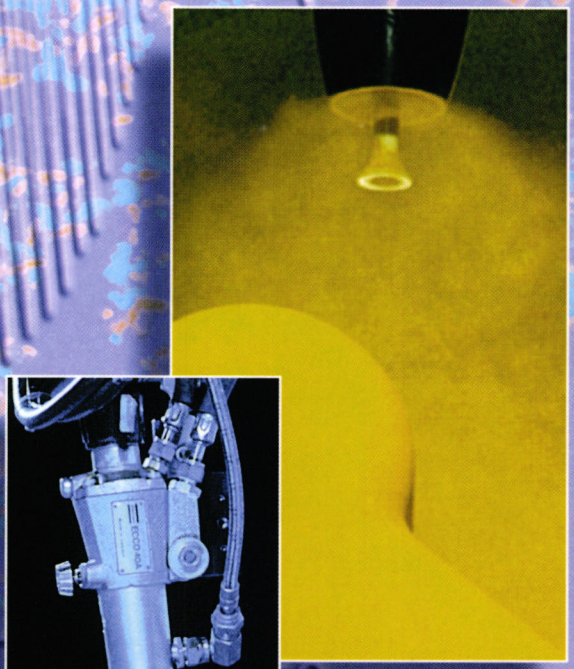
Characterization of Copolymers by Gradient Polymer Elution Chromatography



P.J.C.H. Cools

P109

CHARACTERIZATION OF COPOLYMERS BY GRADIENT POLYMER ELUTION CHROMATOGRAPHY

CIP-DATA LIBRARY TECHNISCHE UNIVERSITEIT EINDHOVEN

Cools, Paul J.C.H.

Characterization of copolymers by gradient polymer elution chromatography / by Paul J.C.H. Cools. - Eindhoven : Technische Universiteit Eindhoven, 1999.
Proefschrift. - ISBN 90-386-0970-1
NUGI 813
Trefwoorden: copolymeren / polymeren; chromatografie / polymeren; chemische analyse / vloeistofchromatografie
Subject headings: copolymers / polymers; chromatography / elution chromatography

Omslagontwerp: Ben Mobach
Druk: Universiteitsdrukkerij Technische Universiteit Eindhoven

Characterization of Copolymers by Gradient Polymer Elution Chromatography

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Technische Universiteit Eindhoven, op gezag van de Rector Magnificus, prof.dr. M. Rem, voor een commissie aangewezen door het College voor Promoties in het openbaar te verdedigen op donderdag 27 mei 1999 om 16.00 uur

door

Paulus Josephus Christiaan Henricus Cools

geboren te Tilburg

Dit proefschrift is goedgekeurd door de promotoren:

prof.dr.ir. A.L. German
en
prof.dr.ir. P.J. Schoenmakers

Copromotor:
dr.ir. B. Klumperman

Akzo Nobel financially supported the work described in this thesis.

"Everybody's looking 4 the ladder
Everybody wants salvation of the soul
The steps U take are no easy road
But the reward is great 4 those who want to go

Everybody's looking 4 the answers
How the story started and how it will end
What's the use in half a story, half a dream
U have 2 climb all of the steps in between"

From ***The Ladder***
Lyrics by John L. Nelson & Prince R. Nelson

Aan mijn ouders

TABLE OF CONTENTS

Chapter 1

Introduction

1.1 Introduction

Due to the increasing complexity and variability of polymer architectures, analytical techniques for the determination (or verification) of their chemical composition have become increasingly important. This stems from the application of different types of monomers, the development of novel polymer architectures, and new polymerization techniques (*e.g.* controlled 'living'). In the specific case of copolymers, the properties of these materials are governed by their microstructure, which is defined by the molar mass distribution (MMD), functional end groups, chemical structure and chemical composition distribution (CCD). The microstructure of copolymers can be investigated by several techniques: size exclusion chromatography (SEC) can be applied to determine the molar mass distribution (MMD), titration can be used to obtain information on functional end groups, and spectroscopic techniques, such as Nuclear Magnetic Resonance (NMR) and Infrared (IR) spectroscopy, reveal information on the average chemical composition of copolymers. However, the properties of a copolymer do not only depend on the molar mass distribution (MMD) and the average chemical composition, but also on the chemical composition distribution (CCD). For example, during batch copolymerizations, composition drift is likely to occur, which may result in a chemically heterogeneous copolymer mixture with inferior mechanical properties [1]. Thus, knowledge about the chemical composition distribution is crucial when copolymers are considered.

Formerly, thin layer chromatography (TLC) was applied to determine the CCD of copolymers [2,3,4]. However, TLC is time-consuming and the reproducibility is poor compared

to gradient elution high performance liquid chromatography (HPLC) [5]. Gradient elution HPLC applied to synthetic homopolymers was already described by *Van der Maeden et al.* [6] in 1978. The authors described the separation of homopolymers, such as poly(ethylene terephthalate) and poly(ethylene oxide), according to molar mass and functionality. The application of gradient HPLC for the separation of copolymers was introduced in 1978 by *Teramachi et al.* [7]. They reported the separation of styrene/methyl methacrylate copolymers, and later published additional work in the field of copolymer analysis [8,9,10]. *Glöckner et al.* [5,11-20] and *Mori et al.* [21-24] also described the separation of several types of copolymers by gradient elution HPLC.

A general name for the analysis of polymers by gradient elution HPLC was introduced by *Staal* [25]; gradient-polymer-elution-chromatography (GPEC). The application of GPEC already has been described for different polymers [26-28]. In this thesis, some applications of GPEC to synthetic polymers will be described. The separation mechanism of GPEC is based on a combination of a precipitation/redissolution mechanism and a mechanism controlled by column interactions (sorption and steric exclusion). The name GPEC does not refer to a specific mechanism, but solely describes the technique (Gradient Elution Chromatography) and the application (Polymers).

GPEC can primarily be applied to determine the CCD [7-10], and the end group distribution (also known as the functional type distribution FTD [29]). Within certain limitations GPEC can also be used for the determination of the MMD [29].

1.2 Outline of the Thesis

In this thesis the application of GPEC for the characterization of copolymers will be discussed. Separation according to molar mass, chemical composition, functional type, and block structures will be presented. The general objective of this study is to investigate and broaden the applicability of GPEC for the analysis of synthetic polymers.

In ***Chapter 2*** important aspects of the GPEC separation are described. The GPEC principles will be discussed based on data from literature and on new experimental data. Examples of the separation of polymers according to the molar mass, functionality, and chemical composition of homopolymers and copolymers will be discussed.

In addition to GPEC, other techniques, such as SEC and mass spectrometric techniques have been used for characterization of the polymers described in this thesis. In ***Chapter 3*** the principles of SEC, electrospray ionization mass spectrometry (ESI-MS) and matrix-assisted-laser-desorption-ionization mass spectrometry (MALDI-MS) will be briefly described.

The GPEC separation of polyesters of neopentyl glycol (NPG) with isophthalic acid (IA) and with terephthalic acid (TA) will be discussed in ***Chapter 4***. Homopolyesters (consisting of one type of acid and NPG) were synthesized as model components. ESI-MS and MALDI-MS were applied to identify the peaks obtained with the GPEC separation, and SEC was used to determine the MMD of the homopolyesters.

The influence of the crystallinity of the homopolyesters on the retention behavior with various solvent/non-solvent combinations is discussed in ***Chapter 5***. Additionally, the (semi-) crystalline behavior of the homopolyesters was investigated by differential scanning calorimetry (DSC), polarized light microscopy and X-ray diffraction.

In ***Chapter 6*** the microstructural analysis of copolyesters by GPEC and ESI-MS is described. Copolyesters with different IA/TA ratios have been synthesized as model polymers. The experimental chemical composition distribution (CCD) of the different copolyester samples has been compared to the CCD calculated from the statistics of the esterfications. Commercial samples containing IA/TA/NPG monomers with similar overall chemical compositions were compared using GPEC.

In ***Chapter 7*** the application of GPEC to block copolymers is described. When block copolymers are synthesized, the blocks can be chemically bonded or when side reactions occur, can be present in the polymer sample as individual homopolymers. The question to what extent the blocks are chemically bonded can be answered by applying GPEC. Spectroscopic techniques only give the average chemical composition of block copolymer samples. GPEC will be used to characterize block copolymers of styrene/butadiene and styrene/isoprene.

Finally, in the epilogue, the status of analytical techniques, and especially GPEC, in modern analytical polymer research will be discussed. The major advantages and drawbacks of GPEC and future possibilities of using GPEC in combination with other analytical techniques will be points of discussion.

1.3 REFERENCES

1. H.A.S. Schoonbrood, "Emulsion Co- and Terpolymerization", *Ph.D. thesis*, Eindhoven University of Technology, *1994*.
2. H. Inagaki, M. Matsuda and F. Kamiyand, *Macromolecules*, **1**, *520 (1968)*.
3. J.C.J.F. Tacx, J.L. Ammerdorffer and A.L. German, *Polymer*, **29**, *2087-2094 (1988)*.
4. J.C.J.F. Tacx and A.L. German, *Polymer*, 30, 918-*927 (1989)*.
5. G. Glöckner, *"Gradient HPLC of Copolymers and Chromatographic Cross Fractionation"*, Springer-Verlag Berlin Heidelberg, *1991*.
6. F.P.B. van der Maeden, M.E.F. Biemond and P.C.G.M. Janssen, *J. Chromatogr.*, **149**, *539-552 (1978)*.
7. S. Teramachi, A. Hasegawa, Y. Shima, M. Akatsuka and M. Nakajima, *Macromolecules*, **12 (5)**, *992-996 (1979)*.
8. S. Teramachi, A. Hasegawa, Y. Shigekuni and S. Natunaga, *Polymer J.*, **21 (10)**, *803-808 (1989)*.
9. S. Teramachi, A. Hasegawa and K. Motoyama, *Polymer J.*, **22 (6)**, *489-496 (1990)*.
10. S. Teramachi, A. Hasegawa, Y. Shigekuni, K. Zenta and M. Hashimoto, *J. Appl. Polym. Sci.: Appl. Polym, Symp.*, **45**, *87-102 (1990)*.
11. G. Glöckner, *Chromatographia*, **23 (7)**, *517-524 (1987)*.
12. G. Glöckner, *Pure & Appl. Chem.*, **55 (10)**, *1553-1562 (1983)*.
13. G. Glöckner and D. Ilchmann, *Acta Polym.*, **35 (11)**, *680-683 (1984)*.
14. G. Glöckner and J.H.M. van den Berg, *J. Chromatogr.*, **384**, *135-144 (1987)*.
15. G. Glöckner, M. Stickler and W. Wunderlich, *Fresenius Z. Anal. Chem.*, **328**, 76-*81 (1987)*.
16. G. Glöckner, *J. Chromatogr.*, **403**, *280-384 (1987)*.

17. G. Glöckner, M. Stickler and W. Wunderlich, *Fresenius Z. Anal. Chem.*, **330**, *46-49 (1988)*.
18. G. Glöckner, M. Stickler and W. Wunderlich, *J. Appl. Polym. Sci.*, **37**, *3147-3161 (1989)*.
19. G. Glöckner and H. G. Barth, *J. Chromatogr.*, **499**, *645-654 (1990)*.
20. G. Glöckner, *J. Appl. Polm. Sci.: Appl. Polym. Symp.*, **52**, *45-54 (1992)*.
21. S. Mori, J. Appl. Polym. Sci.: Appl. Polym. Sci., 43, 65-84 (1989).
22. S. Mori, *J. Liq. Chromatogr.*, **13 (15)**, *3039-3052 (1990)*.
23. S. Mori, *J. Chromatogr.*, **503**, *411-419 (1990)*.
24. S. Mori and H. Taziri, *J. Liq. Chromatogr.*, **17 (14&15)**, *3055-3068 (1994)*.
25. W.J. Staal, *"Gradient Polymer Elution Chromatography", Ph.D. Thesis*, Eindhoven University of Technology, Eindhoven, The Netherlands, *1996*.
26. B. Klumperman, P. Cools, H. Philipsen and W. Staal, *Macromol. Symp.*, **110**, *1-13 (1996)*.
27. H.J.A. Philipsen, B. Klumperman and A.L. German, *J. Chromatogr. A.*, **746**, *211-224 (1996)*.
28. H.J.A. Philipsen, M.R. de Cooker, H.A. Claessens, B. Klumperman and A.L. German, *J. Chromatogr. A*, **761**, *147-162 (1997)*.
29. Philipsen H.J.A., "Mechanisms of Gradient Polymer Elution Chromatography and its Application to (Co)Polyesters", *Ph.D. Thesis*, Eindhoven University of Technology, Eindhoven, The Netherlands, *1998*.

