Size-exclusion Chromatography of Polymers

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Size-exclusion chromatography (SEC) is a standard technique for determining molar mass averages and molar mass distributions (MMDs) of polymers. Sometimes the terms gel permeation chromatography (GPC) or gel filtration chromatography (GFC) are also used, but SEC should be preferred, because this term describes the mechanism much better: polymer molecules are separated according to their hydrodynamic volumes (which can be correlated with molar mass), with the larger size molecules exiting first followed by the smaller. Molar masses are determined either from a calibration or using molar mass sensitive detectors. In the case of copolymers, the knowledge of chemical composition along the MMD is required, which can be obtained from combinations of different concentration detectors. As the hydrodynamic volumes of different polymers are typically somewhat different, molecules with different chemical composition and different molar mass will be eluted in the same slice of the chromatogram. Obviously, a discrimination between such molecules requires a two-dimensional separation, in which one dimension may be SEC, and the other one a chromatographic technique, which separates according to chemical composition rather than molar mass, such as liquid adsorption chromatography (LAC), liquid chromatography at the critical point of adsorption (often also called liquid chromatography under critical conditions, LCCC), supercritical fluid chromatography (SFC), temperature rising elution fractionation (TREF), etc.

In the lower molar mass range, mass spectroscopy competes with SEC. The most frequently used technique is matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI/TOF/MS), which cannot, however, provide quantitatively accurate MMDs. Due to its excellent resolution in molar mass, it can be combined with chromatographic techniques in order to increase the reliability of the analysis.

1 INTRODUCTION

In the characterization of polymers, SEC has become a standard technique for determining molar mass averages and MMDs of polymers. Depending on the field of application, different terms have been used: in biochemistry and related areas the term GFC is usual, while GPC is commonly used in the analysis of (synthetic) polymers.

The principle of SEC is rather easily understood. Due to limited accessibility of the pore volume within the particles of the column packing, polymer molecules are separated according to their hydrodynamic volumes, with the larger size molecules exiting first followed by the smaller. Residence time can be correlated with molar mass. The correlation obtained then depends upon the type of polymer.

1.1 History

The origins of SEC date back to the early 1960s. In 1959, Porath and Flodin described the separation of water-soluble macromolecules on cross-linked poly-dextrane gels. As soon as these gels had become commercially avaliable, they were extensively used for separating biomolecules by the new technique, which was called GFC, typically in low pressure systems.[1]

In 1964, J.C. Moore of the Dow Chemical Company disclosed the separation of synthetic polymers on cross-linked polystyrene (PS) gels in organic mobile phases. The new technique was called GPC and very soon became a standard method for the determination of MMDs.

2 APPLICATIONS

Basically, SEC separates according to the size of a species in solution (the hydrodynamic volume). This species may be a single molecule, a polymer coil, an aggregate, a micelle, etc. Hence, SEC can be applied to determine the molar mass of a polymer and also to study aggregation phenomena in solution.

Typically, SEC is applied to the analysis of synthetic polymers and oligomers,[2–7] coal-derived substances,[8–10] lipids,[11,12] and natural macromolecules (such as proteins,[13–15] poly(ethylene glycol) (PEG)-modified proteins,[16,17] glucans,[18,19] cellulose derivatives,[20,21] humic substances,[22] crude-oil alkanes[23]).

SEC may also be used in studying processes accomplished by a change of the hydrodynamic volume of polymers or small molecules (such as lipids[12,24–26]): degradation,[27,28] hydrolysis,[21,29] refolding of proteins,[30] polymerization,[31–35] aggregation,[36,37] etc.

3 RELIABILITY OF SIZE-EXCLUSION CHROMATOGRAPHY

In the last few years several round-robin tests have been performed with different kinds of polymers[38–45] in order to evaluate the reproducibility of SEC and the precision and accuracy of the results thus obtained.

There may be various sources of error responsible for the differences in the results obtained at different laboratories, as can be easily understood from Figure 1, in which the experimental set-up and the basic steps in obtaining an MMD for a polymer sample are shown schematically. An appropriate mobile phase is delivered to a chromatographic column filled with a suitable stationary phase by a pump at a constant and reproducible
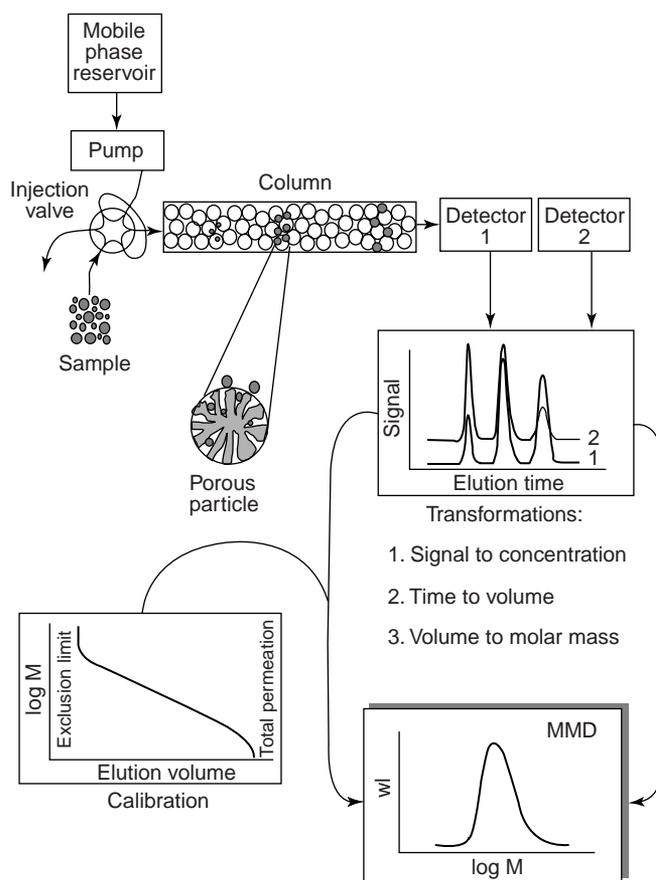
Figure 1 Schematic representation of SEC.

flow rate. Into this solvent stream a small amount (typically 0.01 to 1.0 mg) of the polymer sample is injected.

The separated fractions are detected by at least one detector, the signal of which must represent the concentration of the polymer with good accuracy. From the concentration curve thus obtained the MMD is calculated.

Provided that the separation itself is reliable (which cannot always be taken for granted!), the subsequent transformations are subject to errors:

1. Elution time to elution volume. This requires a highly constant and reproducible flow rate, which means that only high quality pumps should be used.
2. Elution volume to molar mass. The molar mass of a fraction can be obtained either from a calibration or from a molar mass sensitive detector (in addition to the concentration detector).
3. Detector response to polymer concentration. This requires a sufficiently wide linear range, a well defined response of the detector(s) along the entire peak (i.e. for all molar masses within the MMD), and – in the case of copolymers – a second concentration detector.

In the following sections, each step will be referred to in detail. Requirements concerning sample treatment, chromatographic equipment, data acquisition and processing will be discussed and different approaches to the analysis of different types of polymers evaluated.

4 COMPONENTS OF A SIZE-EXCLUSION CHROMATOGRAPHY SYSTEM

As there are considerable differences between SEC and other types of high-performance liquid chromatography (HPLC), the criteria for achieving high performance are somewhat different. In this section, the main components of an SEC system and their influence on the quality of the analysis shall be discussed.

