Characterization of Polymers by Liquid Chromatography◆

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Summary: Liquid chromatography (LC) is well established for the comprehensive characterization of complex macromolecules with multiple distributions. Hyphenated chromatographic methods in their various forms are currently one of the most promising and powerful methods for the fractionation and characterization of complex sample mixtures in different property coordinates. Modern detector technologies open up new ways to investigate various properties with high sensitivity even in the low concentration ranges used in chromatography. This paper discusses possibilities and applications for the advanced characterization of macromolecules.

Keywords: chromatography; characterization; gel permeation chromatography; molecular weight distribution; structure investigation

1. Introduction

Most polymeric materials are highly complex multi component materials. They are composed of macromolecules not only varying in chain length but also in chemical composition, architecture, and/or constitution:

Linear copolymers, for example, can be distributed in molar mass and chemical composition, depending on the polymerization dynamics and kinetics.

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).

Depending on the composition of the monomer feed and on the polymerization procedure, also other types of heterogeneities may become important[1]:

In the synthesis of tailor-made polymers telechelics or macromonomers are used. These oligomers or polymers usually contain functional groups at the polymer chain end. Depending on the preparation procedure, they can have a different number of functional end groups (mono-, bifunctional etc.).

In addition, polymers can be prepared with different architectures, i.e. they can be branched (star- or comb-like), or they can be cyclic.

The structural complexity of macromolecules can be described using the concept of molecular heterogeneity meaning the different aspects of molar mass distribution (MMD), distribution in chemical composition (CCD), functionality type distribution (FTD), and molecular architecture distribution (MAD).

These distributions 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 macromolecules it is necessary to know the molar mass

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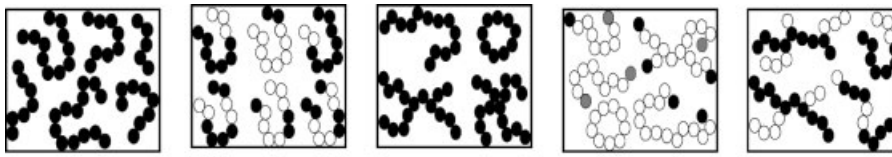

MMD: molar mass distribution

CCD: chemical composition distribution

MAD: molecular architecture distribution

FTD: functional type distribution

STD: structural type distribution

distribution within each other type of heterogeneity.

Analytical Challenges

In contrast to (low molar mass) organic samples, where a set of identical molecules is to be investigated, the analytical task for macromolecules is to determine the distributed property(ies).

Using the traditional methods of polymer analysis, such as infrared spectroscopy (IR) or nuclear magnetic resonance (NMR), one can determine the type and concentration of monomers or functional groups present in the sample. However, these methods do not yield information on how different monomer units or functional groups are distributed in the polymer molecule. On the other hand, these methods in general do not provide molar mass information and the determination of functional end groups lacks sensitivity for long chain molecules because of low concentration. Methods that provide molar mass averages for macromolecules, like static light scattering (LS), osmometry, or viscometry show comparable disadvantages: they measure bulk properties regarding to their detection principle and lack information on distribution information like MMD or CCD.

Chromatographic methods can help to overcome the multi distribution problem of macromolecules since they are able to separate according to distributions present in the investigated sample. The analytical power of chromatographic methods can even be increased by coupling different chromatographic modes in two-dimensional chromatography (a) or by coupling with selective detectors, such as spectroscopic or molar mass-sensitive detectors (b).

a) An efficient approach for characterizing macromolecules with multiple distributions the combination (coupling) of different separation mechanisms. This can be done by coupling two chromatographic systems where each chromatograph must operate in a mode selective towards one type of molecular heterogeneity. This two-dimensional chromatography has been termed ''orthogonal chromatography'' assuming the selectivity of each separation method with respect to one distribution function, e.g. MMD, FTD, or CCD[2]. The first truly automated 2D-chromatography setup for polymer analysis was proposed by Kilz et al. [3], who coupled gradient HPLC and size exclusion chromatography (SEC). The 2D approach is described in more detail in chapter 3.

b) During the last 20 years a number of techniques have been introduced in organic chemistry and applied to polymer analysis, e.g. gas chromatography (GC) combined with spectroscopic detection[4]. GC-MS has been used in polymer analysis [5–12], 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[13,14]. It provides for the analysis of complex polymers with respect to chemical composition. For a number of polymer systems characteristic low molar mass pyrolysis products are obtained, which yield information of the average composition and the ''blockiness'' of the polymer chain. Molar mass information, however, is not available from pyrolysis-GC-MS.

Liquid chromatography (LC) coupled with information rich detectors is also a powerful method: LC has been efficiently coupled to infrared spectroscopy[15–20], to mass spectrometry, and to NMR spectroscopy[21,22].

For molar mass determination molar mass sensitive detectors as on-line light scattering detectors or on-line viscometers have been successfully coupled to SEC during the last 30 years[23–26]. This will be discussed in more detail in chapter 4.

2. Chromatographic Modes of Column Separation

Highly important for polymer analysis are the different techniques of liquid chromatography: Using size exclusion chromatography (SEC), liquid adsorption chromatography (LAC), or liquid chromatography at the critical point of adsorption (LC-CC), polymers can be fractionated with respect to different aspects of molecular heterogeneity, including molar mass, functionality, and chemical composition.

Any chromatographic process relates to the distribution of the analyte in the stationary and mobile phase. The basic principle of chromatography separations can therefore be described by thermodynamics using the distribution coefficient K:

$$K = a_s/a_m = \exp(-\Delta G/RT) \quad (1)$$

a is the activity (concentration) of the molecule in the stationary phase (index s) and the mobile phase (index m)

ΔG is the change in free energy between the species in the stationary phase and the mobile phase

Since $\Delta G = \Delta H - T\,\Delta S$, entropic and/or enthalpic interactions may be influencing the separation process. Therefore two ideal modes of chromatography can be derived

1) size exclusion (SEC) and
2) interaction (HPLC).

1) In ideal SEC separations the enthalpic contribution to the free energy vanishes, when we assume no energetic interaction between analyte and stationary phase:

$$K_{SEC} = \exp(\Delta S/R), \quad 0 < K_{SEC} \leq 1, \quad \Delta H \ll T\Delta S \quad (2)$$

ΔS is the entropy loss when a molecule enters the pore of the stationary phase

2) In the case of ideal HPLC (interaction with stationary phase), the retention can be described by the enthalpic term alone:

$$K_{HPLC} = \exp(-\Delta H/RT), \quad K_{HPLC} \geq 1, \quad T\Delta S \ll \Delta H \quad (3)$$

ΔH is the enthalpy change when a molecule interacts with stationary phase

Another mode of chromatographic behavior exists, if enthalpic and entropic contributions balance out, i.e. when the change in free energy disappears ($\Delta G \approx 0$). This third mode is called liquid adsorption chromatography at the critical adsorption point (LC-CC). The polymeric nature of the sample (that is the repeating units) do not contribute to the retention of the species. Only defects (like end groups, comonomers, branching points) contribute to the separation of the molecule. The following figure illustrates this behavior and shows the retention volume dependence on the molar mass for the different modes of chromatography.

Using this basic theory of separation[27], a modification of experimental conditions allows to shift the separation into one of the chromatographic modes. SEC separations require an interaction-free diffusion of the sample molecule in solution into and out of the pore structure of the column packing material. In many cases this can be achieved by adjusting the polarity of the mobile phase and the stationary phase.

