Nucleosil silica,  $T = 25^\circ\text{C}$ . Detection: UV at 261 nm. From [94] with permission.

Pasch presented the characterization of star shaped poly(L-lactides) (PL) according to the number of arms [99]. Since each arm contained a polar OH group this is in fact a special case of a functionality separation.

During the latest years, much attention has been paid to the analysis of block copolymers by LCCC. This is an important application, since information on block length distributions is very difficult to obtain from other methods. GE-LC for instance is not suited for this purpose. Although blockiness has several times been shown to influence retention time in GE-LC [83,84,100], elution is dominated by a polymers' average chemical composition thus masking block length information.

For block copolymers, critical conditions are established for one of the blocks, using homopolymer standards. The phase system is mostly chosen such that those conditions are *exclusion* conditions for the other block, which is to be investigated. For example: when the most polar block is of interest, critical conditions for the less polar block are established at an RP system. According to theory the 'critical' block becomes 'chromatographically invisible' and elution is solely governed by the block length of the other block. Thus, a BLD can be obtained.

In the early 1990s, Pasch was one of the first to explore this principle, mostly for 'simple' di- and tri-blocks. See [17] for old references. Recent examples deal with the analysis of di- and tri-blocks of PMMA and poly(*tert*-butyl methacrylate) (PtBMA) by Falkenhagen et al. [101] and the characterization of di-blocks of PS and PMMA by Pasch et al. [102]. Unfortunately, the concept of chromatographical invisibility was in most of these cases adopted without being checked. Very recently, however, Falkenhagen et al. obtained experimental proof for this concept from systematic investigations on PMMA-*block*-PtBMA [103]. By varying the length of the PMMA block and keeping the other block constant they found that retention behavior under critical conditions of PMMA was independent of the PMMA block length. In this case, however, both block lengths were in the same order of magnitude.

In contrast, from the same type of experiments on poly(styrene-*block*-isoprene) (PS-*block*-PI) Lee et al. convincingly proved a distinct dependence of elution behavior on the block length of the 'invisible' block [104,105] as can be observed in Fig. 10. A molar mass difference between both blocks of a factor of two led to a molar mass error for the visible block of about 10%. This indicates that more theoretical and thorough experimental research is needed on this topic. Until then results from BLD analysis by LCCC, especially the 'absolute' values must be handled with care.

Examples of LCCC investigations of more complex systems, i.e. star copolymers having three or four arms of different chemical composition were shown by Pasch and co-workers [106,107].

Systems with blocks not widely differing in polarity or of low molar mass can sometimes be characterized in the adsorption mode for the 'visible' block within acceptable

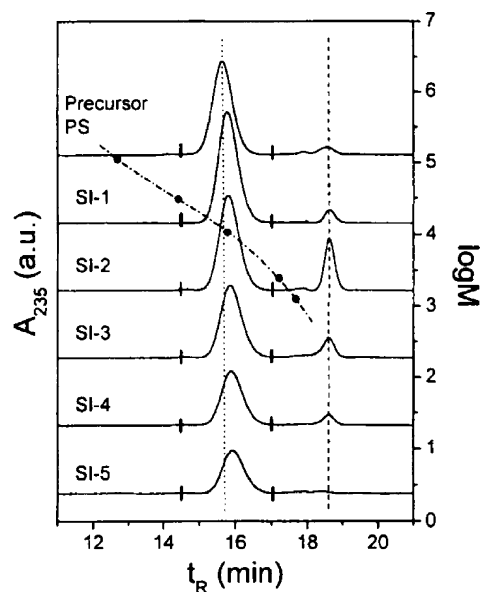


Fig. 10. LCCC chromatograms of PS-*block*-PI samples under the critical conditions of PI. PS block length is constant ( $M = 12.0$ ), PI block length increases with sample number—SI-1:  $M_{PI} = 3.0$ , SI-2:  $M_{PI} = 6.0$ , SI-3:  $M_{PI} = 11.1$ , SI-4:  $M_{PI} = 21.4$ , SI-5:  $M_{PI} = 34.2$  (molar masses in kDa). Notice the distinct effect of PI block length on elution time, despite the critical conditions for PI! LCCC conditions—column: Nucleosil C<sub>18</sub>, 100 + 500 + 1000 Å,  $T = 47^\circ\text{C}$ , eluent: DCM-ACN (78:22 (v/v)). Detection: UV at 235 nm. The vertical dashed line at 18 min indicates the elution time of the critical component (PI). Calibration curve for the calculation of block molecular mass is shown in the plot. Calculated molar masses deviate from 12% for SI-1 to 23% for SI-5 from the expected value (12.0). From [104] with permission. Copyright (2001) American Chemical Society.

retention times. Examples were shown for PEO-PPO blocks [63] and a PEO-co-polymethylene system [108]. Lee et al. studied a tri-block PL-*block*-PEO-*block*-PL system in the adsorption mode and found that when the total molar mass of both PL blocks was kept constant retention was influenced by the distribution of mass between both blocks [109]. This again emphasizes the need for more systematic research on LCCC.

Interestingly, Berek and co-workers demonstrated the potential of LCCC to separate polymers according to tacticity. Syndiotactic and isotactic PMMA [110] and poly(ethyl methacrylate) (PEMA) [111] were successfully separated, where conditions were chosen such that critical conditions for one of the species were exclusion conditions for the other one. The obtained tacticity distribution for unknown samples excellently agreed with values determined from on-line coupling of LCCC to NMR [112].

As was already predicted in the early papers of Russian workers, linear and cyclic species can be separated using LCCC [113]. Furthermore analogously to block copolymers, the application to graft copolymers was described [87,114,115].