4.1 The Mobile Phase

The mobile phase in SEC must be a good solvent for the polymer in order to avoid nonexclusion effects,[46,47] which will be discussed later on. It is also important to dissolve the sample at appropriate temperature and sufficiently long before injecting it in order to allow the coils to swell in the solvent or to break down aggregates.[48] In some cases, the addition of electrolytes can be required to achieve disaggregation.[49] As some polymers – such as polyolefins – are typically analyzed at high temperatures (140–150 °C) in rather toxic mobile phases (trichlorobenzene, etc.), alternative solvents would be desirable.[50]

An important question concerns preferential solvation: When a polymer is dissolved in a mixed solvent, the composition of the latter within the coils can be different from outside because of different interactions of the polymer with the components of the solvent. When the sample is separated on the column from the zone, where the solvent would elute, a system peak (vacancy peak) appears, which is due to the missing component of the mobile phase. Obviously, the missing amount of solvent in the system peak appears in the peak of the polymer, the area of which is now different from what it would be in absence of preferential solvation. Even though this effect has been known for a long time, it is often neglected by chromatographers, because they consider their mobile phase to be a "pure" solvent, which is, however, generally not the case: even HPLC-grade solvents are seldom more than 99.9% pure, and even then the concentration of the sample is in the same order of magnitude as the impurity. Moreover, solvents may take up moisture from the air, form peroxides, etc. (for example, chloroform typically contains 1% of ethanol or 2-methyl-butene as a stabilizer).

Hence it is important to dissolve the sample in the solvent from the reservoir and not from another bottle. If a solvent peak is observed, this is a strong hint for preferential solvation. Preferential solvation is often neglected, which is acceptable if its contribution does not vary along the MMD. If, however, the end groups of the polymer are considerably different from the repeating units, preferential solvation depends on molar mass, as has been shown recently.[51] A similar effect can be expected in copolymers, if their composition varies with molar mass.

4.2 The Pump

As has already been mentioned, a highly constant flow rate has to be maintained during the entire chromatogram. This is very important in SEC: due to the logarithmic relation between molar mass and elution volume a change of the flow rate of only 0.1% can cause an error in molar mass of up to 10%![52] This requires a pump of very good quality or a compensation of flow rate variations. Unfortunately, most pumps can only reproduce the flow rate to 0.2–0.3%, and this precision can be reduced by leakages in the system or increasing back pressure from the column. Moreover, the check valves as well as the pump seals may limit flow rate precision. In-line filters in the solvent reservoir may prevent particles from coming into the pump heads, which might damage the check valves or the pump seals. One should, however, take into account, that even stainless steel filters may corrode in some solvents. It is trivial that rust particles will have the same effect.

There have been attempts to determine the flow rate by measuring the travelling time of a thermal pulse along a capillary, but generally the precision of these devices is not sufficient. The more efficient – and cheaper – approach is the use of a low molecular internal standard in the MMD calibration and in each chromatogram. The corrected flow rate is obtained from the ratio of the elution times of this standard peak.

The absolute flow rate (in the calibration) can also be obtained by measuring the time to fill a calibrated flask or by weighing the solvent passing the system in a defined time.

It must, however, be said, that the knowledge of the absolute flow rate is not absolutely necessary, as long as flow rate variations are compensated by using an internal standard. It is important that such a correction works well only if the flow rate is sufficiently constant within the entire chromatogram!

4.2.1 Types of Pumps

Basically, one has to distinguish between the following types of pumps, the performance of which may differ

considerably (as well as their suitability for high-performance SEC):

- Syringe pumps. This type of pump works like a large syringe, the plunger of which is actuated by a screw-feed drive (usually by a stepper motor). Therefore it delivers a completely pulseless flow, which is especially important for systems using a viscosity detector.

- Reciprocating pumps. This group comprises almost all commercially available pumps: single piston pumps are cheap, but not well suited for SEC; dual piston pumps can have the pistons arranged parallel or in series. The former pumps deliver a smoother flow, the latter are easier to maintain, because they have only two check valves instead of four. The problem of pulsations can be solved by using a pulse dampener.

4.3 The Column(s)

Unlike in other modes of HPLC, the separation efficiency comes only from the stationary phase, while the mobile phase should have no effect. The whole separation occurs within the volume of the pores, which typically equals approximately 40% of the total column volume. This means that long columns or often sets of several columns are required. Therefore, the right choice of the column(s) for a given polymer is the crucial point.

4.3.1 Commercially Available Columns

Basically, there are different types of SEC columns on the market. The typical column diameters are 7.5–8 mm for analytical columns and 22–25 mm for (semi)preparative columns; usual column lengths are 25, 30, 50, and 60 cm. Recently, narrow bore columns with a diameter of 2–3 mm have been introduced, which save time and solvent.

The packings are based on either porous silica or semirigid (highly crosslinked) organic gels, in most cases copolymers of styrene and divinylbenzene. There are, however, other polymer-based packings available, which can be used in different mobile phases.

In general, silica-based packings are rather rugged, while organic packings have to be handled very carefully, as will be pointed out later on.

4.3.2 Selecting Size-exclusion Chromatography Columns

When selecting columns for a given separation problem in SEC, one may choose from a large number of columns from different producers. Many producers offer columns of the same type, which are comparable and sometimes almost equivalent. In general, the following

considerations may lead to the choice of an appropriate column or column set:[53]

- The separation range should be selected carefully, as it does not make sense to use a column with an exclusion limit of 10^6 when analyzing low molecular products. On the other hand, the high molecular end of the MMD should still be below the exclusion limit.

- The particle size, which determines the plate height, has also to be taken into account. Small particles (typically 5 μm) provide a better resolution (higher plate numbers) and achieve the same separation with a smaller overall column length than larger ones (10 μm), but produce a higher back pressure for a given column length. Shorter columns save time and solvent. On the other hand, 5 μm (or even 3 μm) packings are more sensitive towards contamination by samples containing impurities.

- Small particle size packings can sometimes result in shear degradation of large polymer molecules because the space between particles is very narrow. Particles as large as 20 μm have been recommended for very high-molecular-weight polymers. However, axial dispersion (band spreading) effects are then increased.

- Combinations of packings with a different separation range can be achieved by using either columns with different porosity or mixed-bed columns, which typically provide a better linear calibration than combinations of columns.

- When combining columns to a set, one should prefer two 60 cm columns to four 30 cm columns, because the column ends as well as the connections increase peak broadening.

- The chemical nature of a column packing can be crucial: some packings must not be used in certain mobile phases or at higher temperatures, which are required in SEC of polyolefins. Moreover, non-exclusion effects can also be due to an inadequate stationary phase. There may be considerable differences between packings with similar specification, which are mostly due to the residual emulsifiers used in their production.

4.3.3 Handling Size-exclusion Chromatography Columns

Unlike with other HPLC columns, several precautions have to be taken in the use of SEC columns.

- A column set in SEC should be always run in the same mobile phase. This is not only because a different solvent will require a new calibration, but mainly because a solvent change can reduce column life and performance. If, however, a solvent change is

necessary (for example, to remove contamination from the packing), this should be done step-wise (using mixtures of solvents 1 and 2) and at a low flow rate ($0.5\,ml\,min^{-1}$ maximum). For some solvents, a direct change should be avoided by using an intermediate solvent. When switching back to the first mobile phase, the column set should be recalibrated, anyway.

- SEC columns should never be operated in a backward direction, because this may destroy the column packing immediately. Some columns will survive such a procedure, but one should not take that for granted.

- Care should also be taken in connecting columns or in sample injection: one single air bubble injected onto the column can damage the packing!

- Replacing a clogged inlet frit is a dangerous operation, which can also considerably reduce column performance. When analyzing samples, which may contaminate a column, one should always use a precolumn.

- Pulsations from the pump, which can be due to air bubbles in the solvent line, a leakage of one pump seal, or a damaged or dirty check valve, can also reduce column life.

4.3.4 Enhancing Separation Efficiency by Recycling

In SEC, the separation efficiency of a given type of packing depends on the column length, i.e. on the number of columns, which can, however, only be increased to a certain limit, which depends on the resulting back pressure. Reducing the flow rate is not a good solution, because at very low flow rates (far away from the optimum in the van Deemter equation) the plate height increases considerably.

A simple approach towards enhanced separation efficiency is recycling using the alternate pumping method, as shown in Figure 2 for a set of four columns, which are connected to a six-port–two-position valve.[7]

When the peak of interest is still in column 4, the valve is actuated (thus changing the order of the columns to 3-4-1-2), and the peak will leave column 4 to go back to column 1 instead of entering the detector. The overall column length is now 6 instead of four (1-2-3-4-1-2).

