Separation and characterization of functional polymers
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Separation and Characterization of Functional Polymers

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蒋序林
Separation and Characterization of Functional Polymers

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CHAPTER 1

General Introduction and Outline of the Thesis

1.1 Polymer synthesis

1.1.1 Polymer coatings

A coating is a covering layer, which is deposited on a surface for protection, decoration, or identification. It consists of binders, pigments and various additives. The binder materials, which determine the basic properties in coating applications, are mainly organic (co-)polymers called coating resins. Thermosetting acrylic (TSA) polymers have already been used for many years as coating resins, because they are designed to react chemically after application. This so-called post reaction produces a polymer network. At present, there is a strong tendency in the coatings industry to lower the amount of volatile organic compounds (VOC) in the coating formulation. One way to achieve this is to use low-molecular-weight polymers as binders. By using coating ingredients with low molecular weights, high solid concentrations can be achieved, while maintaining reasonable viscosities during application of, for example, automotive top coats. To be reactive, the low-molecular-weight TSA polymers (or oligomers) must possess functional groups, such as hydroxyl or carboxylic acid groups. Molecules without such functional groups will either disappear from the coating through evaporation or leaching, or they will remain in the coating film as plasticizers, probably jeopardizing the film properties. Molecules with one functional group will terminate cross-linking reactions, leaving so-called “dangling ends” in the coating. This will also seriously impair the mechanical properties of the network [1]. Therefore, a minimum of two reactive groups per polymer molecule should be present to ensure incorporation of all the polymer chains in the network. TSA prepolymer s with many functional groups will result in undesirably brittle coatings with a high cross-link density.

Conventionally, functional (cross-linking) polymers are made by random-copolymerization techniques. However, when such techniques are employed to make low-molecular-weight polymers, a fraction of the resulting molecules will have only one or even no functional groups. We aim to obtain well-defined coatings with optimum properties by making TSA prepolymer s with an exact functionality of two (two functional end-groups in each molecule).

By definition, a telechelic (“far-clawed”) polymer has two functional groups, one at each end of the chain. In a coating application, the molecular weights of the telechelic polymers determine the distance between the cross-linking points. The need to obtain a high impact resistance and bending strength in coatings, without inducing brittleness, has made telechelic polymers a significant area of
research [2]. Well-defined telechelic polymers with specific molecular weights, low polydispersities, and high purity (functionality close to 2) are of high interest, because they can be used as ideal model compounds to study the structure-property relationships of tailor-made coatings. Superior performance of a coating can be anticipated if the cross-linking agent is a telechelic (di-functional) polymer with a (very) narrow molecular-weight distribution. This is the main goal of the overall project.

1.1.2 History of the synthesis of telechelic polymers

One of the polymerization methods most often used to obtain telechelic polymers is polycondensation (step-reaction polymerization). In this case two di-functional monomers react with each other to form telechelic polymers, such as carboxyl- or hydroxyl-terminated polyesters, hydroxyl-terminated polyethers, and carboxyl- or amino-terminated polyamides. End-functional polymers, which result as a direct consequence of the polymerization process, possess, in principle, a perfect telechelic structure. However, to obtain identical end-groups at both ends, while controlling the molecular weight, requires very strict reaction conditions, such as highly pure monomers and very accurate monomer ratios. Intra-molecular condensation reactions lead to the formation of cyclic structures, which behave as non-functional plasticizers [3,4]. In practice it is quite complicated to synthesize ideal telechelic polycondensates.

Atthey published a good review on telechelic polymers synthesized by addition-polymerization and their applications [2]. Among these synthetic techniques, living anionic polymerization, using functional initiators and terminators, is widely recognized as a reliable method to obtain appropriate telechelic polymers with predefined molecular weights and narrow molecular-weight distributions (MWDs). The major drawbacks of this technique are the limited number of suitable monomers and the requirement for rigorous exclusion of terminating agents (air, moisture).

One of the most common addition-polymerization mechanisms is free-radical polymerization. It can be used with a broader range of monomers, without the need for stringent reaction conditions and rigorous purification of the reactants. To obtain polymers with functional end-groups, functional reactants, such as functional initiators and chain-transfer agents, must be used. One of the simplest methods is – in theory – to use a functional initiator and ensure that the only chain-stopping events are termination by combination. In general, this is a highly fictitious situation [5], because of the side reactions inherent to growing radicals, such as termination by disproportionation and chain transfer to solvent molecules or monomers. Bergman claimed that 100% telechelic poly(vinyl neononanoate) with hydroxyl end-groups could be obtained at optimal reaction conditions in a bulk polymerization, using a hydroxyl-containing initiator and allyl alcohol as end-capper/chain-transfer agent [5]. The resulting molecular-weight distributions from free-radical polymerization are usually broad (PDI above 1.5).

The end-groups of telechelic polymers may also result from post-polymerization reactions. Any conceivable reaction may, in principle, be used to modify an existing polymer end-group. Esselborn et al. [6] and Reusmann et al. [7] both reported that hydroxyl telechelic polymethacrylates could be produced by radical polymerization using 2-mercaptoethanol as the chain-transfer agent, with
subsequent transesterification of the end-group. However, experience with derivatization reactions on macromolecules indicates that it is not easy to achieve complete conversion. Eventually by-products should be easily removable from the desired reaction products [2,8].

1.1.3 Controlled radical polymerization

Free-radical polymerization is one of most convenient ways to produce polymers on a large (industrial) scale. It can be conducted in the form of bulk, solution, suspension or emulsion polymerizations. A wide variety of monomers, including functional monomers, can be selected and various ratios can be used to provide many different (co)polymers with diverse properties. However, control over the molecular weight and the polymer architecture is difficult to achieve for free-radical polymerization and the resulting MWD is broad.

To retain the advantages of conventional free-radical polymerization while minimizing the disadvantages, controlled radical polymerization (CRP) techniques have been developed. Many kinds of well-defined architectures with predictable molecular weights have been synthesized by CRP [9,10]. The most important methodologies include nitroxide-mediated radical polymerization (NMRP) [11], atom-transfer radical polymerization (ATRP) [12], and, lately, reversible addition-fragmentation chain-transfer (RAFT) polymerization [13]. All these three CRP techniques can produce telechelic polymers. Hawker et al. [14] reported that hydroxyl telechelic polystyrenes could be obtained by NMRP using hydroxyl alkoxyamines, followed by end-group modification (reducing alkoxyamine to obtain another hydroxyl end-group). For ATRP, a one-pot two-step procedure is necessary to obtain telechelic polymers [15,16,17]. RAFT polymerization potentially has the greatest commercial impact, because it only involves organic substances, it has a high tolerance to impurities, and it can be applied for a wide range of monomers, including acrylic acid [10]. We have selected RAFT polymerization for the preparation of bifunctional telechelic (meth)acrylate polymers with a low polydispersity. Therefore, only RAFT techniques will be discussed in more detail below. The mechanisms and applications of NMRP and ATRP can be found in the literature [18].

1.1.4 RAFT polymerization

The RAFT polymerization technique was first reported in 1998 by Rizzardo’s research group in Australia [13,19]. It was found that simple organic compounds with a thiocarbonylthio moiety were effective in controlling the polymerization. The thiocarbonylthio-compound was called a RAFT agent, the general structure of which is Z-C(S)=S-R. R is the homolytic leaving group of the RAFT agent and Z is an activating group, controlling the efficiency of the RAFT agent towards addition and fragmentation. The dithioesters act as reversible addition-fragmentation chain-transfer agents, allowing the formation of polymers with functional end-groups, which originate from the leaving group [19,20]. Most of the polymers obtained at the end of a RAFT polymerization will contain the leaving group of the RAFT agent, but a (small) fraction will contain a fragment of the radical initiator. A simplified mechanism of the RAFT process is given in scheme 3.1 and it is described in Chapter 3. In RAFT polymerization, common free-radical initiators and monomers can be used in
Chapter 1

the common free-radical environments, for example with or without solvent or emulsion medium. However, it is very important to select a suitable RAFT agent, with a chain-transfer activity that is appropriate for the specific monomer. The electronic properties of the activating (Z) and leaving (R) groups determine the chain-transfer activity of the RAFT agent. The influence and the selection of these two groups are discussed in the literature [20]. Note that most of the RAFT agents described in the literature are not yet commercially available.

1.1.5 Design and synthesis of functional telechelic polymers

At the Eindhoven University of Technology (TU/e) Vincent Lima et al. have attempted to use RAFT polymerization to prepare linear α,ω–functional polymers (commonly known as telechelic polymers) with either hydroxyl or carboxyl end-groups and low polydispersities. For polymethacrylates with hydroxyl end-groups, first a hydroxyl-containing initiator [4,4’-azobis(4-cyanopentanol), ACP] and a hydroxyl-containing RAFT agent [4-cyano-1-hydroxypent-4-yl dithiobenzoate, RAFT-ACP] were employed to synthesize polymeric dithioesters with a single hydroxyl end-group. Then a two-step end-group modification (namely aminolysis of the dithioester, followed by a Michael addition on the resulting thiol) was used in an attempt to obtain a second hydroxyl end-group on each polymer chain. In principle, this process results in hydroxyl telechelic polymers (see scheme 1.1). However, this procedure did not result in polymers with a sufficiently high functionality (close to the ideal value of 2), because the end-group modification steps gave rise to side-reactions.

Telechelic carboxyl-functional polyacrylates can be synthesized in one step, according to the procedure described by Lai et al. [10], in which di-carboxyl trithiocarbonate [S,S’-Bis(α,α’-dimethyl-α”-acetic acid)] is used as the RAFT agent (see Scheme 1.2). Our liquid-chromatographic results demonstrated that nearly ideal functionalities (average number of carboxyl end-groups per molecule up to 1.99) were achieved by one-step RAFT polymerization of poly(n-butyl acrylate) (See Chapter 4). Detailed experimental procedures and a discussion on hydroxyl and carboxyl telechelic polymers were presented by Lima et al. [21].
General Introduction and Outline of the Thesis

Scheme 1.1 Synthesis of hydroxyl-telechelic polymethacrylates. Figure courtesy of V. Lima [21]. RAFT-ACP: 4-cyano-1-hydroxypent-4-yl dithiobenzoate, ACP: 4,4'-azobis(4-cyanopentanol), RT: room temperature, HEA: hydroxyethyl acrylate.

Scheme 1.2 Synthesis of carboxyl-telechelic polyacrylates. Figure courtesy of Vincent Lima [21]. ACVA: 4,4'-azobis(4-cyanovaleric acid).
1.2 Separation and characterization of polymers

1.2.1 General polymer analysis

Synthetic polymers are rarely homogeneous chemical species. Rather, they feature multiple distributions in molecular weight, chemical composition, chain architecture, functionality, and so on [22]. In “simple” homopolymers the individual molecules vary unavoidably in the number of polymer repeat units. In this case, only a molecular-weight distribution (MWD) will be present, i.e. a distribution based on the number of repeat units (monomers) in the polymer. The individual molecules in synthetic polymers may be built up from several different repeat units (copolymer). In this case a chemical-composition distribution (CCD) will be present. The CCD is an indication of the average composition of the polymer as well as the shape (and width) of the distribution. The sequence of monomers in the chain is dependent on the properties of the monomers, on the order of addition to the polymerization mixture, as well as on the polymerization method employed [23]. Examples include (a) random copolymers, where the sequence of the different monomers is governed solely by chance, (b) alternating copolymers, which have alternating repeat units, (c) block copolymers, in which each monomer is concentrated in segments, and (d) graft copolymers, that feature side chains of different monomers attached to the main chain. Various hybrids of these possibilities also occur. For example, completely random copolymers are rare. Some degree of “blockiness” is the rule. The individual molecules in synthetic polymers may also possibly be initiated by different compounds or terminated in different ways, to give rise to various end-groups. When functional groups are present on the polymer, either as end-groups or elsewhere along the backbone, a functionality-type distribution (FTD) will be present. Polymeric chains may be linear, branched to variable extents, or even cyclic. In addition, some polymers exhibit variations in chain (stereo-) regularity or “tacticity”. For a precise characterization of synthetic polymers, all the distributions need to be determined, which is a difficult, if not impossible task. Fortunately, in most cases it is sufficient to determine a limited number of molecular characteristics in order to obtain the information required for a given purpose. Nonetheless, it is still nontrivial if distributions for more than one molecular characteristic coexist.

The traditional analytical techniques, such as IR (infrared absorption spectroscopy), UV (ultraviolet spectroscopy), NMR (nuclear-magnetic-resonance spectroscopy), light scattering and viscometry, can give valuable information on the averages of certain polymeric distributions. However, they do not yield any information on the widths and shapes of the distributions. In theory this is not the case for modern mass-spectrometric techniques, such as MALDI-TOF-MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) and ESI-MS (electrospray-ionization mass spectrometry). However, in practice these techniques provide qualitative, rather than quantitative information on distributions, due to variations in the ionization, separation, and detection efficiencies [24]. Since two polymers with the same average molecular weights (or chemical compositions) can have dramatically different properties, it is clear that information on the distributions is necessary. Ideally, some form of separation is applied that is sensitive to one molecular characteristic only (the effect of other molecular characteristics can be suppressed to a
negligible level) [25,26]. Liquid chromatography (LC) is eminent suitable for separating soluble polymers. A number of different mechanisms (size exclusion, adsorption, partition, etc.) can be exploited [27]. For example, size-exclusion chromatography (SEC) can separate molecules based on their size in solution (hydrodynamic volume) and interactive (adsorption or partition) LC can separate molecules based on their chemical and architectural features. Other separation techniques, such as field-flow fractionation (FFF), hydrodynamic chromatography (HDC), and capillary electrophoresis and electrochromatography, are also used for the characterization of polymers [28,29,30,31]. However, since the focus of this thesis is on HPLC, only HPLC techniques will be discussed in more detail below.

1.2.2 HPLC for the characterization of polymers

1.2.2.1 Separation modes in LC of polymers

In LC, porous column packings are generally used as stationary phase. Polymers can (partly) penetrate into the pores of the column packing and subsequently undergo interactions with the active stationary phase inside the pores. Therefore, both steric exclusion and enthalpic interactions (including adsorption and/or partition) play a role in LC of polymers. The retention volume, \( V_r \), can therefore be expressed as [32]:

\[
V_r = V_i + K_{sec} V_p + K_{int} V_s
\]

(1)

where \( V_i \) is the interstitial volume, \( V_p \) the pore volume, and \( V_s \) the stationary-phase volume. \( K_{sec} \) and \( K_{int} \) represent the chromatographic distribution coefficients for steric exclusion and for interaction, respectively.

In case a strong (thermodynamically good) solvent for the polymer is used as the mobile phase, which also effectively suppresses enthalpic interactions with the stationary phase, \( K_{int} = 0 \) and retention is governed by (entropic) exclusion effects. This chromatographic mode is known as size-exclusion chromatography (SEC). \( K_{sec} \) varies from 0 for large molecules, which are totally excluded from the pores (total exclusion), to 1 for small molecules, which can enter all the pores (total permeation). In SEC, retention of polymers decreases with increasing molecular weight.

In contrast, when the mobile phase is a weak or poor solvent [33], enthalpic (adsorptive) interactions start contributing to the total retention. Interactions with the polymer backbone typically increase exponentially with the number of polymeric repeat units \( n \), due to the fact that more monomeric units are available for interactions with the stationary phase. In practice, a linear dependence between the logarithm of the retention factor, \( \log (k) \), and \( n \) is found (the Martin rule [26]). In this mode, retention of polymers increases with increasing molecular weight. Between these two modes, under certain conditions, entropic exclusion effects and enthalpic adsorption effects are (nearly) compensating each other and retention is (almost) independent of the molecular weight. These conditions, commonly referred to as critical conditions, have both been predicted theoretically [e.g. 34] and demonstrated experimentally [33,35] for various polymer systems. The two separation modes and the transition from SEC to interaction (adsorption) chromatography are illustrated in Fig. 1.1.
1.2.2.2 Size-exclusion chromatography

SEC is also referred to as Gel-Permeation Chromatography (GPC) when chromatographic separations of synthetic organic polymers are concerned or as Gel-Filtration Chromatography (GFC) in case of aqueous solutions of mainly biological macromolecules (biopolymers). It has been the technique of choice for the size-based characterization of macromolecules since its development in the late 1950s [36,37]. The eluent in SEC should be strong enough to prevent adsorption of the solute macromolecules on the stationary phase. The volume of the pore that is effectively accessible is greater for small molecules than for large ones. Therefore, larger molecules have shorter retention times in the pores of the packing than smaller ones and are eluted earlier from the column. The volumes corresponding to total permeation (when the molecule is small enough to enter all of the pores) and total exclusion (when the molecule is too large to enter any of the pores) mark the maximum and minimum accessible volumes of packing material. Macromolecules of a size between these two limits can be characterized according to the extent to which they are excluded. The size-based separation is then usually converted to a molecular-weight-based characterization using linear polymer standards of known molecular weights (determined by absolute methods such as light scattering or osmometry) and low polydispersities (“narrow standards”). Although SEC is the most widely accepted and most commonly used analytical method for the measurement of MWDs and molecular-weight averages of biopolymers and synthetic polymers [38], it is not without failings. As an exclusively size-based separation, it does not reflect molecular distributions other than size. Even for a homopolymer the accuracy of the SEC results is highly dependent on the analyte polymer, on the calibration curve established, and on the detector used. For example, polymeric size in solution is influenced by molecular architecture such as branching. Differences in

Fig. 1.1 The separation modes of chromatography for polymers. (a) Drawn line, exclusion mode; (b) dashed line, critical conditions; (c) dash-dotted line, interaction (adsorption) mode.
chemical composition, functionalities (end-groups), tacticity, *etc.*, will either be overlooked or will lead to errors in the determination of the MWD [39,40].

SEC coupled to more than one detector can provide very useful information on the chemical heterogeneity of synthetic polymers. However, it does not reveal a CCD or FTD. Furthermore, it is impossible to distinguish between polymer blends and copolymers, especially when, in the case of a blend, the molecular weights of the polymer components are similar [32].

1.2.2.3 Interactive liquid chromatography (including gradient LC)

When non-size-based distributions are to be characterized, interactive LC is more useful than SEC. Interactive LC separates analyte molecules based on their interaction (adsorption and partition) with the mobile and stationary phases of the chromatographic system [40]. In contrast to SEC, the polymer is not only separated based on its hydrodynamic volume, but also based on its chemical structure, including functionality. Unlike SEC, interactive LC can be optimized by changing the mobile phase (both the constituents and the composition), as well as the stationary phase. It can also be controlled by temperature, pH, *etc.* For the analysis of oligomers, interactive LC is superior when compared to SEC, with respect to selectivity and peak capacity [4]. The retention of polymers in interactive LC usually increases exponentially with the number of polymeric repeat units. However, a number of exceptions to this rule are described in this thesis. Nevertheless, the strong dependence of retention on the mobile-phase composition implies that in most cases gradient-elution LC is required for polymer separations. By definition, in gradient-elution LC the composition of the mobile phase is varied during the separation, so as to increase the solvent strength gradually or in steps [41]. Gradient LC of polymers was first shown by van der Maaden *et al.* [42]. Teramachi *et al.* reported the first example of a separation of a copolymer according to chemical composition by gradient-elution LC [43]. Much pioneering work in this respect was done by Glöckner [26]. To show the usefulness of gradient LC in the characterization of polymer blends (mixtures) and copolymers, we present some examples from our own work.

An example of a hydroxyl-containing functional polymer used in contemporary coating applications results from the copolymerization of methyl methacrylate (MMA) (or ethyl methacrylate, EMA) and hydroxyethyl methacrylate (HEMA). In order to establish suitable conditions for separating the functional acrylate copolymers of MMA and HEMA, the separation of blends of PMMA and PHEMA was accomplished by different forms of LC using various solvents, columns and detectors. PHEMA does not dissolve in common LC solvents, such as tetrahydrofuran (THF) and acetonitrile. It only dissolves in methanol, dimethyl formamide (DMF), or dimethyl sulphoxide (DMSO). However, neither DMF nor DMSO allows UV or evaporative light-scattering detection (ELSD) to be used and high-molecular-weight PMMA does not dissolve in methanol. Therefore, mixed solvents and mobile-phase gradients are needed to separate blends of PMMA and PHEMA. Fig. 1.2 shows that PHEMA and PMMA standards of different molecular weights exhibited different retention times in reversed-phase gradient-elution LC (In reversed-phase LC, the mobile phase is − by definition – more polar than the stationary phase). The retention time of PHEMA was shorter than the dead time ($t_0$), which indicated that PHEMA was separated in the size-exclusion mode. The retention times of the PMMA standards were longer than $t_0$ and increased with increasing molecular

13
weight, which indicated interaction (adsorption, partitioning, or precipitation and redissolution) between the PMMA samples and either the stationary phase (surface of the column packing) or the mobile phase under the investigated (gradient) conditions. Under the same condition copolymers with different HEMA contents, \textit{i.e.} different numbers of OH groups, showed different retention times (Fig. 1.3). The higher the HEMA content, the shorter the retention time of the copolymer. Thus, retention decreased with increasing polarity of the sample. Therefore, blends of PMMA and PHEMA and copolymers with different HEMA contents can be separated, although there was some confounding of molecular-weight and composition effects under these reversed-phase gradient conditions.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.3}
\caption{Gradient elution of different polymer standards (separate injections). Injected sample (the number referred to the molecular weight): 20 \(\mu\)L of 1 mg/ml solutions of each polymer in Methanol/THF 70/30; Dupont Zorbax-C8 column (250 mm x 4.6 mm i.d.; 5-\(\mu\)m particles; 100-\(\AA\) pore size); Flow rate 1 ml/min; Gradient from 30 to 100\% of solvent B (THF) in solvent A (water/methanol 25/75) in 20 minutes.}
\end{figure}

PHEMA and PMMA standards of different molecular weights can also be separated under normal-phase gradient-LC conditions on a cyano-modified silica column ("CN column"; Fig. 1.4) (In normal-phase LC, the stationary phase is – by definition – more polar than the mobile phase). As seen from Fig. 1.4, the retention times of PMMA standards where shorter than \(t_0\) and decreased with increasing molecular weight, which indicated that PMMA was eluted in the size-exclusion mode under these conditions. When we used a bare-silica column instead of a CN column, the PHEMA sample showed a very long retention and a very low recovery. In comparison with the CN column the PHEMA sample showed a somewhat shorter retention time on a C8 column (same dimensions; same gradient). These observations indicated a certain degree of interaction of the PHEMA sample with the CN column and a strong interaction with the bare-silica column.
Fig. 1.3  Chromatograms for copolymers of EMA and HEMA (100% PEMA refers to 0% HEMA). LC conditions as in Fig. 1.2.

Fig. 1.4  Gradient elution of different polymer standards (separate injections). Injected sample: 20 μL of 1 mg/ml solutions of each polymer in Methanol/THF 70/30 (except sample PHEMA, 3 mg/ml); CN column (250 mm x 4.6 mm i.d.; 5-μm particles; 100-Å pore size); Flow rate 1 ml/min; Gradient from 0 to 100% of solvent B (methanol) in solvent A (THF/toluene 50/50) in 20 minutes.
However, under the conditions of Fig. 1.4 copolymers with up to 20% HEMA could not be separated according to their HEMA content, because they were eluted in the SEC mode (mainly according to their molecular weight). In order to separate the copolymers containing different percentages of HEMA up to 20%, the selectivity of the system towards small numbers of OH groups in the polymer chain should be enhanced. To this end, the weak interaction should be exploited, which the OH-containing polymers exhibit with the column under weak eluent conditions (see Fig. 1.5). It can be seen from Fig. 1.5 that copolymers with different HEMA contents (i.e., different numbers of OH groups) showed different retention times under these normal-phase gradient-LC conditions. The higher the HEMA content, the longer the retention time of the copolymer, i.e. retention increased with increasing polarity of the sample. Thus, blends of PMMA and PHEMA and copolymers with different HEMA contents could be separated under normal-phase gradient-LC conditions. However, because the copolymers also possessed chemical-composition distributions, the obtained peaks were broad.

![Graph showing chromatograms for copolymers of EMA and HEMA](image)

**Fig. 1.5** Chromatograms for the copolymers of EMA and HEMA (100% PEMA refers to 0% HEMA). Gradient 0-25% THF in toluene in ten minutes, then to 50% THF in another 16 min, CN column as in Fig. 1.4.

Note: for the sake of clarity, chromatograms of samples 1.6%-HEMA-copolymer and homopolymer PEMA were moved left 2 min.

Gradient LC is more complex than SEC, since retention and selectivity are governed by more than one mechanism [32]. This also explains why different terms were proposed for the same technique, for example liquid-adsorption chromatography (LAC), high-performance precipitation-liquid-chromatography (HPPLC) and gradient polymer-elution chromatography (GPEC). Chang *et al.* have proposed a different gradient technique for polymer separations, in which the column
temperature is varied instead of the eluent composition [44]. Good separations have been published using this so-called temperature-gradient interaction chromatography (TGIC).

1.2.2.4 Critical liquid chromatography (isocratic LC)

Entelis et al. [45] described the basic principles of the so-called "critical conditions" of LC, at which the influence of the molecular weight of the polymer on retention has been minimized, as shown in Fig. 1.1. This occurs when the free-energy effect associated with the addition of a monomeric unit to the polymer \( \Delta G_{\text{mon}} = \Delta H_{\text{mon}} - T \Delta S_{\text{mon}} \) is zero, i.e. the enthalpic and entropic effects of the addition of a monomer are balanced [46,47]. The critical conditions can also be referred to as "conditions of entropy-enthalpy compensation" (CEEC) [48] or as the "exclusion-adsorption transition point" [49]. When the critical conditions are reached, the chain of monomers will neither be retained on the stationary phase, nor excluded, and so it does not have any influence on retention. Retention under these conditions will only be based on the end-groups or other functional groups on the polymer or on any monomeric units present other than the monomer that is at its critical point. This makes critical LC in principle perfectly suitable for studying CCDs and FTDs [32].

The first experimental verification of such a critical point was published by Tennikov and co-workers [50]. Macko and Hunkeler [48] have recently presented a good review, in which all known LC systems under critical conditions reported during the last 25 years were summarized. They listed more than 180 systems, including many examples of applications (and potential applications) of critical LC. End-group separation by critical LC, in which polymers with functional end-groups are retained much more strongly than non-functional polymers, is becoming increasingly popular. Since most functional end-groups are more polar than the polymer backbone, end-group separations are performed in the normal-phase mode in the majority of cases. Examples were described for polyethers [33,51-62], polyesters [45,63-67], polyamides [68], polybutadienes [45,69,70], oligo-carbonates [71], epoxide resins [72], functionalized PS [49,73,74], and functionalized PMMA [75,76,77].

Critical LC can also be used for the characterisation of polymer blends [47,78-84] and copolymers (random and block copolymers) [49,85-98]. For block copolymers, critical conditions are established for one of the blocks, using homopolymer standards. At these conditions one block is "invisible", while the block to be investigated can be in the SEC mode (in the case of long block lengths) or in the interaction mode (in the case of short block length). Due to the exponential increase of retention with chain length, interactive LC is not feasible for long blocks. Thus, most applications so far have concerned the SEC mode [87,90,94,96,97]. An example of the interaction mode was shown for PEO-PPO block copolymers [99]. Pasch and co-workers presented the characterization of more-complex systems, i.e. star copolymers having three or four arms of different chemical composition, by critical LC [100,101]. Berek et al. demonstrated the application of critical LC for separating polymers according to tacticity [102,103,104]. It was predicted by theory [45] and verified experimentally [45,105,106] that linear and cyclic polymers could be separated from each other at the critical conditions.
The above-mentioned critical conditions specifically refer to isocratic elution. Critical conditions (i.e. molecular-weight independent retention) can also be approached in gradient LC of polymers [26]. Such so-called pseudo-critical conditions can only be achieved when the critical solvent composition is above the limiting composition for solubility of the polymer and when the molecular weight of the polymer is above a certain value [98]. Critical LC under isocratic conditions shows distinct advantages over pseudo-critical gradient LC. In the former case, retention can be genuinely independent of the molecular weight, especially for low-molecular-weight polymers. This renders the assignment of chemical structures, especially functionalities, much less ambiguous. However, critical chromatography remains a challenging technique for polymer analysts and many questions pertain as to the validity of the technique [32,35,96,107,108,109]. There remains some controversy as to whether precise co-elution conditions can indeed be achieved [35,96,108]. The finding of a suitable eluent system is still a matter of trial and error. Exact critical conditions can be hard to find and difficult to maintain, rendering the technique experimentally difficult [35,105,107]. The critical conditions always refer to a specific eluent composition. Sometimes small changes in the composition can jeopardize a critical separation [21]. In addition, the critical conditions can be sensitive to the column packing [35,110], the polymer nature [107,111], the temperature [17,21,96], and even to pressure variations [17,107,24]. Critical conditions do not necessarily provide good functionality-based separations [45]. On a given critical system, the retention and separation of polymers with (for example) different end-groups may or may not be satisfactory. Peak broadening for high-molecular-weight polymers may occur [112], resulting in a loss of resolution [35]. Also, reduced recovery has been observed for high-molecular-weight polymers at critical (or near-critical) conditions [107,22]. As a compromise, experiments are often carried out under near-critical isocratic conditions or pseudo-critical gradient conditions [113], which can give sufficient selectivity without the stringent requirements associated with true critical chromatography.

1.2.2.5 Breakthrough of polymers in interactive LC

Due to solubility limitations, many synthetic polymers only offer a limited choice of solvents for LC. The very strong dependence of polymer retention on the mobile-phase elution strength often necessitates the use of gradient elution. A typical gradient runs from a non-solvent (or a weak eluent) to a strong solvent. Therefore, the solvent in which the sample is dissolved and injected is usually stronger than the mobile phase that surrounds it. This is equally true when the polymers are eluted isocratically [24]. In such cases, a fraction of the polymer has often been observed to elute with the solvent peak [see references in Chapters 6 and 7]. This is the so-called “breakthrough”, “solvent-plug” or “sweep-through” effect. Great care should be taken in selecting the injection solvent for polymers. A strong solvent, yet weak eluent is ideal as the injection solvent in the LC of polymers. See Chapter 6 for a detailed discussion.

1.2.3 Hyphenated systems

LC is merely a separation technique, in which the analytes are fractionated into many (infinitely small) portions. Qualitative and quantitative information can be obtained by coupling the LC
column to a detection device, which monitor(s) a change in the property of the effluent when the analyte elutes. The signal(s) from the detector(s) can be related to the mass or the concentration of the analytes. The most frequently used detectors in LC of polymers are ultraviolet-absorbance (UV), refractive-index (RI) and, more recently, evaporative light-scattering detection (ELSD). In most cases some estimate of the mass or concentration of the eluting compounds can be made based on some assumption and/or using a calibration curve. For example, we commonly neglect the effect of molecular weight or end-groups on the sensitivity for polystyrene using UV or RI detections. However, the response of the ELSD is non-linear with analyte concentration, and a calibration curve is necessary.

When absolute molecular-weight or structural information (including end-groups) is required, hyphenation to a more-informative detector, such as a mass spectrometer, is required [114]. Because mass spectrometry (MS) combines fantastic selectivity with high sensitivity and high speed, it is a very powerful tool when coupled with LC in polymer analysis, especially for the identification of functional end-groups. Other coupled systems, such as LC-NMR [40] and LC-IR [115] are also useful for the characterization of (co)polymers. However, since the focus of this work was on functional polymers, hyphenating LC to MS will be discussed below in some more detail.

1.2.3.1 Mass spectrometry

Mass spectrometry plays an increasingly important role in polymer analysis, especially since the development of soft ionization techniques [24,116,117,118], in particular MALDI and electrospray ionization (ESI). Both yield intact polymer ions with little or no fragmentation. The MALDI experiment involves embedding the analyte in a matrix. The matrix will absorb the energy from the laser and transfer it to the analyte, which will be ionized and enter into the gas phase. MALDI can ionize polymer molecules with molecular weights in excess of $10^6$ Da and mainly results in singly charged quasi-molecular ions, with very little fragmentation. It is very useful in polymer analysis. In ESI, the sample is infused into a gas bath as a fine spray of highly charged droplets [119]. A strong electric field is applied to the droplets as they evaporate, resulting in ionization of the molecules. Almost no fragmentation of the molecules occurs reduces the complexity of the resulting spectra. However, sample disparity (in term of molecular weight, chemical composition and functionality), high molecular weights and multiple ionizations all contribute to an increased complexity of the spectra. This, combined with the fact that MALDI is more broadly applicable than ESI, implies that ESI-MS is less commonly applied in synthetic-polymer analysis than MALDI-MS.

Both MALDI-MS and ESI-MS provide good qualitative information (including absolute molecular weights), rather than quantitative results (concentrations). This is because MS sensitivity varies with molecular weight and chemical composition (including end-groups [120,121]), caused by severe discrimination in ionization, transmission and detection in favor of low-molecular-weight species [122-125]. As a result, the application of MALDI for polymer analysis is still limited to polymers with a PDI less than 1.2 [126]. For accurate estimates of MWDs the sample polydispersity should be much smaller. The way to overcome this is the coupling of MS to separation methods, mostly SEC or interactive LC.
1.2.3.2 Hyphenating LC to MS

To overcome discrimination in MALDI-MS, a polydisperse sample (broad MWD) can be fractionated by SEC. Each fraction will have a much narrower MWD, so that it can subsequently be analyzed by MALDI-MS. The coupling of MALDI-MS with SEC is widely reported and applied (mostly off-line, but occasionally on-line). SEC provides MALDI with narrow MWD samples and MALDI provides absolute molecular-weight values to calibrate the SEC system. MALDI is difficult to couple on-line, because of the need to mix the sample with a matrix and then to irradiate the mixture with a laser. Some attempts have been made to realize a semi-on-line coupling [127,128]. However, to date the resulting systems remain limited in their application. In contrast, the on-line coupling of ESI-MS with LC is relatively easy, because the samples are introduced in solution at atmospheric pressure. On-line LC-ESI-MS is quite useful in the analysis of low-molecular-weight polymers with various end-groups. Because on-line LC-MS provides continuous sample detection, it can facilitate better understanding of LC separation mechanisms [113,129]. The separation conditions (the mobile phase and its flow rate) should be suitable for MS operation. In particular, non-volatile additives in the mobile phase should be avoided and the flow rate should be minimized (although state of the art equipment can handle milliliter-per-minute flow rates, performance tends to be better and less maintenance tends to be required when lower flow rates are used) [40].

