

Synthetic polymers are highly complex multicomponent materials. They are composed of macromolecules varying in chain length, chemical composition, and architecture. By definition, complex polymers are heterogeneous in more than one distributed property (for example, linear copolymers are distributed in molar mass and chemical composition).

Properties typically considered important to polymer performance in products may be very diverse and can be divided into simple and distributed properties. Simple properties are the total weight of polymer present, the residual monomer or oligomer content, total weight of microgels or aggregates, and properties that depend only on these measures, such as conversion in the polymerization reaction, monomer composition and average copolymer composition. For other properties, different molecules in the same polymer will have different values of the property. These properties are termed distributed properties, the most important of them in polymer chemistry being the molar mass distribution, the distribution of chemical compositions, the distribution of sequence lengths, the distribution of functional groups and the distribution of molecular topologies.

The end-use application of polymers is most frequently determined not only by their chemical identity but more importantly by the distributions of the key physical and physico-chemical parameters. This is similarly true for synthetic and for biopolymers, for technical polymers used as construction materials and for specialty polymers used in drug delivery and tissue engineering. Adequate understanding and monitoring of polymer distributions helps to improve polymer performance and to predict long term behaviour.

Separation science is an important tool for the determination of polymer distributions. A summary of different separation methods, the accessible macromolecular parameters and representative end-use properties are summarized in Table 1.1. The principles and details of the different separation methods will be discussed in the forthcoming chapters and typical applications will be presented.

**Table 1.1** Polymer distributions, end-use properties and separation methods

Polymer distribution	End-use properties	Separation methods <sup>a</sup>
Molar mass	Elongation, tensile strength, adhesion	SEC, FFF, HDC, TGIC, CEC, SFC
Chemical composition	Toughness, biodegradability, morphology, solubility	Gradient HPLC, TGIC, CEC, LCCC
Long-chain branching	Shear strength, tack, peel, crystallinity	SEC-MALLS, SEC-VISC
Short-chain branching	Haze, stress-crack resistance, crystallinity	SEC-FTIR, SEC-NMR, TREF, CRYSTAF, HPLC
Topology	Flow, viscosity, diffusion	SEC-MALLS-VISC
Tacticity	Crystallinity, toughness, solubility	SEC-NMR, TGIC, LCCC
Copolymer sequence	Miscibility, flexibility, haze	SEC-spectroscopy, gradient HPLC, LCCC, 2D-LC
Polyelectrolyte charge	Flocculation, complexation, transport	SEC-conductivity, CEC

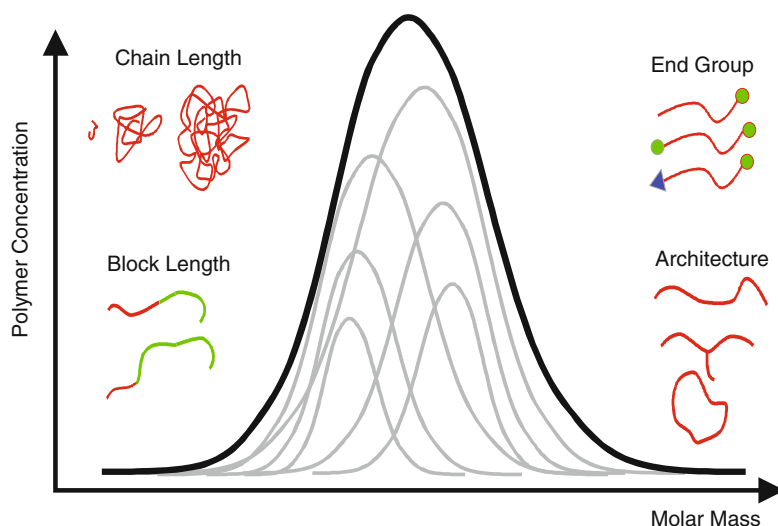
Adapted with permission from [1]. Copyright (2004) American Chemical Society

<sup>a</sup>SEC size exclusion chromatography, FFF field flow fractionation, HDC hydrodynamic chromatography, TGIC temperature gradient interaction chromatography, CEC capillary electrokinetic chromatography, SFC supercritical fluid chromatography, HPLC high performance liquid chromatography, LCCC liquid chromatography at critical conditions, MALLS multi-angle laser light scattering, VISC viscometry, TREF temperature rising elution fractionation, CRYSTAF crystallization fractionation, 2D-LC two-dimensional liquid chromatography

## 1.1 Molecular Heterogeneity of Complex Polymers

In general, the molecular structure of a macromolecule is described by its size, its chemical structure, and its architecture. The chemical structure characterizes the constitution of the macromolecule, its configuration and its conformation. For a complete description of the constitution, the chemical composition of the polymer chain and the chain ends must be known. In addition to the type and quantity of the repeat units, their sequence of incorporation must be described (alternating, random, or block in the case of copolymers). Macromolecules of the same chemical composition can still have different constitutions due to constitutional isomerism (1,2- vs. 1,4-coupling of butadiene, head-to-tail vs. head-to-head coupling, linear vs. branched molecules). Configurational isomers have the same constitution but different steric patterns (cis- vs. trans-configuration; isotactic, syndiotactic and atactic sequences in a polymer chain). Conformational heterogeneity is the result of the ability of fragments of the polymer chain to rotate around single bonds. Depending on the size of these fragments, interactions between different fragments, and a certain energy barrier, more or less stable conformations may be obtained for the same macromolecule (rod-like vs. coil conformation).