Before the peak leaves column 2, the valve is switched again, and the overall column length is again increased by two to yield 8 columns. This procedure can be repeated, as long as the entire peak fits into one half of the column set. Typically, three to four switches are allowed, thus making a column set of 10 to 12 out of 4 with the back pressure of only four columns.

Obviously, a good separation is only one part of a good analysis. Another crucial point is the detection of the fractionated sample leaving the column.
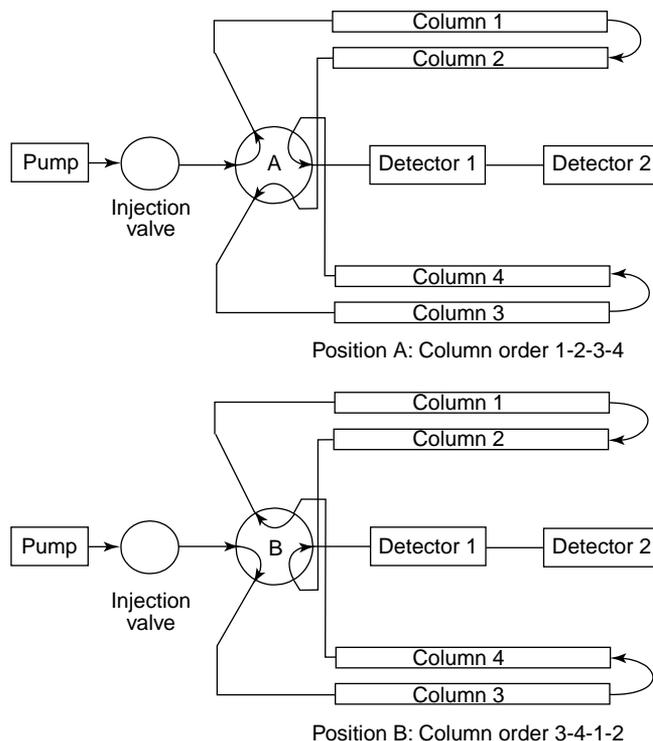
Figure 2 Schematic representation of alternate column recycle SEC.

4.4 Detectors

Among the numerous HPLC detectors, only a limited number can reasonably be applied in SEC. Basically, one has to distinguish the following groups of detectors:

4.4.1 Concentration Sensitive Detectors

It is trivial that at least one concentration sensitive detector has to be used in an SEC system. In the analysis of copolymers, a second concentration sensitive detector is required, the sensitivity of which towards the components of the polymer differs from that of the first detector.

Within the concentration sensitive detectors, one has to distinguish detectors measuring a (bulk) property of the eluate and detectors measuring a property of the solute. Evaporative detectors remove the mobile phase by evaporation prior to detection.

4.4.1.1 Bulk Property Detectors   The most familiar instrument in SEC is the refractive index (RI) detector, which exists in various modifications. Its main advantage is that it can be applied in the analysis of almost any polymer.

The density detector, which has been developed in the group of the author, utilizes the principle of the mechanical oscillator and has been described in several publications.[54–56] It can be used in SEC (as an alternative

to the RI detector) and provides valuable information in the analysis of aliphatic polymers, when combined with the RI detector. This instrument is commercially available from CHROMTECH, Graz, Austria. The measuring cell of such an instrument is an oscillating, U-shaped capillary, the period of which depends on its reduced mass, and thus on the density of its content. Period measurement is performed by counting the periods of a time base (an oven-controlled 10 MHz quartz) during a predetermined number of periods of the measuring cell. The signal of such a detector is thus inherently digital, and its response is integrated over each measuring interval.

4.4.1.2 Solute Property Detectors   The most familiar solute property detector is the ultraviolet (UV) absorption detector, which exists in different modifications and is available from most producers of HPLC instruments. It can be applied to polymers containing groups with double bonds, such as aromatic rings, carbonyl groups, etc., but not to any other polymers. Typical detection wavelengths are in the range of 180–350 nm, which can, however, be utilized only in solvents with a sufficiently low absorbance. Many typical SEC solvents allow detection only above a wavelength of 250 nm.

Infrared (IR) detectors are limited to certain mobile phases that are sufficiently transparent at the detection wavelength.

4.4.1.3 Evaporative Detectors   Evaporative detectors vaporize the mobile phase, and the nonvolatile components of the sample can be detected on-line or off-line.

In the evaporative light scattering detector (ELSD),[12,23,26,57–59] the eluate is nebulized in a stream of pressurized gas and the solvent is evaporated from the droplets. Each droplet containing nonvolatile material forms a particle, which scatters the light of a transversal light beam. The intensity of the scattered light should reflect the concentration of nonvolatile substances in the eluate. There are, however, serious problems in quantification of the signal.[60–63]

It is also possible to use other types of evaporation devices as an interface to a flame ionization detector (FID),[64] a mass spectrometer or a Fourier transform infrared (FTIR) spectrometer.[65–68]

4.4.2 Molar Mass Sensitive Detectors

Molar mass sensitive detectors are very useful in SEC, because they yield the molar mass of each fraction of a polymer peak. As the response of such a detector depends on the concentration as well as the molar mass of the fraction, it has to be combined with a concentration sensitive detector.

Basically, the following types of molar mass sensitive detectors are on the market:

- low angle light scattering (LALS) detectors;[47,69–78]
- multiangle light scattering (MALS) detectors [see references1 21,70,75,77,79–85];
- differential viscometers.[86–90]

The information which can be obtained from such a detector is somewhat different. From light scattering detection, the absolute MMD can be determined directly. With LALS (measuring the scattering intensity at just one angle), no information is obtained on polymer conformation. Using more than one angle, one may also obtain the radius of gyration.

On the other hand, SEC with viscosity detection yields the intrinsic viscosity distribution (IVD). The MMD is, however, determined indirectly (through the universal calibration), and is thus subject to retention errors.

Consequently, it makes sense to combine a light scattering detector with a viscometer detector.[47,69,71–74,76–79] With such a combination, information on branching can be obtained.[89,91–94]

4.4.2.1 Light Scattering Detectors   The scattered light of a laser beam passing the measuring cell is measured at angles different from zero. The (excess) intensity $R(\theta)$ of the scattered light at the angle θ is correlated to the weight average of molar mass M_w of the dissolved macromolecules as shown in Equation (1):

$$\frac{K^*c}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2 c \qquad (1)$$

where c is the concentration of the polymer, A_2 is the second virial coefficient, and $P(\theta)$ describes the scattered light's angular dependence.

K^*, defined in Equation (2), is an optical constant containing Avogadro's number N_A, the wavelength λ_0, RI n_0 of the solvent, and the RI increment dn/dc:

$$K^* = \frac{4\pi^2 n_0^2 (dn/dc)^2}{\lambda_0^4 N_A} \qquad (2)$$

Obviously, there will be problems in copolymer analysis if their composition (and thus the RI increment dn/dc) varies within the MMD. In this case, a second concentration detector will be required, which allows a determination of copolymer composition.

A measurement at more than one angle can provide additional information. In a plot of $K^*c/R(\theta)$ versus $\sin^2(\theta/2)$, M_w can be obtained from the intercept and the radius of gyration from the slope.[70,77,79,81,95]

4.4.2.2 Viscosity Detectors[2,47,69,76,77,86–90,92,93,95–111]
A viscosity detector should yield the intrinsic viscosity $[\eta]$, the so-called limiting viscosity number, given by

Equation (3), which is defined as the limiting value of the ratio of specific viscosity $(\eta_{sp} = (\eta - \eta_0)/\eta_0)$ and concentration c for $c \to 0$:

$$[\eta] = \lim_{c \to 0} \frac{\eta - \eta_0}{\eta_0 c} = \lim_{c \to 0} \frac{\eta_{sp}}{c} \qquad (3)$$

As the concentrations in SEC are typically very low, $[\eta]$ can be approximated by η_{sp}/c. In viscosity detection, one has to determine both the viscosity η of the sample solution as well as the viscosity η_0 of the pure mobile phase, which can be achieved in different ways.