1.2.4 Comprehensive two dimensional LC (LC× SEC)

As discussed above, synthetic polymers are very complex mixtures of many different chemical compounds [27]. Variations in the chemical structure, such as the number of functional groups or end-groups present, in the chemical composition of copolymers, or in architectural features, can have dramatic effects on the properties of the polymer. Clearly, in order to establish relationships between molecular structure and material performance of polymers, we need to obtain information on the average molecular structure, as well as on the underlying distributions. We cannot achieve this using either LC or SEC alone, because these different distributions are in general mutually dependent. To characterize multiple, mutually dependent distributions, multi-dimensional separations are indispensable. For example, all functional polymers exhibit functionality-type distributions (FTD) and all copolymers exhibit chemical-composition distributions (CCD), beside the inevitable MWD. To characterize any two dependent distributions it is necessary to combine two (different) separation mechanisms in a two-dimensional LC system. Ideally, one separation step distinguishes between molecules of different molecular weight, while the other step reveals differences in functionality or chemical composition.

Two-dimensional liquid-chromatographic (2D-LC) systems have been used for many years to separate and characterize synthetic polymers, biomolecules and complex mixtures [130]. 2D-LC can be performed in the off-line or on-line mode. The approach, often called “cross-fractionation” or “heart-cutting”, is simple to use and it has been quite commonly used in earlier studies [24,26,86,94,103,131 - 139]. This technique is very useful for the separation of (a) specific component(s) in a polymer or copolymer, but it requires prior knowledge on the retention of these specific sample components and it is time-consuming.
Heart-cutting can also be performed on-line, but “comprehensive” two-dimensional LC has become the more popular approach recently. In this method, sequential aliquots from the first column are transferred on-line to the second one using an automated switching valve. [140-144]. The transfer volume is taken sufficiently small, so that each chromatographic peak from the first dimension is divided into several fractions. The resulting data is a matrix, usually represented as a contour plot with each chromatographic separation along one axis. Comprehensive two-dimensional operation greatly increases the peak capacity of the LC systems. Also, the information content of the resulting chromatogram is greatly increased. In literature, many impressive examples of comprehensive two-dimensional LC separations of polymers have been reported [74,100,145-153]. With respect to nomenclature, heart-cutting two-dimensional LC is usually referred to as LC-LC, whereas for on-line coupling with complete transfer of the eluate from the first dimension (i.e. comprehensive 2D-LC), the notation LC×LC is used [154].

In LC×LC of polymers several different separation mechanisms can be exploited in the first and second dimensions. The choice for either dimension is dependent on the distributions of interest. A detailed discussion on the principles of and instrumentation for LC×LC can be found in the literature [74]. We have demonstrated the use of comprehensive two-dimensional LC (specifically LC×SEC) to obtain the MWD and FTD of functional poly(methyl methacrylate) (PMMA) polymers. Also, the influence of the molecular weight on the so-called critical conditions in LC was investigated for hydroxyl-functional polymers. More details can be found in Chapter 5.

1.3 Scope of this thesis

Today’s polymer chemists focus much of their attention on the synthesis and application of molecules with very well-defined structures and architectures. This increased emphasis on molecular design also greatly enhances the need for sophisticated characterization tools. This project deals with network-forming propolymers that should have two terminal (“telechelic”) functional groups in every molecule.

This is one of two theses written within the context of project #205 of the Dutch Polymer Institute (DPI), entitled “Network formation of telechelic poly(meth)acrylic polymer resins prepared by ATRP or RAFT; structure properties relationships”. This project was conducted in close cooperation among the Laboratory of Coatings Technology (now called the Laboratory of Materials and Interface Chemistry) of the Eindhoven University of Technology (TU/e), the Institute of Macromolecular Chemistry of the Academy of Sciences of the Czech Republic, and the Polymer-Analysis Group of the University of Amsterdam (UvA). The project was initiated by Prof. Rob van der Linde and lately supervised by Dr. José Brokken-Zijp. All polymers studied in this work were synthesized by Vincent Lima and Gabriëlle van Benthem-van Duuren. The analytical work described in subsequent chapters was performed in Amsterdam.

The specific objectives of this research were to establish methods to obtain (i) quantitative information, such as molecular-weight distributions (MWDs) and functionality-type distributions (FTDs), using LC at near-critical conditions and (ii) qualitative structure information on functional polymers (including end-groups) by mass spectrometry (MS). In addition, the molecular-weight
effect on the retention behavior at near-critical conditions has been studied by LC-ESI-MS and by LC×SEC.

In Chapter 2, the separation of functional poly(methyl methacrylate) (PMMA) prepolymer based on the number of end-groups has been studied using LC at the critical conditions. The functional polymers were successfully separated according to hydroxy (OH) functionality (with zero, one, or two OH groups, respectively) under critical conditions. Fast (five minutes) base-line separations were obtained. Changing the column temperature, flow rate, and mobile-phase composition within certain ranges did not affect the functionality separation. Therefore this isocratic LC separation method proved to be quite robust. Evaporative-light-scattering-detector (ELSD) calibration curves were used for the quantitative analysis of functional PMMA prepolymer.

In Chapter 3, the polymers separated under near-critical conditions were characterized off-line by matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF-MS), and by both offline and on-line LC-ESI-MS. The on-line ESI experiments confirmed a clear base-line separation of the hydroxy-functional prepolymer according to the number of hydroxyl groups. Labile end-groups of PMMA, such as the dithioester group, were lost in the MALDI-TOF-MS experiments, while they were observed intact in the ESI-TOF-MS spectra. This indicates that in the present case ESI is a softer ionization technique than is MALDI. Atmospheric pressure (AP) MALDI has been claimed to be softer than vacuum MALDI, so that this problem may be overcome [155].

In Chapter 4, the (near-) critical solvent compositions were determined for non-, mono- and difunctional (telechelic) functional Poly(n-butyl acrylate) (PnBA) polymers in normal-phase LC, using mixtures of acetonitrile, acetic (or formic) acid, and dichloromethane of varying compositions. The critical solvent compositions obtained were not exactly the same for non-, mono- and difunctional PnBA polymers. Nevertheless, low-molecular-weight PnBA samples were successfully separated according to the carboxyl functionality at (near-) critical conditions, and this was confirmed using MS. The quantitative results proved that nearly ideal functionalities (average number of carboxyl end-groups per molecule up to 1.99) were observed for telechelic PnBAs prepared by one-step RAFT polymerization.

In Chapter 5, comprehensive two-dimensional liquid chromatography (LC×SEC) was investigated to characterize functional PMMA polymers. A simple method to quantify ELSD data was studied. The qualitative and quantitative information obtained on two representative samples (VL37A and VL37B) demonstrated the usefulness of LC×SEC in determining the mutually dependent MWDS and FTDs for functional polymers. The influence of the molecular weight on the so-called critical conditions in LC was investigated for hydroxyl-functional PMMA polymers by LC×SEC.

In Chapter 6, breakthrough phenomena of polymers in interactive LC have been investigated. Three necessary and sufficient conditions are suggested for the breakthrough phenomenon to be observed. Recommendations to avoid the breakthrough phenomenon are given, culminating in a structured method for selecting the best possible sample solvents.

In order to further understand breakthrough phenomena in interactive LC of polymers, simple relationships describing the retention and peak width under isocratic and gradient-elution conditions in reversed-phase LC are used to simulate and quantitatively predict the peak-distortion and peak-
splitting effects that arise when the (composition of the) injection solvent is different from the (composition of the) mobile phase in Chapter 7.

**Note on the text**

The chapters in this thesis have been prepared as articles for publication in scientific journals and can be read independently. Therefore some overlap may occur.

**References**


Chapter 1

General Introduction and Outline of the Thesis

CHAPTER 2

Robust Isocratic LC Separation of Functional Poly(Methyl Methacrylate)

ABSTRACT

The separation of telechelic poly(methyl methacrylate) (PMMA) prepolymer based on the number of end-groups under critical-liquid-chromatography (LC) conditions has been studied using a bare-silica column, which can interact with polar functional groups. The critical solvent compositions for non-functional, mono-functional and di-functional PMMAs were determined in normal-phase LC using mixtures of acetonitrile and dichloromethane of varying composition as the mobile phase. The telechelic prepolymer was successfully separated according to hydroxyl (OH) functionality (with zero, one, or two OH groups, respectively) under the critical conditions, at which fast (five minutes) base-line separations were obtained independent of molecular weight. Changing the column temperature, flow rate, and mobile-phase composition within a certain range did not affect the functionality separation. Therefore this isocratic LC separation method is quite robust. Evaporative-light-scattering-detector (ELSD) calibration curves were used for the quantitative analysis of functional PMMA prepolymer.

Keywords: Critical liquid chromatography, polymer characterisation, hydroxyl polymers, telechelic polymers, RAFT polymers.
2.1 Introduction

Functional (pre)polymers can be used as cross-linking components in coating formulations to create hard, durable films. For example, prepolymers containing two or more hydroxy1 groups react with tri-functional isocyanates to form a urethane network. The mechanical behavior (e.g. strength, brittleness) of the network will be greatly affected by the length of the cross-linking segments, i.e. by the distance between hydroxy1 groups in the functional prepolymer. Therefore, the performance of a coating can be controlled by adjusting the number and positions of the functional groups. Superior performance of a coating may be anticipated if the cross-linking agent is a telechelic (di-functional) polymer with a (very) narrow molecular-weight distribution. However, it is very difficult to produce such telechelic polymers by conventional polymerization methods.

Fortunately, the recent development of controlled/living' radical polymerization has resulted in the synthesis of many well-defined architectures with predictable molecular weights [1,2]. The most important methodologies include nitroxide-mediated radical polymerization [3], atom-transfer radical polymerization (ATRP) [4], and lately reversible addition-fragmentation chain-transfer (RAFT) polymerization [5]. Among these, RAFT might arguably have the greatest commercial impact, because it only involves organic substances and because it works very well with most acrylic derivatives, including acrylic acid [2]. Therefore, this is the method of choice for the preparation of di-functional telechelic acrylate polymers with a low polydispersity index.

The first functional group is incorporated into the polymer chain at the initiating chain end, using functionalized initiators and functionalized RAFT agents, which results in mono-functional polymers [6,7,8]. This is followed by an endcapping reaction to introduce another functional group. However, the efficiencies (yields) of these functionalization reactions are less than 100%, because of the side reactions inherent to growing radicals, such as termination by bimolecular combination or disproportionation. Moreover, chain transfer to solvent molecules or monomers will always occur. Finally, experience with macromolecular reactions has shown that the hydroxyl-endcapping reaction does not proceed with full conversion [7].

The development and optimization of telechelic polymers require methods to determine the functionality-type distribution (FTD) and the molecular-weight distribution (MWD). This is a major challenge in the research on di-functional telechelic polymers. The traditional analytical techniques, such as IR (infrared absorption spectroscopy), UV (ultraviolet spectroscopy), NMR (nuclear-magnetic-resonance spectroscopy), and specific OH titration, by which only the average functionality can be measured, are inadequate for this purpose. The same must still be said for modern mass-spectrometric techniques, MALDI-TOF-MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) and ESI-MS (electrospray ionization mass spectrometry). These techniques provide good qualitative information, rather than quantitative results, due to variations in the ionization, separation, and detection efficiencies. To reach the goals mentioned above, the telechelic polymers must be separated based on the number of end-groups.

LC under critical conditions (at which the retention time of a polymer is independent of the molecular weight) is a relatively new analytical method [9-15]. It can be used to separate polymers
based on the number of functional groups into, for example, non-, mono-, and di-functional polymers [6,16,17,18]. However, the exact critical condition is not easy to obtain experimentally [19,20] and the critical conditions are rather sensitive to eluent composition [21], column packing [19, 22], polymer nature [23,24], temperature [17,21,25], and even to pressure variations [17,23,24]. Critical conditions do not necessarily provide good functionality-based separations [9]. On a given critical system, the retention and separation of polymers with (for example) different end-groups may or may not be satisfactory. In this work, the critical solvent composition for poly(methyl methacrylate) (PMMA) was studied in normal-phase LC. A robust isocratic LC separation of hydroxyl-functional PMMA was developed.

2.2 Experimental

2.2.1 Chemicals

Dichloromethane (DCM), and acetonitrile (both HPLC grades), were from Rathburn Chemicals (Walkerburn, Scotland). Poly(methyl methacrylate) (PMMA) standards were obtained from Polymer Laboratories (Church Stretton, U.K.). The molecular-weight ($M_n$, $M_p$) and polydispersity-index ($PDI$) values were supplied by the manufacturer. The polymers with one hydroxyl (OH) end-group were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization (named RAFT polymers) using a hydroxyl-functional initiator and a hydroxyl-functional RAFT chain-transfer agent [8]. A commercial telechelic PMMA (TEGO DIOL MD-1000X) with two OH groups was obtained from Tego Chemie Service (Essen, Germany). Since well-defined PMMA containing two OH groups is not easy to obtain, a sample VL57 (mainly containing two OH end-groups) was synthesised via end-group modification of a well-defined RAFT polymer with one OH end-group [8]. The molecular weights and molecular-weight distributions were measured by SEC with a Waters (Milford, MA, USA) instrument equipped with a Waters model 510 pump and a model 410 differential refractometer (40°C). THF was used as the eluent at a flow rate of 1.0 ml/min. A set of two linear columns (Mixed-C, Polymer Laboratories, 30 mm x 7.8 mm i.d., 40°C) was used. The calibration curve was prepared with polystyrene (PS) standards, and the molecular weights were estimated based on the universal-calibration principle and Mark-Houwink parameters [PS, $K = 1.14 \times 10^{-4}$ dL g$^{-1}$ and $a = 0.716$; PMMA, $K = 0.944 \times 10^{-4}$ dL g$^{-1}$ and $a = 0.719$] [26,27,28]. The effect of the hydroxyl end-groups on the Mark-Houwink parameters was neglected. All the PMMA standards and samples used are summarized in table 2.1. All the samples were dissolved in DCM unless stated otherwise.

2.2.2 Equipment

A Waters (Milford, MA, USA) 2690 Alliance liquid-chromatography system was used to perform the isocratic LC experiments. This HPLC instrument contained a built-in auto-injector with a sample loop allowing injection of variable sample volumes, and it was equipped with a Waters 996 PDA (photodiode-array detector) and a Sedex 55 evaporative light-scattering detector (ELSD) (temperature 62°C, N$_2$ pressure 2.2 bar). The mobile phase was prepared in-situ using the solvent-
mixing capability of the instrument. The eluent composition is given in volume %. The data collection and the data analysis were handled by Waters Millennium 3.2 software. The columns used (150 mm x 4.6 mm i.d.) were packed in-house with Hypersil Silica (3-μm particles; 100-Å pore size; Shandon, Runcorn, UK).

Also, an HPLC system consisting of a Shimadzu LC-10ADvp pump, a Rheodyne 7010 injector (Berkeley, CA, USA), and an Varex ELSD II A (Burtonsville, Maryland, USA) was used. The columns were contained in a (Millipore) Waters temperature-control module.

**Table 2.1** PMMA samples used in this study. The values for the number-average molecular-weight ($M_n$), the peak molecular weight ($M_p$), and the polydispersity-index ($PDI=M_p/M_n$) were measured by SEC, except for PMMA standards (values supplied by the manufacturer).

<table>
<thead>
<tr>
<th>Sample name</th>
<th>$M_n$</th>
<th>$M_p$</th>
<th>PDI</th>
<th>Intended number of OH end-groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA 1,680$^a$</td>
<td>1.327</td>
<td>1.681</td>
<td>1.15</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 2,990$^a$</td>
<td>2.756</td>
<td>2.991</td>
<td>1.08</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 3,800$^a$</td>
<td>3.437</td>
<td>3.805</td>
<td>1.07</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 6,950$^a$</td>
<td>-</td>
<td>6.950</td>
<td>1.05</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 9,200$^a$</td>
<td>8.502</td>
<td>9.198</td>
<td>1.06</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 13,930$^a$</td>
<td>12.489</td>
<td>13.934</td>
<td>1.06</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 28,300$^a$</td>
<td>26.953</td>
<td>28.304</td>
<td>1.04</td>
<td>0</td>
</tr>
<tr>
<td>PMMA-OH 3,310$^b$</td>
<td>2.425</td>
<td>3.314</td>
<td>1.22</td>
<td>1</td>
</tr>
<tr>
<td>PMMA-OH 6,680$^b$</td>
<td>4.832</td>
<td>6.679</td>
<td>1.25</td>
<td>1</td>
</tr>
<tr>
<td>PMMA-OH 13,950$^b$</td>
<td>10.934</td>
<td>13.946</td>
<td>1.21</td>
<td>1</td>
</tr>
<tr>
<td>PMMA-OH 20,740$^b$</td>
<td>17.043</td>
<td>20.744</td>
<td>1.16</td>
<td>1</td>
</tr>
<tr>
<td>MD-1000X</td>
<td>1.490</td>
<td>2.324</td>
<td>1.64</td>
<td>2</td>
</tr>
<tr>
<td>VL57$^c$</td>
<td>3.577</td>
<td>5.718</td>
<td>1.26</td>
<td>2</td>
</tr>
<tr>
<td>VL37A$^d$</td>
<td>2.587</td>
<td>3.754</td>
<td>1.29</td>
<td>1</td>
</tr>
<tr>
<td>VL37B$^d$</td>
<td>2.853</td>
<td>3.678</td>
<td>1.29</td>
<td>1</td>
</tr>
<tr>
<td>VL47B$^d$</td>
<td>3.312</td>
<td>5.659</td>
<td>1.31</td>
<td>2</td>
</tr>
</tbody>
</table>

$^a$ PMMA standards were obtained from Polymer Laboratories.

$^b$ Polymers with one OH group synthesised by reversible addition-fragmentation chain-transfer (RAFT) polymerization using a hydroxyl-functional initiator and a hydroxyl-functional RAFT chain-transfer agent.

$^c$ Synthesized via end-group modification of well-defined RAFT polymers [8].

$^d$ Synthesized by RAFT polymerization using a 2.2'-azobisisobutyronitrile (AIBN) initiator and a hydroxyl functional RAFT chain-transfer agent [8].
2.3 Results and Discussions

2.3.1 Critical composition for PMMA

Our objective is to establish robust isocratic LC conditions to separate PMMA samples based on the number of hydroxyl end-groups, which means that the separation should not be very sensitive to small changes in solvent composition, flow rate, or column temperature. A bare-silica column, which can interact with polar functional groups, is thought to be a good choice. If one solvent causes the sample (*i.e.* the polymer backbone) to adsorb slightly, and the other solvent just causes the sample to desorb, then we expect the retention of the polymer not to be dramatically affected by the composition of the mobile phase. Dichloromethane (DCM) is a good solvent, but a weak eluent for PMMA on a silica column [29]. It can be used as the injection solvent to avoid breakthrough problems [29]. All the samples were dissolved in DCM in this work unless stated otherwise. Acetonitrile is a more-polar solvent, which can desorb PMMA from the silica column. Therefore these two solvents were used to make up the mobile phase.

The critical solvent composition for PMMA was determined using mixtures of acetonitrile and dichloromethane of varying composition. PMMA samples were used with different molecular weights and different numbers of end-groups, *viz.* PMMA standards with no OH groups, polymers with one OH group synthesised by reversible addition-fragmentation chain-transfer (RAFT) polymerization, and a commercial telechelic PMMA (MD-1000X) with two OH groups (see table 2.1). Since well-defined PMMA containing two OH groups is not easy to obtain, sample VL57, synthesised via end-group modification of a well-defined RAFT polymer [8], was used as another di-functional PMMA with a different molecular weight. The main fraction of VL57 contained two OH end-groups, but the product also contained a small fraction with only one OH end-group [8]. The observed retention times are shown in Figs. 2.1 and 2.2 (for sample VL57, only the retention time of the di-functional fraction was recorded). Critical compositions for PMMAs with no OH groups, one OH group or two OH groups are found to be almost identical at about 43% acetonitrile in dichloromethane. The retention times of PMMA standards (molecular-weight range 1,680-28,300) were almost constant when the mobile phase contained between about 42 and 55% acetonitrile in dichloromethane, as seen in Figs. 2.1A and 2.2A. From a combination of Figs. 2.1A and 2.1B we conclude that the mobile-phase compositions between 42-48% can be used to separate the low-molecular-weight PMMA samples based on the number of hydroxyl-functional end-groups.

Fig. 2.3 shows the representative ELSD (evaporative light-scattering detector) chromatograms of PMMAs under conditions that yield base-line separation according to the number of OH end-groups. The two different PMMA standards (with no OH groups, molecular weights 3,800 and 28,300) co-eluted. The RAFT polymers (with one OH end group, molecular weights 3,300 and 20,000) have identical retention times, but are clearly separated from the PMMA standards. The telechelic di-functional sample is well separated from the mono-functional polymers. Thus, the low-molecular-weight PMMAs were successfully separated according to the hydroxyl functionality under the critical conditions.
Fig. 2.1  

(B) Dependence of retention time on the mobile-phase composition for PMMA-OH and HO-PMMA-OH samples with different molecular weights. Open circles: HO-PMMA-OH 5,720 (VL57, see text), open triangles: MD-1000X; squares: PMMA-OH 20,740, triangles: PMMA-OH 13,950, circles: PMMA-OH 6,680, stars: PMMA-OH 3,310. ELSD detector, home-packed silica column (150 mm x 4.6 mm i.d.; 3-μm particles; 100 Å pore size), flow rate 0.5 ml/min, column temperature 25°C.

Note: Data points with retention times larger than 10 min in figure A (15 min in figure B) were recorded and used to construct the curved lines. However, they are not shown in the figures.
Fig. 2.2  
(A) Calibration plots of log $M_p$ vs. retention time for PMMA standards in different mobile phases. 
(B) Calibration plots of log $M_p$ vs. retention time for PMMA samples with one OH end group in different mobile phases (The values indicated in the figure refer to the percentage of acetonitrile in DCM). LC conditions were the same as in Fig. 2.1. See note under Fig. 2.1.
Fig. 2.3  Representative OH-based separations (chromatograms) of PMMA functional polymers at 25°C. Detector ELSD, mobile phase 43% acetonitrile in DCM, flow rate 0.5 ml/min, injection volume 10 µL, sample concentration 1 mg/ml in DCM, home-packed silica column (150 mm x 4.6 mm i.d.; 3-µm particles; 100 Å pore size).

Mengerink et al. described that the critical composition for polyamide varies when the column temperature or the flow rate changes [17]. Other authors [19,23] also reported that the critical composition is very sensitive to the column temperature and the pressure drop. We investigated the effects of temperature and flow rate (the pressure drop changes when the flow rate varies) on our critical composition. No effect was observed in our experiments when the flow rate was changed from 0.1 to 1.0 ml/min. The same critical composition, 43% acetonitrile in dichloromethane, was obtained when the flow rate used was 0.1, 0.5, or 1.0 ml/min. The temperature had very little effect on the retention of the PMMA standards between 25 and 50°C. Because sample VL37B contained fractions with zero, one, and two OH end-groups [30], it was selected as a sample material in the following discussion. As shown in Fig. 2.4, the base-line separations of sample VL37B according to the hydroxyl functionality were not affected when the column temperature changed from 25 to 50°C, although the retention times of mono-functional and di-functional polymers somewhat decreased when the temperature increased.

The effect of the mobile-phase composition is illustrated in Fig. 2.5. Only one tailing peak was obtained when 70% acetonitrile in dichloromethane was used as mobile phase. Two ill-resolved peaks were observed when the acetonitrile concentration decreased to 60% (not shown). Three barely discernible peaks were obtained at 55%. The separation of these three peaks was improved at 50% acetonitrile in dichloromethane. Base-line separations of sample VL37B based on hydroxyl functionality were achieved when the mobile-phase composition was between 48% and 40% of acetonitrile in dichloromethane. This is a substantial range in composition. Therefore, these “critical” conditions are quite robust. When the mobile-phase composition was below 40% acetonitrile in dichloromethane, di-functional PMMA polymers were strongly retained on the column and molecular-weight (adsorption) effects became apparent.
Fig. 2.4 Effect of the temperature on the separation of a PMMA functional polymer (VL37B). Detector ELSD, mobile phase 45% acetonitrile in DCM, flow rate 1.0 ml/min, injection volume 20 μL, sample concentration 5 mg/ml in DCM, column as in Fig. 2.3.

Fig. 2.5 Effect of mobile-phase composition on the separation of a PMMA functional polymer (VL37B). Detector ELSD, flow rate 1.0 ml/min, injection volume 20 μL, sample concentration 5 mg/ml in DCM at 25°C, column as in Fig. 2.3.
2.3.2 Quantitative aspects

We have already shown that the isocratic separations of PMMA functional prepolymer based on hydroxyl end-groups are quite robust with respect to fluctuations in the LC conditions, such as variations in temperature, mobile-phase composition, and flow rate. The OH base-line separations (the structures of the RAFT polymers and the derivatives) were confirmed by off-line MALDI-TOF-MS and on-line LC-ESI-MS [30]. However, mass spectrometry is not suitable for quantitative analysis due to discrimination in the ionization efficiency. The problem of quantitative analysis arises not only in MS, but also in all chromatographic techniques [31], since many of the typical HPLC detectors are not really useful for the analysis of PMMA samples. For example, a lack of suitable chromophores in PMMA prohibits the use of UV (ultraviolet) and fluorescence detectors in practice. Refraction-index (RI) detectors are not sufficiently sensitive and are easily interfered by the sample solvent and by fluctuations in the mobile-phase compositions due to the great difference in refractive index between the two mobile-phase solvents (acetonitrile, \( n_D^{20} = 1.344 \), and dichloromethane, \( n_D^{20} = 1.424 \)).

In recent years, evaporative light-scattering detection (ELSD) has been used frequently in HPLC, due to its “universal” applicability for all non-volatile analytes [32]. However, quantitative analysis using an ELSD is not easily achieved [22,31,32], because its response depends on operating parameters (mobile phase, gas pressure, temperature) as well as on the nature of the polymer (monomeric unit, molecular weight, end-groups). Moreover, the response of such an instrument is generally not linear with concentration. Instead, it can be expressed by an exponential relationship [6,20,31]. Calibration curves should be established carefully. An exponential calibration curve, such as in equation (1), is often used [31]:

\[
A = a \times m^b
\]

where \( A \) is the ELSD response area, \( m \) is the injected sample amount, and \( a \) and \( b \) are constants. The values of \( a \) and \( b \) can easily be determined from a logarithmic plot, in which the exponent \( b \) is obtained from the slope and the constant \( a \) from the intercept of the regression line. Fig. 2.6 shows ELSD calibration curves of PMMA with different end-groups and very similar molecular weights. The values of \( a \) and \( b \) are shown in table 2.2. It can be seen from Fig. 2.6 and table 2.2 that the OH end-groups had a significant influence on the ELSD response. An exponent \( b \) of 0.999 indicates a good linear ELSD response for PMMA samples with one OH end-group. The mobile-phase flow rate was found to have a clear effect on the ELSD response, as shown in Fig. 2.7 and in table 2.3. The molecular weights of PMMA standards were found to have only a minor effect on the ELSD calibration in our study (molecular-weight range investigated from 1,680 to 28,300, results not shown). This is in agreement with the results reported in the literature [31,33,34,35]. The calibration curves in Fig. 2.6 could therefore be used for the quantitative analysis of functional PMMA prepolymer under the specified LC conditions (42% acetonitrile in dichloromethane at 0.5 ml/min). Some representative results are shown in table 2.4.
**Fig. 2.6** ELSD calibration curves (logarithmic scale) for PMMAs with different end-groups. Circles: PMMA 2,900, open squares: PMMA-OH 3,310, triangles: MD-1000X. Mobile phase: 42% acetonitrile in DCM, flow rate 0.5 ml/min at 25°C, column as in Fig. 2.3.

**Fig. 2.7** Effect of mobile-phase flow rate on ELSD calibration curve (logarithmic scale) for PMMA standard ($M_p$ 9,200). Triangles: 0.1 ml/min., open circles: 0.3 ml/min., open squares: 0.5 ml/min., stars: 0.75 ml/min., squares: 1.0 ml/min.. Mobile phase: 42% acetonitrile in DCM, temperature 25°C, column as in Fig. 2.3.
### Table 2.2 End-group effect on ELSD calibration curves. LC conditions as in Fig. 2.6.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$a$</th>
<th>$b$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA 2,990</td>
<td>556.7</td>
<td>0.921</td>
<td>0.9973</td>
</tr>
<tr>
<td>PMMA-OH 3,310</td>
<td>436.1</td>
<td>0.999</td>
<td>0.9984</td>
</tr>
<tr>
<td>HO-PMMA-OH (MD-1000X)</td>
<td>97.5</td>
<td>1.324</td>
<td>0.9985</td>
</tr>
</tbody>
</table>

### Table 2.3 Flow-rate effect on ELSD calibration curves (PMMA 9,200). LC conditions as in Fig. 2.7.

<table>
<thead>
<tr>
<th>Flow rate</th>
<th>$a$</th>
<th>$b$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ml/min</td>
<td>619.0</td>
<td>0.854</td>
<td>0.9996</td>
</tr>
<tr>
<td>0.3 ml/min</td>
<td>594.2</td>
<td>0.879</td>
<td>0.9999</td>
</tr>
<tr>
<td>0.5 ml/min</td>
<td>522.3</td>
<td>0.930</td>
<td>0.9988</td>
</tr>
<tr>
<td>0.75 ml/min</td>
<td>280.3</td>
<td>1.106</td>
<td>0.9972</td>
</tr>
<tr>
<td>1.0 ml/min</td>
<td>133.8</td>
<td>1.261</td>
<td>0.9960</td>
</tr>
</tbody>
</table>

### Table 2.4 Quantitative analysis of hydroxyl-functional PMMAs by LC-ELSD. Isocratic conditions and column as in Fig. 2.6. Sample volume 20 µl for all injections (samples as indicated in table 2.1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Non-funct. (%)</th>
<th>Mono-funct. (%)</th>
<th>Di-funct. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL37A</td>
<td>10</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>VL37B</td>
<td>6</td>
<td>83</td>
<td>11</td>
</tr>
<tr>
<td>VL47A (PMMA-OH 3,310)</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>VL47B</td>
<td>0</td>
<td>13</td>
<td>87</td>
</tr>
<tr>
<td>VL57</td>
<td>0</td>
<td>12</td>
<td>88</td>
</tr>
</tbody>
</table>
2.4 Conclusions

The critical solvent compositions for non-functional, mono-functional and di-functional hydroxyl PMMAs were determined in normal-phase LC using mixtures of acetonitrile and dichloromethane of varying composition. The low-molecular-weight PMMAs were successfully separated according to hydroxyl functionality (zero, one, or two OH groups, respectively) at the critical conditions. Fast (five minutes) base-line separations were obtained independent of molecular weight. The separations were shown to be quite robust, as changing the column temperature, flow rate, and mobile-phase composition within reasonable ranges did not affect the resolution.

Under appropriate conditions, reliable ELSD calibration curves could be obtained and these were used for the quantitative analysis of hydroxyl-functional PMMA prepolymers at the robust critical condition of 42% acetonitrile in dichloromethane at 0.5 ml/min.

Acknowledgement

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References

CHAPTER 3

Mass-Spectrometric Characterization of Functional Poly(Methyl Methacrylate) in Combination with Critical LC

ABSTRACT

State-of-the-art techniques for the mass-spectrometric characterization of synthetic polymers have been applied to functional poly(methyl methacrylate) (PMMA), synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization. The polymers were first separated effectively according to functionality by liquid chromatography (LC) at the critical conditions (i.e., almost no influence of molecular weight on retention). The separated polymers were characterized off-line by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and both off-line and on-line by LC-electrospray-ionization-quadrupole-TOF-MS (LC-ESI-QTOF-MS). The on-line ESI experiments confirmed a clear baseline separation of the hydroxyl-functional prepolymer according to the number of hydroxyl groups. Labile end-groups of PMMA, such as the dithioester group, were lost in the MALDI-TOF-MS experiments, while they were observed intact in the ESI-QTOF-MS spectra. This indicates that in the present case ESI is a much softer ionization technique than is MALDI. The ESI-MS experiments provided direct evidence that the RAFT polymers still exhibited living characteristics in the form of the dithio moiety.

Keywords: Critical liquid chromatography, hydroxyl group, telechelic polymers, RAFT polymers, LC-ESI-MS, MALDI, fragmentation.

3.1 Introduction

Functional (pre-)polymers can be used as cross-linking components in coating formulations to create hard, durable films. For example, telechelic prepolymers (containing two hydroxyl end-groups) with narrowly distributed molecular weights can react with trifunctional isocyanates to form a well-defined polyurethane network. It is expected that the study of these systems will provide better insights into the relations between the network structure and the properties of cross-linked coating systems. Eventually, this should also lead to coatings with better properties. However, it is very difficult to produce the required telechelic polymers by conventional polymerization methods.

Fortunately, the recent development of controlled 'living' radical polymerization has created possibilities for the synthesis of many well-defined polymers with designed architectures and predictable molecular weights [1,2]. The most important methodologies include nitroxide-mediated radical polymerization [3], atom-transfer radical polymerization (ATRP) [4], and lately reversible addition-fragmentation chain-transfer (RAFT) polymerization [5]. Among these, RAFT polymerization arguably has the greatest commercial impact, because it only involves organic substances, it has a high tolerance to impurities, and it can be applied for a wide range of monomers, including acrylic acid [2]. Therefore, we have selected RAFT polymerization for the preparation of bifunctional telechelic (meth)acrylate polymers with a low polydispersity index.

RAFT polymerization relies on the use of thiocarbonylthio-compounds (or dithioesters) of general structure Z-C(=S)S-R. R is the homolytic leaving group of the RAFT agent; Z is an activating group. The dithioesters act as reversible addition-fragmentation chain-transfer agents, allowing the formation of polymers with functional end-groups [5,6]. A simplified mechanism of the RAFT process is given in scheme 3.1. Initiator-derived primary radicals [I*] react with monomer units to form oligomeric (propagating) radicals [Pn*], which undergo addition to thiocarbonylthio compounds to form adduct radicals. The resulting species fragments into a polymeric thiocarbonylthio compound and a homolytic leaving group. The latter ([R*]) is capable of reinitiating the polymerization to give a new propagating radical [Pn*]. Equilibrium is then established between all the active propagating radicals [Pn* and Pm*] and the dormant polymeric thiocarbonylthio-compounds by way of the intermediate radicals. Most of the polymers obtained at the end of a RAFT polymerization will contain the leaving group of the RAFT agent, but if initiators are used a (small) fraction will contain the initiating radical fragments (I). However, in some experiments [7,8,9] the amount of initiator-derived polymer was too low to be detected. Destarac et al. [10] and Schilli et al. [11] did observe signals corresponding to polymers with initiator end-groups in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) of RAFT polymers.
Mass spectrometry plays an increasingly important role in polymer analysis, because of its high sensitivity, broad dynamic range, specificity, and selectivity \([12,13,14,15]\). Two significant developments in ionization techniques in the late 1980s – MALDI and electrospray ionization (ESI) – greatly enhanced the applicability of MS for polymer characterization. These techniques enable the ionization of large nonvolatile molecules with good integrity, thus allowing determination of the weight of intact polymer molecules by MS. However, to study the heterogeneity of complex polymer systems at the molecular level, it is vital that MALDI or ESI-MS is combined with a suitable separation technique \([16,17,18,19]\). MALDI is the newest and reputedly most promising ionization method for synthetic polymers \([13]\). Thanks to the emergence of MALDI, polymer scientists have taken a greater interest in MS \([13]\). In publications relating to the characterization of synthetic (co-)polymers by mass spectrometry, MALDI is favored as the ionization method, because only singly charged ions are observed \([15]\), producing much clearer spectra.