In order to obtain a ''pure'' SEC separation, the polarity of stationary phase, eluent and the sample have to be matched. This type of ''magic triangle'' is shown in the figure above; dominance of size separation is only maintained in the center of the triangle

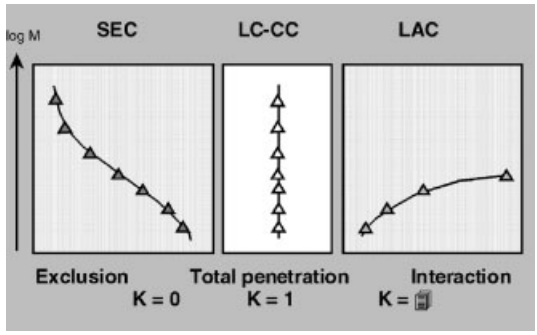

Figure 1.
Different modes of chromatography as seen in the elution order of samples with different molar masses.

where the phase system is balanced. Otherwise specific interactions will occur, which will overlay with the normal GPC elution behavior.

Example:

separation of oligomeric poly(methyl methacrylate) (PMMA) on a non-modified Silica column

In a medium polar eluent (THF) PMMA elutes in size-exclusion mode, because the dipoles of the methyl methacrylate (MMA) repeating units are masked by the dipoles of the THF. Using a non-polar eluent (toluene) on the same column, the separation is governed by adsorption, because the dipoles of the carbonyl group in the PMMA will interact with the dipoles on the surface of the stationary phase.

The separation of PMMA in critical mode of adsorption can be achieved by selecting an appropriate THF/toluene mixture as eluent. In this case all PMMA samples will elute at the same time regardless of their different molar masses. PMMA samples with different end groups will be separated with high selectivity with no overlapping of size separation effects.

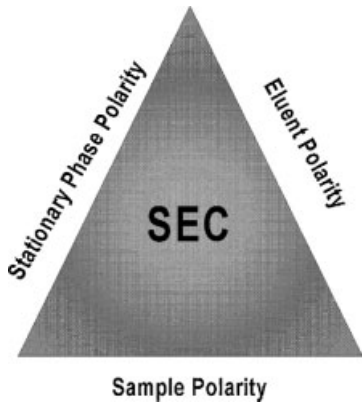

Figure 2.
Balancing polarities of phase system in SEC applications for interaction-free separations.

3. 2-Dimensional Chromatography

Despite the fact that substantial progress has been achieved in recent years in polymer chromatography and column technology[28], the need and use for multi-dimensional separation systems has increased. The main reason for that are the multiple property distributions in more than one parameter (e.g. molar mass *and* chemical composition at the same time). *n* independent properties require *n*-dimensional methods for accurate (independent) characterization of all those parameters. Moreover, the separation efficiency of a single separation method is limited by the efficiency and selectivity of the separation mode, i.e. the plate count of the column and the phase system selected. Adding more columns will not overcome the need to identify more components in a complex sample, due to the limitation of peak capacities. The peak capacity in an isocratic

Table 1.
Comparison of the different LC modes:

Separation technique	Separation governed by	Information content	Potential problems
SEC	- hydrodynamic volume - molecular size in solution diffusion controlled process	molar mass distribution (MMD) chemical composition distribution (CCD)	calibration dilemma specific interactions
LC-CC	- chain inhomogeneity - defect structures - endgroups diffusion and adsorption controlled process	functionality type distribution (FTD) molecular architecture distribution (MAD)	irreversible adsorption determination of critical adsorption point
LAC	- chemical composition - endgroups adsorption controlled process	chemical composition distribution (CCD) functionality type distribution (FTD)	molar mass influence partial adsorption large k′

separation can be described as[29]:

$$n = 1 + \frac{\sqrt{N}}{4} \ln \frac{V_p}{V_0} \tag{4}$$

The corresponding peak capacity in a *n*-dimensional separation is enormously higher due to the fact that each dimension contributes to the total peak capacity as a factor and not as an additive term for single dimension methods:

$$n_{\text{total}} = \Pi\, n_i \cdot \sin^{(i-1)} \vartheta_i \tag{5}$$

where n_{total} represents the total peak capacity, n_i the peak capacity in dimension *i* and ϑ_i is the separation angle between two dimensions. The ''angle'' between dimensions is determined by the independence of the methods; a 90 degree angle is obtained by two methods, which are completely independent of each other and will e.g. separate two properties solely on a single parameter without influencing each other.

Multidimensional chromatography separations can be done in planar systems or coupled-column systems. Examples of planar systems include two-dimensional thin-layer chromatography (TLC) [30,31], where successive one-dimensional TLC experiments are performed at 90° angles with different solvents, and 2D electrophoresis, where gel electrophoresis is run in the first dimension followed by isoelectric focusing in the second dimension[32–34]. Hybrids of these systems where chromatography and electrophoresis are used in each spatial dimension were reported nearly 40 years ago[35].

The main problem using planar methods is the difficulty in detection and collection of fractions among other less critical problems, such as homogeneous preparation of chromatographic media. However, the detection problem exists also for the coupled-column methods, mainly because of fraction dilution by each stage in a multi-dimensional separation system. Another aspect is the adjustment of chromatographic time bases between the different dimensions so that first dimension peaks may be sampled an adequate number of times by the next dimension separation system. This aspect has been studied in detail[36].

In 2D column chromatography systems an aliquot from a column or channel is transferred into the next separation method in a sequential and repetitive manner. Storage of the eluting fraction is typically provided by sampling loops connected to an automated valve. Many variations on this theme exist which use various chromatographic and electrophoretic methods for one of the dimensions. In addition, the simpler ''heart cutting'' mode of operation

takes the eluent from a first dimension peak or a few peaks and manually injects this into another column during the first dimension elution process. A partial compilation of several techniques is given in refs [36–47].

The use of different modes of liquid chromatography facilitates the separation of complex samples selectively with respect to different properties like hydrodynamic volume, molar mass, chemical composition or functionality. Multi-dimensional information on different aspects of molecular heterogeneity can be obtained, when these techniques are used in combination. 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. An overview on different techniques and applications involving combination of SEC and gradient HPLC was published by Glöckner[48].

In SEC mode the separation occurs according to the molecular size of a macromolecule in solution, which is dependent on molar mass, chemical composition, architecture, solvent and temperature. Thus, molecules of the same chain length but different composition may have different hydrodynamic volumes. Since SEC separates according to hydrodynamic volume, SEC in different eluents can separate a copolymer in two diverging directions. This principle of ''orthogonal chromatography'' was suggested by Balke and Patel[49–51]. The authors coupled two SEC instruments using different eluents. As the authors employed mixed mode separation, an independent information on both MMD and CCD could not be obtained from such an experiment.

Since ''orthogonality'' requires that each separation technique is totally selective towards an investigated property, it seems to be more advantageous to use a sequence of methods, in which the first dimension separates according to chemical composition. In this way quantitative information on CCD can be obtained and the resulting fractions eluting from the first dimension are chemically homogeneous. These homogeneous fractions can then be analyzed independently in SEC mode in the second dimension to get the required MMD information. In such cases, SEC separation is strictly separating according to molar mass, and quantitative MMD information can be obtained.