Viscosity measurement in SEC can be performed by measuring the pressure drop across a capillary, which is proportional to the viscosity of the streaming liquid.

Single capillary viscometers (SCVs) using just one capillary and one differential pressure transducer will be strongly affected by the pulsations of a reciprocating pump. Instruments of this type could be used with a syringe pump to eliminate this problem. (This approach is superior to that using additional pulse dampeners.)

A better, but still not perfect approach is the use of two capillaries (C1 and C2) in series, each of which is connected to a differential pressure transducer (DP1 and DP2), and a sufficiently large holdup reservoir (H) in between. The sample viscosity η is thus obtained from the pressure drop across the first capillary, and the solvent viscosity η_0 from the pressure drop across the second capillary. Pulsations are eliminated in this set-up, because they appear in both transducers simultaneously.

A very sophisticated approach is used in another type of differential viscometer, which is commercially available from Viscotek. In this instrument, four capillaries are arranged similar to a Wheatstone bridge.

In Figure 3, both designs are shown schematically. In the Viscotek instrument, a holdup reservoir in front of the reference capillary (C4) ensures that only pure mobile phase flows through the reference capillary, when the peak passes the sample capillary (C3). This design offers several advantages, the most important of which is a higher sensitivity: the detector actually measures the pressure difference ΔP at the differential pressure transducer (DP) between the inlets of the sample capillary and the reference capillary, which have a common outlet, and the overall pressure P at the inlet of the bridge. The specific viscosity $\eta_{sp} = \Delta\eta/\eta$ is thus obtained from $\Delta P/P$.

The main problem in this concept is that the flow in the system must be divided $1:1$ between both arms of the bridge. This shall be achieved by capillaries 1 and 2, which must have a sufficiently high back pressure. Nevertheless, when a peak passes the sample capillary, a slight deviation of the $1:1$ ratio will be observed.
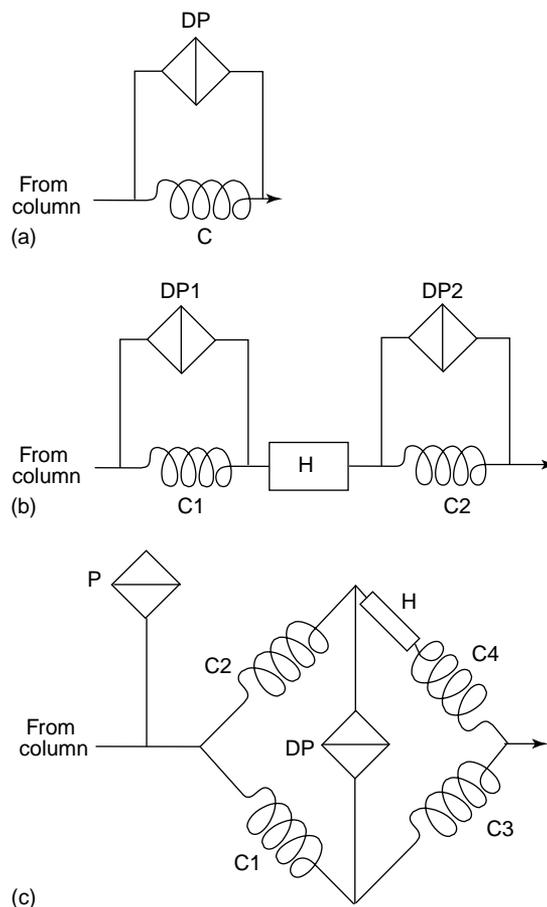
Figure 3 Schematic representation of viscosity detectors: (a) SCV; (b) dual capillary viscometer; (c) Viscotek.

The question of flow rate variations exists, however, also in single or dual capillary viscometers. When the polymer peak passes the measuring capillary, the increasing back pressure leads to a constriction in the system, and thus to a shift of the peak by a weak flow rate fluctuation (Lesec effect).[89,112]

4.5 Data Acquisition and Processing

Software for data acquisition and processing are available from all producers of HPLC equipment. As the requirements of SEC are different from those of other HPLC techniques, standard HPLC software does not fulfill the demands of SEC.

Depending on the nature of samples to be analyzed (whether high or low molecular, homo- or copolymers, etc.) and the equipment used (single or multiple detection), the software should provide special features, which will be discussed in the following sections.

In order to allow calculations not provided by the software, export of data to a spreadsheet or other programs should be possible.

5 THE SEPARATION

In SEC, the separation should be solely governed by size exclusion, which need not always be the case. Aside from an inadequate calibration, nonexclusion effects can cause severe errors. Moreover, low efficiency of the columns or the entire system will cause peak broadening, which also leads to inaccurate results.

5.1 Ideal Size Exclusion

Let us first consider the ideal case, in which size exclusion is governing the separation. As has already been mentioned, the separation in SEC has to be achieved within a volume much smaller than the volume of the column.

It is trivial that no fraction of the sample can be eluted before the interstitial volume V_i (i.e. the volume of the solvent outside the particles of the column packing) has passed the column. This elution volume corresponds to the exclusion limit of the column.

Small molecules, which have access to the entire pore volume V_p, will appear at an elution volume equal to the sum of the interstitial volume V_i and the pore volume V_p.

Molecules of a size between these extremes have access to only a part of the pore volume, hence they will be eluted at an elution volume V_e as shown in Equation (4):

$$V_e = V_i + K_{SEC} V_p \qquad (4)$$

where K_{SEC} is the equilibrium constant of a sample in SEC.

The relation between K and the molar mass of a polymer is determined by a calibration, as will be discussed later on.

5.2 Exclusion versus Nonexclusion Effects

The equilibrium constant of a chromatographic separation can be correlated with thermodynamic parameters. The driving force for a separation at the (absolute) temperature T is the change in Gibbs free energy ΔG, defined in Equation (5), which results from the changes in enthalpy and entropy, ΔH and ΔS, respectively:

$$\Delta G = \Delta H - T \Delta S = -RT \ln K \qquad (5)$$

In ideal SEC, which should be governed solely by entropy, ΔH should equal zero, and the equilibrium constant K_{SEC} should be given by Equation (6):

$$K_{SEC} = e^{\Delta S/R} \qquad (6)$$

where $0 < K_{SEC} < 1$, with $K_{SEC} = 0$ for molecules larger than the largest pore (exclusion limit), $K_{SEC} = 1$ for

small molecules, which have access to the entire pore volume V_p.

According to the theory developed by Casassa, the distribution coefficient of a flexible macromolecule with the root-mean-square end-to-end distance R in a slit-like pore with diameter $2d$ will depend on the ratio of sizes of the macromolecule and the pores. Equation (7) shows:

$$K_{SEC} = 1 - \frac{2}{\sqrt{6\pi}} \frac{R}{d} \qquad (7)$$

In ideal SEC, elution volumes never exceed the void volume $V_0 = V_i + V_p$.

The opposite is true in LAC, where interactions with the stationary phase occur (whether these interactions are adsorption or partition phenomena is not important). If exclusion phenomena can be neglected (which is the case with nonporous stationary phases or in the case of small molecules and stationary phases with large pores), one may write:

$$V_e = V_i + V_p K_{LAC} \qquad (8)$$

The distribution coefficient of LAC is determined by enthalpy:

$$K_{LAC} = e^{-\Delta H/RT} \qquad (9)$$

As ΔH (and thus the probability of being adsorbed) increases with the number of groups capable of being adsorbed, K_{LAC} increases exponentially with the degree of polymerization. Consequently, elution volumes typically exceed the void volume considerably (as $K_{LAC} > 1$).