However, only a few references contain MALDI mass spectra of polymers obtained by RAFT polymerization \([7,8,10,11,20,21]\). The spectra obtained in these reports do not provide direct structural information on, for example, end-groups of the RAFT polymers. Vosloo et al. \([20]\) presented a MALDI-TOF-MS spectrum of RAFT polystyrene, which contained several series of peaks. They stated that it was difficult to obtain useful MALDI-TOF-MS spectra of these polymers. Destarac et al. \([10]\) published a MALDI-TOF-MS spectrum of RAFT poly(vinyl acetate). They observed initiator-derived and hydrogen-terminated polymers. D'Agosto et al. \([21]\) reported the
MALDI-TOF-MS spectrum of poly(N-acyrloylmorpholine), synthesized by RAFT polymerization. They described that in the MALDI instrument the RAFT polymer experienced fragmentation in the laser beam to lose the dithioester moiety. Subsequently, the macroradical formed could abstract a hydrogen from the matrix, giving rise to hydrogen-terminated polymers. Ganachaud et al. [7] and Schilli et al. [11] reported MALDI mass spectra of RAFT poly(N-isopropylacrylamide). Schilli et al. gave a good discussion on the fragmentation of RAFT polymers during ionization. Vana et al. [8] ascribed the multiple peaks within one repeat unit of RAFT poly(methyl acrylate) (PMA) to the complex MALDI ionization and fragmentation processes. Although MALDI was initially described as a "soft ionization" process, a significant extent of fragmentation may occur for MALDI-generated ions [22,23]. This fragmentation usually occurs not only for the RAFT polymers mentioned above, but also for polymers obtained by other controlled/"living" radical-polymerization methods, such as nitroxide-mediated radical polymerization [24,25,26] and ATRP [27-33]. Nonaka et al. [32] suggested that the terminal C-Cl group of PMA obtained by ATRP is relatively stable in comparison with that of poly(methyl methacrylate) (PMMA) during MALDI-TOF-MS analysis.

Musat et al. [23] analyzed some labile low-molecular-weight polyesteramides by field desorption (FD) MS, ESI-MS, and MALDI-MS. They found that ESI yielded the least ion fragmentation and sample decomposition; in other words, they found ESI to be the "softest" ionization method. FD-MS rated second (less soft) and MALDI-MS third (least soft). Because of the reasonable expectation that ESI-MS would yield less fragmentation and therefore clearer spectra, we tried to apply ESI-MS to analyze RAFT polymers and to compare the results with those obtained by MALDI-TOF-MS. Our objective was to obtain mass spectra containing peaks representative of intact molecular ions, to unambiguously assign the polymer end-groups.

In polymer LC, critical conditions refer to a separation in which retention is independent of the number of monomeric units in a homopolymer chain [19]. Under critical conditions, differences in retention (i.e. selectivity) are solely caused by the end-groups and by other (functional) groups present in the polymer molecules. Critical separations are of great potential interest, but critical conditions are typically hard to realize and very hard to maintain through extended series of LC experiments. In a previous study [34], robust critical LC conditions were established for PMMA in normal-phase LC using mixtures of acetonitrile and dichloromethane as the mobile phase on a bare silica column. Baseline separations were obtained for low-molecular-weight RAFT prepolymer, either with or without end-group modification. Separation was thought to be solely based on the number of hydroxyl groups present in the molecules. In this work, off-line LC//MALDI-TOF-MS and both off-line and on-line LC-ESI-QTOF-MS have been used to identify the repeating units and, especially, the end-groups of the polymers. This should allow us to confirm the critical separation of non-functional, mono-functional, and di-functional polymers.
3.2 Experimental

3.2.1 Chemicals

Dichloromethane (DCM), tetrahydrofuran (THF), and acetonitrile (all HPLC grade), were from Rathburn Chemicals (Walkerburn, Scotland). Polymer sample V37A was synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization using a 2,2'-azobisisobutyronitrile (AIBN) initiator and a hydroxyl-functional RAFT chain-transfer agent [35]. Sample V37B was obtained via end-group modification of V37A, with cleavage of the RAFT activating group [35]. The molecular-weight distributions were measured by size-exclusion chromatography (SEC). Calibration is based on polystyrene standards and the molecular weights were recalculated using the universal-calibration principle and Mark-Houwink parameters [34]. The SEC data of samples V37A and V37B are summarized in table 3.1. Sample V37A0 is the non-functional fraction of V37A collected between 1.7 and 2.2 min, V37A1 is the mono-functional fraction of V37A collected between 2.3 and 3.3 min, V37B0 is the non-functional fraction of V37B (1.7-2.2 min), and V37B1 is the mono-functional fraction of V37B (2.3-3.3 min; see Fig. 3.1).

<table>
<thead>
<tr>
<th>Sample name</th>
<th>$M_n$</th>
<th>$M_p$</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>V37A*</td>
<td>2,587</td>
<td>3,754</td>
<td>1.29</td>
</tr>
<tr>
<td>V37B*</td>
<td>2,853</td>
<td>3,678</td>
<td>1.29</td>
</tr>
</tbody>
</table>

* Synthesized by RAFT polymerization using a 2,2'-azobisisobutyronitrile (AIBN) initiator and a hydroxyl-functional RAFT chain-transfer agent [35].

* Synthesized via end-group modification of sample V37A with cleavage of the RAFT activating group [35].

3.2.2 Equipment

The HPLC equipment used was the same as described in the previous paper [34]. The MALDI-TOF-MS measurements were performed with a Voyager De Pro instrument (PerSeptive Biosystems, Framingham, MA, USA) equipped with a 337-nm nitrogen laser. Spectra were acquired by summing the data obtained from 200 laser shots in the reflector mode. The laser energy per pulse was tuned to yield sufficient ionization, while minimizing fragmentation. Absolute intensities were similar to the value of 80 µJ reported by Scriven et al. [36]. α-Cyano-4-hydroxycinnamic acid (about 20 mg/ml in THF) was used as the matrix. The concentration of the polymer sample was about 1 mg/ml in THF.

The off-line ESI-MS experiments were carried out using a Q-TOF Ultima™ Global mass spectrometer (Micromass, Manchester, UK) equipped with an atmospheric-pressure-ionization electrospray interface. The instrument was calibrated with phosphoric acid in the mass range of 98 to 2058 amu. Polymer samples were dissolved in a 4:1 v/v mixture of dichloromethane/methanol at
a concentration of 0.5 mg/ml. The flow rate of the sample introduced into the electrospray interface was 20 μl/min. The sampling cone potential was 31 V and the capillary voltage 3.0 kV. The electrospray-source temperature was 80°C and the desolvation temperature 100°C. Mass spectra were scanned over the range m/z 500-3000 in positive-ion mode. More than 100 scans were summed to produce the final spectrum.

The on-line LC-ESI-QTOF-MS experiments were carried out with an HPLC system consisting of two Shimadzu LC-10ADvp pumps (high-pressure gradient system to prepare the mobile phase in-situ). A post-column addition of methanol at a flow rate of 20 μl/min was delivered by another Shimadzu LC-10ADvp pump to enhance the ionization efficiency. The concentration of the polymer injected was about 1 mg/ml in DCM. Care was taken to avoid breakthrough peaks [37]. The other ESI operation parameters were the same as in the off-line process described above.

Tandem-MS (ESI-MS/MS) experiments were carried out on the selected precursor ions by collision-induced dissociation (CID) using helium as collision gas and a collision energy of about 50 V to obtain the fragmentation-ion spectrum.
3.3 Results and discussion

In a previous study [34] PMMA prepolymer were successfully separated according to hydroxyl functionality under the critical LC conditions. These conditions were used to analyze the functional RAFT polymers. The representative chromatograms of two low-molecular-weight functional-polymer samples are shown in Fig. 3.1. It is relatively straightforward to collect fractions (identified in Fig. 3.1) and to perform off-line mass spectrometry.

3.3.1 Comparison of MALDI-TOF-MS and ESI-QTOF-MS

Because low-molecular-weight materials yield very high MALDI signals, even if present at very low concentrations, the complete spectra are not very useful. This is indicated in Appendix A Fig. Ap-1, where spectra are shown across a broader range, especially for sample V37B1. Fig. 3.2 shows the most useful range of the MALDI-TOF-MS spectra of four different fractions. It can be seen from the spectra in Fig. 3.2 that two different series were observed with a repeating unit of 100.1 amu, which clearly corresponds to a single methyl methacrylate (MMA) monomeric unit, in all of the mass spectra for the fractions V37A0, V37B0, V37A1, V37B1. The main series of peaks result from adducts with one sodium cation (Na⁺). The minor series (seen in Fig. 3.3a and Appendix A Fig. Ap-3a) can be assigned to the potassium-cation (K⁺) series (with mass increments of 16 amu relative to the Na⁺ series). The results are summarized in table 3.2. In addition, we were unable to assign another minor series (with masses 25 amu lower than those of the main peaks) for V37A0 (Fig. 3.3a), for which there were many possibilities. There is another minor series (with masses 16
amu lower than those of the main peaks) that can be seen in Fig. 3.2 for V37B1. These peaks are not likely to be due to the loss of an OH group, as they are not observed for any of the other structures, which contain the same OH moiety. Also, a difference of 16 amu is not expected to result from the loss of a hydroxyl group. More likely, these peaks may originate from oligomeric species B1S undergoing cyclisation to yield oligomers with structure B1Ri (see table 3.2 for abbreviations). Such a mechanism would be analogous to the loss of BrCH$_3$ followed by cyclisation of the final two methyl methacrylate monomer repeat units to yield a lactone end-group, as demonstrated by Borman et al. [30] for PMMA prepared by ATRP.

Fig. 3.2  MALDI-TOF mass spectra for the first two fractions of sample PMMA V37A and V37B. (V37A0) Non-functional fraction of V37A collected at 1.7-2.2 min; (V37A1) Mono-functional fraction of V37A (2.3-3.3 min); (V37B0) Non-functional fraction of V37B (1.7-2.2 min). (V37B1) Mono-functional fraction of V37B (2.3-3.3 min). LC separation conditions were the same as in Fig. 3.1, except a flow rate of 0.5 ml/min.
Table 3.2 Structural assignment of the peaks displayed in the MALDI-TOF and ESI-QTOF mass spectra of the PMMA samples reported in this work.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Structure (abbr.)</th>
<th>Slope</th>
<th>Intercept</th>
<th>End-group, (m/z)</th>
<th>Representative ions (m/z)</th>
<th>Associated ion</th>
<th>Ionization method</th>
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<td>69.06</td>
<td>1492.96 1492.75 (14)</td>
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<td>MALDI</td>
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<td>221.04 221.03</td>
<td>1644.79 1644.76 (14)</td>
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<td>ESI</td>
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<tr>
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<td>1537.08 1536.78 (14)</td>
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<td>MALDI</td>
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<tr>
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<td>69.07 69.06</td>
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<td>69.17 69.06</td>
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<td>136.05</td>
<td>113.06 113.08</td>
<td>1036.55 1036.55 (9)</td>
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<td>145.07 145.06</td>
<td>1005.22 1005.16 (28)</td>
<td>Na(^+)</td>
<td>ESI</td>
</tr>
</tbody>
</table>

1 Abbreviations: CH\(_3\), AOH, B0H: CH\(_3\)-C-[MMA]\(_n\)H
              CN
              CH\(_3\)
              S

A0R: CH\(_3\)-C-[MMA]\(_n\)S-C-
              CN
              CH\(_3\)

B0S: CH\(_3\)-C-[MMA]\(_n\)SH
              CN

A1H, B1H: HO-(CH\(_2\))\(_3\)C-[MMA]\(_n\)H
          CN

CH\(_3\)

A1R: HO-(CH\(_2\))\(_3\)C-[MMA]\(_n\)S-C
          CN
          CH\(_3\)

B1S: HO-(CH\(_2\))\(_3\)C-[MMA]\(_n\)SH
          CN

CH\(_3\)

2 The molar mass of the monomer calculated from peak series in MS, see Appendix A.

3 The total molar mass of polymer-chain end-groups and associated cations calculated from peak series in MS, see Supporting Info.

4 Experimental m/z value of end-groups: intercept minus associated cation.

5 Theoretically calculated m/z value.
Surprisingly, the MALDI-TOF mass spectrum of the fraction V37B0 is very similar to the one of V37A0, and V37B1 is roughly identical to V37A1. Yet, the end-groups of the polymers are known to be different. The UV (ultraviolet) spectrum of sample V37A showed strong absorbance with a maximum at 300 nm in dichloromethane, which is ascribed to the RAFT dithioester moiety [S=C(C₆H₅)S-]. The UV spectrum of sample V37B, which was prepared by removing the RAFT dithioester moiety via end-group modification of the RAFT polymer [35], showed no UV absorbance at 300 nm.

Panels a and b of Fig. 3.3 compare the MALDI and ESI spectra for non-functional PMMAs V37A0 in the mass range of 1,400 to 2,050 amu. The ESI-QTOF mass spectrum is different from the MALDI-TOF spectrum for the same sample. End-groups calculated from the MALDI spectrum of V37A0 are different from those obtained from the ESI spectrum. In the former case the total end-group mass is consistent with hydrogen and initiator moiety [(CH₃)CN-] as the end-groups. In the latter case the end-groups of V37A0 can be assigned to the RAFT dithioester moiety and the initiator moiety, which correspond to the non-functional structure. The fragments lost in the MALDI experimental process account for 152 amu.

We also compare the ESI and MALDI spectra for mono-functional PMMA V37A1 (see Appendix A Fig. Ap-3). Again, the ESI mass spectrum is different from that obtained by MALDI for the fraction V37A1. The total end-group mass calculated from the MALDI spectrum of V37A1 is different (152 amu lower) than that obtained from the ESI spectrum. From the ESI spectra we can conclude that sample V37A1 does contain PMMA with the RAFT dithioester moiety and the leaving group of the RAFT agent [HO(CH₂)₃CH₃CCN-], which together result in a mono-functional structure (one hydroxyl group).

There are also differences between MALDI-MS and ESI-MS spectra for samples V37B0 and V37B1 (see discussion below). The end-groups observed in ESI-MS are different from those observed in MALDI-MS, as shown in table 3.2.

In conclusion, the weakly bonded end-groups were cleaved in the MALDI-MS experimental process, while the polymers were observed intact in ESI-MS. This indicates that in the present case ESI is a much softer ionization technique than MALDI. This is in agreement with the results obtained by Musat et al. [23].

### 3.3.2 Off-line LC//ESI-QTOF-MS and MS²

Fig. 3.4a shows an expanded portion of the ESI spectrum for V37A1 in the range of 980 to 1100 amu. It can be seen clearly from Fig. 3.4a and table 3.2 that singly charged ions, doubly charged ions (difference between isotopic peaks 0.5 amu) and triply charged ions (isotopic peaks separated by 0.33 amu) occur in the ESI spectrum. These different series of peaks arise from the same series of PMMA (same end-groups).
Fig. 3.3  Comparison of MALDI and ESI mass spectra for non-functional RAFT polymer (V37A0). (a) MALDI-TOF-MS; (b) ESI-QTOF-MS.
Fig. 3.4  ESI-QTOF mass spectra of mono-functional RAFT polymer (V37A1). (a) Enlarged part of ESI-QTOF-MS; (b) ESI-MS$^2$ selected at 1088 amu; (c) the proposed fragmentation of the 8-mer in ESI-CID-MS$^2$. 

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To verify the obtained structure, multiple-stage MS (ESI-MS²) experiments were carried out on selected precursor ions with helium as collision gas. This technique takes advantage of the ion-trap mechanism and allows for the fragmentation of a selected precursor ion by collision-induced dissociation (CID) and results in a product-ion spectrum. Fig. 3.4b shows a representative MS² spectrum of the precursor ion with mass-to-charge ratio m/z 1088 corresponding to n = 8 (V37A1) with a collision voltage of 50 V. The RAFT dithioester moiety (152.98 amu) was observed to split off easily. The resulting macroradical (the polymer chain without the RAFT group) loses an additional hydrogen to give rise to the most abundant peak at m/z 934.50. Also a series of fracture ions with m/z 135, 235, 335, and 445 were observed. These correspond to PMMA chains with the RAFT leaving group and a sodium cation. The fragmentation scheme is shown in Fig. 3.4c.

The ESI-CID-MS² spectrum (Appendix A Fig. Ap-4) for the m/z=1044 (n=8) molecular-ion peak of V37A0 shows similar results. The RAFT dithio moiety (152.98 amu) was again observed to split off easily. An additional hydrogen is abstracted from the polymer chain, to give rise to the most abundant peak at m/z 890.63. A series of fracture ions with m/z 191, 291, 391, etc. were observed, which correspond to PMMA prepolymer with an initiator moiety as an end-group.

All our results can be explained from the RAFT polymerization mechanism (Scheme 1). Our results provide direct evidence that the RAFT polymers still present “latent living” (or dormant) characteristics in the form of the dithioester end-group. It can be used for further-chain-extension if new batches of initiator and monomer are added. We can conclude that the dithio moiety very easily splits off from the RAFT polymer, as shown in MALDI-TOF-MS as well as in ESI-CID-MS² experiments.

We proved directly that the RAFT polymer V37A contained not only a fraction V37A1 featuring the leaving group of the RAFT agent, but also a fraction V37A0 featuring initiator moiety. Therefore, an initiator with a radical fragment (I) that has the same structure as the leaving group of the RAFT agent should be used to synthesize RAFT polymers with a well-defined functionality. Based on this knowledge, a new hydroxyl-functional initiator was used to obtain a well-defined mono-functional RAFT polymer with a narrow molecular-weight distribution (see references 34, 35).

In order to characterize sample V37B, fractions V37B0 and V37B1 were also subjected to ESI-QTOF-MS. For the di-functional fraction of V37B2 collected at 3.3-4.3 min, highly complex ESI-MS spectra were obtained, which will not be discussed in the present paper. Fig. 3.5a shows an enlarged part of the ESI spectrum for mono-functional polymer V37B1 in the mass range of 935 to 1040 amu. Despite the rather low ionization efficiency, which is apparent from the relatively high base-line noise, the qualitative information from this spectrum is clear. It can be seen from Fig. 3.5a and table 3.2 that singly, doubly and triply charged ions are all present in the spectrum of V37B1, (somewhat) enhancing the complexity. There are two dominant structures B1H and B1S (see table 3.2 and Appendix A table Ap-1). The ESI-CID-MS² spectrum for the m/z 1036 (n=9) molecular-ion peak, which is shown by way of example in Fig. 3.5b, provides further evidence for the structure of B1H. A series of fracture ions was identified with m/z 135, 235, 335, and 445, which correspond to PMMA polymers with the RAFT leaving group as a chain-end. The most abundant peak at m/z 210
and the associated series correspond to hydrogen as another end-group in sample V37B1. The fragmentation scheme is shown in Fig. 3.5c (see reference 36). The analysis of sample V37B0 via ESI-MS is similar and the results are also summarized in table 3.2.

![ESI-QTOF mass spectra of mono-functional polymer V37B1. (a) Enlarged section (935-1040 amu); (b) ESI-MS² with 1036 as the parent ion; (c) the proposed fragmentation of the 9-mer in ESI-CID-MS².](image_url)
3.3.3 On-line LC-ESI-QTOF-MS

In order to further confirm the hydroxyl-based separation, we used on-line LC-ESI-QTOF-MS to identify the repeating units and, especially, the end-groups. Two narrow-bore columns (2 × 150 mm × 1.0 mm i.d) were used, as these were compatible with the ESI interface without post-column splitting of the effluent (LC eluent flow rate 0.1 ml/min). However, a mobile phase containing 40% acetonitrile in dichloromethane could not be used to elute the PMMA samples from the two columns. The critical solvent composition for the two small columns was about 5% higher in acetonitrile concentration compared with the conventional column (150 mm × 4.6 mm i.d).

An example of the summed mass chromatogram of sample V37A using (near) critical conditions (45% acetonitrile in dichloromethane) is shown in Fig. 3.6. Fig. 3.6a shows the summed chromatogram of masses set at 244+100.06n and 288+100.06n (n is an integral number, which represents the degree of polymerization of the polymer, from 0 to 22 in the present case). These two series correspond to non-functional (244 series, Fig. 3.6c) and mono-functional (288 series, Fig. 3.6b) RAFT polymers, respectively (see Appendix A). It can be seen from Fig. 3.6 that the non-functional polymer (no OH group) eluted earlier (2.17 min.) than the mono-functional polymer (one OH group; 2.67 min). This confirms the base-line separation of polymers according to functionality as seen in Fig. 3.1.

The mass spectra of the two peaks at elution times of 2.2 and 2.6 min (Appendix A Fig. Ap-5) are similar to those in Figs 3a and Ap-3a. The only observed difference in these mass spectra is that next to the sodium-cation (Na⁺) series also a high-intensity potassium-cation (K⁺) series (with a mass difference of 16 amu) was observed in the on-line mass spectra. This is probably due to traces of potassium present in the mobile phase.

On-line MS monitoring of elution profiles of individual oligomeric species can also facilitate a better understanding of LC separation mechanisms [16]. For example, thanks to the high mass accuracy of mass spectrometry [18], on-line LC-ESI-QTOF-MS can be used to investigate the effect of the molecular weights of the polymer on the elution behavior. Fig. 3.7 shows an example of mass chromatograms for various selected masses for sample V37A. It can be seen that the retention times of PMMA oligomers with different molecular weights were almost identical. However, it also can be seen from Fig. 3.7 that the retention time of the polymer increased slightly with increasing molecular weight (from 2.53 to 2.85 minutes for mono-functional PMMAs with n increasing from 0 to 22). The retention time increased from 2.10 to 2.24 minutes for non-functional PMMAs with n between 0 and 20 (Appendix A Fig. Ap-6). This indicates a slight adsorption effect of PMMA, so that the LC conditions are not perfectly critical. We therefore speak of near-critical conditions. The original RAFT agent (n =0) was also observed in the ESI-QTOF-MS spectra of the low-molecular-weight RAFT polymers.
Fig. 3.6 LC-ESI-QTOF-MS summed mass chromatogram of sample 37A. (a) Summed masses set at 244+100.1n and 288+100.1n (n: 0-22); (b) 288 series; (c) 244 series (see explanation in text and Appendix A Fig. Ap-3). Mobile phase: 45% acetonitrile in dichloromethane, two home-packed silica columns (150 mm x 1 mm i.d.; 3-μm particles; 100-Å pore size), flow rate 0.10 ml/min.
MALDI-TOF-MS and on-line LC-ESI-QTOF-MS were performed to analyze hydroxyl-functional PMMA samples synthesized by RAFT polymerization. The end-groups of a non-functional fraction containing the RAFT dithioester moiety \([S=\text{C}(\text{C}_6\text{H}_5)\text{S}]-\) and the initiating-radical fragment \([\text{(CH}_3\text{)}_2\text{CCN}]-\) could be assigned directly from the ESI spectrum. However, the dithioester moiety was lost in the MALDI experimental process. It was also observed to split off easily in collision induced dissociation (ESI-CID-MS\(^2\)) experiments, which further verified the structure of the non-functional fraction of the RAFT polymer. Similar results were obtained with ESI-MS for the mono-functional fraction of the RAFT polymer, which was shown to contain the leaving group of the RAFT agent \([\text{HO(CH}_2\text{)}_2\text{CH}_3\text{CCN}]-\) and the RAFT dithioester moiety. The latter was not observed in the MALDI-MS spectrum. These results provide direct evidence that the RAFT polymers still present latent living character through the presence of the dithioester moiety. This property can be used for further chain extension with addition of fresh monomer and fresh initiator. The structures of non-functional and mono-functional fractions of a derivatized RAFT polymer were also established and discussed. All these results are in agreement with a contemporary interpretation of the RAFT polymerization process and the derivatization reaction. The active (weakly bonded) end-groups, such as the dithioester moiety, were lost in the MALDI-MS experiments, while the
polymeric molecules were observed intact in the ESI-MS spectra. This indicates that in the present case ESI is a much softer ionization method than MALDI.

On-line LC-ESI-QTOF-MS was used to provide further evidence for the structure of the end-groups and to study the mechanism of the LC separation. Specifically the effect of the polymer molar mass on the elution behavior was studied. The results confirmed the base-line separation of the functional prepolymermers according to the number of hydroxyl groups. They also revealed a slight adsorption effect of PMMA under the experimental (near-critical) conditions.

Acknowledgments

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References

Mass Spectrometric Characterization of Functional PMMA in Combination with Critical LC

Appendix A

Additional ESI-MS spectra, assignment, mass chromatograms as well as explanatory texts are provided in this appendix.

Fig. Ap-1  MALDI-TOF mass spectra for the first two fractions of sample PMMA V37A and V37B. (V37A0) Non-functional fraction of V37A collected at 1.7-2.2 min; (V37A1) Mono-functional fraction of V37A (2.3-3.3 min); (V37B0) Non-functional fraction of V37B (1.7-2.2 min). (V37B1) Mono-functional fraction of V37B (2.3-3.3 min). LC separation conditions were the same as in figure 1, except a flow rate of 0.5 ml/min.
The polymer end groups can be calculated from a polymeric series in a mass spectrum using equation 1

$$M_{\text{peak}} = n \times M_{\text{monomer}} + M_{\text{end group}} + M_{\text{counterion}}$$  \hspace{0.5cm} (1)

where $M_{\text{peak}}$ is the molar mass value of the selected peak, $M_{\text{monomer}}$ the molar mass of the monomer, $n$ the (integral) number of monomer repeat units, $M_{\text{end group}}$ the total molar mass of chain end groups (including the initiating group and the end-capping group), and $M_{\text{counterion}}$ the molar mass of the counterion attached in the ionization process.

Plot $M_{\text{peak}}$ vs. $n$ should yield a straight line. The slope of the line represents the value of $M_{\text{monomer}}$. The intercept of the line is the sum of $M_{\text{end group}}$ and $M_{\text{counterion}}$. Figure SI-1 shows an example of the calculation of the total end-group mass ($M_{\text{end group}}$). All the calculation results and structures are summarized in table 2.

![Representative calculation of sum of the two end-groups and the counterion in sample V37A1 from MALDI-TOF-MS and ESI-QTOF-MS data.](image)

Fig. Ap-2

Fig. Ap-4a shows an expanded portion of the ESI spectrum of V37A0 in the range of 930 to 1050 amu. It can be clearly seen from Fig. Ap-4a that singly charged ions exist next to doubly charged ions based on the isotopic peaks separated by 0.5 amu.

Fig. Ap-4b displays the MS$^2$ spectrum for the $m/z$ 1044 ($n = 8$) molecular ion peak. The fragmentation scheme is shown in Fig. Ap-4c.
Fig. Ap-3  Comparison of MALDI and ESI mass spectra for mono-functional RAFT polymer (V37A1). (a) MALDI-TOF-MS; (b) ESI-QTOF-MS.
Fig. Ap-4  ESI-QTOF mass spectra of non-functional RAFT polymer (V37A0). (a) Enlarged part of ESI-QTOF MS; (b) ESI-MS² selected at 1044 amu; (c) the proposed fragmentation of the 8-mer in ESI-CID-MS².
Table Ap-1 Assignments of the peaks in the ESI-QTOF-MS spectrum of V37B1 (Fig. 3.6a).

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The calculated value of B1H: $m/z = [112.08 \text{ (HO(CH}_2)_3\text{CH}_3\text{CCN-)} + n \times 100.06\text{ (PMMA backbone)} + 1.01 (-H) + z \times 22.99 \text{ (Na)}^+]/z$, where $n$ refers to the degree of polymerization, and $m$ to the number of charges (Na$^+$) on the polymers.

The calculated value of B1S: $m/z = [112.08 \text{ (HO(CH}_2)_3\text{CH}_3\text{CCN-)} + n \times 100.06\text{ (PMMA backbone)} + 32.98 (-SH) + z \times 22.99 \text{ (Na)}^+]/z$, where $n$ refers to the degree of polymerization, and $m$ to the number of charges (Na$^+$) on the polymers.

Fig. Ap-5 shows ESI-MS spectra of the non-functional peak eluted at 2.2 min (a) and monofunctional peak eluted at 2.6 min (b) for sample V37A. Two different series were observed with a repeating unit of 100.1 Da (PMMA unit). They result from the counter-ions, sodium cation (Na$^+$) and potassium cation (K$^+$) respectively, with mass difference of 16 amu. The calculated mass of the end groups ($M_{\text{end group}} = 221$ amu) at an elution time of 2.2 min corresponds to polymeric structures with (CH$_3$)$_2$CCN as one end group and SCSC$_6$H$_5$ as the other end group (Fig. Ap-5a). The calculated mass of the end groups ($M_{\text{end group}} = 265$ amu) at an elution time of 2.6 min corresponds to structures with HO(CH$_2$)$_3$CH$_3$CCN as one end group and SCSC$_6$H$_5$ as the other end group (Fig. Ap-5b). These confirm the results obtained in off-line ESI-QTOF-MS and are in agreement with our understanding of the RAFT polymerization process. On this basis, the summed chromatograms selected masses of 244+100.06$n$ and 288+100.06$n$ are shown in Fig. 3.6.
Fig. Ap-5  ESI-MS spectra of the non-functional peak eluted at 2.2 min (a) and mono-functional peak eluted at 2.6 min (b) from sample V37A.
Fig. Ap-6 illustrates the effect of the molecular mass on retention time for non-functional PMMAs. The selected mass-to-charge ratio is shown in the second line of the top-right corner. The value shown in the curve is the retention time.

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**Fig. Ap-6**  Representative (near-critical) LC × MS mass chromatograms for various selected masses of non-functional PMMA from sample V37A. Mobile phase: 45% acetonitrile in dichloromethane, two home-packed silica columns (150 mm × 1 mm i.d.; 3-μm particles; 100-Å pore size), flow rate 0.10 ml/min.
CHAPTER 4

Separation and Characterization of Functional Poly(n-Butyl Acrylate) by Critical LC

ABSTRACT

The separation of functional poly(n-butyl acrylate) (PnBA) polymers based on the number of end-groups under critical-liquid-chromatography (LC) conditions has been studied using a bare-silica column. The (near-) critical solvent compositions for non-, mono-, and di-functional (telechelic) carboxyl-PnBAs were determined in normal-phase LC, using mixtures of acetonitrile, acetic (or formic) acid, and dichloromethane of varying composition. Some formic or acetic acid had to be added to the mobile phase to elute PnBA polymers with carboxyl end-groups. The critical solvent compositions obtained were not exactly the same for non-, mono-, and di-functional PnBA polymers. These were unusual experimental observation, but they were in agreement with theoretic predictions. Nevertheless, low-molecular-weight PnBA samples were successfully separated according to the carboxyl functionality at (near-) critical conditions.

With the aid of mass spectrometry (MS), the (near-) critical separation of low-molecular-weight PnBA polymers was confirmed to be mainly based on the carboxyl functionality. Calibration curves for the evaporative-light-scattering detector (ELSD) were used for quantitative analysis of carboxyl-functional PnBA polymers. The results proved that nearly ideal functionalities (average number of carboxyl end-groups per molecule up to 1.99) were achieved by one-step RAFT polymerization of PnBA.

Keywords: critical liquid chromatography; telechelic polymers; RAFT; functionality; carboxylic-acid end-group.

Chapter 4

4.1 Introduction

Functional (pre-)polymers can be used as precursor (cross-linking) components in coating formulations to create durable films. For a better understanding of the structure-property relationships of such coatings, functional polymers with specific architectures, well-defined molecular weights, and low polydispersities are required. For example, highly pure, linear telechelic poly(meth)acrylates, bearing hydroxyl or carboxyl end-groups and corresponding monofunctional polymers are very desirable. Such polymers can be used to synthesize well-defined networks and, thus, to study the effect of cross-link density and dangling ends on the formation and (mechanical) properties of poly(meth)acrylate networks. However, it is difficult to produce well-defined polymers with the desired end-groups by conventional polymerization methods. The functional polymers in this work have one or two hydroxyl or carboxyl end-groups and their functionality means the number of hydroxyl or carboxyl end-groups.

In the past twenty years numerous research groups have devoted their efforts towards the control of radical-polymerization processes. The most important methodologies include nitroxide-mediated radical polymerization [1], atom-transfer radical polymerization (ATRP) [2], and, most recently, reversible addition-fragmentation chain-transfer (RAFT) polymerization [3]. Among these, RAFT may arguably have the greatest commercial impact, because the process only involves organic substances and because it works very well with most vinyl monomers, including acrylic acid [4]. RAFT can be employed in many polymerization processes (e.g. bulk, solution, suspension, emulsion polymerization [5]).

Therefore, we have selected RAFT polymerization to prepare linear α,ω-functional polymers (commonly known as telechelic polymers) with either hydroxyl or carboxyl end-groups and low polydispersities. Lima et al. [6] have described various reaction schemes and procedures to synthesize well-defined telechelic poly(meth)acrylates using functionalized initiators and RAFT agents. Their work has resulted in polymers with predictable molecular weights and low polydispersities. Ideally, linear telechelic polymers have a functionality of two. It is a serious challenge to approach this limit, mainly because of side reactions inherent to growing radicals, such as termination by bimolecular combination or disproportionation [7]. Moreover, chain transfer to solvent molecules or to monomers will always occur. Finally, in some cases post-polymerization modifications are necessary. Experience with macromolecular reactions indicates that hydroxy-endcapping reactions do not proceed with full conversion [7], and that they are accompanied by side reactions [6,8].