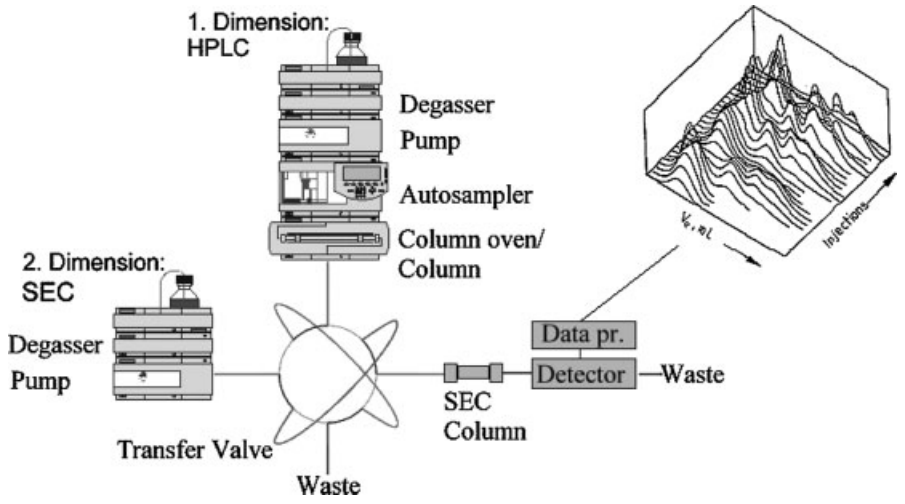

Figure 3.
General experimental design of a 2-dimensional chromatograph.

Experimental Setup of Multi-dimensional Separations

Setting up a 2D chromatographic separation system is easy, if well-known separation methods exist for each dimension. An offline coupled system (a) just requires a fraction collection device and something or someone who reinjects the fractions into the next chromatographic dimension. An on-line 2D system (b) transfers the fractions automatically using a transfer valve[42,52,53]. Figure 3 shows a general setup for an automated 2-dimensional chromatography system.

a) The focal point in 2-dimensional chromatography separations is the transfer of fractions eluting from the first dimension into the second dimension. This can be done in several ways. The simplest approach is collecting fractions from one separation and manual transfer into the second separation system. Obviously, this setup is prone to many errors, labor intense and quite time consuming
b) A more efficient way of fraction transfer can be achieved by using electrically (or pneumatically) actuated valves equipped with two injection loops. Such a setup allows one fraction to be injected from one loop while the next fraction is collected at the same time in the second loop (see Figure 3). Total mass transfer from the first to the second dimension can be guaranteed by proper selection of flow rates in both dimensions[53]. This is a very beneficial situation as compared to heart-cut transfers, since by-products and trace-impurities can be separated even if they are not visible in the first dimension separation.

There are some other important aspects which have to be considered for optimum 2D experiment design:

3.1) Selection of Separation Techniques

A paper by Schure[54] discusses different chromatographic method combinations on the basis of efficiency, sample dilution and detectability. He investigated CE, GC, LC, SEC and FFF in detail, while omitting other methods, which are potential candidates for method hyphenation, e.g. SFC and TREF. Obviously, destructive methods like GC and SFC, which destroy the chromatographic phase system, play a more limited role in multi-dimensional separations as they can only be used in the final separation dimension.

3.2) Sequence of Separation Techniques

The proper sequence of separations methods is important for highest resolution and accurate determination of property distributions. It has been shown that it is best to apply the method with the highest selectivity for one property as the first dimension. This ensures highest purity of eluting fractions being transferred into the subsequent separation. In the case of gradient HPLC and SEC as separation methods, early publications[49–51,55,56] used SEC as the first separation, because it took much longer than a subsequent HPLC analyses. Obviously, this approach is inferior, because the SEC fractions are only monodisperse in hydrodynamic volume, but not in chemical composition, etc. On the other hand, HPLC separations can be fine-tuned using gradients to fractionate only according to a single property, which can then be characterized for molar mass without any bias.

In many cases, interaction chromatography as the first dimension separation method is the best and most adjustable choice. From an experimental point of view, high flexibility is required for the first chromatographic dimension. In general, this is also easier achieved when running the interaction chromatography mode in the first dimension, because

(a) more parameters (mobile phase, mobile phase composition, mobile phase modifiers, stationary phase, temperature etc.) can be used to adjust the separation according to the chemical nature of the sample,
(b) better fine-tuning in interaction chromatography allows for more homogeneous fractions, and

(c) sample load on such columns can be much higher as compared to e.g. SEC columns.

3.3) Detectability and Sensitivity in the Second Dimension

Because of the consecutive dilution of fractions, detectability and sensitivity become important criteria in 2D experiment design. If by-products and trace-impurities have to be determined, only the most sensitive and/or selective detection methods can be employed. Despite several draw-backs evaporative light scattering detectors (ELSD) have been employed due to their high sensitivity for compounds which will not evaporate or sublime under detection conditions. Fluorescence and diode array UV/VIS are also sensitive detection methods, which can pick up samples at nano-gram level. Mass spectrometers have a high potential in this respect too, however, they are currently not developed to a state where they would be generally usable. Only in rare cases refractive index detection, otherwise very popular in SEC, has been used in multi-dimensional separations, because of its low sensitivity and strong dependance on mobile phase composition. As a general rule, the higher the inject band dilution of a given separation method the more sensitive a subsequent detection method has to be. Such type of model calculations can be done easily; refer to the paper by M. Schure[54] for further details.

3.4) Eluent Transfer

The compatibility of mobile phases which are transferred between chromatographic dimensions is an important issue in designing multi-dimensional experiments. Complete miscibility of the mobile phases used in all dimensions is a obvious necessity. Otherwise the separation in the second method is dramatically influenced and the fraction transfer is restricted or completely hindered. In gradient systems, this requirement has to be verified for the total composition range.

In SEC separations the transfer of mixed mobile phases can affect molar mass calibration. In order to get proper molar mass results, the calibration curves have to be measured using the extremes of mobile phase composition and tested for changes in elution behavior and pore-size influence in the SEC column packing. The better the thermodynamic quality of the SEC eluent, the less influence is expected on the SEC calibration, when the transfer of mobile phase from the previous dimension occurs. It has shown advantageous to use the SEC eluent as one component of the mobile phase in the previous dimension to avoid potential interference and mobile phase incompatibility.

3.5) Time Consumption

Time is an important issue when designing multi-dimensional experiments. Setup time itself plays only a minor role, but the time needed for the multi-dimensional separations themselves can be considerable. This is especially true for 2D separations using quantitative mass transfer via dual-loop transfer valves. Heart-cut experiments require much less time and are often sufficient to check out the applicability of the approach. Cutting down on time consumption for multi-dimensional experiments is currently a heavily investigated topic. One approach is the use of recently introduced HighSpeed SEC columns to reduce the analysis time in the SEC dimension by a factor of about 10. This allows 2D experiments with 60 transfer injections completed in about 1 hour without loss of resolution.

Another time requirement in multi-dimensional separations is the time needed for data processing and presentation. With several dozen transfers between dimensions, data reduction and presentation can be very time consuming and has been a real burden for those who performed the first cross-fractionation experiments[49–51,56]. There is a clear need for specialized multi-dimensional software, which does all the data acquisition, fraction transfer, valve switching, data reduction, data consolidation and presentation of results. Currently, there is only one 2-dimensional

chromatography system commercially available[52] which is widely used. A few laboratories use in-house solutions, which are specific to their own chromatography and data capture hardware and specific also to result calculation and report creation.