Chapter 2

Various Aspects of Gradient Polymer Elution Chromatography

In this chapter the separation technique gradient polymer elution chromatography (GPEC) is discussed. Different mechanisms that determine the separation in GPEC, such as the precipitation/redissolution mechanism and the sorption mechanism, will be explained. In addition some applications of GPEC will be given.

2.1 Introduction

Since the introduction of gradient elution high-performance-liquid-chromatography [1,2], this technique has been applied to separate polymer blends [3], random copolymers [4-24] and block copolymers [25-28]. More recently, GPEC has been applied to determine the functional type distribution (FTD), *e.g.* end group distribution, of polyesters [29].

In general the working principle of GPEC can be described as follows. A polymer sample is dissolved in a good solvent. The polymer solution is injected into a non-solvent or a combination of solvent/non-solvent. The initial conditions are poor (in solubility terms) for the polymer molecules and phase separation will occur. Two phases are formed: a polymer rich phase and a highly diluted solvent phase. After phase separation the polymer molecules are retained in the system. After injection a gradient from the initial conditions to the good solvent is applied and during this gradient redissolution of the polymer molecules occurs. The redissolution point (expressed in volume fraction solvent or non-solvent) highly depends on the molar mass and the chemical composition of the polymer molecule. When the polymer molecule is redissolved, interactions with the stationary phase (column interactions) will further control the separation.

Different polymer chromatographic mechanisms are involved, which can be divided into two groups: precipitation/redissolution mechanisms, and mechanisms dominated by column interactions. The column interactions occurring in GPEC are similar to the interactions of the isocratic mechanisms. Unlike in isocratic chromatography in GPEC the conditions (eluent composition) change gradually in time.

Similar to HPLC, different types of chromatography can be applied *viz.* normal-phase mode (NP-GPEC) and reversed-phase mode (RP-GPEC). RP-GPEC is applied with a non-polar column in combination with an eluent decreasing in polarity, *e.g.* a water/tetrahydrofuran gradient on a Silica C18 column. NP-GPEC is performed with a combination of an eluent increasing in polarity and a polar column, e.g. a dichloro methane/tetrahydrofuran gradient on a silica column.

In this chapter different aspects of GPEC will be discussed. The isocratic retention mechanisms will be used to describe the GPEC retention mechanism. At the end of this chapter the GPEC separation based on molar mass and chemical composition will be illustrated by some applications of RP-GPEC and NP-GPEC. However, before the GPEC separation mechanism is described, different GPEC parameters will be briefly explained.

2.2 GPEC Conditions

The separation achieved by GPEC depends on a number of factors, namely the applied solvent system (solvent and non-solvent), the type of column, the column temperature, the applied gradients (gradient curve, flow, initial conditions and end (or final) conditions), and

injection conditions (volume, concentration, and sample solvent). *Philipsen* studied the influence of GPEC conditions on the separation of polyesters in more detail [29]. The contribution of the factors mentioned above will be briefly discussed.

The role of the solvent/non-solvent system is apparent. The solvent has to be a good solvent of high elution strength for the polymer molecules. The non-solvent should have a low solvent strength and/or a low eluent strength. The choice of the column is as important as that of the solvents. In some cases column interactions (sorption and exclusion) are necessary, in other cases column interactions are preferably avoided [16]. Column interactions and the precipitation/redissolution depend highly on the temperature, therefore temperature control is one of the conditions for reproducible polymer separations.

The curve and steepness of the gradient determine the separation. A steep gradient can cause reproducibility and resolution problems. A shallow gradient may cause detection problems (the concentration of the analyte decreases due to sample distribution) and equipment limitation problems (*e.g.* switching of eluent selection valves becomes visible). From previous research it has been found that linear gradients with a steepness of 1 vol% at a flow rate of 0.5 ml/min are the optimal conditions with respect to analysis time and (obtained) resolution [30]. Shallower gradients do not improve the resolution [29]. In general, GPEC separations have a total analysis times of 60 minutes (including the back-to-initial step in order to perform a subsequent injection).

The initial conditions of the gradient should be poor enough in terms of solubility or eluent strength to retain the polymer molecules. This condition can sometimes not be met and the polymer 'elutes' simultaneously with injected solvent molecules. This phenomenon, is called breakthrough [31] and occurs when improper initial conditions are applied or too large an injection volume is injected. Breakthrough at injection can be avoided by decreasing the injection volume, increasing the concentration or by applying poorer initial conditions with respect to solvent and eluent conditions [31].

The injection volume, the sample concentration and the sample solvent have a large influence on the final separation result. The sample load of polymer should not be too high in order to avoid viscosity effects and column blocking [16]. To avoid solvent effects, the injection volume should not be too high. The type of sample solvent can be very important, especially when crystalline polymers are analyzed (see *chapter 5*). The sample solvent can either be a good solvent or a combination of the non-solvent/solvent. Injection of a polymer dispersion can be used to avoid breakthrough of the polymer molecules [31].

2.3 Isocratic Mechanisms

Polymers show completely different chromatographic behavior from small molecules. (In some cases) The isocratic retention mechanisms are based on the same principles, but eventually the effects are totally different. Column interactions of polymers can be divided into two groups: entropy interactions and enthalpy interactions. Small molecules are mainly

subjected to enthalpy interactions. Additional entropy effects are caused by the exclusion or conformation restrictions of the polymer inside the pores and near the walls of the column.

The total column volume can be divided into three volumes: the interstitial volume V_i, the pore volume V_p and the volume of the stationary phase (V_s). The retention behavior of a polymer molecule can be described by the retention equation (Equation 2-1) [16]. An enthalpy contribution ($V_s \cdot K_{enth}$) and an entropy contribution ($V_p \cdot K_{entr}$) can be distinguished.

$$V_{ret} = V_i + V_p \cdot K_{entr} + V_s \cdot K_{enth}$$

Equation 2-1, Retention equation, where V_{ret} [ml] is the retention volume, V_i [ml] is the interstitial column volume, V_p [ml] is the pore volume, V_s [ml] is the volume of the stationary phase, K_{entr} [-] is the distribution coefficient based on entropy effects, and K_{enth} [-] is the distribution coefficient based on enthalpy effects.

The entropy interactions are exclusion and depletion. They will cause an acceleration of the polymer molecules ($0<K_{entr}<1$) in comparison to the eluent molecules ($K_{entr}=1$). The enthalpy contributions are sorption (adsorption and partitioning), resulting in retention of the polymer molecules ($K_{enth}>0$). Different types of mutual interactions between polymer, solvents and stationary phase, such as polymer/solvent and solvent/stationary phase, determine the overall interaction of polymers.

2.3.1 Exclusion/Depletion

Entropy effects are based on the difference in molar mass (or hydrodynamic volume) of the polymer and the eluent, and are based on steric exclusion of the polymer molecules. Where the eluent molecules are small and can enter the pores of the column, the polymer molecules are bigger and can only partly enter the pores. This results in a retention difference between the solvent molecules and the polymer molecules, thus, separation according to molecular size (hydrodynamic volume) is achieved. The HPLC technique that separates polymers according to this principle is size-exclusion-chromatography (SEC).

Another entropy effect that occurs in the case of polymers is depletion. A polymer molecule has fewer conformational possibilities near the surface, which results in depletion of polymer molecules. Due to the depletion at the surface, there is less volume available for the polymer molecule than for the solvent and separation occurs according to molecular size. Since more volume is available for the solvent than for the polymer molecules, the solvent will elute later than the polymer molecule. In general, depletion and steric elution are considered to be identical, therefore entropy effects will be referred to as steric exclusion.

When no enthalpy effects and solely steric exclusion occur during the separation ($K_{enth}=0$) the retention volume (described in Equation 2-1) reduces to;

$$V_{ret} = V_i + V_p \cdot K_{entr}$$

Equation 2-2, Retention equation for steric exclusion, where V_{ret} [ml] is the retention volume, V_i [ml] is the interstitial volume, V_p [ml] is the pore volume, and K_{entr} [-] is the distribution coefficient based on entropy.

The available pore volume, represented by $V_p \cdot K_{entr}$, depends on the molecular size. Small molecules can enter the pores completely (K_{entr} is equal to 1), and elute at the permeation limit of the column ($V_{perm} \approx V_i + V_p$). Large molecules are totally excluded from the pores (K_{entr} is equal to 0) and elute at the exclusion limit of the column ($V_{excl} \approx V_i$). Molecules that can partly enter the pores are separated according to molecular size. In exclusion mode, *i.e.* in entropy driven separations, the dependence of the molecular size on the retention volume yields a curve as depicted in Figure 2-1.

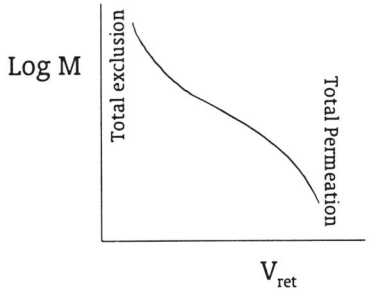


Figure 2-1, A schematic representation of the dependency of molar mass (M) on the retention volume (V_{ret}) in exclusion mode. The total exclusion and total permeation volumes can be seen.

Independent of additional column interactions, exclusion and depletion always occur. Additionally, exclusion influences the amount of surface that is available for sorption of the polymer molecules. Most of the active surface available for enthalpy interactions in an analytical column is present inside the pores, therefore exclusion will have a large influence on the sorption of the polymer.

2.3.2 Sorption

Sorption [32] is a general name for enthalpy effects, and it depends on the applied column material and eluent combination. Similar enthalpy interactions are found for polymers and small molecules, but in contrast to low molar mass analytes, a polymer molecule consists of many groups that can interact with the column material, thus 'irreversible adsorption' of the polymer on the column can occur. Adsorption and partitioning are the most common enthalpy interactions. Adsorption is defined as an interaction of a chemical group of the analyte with a chemical group of the column packing, *e.g.* hydrogen bonding. Adsorption is mainly based on polar interactions, whereas, partitioning (*e.g.* between a polar mobile phase

and non-polar stationary phase, such as C18 chains) is an interaction that is based on interactions, such as Van der Waals-interactions. In general, adsorption interactions are much stronger than partitioning interactions.

Polymer molecules can interact via 'multiple attachments' [16]. Different numbers of segments of the polymer molecules can be attached, which results in a decrease of the number of possible conformations (decrease in entropy). When only a few segments are involved, the polymer molecules will be partly in solution and partly attached to the column material by trains, loops or single point segments. The attachments of different segments do not occur at the same time. The sorption and desorption of the different segments occur simultaneously, which results in a dynamic process. The type of attachment will depend on the type of polymer (homopolymer, copolymer or block copolymer). However, the exact nature of the attachments is still unknown [16].

When only one segment is attached to the column, the total polymer molecule will be retained and thus elution will only occur when all the segments are desorped [16]. The capacity factor (k), defined as the probability ratio of sorption/desorption, depends on the capacity factor of one single segment.

$$\ln k_{pol} = \frac{p \cdot \Delta g_{segm}}{RT} + \frac{\Delta g_{funct}}{RT}$$

$$k_{pol} = "k_{funct}" \cdot (k_{segm})^{p}$$

Equation 2-3, The capacity factor, a measure for the probability of sorption, where Δg_{segm} [J/mol] is the change in Gibbs free energy of a segment of the polymer, Δg_{funct} [J/mol] is the change in Gibbs free energy of the functional groups, k_{pol} [-] is the capacity factor of a polymer molecule, k_{segm} [-] is the capacity factor of a single segment, k_{ends} is capacity factor of the end groups and p [-] is the degree of polymerization [33].