The development and optimization of procedures for the synthesis of well-defined functional polymers is vitally dependent on effective analytical methods to determine the functionality-type distribution (FTD) and the molecular-weight distribution (MWD). The traditional analytical techniques, such as IR (infrared absorption spectroscopy), UV (ultraviolet spectroscopy), NMR (nuclear-magnetic-resonance spectroscopy), and specific titrations of −OH or −COOH groups, are inadequate for this purpose, because only the average functionality can be measured, and not the FTD. Mass spectrometry (MS) can provide good qualitative information on polymer end-groups,
but poor quantitative results. This is because of the variation in the ionization efficiency for different functional polymers. Also mass-discrimination and ion-suppression effects are encountered in polymer MS [9]. To determine accurate FTDs and specific MWDs for molecules of a given functionality, the functional polymers must be separated based on the number of end-groups. The application of critical LC in polymer science and industry is still new and challenging [10-17]. In principle, this technique can be used to separate polymers exclusively according to the number of functional groups, for example into non-, mono-, and di-functional polymers [18,19,20,21]. However, critical conditions do not necessarily provide good functionality-based separations [10]. In a previous study [22], we have established robust critical LC conditions for the separation of hydroxyl-functional PMMA samples. With the aid of mass spectrometry, separation was confirmed [8] to be mainly based on the number of hydroxyl groups present in the low-molecular-weight RAFT polymers, either with or without end-group modification. In this work a similar strategy is employed for a different polymer with different functional end-groups. As model polymers for this study, poly(α-buty l acrylate) (PnBA) polymers with COOH end-groups were synthesized by using one step RAFT polymerization, according to the procedure described by Lai et al. [4]. The resulting polymers had to be separated based on the number of end-groups. Various possible (near-) critical solvent compositions for PnBA were studied in normal-phase LC. Ternary mixtures of acetonitrile, dichloromethane, and acetic (or formic) acid were explored. The temperature was also varied. Subsequently, mass spectrometry was used to identify the repeating units and, especially, the end-groups of the fractionated polymers.

4.2 Experimental

4.2.1 Chemicals

Dichloromethane (DCM), acetonitrile (ACN) (both HPLC grades, from Rathburn Chemicals (Walkerburn, Scotland), formic acid (FA, p.a. grade, Merck, Darmstadt, Germany), and acetic acid (HAc, p.a. grade, Acros Organics, Geel, Belgium) were used without further purification. Non-functional poly(α-buty l acrylate) (PnBA) samples were synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization ("RAFT polymers") using 2,2'-azobisisobutyronitrile (AIBN, Merck, Darmstadt, Germany) as initiator and a non-COOH-functional RAFT chain-transfer agent (2-cyanoprop-2-yl dithiobenzoate, RAFT-AIBN). PnBAs with one COOH group were synthesized by RAFT polymerization using AIBN or carboxyl-terminated azo-initiator 4,4'-Azobis(4-cyanovaleric acid) (ACVA, Aldrich, Milwaukee, WI, USA) as the initiator and a mono-carboxyl-terminated trithiocarbonate derivative, S-1-dodecyl-S'-(α,α'-dimethyl-α''-acetic acid)trithiocarbonate, as the RAFT chain-transfer agent. Linear PnBAs with two COOH groups were synthesized by RAFT polymerization using AIBN or ACVA as the initiator and a di-carboxyl-terminated trithiocarbonate, S,S'-Bis(α,α'-dimethyl-α''-acetic acid)trithiocarbonate, as the RAFT chain-transfer agent [6]. For the sake of clarity, some synthesis data are reported in table 4.1. These data show the origin of structural differences between different functional RAFT-PnBA samples. The details of the synthetic procedures were described in reference 6. The molecular
weights and molecular-weight distributions were measured by SEC with a Waters (Milford, MA, USA) instrument consisting of a Waters model 510 pump and a model 410 differential refractometer (operated at 40°C). THF was used as the eluent at a flow rate of 1.0 ml/min. The columns used were a PLgel guard column (5-µm particles) 50 × 7.5 mm, followed by two PLgel mixed-C (5-µm particles) 300 × 7.5 mm columns (Polymer Laboratories, Church Stretton, Shropshire, UK) kept in an oven at 40°C. The calibration curve was prepared with polystyrene (PS) standards (molecular weights ranging from 580 to 7.1 × 10^6 g mol⁻¹) and the molecular weights were estimated based on the universal-calibration principle and Mark-Houwink parameters (PS, K = 1.14 × 10⁻⁴ dL g⁻¹ and α = 0.716; PnBA: K = 1.220 × 10⁻⁴ dL/g, α = 0.700) [23]. The effect of the carboxyl end-groups on the Mark-Houwink parameters was neglected. All the PnBA samples used are summarized in Table 4.2. All the samples were dissolved in DCM unless stated otherwise.

### Table 4.1 Synthesis data of PnBA samples by RAFT polymerization.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Initiator</th>
<th>CTA°</th>
<th>[RAFT]/[Ini.]</th>
<th>Conversion</th>
<th>M_{n,exp} (M_{n,cal}) (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL068 (PnBA-2COOH 2.510)</td>
<td>AIBN</td>
<td>A</td>
<td>20</td>
<td>99%</td>
<td>2.23 (2.20)</td>
</tr>
<tr>
<td>VL103 (PnBA-2COOH 2.400)</td>
<td>AIBN</td>
<td>A</td>
<td>8</td>
<td>95%</td>
<td>2.09 (2.20)</td>
</tr>
<tr>
<td>VL123 (PnBA-2COOH 3.200)</td>
<td>ACVA</td>
<td>A</td>
<td>8</td>
<td>97%</td>
<td>2.83 (3.20)</td>
</tr>
<tr>
<td>VL127 (PnBA-2COOH 2.500)</td>
<td>ACVA</td>
<td>A</td>
<td>8</td>
<td>99%</td>
<td>2.26 (2.20)</td>
</tr>
<tr>
<td>VL131 (PnBA-COOH 2.610)</td>
<td>ACVA</td>
<td>B</td>
<td>20</td>
<td>99%</td>
<td>2.38 (2.20)</td>
</tr>
</tbody>
</table>

*All polymerizations were carried out at 80°C, in a toluene/acetone (1:1 v/v) mixture under an argon atmosphere.

°CTA: RAFT chain-transfer agent.

A = \text{HOOC}_-\text{S}_-\text{S}_-\text{COOH}  
B = \text{C}_{13}\text{H}_{25}_-\text{S}_-\text{S}_-\text{COOH}

#### 4.2.2 Equipment

A Waters (Milford, MA, USA) 2690 Alliance liquid-chromatography system was used to perform the isocratic LC experiments. This HPLC instrument contained a built-in auto-injector with a sample loop allowing injection of variable sample volumes and it was equipped with a Waters 996 PDA (photodiode-array detector) and a Sedex 55 evaporative light-scattering detector (ELSD; temperature 62°C, N₂ pressure 2.2 bar). The mobile phase was prepared in-situ using the solvent-mixing capability of the instrument. The formic or acetic acid was added in the form of a premixed solution of 10% v/v in DCM. All eluent compositions are given in volume %. The data collection and the data analysis were handled by Waters Millennium 3.2 software. The columns used (150 mm x 4.6 mm i.d.) were packed in-house with Hypersil Silica (3-µm particles; 100-Å pore size; Shandon, Runcorn, UK).

The MALDI-TOF-MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) measurements were performed with a Voyager-De Pro instrument (PerSeptive Biosystems, Framingham, MA, USA) equipped with a 337-nm nitrogen laser. Spectra were acquired by
summing the data obtained from 200 laser shots in the reflector mode. α-Cyano-4-hydroxycinnamic acid (about 20 mg/ml in THF) was used as the matrix. The concentration of the polymer sample was about 1 mg/ml in THF.

**Table 4.2** PnBA samples used in this study. The molecular weight ($M_n$, $M_p$) and polydispersity-index (PDI) values were measured by SEC (Calibration described in the Experimental section).

<table>
<thead>
<tr>
<th>Sample name</th>
<th>$M_n$(kDa)</th>
<th>$M_p$(kDa)</th>
<th>PDI</th>
<th>Intended functionality</th>
</tr>
</thead>
<tbody>
<tr>
<td>PnBA 600$^a$</td>
<td>NA$^b$</td>
<td>6.00</td>
<td>NA$^b$</td>
<td>0</td>
</tr>
<tr>
<td>PnBA 4,690$^a$</td>
<td>3.45</td>
<td>4.69</td>
<td>1.32</td>
<td>0</td>
</tr>
<tr>
<td>PnBA 7,390$^a$</td>
<td>4.24</td>
<td>7.39</td>
<td>1.53</td>
<td>0</td>
</tr>
<tr>
<td>PnBA 32,330$^a$</td>
<td>19.28</td>
<td>32.33</td>
<td>1.49</td>
<td>0</td>
</tr>
<tr>
<td>PnBA-COOH 2,610$^c$</td>
<td>2.38</td>
<td>2.61</td>
<td>1.13</td>
<td>1</td>
</tr>
<tr>
<td>PnBA-COOH 13,260$^{c,d}$</td>
<td>4.61</td>
<td>13.26</td>
<td>2.05</td>
<td>1</td>
</tr>
<tr>
<td>PnBA-COOH 19,090$^{c,d}$</td>
<td>9.54</td>
<td>19.09</td>
<td>1.63</td>
<td>1</td>
</tr>
<tr>
<td>PnBA-2COOH 2,400$^{d,c}$</td>
<td>2.09</td>
<td>2.32</td>
<td>1.13</td>
<td>2</td>
</tr>
<tr>
<td>PnBA-2COOH 2,500$^c$</td>
<td>2.26</td>
<td>2.50</td>
<td>1.11</td>
<td>2</td>
</tr>
<tr>
<td>PnBA-2COOH 2,510$^{d,c}$</td>
<td>2.23</td>
<td>2.51</td>
<td>1.13</td>
<td>2</td>
</tr>
<tr>
<td>PnBA-2COOH 3,200$^c$</td>
<td>2.83</td>
<td>3.20</td>
<td>1.10</td>
<td>2</td>
</tr>
<tr>
<td>PnBA-2COOH 5,540$^{d,c}$</td>
<td>4.41</td>
<td>5.54</td>
<td>1.21</td>
<td>2</td>
</tr>
<tr>
<td>PnBA-2COOH 11,450$^{d,c}$</td>
<td>8.64</td>
<td>11.45</td>
<td>1.22</td>
<td>2</td>
</tr>
</tbody>
</table>

$^a$ Non-functional PnBA samples synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization using a 2,2'-azobisisobutyronitrile (AIBN) initiator and a non-COOH-functional RAFT chain-transfer agent.

$^b$ Because the SEC peak overlaps with the solvent peak, the values of the molecular weights ($M_n$, $M_p$, and PDI) cannot be calculated accurately.

$^c$ PnBAs with one COOH group synthesized by RAFT polymerization. For synthetic procedures see Experimental Section.

$^d$ AIBN initiator used in RAFT polymerization.

$^e$ PnBAs with two COOH groups synthesized by RAFT polymerization. For synthetic procedures see Experimental Section.

The ESI-MS (electrospray-ionization mass spectrometry) experiments were carried out using a Finnigan LCQ Deca XP MAX ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA). Polymer samples were dissolved in a 1:1 v/v mixture of acetonitrile/methanol at a concentration of around 1 mg/ml. The flow rate of the sample introduced into the electrospray interface was 20 μl/min. The electrospray-source voltage was 5.0 kV and the electrospray-source temperature was 275°C. Mass spectra were scanned over the range $m/z$ 500-4000 in the positive-ion mode. More than 100 scans were summed to produce the final spectrum.

ESI-MS/MS experiments were carried out on the selected precursor ions by low-energy collision-induced dissociation (CID) using helium as collision gas to obtain the fragmentation-ion spectrum.
4.3 Results and discussions

4.3.1 Critical conditions for PnBA

Our objective was to establish critical LC conditions to separate linear poly(n-butyl acrylate) (PnBA) samples exclusively based on the number of carboxyl end-groups. In a previous study [22] robust critical LC conditions were established for the separation of hydroxyl-terminated poly(methyl methacrylate) (PMMA) samples using mixtures of acetonitrile (ACN) and dichloromethane (DCM) as the mobile phase. A bare-silica column was used, which gave rise to sufficiently strong interactions with the functional (hydroxyl) groups. DCM is a good solvent for PMMA and also for PnBA, but it is a weak eluent on a silica column [24]. These properties make it a good injection solvent in interactive LC, as breakthrough problems can be avoided [25]. In this study all samples were dissolved in DCM unless stated otherwise. Acetonitrile is a more-polar solvent, which can desorb PnBA from the silica column. Therefore, these two solvents were selected to make up the mobile phase.

Following the method of Cools et al. [12], the obtained retention times for linear PnBA samples of varying molecular weight were plotted against the percentage of acetonitrile (as shown in Fig. 4.1). The intersection point provides an indication of the critical solvent composition, at which the retention time of the polymer is independent of the molecular weight. The critical point for non-functional PnBA was found to be around 11% of acetonitrile in DCM at 25°C (see table 4.3a). However, the PnBA samples with one or two carboxyl end-groups were fully retained on the silica column under these conditions. Increasing the concentration of acetonitrile in the mobile phase, even up to pure acetonitrile did not result in elution of these samples, even though acetonitrile is a good solvent for low-molecular-weight PnBAs with carboxyl end-groups. To overcome this problem, some formic acid (FA) or acetic acid (HAc), added in the form of a premixed solution of 10% v/v in DCM, had to be added to the mobile phase.

Figs. 4.1a, 4.1b and 4.1c show an example of the dependence of the retention time on the mobile-phase composition (percentage of acetonitrile in DCM) at a constant concentration of 0.5% HAc and at 55°C for non-functional PnBA, mono-functional PnBA-COOH, and di-functional PnBA-2COOH samples of varying molecular weights. The critical solvent compositions obtained were about 6.6, 5.8 and 5.5 % v/v acetonitrile in DCM for non-functional PnBA, mono-functional PnBA-COOH, and di-functional PnBA-2COOH polymers, respectively. The obtained critical points for various end-group series were not exactly the same. This was an unusual experimental observation, because, in principle, the critical composition for the PnBA backbone should not change with the end-groups. However, the observed differences could not be ascribed to experimental error. As shown in table 4.3a, a variation in the exact critical composition with variation in the end-groups present was also observed at other temperatures (25 and 40°C). Gorbunov et al. [21] and Skvortsov et al. [26] ratiocinated from theory that the retention of di-functional polymers at the critical point for non-functional polymer depends on the molecular weight. They stated that the distribution coefficient of functional polymers could exceed unity and would decrease with the radius of gyration (molecular weight) if the interaction of end-groups with the stationary phase was strongly attractive. Our observations are in agreement with these predictions. At the critical point of non-
functional PnBA (6.6% ACN), carboxyl end-groups show a strong attractive interaction with the stationary phase. The relative effect of this interaction will decrease with increasing molecular weight and, therefore, the retention of carboxyl-functional PnBA sample decreases. To reduce the effect of the molecular weight on the retention of carboxyl-functional PnBA samples, slightly less acetonitrile should be present in the mobile phase. The amount of acetonitrile required decreases with increasing number of end-groups. An example is shown in Fig. 4.1d, with 6% acetonitrile and 0.5% HAc in DCM as the mobile phase at 55°C. The retention for non-functional PnBA increased with increasing molecular weight. In contrast, the retention for mono-functional PnBA-COOH and di-functional PnBA-2COOH decreased with increasing molecular weight under the same conditions. It also can be seen from Fig. 4.1d that low-molecular-weight PnBA samples could be separated much more easily based on the number of carboxyl end-groups, than could high-molecular-weight PnBA samples.

It also can be seen in Fig. 4.1 that the critical conditions are rather sensitive to the exact eluent composition, which is in agreement with the data reported in reference [27]. Due to the uncertainty surrounding the exact location of the critical point and the residual (slight) variation of retention with molecular weight, we speak of near-critical conditions in this paper. The present near-critical conditions are much-less robust than the critical conditions reported previously for hydroxyl-functional PMMA [22]. From a combination of Figs. 4.1a, 4.1b, 4.1c and 4.1d, we conclude that a mobile phase containing about 6.0% acetonitrile and 0.5% acetic acid in DCM can be used to separate the low-molecular-weight PnBA samples (up to 10,000) based on the number of carboxyl end-groups.

Fig. 4.2 shows representative ELSD (evaporative light-scattering detector) chromatograms of PnBA samples of varying molecular weights and with different numbers of functional end-groups. The two different non-functional PnBA samples (with no COOH groups, molecular weights 4,690 and 32,330) co-eluted. The two mono-functional polymers (with one COOH end-group, molecular weights 2,610 and 13,260) had similar retention times, but were clearly separated from the non-functional PnBA samples. The expectedly telechelic (di-functional) samples (with two COOH end-groups, molecular weights 2,500 and 11,450) were well separated from the mono-functional polymers. Note that there was a small amount of non-functional polymers observed in sample PnBA-COOH 13,260, and a small amount of mono-functional polymers in sample PnBA-2COOH 11,450, because an AIBN initiator was used in the RAFT polymerization (see table 4.2). It also can be seen clearly in Fig. 4.2 that the peaks were broader for the high-molecular-weight samples, which is in agreement with reported observations by Philipson et al. [28] and others (see [17] and references cited therein). The better separation capabilities for low-molecular-weight polymers do not only result from thermodynamics (see Fig. 4.1d) but also from kinetics (peak broadening), as can be seen from Fig. 4.2. In this study we were dealing with low-molecular-weight PnBAs (around 1,000-2,500). These could easily be separated according to carboxyl functionality under the near-critical conditions of Fig. 4.2.
(a) Non-COOH PnBA

(b) Mono-COOH PnBA

Retention time (min)

Acetonitrile (%)
Fig. 4.1 Dependence of retention time on the mobile-phase composition (at 0.5% HAc) for PnBA samples with different molecular weights. (a) Non-functional PnBAs. Squares: PnBA 600, triangles: PnBA 7,390, circles: PnBA 32,330. (b) Mono-functional carboxyl PnBAs. Open Squares: PnBA-COOH 2,610, open triangles: PnBA-COOH 13,260, open circles: PnBA-COOH 19,090 and (c) Di-functional carboxyl PnBAs. Squares: PnBA-2COOH 2,400, stars: PnBA-2COOH 3,200, triangles: PnBA-2COOH 5,540, circles: PnBA-2COOH 11,450. (d) Molecular-weight effect on retention time for non-, mono-, and di-carboxyl functional PnBAs under near-critical conditions (6% ACN and 0.5% HAc in DCM). Squares: non-COOH, triangles: mono-COOH, circles: di-COOH.

ELSD detector, home-packed Hypersil silica column (150 mm x 4.6 mm i.d.; 3-μm particles; 100-Å pore size), flow rate 0.5 ml/min, column temperature 55°C.
Fig. 4.2  Representative separations (chromatograms) of PnBA functional polymers according to COOH end-groups at 55°C. Detector ELSD, mobile phase 5.7% ACN and 0.5% HAc in DCM, flow rate 0.5 ml/min, injection volume 10 μL, sample concentration 1 mg/ml in DCM, column as in Fig. 4.1. For sample identification see table 4.2.

Note: The peak heights of chromatograms were electronically adjusted for the sake of clarity.

We selected three representative low-molecular-weight samples (PnBA 600, PnBA-COOH 2,610, PnBA-2COOH 2,500) and combined these into a single sample, which we then used to investigate the effects of mobile-phase composition and column temperature. Some of the resulting chromatograms are shown in Fig. 4.3. As shown in Fig. 4.3a, the base-line separation of this mixture of low-molecular-weight samples according to the carboxyl functionality was not affected when the column temperature was changed from 25 to 55°C, although the retention times of mono-functional and di-functional polymers decreased somewhat when the temperature increased. However, it should be noted that this result may not be extrapolated to high-molecular-weight PnBAs (see Section 4.3.3 below). The effect of the mobile-phase composition is illustrated in Fig. 4.3b. Overlapping peaks were obtained when a mixture of 10% acetonitrile and 0.5% HAc in dichloromethane was used as the mobile phase. The separation of the three peaks was somewhat improved with 8% acetonitrile (and 0.5% acetic acid) in dichloromethane (not shown). Base-line separations of this mixture according to the carboxyl functionality were achieved when the mobile-phase composition was between 5% and 6% of acetonitrile (and 0.5% HAc) in dichloromethane at 55°C.
Effect of (a) temperature and (b) mobile-phase composition on the separation of a mixture of PnBA with non-, mono- and di-carboxyl functional polymers (PnBA 600, PnBA-COOH 2,610 and PnBA-2COOH 2500). Detector ELSD, flow rate 0.5 ml/min, injection volume 10 μL, sample concentration PnBA 600 0.3 mg/ml, PnBA-COOH 2,610 0.6 mg/ml and PnBA-2COOH 2500 1.2 mg/ml in DCM, column as in Fig. 4.1. Mobile phase: (a) 6% acetonitrile and 0.5% HAc in DCM; (b) varying % of acetonitrile as indicated plus 0.5% HAc in DCM at 55°C.

Note: The peak heights of chromatograms were electronically adjusted for the sake of clarity.
The experimental results concerning near-critical solvent compositions for the PnBA samples at different acetic-acid concentrations and different column temperatures are summarized in table 4.3a. A typical error for the precision in the estimated critical composition is (+/-) 0.3% (see Figs.4.1a, 4.1c). In some cases the confidence interval may be a bit wider (up to +/- 0.6%, see Fig. 4.1b). This implies that differences in the ACN (or DCM) concentrations of 0.5% in table 4.3a are likely to be significant, whereas differences exceeding 1% are almost certainly significant. It can be seen in table 4.3a that at increased acetic-acid concentrations in the mobile phase lower acetonitrile concentrations were required. This seems quite logical, because both HAc and acetonitrile are polar solvents with higher elution strength than DCM on a silica column. Also, the near-critical solvent composition shifted to lower concentrations of acetonitrile with increasing column temperature, because the interaction of the PnBA backbone with the silica column decreased. For example, the approximate critical composition for di-COOH functional PnBA samples was 6.7% acetonitrile and 0.5% HAc in DCM at 25°C, while at 55°C it was 5.5% acetonitrile and 0.5% HAc. A mobile-phase composition of about 6.0% acetonitrile and 0.5% HAc in DCM at 55°C resulted in a slight decrease in retention with increasing molecular weight for di-COOH functional PnBA samples. The same mobile phase at 25°C showed common adsorption behavior, i.e. retention increased with increasing molecular weight [15]. As discussed below, higher temperatures can be used to completely elute high-molecular-weight PnBA samples at the near-critical composition (about 6.0% acetonitrile and 0.5% HAc in DCM).

**Table 4.3a** Approximate critical ("near-critical") compositions for PnBA samples obtained at different acetic-acid concentrations and at different column temperatures.

<table>
<thead>
<tr>
<th>Functionalities</th>
<th>Temperature, °C</th>
<th>HAc, %</th>
<th>ACN, %</th>
<th>DCM, %</th>
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<tbody>
<tr>
<td>Non-COOH</td>
<td>25</td>
<td>0.5</td>
<td>8.9</td>
<td>90.6</td>
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<tr>
<td></td>
<td>25</td>
<td>1.0</td>
<td>7.4</td>
<td>91.6</td>
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<tr>
<td></td>
<td>40</td>
<td>0.5</td>
<td>7.5</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>0.5</td>
<td>6.6</td>
<td>92.9</td>
</tr>
<tr>
<td>Mono-COOH</td>
<td>25</td>
<td>0.5</td>
<td>6.9</td>
<td>92.6</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.5</td>
<td>5.8</td>
<td>93.7</td>
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<td></td>
<td>55</td>
<td>0.5</td>
<td>5.8</td>
<td>93.7</td>
</tr>
<tr>
<td>Di-COOH</td>
<td>25</td>
<td>0.5</td>
<td>6.7</td>
<td>92.8</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1.0</td>
<td>6.1</td>
<td>92.9</td>
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<td>0.5</td>
<td>5.7</td>
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<td></td>
<td>55</td>
<td>0.5</td>
<td>5.5</td>
<td>94</td>
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</tbody>
</table>
Table 4.3b  Approximate critical ("near-critical") compositions for PnBA samples obtained with a mobile phase containing 0.5% formic acid and at different column temperatures.

<table>
<thead>
<tr>
<th>Functionalities</th>
<th>Temperature, °C</th>
<th>ACN, %</th>
<th>DCM, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-COOH</td>
<td>25</td>
<td>10.2</td>
<td>89.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>9.5</td>
<td>90.0</td>
</tr>
<tr>
<td>Mono-COOH</td>
<td>25</td>
<td>8.3</td>
<td>91.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.5</td>
<td>92.0</td>
</tr>
<tr>
<td>Di-COOH</td>
<td>25</td>
<td>8.2</td>
<td>91.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.4</td>
<td>92.1</td>
</tr>
</tbody>
</table>

Formic acid was also tested instead of HAc as the modifier in the mobile phase. Because of the low boiling point of formic acid, it might be more favorable than acetic acid when coupling LC with mass spectrometry. Near-critical solvent compositions for the PnBA samples, using 0.5% formic acid at column temperatures of 25°C and 50°C, are summarized in Table 4.3b. The effect of the formic acid on the retention of carboxyl functional PnBAs was similar to that of HAc.

4.3.2 Quantitative aspects

In order to obtain quantitative information on the FTD of the PnBA samples, as described in reference [22], evaporative light-scattering detection (ELSD) had to be used. Because the ELSD response does not usually increase linearly with the polymer concentration, the calibration curves should be established carefully. An exponential calibration curve, such as in equation (1), is often used

\[
A = a m_i^b
\]  

where \( A \) is the observed peak area, \( m_i \) is the injected sample amount (in mass units), and \( a \) and \( b \) are constants. The values of \( a \) and \( b \) can easily be determined from a logarithmic plot. As shown in Fig. 4.4, the ELSD calibration curves for mono- and di-COOH-functional PBAs established using samples PnBA-COOH 2,610 (VL131) and PnBA-2COOH 2,500 (VL127), respectively, can be described very well by eqn.(1). The values of \( a \) and \( b \) are shown in table 4.4. We did not obtain a similar calibration curve for non-functional PBA, because no sufficiently pure standard was available. It can be seen from Fig. 4.4 and table 4.4 that the COOH end-group had a significant influence on the ELSD response. This is due to the peak broadening encountered in interactive LC, which is greater for di-functional than for mono-functional polymers (see ref. [29] for a more-detailed explanation).
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Fig. 4.4  ELSD calibration curves (logarithmic scale) for PnBAs with one and two carboxyl end-
groups. Triangles: PnBA-COOH 2,610, squares: PnBA-2COOH 2,500. Mobile phase 6% 
acetonitrile and 1.0% HAc in DCM, flow rate 0.5 ml/min, column as in Fig. 4.1.

Table 4.4 End-group effect on ELSD calibration curves for functional PnBAs, LC conditions as in Fig. 4.4.

<table>
<thead>
<tr>
<th>Sample</th>
<th>a</th>
<th>b</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>PnBA-COOH 2,610</td>
<td>11.64</td>
<td>1.64</td>
<td>0.9998</td>
</tr>
<tr>
<td>PnBA-2COOH 2,500</td>
<td>5.53</td>
<td>1.71</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

The calibration curves of Fig. 4.4 were used in the quantitative analysis of COOH-functional PBA 
samples under the specified LC conditions (6% acetonitrile, 1.0% HAc in dichloromethane, with a 
flow rate of 0.5 ml/min at 25°C) assuming a negligible effect of molecular weight on the ELSD 
response in the low-molecular-weight range investigated [22]. Some representative results for 
functional polymers are shown in table 4.5. It can be seen clearly in table 4.5 that all the PnBA 
samples obtained from RAFT polymerization contained predominantly molecules with two 
carboxyl end-groups, as expected. The relative amount of mono-carboxyl polymer chains was 
higher in the case of polymer VL103 than for VL068. This is consistent with the relatively high 
amount of AIBN initiator used in preparing VL103 (see table 4.1), which is more than twice that 
used for preparing VL068 [6]. Higher percentages of di-carboxyl polymer chains in samples VL123 
or VL127, as compared with sample VL103, can also be observed in table 4.5. This result is 
expected from polymer chemistry. The ACVA initiator, which contains two COOH groups, 
was used for preparing both VL123 and VL127 (see table 4.1). The initiator moiety, 
[HOOC(CH₂)₂C(CH₃)CN-] from ACVA, was introduced as one end-groups in some of the obtained 
RAFT polymers. The AIBN initiator, which contains no COOH groups, was used in case of VL103 
[6]. The initiator moiety, [(CH₃)₂CCN-] from AIBN, was introduced as one end-group in some
polymer chains (the other end-group containing the leaving group [HOOC(CH₃)₂C-] of the RAFT agent). This resulted in a relatively high percentage of mono-carboxyl polymers in these samples. All these structures were confirmed by mass spectra (see discussion in Section 4.3.4). To achieve high carboxyl functionalities, either a COOH-containing initiator (ACVA) should be used, or a low concentration of a non-COOH-containing initiator (AIBN) relative to that of RAFT agent. In the latter case the polymerization rate is much lower, which is not favorable in case of industrial application. Nearly ideal functionalities (average number of carboxyl end-groups per molecule up to 1.99) were achieved by one-step RAFT polymerization of PnBA.

Table 4.5 Quantitative analysis of carboxyl-functional PnBAs by LC-ELSD. Isocratic conditions and column as in Fig. 4.4 (samples as indicated in table 4.1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mono-funct.%</th>
<th>Di-funct.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL103 (PnBA-2COOH 2,400)</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>VL123 (PnBA-2COOH 3,200)</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>VL68 (PnBA-2COOH 2,510)</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>VL127 (PnBA-2COOH 2,500)</td>
<td>1</td>
<td>99</td>
</tr>
</tbody>
</table>

4.3.3 Analyte recovery in isocratic LC of polymers

Although critical LC has been successfully employed for the separation of polymer blends [13, 30], block copolymers [15, 31] and for the separation of polymers based on functional groups or end-groups [18,19,20,21,22], there remains some controversy over whether precise co-elution conditions can indeed be achieved [28, 32, 33, 34]. Also, reduced recovery has been observed for high-molecular-weight polymers at critical (or near-critical) conditions [35, 36]. It is generally recommended to verify complete recovery in LC experiments on polymers to avoid misinterpretation. Following an approach described by Mengerink et al. [20], an isocratic mobile phase (near-critical conditions) was first employed during a time exceeding the sum of the dwell time and dead time. Then the elution strength of the mobile phase was increased by programming a gradient to reach genuine exclusion conditions, where all the PnBA samples (either with or without COOH groups) were rapidly eluted. If we only observed peaks before the start of the gradient, no recovery problem was diagnosed, as in the case of Fig.4.5a. All the PnBA samples were fully eluted under the near-critical (isocratic) conditions at 55°C with 6% acetonitrile and 0.5% HAc in DCM as the mobile phase. The retention time of the non-functional low-molecular-weight PnBA was lower than that of non-functional high-molecular-weight PnBA, but the retention time of the di-functional low-molecular-weight PnBA was slightly higher than that of di-functional high-molecular-weight PnBA as shown in Fig. 4.5a. With the same mobile phase at a different temperature of 25°C, we observed peaks after the onset of the gradient for high-molecular-weight PnBA samples, as shown in Fig. 4.5b. We identified this as a recovery problem, because a fraction of the high-molecular-weight sample was not eluted at the initial isocratic critical conditions (during the time allowed).
However, it is important to note that at the same conditions as in Fig. 4.5b, there were no recovery problems for low-molecular-weight PnBA samples (molecular weights up to 7,000). The monohydroxy RAFT-PnBA samples (VL28, $M_p = 12,560$, $M_n = 8,960$, PDI = 1.32 and VL29, $M_p = 5,430$, $M_n = 3,980$, PDI=1.19) were also investigated using 6% acetonitrile and 1.0% HAc in DCM at 25°C. In this case the recovery of the analyte was incomplete (not shown). This indicated that the interaction of OH end-groups with the stationary phase was not weaker than that of COOH end-groups under these conditions.

![Diagram](image)

**Fig. 4.5** Analyte recovery in isocratic LC of polymers. Column temperature (a) 55°C, all samples eluted at isocratic conditions and (b) 25°C, high-molecular-weight samples eluted under gradient conditions, low-molecular-weight samples (top trace) eluted under isocratic condition. Mobile phase: first isocratic, 0-5 min, 6% ACN and 0.5% HAc in DCM; then 5-10 min, linear gradient to 15% ACN and 1.0% HAc in DCM (the dashed line indicates the composition of the mobile phase at the outlet of the column). Detector ELS, flow rate 0.5 ml/min, injection volume 10 µL, low-molecular-weight sample mixture: PnBA 600 0.3 mg/ml, PnBA-COOH 2,610 0.6 mg/ml and PnBA-2COOH 2500 1.2 mg/ml in DCM, high-molecular-weight samples: PnBA 32,330, PnBA-COOH 19,090, PnBA-2COOH 11,450 2 mg/ml, column as in Fig. 4.1. See explanation in text.

Note: The peak heights of chromatograms were electronically adjusted for the sake of clarity.
4.3.4 Mass-spectrometric characterization

The near-critical solvent composition was applied to analyze the carboxyl-functional PnBA polymers obtained by RAFT polymerization. Some representative quantitative results for functional RAFT polymers are shown in table 4.5. To confirm the critical separation of PnBA polymers based on the carboxyl functionality, mass spectrometry (MS) was used. It is relatively straightforward to collect fractions and to perform off-line MS. Our aim was to synthesize telechelic PnBA with two COOH end-groups in one step. We did not observe a signal from the ELSD detector at the position of the non-functional polymer. Therefore, two fractions were collected for each sample, at the positions corresponding to mono- and di-functional polymers (4.5-5.0 min and 5.5-7.5 min, respectively).