Most experiments on chromatographic cross-fractionation have been done by combining SEC with gradient HPLC. In early publications SEC was used as the first separation step followed by HPLC as the second dimension in an offline mode with manual fraction transfer. These investigations demonstrated the efficiency of gradient HPLC for separation by chemical composition. Mixtures of random copolymers of styrene and acrylonitrile were separated by Glöckner et al.[56] in THF in SEC mode followed by a gradient HPLC separation using iso-octane/THF as the eluent. Random copolymers of styrene and 2-methoxyethyl methacrylate were separated similarly using iso-octane/methanol in the HPLC mode[56,57]. Graft copolymers of methyl methacrylate onto EPDM rubber were analyzed by Augenstein and Stickler[58]. Mori published the fractionation of block copolymers of styrene and vinyl acetate[59]. In these experiments true molar mass fractionation was not achieved because SEC is used as the first dimension.

From the theoretical point of view, a better copolymer separation setup is the pre-fractionation through HPLC in the first dimension and subsequent analysis of the fractions by SEC. HPLC was found to be rather insensitive towards molar mass effects and yielded very uniform fractions with respect to chemical composition[60,61].

Example:

2D Separation by LAC-SEC Combination[42]

On-line 2D analysis of a styrene-butadiene star polymers with gradient HPLC-SEC coupling.

Experimental:

The 4-arm star polymers based on poly(styrene-b-butadiene) were prepared by anionic polymerization to give samples with well known structure and molar mass control. In a first reaction step, a poly(styrene-b-butadiene) with a reactive chain end at the butadiene was prepared. This precursor reacted with a tetrafunctional terminating agent to give a mixture of linear (of molar mass M), 2-arm (2M), 3-arm (3M) and 4-arm (4M) species. Four samples with varying butadiene content (about 20, 40, 60, 80 %) were prepared in this way. A mixture of these samples was used for the 2D experiment. Accordingly, a complex mixture of 16 components, resulting from the combination of four different butadiene contents and four different molar masses (M, 2M, 3M, 4M) had to be separated with respect to chemical composition and molar mass.

SEC analysis:

SEC separation of this 16-component star-block copolymer revealed four partially resolved peaks. They correspond to the four molar masses of the sample consisting of species with one to four arms. The molar masses are defined by the number of arms and are M-2M-3M-4M. Despite the appropriate resolution, the SEC chromatogram did not give any indication of the very complex chemical structure of the sample.

LAC analysis:

The same sample mixture run in gradient HPLC mode gave poorly resolved peaks, which might suggest different composition,

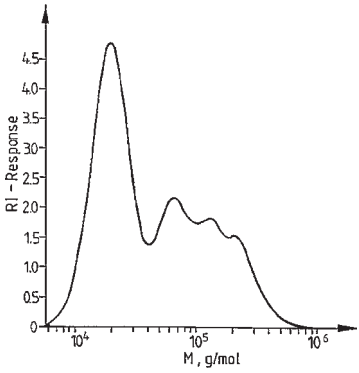

Figure 4.
SEC result of a 4-arm block copolymer.

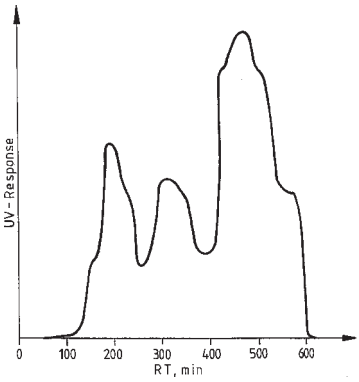

Figure 5.
Gradient LAC separation of 4-arm block copolymer.

but gave no clear indication of different molar mass and topology as shown in Figure 5.

LAC-SEC on-line coupling:
The combination of the two methods in the two-dimensional setup dramatically increased the resolution of the separation system and gave a clear picture of the complex nature of the 16-component sample. A three-dimensional representation of the gradient HPLC-SEC separation shows traces each representing a fraction transferred from HPLC to SEC. It reflects the result of the SEC analysis in the second dimension.

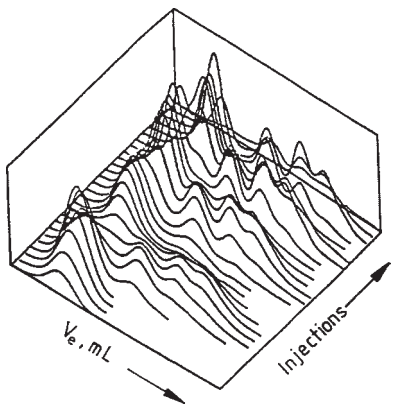

Figure 6.
3D representation of transfer injections of 4-arm block copolymer.

Based on the composition of the sample, a contour map with the coordinates chemical composition and molar mass is expected to show 16 spots, equivalent to the 16 components. Each spot would represent a component which is defined by a single composition and molar mass. The experimental evidence of the improved resolution in the two-dimensional analysis is given in the following figure. This contour plot was calculated from experimental data based on 28 transfer injections.

The contour plot clearly revealed the broad chemical heterogeneity (y-axis, chemical composition) and the wide molar mass distribution (x-axis) of the mixture. The relative concentrations of the components were represented by colors. 16 major peaks were resolved with high selectivity. These correspond directly to the components. For example, peak 1 corresponds to the component with the highest butadiene content (80%) and the lowest molar mass (molar mass 1M) whereas peak 13 relates also to a molecule with 80% butadiene content but a molar mass of 4M. Accordingly, peak 16 is due to the component with the lowest butadiene content and a molar mass of 4M, representing a 4-arm star block copolymer with a styrene-butadiene content of 80:20.

A certain molar mass dependence of the HPLC separation is indicated by a drift of the peaks for components of similar chemical composition (e.g. with peaks 1-5-9-13). This kind of behavior is normal for polymers, because pores in the HPLC stationary phase lead to size-exclusion effects which overlap with the enthalpic interactions at the surface of the stationary phase. Consequently, 2D separations of this type will in general be not orthogonal but skewed, depending on the pore size distribution of the stationary phase and the nature of the sample. The quantitative amount of butadiene in each peak could be determined via an appropriate calibration with samples of known composition. The molar masses could be calculated based on a conventional molar mass calibration of the second dimension.

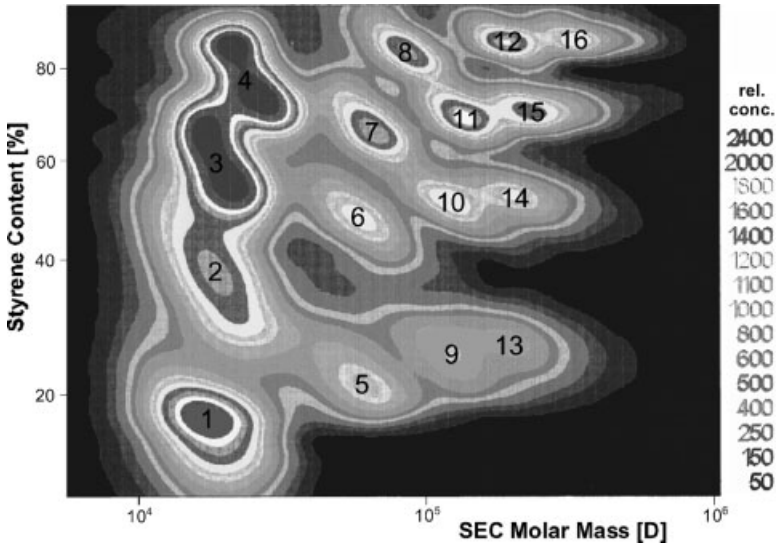

Figure 7.
2 dimensional analysis of 4-arm block copolymer (contour representation), simultaneous CCD and MMD information.