Depending on the composition of the monomer feed and the polymerization procedure, different types of heterogeneities may become important. For example, in the synthesis of tailor-made polymers telechelics or macromonomers are frequently used. These oligomers or polymers usually contain functional groups at the



**Fig. 1.1** Schematic representation of the molecular heterogeneity of complex polymers (reprinted from [2] with permission of Springer Science + Business Media)

polymer chain end. Depending on the preparation procedure, they can have a different number of functional endgroups, i.e., they can be mono-, bifunctional, etc. In addition, polymers can have different architectures, i.e. they can be branched (star- or comb-like), or cyclic.

The structural complexity of synthetic polymers can be described using the concept of molecular heterogeneity, see Fig. 1.1, meaning the different aspects of molar mass distribution (MMD), distribution in chemical composition (CCD, e.g. block length distribution), functionality type (e.g. endgroup) distribution (FTD) and molecular architecture distribution (MAD). They can be superimposed one on another, i.e. bifunctional molecules can be linear or branched, linear molecules can be mono- or bifunctional, copolymers can be block or graft copolymers etc. In order to characterize complex polymers it is necessary to know the molar mass distribution within each other type of heterogeneity.

All synthetic polymers are disperse or heterogeneous in terms of molar mass. The *molar mass distribution* originates from randomness of the polymerization process. In the daily routine synthetic polymers are often characterized by average molar masses, considering the frequencies (numbers) of macromolecules of a certain molar mass  $M_i$  in the sample. Most frequently used are the *number-average* molar mass  $M_n$ , expressing the amount of species in terms of number of moles  $n_i$ , and the *weight-average* molar mass  $M_w$ , considering the mass  $m_i$  of the species. As  $m_i$  is related to  $n_i$  via  $m_i = n_i M_i$ , and for a single species  $M_i = M_o P_i$ , the molar mass averages may be expressed as average degrees of polymerization, where  $P_n$  is the number-average and  $P_w$  is the weight-average degree of polymerization.

$$M_n = \sum n_i M_i / \sum n_i = P_n M_o, \quad (1.1)$$

$$M_w = \sum w_i M_i / \sum w_i = \sum n_i M_i^2 / \sum n_i M_i = P_w M_o. \quad (1.2)$$

Molar masses of polymers may be determined by different methods, SEC being the most important [1–7]. The difference between number and weight average molar masses gives a first estimate of the width of the MMD. The broader the distribution, the larger is the difference between  $M_n$  and  $M_w$ . The ratio of  $M_w/M_n$  is a measure of the breadth of the molar mass and is termed the dispersity.

When two or more monomers of different chemical structures are involved in a polymerization reaction, instead of a chemically homogeneous homopolymer in most cases a chemically heterogeneous copolymer is formed. Depending on the reactivity of the monomers and their sequence of incorporation into the polymer chain, macromolecules can be formed which differ significantly in composition (meaning the amounts of repeat units A, B etc. in the copolymer), and the sequence distribution. With respect to sequence distribution, copolymers can be classified as alternating, random, block and graft copolymers.

Chemical heterogeneity is a consequence of CCD and can be presented as an integral or differential distribution curve of composition vs. molar mass. Consider a random copolymer obtained in a homogeneous reaction from a mixture of A and B monomers. Even under such favourable conditions the resulting macromolecules will differ in chemical structure. There are differences in the sequence of the A and B monomers along the macromolecules, differences in the average chemical composition of the copolymer molecules formed at any instant of the polymerization (instantaneous heterogeneity), and differences due to the depletion of the reaction mixture in one of the monomers.

The sequence distribution of a copolymer chain may be characterized by the number-average lengths of uninterrupted sequences of A and B units in this chain. The average sequence lengths can be measured by physical or chemical methods. The former methods (FTIR or NMR analyses) usually measure the percentage of A and B units inside of triades, pentades etc. whereas the latter methods measure the percentage of A–A, A–B, and B–B linkages. Macromolecules of random copolymers, even if identical in chain length and composition (and thus also in the average sequence length), still offer a great variety with respect to the order of individual sequences in the molecules. Thus, a copolymer sample contains a tremendous number of constituents. In terms of liquid chromatography (LC), a sample of this kind is an extremely complex mixture; it is difficult to separate by size exclusion or interaction chromatography [1].

In addition to the sequence distribution, conversion heterogeneity has to be considered when analyzing copolymers. Only in special cases is the composition of a copolymer identical to the composition of the monomer batch. These cases are azeotropic copolymers or systems whose monomer reactivity ratios equal 1. In general, the instantaneous composition of a copolymer differs from the

composition of the monomer mixture, which causes depletion of the batch of the monomer that is preferably incorporated. Thus, subsequent portions of a copolymer sample are polymerized from mixtures of various compositions and this gives rise to additional chemical heterogeneity. Accordingly, when discussing the CCD of copolymers, *sequence distribution*, *instantaneous heterogeneity* and *conversion heterogeneity* must be considered.