In practice, both exclusion and interaction must be accounted for in LAC. The equilibrium constant K can thus be divided into contributions from ideal size exclusion and adsorption, as shown in Equation (10):

$$V_e = V_i + V_p K_{SEC} K_{LAC} \qquad (10)$$

It must be mentioned that even in the absence of adsorption or partition phenomena the separation can be determined by an effect other than (ideal) size exclusion. This effect is called secondary exclusion. It originates from (electrostatic) repulsion of polar groups and has nothing to do with molar mass.[46,47,113]

Mori and Nishimura[49] observed polyelectrolyte effects in SEC of poly(methyl methacrylate) (PMMA) and polyamides in hexafluoro-2-propanol. The addition of sodium trifluoroacetate as an electrolyte suppressed these effects by breaking down hydrogen bonding.

Under special conditions (mobile phase composition, temperature) the enthalpic and entropic terms in Equation (5) may compensate each other, and all polymer chains with the same structure will elute at the same volume (regardless of their number of repeating units),

which means that the polymer chain becomes "chromatographically invisible". This situation is utilized in LCCC[96,109,114–130] or liquid chromatography at the critical adsorption point (LCCAP),[131,132] which allows a separation according to other criteria (end groups, branching sites, other blocks in copolymers, etc.).

If a polymer contains different structural units (as is the case in block copolymers or functional oligomers), there may be basically four limiting cases:

1. all components are eluted in ideal exclusion mode;

2. main chain in exclusion mode, (weak) adsorption of end groups;

3. critical adsorption point for main chain, separation of end groups by adsorption;

4. critical adsorption point for main chain, separation of second block by exclusion.

Points 3 and 4 are beyond the scope of this chapter, hence they shall not be discussed in detail. An overview is given in a recent book.[133]

Situation 1 would be the most favorable one, which is, however, rare. In many cases, the calibration functions for different polymer homologous series (with the same repeating unit, but different end groups) can be considerably different. In a systematic investigation, Craven et al.[134,135] have studied the elution behavior of polyoxyethylenes with different end groups (diols, mono- and dimethyl ethers) on a Plgel column in different mobile phases. Considerably different calibration lines were found for the different homologous series in different mobile phases. These differences were explained by combinations of exclusion with partition adsorption effects. In the group of the author similar investigations were performed, which led to very similar results.

5.3 The Problem of Peak Dispersion

When a monodisperse sample is analyzed by chromatography, it will appear as a peak more or less of Gaussian shape and not as a rectangular concentration profile (which it was immediately after injection).

The main reasons for the broadening of peaks are diffusion phenomena in the column, the capillaries, and the detector, which can be minimized, but not completely avoided. Additional broadening can be due to high sample loads, interaction of the sample with the column packing, and an imperfect chromatographic system. Void volumes between the connecting capillaries will lead to a dramatically decreased performance of the system.

It is clear that peak broadening will adversely influence the accuracy of results from SEC, where the peak shape is much more important than the area (which is the interesting parameter in most other HPLC applications).

Basically, a chromatographic peak can be described by the function $F(v)$, the detector response at a given elution volume. It must be mentioned that the actual concentration is not always easily obtained from $F(v)$, as will be discussed later.

This function, shown in Equation (11), results from a convolution of two other functions, $G(v, y)$, which is the shape function of a solute eluting at the mean elution volume y, and $W(y)$, the chromatogram corrected for band spreading:

$$F(v) = \int_0^\infty W(y)G_N(v, y)\,\mathrm{d}y \qquad (11)$$

This equation is well known in SEC as the Tung axial dispersion equation. It is clear that the deconvolution – the calculation of $W(y)$ from $F(y)$ and $G_N(v, y)$ – can be problematic, because $G_N(v, y)$ is not easily obtained. Sometimes the so-called convolution integral, given in Equation (12), is used instead of the Tung equation:

$$F(v) = \int_0^\infty W(y)G_N(v - y)\,\mathrm{d}y \qquad (12)$$

Equation (12) is a limiting case of Equation (11), because it explicitly assumes the same normalized shape function for all solutes present and the same spreading (i.e. the same standard deviation in a Gaussian peak). This assumption may not be valid in the SEC of polymers, particularly if very high molecular weight polymers are being analyzed. Different approaches for correcting chromatograms for peak dispersion have been published, which work more or less well.[38,101,111,136–138] Because of the uncertainties in mathematically correcting for axial dispersion, the preferred approach is to utilize a good separation system, which produces low or negligible peak spreading. With today's high resolution columns other sources of error, such as flow variations, an improper baseline, neglect of the molar mass dependence of response factors, etc., are of much more concern.

Mathematical correction of peak spreading makes sense only when molecular weight averages calculated from the chromatograms of standards similar to those of the unknowns to be analyzed do not agree with those known for the standard and provided that other, more likely, sources of error have been minimized.

6 DETERMINATION OF MOLAR MASS

As has already been mentioned, three transformations have to be performed with the chromatographic raw data.

- The first one – time to volume – can be performed very easily using an internal standard, as has already been pointed out.

- The second one – volume to molar mass – requires either a calibration or the use of a molar mass sensitive detector.

- The third one – detector response to concentration (or mass) – will be discussed later. This step is especially important in SEC of copolymers, polymer blends, and oligomers.

6.1 Size-exclusion Chromatography Calibration

As has already been mentioned, the elution volume of a polymer molecule in SEC must be larger than the interstitial volume (exclusion limit) and smaller than the void volume (total permeation). Between these limits, the elution volume increases with decreasing molar mass. Unless a molar mass sensitive detector is used, one has to determine the molar mass of a fraction eluting at the volume V_e from a calibration, which can be obtained in different ways.

6.1.1 Calibration with Narrow Standards

If a series of standards with a narrow MMD is available, their elution volumes have to be determined to establish a calibration, from which the molar mass for a given elution volume is obtained. In classical SEC, a linear relation between $\log M$ and V_e was assumed, which is, however, only a first approximation, the quality of which depends very strongly on the columns used. The calibration function is quite simple in this case, as shown in Equation (13):

$$\log M = A + BV_e \qquad (13)$$

where A and B are constants, which can be determined very easily by linear regression. For many columns, the calibration line is, however, sigmoidal rather than linear. In most cases, a polynomial fit can match the experimental points much better, as Equation (14) shows:

$$\log M = A + BV_e + CV_e^2 + DV_e^3 + EV_e^4 + \cdots \qquad (14)$$

The coefficients A–E in such a relation have to be determined by regression analysis. This feature is provided by many software packages for SEC. The order of the polynomial fit is, however, critical in some cases: if the number of data points (i.e. the number of standards) is too small, a fit of too high an order may produce an erroneous calibration function. A plot of residuals, i.e. a plot of the percent difference in molecular weight provided by the fitted calibration line compared to the experimental data point at a particular retention volume, plotted versus

retention volume is a quick, visual way of evaluating the validity of the fit. The plot reveals whether or not the scatter of data points is random around the fitted line and the magnitude of the difference between the fitted line and the experimental data points.[72]

There can be considerable differences between the calibration lines for different polymers on the same column in the same mobile phase. This is especially important in the analysis of copolymers or polymer blends. Consequently, different molar masses will elute at the same volume when a mixture of two homopolymers is analyzed by SEC. The elution volume of a copolymer should be between the elution volumes of the homopolymers of the same molar mass. If the composition of the copolymer at each point of the peak is known, an approximation will be achieved by interpolation between the calibration lines. The approximation works best for block copolymers.

It must be mentioned that different calibrations for the same polymer will be found on the same column in different mobile phases.

The calibration with narrow standards can be applied to many types of polymers, because appropriate standards have become commercially available for many polymers, and some suppliers provide well characterized standards for speciality polymers.