Glöckner [16] calculated that a polymer molecule with p=100 will be irreversibly attached when the k_{segm} is relatively low (k_{segm}=2). Values for the capacity factor of 2 are normal for low molar mass analytes. Consequently, when the adsorption of a single segment is weak, the polymer can still be irreversibly attached.

In addition to the previously mentioned differences, the diffusion coefficients of polymer molecules are much lower than the diffusion coefficients of low molar mass analytes. This is important for the polymer molecules that can enter the pores of the column material. It is assumed that when polymers penetrate the pores they generally undergo sorption (in the most common case, *e.g.* size exclusion chromatography, sorption does not occur). The penetration of the polymers will result in additional kinetic effects (peak broadening). Steric exclusion effects still occur. However, the sorption effects (enthalpy) are, in general, larger than the steric exclusion effects (entropy). The result is a dependency of the retention volume on the molar mass as can be seen in Figure 2-2.

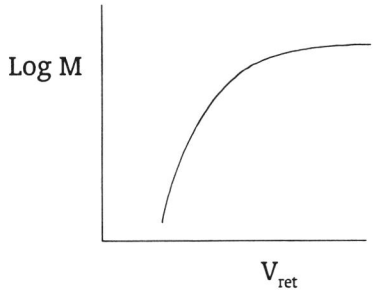


Figure 2-2, Schematic representation of the dependency of the molar mass (M) on the retention volume (V_{ret}) in the sorption mode.

Temperature has a large influence on the sorption of the polymer. The temperature effect is more pronounced for polar interactions and relatively small for non-polar interactions. The separation of polystyrenes with a temperature gradient was published by *Lee et al.* [34,35], who performed temperature-gradient-interaction-chromatography (TGIC) under reversed-phase conditions [34] and normal-phase conditions [35].

2.3.3 *Critical Conditions*

In isocratic chromatography of polymers a cancellation of the exclusion mechanism and the sorption mechanism is possible: the critical conditions [36-45]. At the critical conditions the separation is independent of the molar mass of the polymer and is solely based on the interaction of the functional end groups of the polymer chains with the column. As can be expected the critical conditions depend highly on the temperature [45].

The critical conditions can be determined by investigating the isocratic retention behavior of homopolymers at different eluent composition (see Figure 2-3).

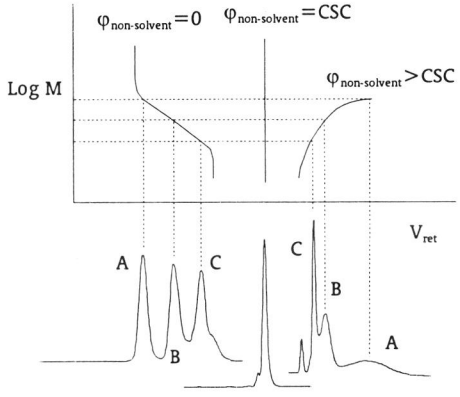


Figure 2-3, Schematic representation of the isocratic retention behavior of PS polymer standards in a specific solvent/non-solvent system on a specific column. The molar masses of the standards are $M_A<M_B<M_C$.

The sequence of elution changes by changing the eluent composition. The non-solvent concentration at which the curve is vertical is the so-called critical solvent composition (CSC), i.e. this is the non-solvent concentration at which all molar masses elute simultaneously [44]. An example is the isocratic retention behavior of polymethyl methacrylate (PMMA) in water/acetonitrile/THF (see Figure 2-4). The PMMA standards show similar behavior as schematically shown in Figure 2-3. The retention sequence of the PMMA standards changes with changing ratio of water/acetonitrile (ACN). The three separation modes can be seen, at the top the sorption mode, at the bottom the exclusion mode and in the middle the approximate critical mode. The determination and the prolongation of the critical conditions are difficult.

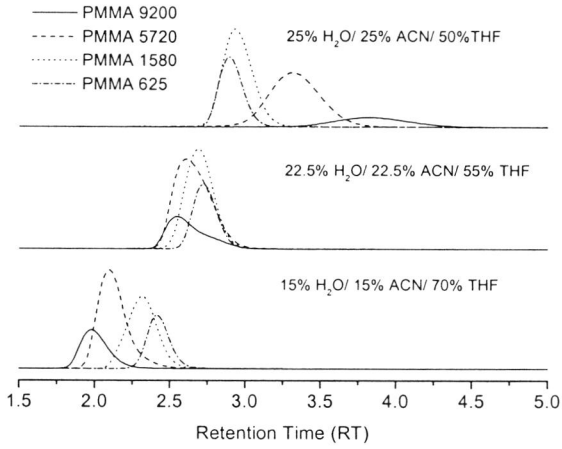


Figure 2-4, Isocratic retention behavior polymethyl methacrylate standards (625, 1580, 5720 and 9200 G/mol) in the solvent system water/acetonitrile/THF on a Symmetry C18 column (35°C). The chromatograms at the top are obtained in sorption mode, the chromatograms at the bottom are obtained in the exclusion mode, and the middle chromatograms are obtained under approximately critical conditions.

At isocratic retention, the Gibbs free energy of a polymer molecule present in the mobile phase and the stationary phase, is described by Equation 2-4.

$$\Delta G = \Delta H_{back} + \Delta H_{funct} - T\Delta S_{back} - T\Delta S_{funct}$$

Equation 2-4, The Gibbs free energy (ΔG) [J/mol] of a molecule with a polymer backbone of identical segments and with different functional end groups at equilibrium conditions, where ΔH_{back} [J/mol] is the enthalpy change of the backbone, ΔS_{back} [J/K·mol] is the entropy change of the backbone, ΔH_{funct} [J/mol] is the enthalpy change of the functional group, ΔS_{funct} [J/K·mol] is the entropy change of the functional group and T [K] is the absolute temperature.

The entropy and the enthalpy can be divided in a contribution from the backbone and from the functional end groups of the polymer chain. The entropy contribution of the functional end groups ΔS_{funct} is negligible ($\Delta S_{funct} << \Delta S_{back}$). For the different isocratic retention modes the following conditions can be defined:

Exclusion:	$\Delta G<0$	$\Delta H_{back}=0,\ \Delta H_{funct}=0$	$\Delta G=-T\Delta S_{back}-T\Delta S_{funct}\approx -T\Delta S_{back}$
Critical conditions:	$\Delta G_{back}=0$	$\Delta H_{back}-T\Delta S_{back}=0$	$\Delta G=\Delta H_{funct}-T\Delta S_{funct}\neq 0$
Sorption:	$\Delta G>0$		$\Delta G=\Delta H_{back}+\Delta H_{funct}$

In exclusion mode the enthalpy contributions of the backbone and the functional end groups ($\Delta H_{back}=0$, $\Delta H_{funct}=0$) are zero. In sorption mode, however, the enthalpy contributions are much higher than the entropy contribution of the backbone. At the critical conditions the contribution of the backbone to the separation is zero. The separation is governed solely by the contributions of the functional groups. The dependence of the molar mass on the retention times can be seen in Figure 2-5.

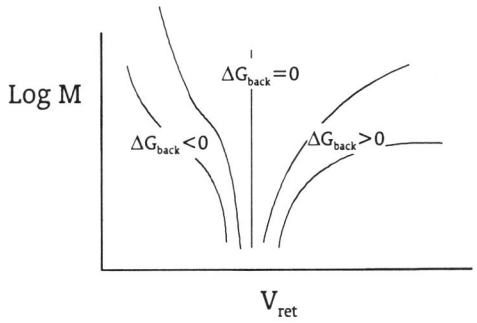


Figure 2-5, The effect of molar mass on the retention volume for different isocratic retention modes.

The critical conditions can be used to separate homopolymers with identical chemical composition of the backbone, but with different end groups. Consequently, a functional type distribution (FTD) of polymers is obtained [42,43]. Also block copolymers can be characterized with liquid chromatography under critical conditions (LCCC) [37-41]. The critical conditions are applied for one block, and the other block will be separated according to block length by sorption or exclusion [41].

LCCC has many drawbacks. The critical point is experimentally difficult to find and practically hard to maintain. The method is very sensitive to impurities in the polymer chain. Another problem of LCCC is the solubility of polymers. In general high molar mass polymers do not dissolve at the CSC. All these drawbacks reduce the applicability of LCCC significantly.

2.4 Gradient Mechanism

In GPEC, separation is based on column interactions alone or on a combination of precipitation/redissolution mechanisms and column interaction mechanisms. The precipitation/redissolution mechanism is directly related to the solubility of polymers, and the column interaction can be described by the isocratic retention mechanisms (see previous section).

2.4.1 Precipitation/Redissolution Mechanism

When a poor solvent is used at the start of the gradient (initial conditions) precipitation/redissolution mechanism will occur. When a polymer solution is injected into an eluent with low solvent strength, phase separation will occur. When a gradient is applied from a poor solvent to a good solvent, the injected polymer molecules will redissolve at a certain solvent composition. The solvent composition at redissolution depends on the molar mass and the chemical composition of the polymer molecules, and this can result in separation of the polymer molecules.

The precipitation/redissolution mechanism is based on the solubility properties of the polymer in the solvent gradient [16,46]. The solubility of polymers is determined by the mutual interactions of the polymer, solvents and the stationary phase. When kinetic effects are neglected, the Gibbs free energy on mixing of a polymer and a solvent can be described by Equation 2-5 [47].

$$\Delta G_{mix} = \Delta H_{mix} - T \cdot \Delta S_{mix}$$

Equation 2-5, Gibbs free energy of mixing, where ΔG_{mix} [J/mol] is the Gibbs free energy of mixing, ΔH_{mix} [J/mol] is the enthalpy of mixing, T [K] is the temperature and ΔS_{mix} [J/K·mol] is the entropy of mixing.

To dissolve the polymer into the solvent the gain in enthalpy (ΔH_{mix}) should be smaller than the loss in entropy ($T\Delta S_{mix}$) or should be negative. Due to this limitation polymers have a restricted number of solvents in which they dissolve. For the change in Gibbs free energy of a binary polymer/solvent mixture Equation 2-6 can be derived, which is known as the Flory-Huggins relation [47]. The first two parts of Equation 2-6 ($\varphi_S \ln\varphi_S + \varphi_P \ln\varphi_P/m_P$) are the entropy contributions and the last part ($\chi_{P/S}\varphi_S\varphi_P$) is the enthalpy contribution.

$$\frac{\Delta G_{mix}}{RTn_{\varphi}} = \varphi_S \ln \varphi_S + \frac{\varphi_P}{m_P} \ln \varphi_P + \chi_{P/S} \varphi_S \varphi_P$$

Equation 2-6, Flory-Huggins relation, where ΔG_{mix} [J/mol²] is the Gibbs free energy of mixing, R [J/mol·K]]is the gas constant, n_{φ} is the total number of lattice places to be filled [mol], φ_S is the volume fraction of solvent [-], φ_P is the volume fraction of polymer [-], m_P [-] is the relative number of lattice places taken by the polymer molecule (relative chain length), and $\chi_{P/S}$ [-] is the Flory-Huggins interaction parameter [47].

The *Flory-Huggins* equation shows that the solubility (mixing) of the polymer is dependent on the concentration (φ), the degree of polymerization (m_P is a function of the degree of polymerization p), the temperature and the interaction parameter of the polymer with the solvent ($\chi_{P/S}$). This interaction parameter for a polymer solvent mixture can be expressed as follows [48].

$$\chi_{P/S} \approx 0.34 + \frac{V_P}{RT}(\delta_P - \delta_S)^2$$

Equation 2-7, Interaction parameter ($\chi_{P/S}$) equation, where V_{pol} is the molar volume of the polymer, R is the gas constant, T is the temperature, and δ_P and δ_S are the Hildebrand solubility parameters of the polymer and the solvent [48].