Oligomers and polymers with reactive functional groups have been used extensively to prepare a great variety of polymeric materials. In many cases, the behaviour and reactivity of these functional homopolymers is largely dependent on the nature and the number of functional groups. In a number of important applications the functional groups are located at the end of the polymer chain. Macromolecules with terminal functional groups are usually termed “telechelics”. Special cases are “macromonomers” which contain one polymerizable endgroup. They can be used as starting materials for the synthesis of graft polymers, combs or brushes.

Molecular functionality,  $f$ , of a telechelic polymer is described as the number of functional groups per molecule. Macromolecules with the same structure of the polymer chain may differ in the number and the nature of the functional groups. When functional homopolymers are synthesized, functionally defective molecules are formed in addition to macromolecules of required functionality. For example, if a target functionality of  $f = 2$  is required, then generally in the normal case species with  $f = 1$ ,  $f = 0$  or higher functionalities are formed as well [3], which may result in a decreased or increased reactivity, cross-linking density, surface activity etc. Each functionality fraction has its own molar mass distribution. Therefore, for a complete description of the molecular structure of a functional homopolymer, the determination of the molar mass distribution and the functionality type distribution is required.

Typically, functionality is quantitatively described as number-average functionality,  $f_n$ , where  $f_n$  is the ratio of the total number of functional groups to the total number of molecules in the system, i.e. the average number of functional groups per initial molecule. The  $f_n$  value provides information on the average functionality but does not characterize the functional dispersity. An average functionality of 1 may be simulated by equal amounts of non-functional and difunctional species, and is therefore ambiguous. The characterization of the width of the functionality type distribution is more informative. In analogy to the average molar masses, number-average and weight-average functionalities may be introduced,

$$f_n = \sum n_i f_i / \sum n_i, \quad (1.3)$$

$$f_w = \sum w_i f_i / \sum w_i = \sum n_i f_i^2 / \sum n_i f_i, \quad (1.4)$$

where  $n_i$  is the number of molecules of functionality  $f_i$ , and  $w_i = n_i f_i$ . For the description of the functional dispersity the term  $f_w/f_n$  may be used. For polymers containing only one type of molecule,  $f_w/f_n = 1$  is obtained. In the case

of a distribution of molecules of different functionality  $f_w/f_n > 1$  is obtained. In the characterization of polymers, a separation according to different distributions is required. This can be achieved using different modes of liquid chromatography.

Using the traditional methods of polymer analysis, such as infrared spectroscopy or nuclear magnetic resonance, one can determine the type of monomers or functional groups present in the sample. However, the determination of functional endgroups is complicated for long chain molecules because of low concentration. On the other hand, these methods do not yield information on how different monomer units or functional groups are distributed in the polymer molecule. Finally, these methods generally do not provide molar mass information.

With respect to methods sensitive to the size of the macromolecule, one can face other difficulties. SEC which is most frequently used to separate polymer molecules from each other according to their molecular size in solution, must be used very carefully when analyzing complex polymers. The molecular size distribution of macromolecules can generally be unambiguously correlated with MMD only within one heterogeneity type. For samples consisting of a mixture of molecules of different functionalities, the distribution obtained represents a sum of distributions of molecules having a different functionality and, therefore, cannot be attributed to a specific functionality type without additional assumptions.

For the analysis of copolymers by SEC either the chemical composition along the molar mass axis must be known or detectors must be used which, instead of providing a concentration information, can provide molar mass information. To this end, SEC has to be coupled to composition-sensitive or molar mass-sensitive detectors. Another option for the analysis of complex polymers is the separation with respect to chemical composition or functionality by means of interaction chromatography. In this case, functionally or chemically homogeneous fractions are obtained which then can be subjected to molar mass determination.

To summarize, for the complete analysis of complex polymers a minimum of two different characterization methods must be used. It is most desirable that each method is selective towards a specific type of heterogeneity. Maximum efficiency can be expected when, similar to the 2D distribution in properties, 2D analytical techniques are used. A possible approach in this respect is the coupling of different chromatographic modes in 2D chromatography or the coupling of a separation technique with selective detectors.

---

## 1.2 Liquid Chromatography of Polymers

Any chromatographic process relates to the selective distribution of an analyte between the mobile and the stationary phase of a given chromatographic system. In LC the separation process can be described by

$$V_e = V_i + V_p K_d, \quad (1.5)$$

where  $V_e$  is the retention volume of the solute,  $V_i$  is the interstitial volume of the column,  $V_p$  is the volume inside the pores of the packing, i.e. the “stationary” volume, and  $K_d$  is the distribution coefficient which is equal to the ratio of the analyte concentration in these fractions of the liquid phase inside the column. In other words, a molecule moves along the column as long it is in the interstitial volume, and it is retained as long as it is inside the pores.

It should be mentioned that the stationary volume can comprise a volume fraction of the mobile phase and the volume of the boundary layer at the surface of the stationary phase, which can have considerably different compositions. In typical reversed phase systems, there is a layer of bonded alkyl chains (which may be swollen or collapsed) as well as an adsorbed layer of almost pure organic component of the mobile phase [8–10], and there is no dividing interface between these layers and the bulk liquid. A similar situation is observed on hydrophilic interaction columns, which contain an aqueous layer close to the surface of the stationary phase [11].