A distinct advantage of LCCC over GE-LC is its molar mass independence, making separation results more unambiguous. The isocratic character allows for a relative

facile on-line coupling to, e.g. SEC in order to obtain additional molar mass information (Section 6) [116]. And next to this, a large variety of detectors, such as RI and LS [105,114] can be used, allowing for direct (average) molar mass determinations.

Hyphenation to spectroscopic techniques is relatively easy, although few practical examples have been shown until now. The on-line coupling to NMR for the tacticity determination of PEMA was demonstrated in [112]. The application of off-line MALDI-MS was described for end group separations of PPO (96,117) and polyamides [76] and the BLD determination for a tri-block PL-*block*-PEO-*block*-PL copolymer [109].

Unfortunately, LCCC suffers from several practical drawbacks [17,118], which, remarkably seem to be neglected in many papers.

- (1) Excessive peak broadening may occur, especially for high molar mass polymers as was found in the authors' lab. for polystyrenes, polyesters and poly(dimethylsiloxanes) [17,119,120].
- (2) Small deviations in composition of sample solvent and eluent sometimes lead to peak splitting [17,119].
- (3) Sample recovery may decrease with increasing molar mass especially on narrow pore column packings [118]. Recently, Berek suggested that this may be caused by strong adsorption effects followed by slow desorption due to reptation of macromolecules into small pores [121].
- (4) LCCC is very sensitive to small deviations in both temperature and eluent composition [17,105] thus hampering sufficient reproducibility.

In some cases, even the principle of molar mass independence is doubtful as was discussed above for block copolymers [104,105] and found from other experiments in our lab [17,119,120,122].

The finding of a suitable eluent system is still a matter of trial and error. Due to the isocratic character of LCCC, separation in a certain system may be insufficient or lead to excessive retention. This is especially the case for functionality separations [94,120]. A practical solution in the latter case may be the combination of LCCC with gradient elution or temperature programming in order to elute highly retained fractions [105,123]. Furthermore one may work slightly 'off-line' critical conditions at slightly higher eluent strength in order to speed up the separation. In the case of sufficient resolution of respective fractions this is advantageous, since in the same analysis also molar mass information can be obtained, as was shown by Falkenhagen et al. [108]. For block copolymers a large polarity difference between both blocks may lead to problems with respect to solubility [103].

Recently, the group of Olesik described the use of so-called enhanced fluidity (EF) solvents, mixtures of liquid CO<sub>2</sub> with an organic solvent, i.e. THF [117,124]. Due to the low viscosity of these solvents, diffusion of

macromolecules is enhanced, leading to a considerable decrease in peak broadening as compared to normal situations [17]. Furthermore, due to relatively low backpressure long columns can be applied which further enhances separation efficiency. Finally it was shown that when using EF solvents, next to temperature also pressure could be used to establish critical conditions. This additional degree of freedom as compared to common systems allows for instance for thermodynamic studies in which temperature must be varied. For above-mentioned reasons the approach is highly interesting and deserves further attention.

In order to overcome some of the drawbacks of LCCC, Berek extensively investigated so called 'barrier methods' [118,125,126]. This term refers to the practice that the sample solvent composition differs from the eluent composition. The differing adsorption/desorption or precipitation/dissolution properties of the sample solvent plug make this plug to act as a 'migration barrier' for polymer molecules. For instance in the 'limiting conditions of adsorption (LCA)' case, the sample is dissolved in a chromatographically strong solvent, which promotes desorption, and subsequently injected on a column containing a weak, adsorption promoting eluent. Due to the solvent plug properties, the polymer sample exhibits a local exclusion regime and therefore migrates faster than the small solvent molecules. At the front of the plug the adsorption promoting eluent is met, causing the sample to be retained due to adsorption. After the solvent plug captures the sample again, this process is repeated, causing accumulation of the polymer molecules irrespective of their molar mass. Thus, like in LCCC molar mass independent elution for homopolymers can be obtained when conditions are chosen properly, as is demonstrated in Fig. 11a.

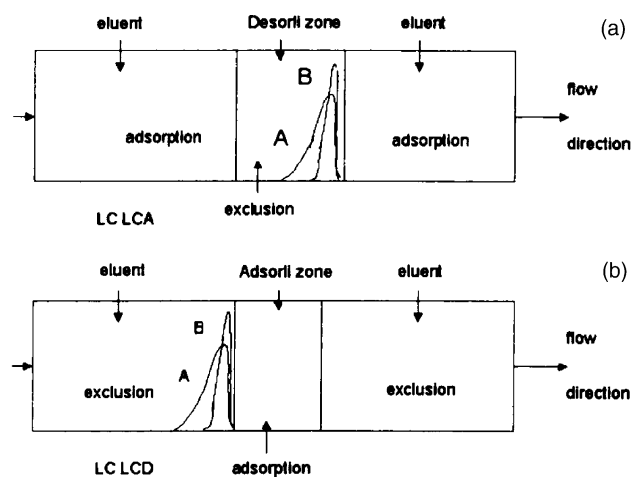


Fig. 11. Schematic representation of liquid chromatography under limiting conditions of adsorption (LC-LCA) (a) and limiting conditions of desorption (LC-LCD) (b). Peak 'A' corresponds to the polymer peak eluted from a wide pore column, at least partially permeated by macromolecules in the SEC mode. Peak 'B' refers to polymer eluted from a narrow pore column packing from which macromolecules are fully excluded in the SEC mode. From [121] with permission.



In the ‘limiting conditions of desorption (LCD)’ case the situation is the other way round, i.e. the sample is dissolved in a weak solvent and the eluent is a strong solvent. This causes the sample to accumulate at the backside of the solvent plug (Fig. 11b). Likewise, solvents promoting dissolution or precipitation, respectively can be used. For a more extensive description of all variants, the reader is referred to [118,125].