The *Hildebrand* parameters are difficult to determine experimentally or to calculate theoretically.

From the Flory-Huggins relation, the upper critical solvent temperature (UCST) can be determined. The typical UCST behavior of a polymer/solvent mixtures can be found in Figure 2-6.

Besides UCST also LCST (lower critical solvent temperature) behavior exists, however, UCST behavior is most common for polymer/solvent mixtures. Two areas can be distinguished: an area where the mixture is homogeneous and an area where the mixture is heterogeneous. For example, the polymer/solvent mixture at T_0 and φ_0 will separate into two phases when T is decreased to T_1: a polymer rich phase with a polymer volume fraction (φ_B) and a solvent rich phase with a polymer volume fraction (φ_A). Figure 2-6 shows that the mixing properties of the polymer and the solvent depend on the temperature.

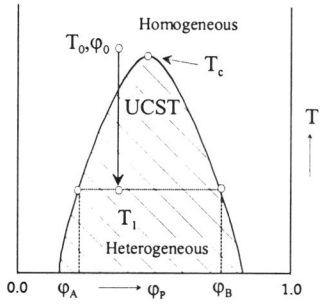


Figure 2-6, T vs. φ phase-diagram of a typical polymer/solvent system with UCST behavior. φ_P is the fraction of polymer in the system and T is the temperature.

The critical interaction parameter χ_{cr}, calculated from the critical conditions (T_{cr} and φ_{cr}), of a binary polymer/solvent mixture (for polymers with high degree of polymerization p) can be derived from Equation 2-6 [16].

$$\chi_{cr} \approx 0.5 + \frac{1}{\sqrt{m_p}}$$

Equation 2-8, Critical interaction parameter of a binary system, where χ_{cr} is the critical interaction parameter and m_p is the relative chain length of the polymer [16].

In GPEC, the thermodynamic system is mostly a ternary system, and sometimes quaternary systems are applied. This makes the de-mixing behavior complex and not simple to describe, which means that the theoretical description of the precipitation/redissolution mechanism is very complicated.

Redissolution of polymers in ternary systems can be studied experimentally by turbidimetric titration [46]. By turbidimetry the cloud point of the polymer is determined by adding (via titration) non-solvent to a polymer solution. The cloud point is the volume fraction of non-solvent (NS) necessary for the solution to become cloudy. At the cloud point, phase separation occurs and two phases are formed: a polymer rich phase and a solvent rich phase (comparable to a binary system). After phase separation, solvent can be added to the polymer dispersion. The solvent/non-solvent composition where the phase separation disappears is the redissolution point. *Staal* [3] showed that the redissolution point and the cloud point can be assumed to be equal. The de-mixing point (cloud point) of a ternary polymer/solvent/non-solvent system at infinite dilution can be approximated by Equation 2-9, which is based on several assumptions [46]. The interaction parameters of polymer/non-solvent ($\chi_{P/NS}$) and polymer solvent ($\chi_{P/S}$) are based on Equation 2-7, which is strictly valid for binary systems.

$$\varphi_{NS} = \frac{0.5 - \chi_{P/S}}{\chi_{P/NS} - \chi_{P/S}} + \frac{1}{(\chi_{P/NS} - \chi_{P/S})\sqrt{m_P}}$$

Equation 2-9, Cloud point of a ternary system, where φ_{NS} is the volume fraction of non-solvent, $\chi_{P/NS}$ is the interaction parameter of polymer/non-solvent, $\chi_{P/S}$ is the interaction parameter of polymer/solvent and m_p is the relative chain length of the polymer [46].

Turbidimetric titration experiments of polycarbonate in methanol/chloroform and isopropanol/methyl chloride solvent/non-solvent systems were performed by Glöckner [46]. The interaction parameters of polycarbonate and the solvents/non-solvents were calculated by using Equation 2-9 and showed reasonably correct values.

However, as can be derived from the Flory-Huggins relation (see Equation 2-6) the cloud point is concentration and molar mass dependent. The concentration dependence can be described by the empirical Equation 2-10 [16].

$$\varphi_S = C_1 + C_2 \log c$$

Equation 2-10, Concentration dependency of cloud point, where φ_S is the cloud point in volume fraction solvent, c is the concentration of polymers, and C_1 and C_2 are constants. φ_S and c are the values at the point of precipitation.

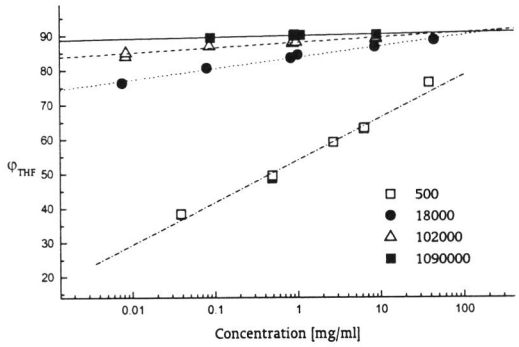


Figure 2-7, Plot of φ_{THF} versus polymer concentration at the cloud points of low polydispersity polystyrene standards (□ 500 g/mol, ● 18,000 g/mol, △102,000 g/mol, ■ 1,090,000 g/mol) obtained by turbidimetry in the system water/tetrahydrofuran.

To illustrate the concentration dependence, the cloud points of low polydispersity polystyrene standards (SEC) were determined in the non-solvent/solvent system water/tetrahydrofuran (THF) at different concentrations (see Figure 2-7) [49]. The data shown in Figure 2-7 can also be presented in another way, as shown in Figure 2-8.

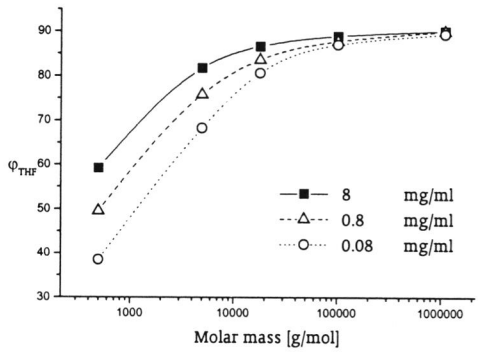


Figure 2-8, The molar mass dependence of the cloud points at different concentrations (■ 8 mg/ml, △ 0.8 mg/ml and ○ 0.08 mg/ml).

From the plots it can be seen that the concentration dependence is more pronounced for the low molar mass polystyrenes, whereas for the high molar mass polymers the concentration dependency is negligible.

The precipitation/redissolution mechanism is kinetically limited. If solely the precipitation/redissolution mechanism would be present and no column interactions would exist, the kinetics of redissolution would affect the (reproducibility of the) separation [50]. In combination with column interactions, the kinetic effects of redissolution may be negligible. However, when the polymer molecules can form a crystalline phase, the redissolution mechanism is affected drastically [51]. The application of GPEC to crystalline polyesters is further discussed in *Chapter 5*.

Glöckner [16] and *Schultz*, and *Engelhardt* [52] have described a precipitation/redissolution mechanism and found empirical relations for the retention of homopolymers in reversed phase systems.

2.4.2 COLUMN INTERACTIONS

During a chromatographic separation of polymers, column interactions always occur. However, depending on the eluent strength, these column interactions will be different. For polymers, column interactions often result in large k factors. Therefore, it is difficult to separate polymers with isocratic chromatography [16]. In order to separate polymer molecules, gradients from poor eluent conditions to good eluent conditions have to be applied. The interaction of a molecule depends on the functional end groups and on the number of repeating segments that can interact with the column material.

Different models to describe the sorption mechanism in GPEC have been described in the literature by various authors. The models have been used to predict the retention of polymers. *Jandera et al.* have described a mechanism where the contribution of the end group

and the repeating unit are separately discussed [53,54]. The mechanism described by *Jandera et al.* is valid for both on isocratic and gradient elution. *Snyder et al.* [32,55,56] and *Schoenmakers et al.* [57,58] related the retention behavior in gradient elution to the volume fraction solvent (φ_s) using different theoretical approaches.

All the models have restrictions with respect to their applicability. All models are based on low molar mass components the retention of high molar mass polymers is not included in the models. The determination of the theoretical parameters used in the models occasionally requires a large number of experiments [29], and in some cases these parameters do not have a clear physical meaning. In addition, the applicability of the models is restricted to a specific polymer/solvent/non-solvent/column system and for each new system the models have to be checked and modified. The possible combinations of polymer/solvent/non-solvent/column are numerous and thus, the general applicability of these models as prediction tools is severely limited.

2.5 Reversed Phase GPEC and Normal Phase GPEC

In general, RP-GPEC is performed on C18 modified silica columns. Although RP-mode is commonly applied, the contribution of column interactions during RP separations with a C18 modified silica columns of polymer molecules, is difficult to determine. A polymer molecule can exhibit two different kinds of interactions with the column: polar interaction as a result of the presence of silica functionalities (silanol, hydroxyl groups), and non-polar partitioning due to C18 tails and the solvent near the surface. This combination makes it hard to generally describe the column characteristics.

The initial conditions in RP-GPEC are in most cases poor in terms of solvent strength. Consequently, the mechanism is a combination of the precipitation/redissolution and column interactions.

RP-GPEC can be used to determine the chemical composition distribution. Several copolymer systems have been studied over the years [16]. Polymer molecules with different chemical compositions will elute at different eluent compositions. The copolymer molecules are separated based on the ratio of the monomers (overall composition). Thus, a chemical composition distribution is obtained. Although RP-GPEC is widely used, the exact mechanism of copolymer separation is still a matter of discussion, especially the effect of the molar mass of the copolymer molecules on the separation [16]. Nevertheless, the effect of the molar mass on the retention is negligible for copolymers with molar masses higher than 50,000 g/mol. The lack of well-defined copolymer standards is often the constraint for a good investigation of the molar mass effect on the retention.

NP-GPEC separation of copolymers was intensively studied by *Mori et al.* [18-21]. They performed chloroform/ethanol gradients to separate acrylate based polymers. In comparison with RP-GPEC a reversed elution order with respect to chemical composition is found. However, a molar mass dependency can be found as described for RP-GPEC. The reversed

elution order can easily be explained by polarity differences of the polymer molecules involved, since RP-GPEC is the reversed technique compares to NP-GPEC with respect to polarity. NP-GPEC provides additional and in some cases essential information [29] about polymer samples.

The separation mechanism of NP-GPEC is based on polar interactions. Therefore, NP-GPEC can be applied to separate polymer molecules according to small chemical differences, *e.g.* functional end groups.

2.6 Retention of Polymers of High Molar Mass

The separation of polystyrene by reversed-phase GPEC (RP-GPEC) has been studied extensively [29,31,59-70], although the studies have mainly been performed with low molar mass polystyrene standards.

The comparison of the cloud points obtained with turbidimetry with the retention compositions obtained with RP-GPEC of polystyrene standards is shown in Figure 2-9 [71]. The retention composition in these figures is related to the retention time according to the following equation.

$$\varphi_S = (t_R - t^0_{grad} - t^0_{col}) \cdot \varphi_{program} + \varphi_{initial}$$

Equation 2-11, The relation between the retention composition (φ_S) and the retention time (t_R), where t^0_{grad} is the gradient dead time, t^0_{col} is the dead time of the column, $\varphi_{program}$ is the gradient program in fraction solvent per minute, and $\varphi_{initial}$ is the initial composition of the solvent at injection.