As it is not possible to determine these fractions of the stationary volume, it does not make sense to consider another distribution coefficient for the partitioning of a solute between the bulk phase inside the pores and the boundary layer at the surface of the stationary phase. In both situations, the molecule will be retained. If there is an enthalpic interaction between the solute and the stationary phase, it will stay longer in the pore, which results in a larger distribution coefficient.

$K_d$  is related to the change in Gibbs free energy  $\Delta G$  related to the analyte partitioning between interstitial and pore volume [12].

$$\Delta G = \Delta H - T\Delta S = -RT \ln K_d. \quad (1.6)$$

In a logarithmic plot of the distribution coefficients as a function of  $1/T$ , one may determine the entropic and enthalpic contributions (van t'Hoff plot). As will be discussed later on:



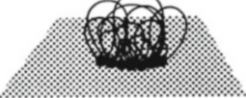
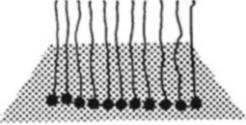

$$\ln K_d = \frac{\Delta S}{R} - \frac{\Delta H}{RT}. \quad (1.7)$$

The change in Gibbs free energy may be due to different effects:

1. Inside the pore, which has limited dimensions, the macromolecule cannot occupy all possible conformations and, therefore, the conformational entropy  $\Delta S$  decreases.
2. When penetrating the pores, the macromolecule may interact with the pore walls resulting in a change in enthalpy  $\Delta H$ . Obviously, the interaction of a polymer chain with the stationary phase has also an entropic contribution: when the chain interacts with the surface, it will lose degrees of freedom. Instead of a random coil, there will be adsorbed trains, loops and free ends, see Table 1.2. Depending on the chromatographic system and the chemical structure of the macromolecule, there may be different entropic or enthalpic contributions.

In SEC separation is accomplished with respect to the hydrodynamic volume of the macromolecules. The stationary phase is a swollen gel with a characteristic pore size distribution, and depending on the size of the macromolecules a larger

**Table 1.2** Model presentation of the adsorption of macromolecules

Model	Relation between adsorbed amount	Molar mass and surface thickness
Flat layer 	Independent of $M$	Independent of $M$
Coil 	Independent of $M$	$\sim M^{0.5}$
Collapsed coil 	$\sim M^{1/3}$	$\sim M^{1/3}$
Brush 	$\sim M$	$\sim M$
Loops and trains 	$\sim M^a$ $0 < a < 0.5$	Independent of $M$

Reprinted from [3] with permission of Springer Science + Business Media

or lesser fraction of the pores is accessible to the macromolecules. Very large molecules, which are excluded from the pores, will elute at the interstitial volume  $V_i$ , while small molecules, which have access to the entire pore volume, will elute at the void volume which is  $V_0 = V_i + V_p$ . Consequently, the separation range is  $0 < K_{\text{SEC}} < 1$ .

In *ideal* SEC, enthalpic interactions are absent, and the distribution coefficients are exclusively determined by the entropy change. In *real* SEC, this may not strictly be fulfilled. Especially with charged polymers it is often difficult to suppress enthalpic interactions completely. These interactions may be attractive or repulsive.

In liquid adsorption chromatography (LAC), where the separation is dominated by enthalpic interactions between the macromolecules and the stationary phase, an ideal and a real case may be defined as well. In *ideal* LAC (which may be observed with small molecules) conformational changes are assumed to be zero ( $\Delta S = 0$ ) and the distribution coefficient is exclusively determined by enthalpic effects. In *real* LAC only a fraction of the pores of the packing is accessible for the polymer



chains, which are more or less deformed, when they interact with the stationary phase. Therefore, entropic contributions must be assumed. Accordingly, the distribution coefficient is a function of  $\Delta H$  and  $\Delta S$ .

Real SEC and real LAC are often mixed-mode chromatographic methods with predominance of entropic or enthalpic interactions. With chemically heterogeneous polymers, effects are even more dramatic because exclusion and adsorption act differently on molecules with different compositions. In a more general sense, the size exclusion mode of LC relates to a separation regime where entropic interactions are predominant, i.e.,  $T\Delta S > \Delta H$ . In the reverse case,  $\Delta H > T\Delta S$ , separation is mainly directed by enthalpic interactions. As both separation modes in the general case are affected by the size of the macromolecule and the pore size, a certain energy of interaction  $\varepsilon$  may be introduced, characterizing the specific interactions of the monomer unit of the macromolecule and the stationary phase.  $\varepsilon$  is a function of the chemical composition of the monomer unit, the composition of the mobile phase of the chromatographic system, the characteristics of the stationary phase and the temperature.