The ESI-MS (electrospray-ionization mass spectrometry) spectra for the two fractions of sample VL103 are shown in Figs. 4.6a and 4.6b. In the spectrum of Fig. 4.6b, a family of triplets is observed, each “triplet” being separated from the next by a mass of 128.1 amu, which clearly corresponds to a single butyl acrylate (BA) monomeric unit. Within the triplets, the peaks are separated by 22 amu. After subtracting the mass of the PnBA chain, the remainder of the main series of peaks (highest peaks of the triplets, one sodium cation) corresponded to the same structure as the RAFT agent used, which contained two leaving groups [HOOC(CH₃)₂C-] of the RAFT agent at the polymer-chain ends and the thioisocarbonate moiety inside the polymer chain. This structure is shown in Fig. 4.7a. It was further confirmed by collision-induced dissociation MS/MS spectrometry (ESI-CID-MS²). The weak C-S bonds between the acrylate chain and the thioisocarbonate group easily break, releasing the thioisocarbonate moiety. The leaving group [HOOC(CH₃)₂C-] stays attached to the acrylate chain. A second sodium ion may exchange with the hydrogen ion of the carboxylic acid group. This does not affect the total charge of the ion, but it results in an increase in the observed mass of the polymer chain by 22 amu. The polymer chains in VL103-2 contain 2 COOH groups. The substitution of 0, 1 or 2 hydrogens of the carboxylic acid groups explains the presence of a family of 3 peaks separated by 22 mass units. Also one minor series of peaks can be observed in the region of low mass-to-charge ratios (towards the left) in Fig. 4.6b. The main peaks in this series are separated by 64 amu. This can be readily attributed to doubly charged ions with the same structure. Therefore the structure of the VL103-2 fraction was confirmed to be poly(butyl acrylate) with di-carboxyl end-groups as shown in Fig.4.7a.
ESI mass spectra for (a) mono-functional fraction VL103-1 collected at 4.5-5.0 min; (b) di-functional fraction VL103-2 collected at 5.5-7.5 min. LC conditions: Mobile phase 6% acetonitrile and 0.5% HAc in DCM, flow rate 0.5 ml/min at 25°C, column as in Fig. 4.1.
In the spectra of the VL103-1 fraction in Fig. 4.6a two different series can be observed with a repeating unit of 128.1 amu, corresponding to a single BA monomeric unit (and singly charged ions). The remainder mass calculated for the main series of peaks (one sodium cation) is consistent with the leaving group [HOOC(CH$_3$)$_2$C-] of the RAFT agent and of the initiator AIBN moiety [(CH$_3$)$_2$CCN-] as the end-groups and a trithiocarbonate moiety inside the polymer chain. This structure is shown in Fig. 4.7b. It has also been confirmed by ESI-CID-MS$^2$ spectrometry. This series was absent in the spectrum of the VL127-1 fraction (not shown), as is expected from polymer chemistry (see discussion in Section 4.3.2). However, we were not sure to assign another series of peaks with mass increments of 128.1 and shifted by +47 amu relative to the main series. The ESI-CID-MS$^2$ spectrum indicated that this structure contained no weak bonds (C–S or other) in the middle of the polymer chain. This series was also present in the spectrum of the VL127-1 fraction. One possibility is that it is a product of chain transfer caused by an impurity in the RAFT agent. In any case, we conclude that the VL103-1 fraction contained poly(butyl acrylate) with one carboxyl end-group.

In a previous paper [8], we reported that the active (weak) bonds, such as dithioester moiety, in the RAFT PMMA samples led to easy fragmentation in MALDI-MS experiments. Nonaka et al. [37] suggested that the terminal C-Cl group of poly(methyl acrylate) (PMA) obtained by ATRP was relatively stable in comparison with that of PMMA during MALDI-TOF-MS analysis. Matyjaszewski et al. [38] reported that only very little fragmentation was observed in the MALDI-TOF-MS spectrum of PnBA synthesized by ATRP. Similarly, we observed less fragmentation in the MALDI-TOF-MS spectra for the RAFT-PnBA samples than for RAFT-PMMA. When the experimental conditions were carefully tuned (reducing the laser energy), intact molecular ions were observed in the MALDI-TOF-MS spectra. An example is shown in Fig. 4.8, which represents the MALDI-TOF-MS spectrum of the di-functional fraction VL127-2. Three different series can be seen in Fig. 4.8 with repeating units of 128.1 amu, again corresponding to the BA monomeric unit. The main series of peaks results from the di-functional PnBA polymers with one sodium cation (Na$^+$). This series contains two leaving groups [HOOC(CH$_3$)$_2$C-] of the RAFT agent at the polymer-chain ends. This is the same structure as that of sample VL103-2 (see Fig.4.7a). The first minor
series can be assigned to the potassium-cation (K\(^{+}\)) series (with mass increments of 16 amu relative to the Na\(^{+}\) series). The second minor series arises from another type of di-functional PnBA polymers with one sodium cation (Na\(^{+}\)), which contains one end-group \([\text{HOOC(CH}_3\text{)}_2\text{C-}]\) from the RAFT agent and one end-group \([\text{HOOC(CH}_2\text{)}_2\text{C(CH}_3\text{)}\text{CN-}]\) from the initiator moiety. This structure, which is expected from polymer chemistry, is shown in Fig. 4.7c. Therefore only di-carboxyl terminated structures were identified in this fraction of VL127-2.

![MALDI-TOF mass spectrum for di-functional fraction VL127-2 collected at 5.5-7.5 min. LC conditions as indicated in Fig. 4.6.](image)

It should be mentioned here that the mono-functional polymers identified in samples VL127 and VL068 could not be clearly observed by ESI-MS or MALDI-TOF-MS without prior LC separation of the sample. Therefore critical LC coupled with MS provides additional information about the polymer structure.
4.4 Conclusions

The (near-) critical solvent compositions for non-functional, mono-functional and di-functional carboxyl-PnBAs were determined in normal-phase LC, using mixtures of acetonitrile, acetic (or formic) acid, and dichloromethane of varying composition and a bare-silica column. Some formic or acetic acid had to be added to the mobile phase to elute PnBA polymers with carboxyl end-groups. The critical solvent compositions obtained were not identical for non-, mono-, and di-functional PnBA-2COOH samples. Because both acetic acid and acetonitrile are polar solvents with higher elution strengths than DCM on a silica column, lower acetonitrile concentrations were required when increasing the acetic-acid concentration in the mobile phase. Formic acid behaved similar to acetic acid. Low-molecular-weight PnBA samples were successfully separated according to carboxyl functionality at (near-) critical conditions (6% acetonitrile and 0.5% HAc in DCM at 55°C or 6% acetonitrile and 1.0% HAc in DCM at 25°C). Isocratic, near-critical LC of high-molecular-weight PnBAs proved feasible, but this required elevated temperatures (55°C).

Under appropriate conditions, reliable ELSD calibration curves could be obtained and these were used for the quantitative analysis of carboxyl-functional RAFT-PnBA prepolymers. The results from LC at the near-critical conditions showed that all the obtained PnBA samples from RAFT polymerization mainly contained (telechelic) molecules with two carboxyl end-groups. Mass spectra (MS) confirmed that the critical separation of PnBA polymers was based on the carboxyl functionality. Critical LC coupled with MS provided a great deal of information on the polymer structure. The quantitative data and MS spectra were consistent with the expected results from the mechanism of the RAFT polymerization. To achieve high carboxyl functionalities, either a COOH-containing initiator (ACVA) or a very low concentration of a non-COOH-containing initiator (AIBN) should be used. In the latter case the polymerization rate is much lower. Near-ideal functionalities (average number of carboxyl end-groups per molecule up to 1.99) were achieved by one-step RAFT polymerization of PnBA.

Acknowledgements

This project is funded by the Dutch Polymer Institute (DPI project 205). We thank Aschwin van der Horst (University of Amsterdam), Rajan Venkatesh and Prof. Rob van der Linde (Eindhoven University of Technology) for their cooperation. Helpful discussions with Profs. A. M. Skvortsov and A. A. Gorbunov from St. Petersburg (Russia) were made possible by INTAS (Project nr. INTAS-OPEN-2000-0031).

References

CHAPTER 5

Comprehensive Two-Dimensional LC for the
Characterization of Functional Acrylate Polymers

ABSTRACT

Comprehensive two-dimensional liquid chromatography – size-exclusion chromatography (LC×SEC) was investigated as a tool for the characterization of functional poly(methyl methacrylate) (PMMA) polymers. Ultraviolet-absorbance and evaporative light-scattering detection (ELSD) were used. A simple method to quantify ELSD data is presented. Each data point from the ELSD chromatogram can be converted into a mass concentration using experimental calibration curves. The qualitative and quantitative information obtained on two representative samples is used to demonstrate the applicability of LC×SEC for determining the mutually dependent molecular-weight distributions (MWD) and functionality-type distributions (FTD) of functional polymers.

The influence of the molecular weight on the retention behavior in LC was investigated using LC×SEC for hydroxyl-functional PMMA polymers. The critical conditions, at which retention is – by definition – independent of molecular weight, were not exactly the same for PMMA series with different end-groups. Our observations are in close agreement with theoretical curves reported in the literature. However, for practical applications of LC×SEC it is not strictly necessary to work at the exact critical solvent composition. Near-critical conditions are often sufficient to determine the mutually dependent distributions (MWD and FTD) of functional polymers.

Keywords: critical liquid chromatography; comprehensive 2D-LC; RAFT; polymer functionality; LC×SEC; ELSD quantification.

5.1 Introduction

Synthetic polymers are very complex mixtures of many different chemical compounds [1]. In "simple" homopolymers the individual molecules vary unavoidably in the number of polymer repeat units. The individual molecules in synthetic polymers may be built up from several different repeat units (copolymer), and possibly be initiated by different compounds or terminated in different ways, to give rise to various end groups. Polymeric chains may be linear, branched to variable extents, or even cyclic. In addition, some polymers exhibit variations in chain (stereo-) regularity or "tacticity". Variations in the chemical structure, such as the number of functional groups or end-groups present or the chemical composition of copolymers, can have dramatic effects on the properties of the polymer. Clearly, in order to establish relationships between molecular structure and material performance of polymers, we need to obtain information on the average molecular structure, as well as on the underlying distributions.

Liquid chromatography (LC) is eminently suitable for separating soluble polymers. A number of different mechanisms (size exclusion, adsorption, partition, etc.) can be exploited [1]. Size-exclusion chromatography (SEC) is the most commonly applied technique for separating polymers based on the size (hydrodynamic volume) of molecules in solution and the extent to which they are excluded from porous particles. The molecular-weight averages and the molecular-weight distribution (MWD) can be obtained using a calibration curve that relates the (logarithm of the) molecular weight to the retention time or volume. "Interactive" LC, which is based on molecular interactions between the polymer molecules and the mobile and stationary phases in the column, can be used to separate polymers based on chemical composition or functionality (functional groups or end-groups). As in conventional LC techniques, the composition of the mobile phase is varied to achieve the desired separation. Gradient elution is often needed to elute a variety of polymer molecules within a reasonable time, because the molecular interactions vary dramatically with the size and structure of polymer molecules. Between (or beside) the SEC mode and the interactive mode, there is a specific mode of isocratic LC, in which retention is independent of molecular weight and solely influenced by the chemical composition or functionality of the molecules. These so-called critical conditions are hard to achieve and maintain, but they are extremely useful for separating polymer molecules according to the number of functional groups present.

Complex polymers feature several simultaneous distributions. For example, all functional polymers exhibit functionality-type distributions (FTD) and all copolymers exhibit chemical-composition distributions (CCD). As a rule, the different distributions are mutually dependent. To characterize multiple, mutually dependent distributions, multi-dimensional separations are indispensable. In this work, functional polymers featuring an MWD and an FTD will be analyzed. To characterize these two dependent distributions, we need a two-dimensional separation. Ideally, but not necessarily, one separation step distinguishes between molecules of different molecular weight, while the other step reveals differences in functionality.

Two-dimensional liquid-chromatographic (2D-LC) systems have been used for many years to separate and characterize synthetic polymers, biomolecules and complex mixtures [2]. The most common form of 2D-LC in the earlier studies was an off-line approach [3,4,5]. In this so-called
Two-Dimensional LC for the Characterization of Functional Polymers

"cross-fractionation" or "heart-cut" method, a few fractions from the first-dimension column were collected and re-injected into a second liquid-chromatographic system. The resulting data are two or more chromatograms. This technique requires knowledge of the retention of specific sample components, before the fractionation can take place. It is very useful for the separation of (a) specific component(s) in a polymer or copolymer.

During the 1980s Erni and Frei [6] were probably the first to explore the on-line approach, which has become known as "comprehensive" two-dimensional LC. In this method, sequential aliquots from the first column are transferred on-line to the second one using an automated switching valve [5-10]. The transfer volume is taken sufficiently small, so that each chromatographic peak from the first dimension is divided into several fractions of equal volume. The resulting data is a three-dimensional matrix, usually represented as a contour plot, with each chromatographic retention time along one axis and the detector signal as the intensity parameter. Comprehensive two-dimensional operation greatly increases the peak capacity of LC systems. Consequently, the information content of the resulting chromatogram is greatly enhanced. Several other research groups have contributed significantly to the development of two-dimensional separations of polymers. Especially relevant in the context of the present work are the studies from the group of Pasch [11,12]. With respect to nomenclature, heart-cut two-dimensional liquid chromatography is usually referred to as LC-LC, whereas for on-line coupling with complete transfer of the eluate from the first dimension (i.e. comprehensive 2D-LC) the notation LC×LC is preferred [13].

In LC×LC of polymers several different separation mechanisms can be exploited in the first and second dimensions. The choice for either dimension is dependent on the distributions of interest. Following the method of van der Horst et al. [5], we used LC×SEC in this work to investigate the FTD and MWD of functional polymers. The total analysis time is the product of the analysis time in the second dimension and the number of fractions collected from the first dimension effluent. To limit the total analysis time in LC×SEC and to conserve the chromatographic separation (resolution) obtained in the first dimension, it is very important that the second dimension be fast. We opted for size exclusion in the second dimension and fast SEC analyses were performed, using short columns packed with small particles.

In a comprehensive set-up, a 10-port switching valve equipped with two loops was used in a symmetrical configuration [5]. While one loop is being filled with the first-dimension eluate, the fraction that has previously been collected in the second loop is analyzed in the second-dimension separation. The collection time for each fraction in the first dimension is equal to the analysis time in the second dimension. As a consequence, the analysis time in the second dimension and the loop volume together determine the (maximum) flow rate for the first-dimension separation. Therefore, to realize truly comprehensive LC×LC (i.e. without splitting after the first column), the flow rate of the first-dimension separation cannot be very high. We prefer to use a micro-bore LC column for the first-dimension separation. This ensures comprehensive operation of the system [5].

In this work, we demonstrate the use of comprehensive two-dimensional LC (specifically LC×SEC) to obtain the MWD and FTD for functional poly(methyl methacrylate) (PMMA) polymers. Also,
the influence of the molecular weight on the so-called critical conditions in LC was investigated for hydroxyl-functional polymers.

5.2 Experimental

5.2.1 Chemicals

Dichloromethane (DCM) and acetonitrile (both HPLC grades) were from Rathburn Chemicals (Walkerburn, Scotland). Non-stabilized tetrahydrofuran (THF, Biosolve, Valkenswaard, The Netherlands) was used as the mobile phase in size-exclusion chromatography (SEC). Poly(methyl methacrylate) (PMMA) standards were obtained from Polymer Laboratories (Church Stretton, Shropshire, UK). The molecular-weight ($M_n$, $M_p$) and polydispersity-index (PDI) values were specified by the manufacturer. The ("RAFT") polymers with one hydroxy (OH) end-group were synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization, using a hydroxy-functional initiator and a hydroxy-functional RAFT chain-transfer agent [14]. A commercial telechelic PMMA (TEGO DIOL MD-1000X) with two OH groups was obtained from Tego Chemie Service (Essen, Germany). The molecular weights and molecular-weight distributions were measured by SEC using a Waters (Milford, MA, USA) instrument equipped with a Waters model 510 pump and a model 410 differential refractometer (40°C). A set of two linear columns (Mixed-C, Polymer Laboratories, 300 mm × 7.5 mm i.d., 40°C) was used. The calibration curve was prepared with polystyrene (PS) standards and the molecular weights were estimated based on the universal-calibration principle and Mark-Houwink parameters [PS, $K = 1.14 \times 10^{-4}$ dL g$^{-1}$ and $\alpha = 0.716$; PMMA, $K = 0.944 \times 10^{-4}$ dL g$^{-1}$ and $\alpha = 0.719$] [15,16,17]. The effect of the hydroxyl end-groups on the Mark-Houwink parameters was neglected. All the PMMA standards and samples used are summarized in table 5.1. Numbers after PMMA (or PMMA-OH, or PMMA-2OH) refer to the peak molecular weight. All the samples injected in the first dimension were dissolved in DCM, which is a good solvent for PMMA, but a weak eluent on bare silica. In this way breakthrough peaks were avoided [18]. All the samples analyzed by SEC (as a stand-alone technique) were dissolved in THF (Samples analyzed in SEC as a second-dimension separation were dissolved in the first-dimension effluent.)
Table 5.1  PMMA samples used in this study. The molecular-weight \((M_n, M_p)\) and polydispersity-index (PDI) values of samples were measured by SEC. The values for PMMA standards were supplied by the manufacturer.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>(M_n)</th>
<th>(M_p)</th>
<th>PDI</th>
<th>Intended number of OH end groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA .620(^a)</td>
<td>550</td>
<td>620</td>
<td>1.35</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 1,310(^a)</td>
<td>1,160</td>
<td>1,310</td>
<td>1.12</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 1,680(^a)</td>
<td>1,327</td>
<td>1,681</td>
<td>1.15</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 1,990(^a)</td>
<td>1,840</td>
<td>1,990</td>
<td>1.09</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 2,990(^a)</td>
<td>2,756</td>
<td>2,991</td>
<td>1.08</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 3,800(^a)</td>
<td>3,437</td>
<td>3,805</td>
<td>1.07</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 5,270(^a)</td>
<td>4,977</td>
<td>5,270</td>
<td>1.06</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 6,950(^a)</td>
<td>6,950</td>
<td></td>
<td>1.05</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 9,200(^a)</td>
<td>8,502</td>
<td>9,198</td>
<td>1.06</td>
<td>0</td>
</tr>
<tr>
<td>PMMA 13,930(^b)</td>
<td>12,489</td>
<td>13,934</td>
<td>1.06</td>
<td>0</td>
</tr>
<tr>
<td>PMMA-OH 3,310(^b)</td>
<td>2,425</td>
<td>3,314</td>
<td>1.22</td>
<td>1</td>
</tr>
<tr>
<td>PMMA-OH 13,950(^b)</td>
<td>10,934</td>
<td>13,946</td>
<td>1.21</td>
<td>1</td>
</tr>
<tr>
<td>MD-1000X</td>
<td>1,490</td>
<td>2324</td>
<td>1.64</td>
<td>2</td>
</tr>
<tr>
<td>VL37A(^c)</td>
<td>2,587</td>
<td>3,754</td>
<td>1.29</td>
<td>1</td>
</tr>
<tr>
<td>VL37B(^d)</td>
<td>2853</td>
<td>3678</td>
<td>1.29</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\) PMMA standards obtained from Polymer Laboratories.

\(^b\) Polymers with one OH group synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization using a hydroxy-functional initiator and a hydroxy-functional RAFT chain-transfer agent.

\(^c\) Synthesized via end-group modification of well-defined RAFT polymers [14].

\(^d\) Synthesized by RAFT polymerization using a 2,2'-azobis(isobutyronitrile (AIBN) initiator and a hydroxy-functional RAFT chain-transfer agent [14].

5.2.2 Instrumentation

The first-dimension LC system used consisted of a Shimadzu LC-10ADvp solvent-delivery unit (Shimadzu, 's Hertogenbosch, The Netherlands) and a Rheodyne two-position six-port injection valve (Berkeley, CA, USA) equipped with a 1-\(\mu\)l or a 10-\(\mu\)l loop. Two home-packed Hypersil "bare" silica columns (150 mm \(\times\) 1.0 mm i.d.; 3-\(\mu\)m particles; 100-Å pore size; ThermoQuest, Breda, The Netherlands) were used in the first (LC) dimension at room temperature. The second-
dimension (SEC) system consisted of a Kratos Spectroflow 400 pump (ABI, Ramsey, NJ, USA), a Kratos Spectroflow 757 UV-absorbance detector (ABI) operated at a wavelength of 220 or 300 nm, and a Sedex 55 evaporative light-scattering detector (ELSD; temperature 62°C, N₂ pressure 2.2 bar). One or two 50 mm × 4.6 mm i.d. PLgel columns (Polymer Laboratories, 5-μm particles with 100 Å pore size and/or 6-μm oligoPore particles with 100-Å pore size, 25°C) were used with THF at a flow rate of 0.9 ml/min in the SEC systems. The LC and SEC systems were coupled with an air-actuated VICI two-position 10-port valve (Valco, Schenkon, Switzerland) [5]. This valve was operated using a high-speed switching accessory (switching-time of 20 ms using nitrogen) and dual injection loops of equal volume (various sizes between 4 and 40 μl) were used.

### 5.2.3 Instrument control

A personal computer with Windows NT was equipped with a Keithley KNM-DCV 12 Smartlink interface (Cleveland, OH, USA). Two-dimensional plots and distribution data were calculated with an in-house program written in a Matlab (Natick, MA, USA) software environment [5]. This program enabled us to register and control the valve-switching time for the two-dimensional separations. The program options also allowed us to extract LC and SEC chromatograms at any positions in the LC×SEC contour-plot. Furthermore, the software was able to carry out quantification by computing peak volumes for specified retention ranges and to calculate MWDs for different polymers, using SEC calibration curves, provided that the detection response was linear or linearized (see the text in Section 5.3.3).
5.3 Results and discussion

5.3.1 Optimization of SEC flow rate

The second-dimension separation in LC×SEC needs to be fast, while sufficient separation efficiency needs to be maintained. Because a conventional SEC analysis, for example, using two 300 mm × 4.6 mm i.d. columns at 0.5 ml/min, shows a typical analysis time of 10 to 15 minutes, it is not useful for LC×SEC. Instead, fast-SEC column(s) should be selected and relatively high flow rates should be employed. There is a trend to perform fast-SEC separations on short (typically 50 mm) columns with analysis times of 1 to 3 minutes. The flow rate is dependent on the column diameter (e.g. 0.5 ml/min for a 4.6-mm i.d. column or 1.0 ml/min for a 7.5-mm i.d. column). However, recent studies from our group [19,20,21] provide theoretical and practical support for the use of longer columns and higher flow rates in fast SEC. To keep the analysis time in the second-dimension SEC separation similar (within 2 minutes), we selected one column at a flow rate of 0.45 ml/min, or two columns of the same dimension at a flow rate of 0.9 ml/min. For comparison of the SEC resolution, a mixture of Standards PMMA 620 and PMMA 6,950 was injected using the second-dimension part of the LC×SEC set-up (same 10-port switching valve equipped with two 40-μl loops) with the UV and ELSD detectors as a stand-alone system. As can be seen clearly from Fig. 5.1, better resolution was obtained when two columns were used at a flow rate of 0.9 ml/min. The PMMA 620 peak (2) was baseline separated from the solvent peak (3) (UV at 220 nm, see Fig. 5.1b). The former was also nearly baseline separated from the PMMA 6,950 peak (1). Therefore longer columns with higher flow rates are seen to provide better resolution in fast SEC. However, when using even longer columns, the pressure drop may become prohibitive. Also, the higher flow rates required would induce higher solvent costs and might impart the sustained stable operation of the pump [22].

In the present case, the selection of columns (one regular PLGel 100-Å column and one 100-Å OligoPore column) was also tailored to the separation of low-molecular-weight prepolymers. Therefore, the two 50 mm columns were used in series in the second-dimension SEC separation for the LC×SEC experiments reported in this work.
Fig. 5.1  SEC chromatograms obtained from a standard mixture of PMMA 620 and PMMA 6,950. Dotted line, ELSD response; drawn line, UV response at 220 nm. (a) One 50 mm × 4.6 mm i.d. PLgel column (5-μm particles with 100 Å pore size), flow rate 0.45 ml/min. (b) Two 50 mm × 4.6 mm i.d. PLgel columns (5-μm particles, 100 Å pore size and 6-μm oligoPore particles, 100 Å pore size), flow rate 0.9 ml/min. Peak 1: PMMA 6,950; peak 2: PMMA 620; peak 3: THF. Mobile phase was fresh non-stabilized THF.

5.3.2 Molecular-weight effects at near-critical LC

In Chapter 2 we have shown the robust critical separation of PMMA using a conventional bare-silica column (150×4.6 mm). The PMMA samples could be separated according to the number of hydroxyl end-groups with negligible influence of the molecular weight [23]. However, this column cannot be coupled directly on-line to electrospray ionization-mass spectrometry (ESI-MS) without splitting. Two micro-bore columns (150×1.0 mm) were used to study the effect of molecular weight on retention time at near-critical conditions [24]. Because of the limited (low) molecular-weight range of ESI-MS and its limited range of applicability to specific (polar) types of polymers [25], LC×SEC was investigated in this work as an alternative approach to study the molecular-weight effect. LC×SEC is applicable to all soluble polymers across a broad range in molecular weight. It
Comprehensive Two-Dimensional LC for the Characterization of Functional Polymers

also allows quantitative data to be obtained on mutually dependent distributions, such as the FTD and MWD of functional polymers.

As shown in Fig. 5.2, a mixture of non-, mono- and di-functional PMMA polymers of various (relatively low) molecular weights yielded clearly separated peaks using 48% acetonitrile in DCM as the mobile-phase in the first dimension (LC). Some variation in the retention time with molecular weight can be observed in Fig. 5.2. The retention times of non- and mono-functional PMMA polymers increased slightly with increasing molecular weight, displaying a "banana" shape, which indicated that the mobile-phase was on the adsorption side of the critical point. This is in agreement with what van der Horst et al. have reported [5]. However, the retention of di-functional PMMA polymers seemed to decrease slightly with increasing molecular weight, suggesting an opposite molecular-weight effect at the same mobile-phase composition. Despite the slight molecular-weight effect, we could obtain the molecular-weight and MWD information for individual peaks using the SEC calibration curve. Subsequently, we could obtain the FTD and MWD for the functional polymers by LC×SEC at a near-critical composition (see discussion below in Section 5.3.4). If only one dimensional chromatography (either LC or SEC) were used, we would have obtained overlapping peaks, as is evident from the one-dimensional projections of the chromatograms in the LC dimension (shown as Fig. 5.3a) and in the SEC dimension (shown as Fig. 5.3b). A small peak was observed in the UV chromatogram (\(t_R = 0.6 \text{ hr}, t_R = 1.2 \text{ min}\)), but not seen with ELSD detection. This was probably an unreacted OH-RAFT agent (see discussion in Section 5.3.4).

Acetonitrile is a more-polar solvent than DCM. It can desorb PMMA from the silica column. When the concentration of acetonitrile in the mobile phase increases, the retention of non-functional and hydroxyl-functional PMMA polymers will decrease. When the concentration of acetonitrile in the mobile phase was 52% in DCM, the two mono-functional PMMA polymers with different molecular weights showed the same retention time, which suggested that this was the critical solvent composition for mono-hydroxyl PMMA samples (results not shown). However, the retention of non-functional PMMA standards still increased slightly with increasing molecular weight, displaying a "banana" shape. This suggested that the mobile phase was still on the adsorption side of the critical point. At the same time the retention of di-functional PMMA polymers decreased slightly with increasing molecular weight. One should note that this is not typical exclusion behavior, because the retention volumes far exceed the total volume of mobile phase in the column.

As shown in Fig. 5.4, with a higher acetonitrile concentration (56% ACN in DCM), the retention of non-functional PMMA standards in the first dimension still increased slightly with increasing molecular weight, displaying a "banana" shape, which indicated that the mobile phase was still on the adsorption side of the critical point. However, the retentions of mono- and di-functional PMMA polymers decreased slightly with increasing molecular weight. When the concentration of acetonitrile in the mobile phase was 70% or above, the retention of all PMMA polymers decreased with increasing molecular weight and typical exclusion behavior was observed.
Fig. 5.2 LC×SEC chromatograms of a mixture of PMMA standards (peak 1: PMMA 620, peak 2: PMMA 5,270; non-functional), mono-functional RAFT polymers (peak 3: PMMA-OH 3,310, peak 4: PMMA-OH 13,950) and di-functional PMMA (peak 5: MD-1000X) at near-critical conditions. (a) UV 220 nm, (b) ELSD. LC columns: two 150×1.0 mm i.d., 3-μm, 100-Å bare silica; 48% ACN in DCM, 8 μl/min. SEC columns: 5-μm 100 Å plus 6-μm oligopore, 2 times 50×4.6 mm i.d.; fresh non-stabilized THF, 0.9 ml/min.
We may conclude that the critical conditions, at which retention is independent of molecular weight, are not the same for PMMA series with different end-groups. Apparently, the critical composition is (slightly) less than 48% ACN for di-functional PMMA, about 52% for mono-functional PMMA and more than 56% ACN for non-functional PMMA. This may be surprising, because, in principle, the critical composition for the PMMA backbone should not change with the end-groups. Variations in the exact critical composition with different end-groups has also been observed for poly(n-butyl acrylate) polymers [26].

Gorbunov et al. [27] and Skvortsov et al. [28] developed a unified theory of the combination of interaction (adsorption) LC and SEC for polymers. Theoretical models suggested that the retention of di-functional polymers at the critical point (Fig. 15 in ref. 28) or at conditions of very weak adsorption (Fig. 14 in ref. 28) for non-functional polymers should depend on the molecular weight. Gorbunov et al. [27] reported some experimental and theoretical results indicating that the retention of di-functional polymer at the critical conditions of the non-functional polymer decreased with increasing molecular weight. They stated that the distribution coefficient of functional polymers...
could exceed unity and that it would decrease with increasing radius of gyration (molecular weight) if the interaction of end-groups with the stationary phase was strongly attractive. Our observations in Fig. 5.2 are in close agreement with the theoretical curves reported in Fig. 14 of ref. 28 for the low-molecular-weight range. The results shown in Fig. 5.4 of the present paper are similar to the data published in Fig. 4 of ref. 27. It would be interesting to study the molecular-weight effect for high-molecular-weight polymers by LC×SEC, if samples with various molecular weights and end-groups were available.

Fig. 5.4 LC×SEC (a) UV (220 nm) and (b) ELSD chromatograms of the same mixture of PMMA samples as in Fig. 5.2. The conditions were identical as for Fig. 5.2, except that the LC mobile phase was 56% ACN in DCM and the flow rate was 4 μl/min in the first dimension.
At this point it is worth noticing that for practical applications of LC×SEC it is not strictly necessary to work at the exact critical solvent composition. Near-critical conditions often suffice to determine the mutually dependent MWD and FTD for functional polymers.

### 5.3.3 Quantitative aspects

In LC×SEC, the detector monitors the signal after SEC. Therefore the detectors used in common SEC can, in principle, be used in LC×SEC. The most frequently used detectors in SEC of polymers are refractive-index (RI) and ultraviolet absorbance (UV) detectors. However, both of them have their limitations. For instance, RI detectors exhibit a low sensitivity. Because two-dimensional separation gives rise to a strong dilution of the analytes, it is not easy to use RI detectors in LC×SEC. Moreover, a significant dependence of RI on molecular weight was observed for samples with low molecular weight [29], due to influence of the end-groups. Since in our case low-molecular-weight RAFT-polymers and the corresponding non-RAFT polymers (lost RAFT end-group) must be analyzed, the effect of the RAFT end-group on the RI response greatly complicates the quantitative analysis.

UV detection is limited to UV-active polymers. It can only be used when chromophores, which may be the repeating unit, the end-groups, or both, are present in the analyte. In case both the polymer backbone and the end-groups show a high UV absorbance at the selected wavelength, it is also difficult to obtain accurate quantitative results for low-molecular-weight samples. Tetrahydrofuran (THF) is a common solvent in SEC, but it is not transparent at short wavelengths, especially due to its oxidization in air. Acrylate polymers exhibit UV absorbance only at short wavelengths (210-235 nm). The UV absorbance at 220 nm for fresh non-stabilized THF was 0.47 AU with water as a reference. As an example, the UV absorbance at 220 nm was 0.83 AU for standard PMMA 1,680 at a concentration of 0.5 mg/ml with non-stabilized THF as the reference. When fresh, helium-covered non-stabilized THF was used as SEC mobile phase, UV detection with a selected wavelength of 215-233 nm clearly showed peaks of acrylate polymers. As shown above in Fig. 5.1 and Fig. 5.2a, the UV signal at 220 nm provided a good qualitative impression of the PMMA samples. However, this signal could not be used to obtain accurate quantitative results on functional PMMA polymers, because the response was due to the polymer backbone as well as to end-groups. Both contributions are significant and variable in case of low-molecular-weight samples with different end-groups. The response neither reflects the sample mass, nor the number of the polymer chains. For example, the UV absorbance at 220 nm was 0.53 AU for RAFT polymer PMMA-OH 3,310 at a concentration of 0.1 mg/ml with non-stabilized THF as the reference. However, it was approximately 3 for the OH-containing RAFT agent (with the same end-groups as those of PMMA-OH 3,310 polymer, but without MMA units) at the same concentration. In the functional RAFT polymer VL37A every polymer chain has a RAFT group, which exhibits UV absorbance at high wavelengths (300 nm). It is reasonable to assume that the UV absorbance at this wavelength is proportional to the number of RAFT polymer chains with a negligible effect of molecular weight. For such a polymer we can obtain quantitative information by LC×SEC (see Section 5.3.4).
Infrared (IR) spectrometry has proven to be a powerful tool for the selective detection of (either UV-active or non-UV-active) functional groups in polymers [22]. However, the practical use of IR detection in LC is still quite limited, because of the possible occurrence of inconsistent absorption-band intensities in solvent-elimination LC-IR spectra, the low detection limits for flow-cell interfaces and other reasons [22].

Evaporative light-scattering detection (ELSD) has become increasing popular in HPLC, due to its “universal” applicability and high sensitivity for all non-volatile analytes [30]. However, quantitative analysis using an ELSD is not easily achieved [30,31,32], because the ELSD response does not usually increase linearly with the polymer concentration. Calibration curves should be established and applied carefully. An exponential calibration curve, such as in equation (1), is often used [32,33,34]:

\[ A = a \times m^b \]  

(1)

where \( A \) is the ELSD response area, \( m \) is the injected mass of sample, and \( a \) and \( b \) are constants. The values of \( a \) and \( b \) can easily be determined from a logarithmic plot, in which the exponent \( b \) is obtained from the slope and the constant \( a \) from the intercept of the regression line.