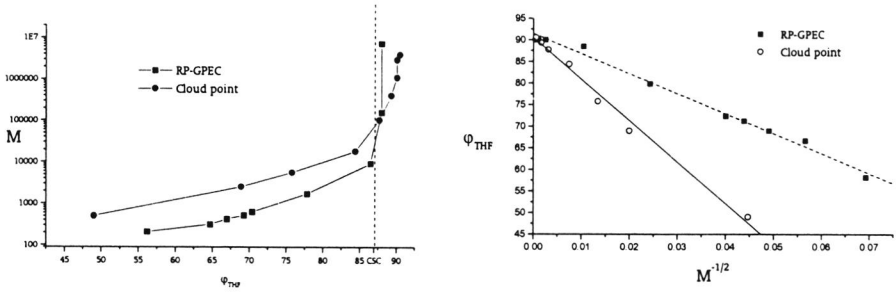


Figure 2-9, Comparison of the effects of the molar mass (M) of polystyrene in the system water/THF on the cloud points (turbidimetry, ■) and RP-GPEC elution composition (1 %/min, Symmetry C18 column, ○) (φ_S). Left: M vs. φ_{THF}; Right: φ_{THF} vs. $M^{-1/2}$.

For the lower molar masses, the RP-GPEC elution composition was richer in solvent than the cloud point. According to the redissolution mechanism the standards should elute at elution compositions containing less solvent. However, due to sorption additional retention occurred.

The difference between φ_{THF} from RP-GPEC and the cloud points decreases with increasing molar mass. This is due to the increase in elution strength. As long as the polymer dissolves in the sorption mode, normal retention behavior is found.

For the higher molar mass, RP-GPEC retention compositions were higher in solvent content than the cloud points. The high molar mass polystyrene dissolved at a higher solvent fraction than the critical conditions (specific for the used system solvents/column/temperature). Since relatively little sorption will take place, the polymer molecules will dissolve and exclusion will occur. Less column volume is available for the polymer molecules. Therefore the polymer molecules will elute faster through the column than the eluent in which they initially redissolved. Eventually phase separation will occur, when the molecules reach the eluent composition that has the composition of their cloud point [16]. The formation of a molecular gel results in strange peak forms and even splitting of the peaks [16]. The signal of the Ultraviolet (UV) detector can detect the de-mixing of high molar mass molecules. When s polymer dispersion passes through the detector, the light scattering of the polymer dispersion will give an increase in the signal. Due to this light scattering the UV detector can detect polymers without any UV absorbance, such as polymethyl methacrylate. Note that in this case the UV detector cannot be used quantitatively.

2.7 Applications of GPEC

GPEC can be used to separate a polymer sample into monomers, additives, oligomers and polymer molecules [72]. Additionally polymer molecules can be separated according to molar mass, chemical composition and functional end group.

The separation of polymer blends can be performed easily by GPEC [3]. The differences in solubility and specific column interactions of polymers with different chemical composition can be used to separate polymer blends into the separate homopolymers and/or copolymers. RP-GPEC, as well as NP-GPEC, can be applied. The separation of polystyrene (PS) and polyisoprene (PiP) as described in *Chapter 7* will be used to illustrate separation based on chemical composition. The molar mass dependencies of PS and PiP in the RP-GPEC separation in the system acetonitrile (ACN)/THF are shown in Figure 2-10. The PS and PiP samples were SEC standards with a well-defined molar mass and a low polydispersity.

The two homopolymers did not dissolve in ACN, but both dissolved in THF. The homopolymers PiP and PS were easily separated by the system described in Figure 2-10 (the polymer molecules of PS and PiP do not co-elute). Above molar mass of 50,000 g/mol, the molar mass dependency becomes negligible [16].

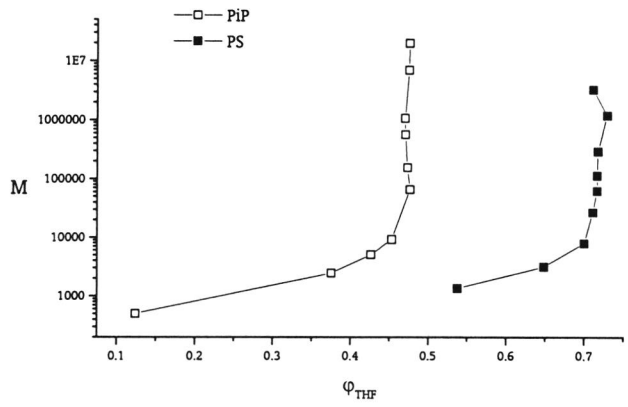


Figure 2-10, The molar mass dependencies of polystyrene (PS, ■, 500, 2450, 5050, 92k, 66k, 156k, 570k, 1075k, 7100k, and 20000k g/mol) and polyisoprene (PiP, □, 1350, 3200, 8k, 27k, 64k, 115k, 295k, 1200k, and 3310k g/mol) homopolymers on the retention composition (φ_{THF}) in the RP-GPEC system acetonitrile/THF on a Si-C18 column.

The NP-GPEC molar mass dependencies for the system heptane/THF on a silica column are shown in Figure 2-11. The PiP standards dissolved in heptane and were eluted before the gradient reached the column. However, the NP-GPEC chromatograms are in general not as clear as the RP-GPEC chromatograms, and many ghost peaks have been observed [16].

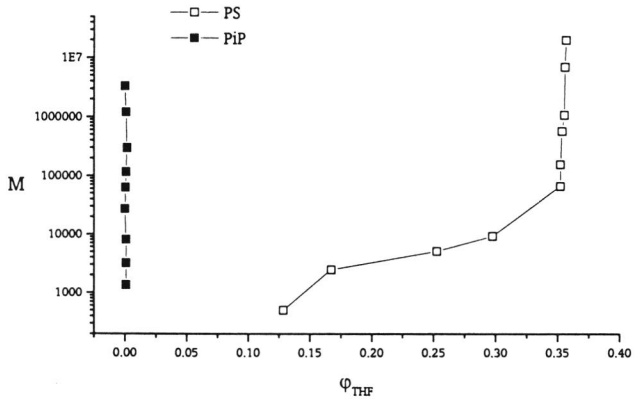


Figure 2-11, The molar mass dependencies of polystyrene (PS, ■, 500, 2450, 5050, 92k, 66k, 156k, 570k, 1075k, 7100k, and 20000k g/mol) and polyisoprene (PiP, □, 1350, 3200, 8k, 27k, 64k, 115k, 295k, 1200k, and 3310k g/mol) homopolymers on the retention composition (φ_{THF}) in the NP-GPEC system heptane/THF on a Silica column

The separation of copolymers is a well-known application of GPEC. The most extensively studied copolymer systems are styrene/acrylonitrile and styrene/acrylate copolymers [16]. Similar to SEC, GPEC is a relative method. By using copolymer standards, the retention in GPEC has to be related to the chemical composition. This relation is called the GPEC calibration curve. With the GPEC calibration curve the chemical composition distribution of similar copolymers can be calculated on the basis of a chromatogram [22-24]. The effect of the chemical composition on the retention composition of random copolymers is found to be linear. This means that the separation is based on the ratio of monomers in the molecule and not on the number of monomer segments in the polymer chain. However, the effect of the molar mass of the copolymer on the separation has to be studied in more detail. As mentioned before, the lack of copolymer standards often limits the application and the understanding of the mechanism. Not many copolymer standards are commercially available. Thus, the effect of the chemical composition on the separation of copolymers is often not known. Nevertheless, it is possible to apply on-line infrared analyses to determine the chemical composition of separated components.

Unfortunately, for the system PS and PiP, no copolymer standards are available. Still, the RP-GPEC conditions of Figure 2-10 can be successfully used to achieve a separation according to chemical composition. Nevertheless, only qualitative rather than quantitative results can be obtained with respect to the chemical composition.

Besides random copolymers, also block copolymers can be separated by GPEC. In contrast to random copolymers, the relation of the elution volume versus the chemical composition is not linear [16]. The solubility and sorption behavior of block copolymers will differ from random copolymers. Also in this case, the lack of acceptable standards prevents reliable studies on the retention mechanism and on applications. However, the development of new polymerization techniques, such as atom transfer radical polymerization [73], will provide well-defined block copolymers, which can be used to calibrate the GPEC separation.

2.8 GPEC IN COMBINATION WITH OTHER ANALYTICAL TECHNIQUES

HPLC techniques, such as GPEC, can be hyphenated with other analytical techniques, such as infrared spectroscopy or mass spectrometry. With the introduction of new coupling techniques, additional information about the microstructure of polymer molecules can be obtained. This has, for instance, been shown by the coupling of HPLC with Fourier-Transform-Infrared (FTIR) [74]. Even the coupling of HPLC with NMR has already been achieved [75]. However, the applications of LC-NMR are still very limited.

From the coupling of two HPLC techniques additional information can be obtained. The term used for the coupling of HPLC techniques is cross-fractionation [16,76-79] or orthogonal chromatography [42,80]. The different HPLC techniques that can be coupled are GPEC (for the separation according to chemical composition and functional type), SEC (separation on molecular size), and LCCC (for the separation according to functional type). The couplings

SEC–GPEC [76,79,29], LCCC–SEC [42], and NP-GPEC–RP-GPEC [29] have been successfully applied and the results can be presented in three dimensional (3D) graphs or contour plots. For SEC–GPEC a molar-mass-chemical composition-distribution (MMCCD) is obtained [79,29] and molar-mass-functional-type-distributions (MMFTD) can be obtained when SEC is coupled with LCCC or GPEC [29].

In the last decade the application of mass spectroscopy (MS) for polymers has increased intensively. GPEC hyphenated with MS will be discussed in detail in *Chapter 4* and *Chapter 6*.

2.9 Conclusions

GPEC can be applied to separate polymers according to chemical composition and functional end group. Depending on the type of GPEC (reversed-phase or normal-phase), different types of interactions can lead to separation. The mechanism is highly dependent on the conditions used and on the applied polymer. Therefore, the development of a universal model or theory is difficult. The conditions have to be optimized for every new GPEC separation. This makes GPEC a time consuming method with an art-like character. Despite this drawback, GPEC in many cases reveals differences between samples that cannot be obtained by any spectroscopic technique.

The most important application of GPEC is the separation of the polymer molecules according to chemical composition. Although the separation is seldom based on chemical composition differences only, the information obtained with GPEC can be conclusive, crucial and in some cases critical.

2.10 References

1. F.P.B. van der Maeden, M.E.F. Biemond and P.C.G.M. Janssen, *J. Chromatogr.*, **149**, *539-552 (1978)*.
2. S. Teramachi, A. Hasegawa, Y. Shima, M. Akatsuka and M. Nakajima, *Macromolecules*, **12 (5)**, *992-996 (1979)*.
3. W.J. Staal, *"Gradient Polymer Elution Chromatography", Ph.D. Thesis*, Eindhoven University of Technology, Eindhoven, The Netherlands, *1996*.
4. S. Teramachi, A. Hasegawa, Y. Shigekuni and S. Natunaga, *Polymer J.*, **21 (10)**, *803-808 (1989)*.
5. S. Teramachi, A. Hasegawa and K. Motoyama, *Polymer J.*, **22 (6)**, *489-496 (1990)*.
6. S. Teramachi, A. Hasegawa, Y. Shigekuni, K. Zenta and M. Hashimoto, *J. Appl. Polym. Sci.: Appl. Polym, Symp.*, **45**, *87-102 (1990)*.
7. G. Glöckner, *Chromatographia*, **23 (7)**, *517-524 (1987)*.
8. G. Glöckner, *Pure & Appl. Chem.*, **55 (10)**, *1553-1562 (1983)*.
9. G. Glöckner and D. Ilchmann, *Acta Polym.*, **35 (11)**, *680-683 (1984)*.
10. G. Glöckner and J.H.M. van den Berg, *J. Chromatogr.*, **384**, *135-144 (1987)*.
11. G. Glöckner, M. Stickler and W. Wunderlich, *Fresenius Z. Anal. Chem.*, **328**, *76-81 (1987)*.
12. G. Glöckner, *J. Chromatogr.*, **403**, *280-384 (1987)*.
13. G. Glöckner, M. Stickler and W. Wunderlich, *Fresenius Z. Anal. Chem.*, **330**, *46-49 (1988)*.
14. G. Glöckner, M. Stickler and W. Wunderlich, *J. Appl. Polym. Sci.*, **37**, *3147-3161 (1989)*.