In the low molecular range, additional data points can be taken from the maxima of oligomer peaks, which are at least partially resolved. If one of these peaks can be identified, this is also possible for the higher oligomers. An extension to even higher molar masses can be achieved by semipreparative separation of oligomers by LAC.[139]

In the analysis of samples for which no narrow MMD standards are available, different approaches have been described in the literature. The most feasible one is the use of molar mass sensitive detectors. Alternatively, mass spectrometric techniques (such as MALDI/TOF/MS) can also be applied in establishing a calibration function.[10,23,140–147]

6.1.2 Calibration with Broad Standards

If a well characterized sample with broad MMD is available, one may use different procedures to establish a calibration fitting these averages. The integral MMD method can be applied if the entire MMD of the standard is known with high accuracy (which is, however, seldom the case). The method may assume that the MMD of the sample can be described by the most probable distribution function, and matches the calibration to this distribution. No assumptions on the shape of the calibration are made; the precision of the method is, however, rather poor at points corresponding to the tails of the distribution.

If only the molar mass averages of the sample are known from independent methods (light scattering or

osmometry), linear calibration methods can be applied. It is clear that with two known parameters only a linear calibration which is defined by two parameters (slope and intercept) can be obtained. However, this method has been expanded to nonlinear calibration curves through the use of more than one different standard. Also, it has been combined with axial dispersion correction theory to provide both a band spreading parameter (i.e. sigma) and a calibration curve.

6.1.3 Universal Calibration

A very elegant approach is based on the fact that in SEC the elution volume V_e of a polymer depends on its hydrodynamic volume, which is proportional to the product of its molar mass M and intrinsic viscosity $[\eta]$.

In a plot of log $(M[\eta])$ versus V_e (on the same column), identical calibration lines should be found for two polymers (1 and 2), which can be considered as universal calibration,[148] as shown in Equation (15):

$$M_1[\eta_1] = M_2[\eta_2] \qquad (15)$$

The intrinsic viscosity is a function of molar mass, which is described by the Mark–Houwink relationship, where K and a are constants for a given polymer in a given solvent (at a given temperature), as shown in Equation (16):

$$[\eta] = KM^a \qquad (16)$$

Combination of these equations yields Equation (17):

$$K_1 M_1^{a_1+1} = K_2 M_2^{a_2+1} \qquad (17)$$

If a column has been calibrated with polymer 1 (e.g. PS), the calibration line for another polymer (2) can be calculated, provided that the constants K and a are known for both polymers with sufficient accuracy, as shown in Equation (18):

$$\ln M_2 = \frac{1}{1+a_2} \ln \frac{K_1}{K_2} + \frac{1+a_1}{1+a_2} \ln M_1 \qquad (18)$$

The concept of the universal calibration would provide an appropriate calibration also for polymers for which no narrow standards exist.

For lower molar mass samples the Dondos–Benoit relation,[2,149] shown in Equation (19), is used, which is linear in this region:

$$\frac{1}{[\eta]} = -A_2 + \frac{A_1}{\sqrt{M}} \qquad (19)$$

The main problem is the accuracy of K and a, which is rather limited even in the case of polymers for which a sufficient number of well defined standards exists: there are very high variations in the values reported in literature. If one has to rely on these data, there is the question which set of constants would yield an appropriate calibration.

After all, the expense of buying (even costly) narrow standards would be worthwhile in most cases. If such standards are not available, the method of choice will be the use of molar mass sensitive detectors.

7 QUANTIFICATION IN SIZE-EXCLUSION CHROMATOGRAPHY

Once the first two transformations (time to volume and volume to molar mass) have been performed, there remains the third transformation (detector response to amount of polymer in a fraction), which can also be subject to errors, depending on the nature of the samples. In the following section, the particular problems are referred to with respect to the type of polymer to be analyzed.

7.1 Homopolymers and Oligomers

In SEC of polymers, most chromatographers assume a constant response factor within the entire MMD, which is, however, justified only in the analysis of homopolymers with sufficiently high molar mass.

7.1.1 Molar Mass Dependence of Response Factors

The most frequently used detectors in SEC are the UV and the RI detectors. Recently, we have introduced the density detector, which is useful in the analysis of non-UV absorbing polymers.

The UV detector "sees" UV-absorbing groups in the polymer, which may be the repeating unit, the end groups, or both. Basically, there may be two limiting cases:

- If the repeating unit absorbs at the detection wavelength, the signal reflects the weight concentration of the polymer.
- If the end groups can be detected at a wavelength where the repeating units do no absorb, the signal reflects the number concentration of the polymer (provided that the functionality is known). This can be utilized for determining the number of functional groups in oligomers by derivatization with UV-active reagents (as phenyl isocyanate).[150,151]

RI and density detector measure a property of the entire eluate, that means, they are sensitive towards a specific property of the sample (the RI increment or the apparent specific volume, respectively).

It is a well known fact that specific properties are related to molar mass, as shown in Equation (20):

$$x_i = x_\infty + \frac{K}{M_i} \qquad (20)$$

where x_i is the property of a polymer with molecular weight M_i, x_∞ is the property of a polymer with infinite (or at least very high) molecular weight, and K is a constant reflecting the influence of the end groups. A similar relation holds for the response factors for RI and density detection, as shown in Equation (21):

$$f_i = f_\infty + \frac{K}{M_i} \qquad (21)$$

In a plot of the response factor f_i versus the molecular weight M_i of a polymer homologous series (with the same end groups) one will obtain a straight line with the intercept f_∞ (the response factor of a polymer with very high molecular weight, or the response factor of the repeating unit) and the slope K, which represents the influence of the end groups.[7,152–154]

Different methods can be applied for the determination of f_∞ and K:[155]

- If a sufficient number of monodisperse oligomers is available (as is the case with PEG), linear regression will be the method of choice.

- If at least one sample with very high molecular weight (from which the intercept f_∞ can be obtained) and a polydisperse sample with low molecular weight are available, an iteration procedure can be used to determine K.

Once f_∞ and K are known, the correct response factors for each fraction eluting from an SEC column can be calculated (with the molar mass obtained from the SEC calibration).

Molar mass dependence of response factors – unless compensated – can lead to severe errors, as has been shown in another paper.[7] Ethoxylated fatty alcohols were analyzed using SEC with coupled density and RI detection. While the chromatograms looked quite normal in density detection, the sign of the response for the lower oligomers changed in RI detection: the alkanols and the monoethoxylates appeared as negative peaks, and the diethoxylate was almost invisible.

7.2 Copolymers and Polymer Blends

In the analysis of copolymers, the use of multiple detection is generally inevitable. If the response factors of the detectors for the components of the polymer are sufficiently different, the chemical composition along the MMD can be determined from the detector signals.

Typically, a combination of UV and RI detection is used,[156] but other detector combinations have also been described. If the components of the copolymer have different UV spectra, a diode array detector will be the instrument of choice. One has, however, to keep in mind that nonlinear detector response may also occur with UV detection, as Mori and Suzuki[157] have shown. They analyzed PS and copolymers of styrene with methyl methacrylate by SEC with RI and UV detection (at 254 nm) on PS gels in chloroform as mobile phase, and found that the ratio of UV and RI signals increased at the extreme parts of the MMD. Peak dispersion between the detectors, which might have caused a similar effect, was obviously not, or not alone, responsible for the deviations. In a concentration series of PSs, a nonlinear relation between sample size and peak area was found. Lukyanchikov et al.[158] described similar deviations in the analysis of butadiene–styrene copolymers and PS blends with polybutadiene (PB) and poly(dimethylsiloxane) (PDMS) using SEC with UV and refractometric detectors.

In the case of non-UV absorbing polymers, a combination of RI and density detection yields the desired information on chemical composition.[120,124,154,155,159–161] The ELSD cannot be applied because of its poor linearity and its unclear response to copolymers.