It should be noted that equation (1) was established and proven in one-dimensional LC. There is a serious difficulty in deriving quantitative data from comprehensive two-dimensional LC using a non-linear detector. In this discussion we assume that the chromatographic profile (analyte concentration vs. time) is Gaussian, an approximation that is often satisfactory. If the detector response is such that at any time the signal, \( Y \), is related to the analyze concentration (\( C \)) (see plateau method below) by

\[ Y = a' \times e^{b'c} \]  

(2)

where \( a' \) is the response factor, then the recorded peak profile by ELSD is also a Gaussian curve, because of the properties of the exponential [35]. Only the standard deviation observed by ELSD (\( \sigma_{ELSD} \)) is changed from the standard deviation \( \sigma \) obtained by using a linear detector to

\[ \sigma_{ELSD} = \sigma^2 / b' \]

(3)

The power constants \( b \) used in eqn. 1 and \( b' \) in eqn. 2 are identical [34,35]. The constants \( a \) in eqn. 1 and \( a' \) in eqn. 2 are related by [34,35]

\[ a = a' / \left[ \sqrt{b} \times F^{-b} \times (\sigma \times \sqrt{2\pi})^{-b'} \right] \]

(4)

where \( F \) is the flow rate. The constant \( a \) will not vary when the LC conditions do not change. Therefore, the calibration curves can be established based on the measured individual response (eqn. 2, see plateau method below) using ELSD (column is not necessary), on the integrated peak area (eqn. 1, see method 2 below) or on the integrated peak volume (see method 1 below). As a consequence, the calibration curves can be established in three different ways in LC×SEC. First, by individually injecting each standard in various amounts into the LC×SEC, we obtain a series of integrated peak volumes (equivalent to summing the peak areas obtained from each fraction of the
first dimension in LC×SEC, similar to summing all data points in one-dimensional LC; the power constant \( b \) will not change). Second, by injecting each standard in various amounts only into the second-dimension SEC system we can obtain the calibration curves based on integrated peak areas from SEC. Third, by injecting each standard in various concentrations directly into the ELSD to get the stationary (plateau) signal using the 10-port switching valve equipped with two big loops, such that a flat peak (plateau) can be obtained. The height of the plateau refers to the real injected analyte concentration, without dispersion in the connecting capillary tubes between the injector and the detector. Thus, the ELSD calibration curves can be obtained (eqn. 2). Each response (data point) from the ELSD chromatogram can be converted into the analyte concentration using the above calibration curves. The same power constant \( b \) or \( b' \) should be obtained in any case. However, it is time-consuming to obtain the calibration curves by the first method due to the long analysis time in LC×SEC. The third method is fast and allows easy data processing. Therefore, it is recommended.

The above discussion refers to one compound. However, polymers are mixtures of (large) series of molecules with different molecular weights, which strongly overlap even after elution from the SEC column. It is impossible to calculate the responses for the individual molecules and add them up, due to the non-linear characteristics of the ELSD. Therefore, we assume polymers to consist of one compound, neglecting the molecular-weight effect on the ELSD response. This can be justified, because no obvious molecular-weight effect was observed in previous studies [23,32,36,37,38,39]. Most of these papers deal with high-molecular-weight polymers. In our work we deal with relatively low-molecular-weight polymers, where the effect of molecular weight on the response may be greater. In our own work, some variation in response was discerned, but no systematic trend could be observed.

We selected the third method described above to obtain calibration curves. Fig. 5.5 shows ELSD calibration curves for PMMAs with different end-groups. The values of \( a' \) and \( b' \) are shown in table 5.2. It can be seen from Fig. 5.5 and table 5.2 that the OH end-groups had a smaller influence on the ELSD detection in SEC than on the observed (peak area) in critical LC [23]. This is mainly due to the use of integrated peak areas in the latter case. The standard deviation (eqn.3) observed for the di-functional polymer was larger than those of the non- and mono-functional polymers. The calibration curves in Fig.5.5 were used for quantitative analysis of functional PMMA prepolymer under the specified SEC conditions (see Section 5.3.4).

<table>
<thead>
<tr>
<th>Sample</th>
<th>( a' )</th>
<th>( b' )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA standards*</td>
<td>0.066</td>
<td>1.52</td>
<td>0.9929</td>
</tr>
<tr>
<td>PMMA-OH 3,310</td>
<td>0.076</td>
<td>1.49</td>
<td>0.9964</td>
</tr>
<tr>
<td>HO-PMMA-OH (MD-1000X)</td>
<td>0.167</td>
<td>1.33</td>
<td>0.9995</td>
</tr>
</tbody>
</table>

*Parameters in Equation (2)

*PMMA standards used include PMMA 620, PMMA 1,990, PMMA 3,800, PMMA 9,200.
Chapter 5

![Graph](image)

**Fig. 5.5** ELSD calibration curves (plateau height versus injection concentration, logarithmic scale) for PMMAs with different end-groups. Open squares, drawn line: PMMA standards (non-functional); circles, dashed line: PMMA-OH 3,310 (mono-functional); triangles, dotted line: MD-1000X (di-functional). Mobile phase: THF, flow rate 0.9 ml/min at 25°C; injection loop volume 800 µl; no column used.

5.3.4 Application

Fig. 5.6 shows an example of LC×SEC for a real RAFT sample (VL37A). In the ELSD trace of Fig. 5.6b two peaks are observed. Peak 1 represents non-functional PMMA and peak 2 mono-OH PMMA. Both peaks are in agreement with the observations in one-dimensional near-critical LC [24]. However, four separate peaks were detected by UV at 300 nm, as shown in Fig. 5.6a (similar results were obtained using UV at 220 nm). Peaks 1 and 2 in Fig. 5.6a are similar to those in Fig. 5.6b obtained using ELSD. Peak 3 in Fig. 5.6a represents molecules with a very low molecular weight and without OH end-groups; peak 4 may represent residual (unreacted) RAFT agent used in the polymerization process. This latter peak was not observed if PMMA standards were subjected to LC×SEC. If only one-dimensional LC or SEC is used, peaks 3 and 4 are hard to separate completely. Therefore, LC×SEC provides more information and is demonstrated to be useful in polymer analysis.

On-line LC-ESI-MS confirmed that non-functional RAFT ($m/z$ 244 + 100$n$ with sodium cation) and OH-RAFT polymers ($m/z$ 288 + 100$n$) existed in the VL37A sample. On-line LC-ESI-MS showed that the 288 peak (without MMA unit) had a higher intensity than the 388 (1 MMA unit) and 488 peaks (2 MMA units) and that the 444 peak had a higher intensity than the 244, 344, and 544 peaks. We also injected the pure non-functional RAFT and mono-functional OH-RAFT agents. The results support the peak identification above.
The quantitative results obtained for sample VL37A by LC×SEC are summarized in table 5.3. Because every polymer chain possesses a RAFT end-group, which exhibits UV absorbance at high wavelengths (300 nm), we can calculate the relative molar concentration for each peak shown in Fig. 5.6a directly from the UV chromatogram at this wavelength. The concentration of molecules in peaks 3 and 4 in Fig. 5.6 combined was only about 1 mole-% in sample VL37A. The average molecular weights (\(M_n\) and \(M_w\)) and the molecular weight distribution (MWD or PDI) calculated
from all four peaks together (1, 2, 3 and 4, in Fig. 5.6) were very close to those obtained summing only peaks 1 and 2 (results not shown). Since we want to compare the results from ELSD and UV and since the lowest molecular weight of the PMMA standards used in the second-dimension calibration is 620, quantitative results for peaks 3 and 4 in Fig. 5.6a are not included in table 5.3. As seen from table 5.3, there was a small difference between the molecular weight information ($M_n$, $M_p$ and PDI) obtained for the non-functional PMMA (peak 1) and for the mono-OH RAFT (peak 2). The concentration of non-functional PMMA (peak 1) was 16 mole-% in sample VL37A, calculated directly from the UV chromogram. The second-dimension molecular-weight calibration (retention time converted into molecular weight) can be used to convert this number to a weight-%. A value of 13 weight-% was obtained for the non-functional PMMA (peak 1).

To compare UV and ELSD detection, quantitative results were obtained from the ELSD chromatogram. Each data point of the ELSD chromatogram was converted into a concentration using the ELSD calibration curves shown in Fig. 5.5 and table 5.2. Similarly, we obtained the molecular weight information ($M_n$, $M_p$ and PDI) for each peak and for the total sample, as well as relative amounts in weight-% or mole-% (indirectly) as shown in table 5.3. It can be seen from table 5.3 that the molecular weight values ($M_n$, $M_p$ and PDI) for each peak and for the total sample obtained from the UV chromatogram were close to those obtained from the calibrated ELSD chromatogram. However, there were some differences between the calculated relative amounts of non-functional PMMA in mole-% when calculated directly using UV and when calculated indirectly using ELSD and the second-dimension molecular-weight calibration. Likewise, there were some differences when calculating the weight-% directly (from ELSD) and indirectly (from UV). This is likely due to uncertainties in the SEC calibration curves.

<table>
<thead>
<tr>
<th>Peak name</th>
<th>$M_n$ kg/mol</th>
<th>$M_p$ kg/mol</th>
<th>PDI</th>
<th>Conc. (mol %)</th>
<th>$M_n$ kg/mol</th>
<th>$M_p$ kg/mol</th>
<th>PDI</th>
<th>Conc. (mol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 1 (non-OH)</td>
<td>2.3</td>
<td>2.3</td>
<td>1.28</td>
<td>16 (13°)</td>
<td>2.1</td>
<td>2.5</td>
<td>1.24</td>
<td>(10°) 9</td>
</tr>
<tr>
<td>Peak 2 (mono-OH)</td>
<td>2.7</td>
<td>3.2</td>
<td>1.28</td>
<td>84 (87°)</td>
<td>2.4</td>
<td>2.9</td>
<td>1.27</td>
<td>(90°) 91</td>
</tr>
<tr>
<td>Peaks 1 and 2 (combined)</td>
<td>2.6</td>
<td>3.0</td>
<td>1.30</td>
<td>N/A</td>
<td>2.3</td>
<td>2.9</td>
<td>1.28</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Indirect estimate.

The relative amount of non-functional PMMA in weight-% is close to that obtained by (one-dimension) critical LC (10%, obtained using ELSD, see table 2.4 and ref. [23]). When the ELSD response was assumed to be proportional to the mass (an assumption that is likely to be incorrect), the calculated average molecular weights ($M_n$ and $M_p$) from the original ELSD chromatogram were close to those obtained from the UV chromatogram or from the calibrated ELSD chromatogram. However, the PDI value calculated from the original ELSD chromatogram (1.21) was much lower.
than that from the UV chromatogram (1.30) or that from the calibrated ELSD chromatogram (1.28). This is logical when we consider eqn. 3, because the power constant $b$ is usually larger than 1 (see table 5.2). Similar results were observed for other samples (results not shown). Therefore, we can use the original ELSD chromatogram to get the approximate molecular-weight values ($M_n$ and $M_p$), but we have to bear in mind that the PDI (and, thus, also the weight-average molecular weight, $M_w$) is somewhat underestimated.

Fig. 5.7 shows an example of LC×SEC for a real sample without UV-active groups (VL37B). Only the ELSD chromatogram could be used for quantitative analysis, as summarized in table 5.4. As seen from this table, there were small difference between the molecular weight characteristics ($M_n$, $M_p$ and PDI) of the various functional polymers [non-functional (peak 1), mono-OH (peak 2) and di-OH PMMA (peak 3)]. This was especially true for the di-functional PMMA, which showed the highest molecular weight. The relative amounts of non-, mono-, and di-functional polymers in weight-%, calculated directly from the calibrated ELSD chromatogram, are close to those obtained by (one-dimension) critical LC (non-funct. 6%, mono-funct. 83% and di-funct. 11% obtained using ELSD, see table 2.4 and ref. [23]). Using the second-dimension molecular-weight calibration, the concentrations by weight can be converted into molar concentrations for individual peaks. As seen in table 5.4, the molar concentration was larger than the weight concentration for non-functional polymer (and *vice versa* for the di-functional polymer), because the molecular weight of the former was lower than that of the latter. It also can be seen from tables 5.3 and 5.4 that the molecular weight of the non-functional fraction was very similar for samples VL37A and VL37B. It was also similar for the mono-OH PMMA fraction. Therefore we established a method to calculate the molecular weight information ($M_n$, $M_p$ and PDI) and the relative amount (mole or weight percentages) for any fraction or peak(s) in LC×SEC to obtain the MMD and FTD for functional polymers.

![ELSD chromatogram](image)

**Fig. 5.7** LC×SEC chromatogram of sample VL37B detected by ELSD. The conditions were identical to those of Fig. 5.6.
Table 5.4 Quantitative results obtained for sample VL37B using calibrated ELSD. The conditions and the peak numbers are as in Fig. 5.7.

<table>
<thead>
<tr>
<th>Peak name</th>
<th>$M_n$ g mol</th>
<th>$M_p$ g mol</th>
<th>PDI</th>
<th>Conc. (mol %) w %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 1 (non-OH)</td>
<td>2.4</td>
<td>2.5</td>
<td>1.25</td>
<td>(7') 6</td>
</tr>
<tr>
<td>Peak 2 (mono-OH)</td>
<td>2.6</td>
<td>3.0</td>
<td>1.31</td>
<td>(86') 85</td>
</tr>
<tr>
<td>Peak 3 (di-OH)</td>
<td>3.2</td>
<td>5.2</td>
<td>1.35</td>
<td>(7') 9</td>
</tr>
<tr>
<td>Peaks 1, 2 and 3 (combined)</td>
<td>2.7</td>
<td>3.0</td>
<td>1.32</td>
<td>-</td>
</tr>
</tbody>
</table>

*Indirect estimate.

5.4 Conclusions

Comprehensive two-dimensional liquid chromatography (LC×SEC) was investigated to determine the mutually dependent molecular-weight distributions (MWD) and functionality-type distributions (FTD) of functional poly(methyl methacrylate) (PMMA) polymers. Experimental results confirmed that LC×SEC may benefit from the use of longer columns and higher flow rates, to maintain sufficient separation efficiency in the second (fast-SEC) dimension. The complications of quantitative analysis for functional low-molecular-weight PMMA polymers by LC×SEC was discussed for various detection techniques, including refractive-index (RI), ultraviolet-absorbance (UV), and evaporative light-scattering detection (ELSD). A simple method to establish ELSD calibration curves was presented. Each response (data point) from the ELSD chromatogram could be converted into the corresponding mass concentration, using calibration curves obtained by injecting each standard directly into the ELSD without a column. The height of the flat area (plateau) is related to the injected concentration.

Qualitative and quantitative information was obtained on real samples (VL37A and VL37B). This demonstrated the usefulness of LC×SEC in determining the MWD and FTD for functional polymers. The peak capacity was greatly enhanced by LC×SEC in comparison with one-dimensional separations and accurate molecular-weight information ($M_n$, $M_w$, $M_p$ and PDI) could be obtained for individual peaks or for combinations of peaks. Experimental results suggested that the original (uncorrected) ELSD chromatograms could be used to obtain the approximate molecular-weight values ($M_n$ and $M_w$), but that the resulting PDI and $M_w$ were somewhat underestimated.

The influence of the molecular weight on the retention behavior in LC was also investigated for hydroxyl-functional PMMA polymers using LC×SEC. The critical conditions – by definition - independent of molecular weight were not exactly the same for PMMA series with different end-groups. Our observations are in close agreement with theoretical curves reported in the literature.
However, for practical applications of LC\texttimes SEC it is not strictly necessary to work at the exact critical solvent composition. Near-critical conditions often suffice to determine the mutually dependent MWD and FTD of functional polymers. Quantitative results obtained by LC\texttimes SEC are still subjected to some error, especially in case ELSD is required. This is the subject of ongoing research.

Acknowledgements

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References

Chapter 5

CHAPTER 6

Breakthrough of Polymers in Interactive LC

ABSTRACT

Two separate peaks are observed for narrow polymer standards in both isocratic and gradient HPLC. One peak appears around the solvent front (the “solvent-plug peak” or “breakthrough peak”), whereas the second peak is retained significantly – or even highly. Although the effect has been observed many times before, it has never been rigorously explained. In this paper we provide a detailed explanation for the breakthrough peak. The two completely separated peaks are demonstrated not to represent two different fractions of the sample (e.g. the low- and high-molecular-weight parts of the distribution). Both peaks are representative of the entire polymeric sample for narrow polymer standards. Because the amount of the polymer in the breakthrough peak may vary, the quantitative analysis of the polymers by LC is jeopardized. The effects of the sample solvent, the (initial) mobile-phase composition, the injection volume, the injected sample concentration, the column temperature, and the analyte structure and molecular weight on the breakthrough peak were investigated in LC experiments involving standards of polystyrene (PS) and poly(methyl methacrylate) (PMMA). Three necessary and sufficient conditions are suggested for the breakthrough phenomenon to be observed. Recommendations to avoid the breakthrough phenomenon are given, culminating in a structured method for selecting the best possible sample solvents.

Keywords: liquid chromatography, solvent-plug peak, breakthrough, PS, PMMA

6.1 Introduction

Synthetic polymers present many unique separation challenges, because they consist of a
distribution of structurally different chains [1]. Due to the solution properties of macromolecules,
the liquid chromatography of specific synthetic polymers is restricted to a narrow selection of
solvents. The huge dependence of polymer retention on the mobile-phase elution strength often
necessitates the use of gradient elution. A typical gradient runs from a non-solvent (or a weak
solvent) to a strong solvent. Therefore the solvent in which the sample is dissolved and injected is
usually stronger than the mobile phase that surrounds it. This is equally true when the polymers are
eluted isocratically [2]. Armstrong and Bui [3], Larmann et al. [4], Glöckner [5], Lochmüller and
McGranaghan [6], Schultz and Engelhardt [7], Shalliker et al. [8], Northrop et al. [9], and Philipsen
et al. [10], have all observed that single, narrow polystyrene standards of high molecular weight
eluted as multiple peaks in a binary mobile phase. This “breakthrough”, “solvent-plug” or “sweep-
through” effect may jeopardize the qualitative and quantitative analysis of polymeric samples.
Although the above authors have identified the problem, the significance of the sample solvent is
still frequently overlooked, both in scientific literature and, arguably, in common practice.

Lochmüller and McGranaghan [6] reported that for the isocratic elution of polystyrenes in
a binary mobile-phase mixture of tetrahydrofuran (THF) and water, a fraction of the polymer eluted
with the solvent front. They suggested that a mixing chamber between the injector and the column
would improve the mixing of the sample with the mobile phase, thus eliminating this anomalous
behaviour. Shalliker et al. [11,12] observed double peaks in gradient-elution chromatograms of
high-molecular-weight polystyrenes using mobile phases consisting of dichloromethane and
methanol. However, when they used a dichloromethane-acetonitrile solvent system, they observed a
single, symmetrical peak. They concluded that the amount of polymer eluting together with the
sample solvent was considerably reduced when smaller packing particles were used. However,
these observations are difficult to interpret, because many parameters (column length, particle size,
porosity) were changed simultaneously [11].

Multiple peaks can also be seen in chromatograms of copolymers published in the literature [13-
20]. Augenstein and Stickler [17] observed that an additional peak, coinciding with the
breakthrough of the solvent in the case of UV or RI detection, could also be detected with an
evaporative light-scattering detector (ELSD). Therefore, this additional peak was thought to be due
to the polymer sample. They suggested that the breakthrough peak (the additional peak) was due to
incomplete precipitation of the injected polymer [17].

In our experiments, the gradient elution of low-molecular-weight ($M_p 2,990$) and high-molecular-
weight ($M_p 34,500$) PMMA standards with low polydispersity (PDI < 1.1) produced two separated
peaks (with ELSD detection). A precipitation phenomenon could not have played a role in this case,
because the PMMA could be dissolved in the initial mobile phase (consisting of 2% methanol in
toluene).

The crucial question is whether the polymer is separated into two fractions that differ in (for
example) their molecular weights. If the two fractions differ, the fraction that is eluted at the
(physically) correct retention time is not representative of the entire polymeric sample [13]. In that case many incorrect conclusions on the composition and distribution(s) of synthetic polymers may be drawn from LC experiments. If the fractions are both representative of the entire sample, then quantitative analyses will be jeopardized. In the latter case, we would also be left to explain the mechanisms that give rise to the two vastly separated peaks.

We set out to establish a sound explanation for the breakthrough effect, to establish which parameter has a significant effect on this phenomenon and, ultimately, to determine how it can be avoided or overcome. To this end we investigated the sample solvent, the (initial) mobile-phase composition, the injection volume, the injected sample concentration, and the effects of the column temperature, of the analyte structure and of the molecular weight on the breakthrough phenomenon.

6.2 Experimental

6.2.1 Chemicals

Non-stabilized tetrahydrofuran (THF), HPLC grade, was obtained from Biosolve (Valkenswaard, The Netherlands). Toluene and n-hexane (both glass-distilled grade), and methanol (HPLC grade), were from Rathburn Chemicals (Walkeburn, Scotland). THF was used as obtained (without further purification by, e.g., distillation). Water for use in HPLC was doubly distilled in house. The polystyrene (PS) and poly(methyl methacrylate) (PMMA) standards were obtained from Polymer Laboratories (Church Stretton, U.K.). The molecular-weight values ($M_w$) were supplied by the manufacturer. The polydispersity, PDI, of all standards was lower than 1.10.

6.2.2 Equipment

A Waters (Milford, MA, USA) 2690 Alliance liquid-chromatography system was used to perform the isocratic LC experiments on PS. This HPLC instrument contained a built-in auto-injector with a sample loop allowing injection of variable sample volumes, and was equipped with a Waters 996 PDA (photodiode-array detector) and a Sedex 55 evaporative light-scattering detector (ELSD) (temperature 62°C, $N_2$ pressure 2.2 bar). THF was flushed with helium in order to prevent the formation of explosive peroxides. The mobile phase was prepared in-situ using the solvent-mixing capability of the instrument. The data collection and the data analysis were handled by Waters Millennium 3.2 software. The columns used (150 mm x 4.6 mm i.d.) were packed in-house with Hypersil Silica (3-μm particles; 100-Å pore size; Shandon, Runcorn, UK). The columns used to measure the molecular weight in SEC were three PLgel columns (300 mm x 7.6 mm i.d.) with pore sizes of 100 Å, 100 Å, $10^5$ Å, respectively.

The HPLC system used in the gradient LC of PMMA standards consisted of two Gynkotek (Germering, Munich, Germany) Model 300C high-precision pumps and a Rheodyne 7010 injector (Berkeley, CA, USA). The detectors were a variable-wavelength UV-VIS spectrometer (Spectroflow 757, Applied Biosystems, Ramsey, NJ, USA) set at 254 nm and a Varex ELSD II A (Burtonsville, Maryland, USA). A Dupont Zorbax C8 column (250 mm x 4.6 mm i.d.; 5-μm particles; 100-Å pore size; Rockland Technologies, PA, USA) and a home-packed column (150 mm
x 4.6 mm i.d.; 3-μm Hypersil Silica particles; 100-Å pore size) were used. The columns were contained in a (Millipore) Waters temperature-control module.

6.3 Results

Breakthrough peaks have been observed in many different systems [2-22]. Here we chose to study a column/eluent combination that we used for the separation of blends of PMMA and poly(hydroxyethyl methacrylate) (PHEMA) and their copolymers [21]. An evaporative light-scattering detector (ELSD) is most appropriate for this purpose. This system showed clear breakthrough peaks, allowing the phenomenon to be studied thoroughly. To study quantitative aspects of the breakthrough phenomena, some experiments involving UV detection and polystyrene (PS) as the sample are also described.

6.3.1 Sample solvent

A series of reversed-phase gradient-elution chromatograms were recorded for a PMMA standard ($M_p$ 34,500, PDI 1.04), dissolved in mixtures of tetrahydrofuran (THF) with methanol. The mobile-phase composition was programmed from 39.5 to 100% THF in the weak solvent A (water/methanol 25/75) in 20 minutes and the C8 column was used. The flow rate was 0.6 ml/min. All chromatograms obtained with binary THF/methanol mixtures as sample solvents showed two distinctly different peaks (top four traces in Fig. 6.1). When the sample solvent was a weak eluent, such as THF/solvent A (50/50) (viz. THF/methanol/water 50/37.5/12.5; bottom trace), only one peak was observed for PMMA. The peak eluting with a retention time of about 9.5 minutes was observed with all sample solvents. We believe that this peak represents the true chromatographic retention time of the PMMA standard in the present system (see discussion in section 6.4.2), which is why we refer to it as the "real" peak. The other peak was approximately unretained. Following Philipsen et al. [10] we refer to this peak as the "breakthrough peak". Other authors have observed similar unretained signals and referred to them as solvent-plug [22] or swept-through peaks [17]. As can be seen from Fig. 6.1, the size of the breakthrough peak decreases when the strength of the sample solvent decreases (chromatograms from top to bottom). At the same time, the size of the "real" (retained) peak increases. Using mixtures of THF and methanol as sample solvents, the occurrence of a breakthrough peak cannot be avoided. The PMMA standards do not dissolve in pure methanol. In the weakest possible injection solvent (about 35/65 THF/methanol) a breakthrough peak is still observed (Fig. 6.1). A breakthrough peak is not observed when a ternary mixture of THF, methanol, and water is used as the sample solvent. This mixture is a solvent for the PMMA standard, but it is a weaker eluent than the THF/methanol mixtures. We will discuss later (section 6.4.2) how a solvent can be selected in order to avoid the breakthrough peak.
Fig. 6.1 Chromatograms of PMMA ($M_n$ 34,500, PDI 1.04) dissolved in mixtures of methanol, THF (and water) indicated in the figure at 25°C (from top to bottom sample solvent: pure THF, THF/methanol 80/20, THF/methanol 50/50, THF/methanol 35/35, THF/methanol /water 50/37.5/12.5). Detector: ELSD; flow rate 0.6 ml/min; injected sample 20 µl of 1 mg/ml; Dupont Zorbax C8 column (250 mm x 4.6 mm i.d.; 5 µm particles; 100 Å pore size); gradient from 39.5% of solvent B (THF) in solvent A (water/methanol 25/75) to 100% of solvent B (THF) in 20 min.

As seen from Fig. 6.1, when the sample solvent consisted of 50/50 or 35/65 THF/methanol, the breakthrough peak elutes somewhat earlier than with pure THF as sample solvent. This indicates that with pure THF as the eluent as is the case in the sample-solvent zone, a greater amount of THF is adsorbed on the stationary phase. Different sample solvents as eluents are known to show different retention times (hold-up time for the column) in reversed-phase liquid chromatography (RPLC) [23]. In normal-phase liquid chromatography (NPLC), we also observed a significant effect of the sample solvent on the occurrence, the size, and the elution time of the breakthrough peak (see discussion in section 6.4.2).

6.3.2 Initial mobile-phase composition

To investigate the influence of the initial mobile-phase composition on the breakthrough effect, we recorded a series of chromatograms using different initial percentages of the strong solvent B (THF) in the weak solvent A (water/methanol 25/75). The same PMMA standard (dissolved in THF) and the same reversed-phase gradient-elution system where used as in Fig. 6.1. The gradient then ran from the indicated composition to 100% of THF in 20 minutes. As seen in Fig. 6.2, we observed only one peak when the initial mobile phase contained 35% of THF (65% of mixture A). When the initial mobile phase contained 38% to 40% of THF, we observed two completely separated peaks for PMMA standards. When we subsequently collected these two peaks, evaporated them to dryness, re-dissolved the residues in THF, and injected the resulting solutions in a SEC system, identical molecular-size distributions were found for the material contained in the two peaks (see section 6.4.1 below).
As seen in Fig. 6.2, the retention time of the second peak (retention peak) increased with decreasing THF composition in the initial mobile phase. This is due to the change in the gradient slope. The lower the THF content in the initial mobile phase, the longer it takes until the mobile-phase composition reaches the elution composition of the PMMA standard. The location of the breakthrough peak is also affected by the initial composition. If the initial mobile phase is very strong (above the critical composition, e.g. 50% THF), then the polymer sample may be eluted in the size-exclusion mode before the column dead time, t₀. In this case, only one (partially) excluded peak can be observed.

6.3.3 Injection volume

To investigate the effect of the injection volume on the breakthrough phenomenon, we recorded a series of chromatograms for the same PMMA standard as used in Figs. 6.1 and 6.2 using one of the reversed-phase gradients also used in Fig. 6.2 (gradient from 38% to 100% of solvent B, THF, in weak solvent A, water/methanol 25/75, in 20 minutes; C8 column; flow rate 0.6 ml/min). It is clearly seen in Fig. 6.3 that the size of the breakthrough peak increased sharply when the injection volume was increased from 10 to 30 μl. If the injection volume is large, the breakthrough peak is asymmetrical, with a sharp front and a slower tail. When the injection volume becomes very large, we even observe double peaks around the breakthrough volume.

Because the PMMA sample cannot be detected with a UV detector with THF in the mobile phase, a PS standard was used to quantitatively investigate the effects of the injection volume and of the sample concentration on the breakthrough phenomenon. To minimize the effect of mobile-phase UV absorption, isocratic elution was used instead of gradient elution. We recorded a series of isocratic chromatograms of PS standards (Mₚ 11,600, PDI 1.03) with THF as the sample solvent and 25% THF in hexane as the mobile phase. The home-packed silica column was used and the flow
rate was 1.0 ml/min. The injection volume was varied from 12 to 40 μl. The sample concentration was constant. As shown in Fig. 6.4, we observed two peaks in every chromatogram. One was the breakthrough peak, eluting near t₀; the other was the real retention peak, which was always very broad under isocratic conditions. The online UV spectra recorded for these two peaks were found to be almost identical and appeared to indicate the dominant presence of PS (obvious absorption bands at 261.5 nm in the UV spectrum). It is also apparent from Fig. 6.4 that the relative size of the breakthrough peak increased as the injection volume increased. As can be seen in Fig. 6.5a, when the injected volume exceeded about 12 μl, the relative area of the breakthrough peak increased sharply. At the same time, the observed elution time for the real retained peak showed a distinct minimum (Fig. 6.5b). This can be explained by a gradual diminishing of the breakthrough effect at the top of the column (cf. Fig. 6.8 below) and the real retention peaks are broadened.

![Fig. 6.3 Effect of the injection volume on the breakthrough phenomenon for PMMA (Mₚ 34,500, PDI 1.04) in reversed-phase gradient-elution LC with THF as the sample solvent at 25°C. Detector: ELSD; flow rate 0.6 ml/min; 1 mg/ml of sample dissolved in THF; Dupont Zorbax C8 column (250 mm x 4.6 mm i.d.; 5-μm particles; 100-Å pore size); gradient from 38% of solvent B (THF) in solvent A (water/methanol 25/75) to 100% of solvent B (THF) in 20 min.](image)

To investigate whether the sample-size effect can be eliminated, we injected the same amount (40 μg) of PS in different volumes and concentrations (table 6.1). The results again showed that the size of the breakthrough peak increased as the injection volume increased. The retention time of the second analyte peak again showed a minimum at intermediate injection volumes. It should be noted that the observed peaks were very broad in all these isocratic chromatograms. Because ELSD is not convenient for quantitative analysis, the quantitative results in table 6.1 are based on the UV response at 254 nm (UV254). However, the ELSD response is shown as an indication of the presence of polymer in the peak. There is some variation in the observed total UV areas in table 6.1. In part this is related to variations in the injected volume and to inaccuracies in the (approximate) concentrations specified. Also, these data refer to isocratic experiments, where the real-retention peaks are very broad.
Fig. 6.4  Effect of the injection volume on the breakthrough peak for the isocratic elution of a PS (11,600, PDI 1.03) standard on a home-packed column (150 mm x 4.6 mm i.d.; 5-µm Hypersil silica particles; 100-A pore size) at 25°C. Detector: ELSD; flow rate 1 ml/min; mobile phase 25% THF in hexane; constant sample concentration of 5 mg/ml of dissolved in THF. The inserted part shows the enlarged 2-µl-injection-volume chromatogram.
(Note: Successive chromatograms are shifted by 1 minute and 100 ELSD units)

Fig. 6.5  (a) The relative area of the breakthrough peak and (b) the retention time of the "real" retention peak detected by UV at 254 nm for different injection volumes. LC conditions and PS standard used were the same as in Fig. 6.4.
6.3.4 Sample concentration

In order to investigate the effect of the polymer concentration injected on the breakthrough peak, we measured the ratio of the areas of the two peaks for a series of injections of equal volume, but with different concentrations, using the same PS standard and same conditions as in Figs. 4 and 5 and table 6.1. Table 6.2 shows that the fractional area of the breakthrough peak decreases with increasing sample concentration, but that this effect is small until very high concentrations (20 mg/ml) are reached. It should be noted that the observed peaks are very broad in all these cases.

### Table 6.2

<table>
<thead>
<tr>
<th>Polymer concentration (mg/ml)</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total area (UV254)</td>
<td>851</td>
<td>1444</td>
<td>3563</td>
<td>6621</td>
<td>13887</td>
</tr>
<tr>
<td>first peak area (%) (UV254)</td>
<td>67</td>
<td>65</td>
<td>65</td>
<td>63</td>
<td>14</td>
</tr>
<tr>
<td>time for 1st peak (min) (UV254)</td>
<td>1.9</td>
<td>1.9</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>time for 2nd peak (min) (UV254)</td>
<td>6.9</td>
<td>6.8</td>
<td>6.6</td>
<td>6.2</td>
<td>4.9</td>
</tr>
<tr>
<td>first peak area (%) (ELSD)</td>
<td>97</td>
<td>93</td>
<td>84</td>
<td>74</td>
<td>8</td>
</tr>
</tbody>
</table>

6.3.5 Column temperature

The effect of the temperature was studied from a series of reversed-phase gradient-elution chromatograms recorded at different column temperatures using the same PMMA standard as in Figs. 1 and 2. The same C8 column was used, but the flow rate was slightly lower (0.5 ml/min). The gradient ran from 30% to 100% of solvent B (THF) in solvent A (methanol/water 75/25) in 20 minutes. As shown in Fig. 6.6, the retention time of the real PMMA peak decreased with increasing column temperature. Concomitantly, the column temperature was seen to influence the size of the breakthrough peak. At room temperature (25°C), only one peak was observed. However, when the column temperature was increased, the magnitude of the observed breakthrough peak increased, while the height of the second peak ("real" retention peak) decreased.
6.3.6 Effect of analyte structure and molecular weight

Chromatograms were recorded for a series of PMMA standards of different molecular-weight and one PS standard ($M_p 11,600$, PDI 1.03) under the same conditions as those of Fig. 6.2. As shown in Fig. 6.7, low-molecular-weight PMMA standards gave rise to a very large breakthrough peak. A PMMA standard with a high molecular-weight only yielded a small breakthrough peak, whereas very-high-molecular-weight PMMA standards ($\geq 500,000$ Da) and the PS standard ($M_p 11,600$, PDI 1.03) did not give rise to a breakthrough peak (not shown). Thus, the occurrence of a breakthrough peak and the ratio of the areas of the breakthrough peak and the real retention peak depend not only on the LC conditions, but also on the molecular weight and chemical structure of the analyte polymers. As seen in Fig. 6.7, low-molecular-weight samples (below 34,500) showed relatively short retention times, which strongly depended on molecular weight. In contrast, PMMA samples with molecular weights of 34,500, 67,000 (not shown), and 127,000, showed practically the same retention time. This is all in agreement with contemporary studies on the retention behaviour of polymers in LC, with the high-molecular-weight standards being eluted at approximately the critical composition for PMMA [24]. The retention time of the 127,000 PMMA sample was less than that of the 34,500 PMMA sample. This may well be due to a size-exclusion effect. In gradient elution, when the polymers are eluted near the critical point, high-MW polymers may be affected by SEC phenomena and may be eluted somewhat earlier [24]. However, when the molecular weight of PMMA was higher than 480,000, the peaks shifted to somewhat higher retention times (around 11 minutes). At this point other factors will start playing a role, such as the diameter of the pores (100 Å) in relation to the effective radius of the analyte molecules.
6.4 Discussion

6.4.1 Nature of the two peaks

In the chromatographic experiments described above, narrow polymer standards produced two completely separate peaks. The position of the first peak was near $t_0$, the dead time of the column. As will be explained below (Fig. 6.8), we expect the polymer-breakthrough to coincide with the beginning of the solvent peak. The actual peak maximum of the breakthrough peak may occur a bit earlier than that of the solvent peak. The exact time of elution of the latter is somewhat dependent on the actual mobile-phase composition [23]. When using UV detection this first peak is often obscured by a strong solvent peak and therefore overlooked. In a detailed quantitative study Glöckner [13] assumed that the peaks eluted around the solvent peak formed part of the polymer sample, but since he only used UV detection he could not prove conclusively that the first peak represented a true polymer. He concluded that it was a “crucial question” whether both peaks were representative of the entire polymer, the alternative hypothesis being that the polymer was separated in two different fractions (e.g. one fraction of low molecular weight and one of high molecular weight).