Since the adsorption or precipitation processes at the edges of the plug depend on chemical composition it is imaginable that composition distribution information can be obtained from barrier methods. Critical parameters in this respect are the volume of the barrier plug and the difference in strength between sample solvent and eluent [121].

Claimed advantages as compared to LCCC and depending on the chosen barrier method [121,127,128] are improved recovery, better peak shape and improved tolerance towards small deviations in eluent composition or temperature. The number of practical applications is, however, very limited until now. Hunkeler and co-workers demonstrated in several papers the determination of CCD of random PS-co-MMA [127–129]. They found that this application is limited to a restricted chemical composition range. Nevertheless, it is the authors’ opinion that barrier methods deserve serious attention as a variant to LCCC for chemical composition distribution determinations of synthetic polymers.

## 6. Two-dimensional liquid chromatography

For complex polymers being disperse in more than one compositional feature, a combination of separation methods must be used to completely unravel their structure. Only in specific cases a combination of mechanisms in one separation step allows a separation with respect to more than one feature, as was shown in [108]. In [130], the group of Berek described a combination of adsorption and exclusion mechanisms in one system to completely separate a polymer blend consisting of poly(butyl methacrylate) (PBMA), PMMA, poly(tetrahydrofuran) (PTHF) and poly(vinyl acetate) (PVAc) and subsequently characterize the respective components according to their individual MMD. Lee and Chang used temperature programming (see also Section 8) to separate a mixture of PMMA and PS and to simultaneously obtain MMD information of the individual polymers [123]. The present author demonstrated the separation of functional copolyesters by NPLC, where both the CCD of the polyester backbone and the FTD could be determined in one separation step [69]. In order to obtain the complete MM-FT-CCD, however, a preceding step by SEC was necessary [68].

Although in principle all types of separation methods can be coupled, for polymer analysis almost exclusively a combination of LC methods is used. This is in (rough) agreement with the findings of Schure who predicted the potentials of different two-dimensional (2D) combinations based on the-

ory [131]. The combination of methods should be chosen such that their orthogonality is as large as possible, in order to maximize the total peak capacity [10]. Ideally the methods are completely independent, each of them responding to only one specific molecular characteristic. In practice, however, this is hardly ever possible. SEC for instance is always affected by chemical composition differences since hydrodynamic volume is a function of composition, and GE-LC suffers from molar mass influences.

Coupling of LC techniques, mainly off-line, for copolymer characterization have been described since the early 80’s. Much pioneers work was done by Glöckner [6]. In his book he presented several examples of MM-CCD determinations of mainly statistical copolymers, via off-line coupling of SEC with GE-LC, which he called ‘cross-fractionation’. Recent examples regard the full characterization of surfactants (fatty polyethers). To this end, Trathnigg et al. used the combination of LCCC under RP conditions with GE-LC in the NP mode [98]. By LCCC, the polyethers were separated according to the length of the fatty alkyl chain and subsequently the obtained fractions were further separated according to the polyether length by GE-LC (see Fig. 12).

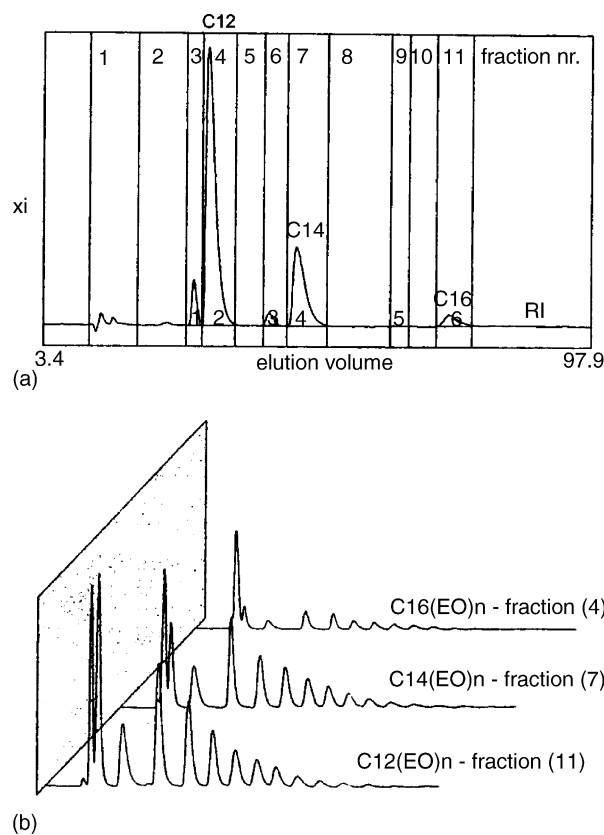


Fig. 12. Fractionation of a fatty alcohol ethylate macromonomer by LCCC (a) and subsequent analysis of LCCC fractions (as indicated in (a)) by GE-LC (b). LCCC conditions—column: Spherisorb ODS2, eluent: MeOH–water (90:10 (v/v)). Detection: RI. GE-LC conditions: Spherisorb S3W, eluent: acetone–water (100:0 (v/v)) to 80/20 linear over 50 min. Detection: ELSD. From [98] with permission. Copyright (2001) American Chemical Society.



To obtain a comparable result Murphy et al. explored a combination of two GE-LC systems, i.e. RP followed by NP [132]. The off-line combination of two GE-LC systems was also used by Kawai et al. for the characterization of a terpolymer system consisting of styrene, methyl methacrylate and acrylonitrile [65].

At this moment, most papers dealing with coupled techniques describe the on-line approach. To this end, an automated injection valve is used for the transfer of fractions from the first into the second dimension, according to the approach of Kilz et al. [133].