The theory of adsorption at porous adsorbents predicts the existence of a finite critical energy of adsorption  $\varepsilon_c$ , where the macromolecule starts to adsorb at the stationary phase. Thus, at  $\varepsilon > \varepsilon_c$  the macromolecule is adsorbed, whereas at  $\varepsilon < \varepsilon_c$  the macromolecule remains non-adsorbed. At  $\varepsilon = \varepsilon_c$  the transition from the non-adsorbed to the adsorbed state takes place, corresponding to a transition from SEC to LAC. This transition is termed “critical point of adsorption” or “critical adsorption point” (CAP) and relates to a situation, where the adsorption forces are exactly compensated by entropy losses [13–15].

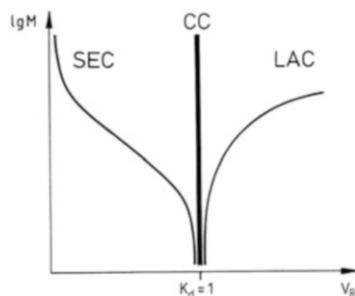
$$T\Delta S = \Delta H. \quad (1.8)$$

Accordingly, at the critical point of adsorption the Gibbs free energy is constant ( $\Delta G = 0$ ) and the distribution coefficient is  $K_d = 1$ , irrespective of the molar mass of the macromolecules and the pore size of the stationary phase.

The critical point of adsorption relates to a very narrow range between the size exclusion and adsorption modes of LC, a region which is very sensitive towards temperature and mobile phase composition. The transition from one to another chromatographic separation mode by changing the temperature or the composition of the mobile phase was reported for the first time by Tennikov et al. [16] and Belenkii et al. [13, 17]. They showed that a sudden change in elution behaviour may occur by small variations in the solvent strength. Thus, just by simply gradually changing the eluent composition, a transition from the SEC to the LAC mode and vice versa may be achieved. The point of transition from SEC to LAC is the critical point of adsorption and chromatographic separations at this point are termed *liquid chromatography at the critical point of adsorption* or liquid chromatography at critical conditions (LCCC).

The retention behaviour of linear homopolymers in these separation modes is shown schematically in Fig. 1.2: with increasing molar mass, retention decreases in SEC, increases in LAC, while it is constant in LCCC. The separation modes

**Fig. 1.2** Molar mass vs. retention volume behaviour in different chromatographic modes



described above can be combined in various ways in order to separate polymers according to the distributions of molar mass, chemical composition and functionality.

For SEC as the most important and well established separation method a number of approaches are in place to obtain chemical composition and molar mass information in the same chromatographic run [18]:

1. Multiple detection SEC systems:  $n$  independent detector signals (different responses by components) allow the composition calculation of  $n$  components in the sample.
2. Universal calibration: measurement of Mark-Houwink coefficients for copolymers with homogeneous and known composition will give copolymer molar masses.
3. SEC with on-line viscometric detection: on-line measurement of Mark-Houwink coefficients for copolymers of various architectures. Here, copolymer  $M_n$  measurement according to the Goldwasser approach [19] is an additional benefit.
4. SEC with light-scattering detection: direct measurement of copolymer molar masses for chemically homogeneous and segmented copolymers independent of their chemical structure.

The different approaches of copolymer analysis, their requirements, benefits, and limitations are summarized in Table 1.3.

Although these approaches are most useful, they are not based on a chemical composition separation as in the case of interaction chromatography. SEC is, therefore, intrinsically not able to provide a CCD. The chemical composition information that is obtained is an average value that relates to a given molar mass fraction.

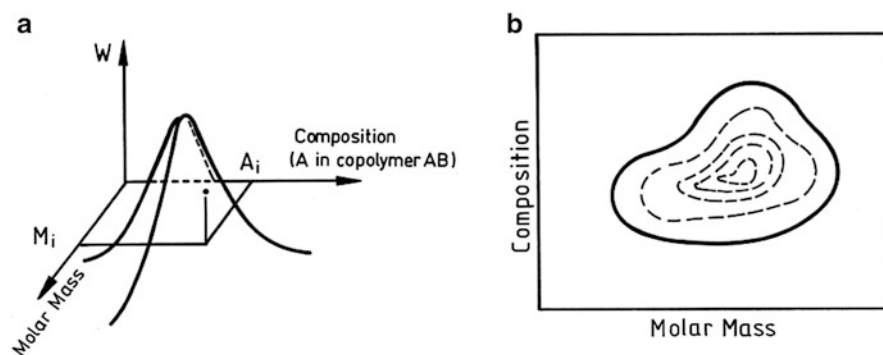
### 1.3 Multidimensional Separation of Complex Polymers

Complex polymers are distributed in more than one direction of molecular heterogeneity. Copolymers are characterized by the molar mass distribution and the chemical heterogeneity, whereas functional homopolymers are distributed in molar mass and functionality. Hence, the experimental evaluation of the different distribution functions requires analysis in more than one direction. The molecular

**Table 1.3** Chromatographic methods for copolymer and blend analysis

Method	Requirements	Preconditions	Advantages	Limitations
Multiple detection	Two or more detectors, proper calibrants	No segment-segment interactions, no neighboring group effects	Bulk composition and compositional distribution, broad applicability, no additional sample preparation	Statistical copolymers, densely grafted polymer chains
Universal calibration	Base calibration, $[\eta]$ - $M$ relationship	Validity of universal calibration, homogeneous sample composition	Simple and accurate MMD	Chemically homogeneous samples only, detailed information about sample required
Light-scattering detection	Light-scattering and concentration detectors	Known $dn/dc$ as a function of elution volume	Direct MMD measurement, no calibration, independent of architecture	No CCD information
Viscometric detection	Viscometric and concentration detectors	Validity of universal calibration	Easy MMD calculation, independent of architecture, $K$ and $a$ for copolymers	No CCD information, no heterogeneous samples