In our experiments we used both UV and ELSD detection. A blank injection of pure solvent did not give rise to a peak on the ELSD trace. If the sample solvent was different from the mobile phase, the UV detector yielded a large solvent peak. The two peaks observed for the PMMA and PS standards were seen with both detectors. The PDA (photodiode-array detector) signals for the two separate peaks of PS featured almost identical UV spectra (PMMA does not strongly absorb in the UV region). We then collected two effluent fractions for the PMMA standard ($M_p=34,500$, PDI=1.04) corresponding to the two peaks. After evaporation of the eluent, the non-volatile analytes were re-dissolved and injected on a SEC system. The result (not shown) indicated that the two fractions obtained from a single standard had identical molecular-weight distributions. When we re-injected the collected peaks on the original LC system (C8 column, see section 3.3), we again
obtained two peaks for each of the fractions under the same gradient conditions. Therefore, we may conclude unequivocally that the two vastly separated peaks do not represent different parts of the (narrowly distributed) sample. Rather, the material responsible for either peak is representative of the entire polymeric sample. It should be noted that the above experiments concerned a very narrow standard. If a (very) broad polymer sample or a copolymer sample is injected, we may easily obtain two separate peaks that represent different parts of the sample (see section 6.3.6).

6.4.2 Explanation for the breakthrough phenomenon

The solvent zone as it may exist in the column after the injection of the sample is shown in Fig. 6.8. The sample solvent A is assumed to be a strong solvent and the mobile phase B is assumed to be a weak solvent or non-solvent. Mixtures of A and B should have an eluent strength somewhere between that of A and B. At some composition \((\varphi)\) the migration velocity of polymers without strongly interacting end groups is approximately the same as that of the solvent. This is known as the critical composition, where — by definition — retention is independent of molecular weight and all members of a polymeric series co-elute. The horizontal dashed line in Fig. 6.8 represents the critical composition \((\varphi).\)

If the concentration of strong solvent in the mobile phase is higher than the value of \(\varphi\), we are in the size-exclusion (SEC) mode and only one peak will be observed. If both the sample solvent and the mobile phase are weaker than the critical composition, we are in the adsorption mode and the polymer will be retained on the column, again producing only one peak (Fig. 6.8b). The retention factor \((k_i)\) of this peak is related to the thermodynamic partition coefficient of the analyte across the two phases \((K_{c,i})\) by the conventional relationship

\[
k_i = \frac{c\text{,eq}_s V_s}{c\text{,eq}_m V_m} = K_{c,i} \frac{V_s}{V_m}
\]

where \(c\text{,eq}_s\) is the equilibrium concentration of the analyte in the stationary phase \((s)\), and \(c\text{,eq}_m\) is its equilibrium concentration in the mobile phase \((m)\). \(V_s\) and \(V_m\) are the respective volumes of the two phases in the chromatographic column. Because this second peak obeys the conventional laws of chromatography, we have referred to it as the “real retention peak” in the present paper.

Only when the mobile phase is weaker than the critical composition and the sample solvent is stronger is it possible to observe the breakthrough effect.

How do the two peaks come about? As shown in Fig. 6.8a, when the polymer sample is injected into the column, polymer will be present throughout the sample zone. Those molecules that are ahead of the solvent plug will move more slowly (the mobile phase is a weak eluent), so that they will soon be caught up by the solvent front. Polymers present in the centre of the solvent plug will experience exclusion conditions and they will move faster than the solvent, until they reach the critical point. Thus, we expect a focussing of the polymer molecules within this solvent zone at the exact location of the critical point (Fig. 6.8a). However, those polymer molecules that are in the tail of the solvent plug, where the composition is below the critical value, will rapidly fall behind the
solvent plug and they will be retained properly, giving rise to the second ("real retention") peak (Fig. 6.8a).

Fig. 6.8 Simulated representations of the solvent zone in the column. The horizontal dashed line represents the critical composition ($\phi_c$). (a) A focusing of the polymer molecules within the solvent zone at the exact location of the critical point is expected and polymers in the tail of the solvent plug will be retained properly, giving rise to the second ("real retention") peak. (b) When both the sample solvent and the mobile phase are weaker than the critical composition, we are in the adsorption mode and the polymer will be retained on the column, producing only one peak. (c) the percentage of A solvent in the solvent zone will decrease and the width of the solvent zone will increase along the column.
Due to kinetic diffusion and other dispersive processes, the percentage of A solvent in the solvent zone will decrease and the extent (width) of the solvent zone will increase along the column as shown in Fig. 6.8b and 6.8c. If the solvent plug is dispersed (or the composition at its “peak” falls below the critical value) the polymer molecules will be gradually left behind and the two peaks will not be completely separated (cf. the isocratic LC experiments shown in Fig. 6.4). When the injection volume increases (cf. section 6.3.3), the solvent zone stays essentially intact and a long plug of strong solvent will leave the column, increasing the size of the breakthrough peak. If the polymer concentration in the injected sample increases, a longer zone of strong solvent will be required to push the (viscous) plug of concentrated polymer solution through the column. A greater fraction of the polymer is likely to fall behind and the solvent plug may break up more easily. Therefore, when the polymer concentration is increased without increasing the volume of strong solvent injected, the breakthrough peak becomes smaller relative to the “real” retention peak (cf. table 6.2).

One tentative explanation for the observations in Fig. 6.5 may be that at injection volumes smaller than about 12 µl only small patches or droplets of the injection solvent reach the detector without being diluted by the mobile phase to a (maximum) concentration below the critical composition. Large volumes (> about 12 µl) may result in a single, coherent plug of the injection solvent reaching the end of the column. The analyte molecules have a greater chance to “escape” from a diffuse (droplet) solvent plug than from a coherent one, resulting in a peak with a retention time in between that of the real retention peak and the breakthrough peak. The excessive peak broadening observed for the analyte peaks, especially at intermediate injection volumes (between 10 and 20 µl; see Fig. 6.4), provides some support for such a model.

Different polymers (e.g. PS, PMMA) have different critical compositions. Thus, it is easy to understand that the breakthrough phenomenon depends on the chemical composition of a (co)polymer. As to the molecular-weight dependence shown in Fig. 6.7, one of the possible reasons is that the viscosity of the polymer in solvent zone increases with the molecular-weight. As was the case when increasing the polymer concentration (table 6.2), a longer zone of strong solvent will be required to push the viscous polymer-solution plug through the column. A greater fraction of the polymer is likely to fall behind the solvent plug. Another reason is that the retention factor increases with increasing molecular weight. The greater the retention factor, the lower the tendency for the analyte peak to be distorted when the injection solvent is stronger than the mobile phase [22].

Thus, an essential condition that needs to be fulfilled for a breakthrough peak to be observed is that the solvent plug stays intact until the end of the column and that the eluent strength in the centre of this solvent zone remains stronger than the critical value (Fig. 6.8c). In summary, the three necessary conditions for separating a single, narrow (or even monodisperse) polymer standard into two vastly separated peaks are

1. The mobile phase should be weaker than the critical composition;
2. The sample solvent should be stronger than the critical composition;
3. A plug of sample solvent with a concentration higher than the critical composition should remain intact throughout the column.

Although these conditions seem rather restrictive, they are, unfortunately, often met in practice. If we wish to separate polymers according to their chemical composition (ratio of different monomers) or functionality (number and type of end groups or functional groups), we must rely on interactions between the polymer and the stationary and mobile phases, rather than on exclusion effects. Thus, condition 1 will be met. Condition 2 is hard to avoid in practice, because it is very much easier to inject a polymer in a strong solvent than in a weak solvent. Especially under gradient-elution conditions, the initial mobile phase tends to be a weak solvent, which is not suitable for preparing and injecting samples. The third condition is likely to be met with contemporary HPLC systems and columns, both of which have been designed with the intention to minimize zone dispersion. The breakthrough phenomenon may be circumvented by inserting a mixer after the injector [6], but this does lead to additional band broadening. It is, therefore, not an attractive option from the perspective of chromatographic separation.

Berek et al. [25] measured the zones of the injection solvent as they left the column. The sample solvent appeared to always overload the column (250 mm x 4.6 mm i.d.) for injection volumes of 10 µl, corresponding to about 10 mg of solvent [26]. A zone of pure injection solvent ("solvent plug") pertains throughout the column. However, the conditions for the solvent zone to remain intact are affected by the packing of the column, the viscosity of the mobile phase, the polarity difference between the components of the injection solvent and the mobile phase, the kinetic diffusion of the polymer sample, etc..

A very important factor is the location of the critical point (composition). When the column temperature changes, the critical point will change and the breakthrough peak will vary as shown in Fig. 6.6.

There are several ways in which the occurrence of breakthrough peaks can be avoided. As discussed above, condition 1 cannot be avoided. However, conditions 2 and 3 can be avoided by a judicious choice of conditions and sample solvents. Fundamentally, it is best to use the mobile phase (or an even weaker solvent) as the sample solvent. The injection of turbid samples, containing finely dispersed (rather than dissolved) polymer in weak solvents, reputedly yields good results in polymer LC [27,28]. In any case the weakest possible solvent (whether weaker or stronger than the mobile phase) should be used in the smallest possible volume. It must be noted that the sample solvent is not necessarily a (mixture of) eluent component(s). It is quite possible that a third solvent can be found which (i) dissolves the polymer, (ii) mixes with the (initial) eluent and (iii) is a relatively weak eluent itself. According to the conventional liquid–solid chromatography (LSC) retention model of competitive adsorption [29], desirable sample solvents interact favourably with the polymer, but not with the stationary surface.

For example, in RPLC a relatively weak eluent should have a high polarity parameter ($P'$, reference [30], p.285). Examples include water (10.2), DMSO (dimethyl sulfoxide, 7.2), DMF (dimethylfomamide, 6.4), acetonitrile (5.8), and methanol (5.1). Of all these, only DMSO and DMF are good solvents for PMMA. The same PMMA standard and the same RPLC conditions as in Fig.
6.1 were used in the following examples. When pure DMSO or pure DMF was used as the sample solvent, no breakthrough was observed. As shown in Fig. 6.1 (bottom trace), also no breakthrough was observed when the sample solvent contained 12.5% of water (a very weak eluent), 37.5% methanol and 50% THF.

(a): Reversed-phase LC

<table>
<thead>
<tr>
<th>Strong eluent</th>
<th>Weak eluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polarity parameter $P'$</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hexane (0.1)</th>
<th>Toluene (2.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM (3.1)</td>
<td>THF (4.0)</td>
</tr>
<tr>
<td>Methanol (5.1)</td>
<td>Dioxane (4.8)</td>
</tr>
<tr>
<td>Acetonitrile (5.8)</td>
<td>Acetone (5.1)</td>
</tr>
<tr>
<td>Water (10.2)</td>
<td>DMF (6.4)</td>
</tr>
<tr>
<td></td>
<td>DMSO (7.2)</td>
</tr>
</tbody>
</table>

(b): Normal-phase LC

<table>
<thead>
<tr>
<th>Strong eluent</th>
<th>Weak eluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluent-strength parameter $\varepsilon^0$</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Water</th>
<th>DMF (0.80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol (0.95)</td>
<td>DMSO (0.75)</td>
</tr>
<tr>
<td>Acetonitrile (0.65)</td>
<td>Acetone (0.56)</td>
</tr>
<tr>
<td></td>
<td>THF (0.57)</td>
</tr>
<tr>
<td>Hexane (0.01)</td>
<td>DCM (0.42)</td>
</tr>
<tr>
<td></td>
<td>Toluene (0.29)</td>
</tr>
</tbody>
</table>

Fig. 6.9 Solvent selection scheme for PMMA: when the injection solvent is a strong solvent for the polymer and a strong eluent (top-right corner), a breakthrough peak will appear; the injection solvent should be a strong solvent for the sample and a weak eluent (bottom-right corner) to avoid the breakthrough phenomenon. (a) Reversed-phase LC (b) Normal-phase LC.

Fig. 6.9 illustrates a concept for selecting suitable sample solvents. Ideal injection solvents dissolve the sample easily (right-hand-side of the figure), but are poor eluents (bottom half of the figure). The best solvents to avoid breakthrough effects are thus situated in the bottom-right corner of the diagrams in Fig. 6.9. Fig. 6.9a shows the relevant characteristics for a number of solvents for PMMA in RPLC. The polarity parameter ($P'$, [30]) is used to indicate the solvent strength. Qualitative information on the quality of the suggested solvents for dissolving PMMA was obtained.
from ref. [31]. Fig. 6.9b applies to normal-phase LC. Snyder's eluent-strength parameter \( (s^\theta) \), ref. [30], p.365) is now plotted on the vertical axis.

Fig. 6.10 provides evidence for the usefulness of Fig. 6.9a to select an appropriate injection solvent for the PMMA samples of Fig. 6.1. Usually, a strong sample solvent is selected that is also a strong eluent, such as THF (top right corner in Fig. 6.9a). The presence of THF in the sample solvent ensures sample solubility and compatibility with the eluent. When adding sample solvents from the top left corner in Fig. 6.9a to THF, large breakthrough peaks are observed, as exemplified by the mixture of 33% hexane and 67% THF (Fig. 6.10 a). Hexane is a non-solvent for PMMA. Because the polarity parameter of hexane is very low (0.1), it is a strong eluent in reversed-phase LC. Selecting a solvent from the bottom left corner in Fig. 6.9a leads to less breakthrough. Thus, mixtures of acetonitrile and THF lead to better results (Fig. 6.10 b and c). However, using mixtures of THF and acetonitrile as sample solvents, the occurrence of a breakthrough peak could not be avoided. The PMMA standards do not dissolve in pure acetonitrile. In the weakest possible injection solvent (about 10/90 THF/acetonitrile) a breakthrough peak was still observed. The best results can be obtained with solvents from the bottom right corner in Fig. 6.9a, such as DMSO (Fig. 6.10 d and e). No breakthrough peak was observed with DMSO/THF 70/30 as the sample solvent, but breakthrough did occur when the sample solvent contained 50% or more of THF in DMSO. Pure DMSO is a good solvent for PMMA and it does not show any breakthrough. However, it is less attractive due to its high viscosity.

To demonstrate the usefulness of Fig. 6.9b, a series of NPLC chromatograms were recorded for a PMMA standard \( (M_p 5,270, \text{PDI 1.06}) \) dissolved in various mixtures of toluene and methanol. 20 \( \mu \)l of 5 mg/ml sample solution were injected. The home-packed silica column was used and the flow rate was 0.9 ml/min. The mobile-phase composition was programmed from 2 to 100% methanol in toluene in 20 minutes. When the sample solvent was pure toluene (bottom-right corner in Fig. 6.9b), only one peak was observed for PMMA with a retention time of about 4½ min (not shown). Solvents in the top-left corner are least attractive. When a significant amount (≥ 20%) of methanol was added to toluene severe breakthrough was observed. When dichloromethane (another example from the bottom-right corner) was used as the sample solvent, no breakthrough was observed.

Injecting the same amount of polymer, a small injection volume and a relatively high analyte concentration are favourable from the point of view of avoiding breakthrough peaks. In isocratic separations, we cannot vary the mobile phase, as its composition is dictated by the desired separation. In gradient-elution experiments the use of weak initial solvents is recommended. The weaker the initial solvent, the more effectively the solvent plug can be dispersed during the early stages of the experiment. This effect can be consciously exploited by using the “sandwich” injection method proposed by Mengerink et al. [32]. Measures to enhance mixing between the (initial) mobile phase and the sample solution [6] reduce the risk of breakthrough peaks. However, they may result in additional band broadening, especially in the case of isocratic LC.
Chapter 6

Conclusions

In the chromatographic experiments described above, narrow polymer standards produced two completely separate peaks. These two peaks showed identical molecular weight distributions upon re-injection. They are both representative of the entire polymeric (narrow-standard) sample. However, whether the breakthrough peak exists (and how large the ratio of the breakthrough peak to the real retention peak is) depends not only on the LC conditions, but also on the molecular weight and chemical composition of the analyte polymer. This implies that if a (very) broad polymer sample or a copolymer sample is injected, we may easily obtain two separate peaks that represent different parts of the sample.

The sample solvent, the (initial) mobile-phase composition, the injection volume, the injected sample concentration, and the effect of the column temperature on the breakthrough phenomenon were investigated in the LC of PS and PMMA. The results showed that the breakthrough peak increased as the injection volume, the column temperature, the strength of the sample solvent injected, and the strength of the initial mobile phase in gradient LC increased, or as the polymer concentration decreased.

An explanation for breakthrough phenomena in polymer LC was proposed and discussed in detail. The three necessary conditions for separating a single, narrow (or even monodisperse) polymer standard into two vastly separated peaks are

1. The mobile phase should be weaker than the critical composition;
2. The sample solvent should be stronger than the critical composition;
3. A plug of sample solvent should remain intact throughout the column.

In order to avoid the breakthrough phenomenon, the chromatographer should
i. Use an injection solvent that is as weak an eluent as possible
   a. In case of reversed-phase LC a solvent with a high polarity parameter \( P' \) should be selected as
      the sample solvent (or as one of the components of the injection solvent);
   b. In case of normal-phase LC a solvent with a low solvent-strength parameter \( \varepsilon^0 \) should be
      selected as the sample solvent (or as one of the components of the injection solvent);
ii. Inject (relatively) high concentrations in (relatively) small volumes
iii. a. In case of gradient elution, use the weakest possible initial solvent
   b. In case of isocratic elution use the “sandwich” injection method \cite{32} when necessary, \textit{i.e.}
      when the above remedies (ia and ib) fail to yield satisfactory results.

For the case of PMMA, the rules for selecting sample solvents in RPLC and NPLC have been
summarized in simple diagrams (Fig. 6.9), the validity of which has been demonstrated by selecting
suitable sample solvents for RPLC (DMF or DMSO) and NPLC (dichloromethane or toluene)
separations. To prepare similar figures for other polymers, only some (qualitative) information on
the solubility of the polymer in various solvents is required.

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their cooperation.

References

CHAPTER 7

Computer Simulation of the Effect of the Injection Solvent on the Peak Profile in Reversed-Phase LC under Isocratic and Gradient Conditions

ABSTRACT

Simple relationships describing the retention and peak width under isocratic and gradient-elution conditions in reversed-phase liquid chromatography (RPLC) are used to simulate and quantitatively explain the peak-distortion and peak-splitting effects that arise when the (composition of the) injection solvent is different from the (composition of the) mobile phase. Special attention is paid to the LC separation of polymers, where breakthrough peaks form a serious problem. The results of simulation of the effects of the sample solvent (composition), the injection volume, and the column length on the peak distortion and eventual peak splitting corresponded well with experimental results and with numerous observations reported in the literature. Furthermore, the simulation data showed that the strength of the injection solvent had a much greater influence on the peak profile compared with that of the mobile phase.

Keywords: liquid chromatography, computer simulation, peak profile, peak splitting, breakthrough peak.

Chapter 7

7.1 Introduction

In liquid chromatography, the sample solvent (or injection solvent) is preferably the same as the mobile phase (minimizing disturbances of the base line) or a weaker eluent (minimizing analyte band widths, so-called on-column focusing [1,2,3,4]). However, it may be necessary to use a stronger solvent in order to increase the solubility of the sample [5]. The latter may, for instance, be the case in separations of polymers or bio-materials. In reversed-phase HPLC, many researchers [6-15] have observed peak distortions or multiple peaks for single components. Such phenomena are generally ascribed to the use of a sample solvent stronger than the mobile phase.

Tseng and Rogers [5], using a C_{18}-derivatized silica column, reported that a single component might produce two sharp peaks or a sharp peak with a shoulder when the injected sample solvent was either more polar or less polar than the mobile phase. However, the analytes (o-, m- and p-dihydroxybenzenes) only gave single, symmetrical peaks when the solvent injected was identical to the mobile phase. Kirschbaum et al. [7] observed that a sample solution of hydrochlorothiazide dissolved in methanol gave rise to a doublet peak, while a single sharp peak was obtained when the mobile phase was used as the sample solvent. Williams et al. [6] found that the peak profile changed and that the observed number of theoretical plates decreased in the reversed-phase HPLC of Aspirin and related analgesics when the percentage of organic solvent in the sample solution was increased. Perlman and Kirschbaum [10] connected peak shape with hydrogen bonding, but Berridge [11] presented opposing arguments and Chan and Yeung [12] believed that the observations could be readily explained as being the result of dynamic gradient elution arising between sample solvent and mobile phase. Khachik et al. [13] concluded that the formation of multiple peaks in HPLC depended on the relative solubility of the analytes (carotenoids) in the sample solvent and the mobile phase. Vukmanic and Chiba [14] reported systematic studies into the effects of the type and percentage of organic solvents in the sample solutions and of the injection volumes on the retention times and peak profiles observed for two benzimidazole compounds. They believed that the peak profiles observed for different analytes might be influenced in different ways by the type and percentage of organic solvents used in the sample solutions. Hoffman et al. [15] observed peak distortions ranging from a single, fronting peak to a multitude of peaks. They suggested that this observation was due to slow dilution of the injected plug of strong solvent with the surrounding (weaker) mobile phase. Simulation and computation of this phenomenon was attempted [16,17]. However, the behaviour of the solute zone under these conditions proved difficult to predict [18].

Peak distortion and peak splitting due to the sample solvent could be avoided by injecting very small sample volumes in analytical HPLC. However, large volumes of sample are required in preparative HPLC, so as to achieve the maximum throughput of the purified material. The effects of the sample solvent on the observed peak profiles in preparative HPLC have also been reported by several workers [19,20,21].

In our previous work [22] and the references cited therein, two separate peaks were observed when a strong sample solvent was used in polymer separations. We proved that this breakthrough peak arose from the sample instead of the injected solvent or mobile phase. A qualitative explanation was
proposed for the breakthrough phenomenon in the interactive LC of polymers. Several ways in which the occurrence of breakthrough peaks can be avoided were suggested.

In this work we wish to use well-documented, simple relationships to describe the retention and peak width in isocratic and gradient-elution LC and to quantitatively describe, simulate and predict peak distortion effects and on-column focusing effects encountered in RPLC.

7.2 Theory and Method

Various empirical and theoretical equations to describe the dependence of solute retention on mobile-phase composition in reversed-phase chromatography have been reviewed and compared by Valko et al. [23]. One of the most useful, but approximate equations is the logarithmic-linear relationship given in eqn. 1:

\[
\ln k = \ln k_0 - S\varphi \quad (1)
\]

where \(k\) is the solute retention factor at a specific mobile-phase composition (\(\varphi\)), \(k_0\) is the retention factor in the pure weak solvent (usually water, \(\varphi = 0\)), and \(S\) is the slope. It has been shown that a quadratic form derived from solubility-parameter theory [24], given in eqn. 2, generally provides a better statistical fit [25,26].

\[
\ln k = A\varphi^2 + B\varphi + C \quad (2)
\]

Eqn. 1 is adequate over a sufficiently narrow range in mobile-phase composition [26]. Therefore, it is preferred in many practical situations, such as for the prediction of gradient-elution retention times from isocratic data. To simplify the simulations, we use eqn. 1 in this paper, although eqn. 2 can easily be accommodated in the software programs and it can be used if sufficient experimental data are available.

The Craig model was used to set up the simulations [27,28,29]. The column is assumed to be divided into a number of plates, the mobile-phase volume, of which must be equal to or smaller than the injection volume [17]. Initially, the entire column is in equilibrium. The progression of time is simulated by moving the (non-stagnant) mobile phase by one plate. Next, we calculate the equilibrium concentrations at each plate, to obtain the profiles of the weak solvent, the strong solvent, and the analyte along the column length. After which a new time step is taken. The chromatogram can be obtained by monitoring the analyte concentration in the effluent of the last plate in the column.

We assume that the strong solvent (present in the sample, as well as in the mobile phase) moves rapidly through the column. However, the strong solvent is not necessarily completely unretained, as we allow it to adsorb on the stationary phase according to the classic Langmuir adsorption model:

\[
q_S = a\varphi_m/(1+b\varphi_m) \quad (3)
\]

where \(q_S\) is the amount of the strong solvent adsorbed on the stationary surface and \(\varphi_m\) is the volume fraction of the strong solvent in the mobile phase in any given plate in the column, and \(a\) and \(b\) are
empirical constants related to the strong solvent, the weak solvent, and the stationary surface. Using eqn. 3, the concentration of the strong solvent can be calculated on a given plate at a given time and the profile of the strong solvent arising from the injection can be provided. Concomitantly, the solute retention factors can be calculated using eqn. 1 (or 2) for the various plates at different time. The amount of the solute on any given plate at any given time can be calculated from

\[ k = \frac{Q_s}{(Q_r - Q_s)} = \frac{Q_s}{Q_m} \quad (4) \]

where \( k \) is the solute retention factor at a specific mobile phase composition, \( Q_r \) is the total weight of the solute in a plate and \( Q_s \) is the weight of the solute in the stationary phase in a plate. By calculating the weight of solute in the mobile phase \( (Q_m) \) as a function of time, we can get the solute profile at any plate, viz. the chromatographic peak as it would appear from any intermediate length of column.

### 7.3 Experimental

The spreadsheet workbooks were first written in Excel 97 from Microsoft (Seattle, WA, USA) and then re-implemented in MatLab 6.1 (Mathwork, Natick, MA, USA). It runs on various personal computers (equipped with minimally a Pentium II processor). Table 7.1 provides a summary of all the inputs required by and results obtained from the software.

Table 7.1 | Input and output of Excel spreadsheet (or in the Matlab computer program) for simulating solvent effect on the peak profile under isocratic and gradient-elution conditions in LC.
<table>
<thead>
<tr>
<th>Input data</th>
<th>Output data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical constants in eqn. 3, ( a ) and ( b )</td>
<td>( t_0 ) (retention time of unretained solvent)</td>
</tr>
<tr>
<td>Two of the parameters ( \ln k_0 ), ( S ) and ( \ln k_f )</td>
<td>Isocratic model parameters ( (e.g., S, \ln k_0) )</td>
</tr>
<tr>
<td>Injected solvent composition, volume, amount of solute injected</td>
<td>Retention factor in the injection solvent ( (k_s) )</td>
</tr>
<tr>
<td>Gradient parameters</td>
<td>Gradient slope ( (k_{\text{grad}}) )</td>
</tr>
<tr>
<td>Total dwell time, gradient duration, initial mobile-phase composition,</td>
<td>Retention time ( (t_R) )</td>
</tr>
<tr>
<td>final mobile-phase composition</td>
<td>Elution composition ( (\varphi) )</td>
</tr>
<tr>
<td>Or isocratic mobile phase composition</td>
<td>Elution time of (eventual breakthrough peak ( (t_e) )</td>
</tr>
<tr>
<td></td>
<td>Retention factor in the mobile phase ( (k_m) )</td>
</tr>
<tr>
<td></td>
<td>Peak profile</td>
</tr>
</tbody>
</table>

### 7.3.1 Parameters related to the injection solvent, the mobile phase and the sample

Adsorption of the strong solvent on the stationary phase can be accounted for. This is especially important if we wish to study the solvent peak [22]. As stated above, parameters \( a \) and \( b \) in eqn. 3 are empirical constants related to the strong solvent, the weak solvent and the packing material used. If \( a = 0 \), adsorption effects are neglected. For reasonable values \( (a = 0.01 \) or 0.001) we found
little influence on the peak profile. In this paper, we use \( a = 0.001 \) and \( b = 0.2 \), unless stated otherwise.

Two of the three parameters that describe the retention of the analyte as a function of mobile-phase composition (\( \ln k_0, S \) and \( \ln k_f \)) have to be provided. There are many ways to obtain values for these parameters. The conceptually most simple and perhaps most accurate method uses measured isocratic \( k \) values at two or more compositions. A more convenient method uses retention data obtained in two (or more) different gradient runs.

7.3.2 Gradient parameters

Gradient parameters include the total dwell time (equal to the sum of the instrument dwell time and the initial time, viz. the programmed hold time at the initial composition), the initial and final mobile-phase compositions, and the duration of the (linear segment of the) gradient. It is assumed that the final composition is held indefinitely. Incomplete elution of the solute is usually evident from the predicted chromatogram. Recovery and efficiency can be easily calculated in the program. It is also possible to calculate how much of the analyte is left in the column (and where). Isocratic conditions can be accommodated, by entering a very long initial time or by choosing equal values of the initial and final mobile-phase compositions [30].

7.3.3 Injected solvent composition, volume, solute amount

The volume of sample injected is expressed as an integer number of plates. This can readily be converted to a volume. For example, if we set up a total of 200 plates and if the column dead volume is 1 ml, then an injection on two plates corresponds to an injection volume of 10 \( \mu l \). The total amount of solute injected can also be varied. This is usually set at a (arbitrary) value of 100 (in our model, solute retention is independent of its concentration). We can use different percentages of the strong solvent (from 0-100%) in the sample solvent. This allows us to inspect the different peak profiles that are obtained using a weak solvent or a strong eluent to dissolve (and inject) the samples (see table 7.4 below).

7.3.4 Program outputs

After providing data to the program as outlined above, the analyst is provided with a complete elution profile of the analyte. This is usually one peak, but depending on the input parameters peak splitting may be observed. All information about the peaks is available from the program, including the retention time of the peak, the peak height, and the peak area. The user may immediately observe the predicted effects of changes in the injection solvent (volume and composition), changes in the gradient parameters or in the isocratic composition, changes in the properties (retention parameters) of the sample, etc. The chromatographer also may be provided with the solvent and/or analyte profiles as a function of time at any particular plate in the column, or with the profiles as a function of the column length at any given time. This program can be used to predict the peak profile in analytical HPLC or (with slight modification to account for the variation of analyte retention with concentration) in preparative HPLC.
7.4 Results and Discussion

We have studied the influence of the injection volume, the solvent composition (including composition of the sample solvent and of the mobile phase) and the column length on the peak profile (and on possible peak splitting). The effects of the sample solvent and the mobile-phase composition on the peak splitting have been compared for various solute retention factors. Our aim in this work is essentially to relate the peak-splitting phenomenon quantitatively to the underlying parameters.

7.4.1 Effect of injection volume

The data in Fig. 7.1 shows the variation in peak profiles caused by an increase in the injection volume, using injection solvents stronger than the mobile phases under isocratic conditions. The mass of analyte injected (injection volume times sample concentration) was the same for all profiles in Fig. 7.1. Some quantitative data are collected in Table 7.2. As seen in Table 7.2, upon increasing the injection volume, the height and the retention time of the "real" retention peak [22] and the calculated retention factor, \( k \), decreased. At the same time, the height of the first peak, which appeared near the solvent peak, increased. This has been observed in many experimental studies [14,15,19,21,22] and it has been suggested to inject the smallest possible volumes. Indeed, no breakthrough peak (the first peak in the chromatogram [22]) can be seen in Fig. 7.1 at the smallest injection volume (0.5% injection volume of the total dead volume for the column). However, this is merely a matter of scaling. If the chromatogram is sufficiently enlarged, a small first peak can be observed. Also, even at the smallest injection volume the observed peak height of the second peak is about 25% lower \( (h = 7.3) \) when the sample is dissolved in the strong solvent, than it is when the sample is dissolved in the mobile phase \( (h = 9.8) \). In some cases, even sample volumes as low as 0.5% of the column can give rise to serious peak splitting (see Figs. 7.3, 7.4). Also there was a small difference between the retention factors calculated (2.3 and 2.4) in Table 7.2, because the injection solvent was stronger than the mobile phase. This teaches us that the strength of sample solvent should be the same as that of the mobile phase if we are to determine the solute retention factor accurately.
Fig. 7.1 Simulation effect of the injected volume on the peak profile under isocratic condition. The injected volumes are indicated in the figure, chromatographic conditions and parameters are same as in table 7.2.

* Reference injection of minimal volume (1) with the sample-solvent composition being identical to the mobile-phase composition (20% strong solvent).

Table 7.2 Simulated effect of the injected volume on the peak profile detected on the 201st plate under isocratic condition; mobile phase with 20% strong solvent, injected sample solvent composition 85%, $k_i=8$, $S=6$, total injected sample amount 1000 (injected sample concentration inversely proportional to the injected volume).

<table>
<thead>
<tr>
<th>injected volume</th>
<th>Ref.*</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>height of the retention peak</td>
<td>9.8</td>
<td>7.3</td>
<td>5.2</td>
<td>3.3</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>retention time</td>
<td>682</td>
<td>652</td>
<td>629</td>
<td>601</td>
<td>570</td>
<td>514</td>
</tr>
<tr>
<td>$k$ calculated</td>
<td>2.4</td>
<td>2.3</td>
<td>2.1</td>
<td>2.0</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>height of the first peak</td>
<td>0</td>
<td>0.067</td>
<td>1.3</td>
<td>13.3</td>
<td>48.5</td>
<td>49.8</td>
</tr>
</tbody>
</table>

* Reference injection of minimal volume (1) with the sample-solvent composition being identical to the mobile-phase composition (20% strong solvent).

Fig. 7.2 and table 7.3 show the simulated effect of the volume of an injection solvent that is stronger than the initial mobile phase on the peak profile under gradient-elution conditions. Again, the mass of analyte injected was kept constant. As seen from Fig. 7.2 and table 7.3, upon increasing the injection volume the height of the “real” retention peak and the recovery decreased, while the height of the first peak, which appeared near the solvent peak, increased. However, the position of the second (real retention) peak did not change significantly. This is due to the fact that the gradient mobile-phase composition controls the sample elution. Also, the recovery computed on the 101st plate was always lower than that on the 201st plate. The simulated peak profiles as shown in Fig.
7.2 are similar to the experimental one shown in Fig. 6.3. Estimates of the recovery on the 101st plate were obtained by integrating the response values from the 150th to the 1009th time steps. This was done because \( t_0 \) appears after 101 time steps and we should allow some time for the signal to return to baseline. Values of less than 1% of the peak top were ignored in this calculation, to mimic real chromatography. Because there are some situations in which the real analyte peak is very close to the solvent peak (not base-line separated and/or starting to elute before time step 150), the recovery data should be treated carefully and –when necessary– verified by visual inspection of the chromatogram.