An essential difference with off-line coupling where only a few fractions according to the method of ‘heart-cutting’ are selected is the transfer of the complete eluate of the first dimension into the second one. The transfer volume is taken sufficiently small such that each chromatographic peak from the first dimension is divided into several fractions. This

largely enhances the amount of information to be obtained from samples thus visualizing aspects that remain hidden when the separation techniques are used ‘stand alone’. With respect to nomenclature, off-line coupling is often referred to as LC–LC, whereas for on-line coupling with complete transfer of the eluate from the first dimension, the notation LC  $\times$  LC is used [134].

As a rule of thumb the method with highest selectivity for the separation of *only one* structural feature and no (or minor) selectivity towards the other feature(s) should be chosen as the first dimension [10,11]. For high molar mass copolymers this is often GE-LC, since molar mass effects are negligible in many cases. This is also advantageous due to the adjustable character of GE-LC, allowing for optimization for a specific polymer. SEC is in the majority of cases selected for the second dimension, which allows for the selection of many different detectors. For low molar mass copolymers,

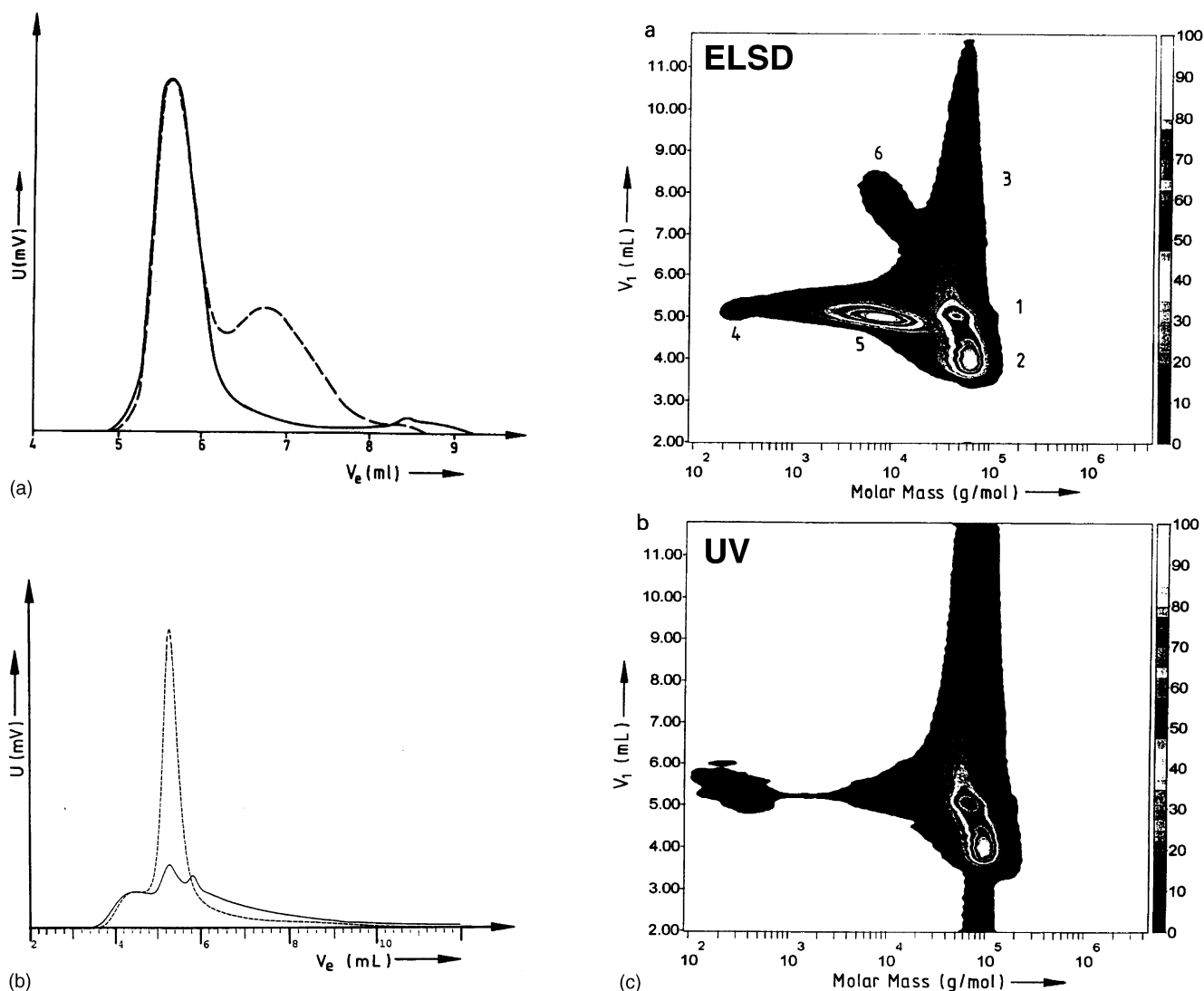


Fig. 13. Analysis of poly(styrene–block-butadiene)-graft-butyl acrylate by SEC (a), LCCC (b) and LCCC  $\times$  SEC (c). SEC conditions—column: PL Mixed D, eluent: THF. Detection: UV at 254 nm (—) and ELSD (---). LCCC conditions—column: Si-300 + 1000 Å, eluent: THF–cyclohexane 15.5:84.5 (v/v), detection: UV at 254 nm (—) and ELSD (---). LCCC  $\times$  SEC contour plots: ELSD (upper) and UV at 254 nm (lower). From [115] with permission.

however, SEC may be a better choice for the first dimension due to severe molar mass dependence in interaction based separations as was shown by the present author [68]. Whatever the order of methods, compatibility of solvents used in both systems always needs attention in order to avoid, e.g. breakthrough effects. Further practical considerations for LC  $\times$  LC can be found in [10,11,116].