15. G. Glöckner and H. G. Barth, *J. Chromatogr.*, **499**, *645-654 (1990).*
16. G. Glöckner, *"Gradient HPLC of Copolymers and Chromatographic Cross Fractionation"*, Springer-Verlag Berlin Heidelberg, *1991.*
17. G. Glöckner, *J. Appl. Polm. Sci.: Appl. Polym. Symp.*, **52**, *45-54 (1992).*
18. S. Mori, J. Appl. Polym. Sci.: Appl. Polym. Sci., 43, 65-84 (1989).
19. S. Mori, *J. Liq. Chromatogr.*, **13 (15)**, *3039-3052 (1990).*
20. S. Mori, *J. Chromatogr.*, **503**, *411-419 (1990).*
21. S. Mori and H. Taziri, *J. Liq. Chromatogr.*, **17 (14&15)**, *3055-3068 (1994).*
22. P.J.C.H. Cools, F. Maesen, B. Klumperman, A.M. van Herk and A.L. German, *J. Chromatogr. A*, **736**, *125-130 (1996).*
23. R.W. Sparidans, H.A. Claessens, G.H.J. van Doremaele and A.M. van Herk, *J. Chromatogr.*, **508**, *319-331 (1990).*
24. H.A.S. Schoonbrood, *"Emulsion Co- and Terpolymerization"*, *Ph.D. Thesis*, Eindhoven University of Technology, Eindhoven, The Netherlands, *1994.*
25. T. Tanaka, M. Omoto, N. Donkai and H. Inagaki, *J. Macromol. Sci.-Phys.*, **B17(2)**, *211-228 (1980).*
26. M. Augenstein and M.A. Müller , *Makromol. Chem.*, **191**, *2151-2172 (1990).*
27. G. Glöckner and J.H.M. van den Berg, *J. Chromatogr.*, **550**, *629-638 (1991).*
28. G. Glöckner and A.H.E. Müller, *J. Appl. Polym. Sci.*, **38**, *1761-1774 (1989).*
29. Philipsen H.J.A., *"Mechanisms of Gradient Polymer Elution Chromatography and its Application to (Co)Polyesters"*, *Ph.D. Thesis*, Eindhoven University of Technology, Eindhoven, The Netherlands, *1998.*
30. P.J.C.H. Cools unpublished results.
31. T.L.J. Willems, M.Sc. Thesis, Eindhoven University of Technology, Eindhoven, The Netherlands (1993).
32. M.A. Quarry, M.A. Stadalius, T.H. Mourey, and L.R. Snyder, *J. Chromatogr.*, **358**, *1-16 (1986).*
33. P.J. Schoenmakers, *Personal conversation.*
34. H.C. Lee and T. Chang, *Polymer*, **37(25)**, *5747-5749 (1996).*
35. H.C. Lee and T. Chang, *Macromol. Symp.*, **118**, *261-265 (1997).*
36. A.M. Skvortsov and A.A. Gorbunov, *J. Chromatogr.*, **507**, *487-496 (1990).*
37. T.M. Zimina, Y.Y. Kever, Y.Y. Melenevskaya, V.N. Zgonnik and B.G. Belen'kii, *Polym. Sci.*, **33(6)**, *12050-1254 (1991).*
38. T.M. Zimina, J.J. Kever, E.Y. Melenevskaya and A.F. Fell, J. Chromatogr., 593, 233-241 (1992).
39. H. Pash, H. Much and G. Schulz, *J. Appl. Polym. Sci: Appl. Polym. Symp.*, **52**, *79-90 (1993).*
40. T.M. Zimina, A.F. Fell and J.B. Castledine, *J. Chromatogr. Sci.*, **31**, *455-460 (1993).*
41. H. Pasch, C. Brinkmann and T. Gallot, *Polymer*, **34 (19)**, *4100-4104 (1993).*
42. R.P. Krüger, H. Much and G. Schulz, *J. Liq. Chromatogr.*, **17 (14&15)**, *3069-3090 (1994).*
43. A.V. Gorshkov, T.N. Prudskova, V.V. Gur'yanova and V.V. Evreinov, *Polym. Bulletin*, **15**, *465-468 (1986).*
44. P.J.C.H. Cools, A.M. van Herk, A.L. German and W. Staal, *J. Liq. Chromatogr.*, **17(14&15)**, *3133-3143 (1994).*
45. H.J.A. Philipsen, B. Klumperman, A.M. van Herk and A.L. German, *J. Chromatogr. A*, **727**, *13-25 (1996).*
46. G. Glöckner, *Z. Phys. Chem.*, **229**, *98-109 (1965).*
47. P. Flory, *"Principles of Polymer Chemistry"*, Cornell University Press, Ithaca, New York, *1953.*
48. D.W. van Krevelen, *"Properties of Polymers"*, 3rd Edition, Elsevier, Amsterdam, *1990.*
49. P.J.C.H. Cools *unpublished results.*
50. H.J.A. Philipsen, M.R. de Cooker, H.A. Claessens, B. Klumperman and A.L. German, *J. Chromatogr. A*, **761**, *147-162 (1997).*
51. H.J.A. Philipsen, M. Oestreich, B. Klumperman and A.L. German, *J. Chromatogr. A*, **775**, *157-177 (1997).*
52. R. Schultz and H. Engelhardt, *Chromatographia*, **29(5/6)**, *205-213 (1990).*
53. P. Jandera and J. Rozkošá, *J. Chromatogr.*, **362**, *325-343 (1986).*
54. P. Jandera, *Chromatographia*. **26**, *417-422 (1988).*
55. L.R. Snyder, M.A. Stadalius and M.A. Quarry, *Anal. Chem.*, **55(14)**, *1414A-1430A (1983).*

56. M.A. Stadalius, M.A. Quarry, Th. Mourey and L.R. Snyder, *J. Chromatogr.*, **358**, *17-37 (1986)*.
57. P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen and L. De Galan, *J. Chromatogr.*, **149**, *519-537 (1978)*.
58. P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen and L. De Galan, *J. Chromatogr.*, **185**, *179-195 (1979)*.
59. S.T. Lai and D.C. Locke, *J. Chromatogr.*, **252**, *325-330 (1982)*.
60. J.J. Lewis, L.B. Rogers and R.E. Pauls, *J. Chromatogr.*, **264**, *339-356 (1983)*.
61. C.H. Lochmüller and M.B. McGranaghan, *Anal. Chem.*, **61**, *2449-2455 (1989)*.
62. R.A. Shalliker, P.E. Kavanagh, I.M. Russell and D.G. Hawthorne, *Chromatographia*, **33(9/10)**, *427-433 (1992)*.
63. G. Glöckner, *Chromatographia*, **25(10)**, *854-860 (1988)*.
64. R.A. Shalliker, P.E. Kavanagh and I.M. Russell, *J. Chromatogr.*, **543**, *157-169 (1991)*.
65. D.M. Northrop, D.E. Martire and R.P.W. Scott, *Anal. Chem.*, **64**, *16-21 (1992)*.
66. R.A. Shalliker, P.E. Kavanagh and I.M. Russell, *J. Chromatogr. A*, **664**, *221-228 (1994)*.
67. R.A. Shalliker, P.E. Kavanagh and I.M. Russell, *J. Chromatogr.*, **558**, *440-445 (1991)*.
68. P. Jandera, *J. Chromatogr.*, **449**, *361-389 (1988)*.
69. J.P. Larmann, J.J. DeStefano, AP. Goldberg, R.W. Stout, L.R. Snyder and M.A. Stadalius, *J. Chromatogr.*, **255**, *163-189 (1983)*.
70. S.T. Lai, L. Sangermano and D. C. Locke, *J. Chromatogr.*, **312**, *313-326 (1984)*.
71. P.J.C.H. Cools *unpublished results*.
72. W.J. Staal, P.J.C.H. Cools, A.M. van Herk and A.L. German, *Chromatographia*, **37(3/4)**, *218-220 (1993)*.
73. K. Matyjaszewski, T.E. Patten and J. Xia, *J. Am. Chem. Soc.*, **119**, *674 (1997)*.
74. L.M. Wheeler and J.N. Willis, *Appl. Spectrosc.*, **47**, *1128 (1993)*.
75. H. Buskens, *Chemisch-2-Weekblad*, **1**, *18 (1999)*.
76. G. Glöckner, J.H.M. van den Berg, N.L.J. Meijerink, Th.G. Scholte and R. Koningsveld, *J. Chromatography*, **317**, *615-624 (1984)*.
77. G. Glöckner and J.H.M. van den Berg, *J. Chromatography*, **381**, *135-144 (1987)*.
78. G. Glöckner, J. Stejskal and P. Kratochvíl, *Makromol. Chem.*, **190**, *427-435 (1989)*.
79. G.H.J. van Doremaele, F.H.J.M. Geerts, L.J. aan de Meulen and A.L. German, *Polymer*, **33(7)**, *1512-1518 (1992)*.
80. G. Schulz, H. Much, H. Krüger and C. Wehrstedt, *J. Liq. Chromatogr.*, **13(9)**, *1745-1763 (1990)*.

Chapter 3

Other Analytical Techniques Applied to Characterize Copolymers

In this chapter, the applied analytical techniques, beside gradient polymer elution chromatography (GPEC), are described. Size exclusion chromatography (SEC) using the universal calibration method is described. Additionally, electrospray-ionization mass spectrometry (ESI-MS) and matrix-assisted-laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) are described.

3.1 INTRODUCTION

Analytical techniques such as gradient polymer elution chromatography, size exclusion chromatography, electrospray ionization mass spectrometry and matrix assisted laser desorption ionization mass spectrometry have been applied in order to characterize the polymers described in this thesis. The various aspects of GPEC have already been described in Chapter 2, and in this chapter the relevant principles of SEC, ESI-MS and MALDI-MS will be described briefly.

3.2 SIZE EXCLUSION CHROMATOGRAPHY [1]

The separation in size exclusion chromatography (SEC) is based on the steric exclusion. The principle of steric exclusion is already discussed in *Chapter 2 (pages 8/9)*.

The combination of SEC with molecular weight sensitive detectors, such as the viscosity detector, can be used to detect small differences in molar mass or in chemical structure (block structures and branching can be detected).

The viscosity detector measures a pressure difference over a specific capillary. The intrinsic viscosity [η] of an eluting component can be calculated from this pressure difference. The effect of molar mass on the intrinsic viscosity [η] can be described by the *Mark-Houwink* relation [1].

$$[\eta] = K_{\eta} \cdot M^{a}$$

Equation 3-1, Mark-Houwink relation, where [η] is the intrinsic viscosity [dl/g], K_{η} [dl·mol/g²] and a [-] are Mark-Houwink constants, M is the molar mass [g/mol].

The *Mark-Houwink* constants are specific for the polymer/eluent combination. The intrinsic viscosity [η] can be determined by the viscosity signal (DP or Δp). The intrinsic viscosity [η] can be calculated (via the specific viscosity η_{sp}) from the DP signal according to Equation 3-2.

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c} \approx \frac{\eta_{sp}}{c} = \frac{f(\Delta p)}{c}$$

Equation 3-2, Intrinsic viscosity [η] relation, where Δp = the pressure difference, c = concentration [g/l], and η_{sp} is the specific viscosity [-] which can be directly calculated from the measured differential pressure Δp.

When the separation in SEC solely depends on entropy effects, the separation is based on the hydrodynamic volume (V_h). According to Einstein's viscosity law [2], V_h can be expressed as;

$$V_h \propto [\eta]M$$

Equation 3-3, Hydrodynamic volume [l/mol].

By the universal calibration method (UC) the molar mass of polymers can be determined when log [η]M *versus* retention volume of the system is known and [η] is measured [3]. The following equation can be used to calculate the molar mass for a specific retention volume, *i.e.* a specific V_h.

$$[\eta]_1 M_1 = [\eta]_2 M_2$$

Equation 3-4, Universal calibration relation, where $[\eta]_i$ is the intrinsic viscosity of polymer i and M_i is the molar mass of polymer i.