4. Detection Techniques in SEC

Size exclusion chromatography is the premier polymer characterization method for determining molar mass distributions (MMD). For linear homopolymers, condensation polymers and strictly alternating copolymers, there is an unequivocal relationship between elution volume and molar mass. Thus, chemically similar polymer standards of known molar mass can be used for calibration.

However, SEC separates according to hydrodynamic volume and not according to molar mass. For random and block copolymers as well as for branched polymers the simple approach using calibration standards fails, even when only MMD should be determined. The distribution of macromolecules can in general be unambiguously correlated with MMD only within one heterogeneity type. For samples consisting of molecules of different chemical composition (CCD), the distribution obtained represents an average of distributions of molecules having a different composition and, therefore, cannot be attributed to a certain type of macromolecules[62]. The same is valid for branched polymers where a molecular architecture distribution (MAD) is present.

For these polymers advanced methods for the determination of MMD, CCD, and/or MAD can be used where several detectors are attached to the SEC system.

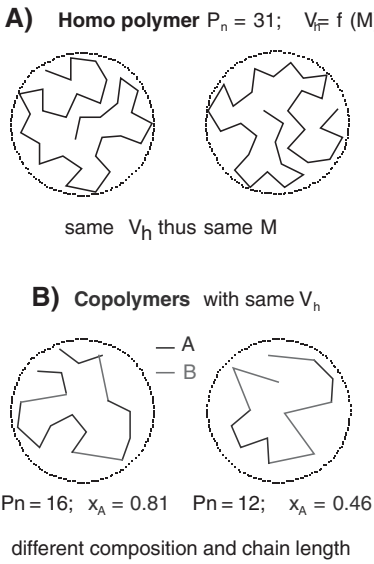

Figure 8.
Dependance of molecular size on chain length (A) and chemical composition (B) in SEC separations.

Nevertheless, it is important to keep in mind that for such setups both parts, separation part and detection part, have their method related requirements. For light scattering (LS) measurements e.g. the refractive index increment (dn/dc) for the polymer/solvent system has to be known. Also, even more important, if there is no or not efficient enough separation due to multiple distributions, only averaged primary information and no distribution information is obtained.

Detection Systems for Liquid Chromatography

In SEC several detection systems are in use. The signal intensity, U, of the on-line detectors can be expressed using the following equation:

$$U_D = K_D \times \sum_i (k_{Sample} \times c_{Sample} \times M^x) \quad (6)$$

where k_{sample} is a sample dependent parameter

for UV detectors: k_{sample} = extinction coefficient

for refractive index (RI) detectors: k_{sample} = refractive index increment dn/dc

The exponent x for the molar mass dependence is related to the kind of detector used:

for RI, UV, ELSD: $X = 0$

for on-line light scattering detectors: $X = 1$

for on-line viscosimeters: X = Mark Houwink coefficient α

for on-line osmometers*: $X = -1$

*not commercially available at the moment

Detectors with an exponent of 0 are concentration detectors since their signal intensity depends only on the injected mass. Detectors with an exponent $\neq 0$ are called molar mass sensitive detectors since their signal intensity is also correlated with the molar mass of the investigated macromolecule.

The signals measured in SEC depend therefore on the detection system used (Figure 9).

The combination of different detectors or detector types however can allow the

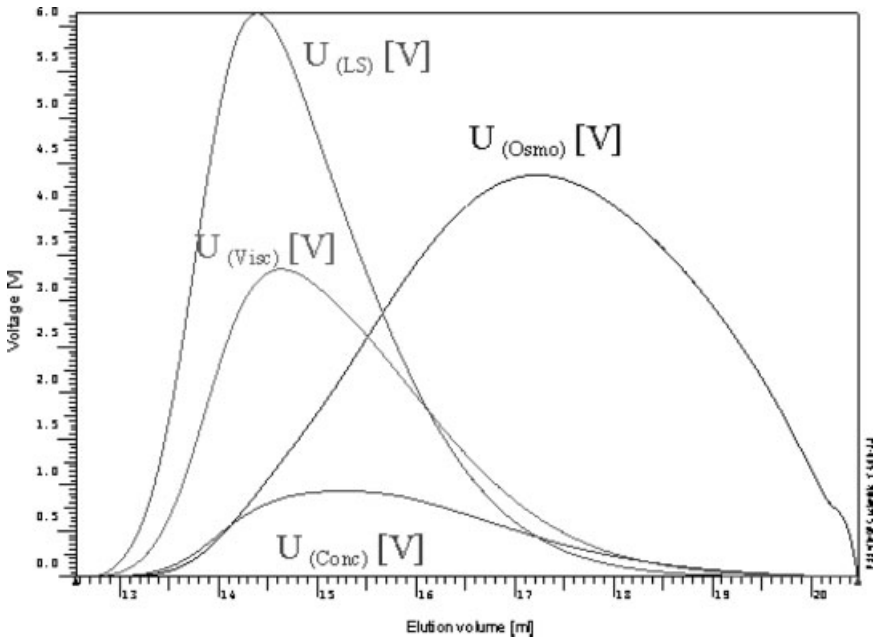

Figure 9.
Detector signals for different detector types.

investigation of more than one distribution, if the separation is performed properly according to molecular size in solution. Three on-line detection methods are used to try to characterize copolymers by SEC with respect to MMD, MAD, and/or CCD:

4.1. Conventional SEC utilizing multiple concentration detection for determination of MMD and CCD
4.2.1 On-line analysis of SEC fractions with a light scattering detector for determination of MMD and MAD
4.2.2. On-line analysis of SEC fractions with a viscometer for determination of MMD and MAD
4.3. Simultaneous separation and identification by LC-FTIR coupling

4.1) Conventional SEC Utilizing Multiple Concentration Detection

In conventional SEC experiments with only one concentration detector it is not possible to determine important polymer properties like copolymer composition or copolymer molar mass. The reason is that the SEC separation is based on hydrodynamic volume rather than the molar mass of the polymer and that molar mass calibration data are only valid for polymers of identical structure. This means that polymer topology (e.g. linear, star-shaped, comb, ring or branched polymers), copolymer composition and chain conformation (isomerization, tacticity, etc.) determine the *apparent* molecular weight. The main problem of copolymer analysis is the calibration of the SEC instrument for copolymers with varying comonomer compositions. But even if the bulk composition is constant, second order chemical heterogeneity has to be taken into account, i.e. composition will vary for a given chain length in general.

Several attempts have been made to solve the calibration dilemma. Some are based on the universal calibration concept which has been extended for copolymers another approach to copolymer calibration is multiple detection. The advantage of multiple detection can be seen in its flexibility and yielding the composition distribution as well as molar masses for the copolymer under investigation[63–65]. This method requires the molar mass calibration and an additional detector response calibration to determine chemical composition at each point of the elution profile. No other kind of information, parameters or special equipment are necessary to do this kind of analysis and calculate compositional drift, bulk composition and copolymer molar mass[64].

In order to characterize the composition of a copolymer of k comonomers the same number of independent detector signals d are necessary in the SEC experiment; e.g. in the case of a binary copolymer two independent concentration detectors (e.g. UV and RI) are required to calculate the composition distribution $w_k(M)$ and the overall (bulk) composition w_k. The detector output U_d of each detector d is the superposition of all individual responses from all comonomers present in the detector cell at a given elution volume V. Therefore,

$$U_d(V) = \sum_d f_{dk} \cdot c_k(V) \tag{7}$$

with f_{dk} being the response factor of comonomer k in detector d and c_k the true concentration of comonomer k in the detector cell at elution volume V. The detector response factors are determined in the usual way by injecting homopolymers for each comonomer of known concentration and correlating that with the area of the corresponding peak. If no homopolymers are available model compounds have been used to estimate the detector response factors.