Recent LC  $\times$  LC examples mainly describe the use of LCCC in the first dimension, combined with SEC [87,99,101–103,111,115]. A major advantage of this combination is the molar mass independence in LCCC. The rather low peak capacity of this method is overcome by the 2D approach. LCCC  $\times$  SEC was applied for block copolymers to obtain the complete BL-MMD [101–103], tacticity separations [111] and complex, grafted systems [87,115]. In Fig. 13, results from LCCC, SEC and LCCC  $\times$  SEC for poly(styrene-*block*-butadiene-graft-butyl acrylate) are shown [115]. From the 2D plots (Fig. 13c), a larger compositional heterogeneity can be deduced than would have been expected from 'the sum' of both techniques alone (Fig. 13a and b).

## 7. Mass spectrometry

Until a decade ago, MS of polymers was mainly restricted to methods based on chemical or thermal degradation preceding to the MS step itself. This approach only reveals information on the constituting monomers and the average chemical composition, but not on the polymer distributions. This situation changed with the introduction of soft ionization techniques, i.e. MALDI and ESI, affording the analysis of intact polymer ions with no or little fragmentation [8,9]. Since MS is a separation technique, information on polymer distributions can in principle be obtained.

For MALDI, a polymer solution is mixed with a matrix and, in some cases, with an additional salt. After drying on a target, the sample is brought in high vacuum and part of it is desorbed by a laser pulse, the energy of which is absorbed by the matrix. This matrix also donates the charge to the analyte, although the exact mechanism of ionization is still unclear [135]. In most cases, final products of ionization are protonated or cationized (e.g. sodiated) species. The matrix can be added before, during or after deposition of the sample solution. Both the order and the method of deposition influence final results with respect to ionization efficiency and observed polymer distributions [136,137]. MALDI suffers from bad reproducibility, even from shot to shot in the same sample spot and the selection of an adequate matrix is still a matter of trial and error. MALDI is mostly coupled to a time-of-flight (TOF) type mass spectrometer, which in principle is unlimited in mass range. In an extensive review by Nielen, detailed information with respect to practical aspects, including matrix selection is given [9]. Rather new is the development of atmospheric pressure MALDI (AP-MALDI) allowing an easy combination in one

instrument with other AP ionization techniques such as ESI [138,139]. Benefits and drawbacks of this method are still under investigation. MALDI is nowadays extensively applied to bio-macromolecules, but has also become popular for synthetic polymers, both polar and moderately to low polar such as PS and poly(isobutylene)[140].

In ESI, a liquid stream is broken down into fine droplets by an electrospray. After evaporation of the solvent, (associates of) intact (macro) molecules remain, carrying one or more charges [141]. ESI is even a softer ionization technique than MALDI, although this is not a general rule. In contrast to MALDI, ionization occurs in atmospheric conditions, after which ions are transferred (in two or three steps) to the vacuum analyzer. Like in MALDI, ionization is mostly due to protonation or cationization. For ESI various analyzer types can be used, i.e. quadrupole, ion trap, TOF and Fourier transform ion cyclotron resonance (FT-ICR) instruments. For polymer analysis in most cases TOF instruments are used [80,142,143], due to their high molar mass range. An additional advantage is the relatively high resolution, which is especially important for copolymer analysis where the number of different product grows exponentially with molar mass. Even better in this respect are FT-ICR instruments [144–146], which, however, are very expensive.

The molar mass range of ESI is considerably lower than MALDI. The frequent occurrence of multiple charged species in ESI extends this range but also complicates the spectra. In practice, ESI is mainly applied for relatively low molar mass polymers—up to mass values of approximately 10 000. The polarity range of ESI is somewhat less broad than MALDI varying from polar polymers such as poly(ethylene glycol) [80] to moderately polar products such as polyesters [80] and PS [147]. The most critical parameters influencing ion intensity and observed polymer distribution are the solvent type, cone voltage and the type of salt used to enhance ionization [148–151].

A major drawback of both ESI and MALDI for the analysis of synthetic polymers is the fact that the observed distributions next to several practical parameters (see above) largely depend on the polydispersity. This is due to the fact that MS sensitivity varies with molar mass and chemical composition caused by severe discrimination in ionization, transmission and detection towards high molar mass species [8,9,42,146,150,151]. The way to overcome this is the coupling of MS to separation methods, mostly LC. From the LC pre-separation according to a specific structural feature (in many cases molar mass) quantitative information from a concentration detector is obtained and fractions of sufficiently low dispersity become available for further MS analysis. By MS the sample is subsequently further separated without the drawback of suppression effects allowing for qualitative and, to some extent, quantitative evaluation.

The need in polymer analysis for coupling MS to a pre-separation is nowadays widely accepted. Nevertheless some recent papers still describe efforts to characterize polydisperse systems solely by MS. In some cases, this

may be sufficient such as the qualitative characterization of different end group types [152,153] or macrocycles [154] in disperse, hyperbranched polymers. Furthermore, for products with polydispersity < 1.1–1.2 (exact value depends on the molar mass of the polymer) fair agreement is generally found between molar mass averages from SEC coupled to DV or LS and MALDI [152,153], thus eliminating the direct need for pre-separation. In other cases, a correction for discrimination effects is made. Van Rooij et al. described such method for an FT-ICR system, by which correct MMD values for PEO–PPO block copolymers could be determined. From this information, the BLD for the respective blocks were derived [155]. An extensive review on copolymer analysis by MS including chemical distribution analysis recently appeared from Montaudo [156]. Murgasova and Hercules reviewed the developments on MALDI for synthetic polymers in general, for the period 1999–2003 [157].