The technique can also be applied to oligomers instead of compensating for the molar mass dependence of detector response: in SEC of fatty alcohol ethoxylates or PEG macromonomers, a combination of density and RI detection can be applied as well and yields consistent results.[7,154,161]

The principle of dual detection is rather simple: when a mass m_i of a copolymer, which contains the weight fractions w_A and w_B $(= 1 - w_A)$ of the monomers A and B, is eluted in the slice i of the peak, it will cause a signal $x_{i,j}$ in the detectors, the magnitude of which depends on the corresponding response factors $f_{j,A}$ and $f_{j,B}$, where j denotes the individual detectors. This is shown in Equation (22):

$$x_{i,j} = m_i(w_A f_{A,j} + w_B f_{B,j}) \qquad (22)$$

The weight fractions w_A and w_B of the monomers can be calculated using Equation (23):

$$\frac{1}{w_A} = 1 - \frac{(x_1/x_2)f_{2,A} - f_{1,A}}{(x_1/x_2)f_{2,B} - f_{1,B}} \qquad (23)$$

Once the weight fractions of the monomers are known, the correct mass of polymer in the slice can be calculated using Equation (24):

$$m_i = \frac{x_i}{w_A(f_{1,A} - f_{1,B}) + f_{1,B}} \qquad (24)$$

and the molecular weight M_C of the copolymer is obtained by interpolation between the calibration lines of the homopolymers, as shown in Equation (25):

$$M_C = M_B + w_A(M_A - M_B) \qquad (25)$$

where M_A and M_B are the molecular weights of the homopolymers, which would elute in this slice.

The interpolation between the calibration lines cannot be applied to mixtures of polymers: If the calibration lines of the homopolymers are different, different molecular weights of the homopolymers will elute at the same volume. The universal calibration is not capable of eliminating the errors originating from the simultaneous elution of two polymer fractions with the same hydrodynamic volume but different composition and molecular weight![154]

As the molar masses of different polymers eluting at the same elution volume are given by the corresponding constants K and a in the Mark–Houwink equation, one may calculate the molar masses of the homopolymers in a polymer blend, which will be eluted in the same interval, using Equation (26):

$$\ln M = \frac{AV_e}{1+a} + \frac{B - \ln K}{1+a} \qquad (26)$$

Basically, in SEC there will always be local polydispersity[162] in each slice of the polymer peak: in the case of homopolymers because of peak spreading, in the case of copolymers and polymer blends because of overlapping chemical composition distribution (CCD) and MMD.[163]

Nevertheless, a discrimination of copolymers and polymer blends is impossible with one-dimensional chromatography! Moreover, the architecture of a copolymer (random, block, graft) has to be taken into account, as Revillon[164] has shown by SEC with RI, UV, and viscosity detection. Intrinsic viscosity varies largely with molar mass according to the type of polymer, its composition, and the nature of its components.

Obviously it is feasible to use a combination of molar mass sensitive detectors, such as a LALS, MALS and viscosity detector with two concentration detectors,[72,163,165] from which the (average) composition for each fraction can be obtained, and thus the amount of polymer in the fraction.[166] When using multiple detection, one has to be aware of errors arising from inaccurate interdetector volume[74,101,108,137,166,167] and peak spreading between the detectors.[133] Bielsa and Meira[136] have studied the influence on instrumental broadening in copolymer analysis with dual-detection SEC, and demonstrated the effect of different corrections. Concentration errors may also influence the reliability of the results.[168] Mourey and Balke[72] have proposed a "systematic approach" for setting up multidetector systems. The approach is needed because, as Mourey and Balke show, in such systems, multiple sources of error are present and often the same error can originate from two different sources. The approach emphasizes the idea of ensuring that each detector alone is functioning correctly by comparing results calculated using only data from that detector with the values known for a standard before using detectors in combination. It also employs a superposition of calibration curves obtained from narrow standards and from molecular weight sensitive detectors to determine the effective volume of tubing between detectors (the effective "inter-detector volume"). This method works very well for broad molelcular weight distribution polymers but not for those with a narrow molecular weight distribution. The configuration of the detector system (whether series or parallel) was not important for broad molecular weight distribution results. It has recently been found that the inter-detector volume as measured from the difference in peak retention volumes of narrow molecular weight distribution standards from one detector to another varied with molecular weight when the detectors were in the parallel configuration and the differential viscometer (DV) was one of the detectors.[169,170] In the series configuration no such dependence was observed. This could partly account for difficulties in analyzing narrow molecular weight distribution polymers in parallel configuration systems and may be due to flow rate variation in different branches of the parallel configuration during elution of a sample.

8 COMPARISON WITH OTHER TECHNIQUES

As the analysis of polymers is a difficult task, different techniques can be applied, some of which yield similar information, while others are rather complementary to SEC.[133,171]

In oligomer analysis, SEC competes with LAC and MALDI/TOF/MS: all three techniques can be applied to determine the MMD and yield comparable results.[172]

8.1 Other Types of Chromatography

Capillary SFC and capillary high-temperature gas chromatography (HTGC) can be applied for the quantitative characterization of nonionic alcohol ethoxylate surfactants[173–176] and other oligomers.[177,178] SFC is also very useful in the analysis of carbohydrates[179] and glycerides,[180] etc.

LAC can be performed in isocratic or gradient mode. While isocratic separations[139,172,181–184] are typically limited to oligomers with a narrow MMD, gradient LAC allows also a separation of higher molar mass samples.

In some cases, chromatograms with fully resolved peaks can be obtained. PEGs can be separated on normal or reversed-phase packings,[185–188] while the separation of surfactants according to their degree of ethoxylation is only possible on normal phases.[189–193] Under similar conditions, polyesters,[194,195] PS[195–197] and other polymers can also be separated according to their degree of polymerization.

On the other hand, LAC is a technique complementary to SEC, which can be used to separate copolymers or polymer blends according to their chemical composition.[61,171,194,198–202]

Gradient elution does not necessarily mean a gradient of solvent composition: recently, temperature gradients have successfully been applied in a new technique called temperature gradient interaction chromatography (TGIC).[203,204]

LCCC allows a separation according to groups (or blocks) different from the polymer chain, which is chromatographically invisible under these special conditions. This technique is highly important in two-dimensional separations, hence it will be discussed there.

TREF can be employed to separate according to quite different criteria: the fractionation process depends on melting temperature, melting enthalpy, average crystallinity, average crystallizable sequence length, and polymer–solvent interaction parameter.[205] It is very useful in the analysis of polyolefins.[42,206] Additional information is obtained by coupling TREF with NMR spectroscopy.[206]

Field flow fractionation in various modifications can also be applied. It has been shown that the results obtained for block copolymers – poly(styrene-*b*-*p*-methoxystyrene-*b*-styrene), poly(styrene-*b*-*p*-methylstyrene-*b*-styrene) and poly(styrene-*b*-*p*-cyanostyrene) – using thermal field-flow fractionation (ThFFF), SEC and light scattering were in satisfactory agreement. ThFFF can also be used to determine the thermal-diffusion coefficients for polydisperse polymers and microgels.[84]

Capillary electrophoresis (CE)[207] can be applied in the separation of PEGs and ethoxylated surfactants.[208] Samples containing no charged group have to be derivatized prior to CE analysis with phthalic anhydride[209–211] or 1,2,4-benzenetricarboxylic anhydride[212] to impart charge and detectability on the neutral polymer.

8.2 Mass Spectroscopy

In the analysis of oligomers (such as nonionic surfactants), fast atom bombardment (FAB), time-of-flight secondary ion mass spectrometry, MALDI, electrospray ionization, and field desorption can be applied.[213] The most frequently used mass spectroscopic technique is MALDI/TOF/MS, which has been applied successfully in the analysis of poly((*R*)-3-hydroxybutanoates),[214] coal-derived liquids[8] and many other oligomers and polymers.

The technique has some considerable advantages. It is rapid, requires very small sample amounts, and its resolution and mass-accuracy are marvellous.