In the case of a binary copolymer the weight fraction, w_A, of comonomer A is then given by:

$$W_A(V) = \left[1 + \frac{\left[U_1(V) - \frac{f_{1B} \cdot U_2(V)}{f_{2B}}\right]\left[f_{1A} - \frac{f_{1B}}{f_{2B}} \cdot f_{2A}\right]}{\left[U_1(V) - \frac{f_{1A}}{f_{2A}} + U_2(V)\right]\left[f_{1B} - \frac{f_{1A}}{f_{2A}} \cdot f_{2B}\right]}\right]^{-1} \tag{8}$$

Obviously, the sum of all comonomer weight fractions is unity. The accurate

copolymer concentration and the distribution of the comonomers across the chromatogram can be calculated from the apparent chromatogram and the individual comonomer concentrations.

The accuracy of the compositional information is not affected by the polymer architecture. Deviations from the true comonomer ratios are only possible, if the detected property is dependent on the local environment. This is the case if neighbor-group effects will exist. The possibility of electronic interactions causing such deviations is very low, because there are too many chemical bonds between two different monomer units. Other types of interactions especially those which proceed across space (e.g. charge-transfer interactions) may influence composition accuracy.

The major difficulty in the determination of the copolymer molar mass distribution is the fact, that the SEC separation is based on the molecular size of the copolymer chain. Its hydrodynamic radius, however, is dependent on the type of the comonomers incorporated into the macromolecule and their placement (sequence distribution). Consequently, there can be a coelution of species possessing different chain length *and* chemical composition. The influence of different comonomers copolymerized into the macromolecule on the chain size can be measured by the SEC elution of homopolymer standards of this comonomer. Unfortunately, the influence of the comonomer sequence distribution on the hydrodynamic radius cannot be described explicitly by any theory at present. However, there are limiting cases which can be discussed to evaluate the influence of the comonomer placement in a macromolecular chain.

From a SEC point-of-view the most simple copolymer is an alternating copolymer $(AB)_n$, which can be treated exactly like a homopolymer with a repeating unit (AB). The next simple copolymer architecture is a AB block copolymer, where a sequence of comonomer A is followed by a block of B units. The only heterocontact in this chain is the A-B link, which can influence the size of the macromolecule. The A segment and the B segment of the AB block copolymer will hydrodynamically behave like a pure homopolymer of the same chain length. In the case of long A and B segments in the AB block copolymer the only A-B link acts as a defect position and will not change the overall hydrodynamic behavior of the AB block copolymer chain. Consequently, the molar mass of the copolymer chain can be approximated by the molar masses of the respective segments. Similar considerations are true for ABA, ABC and other types of block structures and for comb-shaped copolymers with low side-chain densities.

In such cases the copolymer molar mass M_c can be determined from the interpolation of homopolymer calibration curves $M_k(V)$ and the weight fractions w_k of the comonomers k according to

$$lgM_c(V) = \sum_k w_k(V) \cdot lgM_k(V) \qquad (9)$$

The calculation of copolymer molar mass averages $M_{n,c}$, $M_{w,c}$, etc and copolymer polydispersity D_c is done as in conventional GPC calculations using the copolymer molar mass.

In cases where the number of heterocontacts can no longer be neglected, this simplified reasoning breaks down and copolymer molar masses cannot be measured accurately by SEC alone. This is the case with statistical copolymers, polymers with only short comonomer sequences and high side chain densities. In such cases more powerful and universal methods have to be employed, e.g. the above discussed 2D separations.

Block copolymers are an important class of polymers used in many applications from thermoplastic elastomers to polymer blend compatibilizers. The synthesis is most often done by ionic polymerization, which is both costly and sometimes difficult to control. However, block copolymer properties strongly depend e.g. on the exact chemical composition, block molar mass and block yield. These parameters can be evaluated in

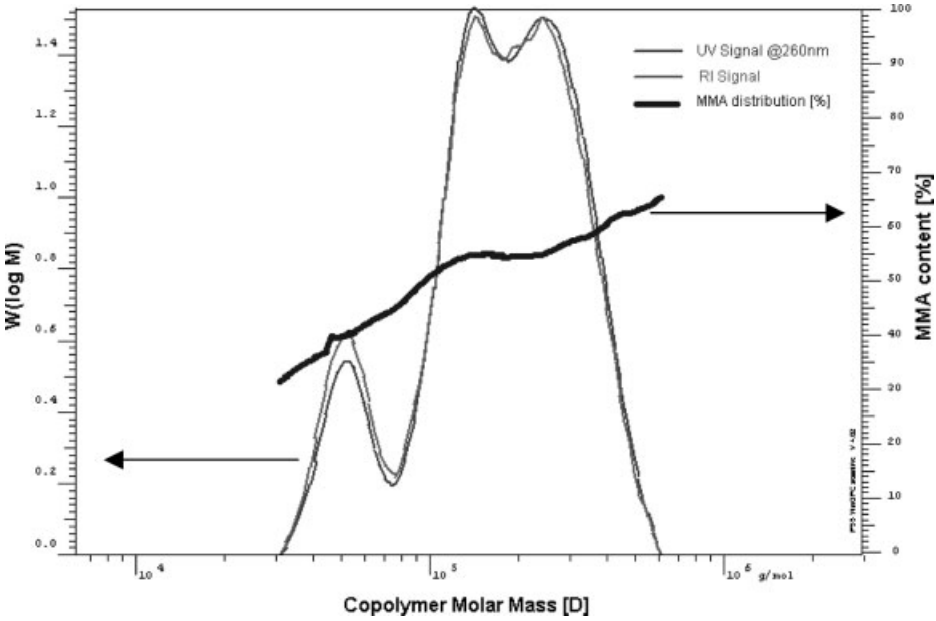

Figure 10.
Simultaneous determination of molar mass and chemical composition distributions of a complex styrene/MMA block copolymer using GPC in combination with RI and UV detection.

a single experiment using copolymer SEC with multiple detection.

Example:

copolymer SEC analysis by multiple detection with UV and RI

Figure 10 shows the measured molar mass distribution of an styrene/MMA block copolymer using RI and UV detection. The RI responds to the styrene and MMA units, whereas the UV tuned to 260 nm predominantly picks up the presence of styrene in the copolymer. After detector calibration the styrene and MMA content in each fraction can be measured. The MMA content distribution (blue solid line) is superimposed to the MWD of the product in Fig. 10. It is obvious that the MMA content is not constant throughout the MWD, but continuously increases with the molar mass. The trimodal MWD itself only shows the presence of three different species. The MMA content information clearly reveals that the copolymerization process was not producing block structure, but that the MMA was added to chains of different styrene molar mass.

4.2) Application of Molar Mass Sensitive Detectors in GPC

To overcome the problems related to classical SEC of polymers, molar mass sensitive detectors were introduced into SEC instruments. Since the response of such detectors depends on both concentration and molar mass, they have to be combined with a concentration detector. It allows the direct measurement of molar mass in each analytical fraction and no longer relies on a calibration curve generated from reference polymer standards. This can be done by using molar mass sensitive detectors based on Rayleigh light scattering or intrinsic viscosity measurements[66,67].