Because of easy practical implementation, the coupling of ESI to chromatographic methods nowadays usually is performed on-line. Since optimum flow rates for ESI are in the order of magnitude 30  $\mu\text{L}/\text{min}$  the use of conventional scale LC columns requires post column splitting [80]. In contrast, the vast majority of LC–MALDI–MS uses off-line coupling, examples of on-line coupling are rare [158]. Even off-line coupling is still immature and suffers from bad reproducibility. Lou et al. described a theoretical and experimental study to the number of (SEC) fractions needed to obtain reliable distributional information from MALDI [159], which was found to be remarkably low. Like for LC–FT-IR, a commercial interface is available for LC–MALDI–MS using targets pre-coated with matrix. Practical aspects on the use of this interface were studied by Esser et al. [160].

Although most papers describe the coupling of SEC to MS until now, coupling of GE-LC techniques and LCCC is very well possible [80,81] and some cases even more advantageous due their larger orthogonality compared to MS. In

Table 1, some characteristics of the coupling of separation techniques to MS are given [143].

LC–MS can be used for studying various types of chemical composition distribution. The detection of cyclics formation in polyesters by SEC–ESI–MS or SEC–MALDI–MS was described in [41,42,150]. Studies to the FTD and the formation of various end group types by LC–ESI–MS and LC–MALDI–MS was shown for functional polyesters [80] and polyethers [80,97,117,142,144], acrylic and epoxide resins [144] and polycarbonates [43]. In [80,97], LCCC was used as separation mode for this purpose.

Applications of LC–ESI–MS and LC–MALDI–MS for the CCD of the copolymer backbone were described for acrylic based copolymers [145,160], epoxide resins [161] and copolyesters [45]. Also in these cases the preferred LC mode was SEC. However, an example obtained by GE-RPLC on-line coupled to ESI-TOF-MS from the authors' lab is shown in Fig. 14, demonstrating the CCD of a copolyester with a specific degree of polymerization ( $p = 3$ ) and a specific end group combination. In this example, next to the chemical composition distribution of the polyester backbone, an additional distribution according to degree of propoxylation of the diol was found. See also [45].

Determination of the BLD by LC–MS is until now only possible for relatively simple di-block systems of low molar mass (up to a few thousands Dalton) and published applications are rare [63]. More complex systems such as tri-blocks require the use of MS–MS methods as was demonstrated for tri-blocks based on PEO–PPO [63,109] and PEO–PL [162]. Alternatively, a combination of MS with more sophisticated LC methods such as LCCC being able to discriminate against differences in block length [63] can be used. For more randomized systems successful attempts in determining the copolymer sequence using MS in combination with chain statistics [163,164] or MS–MS [165,166] were described. Nevertheless, for complex systems even the possibilities of MS–MS methods coupled to LC in this respect

Table 1  
Overview of separation techniques for synthetic polymers and their characteristics for coupling with MS

Family	Technique <sup>2</sup>	Separation according to	On-line ESI–MS	Off-line MALDI–MS
LC	SEC	Molecular size	Large columns (i.d. $\geq$ 4.6 mm) are typically used	Very narrow $M_r$ fractions
	Gradient LC	Chemical composition; chemical functionality	Separation mechanism very different from that of MS ('orthogonal') Great variations in mobile phase composition (polar and apolar)	Fractions vary mainly main in molecular mass (Optimum) deposition conditions vary with time
	Isocratic ('critical') LC	Chemical functionality	Constant mobile-phase composition Typically short retention times  Limited resolution	Constant deposition conditions Complex fractions are obtained (in which $M_r$ varies) Retention times may vary with fluctuations in mobile phase composition and temperature
FFF	Thermal FFF	Molecular size and chemical composition (confounded)	Used mainly for very large macromolecules ( $M_r \geq 10^5$ )	Very narrow $M_r$ fractions

From [143] with permission.