Reprinted with permission from [18]. Copyright (1995) American Chemical Society



**Fig. 1.3** Representation of the molecular heterogeneity of a random copolymer, (a) three-dimensional diagram, (b) contour diagram;  $A_i$  and  $M_i$  indicate the average composition and molar mass, respectively (reprinted from [3] with permission of Springer Science + Business Media)

heterogeneity of a random AB copolymer is presented in Fig. 1.3 showing the distributions in molar mass and chemical composition.

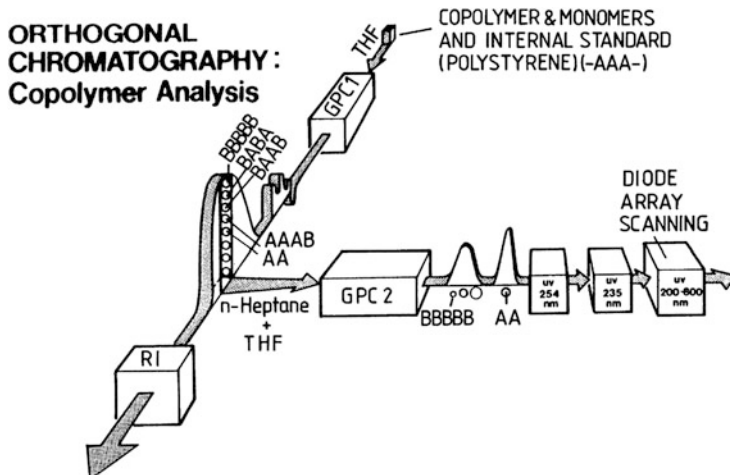
For a complete analysis both distributions must be determined. The classical approach is based upon the dependence of copolymer solubility on composition and chain length. A solvent/non-solvent combination fractionating solely by molar

mass would be appropriate for the evaluation of MMD, another one separating with respect to chemical composition would be suited for determining CCD. Unfortunately, in most cases precipitation fractionation yields fractions which vary both in molar mass and chemical composition. Even high resolution fractionation would not improve the result and it is nearly impossible to obtain perfectly homogeneous fractions.

By the use of different modes of liquid chromatography it is possible to separate polymers selectively with respect to hydrodynamic volume (molar mass), chemical composition or functionality. Using these techniques and combining them with each other or with a selective detector, one can obtain two-dimensional information on different aspects of molecular heterogeneity. If, for example, two different chromatographic techniques are combined in a “cross-fractionation” mode, information on CCD and MMD can be obtained. Literally, the term “chromatographic cross-fractionation” refers to any combination of chromatographic methods capable of evaluating the distribution in size and composition of copolymers.

First attempts to make use of orthogonal chromatography were presented by Balke et al. [20] and Glöckner [12] in the 1980s. Balke et al. used the fact that macromolecules of the same chain length but different composition have different hydrodynamic volumes. Since SEC separates according to hydrodynamic volume, SEC in different eluents can separate a copolymer in two diverging directions. The authors coupled two SEC instruments together so that the eluent from the first one flowed through the injection valve of the second one. At any desired retention time the flow through SEC 1 could be stopped and an injection made into SEC 2. The first instrument was operated with THF as the eluent and polystyrene gel as the packing, whereas for SEC 2 polyether bonded-phase columns and THF-heptane were used. The schematic presentation of this system is given in Fig. 1.4. Both instruments utilized SEC columns. However, whereas the first SEC was operating so as to achieve conventional molecular size separation, the second SEC was used to fractionate by composition, utilizing a mixed solvent to encourage adsorption and partition effects in addition to size exclusion.

Much work on chromatographic cross-fractionation was carried out with respect to combination of SEC and gradient HPLC. In most cases SEC was used as the first separation step, followed by HPLC. In a number of early papers the cross-fractionation of model mixtures was discussed. Investigations of this kind demonstrated the efficiency of gradient HPLC for separation by chemical composition. Mixtures of statistical copolymers of styrene and acrylonitrile were separated by Glöckner et al. [21]. In the first dimension a SEC separation was carried out using THF as the eluent and polystyrene gel as the packing. In total, about 10 fractions were collected and subjected to the second dimension, which was gradient HPLC on a CN bonded-phase using isooctane-THF as the mobile phase. Model mixtures of statistical copolymers of styrene and 2-methoxyethyl methacrylate were separated in a similar way, the mobile phase of the HPLC mode being isooctane-methanol in this case [22]. Graft copolymers of methyl methacrylate onto EPDM rubber were analyzed by Augenstein and Stickler [23] whereas Mori reported on the fractionation of block copolymers of styrene and vinyl acetate [24].