![Simulation effect of the injected volume on the peak profile under gradient-elution condition. The injected volumes are indicated in the figure. Gradient conditions and parameters used are same as in table 7.3.](image)

**Table 7.3** Simulated effect of the injected volume on the peak profile under gradient-elution condition. Gradient from 0% to 100% strong solvent in 600 time units, lag time 100 units, injected sample solvent composition 100%, \( k_0 =16.8, S =20 \), total injected sample amount 100 (injected sample concentration inversely proportional to the injected volume).

<table>
<thead>
<tr>
<th>Injection volume</th>
<th>20</th>
<th>12</th>
<th>8</th>
<th>5</th>
<th>3</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height of first peak on the 201st plate</td>
<td>5.00</td>
<td>7.24</td>
<td>5.36</td>
<td>1.85</td>
<td>0.41</td>
<td>0.01</td>
</tr>
<tr>
<td>Height of retention peak on the 201st plate</td>
<td>1.01</td>
<td>1.69</td>
<td>2.52</td>
<td>3.83</td>
<td>5.26</td>
<td>7.14</td>
</tr>
<tr>
<td>Recovery on the 101st plate</td>
<td>0.204</td>
<td>0.341</td>
<td>0.505</td>
<td>0.724</td>
<td>0.881</td>
<td>0.973</td>
</tr>
<tr>
<td>Recovery on the 201st plate</td>
<td>0.407</td>
<td>0.664</td>
<td>0.861</td>
<td>0.960</td>
<td>0.984</td>
<td>0.993</td>
</tr>
</tbody>
</table>
7.4.2 Effect of the sample solvent

From Fig. 7.3 it is apparent that the retention peak appears earlier when the concentration of the strong solvent in the sample increases. In this case the leading region developed into a breakthrough peak [15] when the composition of the sample solvent reached 55%. Table 7.4 compares the heights, retention times, and retention factors (under isocratic conditions) of the real peaks and the heights of the breakthrough peak (first peak) using different solvent compositions. As seen as in table 7.4, when the composition (and elutropic strength) of the sample solvent increased, the height and retention time of the “real” retention peak [22] and the calculated sample retention factor, $k$, decreased, while the height of the first peak increased. This is agreement with the experimental studies [14,15,22].

![Graph](image)

**Fig. 7.3** Simulation effect of the injected sample solvent on the peak profile under isocratic condition. The compositions of the injected sample solvent are indicated in the figure, chromatographic conditions and parameters used are same as in table 7.4.

**Table 7.4** Simulated effect of the injected sample solvent on the peak profile detected on the 201st plate under isocratic condition; mobile phase 0% strong solvent, injected volume 1, $k_R=3.5$, $S=8$, total injected sample amount 600.

<table>
<thead>
<tr>
<th>injected sample solvent</th>
<th>0%</th>
<th>40%</th>
<th>50%</th>
<th>55%</th>
<th>60%</th>
<th>70%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>height of the retention peak</td>
<td>4.3</td>
<td>4.0</td>
<td>3.4</td>
<td>3.0</td>
<td>2.5</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td>retention time</td>
<td>901.0</td>
<td>881</td>
<td>865</td>
<td>855</td>
<td>845</td>
<td>823</td>
<td>763.0</td>
</tr>
<tr>
<td>$k$ calculated</td>
<td>3.5</td>
<td>3.4</td>
<td>3.30</td>
<td>3.25</td>
<td>3.2</td>
<td>3.1</td>
<td>2.8</td>
</tr>
<tr>
<td>height of the first peak</td>
<td>0.00000</td>
<td>0.000</td>
<td>0.0014</td>
<td>0.093</td>
<td>1.6</td>
<td>41.0</td>
<td>469.5</td>
</tr>
</tbody>
</table>
Vukmanic and Chiba [14] and Porsch [20] believed that the highest efficiency is reached when the eluting power of the sample solvent is substantially less than that of the mobile phase. In the so-called on-column focusing, a weak injection solvent and a large injection volume are used. Our model can be used to simulate the influence of injection solvents weaker than the mobile phase on the height of the retention peak ($h$), its retention time ($t_R$), and the column efficiency ($N$) calculated according to eqn. 5 for different injection volumes.

$$N = 5.54 \times (t_R/w_h)^2$$

where $w_h$ is the width in the half height of the retention peak. The results are shown in table 7.5.

The analyte retention factor in the weak solvent, $k_s$, is lower than that in the mobile phase, $k_m$, when the mobile phase is stronger than the injection solvent. We observed only one single peak in our simulations when $k_s \geq k_m$, which is in agreement with experimental results. As seen from table 7.5, when $k_s$ increased, $t_R$ never decreased. However, the height of the retention peak ($h$) and the efficiency $N$ increased. Similar results (not shown) were obtained when larger injections volumes were used.

Table 7.5 Simulated effect of the injected sample solvent weaker than the mobile phase under isocratic condition: injected volume 1 unit (the dead volume of the column is 201 units). total injected sample amount 100, the value $a$ is the isotherm constant in eqn. 3.

<table>
<thead>
<tr>
<th>$k_m$ = 0.2</th>
<th>$a$ = 0.001</th>
<th>$a$ = 0.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_s$</td>
<td>$h$</td>
<td>$t_R$</td>
</tr>
<tr>
<td>0.2</td>
<td>5.746</td>
<td>240</td>
</tr>
<tr>
<td>2.0</td>
<td>5.757</td>
<td>241</td>
</tr>
<tr>
<td>10</td>
<td>5.748</td>
<td>241</td>
</tr>
<tr>
<td>20</td>
<td>5.747</td>
<td>241</td>
</tr>
<tr>
<td>50</td>
<td>5.747</td>
<td>241</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$k_m$ = 0.8</th>
<th>$a$ = 0.3</th>
<th>$a$ = 1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_s$</td>
<td>$h$</td>
<td>$t_R$</td>
</tr>
<tr>
<td>0.8</td>
<td>2.349</td>
<td>360</td>
</tr>
<tr>
<td>8</td>
<td>2.352</td>
<td>362</td>
</tr>
<tr>
<td>20</td>
<td>2.353</td>
<td>362</td>
</tr>
<tr>
<td>50</td>
<td>2.354</td>
<td>362</td>
</tr>
</tbody>
</table>

7.4.3 Effect of the column length

Fig. 7.4 shows the effect of the column length on the peak profile under isocratic and gradient conditions. The peak splitting, under both isocratic (Fig. 7.4a) and gradient-elution conditions (Fig. 7.4b), can clearly be observed from the response detected on the 100th, 200th and 400th plate. The effect becomes less visible on the 600th and 800th plates and it can hardly be noticed on the 1000th plate. The height of the first peak (breakthrough peak) decreases with increasing the column length shown in Figs. 7.4a and 7.4b. This has been observed in an experimental study [31] and the peak splitting was attributed to poor mixing of the sample and the mobile phase along the column. It is
seen from table 7.3, that the calculated recovery on the 101\textsuperscript{st} plate was always lower than that on the 201\textsuperscript{st} plate. The recovery on the 101\textsuperscript{st} plate sharply decreased upon increasing the injection volume. Thus, increasing the column length may help to reduce the chances of peak splitting and increase the product recovery in the preparative HPLC if the strong solvent has to be used.

**Fig. 7.4a** Simulation effect of the column length on the peak profile under isocratic condition. The detected positions are indicated in figure, mobile phase composition 0\% strong solvent, injected sample solvent composition 100\%, $k_o=2.5$, $S=5.5$, total injected sample amount 600, injected volume 1 unit.

**Fig. 7.4b** Simulation effect of the column length on the peak profile under gradient-elution condition. The detected positions are indicated in figure, gradient from 0\% to 40\% strong solvent in 600 time units, lag time 100 units, injected sample solvent composition 100\%, $k_o=3.0$, $S=5.8$, total injected sample amount 600, injected volume 1 unit.
7.4.4 Comparison of the \(k_s\) and \(k_m\) influences on the breakthrough peak

We have earlier [22] observed that both the strength of the injection solvent and the mobile-phase composition can influence peak distortion and peak splitting. However, it seems reasonable to expect a greater influence from the mobile phase than from the injected solvent, because of the much larger amount of the former present in the column. Our experiments, however, indicated a much greater effect of the injection solvent [22]. In table 7.6 the influence of the solute retention factor in the injection solvent, \(k_s\), and that in the mobile phase, \(k_m\), are compared based on the heights of breakthrough peak. This is a useful comparison, because the recovery (area of the retention peak) will decrease if the breakthrough-peak height increases. As seen in table 7.6, the height of the breakthrough peak decreases when either \(k_s\) or \(k_m\) increases. However, the height of the breakthrough peak decreases more sharply as \(k_s\) increases (i.e., the injection solvent becomes weaker). The value of \(k_m\) does not appear critical when large (≥ 10 plates) or small (1 plate) injection volumes are used, i.e., the peak distortion and peak splitting are greatly dependent on the strength of the injection solvent and largely independent of that of the mobile phase [17]. Therefore the selection of the sample solvent is of eminent importance to avoid or reduce the peak distortion and peak splitting.

Table 7.6 Comparison of the \(k_s\) and \(k_m\) influences on the breakthrough peak height under isocratic elution: the dead volume of the column is 201 units, total injected sample amount 100.

<table>
<thead>
<tr>
<th>Injected volume</th>
<th>(k_s) 0.5</th>
<th>(k_m) 5</th>
<th>(k_m) 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 unit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>13.03</td>
<td>12.74</td>
<td>12.45</td>
</tr>
<tr>
<td>0.02</td>
<td>1.756</td>
<td>1.679</td>
<td>1.605</td>
</tr>
<tr>
<td>0.03</td>
<td>0.243</td>
<td>0.227</td>
<td>0.212</td>
</tr>
<tr>
<td>0.04</td>
<td>0.0343</td>
<td>0.0314</td>
<td>0.0287</td>
</tr>
<tr>
<td>0.05</td>
<td>0.0049</td>
<td>0.0044</td>
<td>0.0040</td>
</tr>
<tr>
<td>10 units</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>4.548</td>
<td>4.546</td>
<td>4.545</td>
</tr>
<tr>
<td>0.06</td>
<td>2.447</td>
<td>2.446</td>
<td>2.445</td>
</tr>
<tr>
<td>0.08</td>
<td>0.4743</td>
<td>0.4737</td>
<td>0.4731</td>
</tr>
<tr>
<td>0.10</td>
<td>0.0636</td>
<td>0.0634</td>
<td>0.0633</td>
</tr>
<tr>
<td>0.12</td>
<td>0.00673</td>
<td>0.00671</td>
<td>0.00669</td>
</tr>
</tbody>
</table>
Conclusions

Simple relationships to describe the retention and peak width under isocratic and gradient-elution reversed-phase liquid chromatography (RPLC) are used to simulate and quantitatively explain the peak distortion and peak splitting that occurs when the injection solvent (composition) is different from the mobile-phase composition. The general features of the peak distortion and peak splitting obtained by computer simulation are similar to the results observed and documented in many experimental studies. The observed solute retention factor is predicted to decrease with both increasing strength and increasing volume of the injection solvent. In order to determine the solute retention factor accurately, the strength of sample solvent should be the same as that of the mobile phase. Furthermore, the simulated data showed that the strength of the injection solvent had a much greater influence on the peak profile than that of the mobile phase. In order to fully understand and explain the observations in polymer LC, all active mechanisms (adsorption, partition and exclusion) must be incorporated in the plate model. Using the present simple model, we can explain many, but not all observation related to polymer LC.

Acknowledgement

This project is funded by the Dutch Polymer Institute (DPI project 205). We thank Wim Kok (University of Amsterdam) and Prof. Rob van der Linde (Eindhoven University of Technology) for their co-operation.
References

Summary

The research described in this thesis is part of project #205 of the Dutch Polymer Institute (DPI), entitled “Network formation of telechelic poly(meth)acrylic polymer resins prepared by ATRP or RAFT; structure properties relationships”. The primary goal of the overall project was to study network formation and structure-property relationships of tailor-made network coatings using well-defined telechelic polymers with specific molecular weights, low polydispersities, and high purity (functionality close to two). To analyze the functional polymers, new and better analytical tools, such as hyphenated LC-MS and comprehensive two-dimensional LC systems, were needed.

The specific objectives of this research were to establish analytical methods to obtain (i) quantitative information, such as molecular-weight distributions (MWDs) and functionality-type distributions (FTDs), using LC at near-critical conditions and (ii) qualitative structure information on functional polymers (including end-groups) by MS. These objectives were achieved and some significant progress was made in separation and characterization of functional polymers. In addition, the effects of molecular weight at near-critical conditions have been studied by LC-ESI-MS and by LC×SEC.

In Chapter 1, a brief description of polymer synthesis and analysis is presented. The history, the application and the synthetic routes of telechelic polymers are discussed. The separation modes in LC of polymers, LC hyphenated techniques (especially LC-MS), and comprehensive two-dimensional LC systems are introduced. Some examples from our own work are presented to show the usefulness of gradient LC in the characterization of polymer blends (mixtures) and functional copolymers. Critical LC and its applications in polymer analysis are reviewed.

In Chapter 2, the separation of functional poly(methyl methacrylate) (PMMA) prepolymer based on the number of end-groups has been studied using LC at the critical conditions. The functional polymers were successfully separated according to hydroxyl functionality at the critical conditions. This separation was confirmed by MS in Chapter 3. On-line LC-ESI-MS was used to provide further evidence for the structures of the end-groups and to study the separation mechanism of the

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1 ATRP = atom-transfer radical polymerization; RAFT = reversible addition-fragmentation chain-transfer.
2 Telechelic polymers are linear polymers with two functional end-groups.
3 LC = liquid chromatography; MS = mass spectrometry.
4 ESI = electrospray ionization; SEC = size-exclusion chromatography.
Summary

polymer LC. Specifically, the effect of the polymer molecular weight on the elution behaviour was studied. The separations were shown to be quite robust, as changing the column temperature, flow rate, and mobile-phase composition within reasonable ranges did not affect the resolution. Quantitative results on functional PMMA prepolymer were obtained by careful calibration of an evaporative-light-scattering detector (ELSD). In the work described in Chapter 3, the active (weakly bonded) end-groups, such as the dithioester and thiol groups, were lost in the MALDI-MS\(^1\) experiments, while the polymeric molecules were observed intact in the ESI-MS spectra. This indicates that in the present case ESI is a much softer ionization method than MALDI.

In Chapter 4, the (near-) critical solvent compositions were determined for non-, mono-, and difunctional (telechelic) poly(n-butyl acrylate) (PnBA) polymers with carboxyl end-groups. Normal-phase LC was used, with mixtures of acetonitrile, acetic (or formic) acid, and dichloromethane of varying compositions. Some formic or acetic acid had to be added to the mobile phase to elute these polymers. The critical solvent compositions obtained were not identical for non-, mono-, and difunctional PnBA polymers. This was an unusual experimental observation, but it was in agreement with certain theoretical predictions. Nevertheless, low-molecular-weight PnBA samples were successfully separated according to the carboxyl functionality at (near-) critical conditions, and this was confirmed by MS. Isocratic, near-critical LC of high-molecular-weight PnBAs proved feasible, but this required elevated temperatures (55°C).

The quantitative data and mass spectra were consistent with the results expected from the mechanism of the RAFT polymerization. To achieve ideal carboxyl functionalities, either a COOH-containing initiator should be used, or a very low concentration of a non-COOH-containing initiator. In the latter case the polymerization rate is much lower. Near-ideal functionalities (average number of carboxyl end-groups per molecule up to 1.99) were observed for telechelic PnBAs prepared by one-step RAFT polymerization.

In Chapter 5, comprehensive two-dimensional LC (LC×SEC) was investigated to determine the mutually dependent MWDs and FTDs of functional PMMA polymers. Experimental results confirmed that LC×SEC may benefit from the use of longer columns and higher flow rates, to maintain sufficient separation efficiency in the second (fast-SEC) dimension. A simple method to establish ELSD calibration curves was presented. Each response (data point) from the ELSD chromatogram could be converted into the corresponding mass concentration, using calibration curves obtained by injecting each standard directly into the ELSD without a column. The height of the flat area (plateau) is related to the injected concentration.

Qualitative and quantitative information was obtained on real samples. This demonstrated the

\(^1\)MALDI = matrix-assisted laser desorption/ionization.
usefulness of LC×SEC for determining the MWD and FTD for functional polymers. The peak capacity was greatly enhanced by LC×SEC in comparison with one-dimensional separations and accurate molecular-weight information could be obtained for individual peaks or for combinations of peaks.

The influence of the molecular weight on the retention behavior in LC was also investigated for hydroxyl-functional PMMA polymers using LC×SEC. The critical conditions, where retention is – by definition – independent of molecular weight, were not exactly the same for PMMA series with different end-groups. This supported the results obtained in Chapter 4 and again there was close agreement with theoretical curves reported in the literature. However, for practical applications of LC×SEC it is not strictly necessary to work at the exact critical solvent composition. Near-critical conditions often suffice to determine the mutually dependent MWD and FTD of functional polymers.

In interactive LC of polymers, part of the sample is often seen to elute with the solvent front. In Chapter 6, such breakthrough phenomena were investigated. The results showed that the size of the breakthrough peak increased as the injection volume, the column temperature, the strength of the sample solvent, and the strength of the initial mobile phase in gradient LC increased, or as the polymer concentration decreased. Whether the breakthrough peak exists (and how large the ratio of the breakthrough peak to the real retention peak is) depends not only on the LC conditions, but also on the molecular weight and chemical composition of the analyte polymer. Three necessary and sufficient conditions were suggested for the breakthrough phenomenon to be observed. Recommendations to avoid the breakthrough phenomenon were given, culminating in a structured method for selecting the best possible sample solvents.

In Chapter 7, simple relationships describing the retention and peak width under isocratic and gradient-elution conditions in reversed-phase LC were used to simulate and quantitatively predict the peak-distortion and peak-splitting effects that arise when the (composition of the) injection solvent is different from the (composition of the) mobile phase. The results of simulations of the effects of the sample solvent (composition), the injection volume, and the column length on the peak distortion and eventual peak splitting corresponded well with experimental results and with numerous observations reported in the literature.
Samenvatting

Het onderzoek dat in dit proefschrift wordt beschreven is een onderdeel van project #205 van het Dutch Polymer Institute (DPI), getiteld “Network formation of telechelic poly(meth)acrylic polymer resins prepared by ATRP or RAFT; structure properties relationships”. De voornaamste doelstelling van dit project in zijn geheel was het bestuderen van de relatie tussen de netwerkstructuur van specifieke coatings en de daaruit voortvloeiende eigenschappen. Daartoe is gekozen voor het gebruik van goed gedefinieerde, telechelische polymeren met gespecificeerde molgewichten, lage dispersiteiten en hoge zuiverheid (functionaliteit dicht bij twee). Om deze polymeren te kunnen analyseren waren nieuwe en betere analytische methoden nodig, zoals gekoppelde LC-MS systemen en volledig twee-dimensionale vloeistofchromatografie (LC×SEC).

De specifieke doelstelling van het hier beschreven onderzoek was de ontwikkeling van analytische methodes voor het verkrijgen van zowel kwalitatieve als kwantitatieve informatie over polymere netwerken. Met behulp van LC onder (nagenoeg) kritische condities zijn molgewichtsverdelingen (MWDs) en functionaliteitsverdelingen (FTDs) bestudeerd. MS is toegepast om inzicht in de eindgroepen van de functionele polymeren te verkrijgen. Aanzienlijke vooruitgang werd geboekt op het terrein van de scheiding en karakterisering van functionele polymeren. Bovendien is de invloed van het molgewicht in de nabijheid van het kritische punt bestudeerd m.b.v. LC-ESI-MS en LC×SEC.

In Hoofdstuk 1 wordt een korte beschrijving gegeven van de synthese en analyse van polymeren. De geschiedenis, toepassing en synthese van telechelische polymeren worden besproken. Verder wordt een inleiding gegeven over vloeistofchromatografische scheidingsmethoden voor polymeren. Gekoppelde systemen (met name LC-MS) en twee-dimensionale LC technieken komen hier aan bod. Sommige voorbeelden uit ons eigen werk worden besproken om de bruikbaarheid van gradiënt LC voor de karakterisering van mengpolymeren en functionele polymeren aan te tonen. Tenslotte wordt een overzicht gegeven van kritische vloeistofchromatografie en de toepassingen

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1 Netwerkvorming van telechelische poly(meth)acrylaat polymeerharsen gemaakt via ATRP of RAFT; structuur-eigenschap relaties. ATRP = “atom-transfer” radicaalpolymerisatie; RAFT = reversible addition-fragmentation chain-transfer.
2 Telechelische polymeren zijn lineaire polymeren met twee functionele eindgroepen.
3 LC = vloeistofchromatografie; MS = massa spectrometrie.
4 ESI = “electrospray” ionisatie; SEC = “size-exclusion” chromatografie.
Summary in Dutch

hiervan binnen de polymeeranalyse.

In Hoofdstuk 2 wordt een studie beschreven naar de scheiding van functionele poly(methylmethacrylaat) (PMMA) polymeren met kritische LC. De functionele polymeren met een laag molgewicht werden met succes gescheiden op basis van het aantal functionele hydroxyeindgroepen. Deze scheiding werd bevestigd m.b.v. MS, zoals beschreven in Hoofdstuk 3. On-line LC-ESI-MS werd gebruikt om aanvullend bewijs te leveren voor de structuur van de eindgroepen en voor het mechanisme dat aan de LC scheiding ten grondslag ligt. In het bijzonder werd het effect van het molgewicht van het polymer op het retentiedrag bestudeerd. De scheidingen bleken zeer robuust te zijn, omdat veranderingen binnen redelijke grenzen in de kolomtemperatuur, het eluensdebit en in de samenstelling van de mobiele fase geen invloed bleken te hebben op de resolutie. Kwantitatieve resultaten voor functionele PMMA polymeren met een laag molgewicht werden verkregen door zorgvuldige ijking van een ELSD instrument, waarmee de lichtverstrooiing na verdamping gemeten werd. Uit het onderzoek dat beschreven wordt in Hoofdstuk 3 bleek dat bij gebruik van MALDI-MS actieve eindgroepen, zoals de dithioester en de thiol groepen, tijdens de analyse verdwenen. Bij gebruik van ESI-MS daarentegen konden de intacte polymeermoleculen wel worden geïdentificeerd in de spectra. Dit wijst erop dat in het onderhavige geval ESI een aanzienlijk zachtere ionisatiemethode was dan MALDI.

In Hoofdstuk 4 worden de (bij benadering) kritische mobiele-fase samenstellingen beschreven voor niet-, mono- en di-functionele (telechele) poly(n-butylacrylaat) (PnBA) polymeren met carbonzuureindgroepen. Normal-phase LC werd toegepast met als mobiele fasen verschillende mengsels van acetonitril, azijnzuur (of mierenzuur) en dichloormethaan. Het zuur werd toegevoegd om deze polymeren te kunnen elueren. De gevonden kritische samenstellingen waren niet identiek voor de niet-, mono- en di-functionele PnBA polymeren. Dit is een ongewone waarneming, maar het stemt wel overeen met bepaalde theoretische voorspellingen. Niettemin werden PnBA monsters met een laag molgewicht met succes gescheiden op basis van het aantal carbonzuurgroepen. Dit werd bevestigd m.b.v. MS. Isocratische, nagenoeg kritische LC van PnBAs met een hoog molgewicht bleek ook mogelijk, maar hiervoor waren hogere temperaturen (55°C) vereist.

De kwantitatieve resultaten en de massaspectra stemden overeen met de verwachtingen gebaseerd op het mechanisme van de RAFT polymerisatie. Om een hoge functionaliteit (twee carboxylgroepen per molecule) te bereiken, moet hetzij een initiator gebruikt worden die een COOH-groep bevat, hetzij een hele lage concentratie toegepast worden van een initiator zonder een dergelijke groep. In het laatste geval zal de polymerisatie veel trager verlopen. Nagenoeg ideale

1 ELSD = Evaporative light-scattering detection.
2 MALDI = matrix-assisted laser desorptie/ionisatie.
functionaliteiten (tot aan 1.99 carboxylgroepen per molecule) werden gevonden voor telechelische PnBA polymeren, die waren bereid via een ééntaps RAFT polymerisatie.

In Hoofdstuk 5 wordt een methode beschreven voor de analyse van functionele PMMA polymeren: Onderling afhankelijke molgewichtsverdelingen (MWDs) en functionaliteitsverdelingen (FTDs) zijn bepaald door gebruik te maken van twee-dimensionale vloeistofchromatografie (LC×SEC). Experimenteel werd bevestigd dat het gebruik van relatief lange kolommen en relatief hoge debieten voordelen biedt als het gaat om het behoud van de scheidingskoefficiëntie in de tweede (snelle SEC) dimensie van LC×SEC. Een eenvoudige methode om ELSD ijekurves te bepalen wordt gepresenteerd. Ieder signaal (datapunt) uit het ELSD chromatogram kan omgezet worden in de overeenkomstige (gewichts-) concentraitie door gebruik te maken van ijkcurves, die werden verkregen door iedere standaard rechtstreeks in de ELSD te injecteren, zonder gebruik te maken van een kolom. De hoogte van het constante (plateau-) signaal kon worden gerelateerd aan de geïnjecteerde concentratie.

Kwalitatieve en kwantitatieve informatie werd verkregen voor echte monsters. Daarmee werd de bruikbaarheid van LC×SEC voor het bepalen van de MWD en FTD van functionele polymeren aangetoond. De piekcapaciteit van LC×SEC was aanzienlijk groter dan die van één-dimensionale scheidingen en juiste informatie over molgewichten kon worden verkregen voor afzonderlijke pieken, evenals voor combinaties van pieken.

De invloed van het molgewicht op het retentiegedrag in LC werd ook onderzocht met LC×SEC voor hydroxy-functionele PMMA polymeren. De kritische omstandigheden, waarbij retentie – per definitie – onafhankelijk is van het molgewicht, waren niet precies gelijk voor reeksen PMMA moleculen met verschillende eindgroepen. Hiermee werden de resultaten die in Hoofdstuk 4 waren behaald onderschreven. Opnieuw was er goede overeenstemming met theoretische curves in de literatuur. Voor praktische toepassingen van LC×SEC is het niet strikt noodzakelijk om bij de exacte kritische omstandigheden te werken. Om-en-nabij kritische condities zijn vaak afdoende om de onderling afhankelijke MWD and FTD van functionele polymeren te bepalen.

Bij toepassing van interactieve LC voor de analyse van polymeren wordt vaak een deel van het monster samen met het oplosmiddel geëlleueerd. Dergelijke doorbraakverschijnselen worden in Hoofdstuk 6 onderzocht. De resultaten laten zien dat de doorbraakpiek groter werd bij toename van het injectievolume, de kolomtemperatuur, de elutiesterke van het oplosmiddel, de elutiesterke van het eluens aan het begin van de (gradiënt-) analyse en de verdunning van het polymeer. Of doorbraak daadwerkelijk optreedt (en hoe groot de verhouding is tussen de doorbraakpiek en de echte, vertraagde piek) hangt niet alleen af van de LC condities, maar ook van het molgewicht en de chemische samenstelling van het te analyseren polymeer. Drie criteria worden voorgesteld, die zowel noodzakelijk als voldoende zijn om het verschijnsel van doorbraak te verklaren. Aanbevelingen worden gegeven om doorbraak te vermijden. Uiteindelijk mond dit uit in een
gestructureerde methode om de best mogelijke oplosmiddelen voor het monster te kiezen.

In reversed-phase LC treedt verstoring en opsplitsing van pieken op als de samenstelling van het oplosmiddel van het monster anders is dan de samenstelling van de mobiele fase. In Hoofdstuk 7 worden eenvoudige formules voor de retentie en de piekbreedte gebruikt om deze effecten te simuleren en kwantitatief te voorspellen. De resultaten van simulaties van de effecten van de oplosmiddelsamenstelling, het injectievolume en de kolomlengte op de piekverstoring (en uiteindelijk de opsplitsing van pieken) kwamen goed overeen met experimentele resultaten en met talrijke in de literatuur gerapporteerde waarnemingen.
功能高分子的分离和表征

摘要

本课题是荷兰高分子研究院 (DPI) 第 205 号项目的一部分。该项目要求用 ATRP 或 RAFT 方法合成结构明确的、窄分子量分布的溴化 (甲基) 丙烯酸类高分子树脂。1 由这种树脂制成涂料交联成可控的网络结构，并研究其结构与性能的关系。为了更好地表征这类功能性溴化高分子，必须使用先进的分析方法，如液质联用 (LC-MS) 及全二维液相色谱体系。

本工作的主要目标是建立合适的分析方法: (1) 研究高分子的临界液相色谱条件 (即保留时间不随分子量变化) 并用于定量分析功能性高分子，如分子量分布 (MWD) 和官能团分布 (FTD), (2) 用质谱得到功能高分子的定性结构信息 (包括端基)。该目标已经达成，并在功能高分子的分离和表征方面取得了一些重要进展。另外，运用 LC-ESI-MS 和 LC×SEC 研究了在近似临界液相色谱条件下分子量的影响。2

第一章简介了高分子合成和分析，概述了溴化高分子的历史、应用和合成路线，对高分子液相色谱的分离方法、液相色谱的联用技术 (特别是 LC-MS)、全二维液相色谱体系进行了讨论。通过自己的实验例子表明了梯度洗脱液相色谱在高分子共混物 (混合物) 和共聚物方面表征的有效性，综述了临界液相色谱及其在高分子分析上的应用。

第二章应用临界液相色谱条件研究了功能性聚甲基丙烯酸甲酯 (PMMA) 的分离。在临界液相色谱条件下，成功地按照羟基官能团分离了功能性高分子，并用质谱证实了该分离结果 (第三章)。该分离方法可靠，不受柱温、流速、流动相组成在合理范围内波动的影响。通过蒸发激光散射检测器 (ELSD) 建立的标准曲线，对功能性 PMMA 高分子进行了定量分析。

在线 LC-ESI-MS 能提供进一步的基团结构信息，并可用于研究高分子液相色谱的分离机理，特别是应用于研究高分子的分子量对保留行为的影响：活泼的 (弱键合的) 端基如二硫酚和硫醇端基，在基质辅助激光解吸附—质谱 (MALDI-MS) 的实验中断裂，而在 ESI-MS 中可得到不断裂的高分子完整谱图 (第三章)。这表明 ESI 相对于 MALDI 是一种更软的离子化方法。

第四章应用乙腈、乙酸 (或甲酸)、二氯甲烷混合物作流动相，在正相液相色谱中测定了带不同端基官能团系列的功能性聚丙烯酸正丁酯 (PnBA) 的临界液相色谱溶剂组成。一些甲酸或乙酸必须加入到流动相中使带羧基端基的 PnBA 高分子洗脱出来。对于带不同端基官能

1 ATRP = 原子转移自由基聚合; RAFT = 可逆加成——断裂链转移自由基聚合; 溴化高分子 = 带有两个功能性端基的线型高分子。
2 LC-ESI-MS = 在线液相色谱—电喷雾离子化质谱; LC×SEC = 全二维液相色谱×体积排阻色谱。
团系列的 PnBA 高分子，得到的该临界溶剂组成不相同。这是不常见的实验结果，但符合理论预测，且在近似临界条件下，低分子量的 PnBA 样品成功地依据羧基官能团得到分离，并得到质谱证实。等度的近似临界条件对较高分子量的 PnBA 样品也可行，但要求更高的温度 (55°C)。

定量分析得到的数据及质谱图符合 RAFT 聚合机理预期的结果。为了达到理想的羧基官能团数 (2 个)，需使用含羧基的引发剂或使用非常低浓度的不含羧基的引发剂，后者聚合速度较慢。通过一步法 RAFT 聚合得到的羧基 PnBA 高分子具有接近理想的羧基末端 (平均每个分子达 1.99 个羧基端基)。

第五章用 LC×SEC 测得了功能性 PMMA 高分子相互依赖的分子量分布和官能团分布。实验表明在第二维快速 SEC 中使用较长的柱子和较高的流速有利于获得较高的分离效率。引入了一个简单的建立 ELSD 标准曲线的方法，并以此标准曲线将每个样品讯号数据点转换成样品浓度。实际样品的测试结果表明了 LC×SEC 在定量分析功能性高分子 (MWD 和 FTD) 中的有效性。与一维分离相比，LC×SEC 大大提高了分离能力，对每个峰或者多个峰的结合都可得到较准确的分子量信息。

利用 LC×SEC 研究了羟基化功能化 PMMA 高分子的分子量对液相色谱保留行为的影响。依定义在临界条件下保留值与分子量无关，但是带不同羟基基的功能化 PMMA 系列有不同的临界条件。这支持第四章不平常的实验结果，并接近文献中的理论曲线。然而，对 LC×SEC 的实际应用，不必在非常严格的临界溶剂组成中分离。近似的临界条件足以测得功能性高分子的相互依赖的分子量分布和官能团分布。

在高分子相互作用液相色谱法中 (interactive LC of polymers)，常发现部分样品与溶剂同时出峰，本论文第六章研究了这种称为“流穿” (breakthrough) 的现象。实验结果表明“流穿”峰的大小随进样体积、柱温、进样溶剂强度、梯度洗脱中的初始流动相强度的增加而增大，随高分子样品浓度的增加而减小。“流穿”峰是否出现及该峰与正常保留峰的比例不仅取决于液相色谱条件，也取决于被分析的高分子的分子量和化学组成。讨论了“流穿”峰出现的原因及如何避免该峰的出现，并提出了选用合适样品溶剂解决此问题的系统方法。

第七章，利用描述反相液相色谱的等度洗脱和梯度洗脱中的保留及峰宽的简单公式，模拟并定量解释了当进样溶剂不同于流动相组成时峰的变形和峰分离的情况。模拟结果与实验及文献报道的进样溶剂、进样体积、柱长对峰变形和峰分裂 ("流穿" 峰) 的影响基本一致。
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Reviewed Publications Dating From 1999

1. X.-L. Jiang, P. J. Schoenmakers, “Computer Simulation of the Effect of the Injection Solvent on the Peak Profile in Reversed-Phase LC under Isocratic and Gradient Conditions”, in preparation. (Chapter 7 of this thesis)