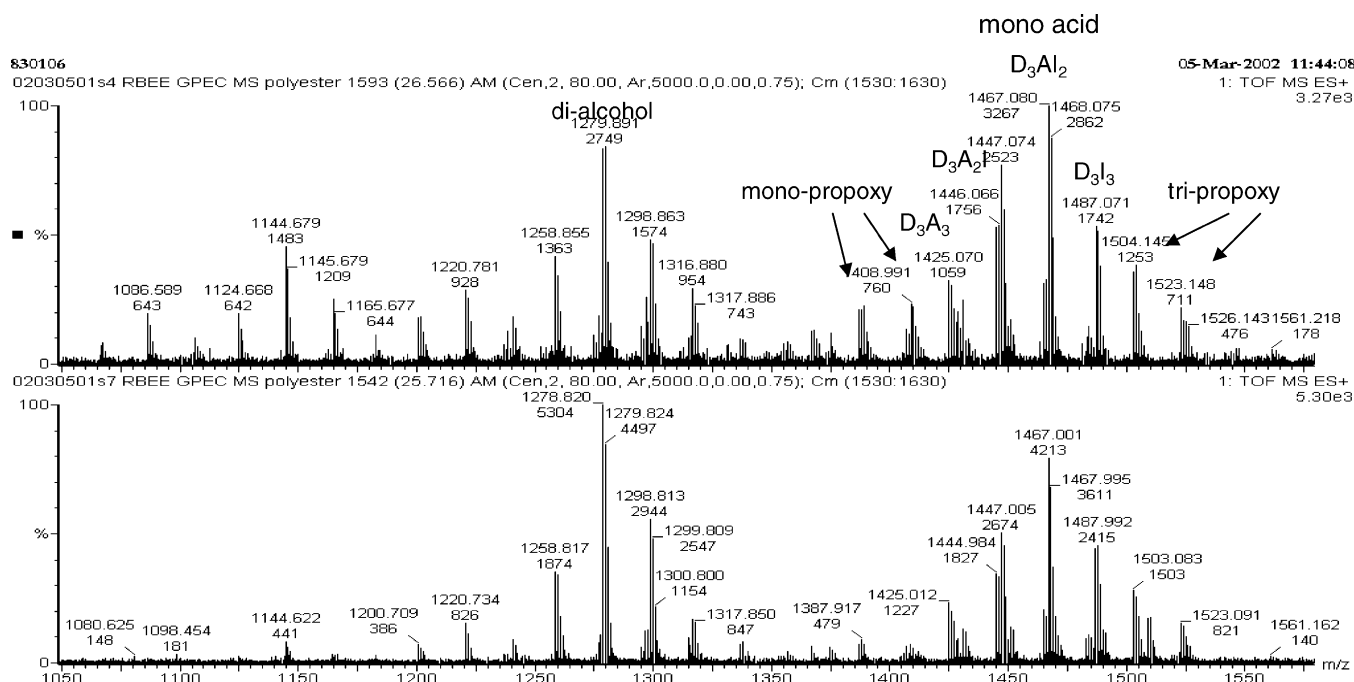


Fig. 14. RP-GE-LC-ESI-TOF-MS spectra (positive mode) for copolyester oligomers  $p = 3$  of two batches of the same copolyesters type. GE-LC conditions—column: Nova-Pak  $C_{18}$ , eluent: THF–water (35:65) to (15:85), linear in 50 min. Detection: UV at 277 nm. MS conditions—ESI capillary: 3 kV, sample cone: 120 V, RF-lens: 1000 V, source temperature: 100 °C, desolvation temperature: 300 °C, desolvation gas: nitrogen at 350 l h<sup>-1</sup>.

are limited as was recently demonstrated by Koster and co-workers for, e.g. copolyesters [146,167].

Further development of sophisticated separation methods and methods relying on partial chemical degradation in combination with MS–MS is necessary to gain further insight in block length and sequence distributions of complex systems.

Recently, the coupling of ESI and MALDI to ion mobility measurements was described [168,169]. Since ion mobility is related to the size and shape of an ion, this may allow for the determination of composition distributions based on conformational differences such as tacticity and branching, in the near future.

## 8. Other methods

Finally, in this chapter three less commonly used methods able to provide chemical composition distribution information in specific cases will be briefly discussed.

### 8.1. Temperature gradient interaction chromatography (TGIC)

TGIC was recently developed by Chang and his coworkers. This LC method is operated isocratically, near the critical conditions, in slight adsorption mode. By applying an appropriate temperature gradient, high resolution according to molar mass, far superior to SEC, can be obtained. This was observed for PS, PMMA and PI [170–172].

Regarding chemical composition separations, the method can be used for the separation of polymer blends. To this end, conditions are chosen such that one of the components elutes in SEC mode whereas near critical conditions are established for the other component. Elution for the latter component is performed by TGIC as was shown for blends of PS and PI, and PS and PMMA [123,172,173]. Next to this, TGIC is suited for functionality type based separations as was demonstrated for functionalized PS using the NP mode [174]. Temperature programming may overcome the problem of highly retained end group fractions, which is frequently encountered in LCCC [94,120]. An advantage of TGIC that should not be underestimated is the applicability of detectors like RI and LS [171,175], which is not possible in GE-LC.

To the authors' opinion TGIC is a valuable addition to the more commonly used LC methods for polymers that should be further explored. For instance, TGIC may be suitable for chemical composition separations of copolymers consisting of strongly resembling monomers showing little differences in adsorption properties. Furthermore, tacticity separations that were shown to be possible using LCCC [110,111] may be performed with much higher resolution in TGIC, although this is speculative. For a specific review on TGIC the reader is referred to [176].

### 8.2. Thermal field flow fractionation (ThFFF)

FFF was originally developed by Giddings [177]. Like in chromatography, residence time of analytes, in all cases

polymers, is measured and related to a polymers' structural feature. Analytes are forced through a narrow, ribbon shaped channel. Perpendicular to the flow direction an external field is applied, which influences residence time and enhances separation selectivity. The field type characterizes the form of FFF that is dealt with [178].

One of the few commercialized FFF types is thermal FFF (ThFFF). This technique is especially suited for lipophilic polymers. Residence time is determined by the Soret coefficient, which is equal to  $(D_T/D)$ .  $D$  is the components' diffusion coefficient, which mainly depends on molar mass, whereas  $D_T$ , the thermal diffusion coefficient, is determined by chemical composition and independent of molar mass.

This implies that ThFFF, next to the determination of MMD of mainly high molar mass homopolymers ( $M > 10^4$ ) can also be used for CCD measurements of random copolymers. For the latter purpose, an (off-line) coupling to SEC is needed for a pre-separation to molar mass. A recent example of this approach was shown in [179]. Unfortunately the method has its limitations for branched and block copolymers since  $D_T$  values in these cases fail to change in a predictable fashion with copolymer composition [180]. This together with the moderate fractionating power, little advantage to more commonly used techniques and experimental difficulties make ThFFF a rarely applied method. More details regarding the technique can be found in a recent review by Schimpf [181].

### 8.3. Temperature rising elution fractionation (TREF)

TREF is a non-chromatographic separation technique, which fractionates polymers according to differences in crystallizability [182]. Crystallizability is determined by branching, tacticity, chemical composition in copolymers and, for low molar mass polymers ( $M < 10^4$ ), molar mass. This implies that TREF is a good means for obtaining information on chemical composition distributions, e.g. CCD, BLD and SD. Its applications are limited to (semi) crystalline polymers, in practice always polyalkenes. These polymers types are less or not amenable to GE-LC or LCCC techniques due to solubility difficulties and to small polarity differences between the various monomer types. Therefore, TREF is a technique of great value to polyalkenes.