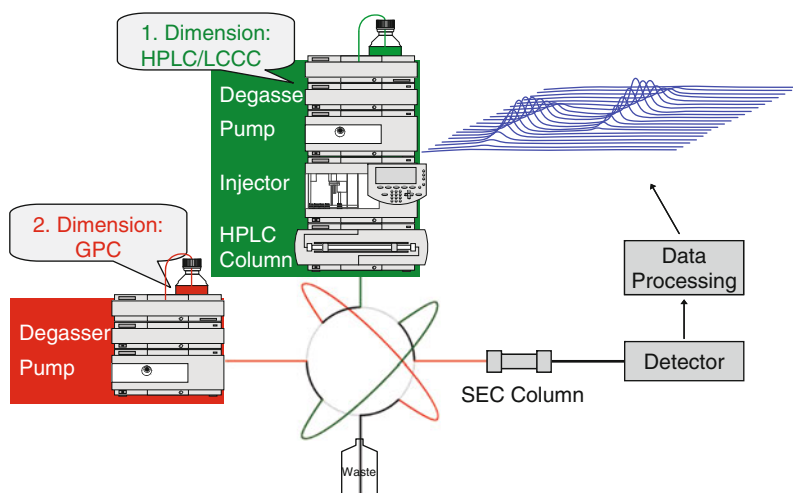
**Fig. 1.4** Schematic representation of an orthogonal chromatographic system showing size fractionation of a linear copolymer by SEC 1 and the variety of molecules of the same molecular size within a chromatogram slice, A-styrene and B-butyl methacrylate units (reprinted from [3] with permission of Springer Science + Business Media)

A more feasible way of analyzing copolymers is the pre-fractionation through HPLC in the first dimension and subsequent analysis of the fractions by SEC [25, 26]. HPLC was found to be rather insensitive towards molar mass effects and yields very uniform fractions with respect to chemical composition. Principal considerations of LC couplings will be discussed more in detail in Chapter 6.

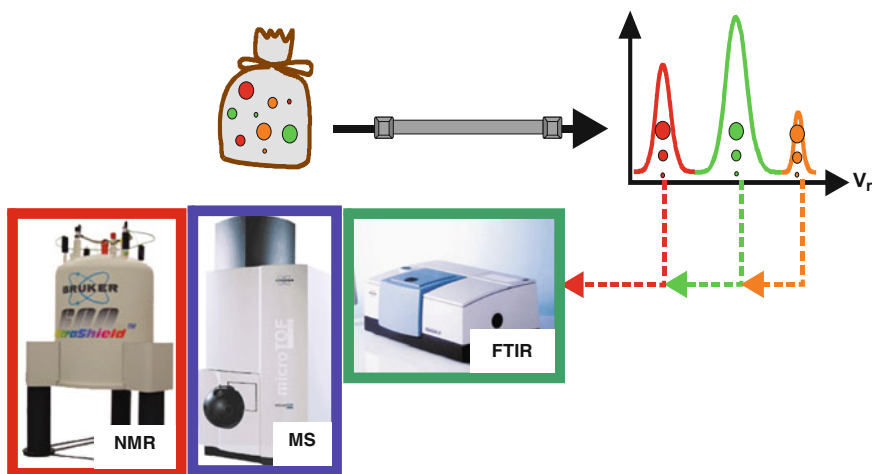
The major disadvantage of all early investigations on chromatographic cross-fractionation was related to the fact that both separation modes were combined to each other either off-line or in a stop-flow mode. Regardless of the separation order SEC vs. HPLC or HPLC vs. SEC, in the first separation step fractions were collected, isolated, and then subjected to the second separation step. This procedure was very time-consuming and the reliability of the results at least to a certain extent depended on the skills of the operator.

A fully automated two-dimensional chromatographic system was developed by Kilz et al. [27–29] in the 1990s. It consists of two chromatographs, one which separates by chemical composition or functionality and a SEC instrument for subsequent separation by size. Via a storage loop system, fractions from the first separation step are automatically transferred into the second separation system. The operation of the column switching device is automatically driven by the software, which at the same time organizes the data collection from the detector. The design of a typical system is presented schematically in Fig. 1.5.

Another option to address the molecular heterogeneity of complex polymers is the combination of selective fractionation methods with information-rich detectors, see Fig. 1.6.



**Fig. 1.5** Schematic representation of an automated two-dimensional chromatographic system (reprinted from [30] with permission of Elsevier)



**Fig. 1.6** Schematic representation of the hyphenation of a selective chromatographic separation and spectroscopic analysis for the analysis of a sample that is distributed regarding composition (different colours) and molar masses (different sizes)

During the last two decades a number of techniques have been introduced in organic chemistry and applied to polymer analysis, combining chromatographic separation with spectroscopic detection [31]. GC-MS has been used in polymer analysis, but, due to the low volatility of high molar mass compounds it is limited to the oligomer region. The combination of pyrolysis and GC-MS, however, is of great value for polymer characterization [32, 33]. It provides for the analysis of complex

polymers with respect to chemical composition. Much more important are the different techniques of liquid chromatography. Using SEC, liquid adsorption chromatography (LAC), or liquid chromatography at the critical point of adsorption (LCCC) polymers can be fractionated with respect to different aspects of molecular heterogeneity, including molar mass, functionality, and chemical composition. As will be shown in the next chapters, liquid chromatography can be efficiently coupled to infrared spectroscopy [34–39], to mass spectrometry, and to nuclear magnetic resonance spectroscopy [40, 41]. Another most feasible approach is multidetector SEC where molar mass separation is hyphenated with molar mass sensitive detectors like on-line viscometry and on-line static light scattering [1, 42].