With the experimental data of an arbitrary polymer (polymer '1') (log[η]M *vs.* retention volume, the universal calibration curve), the molar mass (M) of the polymer of interest (polymer '2') can be calculated by measuring [η] or by using known Mark-Houwink parameters of polymer '2' (see Equation 4-1) [2]. Thus, SEC in combination with a viscometry detector allows the use of the UC method. In the following chapters UC-SEC will be applied to characterize polyesters.

3.3 Introduction to Mass Spectrometry of Polymers

Mass spectroscopy (MS) is an analytical technique that has already been used for many years. The applications of MS were mainly the analyses of low molar mass chemicals. Lately, the emphasis has been put more and more on the identification of macromolecules [4-7], and especially on the identification of bio-polymers. In this and the following paragraphs the applications of MS in the field of polymer analysis will be briefly discussed.

MS techniques can be divided into four parts: the sample introduction, the ionization, the analysis, and the detection. The sample introduction can, for example, be liquid chromatography or a syringe. In the ionization step, molecules of the sample are ionized, and the analyzer separates the polymer molecules according to mass number (m) over charge number (z), m/z. After separation the molecules are detected.

The ionization step is the most crucial part of MS. The sample can be fragmented by a destructive ionization method, such as for example electron impact ionization or fast atom bombardment [7]. Fragmentation MS provides information about the overall chemical composition (presence of specific groups) of the sample. However, this technique offers no information on the molar mass or the distribution of certain groups over the polymer chain. Thus, ionization techniques involving fragmentation are applicable for polymers, but little additional information is obtained as compared to *e.g.* NMR.

Since the introduction of the so-called soft ionization techniques, such as electrospray-ionization (ESI) and matrix-assisted-laser-desorption-ionization (MALDI), the number of applications of MS in the field of polymer characterization has increased enormously [3,4,8,9]. These soft ionization techniques are characterized by no or little fragmentation, *i.e.* the polymer molecules become charged but remain intact.

The most commonly used analyzers for MS are quadrupole, ion trap, and time-of-flight (TOF) analyzers. Each analyzer has its specific advantages and drawbacks. For instance, the TOF analyzer can only be used in combination with pulsed ionization techniques, such as MALDI, where charged samples are introduced via laser pulses [10]. Different type of detectors can be used. The photographic plate and the Faraday cup measure the ions directly. Electron or photon multipliers amplify the signal of the analyte [10].

3.3.1 Electrospray Ionization Mass Spectrometry

ESI-MS is a soft ionization technique, resulting in no or little fragmentation of the polymer molecules during ionization. The principle of ESI-MS will be discussed briefly [10]. The polymer sample is dissolved, and sprayed through a capillary (see Figure 3-1). The polymer solution flows with a constant flow (1-10μl/min) through the capillary, and is nebulized by the nebulizing gas. The formed aerosol is charged by the potential difference (ΔV=3-6 kV) between the capillary and the electrode (see Figure 3-1). The electrode is a thick metal plate with a small orifice (5μm). The analytes are transported through this orifice due to the pressure difference between the two sides of the plate.

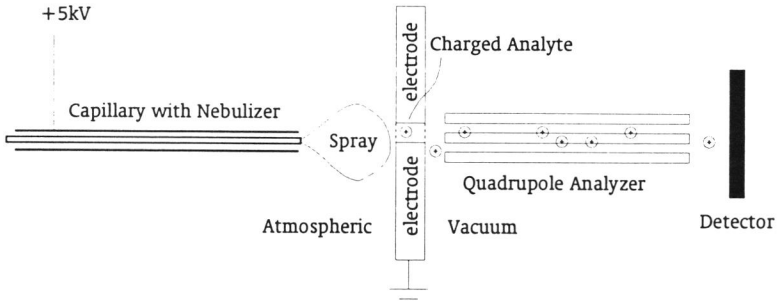


Figure 3-1, A schematic representation of the principle of electrospray ionization (ESI).

The solvent is evaporated and the charge (either anionic or cationic) is transferred to the polymer molecule. Cationic ionization of a polymer molecule is shown Figure 3-2. During spraying droplets are formed, which contain multiple polymer molecules. The droplet will have a high charge. The solvent will evaporate until the repelling coulombic forces (the droplet surface decreases as the solvent evaporates) will equal the cohesion forces of the

droplet, and the initial droplet will explode into smaller droplets. These smaller droplets will have less charge, and eventually, after total evaporation of the solvent, charged molecules, such as $(M\text{-}NH_4)^+$ or $(M\text{-}Ag)^+$ adducts, will be the result. A simplified scheme of this process can be found in Figure 3-2. This description of the ionization is one of several possible mechanisms. The exact mechanism of ionization is not fully known [10].

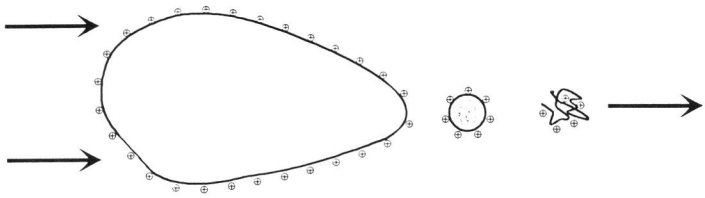


Figure 3-2, Simplified scheme of the cationic ionization of a polymer molecule by electrospray ionization.

Specific groups, such as the repeating units of the polymer molecule or a functional end group will be charged. Therefore, the polymer molecule can have multiple charges. After ionization it is introduced into the vacuum of the MS and will be accelerated to the analyzer by electric lenses.

In general, and also in this study described in *Chapters 4 and 6*, ESI-MS is used in combination with a quadrupole analyzer. A major drawback of the quadrupole analyzer is the relatively small m over z detection window ($m/z<4000$ g/mol) [10]. Since with ESI the analytes can be multiply charged, the highest molar mass that can be analyzed by a quadrupole analyzer is higher. One of the latest developments in ESI-MS is the use of a TOF analyzer for the analysis of polymers. The TOF analyzer can separate high molar mass molecules, which makes the applicability of ESI-MS for higher molar mass polymers accessible [11].

Beside the above advantage, multiple charged analytes also result in a disadvantage. Each oligomer with mass m will occur in MS spectra as a distribution of peaks with different m/z values, and not as one single m/z peak. Algorithms have been developed to interpret the mass spectra obtained with ESI-MS. Nevertheless, when a polymer with a broad distribution of oligomers is analyzed directly, the interpretation of the MS spectrum is very difficult.

ESI-MS can be coupled on-line with liquid chromatography [8,9,12,13]. LC can easily provide the constant flow required for ESI-MS. The flow required for ESI-MS (approximately 1 μl/min depending on the electrospray method) is much lower than the conventional LC flow (0.5-1.0 ml/min), therefore, flow splitters are commonly used [8].

3.3.2 Matrix Assisted Laser Desorption Ionization Mass Spectrometry

Another soft ionization method is matrix-assisted-laser-desorption-ionization (MALDI). In Figure 3-3 the ionization principle of MALDI MS is shown.

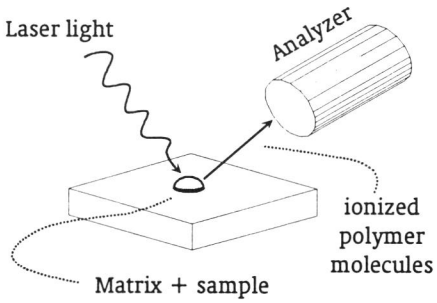


Figure 3-3, Ionization principle of MALDI.

The sample preparation is more crucial for MALDI-MS than for ESI-MS. A polymer solution is mixed with a specific matrix. This matrix can be a salt and/or an acid and has to be able to absorb the laser light, which in general has a wavelength of 337 nm. In addition, the type of the matrix is very important, since different matrices have to be used for different types of polymers [4]. During the evaporation of the solvent the matrix crystallizes, and the polymer molecules are, in the ideal case, incorporated in the crystalline matrix as isolated coils. Also the matrix will need to transfer a charge to the polymer, which generally is not easily ionized. The probe, containing sample and matrix, will be pulsed by laser-light, the matrix absorbs the laser-light, and the polymer molecules desorb. The exact ionization process is not known, but the polymer molecules get ionized. In general, the polymer molecules will be charged by one single proton or cation from the matrix.

By applying electrical lenses, the charged molecules (matrix and polymer) are transported to the analyzer. The ionization step of MALDI is not as soft as that of ESI, because the high-energy laser light can still fragment the polymer molecules. The most common analyzer used in combination with MALDI is the time-of-flight (TOF) analyzer. Since low molar masses will be accelerated to a higher speed than the higher molar masses, the TOF analyzer separates the analyte from low to high molar masses. The TOF analyzer has a specific length, each ion will have a characteristic time of flight, depending on the length of the analyzer and on the m/z of the ion. The TOF analyzer analyzes plugs or pulses of the sample according to the speed (time of flight) of the analyte. The TOF determination of one pulse/plug of the sample takes a fraction of a microsecond [10].

There are certain drawbacks associated with MALDI-TOF MS. MALDI-TOF MS is more sensitive for low molar mass molecules [9], resulting in an overestimation of low molar mass molecules compared to the high molar mass molecules. Desorption of higher molar masses from the matrix is more difficult than that of low molar masses. Consequently, when a

polymer sample with a broad molar mass distribution (high polydispersity) is analyzed with MALDI MS, the low molar mass part will dominate the spectra and the high molar mass species will not be detected. Only polymers with a low polydispersity ($< \pm 1.2$) can be correctly identified with MALDI-MS [9] and since commercial polymers rarely have polydispersities lower than 1.2, MALDI-MS is not directly useful for commercial polymers. However, SEC or GPEC can be applied to obtain low polydispersity fractions. The separated SEC or GPEC fractions can thereafter be analyzed off-line with MALDI-MS. Since the sample has to be incorporated into the matrix, MALDI-MS cannot easily be used on-line. Nevertheless, recent developments in the field of on-line interfaces will probably make on-line MALDI-MS practicable in the near future.

3.4 *REFERENCES*

1. B.J. Hunt and S.R. Holding, *"Size Exclusion Chromatography"*, Blackie and Son Ltd., London, *1989*.
2. G. Glöckner, *"Gradient HPLC of Copolymers and Chromatographic Cross Fractionation"*, Springer-Verlag Berlin Heidelberg, *1991*.
3. Z. Grubisic, P. Remmp and H. Beniot, *J. Polym. Sci. B*, **Polym. Letters 5**, *753 (1967)*.
4. G. Montaudo, *Trends Polym. Sci.*, **4**, *81-86 (1996)*.
5. A.L. Burlingame, R.K. Boyd, and S.J. Gaskell, *Anal. Chem.*, **70**, *647R-715R (1998)*.
6. R. Saft, C. Mirtl and K. Hummel, *Acta Polymer*, **48**, *513-526 (1979)*.
7. J. Throck Watson, *"Introduction to Mass Spectrometry"*, third edition, Lippincott-Raven Publishers, Philidelphia, *1997*.
8. M.W.F. Nielen, *Rapid Commun. Mass Spectrom.*, **10**, *1652-1660 (1996)*.
9. M.W.F. Nielen and S. Malucha, *Rapid Commun. Mass Spectrom.*, **11**, *1194-1204 (1997)*.
10. E. De Hoffman, J. Charette and V. Stroobant, *"Mass Spectrometry, Principles and Applications'*, John Wiley & Sons, New York, *1996*.
11. M.W.F. Nielen and F.A. Buijtenhuijs, *Anal. Chem.*, in press.
12. W.M.A. Niessen and A.P. Tinke, *J. Chromatogr. A*, **703**, *37-57 (1995)*.
13. R.P Krüger, H. Much and G. Schultz, *Macromol. Symp.*, **110**, *155-176 (1996)*.