The following types of molar mass sensitive detectors are used frequently:

– low-angle laser light scattering detector (LALLS)
– right-angle laser light scattering detector (RALLS)

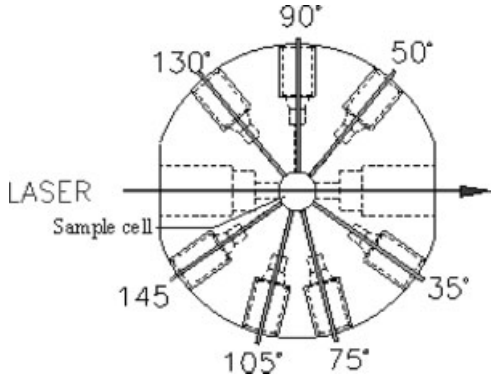

Figure 11.
MALLS detector cell: the scattering intensity is measured simultaneously at 7 different angles.

– multi-angle laser light scattering detector (MALLS)
– differential viscometer

4.2.1. On-line Analysis of SEC Fractions with a Light Scattering Detector

On-line light scattering measurements overcome the calibration dilemma in SEC analyses by direct determination of molar masses independent of the nature of the sample or its architecture. In such setups there is no longer a need for using reference polymer standards as calibrants to relate molecular size to molar mass.

Light scattering detectors measure the scattered light of a laser beam passing through the detector cell. Different kinds of light scattering instruments have been used for SEC analyses. Right angle light scattering instruments with only a single 90° angle are less universally applicable and limited to certain molar mass ranges or sample types. Multi angle light scattering detectors measure the intensity of the scattered light at various observation angles simultaneously.

The (excess) intensity R(q) of the scattered light at an angle θ is related to the weight-average of molar mass M_w:

$$K^*c/R(\theta) = [1/M_w P(\theta)] + 2A_2c \qquad (10)$$

wherein c is the concentration of the polymer, A_2 is the second virial coefficient, and $P(\theta)$ describes the scattered light angular dependence. K^* is the optical constant containing Avogadrós number N_A, the wavelength λ_0, the refractive index n_0 of the solvent, and the refractive index increment dn/dc of the sample:

$$K^* = 4\pi^2 n_0^2 (dn/dc)^2/(\lambda_0^4 N_A) \qquad (11)$$

In a plot of $K^*c/R(\theta)$ versus $\sin^2(\theta/2)$, M can be obtained from the intercept. If an MALLS detector is used the radius of gyration, R_g, can be derived from the slope of the angular dependence of the scattering intensity. Therefore a multi-angle measurement provides additional information on molecular size, structure, conformation and aggregation state.

Example:

SEC-MALLS results for PVB by advanced detection

Figure 12 illustrates the direct molar mass measurement in a SEC experiment by light scattering for a poly(vinyl butyral) sample. For each analytical fraction (elution volume slice) the LS detector signal combined with the concentration detector signal determines the correct slice M and subsequently the molar mass distribution can be determined without any further assumptions. Obviously, this sample contains species of very different molar mass *and* structure as can be further elucidated by molecular size measurement, which is

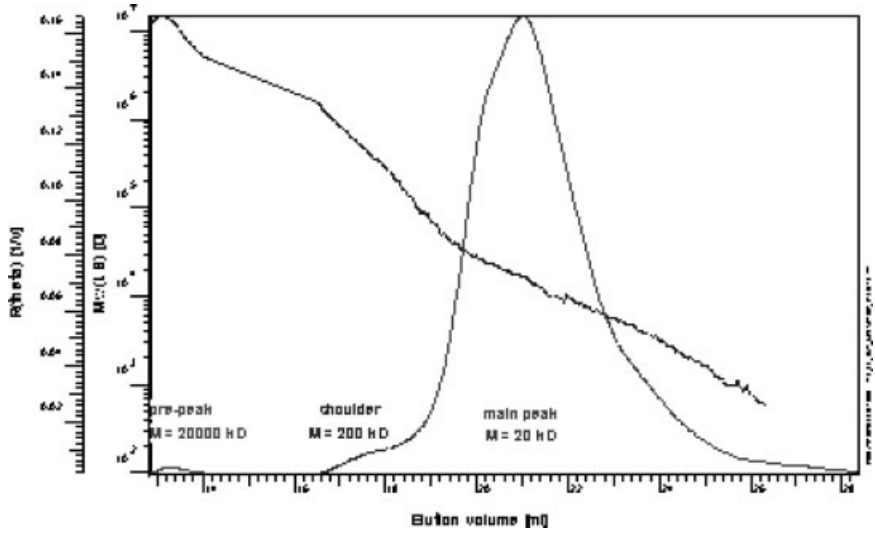

Figure 12.
Direct measurement of molar mass (blue line) of a PVB sample by SEC-MALLS independent of the structural differences in the sample.

possible from the multi-angle light scattering data analysis (cf. Figure 13).

4.2.2 On-line Analysis of SEC Fractions with a Viscometer

Another very useful approach to molar mass information of complex polymers is the coupling of SEC to a viscosity detector. The viscosity of a polymer solution is closely related to the molar mass (and architecture) of the polymer molecules. The product of polymer intrinsic viscosity $[\eta]$ and molar mass M is proportional to the size of the polymer molecule (the hydrodynamic volume). Based on the Einstein-

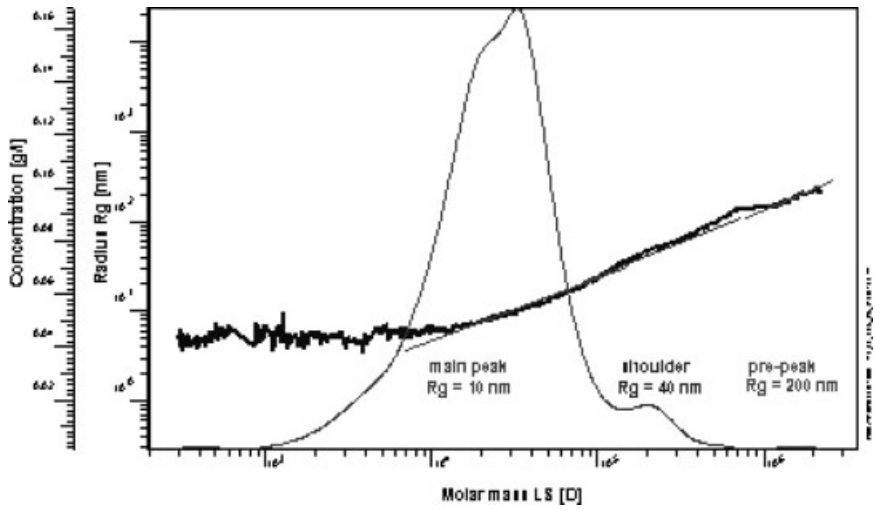

Figure 13.
Structure analysis by SEC coupled with multi-angle light scattering by direct measurement of molar mass and size (Rg).

Stokes theory the molar mass of an unknown sample can be determined directly in a SEC experiment from the known molar mass of a polymer standard and their (on-line measured) intrinsic viscosities independent on sample type or architecture.

$$M_{unknown} = \frac{M_{molarmasss\,\tan dard} \cdot [\eta]_{molarmasss\,\tan dard}}{[\eta]_{unknown}} \quad (12)$$

This behavior is generally referred to as "universal calibration"[68]. In conventional calibrations the logarithm of molar mass is plotted versus elution volume; different samples yield different calibration curves. In a universal calibration the logarithm of molar mass times intrinsic viscosity is plotted versus elution volume. In this plot the calibration curves of very different samples all fall on a single line (so-called universal calibration curve).