---

## References

1. Striegel AM (2004) In: Striegel AM (ed) Multiple detection in size exclusion chromatography, vol 893, ACS Symposium Series. ACS, Washington, DC
2. Pasch H (2000) Adv Polym Sci 150:1
3. Pasch H, Trathnigg B (1998) HPLC of polymers. Springer, Berlin
4. Yau WW, Kirkland JJ, Bly DD (1979) Modern size exclusion chromatography. Practice of gel permeation and gel filtration chromatography. Wiley Interscience, New York
5. Janca J (1984) Steric exclusion liquid chromatography. Dekker, New York
6. Glöckner G (1987) Polymer characterization by liquid chromatography. Elsevier, Amsterdam
7. Mori S, Barth HG (1999) Size exclusion chromatography. Springer, Berlin
8. Rustamov I, Farcas T, Ahmed F, Chan F, LoBrutto R, McNair HM, Kazakevich YV (2001) J Chromatogr A 913:49
9. Kazakevich YV, LoBrutto R, Chan F, Patel T (2001) J Chromatogr A 913:75
10. Bocian S, Vajda P, Felinger A, Buszewski B (2008) J Chromatogr A 1204:35
11. Jandera P (2008) J Sep Sci 31:1421
12. Glöckner G (1991) Gradient HPLC and chromatographic cross-fractionation. Springer, Berlin
13. Belenkii BG, Gankina ES, Tennikov MB, Vilenchik LZ (1978) J Chromatogr 147:99
14. Entelis SG, Evreinov VV, Gorshkov AV (1986) Adv Polym Sci 76:129
15. Entelis SG, Evreinov VV, Kuzaev AI (1985) Reactive oligomers. Khimiya, Moscow
16. Tennikov MB, Nefedov PP, Lazareva MA, Frenkel SJ (1977) Vysokomol Soedin A19:657
17. Belenkii BG, Gankina ES, Tennikov MB, Vilenchik LZ (1976) Dokl Acad Nauk USSR 231:1147
18. Kilz P, Krüger RP, Much H, Schulz G (1995) In: Provder T, Urban MW, Barth HG (eds) Chromatographic characterization of polymers: hyphenated and multidimensional techniques, vol 247, Advances in Chemistry Series. American Chemical Society, Washington, DC
19. Goldwasser JM, Rudin A (1983) J Liq Chromatogr 6:2433
20. Balke ST, Patel RD (1980) J Polym Sci B Polym Lett 18:453
21. Glöckner G, van den Berg JHM, Meijerink NL, Scholte TG (1986) In: Kleintjens I, Lemstra P (eds) Integration of fundamental polymer science and technology. Elsevier Applied Science, Barking
22. Glöckner G, Stickler M, Wunderlich W (1989) J Appl Polym Sci 37:3147
23. Augenstein M, Stickler M (1990) Makromol Chem 191:415
24. Mori S (1990) J Chromatogr 503:411
25. Mori S (1988) Anal Chem 60:1125
26. Mori S (1981) Anal Chem 53:1813
27. Kilz P (1993) Labor Praxis 6:64
28. Kilz P, Krüger R-P, Much H, Schulz G (1995) ACS Adv Chem 247:223
29. Kilz P, Krüger R-P, Much H, Schulz G (1993) PMSE Preprints 69:114

30. Pasch H (2012) In: Matyjaszewski K, Moeller M (eds) *Comprehensive polymer science*, 2nd edn. Elsevier, Amsterdam
31. Cortes HJ (1992) *J Chromatogr* 626:3
32. Liebman S, Levy E (1985) *Pyrolysis and GC in polymer analysis*. Dekker, New York
33. Geißler M (1997) *Kunststoffe* 87:194
34. Sabo M (1985) *Anal Chem* 57:1822
35. Hellgeth JW, Taylor LT (1987) *Anal Chem* 59:295
36. Hellgeth JW, Taylor LT (1986) *J Chromatogr Sci* 24:519
37. Griffiths PR, Conroy CM (1986) *Adv Chromatogr* 25:105
38. Gagel JJ, Biemann K (1986) *Anal Chem* 58:2184
39. Wheeler LM, Willis J (1993) *Appl Spectrosc* 47:1128
40. Lindon JC (1996) *Progr Nucl Magn Res Spectrosc* 29:1
41. Albert K (1995) *J Chromatogr A* 703:123
42. Striegel AM, Yau WW, Kirkland JJ, Bly DD (2009) *Modern size-exclusion liquid chromatography. Practice of gel permeation and gel filtration chromatography*. Wiley, Hoboken, NJ



Multidimensional HPLC of Polymers

Pasch, H.; Trathnigg, B.

2013, XIV, 280 p. 181 illus., 24 illus. in color., Hardcover

ISBN: 978-3-642-36079-4