A TREF analysis starts by gently cooling down a heated polymer solution causing gradual precipitation/crystallization on a packed column. Subsequently, a good solvent is pumped through the column, which is subsequently slowly heated again. This causes gradual redissolution depending on crystallizability, e.g. branching, chemical composition, etc. The final elution is monitored by a detector.

After calibration with well-defined standards, the elugram can be translated into a chemical composition distribution. In many cases, the obtained fractions are further characterized by SEC and spectroscopic techniques. Some recent examples describe the characterization of propylene-butene-1 [183],

ethylene-propylene [184] and ethylene-1-hexene copolymers [185]. For further details on TREF and its applications the reader is referred to [182].

## 9. Conclusions and future developments

During the past decade large progress has been made in the development of analytical tools for the characterization of chemical composition distributions of all types in synthetic polymers. One of the most important steps in this respect was the introduction of soft ionization techniques in mass spectrometry and further developments in this direction are to be expected in the near future. Important topics are the on-line coupling of MALDI-MS to LC, ionization techniques for relatively apolar polymers such as, e.g. atmospheric pressure photoionization [186], and MS-MS to obtain information on sequence distributions. Ion mobility measurements coupled to MS may provide a new fashion of studying topology differences in polymer systems providing insight in tacticity and branching distributions. Apart from this a major topic to deal with is data treatment. LC-MS is engaged with the generation of huge amounts of data. Although routines for chemometric reduction of LC-MS data in general have become available recently [187], the development of procedures for treatment of polymer data is only in its infancy [188].

Next to MS, the understanding of the chromatography of polymers has largely increased. LCCC is developing rapidly to a widely used technique, especially in 2D-LC applications where its main drawback of limited peak capacity is largely overcome. Enhanced fluidity solvents as a means to reduce some of the practical drawbacks of LCCC deserve serious attention. Further efforts should be also made to explore the advantages (and drawbacks) of barrier methods in comparison to LCCC and the same holds for TGIC.

2D-LC for the simultaneous determination of multiple distributions is becoming popular, especially since commercial software for (quantitative) data treatment became available. Nevertheless, its final success will mainly depend on factors like flexibility, versatility and repeatability, which were hardly discussed in literature until now. Next to this, the answer to the question how generic a chosen 2D-LC setup can be used will largely affect its final adaptation in polymer analysis.

The development of new column packings introduces new possibilities in polymer LC. Monolithic columns were already shown to provide impressive oligomer separations [76]. Flow through particles seem very well suited for fast SEC separations, which is especially of interest for 2D-LC [116]. Functionality separations by LCCC mostly require NPLC. Unfortunately the number of commercially available, essentially different column packings is very limited until now [189]. Further development of tailor made column materials for NPLC would be of great help for polymer chromatographers.



Detection remains an issue of interest in LC, since still no real universal detector exists. ELSD comes close to this ideal but suffers from quantification problems. Therefore, developments like the argon ionization detector [190] are of special interest for polymer LC.

Hyphenation of LC, or even better: LC–MS to NMR [191] has not reached wide acceptance yet, mainly due to its costs. Nevertheless due its unsurpassed possibilities it seems only a matter of time before this combination will dominate polymer analysis. The final addition of FT-IR, which is already possible since a decade may then complete the ‘hyphenation’ towards what has already been called the ‘Total Organic Analysis Device’ [192].

## 10. Nomenclature

$c_s$	concentration of a solute in the stationary phase ( $\text{mol l}^{-1}$ )
$c_m$	concentration of a solute in the mobile phase ( $\text{mol l}^{-1}$ )
$D$	diffusion coefficient ( $\text{cm}^2 \text{s}^{-1}$ )
$D_T$	thermal diffusion coefficient ( $\text{cm}^2 \text{s}^{-1}$ )
$dn/dc$	refractive index increment
$g$	branching parameter (quotient of radii of gyration)
$g'$	branching parameter (quotient of intrinsic viscosities)
$k$	retention factor
$K_{\text{ads}}$	chromatographic distribution constant for adsorption
$K_D$	overall chromatographic distribution constant
$K_{\text{sec}}$	chromatographic distribution constant for size exclusion
$M_n$	number-average molar mass
$M_w$	weight-average molar mass
$p$	degree of polymerization
$P(\theta)$	angular dependence of the Rayleigh scattering
$R_g$	radius of gyration
$T$	temperature (K)
$V_i$	interstitial volume (ml)
$V_m$	volume of the mobile phase (column dead volume) (ml)
$V_p$	pore volume (ml)
$V_r$	retention volume (ml)
$V_s$	volume of the stationary phase (ml)
$\Delta h$	partial molar enthalpy change ( $\text{kJ mol}^{-1}$ )
$\Delta s$	partial molar entropy change ( $\text{J mol}^{-1} \text{K}^{-1}$ )

### Greek letters

$\varepsilon$	conformation parameter
$\Phi_{\text{cr}}$	fraction strong solvent at the critical conditions
$\Phi_{\text{sol}}$	fraction strong solvent at the point of complete solubility
$\eta$	viscosity of the sample solution
$[\eta]$	intrinsic viscosity ( $\text{dl g}^{-1}$ )

$\eta_0$	viscosity of the solvent
$\eta_{\text{sp}}$	specific viscosity
$\lambda_0$	wavelength of the scattered light in vacuum (nm)
$\Delta\mu$	partial molar free energy change ( $\text{kJ mol}^{-1}$ )

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