Viscosity measurements in SEC can be performed by measuring the pressure drop across a capillary, which is proportional to the viscosity η of the flowing liquid (the viscosity of the pure mobile phase is denoted as η_0). The relevant parameter, intrinsic viscosity $[\eta]$, is defined as the limiting value of the ratio of specific viscosity ($\eta_{sp} = (\eta - \eta_0)/\eta_0$) and vanishing concentration c:

$$[\eta] = \lim(\eta - \eta_0)/\eta_0 c = \lim \eta_{sp}/c \quad \text{for } c \to 0 \quad (13)$$

Thus, the concept of universal calibration provides an appropriate calibration also for polymers for which no calibration standards exist.

Various experimental designs of on-line viscometers have been investigated since the late 60-ies. The most useful viscometry detection technique is based on a balanced or non-balanced 4 capillary bridge design[68]. Signal artifacts and sometimes strong dependence on flow rate changes make other experimental configurations less attractive for general use.

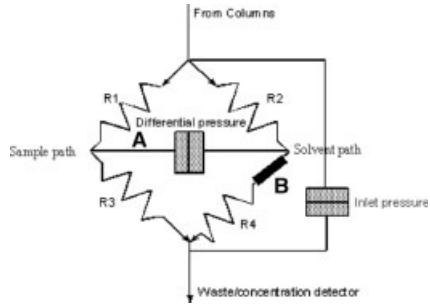

Figure 14.
Symmetric 4 capillary viscometer bridge for measuring the specific viscosity.

Triple Detection

Due to the limitations encountered with SEC-RALLS and SEC-viscometry, a combination of these techniques has been developed, where three on-line detectors are incorporated into a single SEC system. In addition to the concentration detector, an on-line viscometer and a RALLS instrument are used in the SEC (so-called triple detection). This allows for the absolute molar mass determination for polymers that are very different in chemical composition and molecular conformation. The usefulness of this approach has been demonstrated in a number of applications[69].

4.3) Simultaneous Separation and Identification by LC-FTIR Coupling

As shown above LC is a powerful separation tool to fractionate macromolecules by size. However, with the detectors mentioned above the ability to identify sample constituents like additives, processing agents, residual monomers and solvents, etc is limited. Infrared spectroscopy is a perfect companion as it is strong in the determination of different structural elements (functional groups, conformations, etc.) as long as they are present in pure state. The combination of LC as a separation technique with FTIR-detection as an identification method allows on-line deformulation of complex compounds and mixtures. Unfortunately, most LC solvents show strong IR absorptions bands. Consequently, useful IR spectra from flow-through cells are obtained in favorable

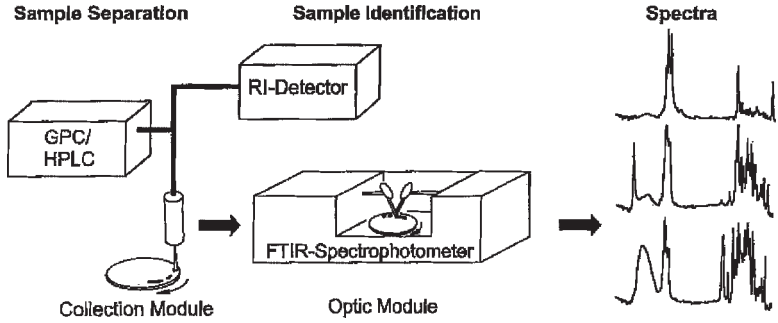

Figure 15.
Experimental setup of HPLC-FTIR detection using an on-line sample collection on a germanium disk and an offline spectroscopic analysis.

LC solvents like methylene chloride, chloroform and tetrachloro methane.

More universal FTIR-detection can be achieved when the mobile phase is removed from the sample prior to spectral analysis. In this case the sample fractions are measured in pure state without interference from solvents[1,70–72]. For this a commercial available LC-FTIR interface can be used. The so called LC-Transform strips volatile mobile phases by nebulizing the mobile phase and spraying it on a rotating Germanium (IR transparent) disk forming a solid time-resolved deposit. In an off-line second step the disk is placed in a the sample compartment of a standard FTIR spectrometer and IR spectra with KBr quality can be recorded at each position in the reflection mode. The instrument design of the interface is shown in Figure 15.

Example:

de-formulation of blister foil by coupling SEC with FTIR detection:

Figure 16 shows the de-formulation of a blister foil which is used in food packaging. In a single GPC-FTIR run the following information could be obtained:

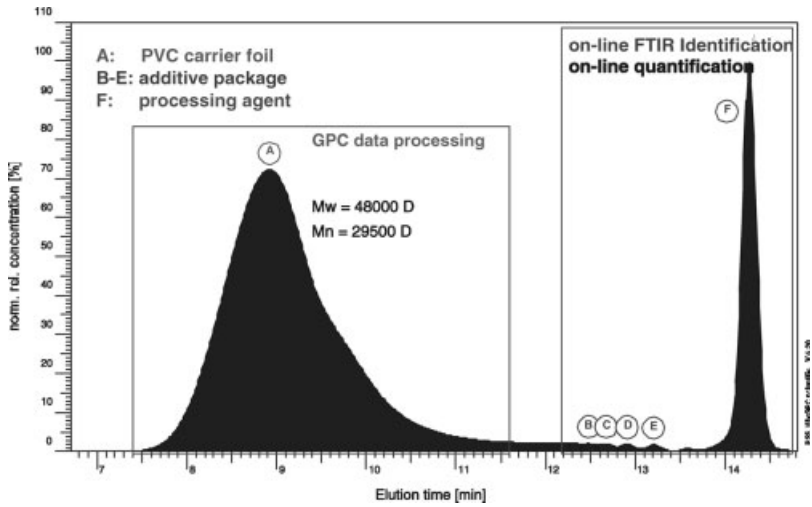

Figure 16.
Simultaneous determination of molar mass, compound identification and quantification of a PVC food packaging foil by coupling a separation method (SEC) with FTIR spectroscopy detection.

- type and nature of the polymer used (peak A: PVC)
- molar masses and molar mass distribution of the polymer (peak A)
- identification of the additives (peaks B – E)
- quantification of all additives in the packaging foil
- identification and quantification of the processing agent (peak F)

Coupled SEC-FTIR becomes an inevitable tool when blends comprising copolymers have to be analyzed. Very frequently components of similar molar masses are used in polymer blends. In these cases resolution of GPC is not sufficient to resolve all component peaks.

Many groups applied this synergistic combination of SEC separation and IR detection for various samples in many application areas; details can be found in[1,70]. Willis and Wheeler demonstrated the determination of the vinyl acetate distribution in ethylene-vinyl acetate copolymers, the analysis of branching in high-density polyethylene, and the analysis of the chemical composition of a jet oil lubricant[71]. Provder et al. showed that in powder coatings all additives were positively identified by SEC-FTIR through comparison of the known spectra[72]. Even biocides could be analyzed in commercial house paints. The comparison of a PS-PMMA blend with a corresponding copolymer gave information on the chemical drift. In the analysis of a competitive modified vinyl polymer sample by SEC/FTIR some of the components of the binder could be identified readily (vinyl chloride, ethyl methacrylate and acrylonitrile), and an epoxidized drying oil additive was detected. The analysis of styrene-butadiene copolymers, the determination of the styrene/butadiene ratio and the microstructure of the butadiene units (cis/trans-, 1,2/1,4-units) was performed by Pasch et al.

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