On the other hand, there are serious concerns about the quantitation, for the following reasons:

- Sample preparation and desorption/ionization can introduce serious mass biasing that appears to be due to the characteristics of the MALDI process.[215] There are pronounced effects of solvents, particularly solvent mixtures, used to prepare polymer, matrix, and cationization reagent solutions, on MALDI analysis:[216] solvent mixtures containing a polymer nonsolvent can affect the signal reproducibility and cause errors in average weight measurement. Hence it is important to select a solvent system that will allow matrix crystallization to take place prior to polymer precipitation. If these preconditions are fulfilled, MALDI mass spectrometry can provide accurate molecular weight and molecular weight distribution information for narrow polydispersity polymers.[217]

- Serious problems arise in the analysis of polymers with wide polydispersity: the highest mass molecules in the distribution are not observed unless the more abundant lower mass ions are deflected from reaching the detector.[218]

Polydisperse polymers can be analyzed by a combination of MALDI/TOF/MS with SEC, which can be used to obtain fractions with a narrow MMD.[141,143] Microscale SEC can even be coupled on-line to MALDI/TOF/MS with a robotic interface.[142]

Time-lag focusing MALDI mass spectrometry has been employed to analyse PMMA polymers of industrial relevance.[219] This technique also enables the differentiation of end groups.

9 HYPHENATED TECHNIQUES

The analysis of complex polymers and oligomers is complicated by the fact that there may be several distributions in such samples: MMD, CCD, and type of functionality, eventually also architecture (tacticity, branching, blockiness, etc.). Recently, a combination of SEC with 750 MHz NMR has been successfully applied to determine the MMD and the tacticity of PMMA. The molar mass of the polymer in flowing eluate was determined directly (without a conventional calibration procedure) from the relative intensity of NMR signals due to the end-group and repeating units.[146]

Obviously, a full characterization of such samples is very difficult, if it is possible at all. Anyway, it cannot be achieved by simple analytical techniques.

The goal of a full characterization may be approached in several steps, each of which represents a more or less sufficient approximation and will be subject to particular sources of error, as has already been pointed out in the previous sections.

Concerning the particular case of SEC, the following limitations have to be observed:

- One-dimensional separations with one concentration detector may be applied to homopolymers, where calibration standards are available.
- One-dimensional separations with two concentration detectors may be applied to copolymers, where calibration standards are available for both homopolymers.
- One-dimensional separations with one concentration detector and one molar mass detector may be applied to homopolymers of any type. In the case of copolymers, the chemical composition is required for each molar mass. This can be achieved by a second concentration detector.
- One-dimensional separations with two concentration detectors and one molar mass detector may be applied to copolymers with the same architecture. The determination of molar mass and branching requires, however, one more molar mass detector.
- One-dimensional separations with two concentration detectors and two molar mass detectors (viscometer plus LALS or MALS) may be applied to all copolymers. No discrimination between copolymers and polymer blends is possible even in this case.

Basically, multiple detection always yields only the average composition or molar mass of each fraction: the CCD or type of functionality in addition to the MMD can only be obtained by two-dimensional separations (in some cases, even three or more dimensions would be required, which is, however, not yet possible in practice).

The chromatographic and mass spectroscopic techniques described above (SEC, LAC, LCCC, SFC, field-flow fractionation, and MALDI/TOF/MS), which yield different kinds of information, can be combined in different ways:

- When applied independently, they yield different projections of a three-dimensional surface, which describe complex polymers and oligomers: in the case of copolymers with the axes molar mass, chemical composition, and (weight) fraction (as altitude), in the case of functional oligomers with functionality instead of composition.

- Two-dimensional separations, which allow an independent determination of two distributions, can be achieved by combining different modes of chromatography or by coupling a chromatographic separation to a mass spectrometer (preferably MALDI/TOF/MS).[129]

9.1 Multidimensional Chromatography

The distributions of molar mass and functionality can be determined by orthogonal chromatography.[220,221] This technique was also applied to determine MMD and CCD of poly(ethylene oxide-*b*-propylene oxide)s (with LCCC as the first dimension and SEC or SFC as the second one).[116] The application of SEC and nonexclusion liquid chromatography in the characterization of styrene copolymers was described by Mori.[222] Nonexclusion liquid chromatography for polymer separation can be divided into five separation techniques: adsorption, precipitation (solubility), normal and reversed phases, orthogonal, and adsorption at a critical point.[223]

Methyl methacrylate–methacrylic acid copolymers were analyzed by a combination of normal-phase LAC with gradient elution and SEC.[224]

Random copolymers of *N*-vinylpyrrolidone and 2-methyl-5-vinylpyridine were analyzed by SEC–reversed-phase LAC.[105]

A quantitatively accurate mapping of fatty alcohol ethoxylates can be achieved by a combination of LCCC and SEC with coupled density and RI detection in both dimensions.[225] Alternatively, normal-phase LAC may be used as the second dimension.[226]

On-line coupling of SEC, normal-phase liquid chromatography, and gas chromatography was applied in the characterization of complex hydrocarbon mixtures.[227]

Cross-fractionation of a PS sample blended with a PB, and of butadiene– and styrene–methylmethacrylate copolymers by combining SEC with ThFFF has been described.[228]

PS–poly(ethylene oxide) blends and copolymers were analyzed with respect to CCD and MMD using two-dimensional SEC/ThFFF.[229]

A two-dimensional separation of peptides by SEC/reversed-phase liquid chromatography coupled to mass spectrometry has been described recently.[15]

SEC has also been coupled to anion-exchange chromatography in the analysis of polysaccharides and oligosaccharides.[230]

Coupling of full adsorption–desorption and SEC has been applied to the separation and molecular characterization of polymer blends.[231–234]

9.2 Combination of Size-exclusion Chromatography with Mass Spectroscopy

As has already been pointed out, MALDI/TOF/MS can only be applied to polymers with a narrow MMD. Polydisperse polymers can be analyzed with good accuracy by an SEC fractionation (which yields narrow MMD fractions) prior to mass spectroscopy.[141,143] On the other hand, MALDI/TOF/MS is an excellent tool for establishing SEC calibration functions.[145,147,235] In LCCC of oligomers, it yields information on the type of the functionality as well as on the quality of the chromatographic separation.[129,221]

10 SUMMARY

The potential of SEC in polymer characterization is very high, especially when this technique is combined with other modes (LAC, LCCC, SFC) or with mass spectrometric techniques, such as MALDI/TOF/MS.

Multiple detection is in most cases inevitable: combinations of different concentration detectors provide information on copolymer composition, and with molar mass sensitive detectors one may avoid errors with inadequate calibrations.

For complex polymers (with distributions in molar mass, chemical composition, functionality, etc.) one-dimensional techniques can, however, only provide part of the desired information. For these samples, multidimensional separations will be required. In most cases, one of the dimensions will be SEC, while the other(s) could be (gradient) LAC or LCCC.

ABBREVIATIONS AND ACRONYMS

CCD	Chemical Composition Distribution
CE	Capillary Electrophoresis
DV	Differential Viscometer
ELSD	Evaporative Light Scattering Detector
FAB	Fast Atom Bombardment
FID	Flame Ionization Detector
FTIR	Fourier Transform Infrared
GFC	Gel Filtration Chromatography
GPC	Gel Permeation Chromatography
HPLC	High-performance Liquid Chromatography
HTGC	High-temperature Gas Chromatography
IR	Infrared
IVD	Intrinsic Viscosity Distribution
LAC	Liquid Adsorption Chromatography
LALS	Low Angle Light Scattering
LCCAP	Liquid Chromatography at the Critical Adsorption Point
LCCC	Liquid Chromatography Under Critical Conditions
MALDI/TOF/MS	Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass Spectroscopy
MALS	Multiangle Light Scattering
MMD	Molar Mass Distribution
PB	Polybutadiene
PDMS	Poly(dimethylsiloxane)
PEG	Poly(ethylene Glycol)
PMMA	Poly(methyl Methacrylate)
PS	Polystyrene
RI	Refractive Index
SCV	Single Capillary Viscometer
SEC	Size-exclusion Chromatography
SFC	Supercritical Fluid Chromatography
TGIC	Temperature Gradient Interaction Chromatography
ThFFF	Thermal Field-flow Fractionation
TREF	Temperature Rising Elution Fractionation
UV	Ultraviolet

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