Chapter 4

The Microstructural Analysis of Homopolyesters of Neopentyl Glycol with Isophthalic Acid and Terephthalic Acid

Various analytical tools have been used to identify the microstructure of homopolyester samples consisting of neopentyl glycol with isophthalic acid, or terephthalic acid. Size exclusion chromatography (SEC) was coupled with a viscometry detector to allow the application of universal calibration method. Thus, the molar mass distribution (MMD) of the polyester samples was determined. Gradient polymer elution chromatography (GPEC) was used to separate the polyesters according to chemical composition and functional end groups. Reversed-phase gradients of water, acetonitrile, and tetrahydrofuran were applied to obtain an optimal separation. Electrospray-ionization mass spectrometry and matrix-assisted-laser-desorption-ionization mass spectrometry were successfully applied for the identification of the polyester oligomers. The combination of analytical techniques provides unique and crucial information on the structure of the polymer.

4.1 INTRODUCTION

In literature different LC techniques have been described to characterize the microstructure of polyesters [1-11]. Special credit is due to *Van der Maeden et al.* [1], since these authors have been the first to report gradient elution LC of polyesters. In order to determine the molar mass distribution (MMD) of polymers, size exclusion chromatography (SEC) can be used. Gradient polymer elution chromatography (GPEC) can also be used to obtain an MMD [7], but the application is limited to low molecular weight polyesters (up to molar masses of approximately 5000 g/mol). GPEC is mainly applied to determine the chemical composition distribution (CCD) [11-15] and the functionality type distribution (FTD) [11]. The MMD, the CCD and the end group distribution are referred to as the chemical microstructure of the polyesters. Polyesters consisting of neopentyl glycol (NPG) with isophthalic acid (IA) and/or terephthalic acid (TA) (see Figure 4-1) are used to study the applicability of GPEC. Since these are complex polymers with complex microstructure, the separation by GPEC and the interpretation of the GPEC chromatograms will be complicated.

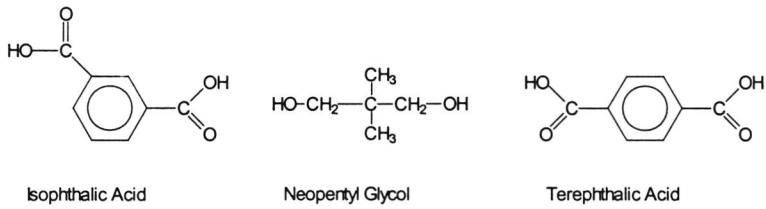


Figure 4-1, Chemical Structures of isophthalic acid, neopentyl glycol and terephthalic acid.

Copolyesters of NPG, TA and IA are applied as powder coatings. Powder coatings are a solvent free coating system, which can be applied on different types of substrates, such as metal or polymer surfaces. A powder coating consists of a polymer matrix, which forms the basis of the coatings and additives, which are added to adjust several properties, such as electrostatic attraction or conductivity. A cross-linking agent is added to obtain a cross-linked polymer matrix based on NPG/TA/IA copolyesters. The combination NPG/TA improves the flexibility and graining properties of the coating and IA is added (up to 33w%) to improve the resistance with respect to aging. Powder coatings are applied by electrospray or by a fluidized bed, and are cured in an oven. During curing the polymer matrix is cross-linked by a reaction of the end groups of the copolyesters. Consequently, information on the microstructure of the polymer matrix is crucial.

Rissler [10] already described the RP-GPEC separation of commercial polyesters containing NPG, IA, and TA. He used slow gradient steps and separation according to molar mass was achieved for molar masses up to 10,000 g/mol. However, when slow gradients were performed the reproducibility became poor, and baseline drift occurred. He focussed on the

separation of the oligomers according to molar mass, especially of the high molar mass oligomers, and not on the separation according to the chemical composition. He did not identify the peaks and therefore detailed information about the separation and the polyesters was not obtained.

The application of GPEC for polyesters containing di-propoxylated bisphenol-A, isophthalic acid, adipic acid and maleic acid, has been studied by *Philipsen* [11]. He studied the RP-GPEC retention mechanisms of the polyesters and applied RP-GPEC and NP-GPEC to determine the CCD and FTD of the copolyesters.

Polyesters are synthesized by a polycondensation reaction, defined as a step reaction, where the functional groups of the monomers, oligomers and polymers react. The polyesters used in this study are formed from a di-alcohol monomer (D) and a di-acid monomer (A). A functional acid group of A reacts with a functional alcohol group of D, releasing water, giving rise to the name polycondensation. The condensation reaction is schematically shown in Figure 4-2.

Figure 4-2, Scheme of (poly)condensation reaction.

The reaction of an acid group with an alcohol group is also known as esterfication. The side product, water, is eliminated by distillation. This means that the equilibrium, as shown in Figure 4-2, is forced to the right hand side. Esterfication can occur with monomers as well as with already formed oligomers. Therefore, the R_1 and R_2 groups can be a monomer or an oligomer. Consequently, the polymer molecules grow by combination of the oligomers and monomers, in contrast to a chain polymerization, where solely the monomer units are added to the active site of a growing polymer chain.

Depending on the monomer unit at both ends of the chain, the end groups of the polyesters can either be diol (D~D), acid~hydroxy (A~D), or di-acid (A~A). Also, cyclic species can be formed. These cyclic oligomers have no functional group with respect to the hydroxy and acid end groups. The number and type of end groups play an important role in the processing of the polyesters. Thus, information about the end group distribution is essential for a proper understanding of the properties. Besides the differences in functional end groups, the polyester will also contain oligomers with varying chemical composition (ratio and sequence of IA and TA).

In this chapter the separation of the homopolyesters by GPEC will be discussed. In order to separate the copolyesters by GPEC (see Chapter 6), the retention behavior of the homopolyesters is studied. GPEC is solely a separation technique, and in general other analytical techniques have to be applied to identify the separated components. Therefore mass spectrometry techniques, such as electrospray-ionization-mass-spectroscopy (ESI-MS)

and matrix-assisted-laser-desorption-ionization-mass-spectrometry (MALDI-MS) were applied to determine the molar mass of the separated components (oligomers with different molar mass and functional groups). ESI-MS was used on-line for the identification in reversed-phase GPEC. MALDI-MS was applied off-line for the normal-phase GPEC experiments. Size exclusion chromatography (SEC) was applied to determine the MMDs of the homopolyesters.

4.2 EXPERIMENTAL

4.2.1 SYNTHESIS OF POLYESTERS

In order to study the separation of polyesters by GPEC, homopolyesters were synthesized in bulk. The applied monomers were neo-pentyl-glycol (NPG), isophthalic acid (IA), and terephthalic acid (TA). Three different homopolyesters were synthesized: a polyester containing IA and NPG (sample 14), a polyester containing TA and NPG (sample 15) and the alkylated polyesters (sample 15M) of the TA/NPG homopolyester. The homopolyesters were synthesized under melting conditions using the following procedure.

Initially, 50g of water and 500g NPG were mixed in a two liter reactor and heated to a temperature of 80°C (water was added to melt the NPG at a lower temperature). Dibutyl tin oxide was added (1.5g) as a catalyst for the esterfication reaction. When the NPG was melted, 900g of acid monomer, either IA or TA was added to the reactor, and the reactor was purged with nitrogen and slowly heated to a temperature of 240°C in order to melt the acid monomer. The water formed during the esterfication was distilled off. After an acid value (AV), determined by titration, of approx. 35 [mg KOH/g] was reached, the reactor was cooled to 180°C. The total reaction times were not the same for the two homopolymers (see Table 4-1).

The homopolyester obtained with NPG and TA (sample 15) was also alkylated with diazomethane using standard procedures and was coded sample 15M [16]. The compositions of the homopolyester samples used are shown in Table 4-1.

Table 4-1, Polyester Samples with different mol fractions IA and TA. The used mol fraction acid (0.47)was not changed

Sample Code	Mol Fraction NPG	IA	TA	Reaction Time Hours
14	0.47	0.53	0.0	9
15	0.47	0.0	0.53	17
15M	0.47	0.0	0.53	-

4.2.2 SIZE EXCLUSION CHROMATOGRAPHY

The SEC experiments were performed with a Spectroflow 400 solvent delivery system, a Gaston GT-103 on-line degassing device, an ABI 757 multiple wavelength UV detector (λ=275

nm), a Waters 410 DRI detector, a Viscotek model 502 DP detector, and a Separations Triathlon automatic sample injector with a calibrated 50 μL sample loop. The established flow was 0.4 ml/min of hexafluoro iso-propanol (HFIP, Matco N.V.) with 0.02 M potassium trifluoro acetate (KTFA was added to avoid repelling and adsorption effects). The columns used were two silica diol columns (Alltech, 100Å and 1000Å, 300x8mm, temperature 30°C). The polymer samples were dissolved in HFIP + 0.02 M KTFA at different concentrations (± 1 mg/ml). The concentration of the injected samples had to be determined accurately in order to apply the universal calibration method. The columns were calibrated in tetrahydrofuran with polystyrene SEC standards from Polymer Laboratories. The silica diol columns could be calibrated with a different solvent system since the column packing was not polymer based and, therefore, does not shrink or swell when different solvents were used. The molar masses were determined with the conventional method (polystyrene equivalent) and with the universal calibration method. The SEC data were acquired and processed with the Viscotek Trisec software (version 3.0).

4.2.3 Gradient Polymer Elution Chromatography

The reversed phase GPEC experiments were performed with a Waters 616 Gradient Pump, a Waters 490 multiple wavelength UV detector (260nm, 275nm, and 305nm), an Hewlett Packard 1010 photodiode array detector (DAD), a Gaston GT-103 on-line degassing device, a Separations Mistral column oven and a Waters 717 WISP Autosampler with variable injection volume. All the experiments were performed using a Waters 490 multiple UV detector (275nm), except in the explicitly mentioned experiments in which the HP 1010 DAD detector was used. The applied solvents were acetonitrile (ACN, gradient grade, Merck), THF (HPLC grade, unstabilized, Merck), H_2O (MilliQ system, Millipore) and HFIP (UV quality, Matco BV). Acetic acid (1 vol%) was added to the eluents in order to protonate the acid end groups of the polyesters, thus avoiding repelling and sorption effects. The applied gradients are described in Table 4-2 and Table 4-3, and schematically represented in Figure 4-3. The column applied for the RP-GPEC experiments, was a Waters Symmetry Si-C18. The flow was 0.5 ml/min. The concentration was 10 mg/ml and the injection volume 25μl. The column temperature was established at 55 °C. Gradient A was also applied with HFIP instead of THF (see Table 4-2). The optimization of the gradient A was performed by trial and error.

Table 4-2, Gradient A, a combiantion of binary gradients and isocratic steps, used for reversed-phase GPEC with THF and HFIP. The eluent compositions are given in volume fraction (φ), and $\Delta\varphi_{gradien}$ is the gradient steepness of the applied step.

Gradient A

Step	Time [min]	φ_{water} -	φ_{ACN} -	φ_{THF} or φ_{HFIP} -	$\Delta\varphi_{gradient}$ Min^{-1}
	Initial	0.5	0.5	0.0	-
1	10	0.5	0.5	0.0	Isocratic
2	35	0.0	1.0	0.0	0.02
3	45	0.0	1.0	0.0	Isocratic
4	95	0.0	0.0	1.0	0.02
5	110	0.0	0.0	1.0	-
6	115	0.0	1.0	0.0	-
7	120	0.5	0.5	0.0	-

Table 4-3, Gradient B, one single linear ternary gradient, used for reversed-phase GPEC. The eluent compositions are given in volume fraction (φ), and $\Delta\varphi_{gradien}$ is the gradient steepness of the applied step.

Gradient B

Step	Time [min]	φ_{water} -	φ_{ACN} -	φ_{THF} or φ_{HFIP} -	$\Delta\varphi_{gradient}$ Min^{-1}
	Initial	0.25	0.25	0.0	-
1	100	0.0	0.0	1.0	0.01
2	115	0.0	0.0	1.0	-
3	120	0.0	1.0	0.0	-
4	125	0.25	0.25	0.0	-

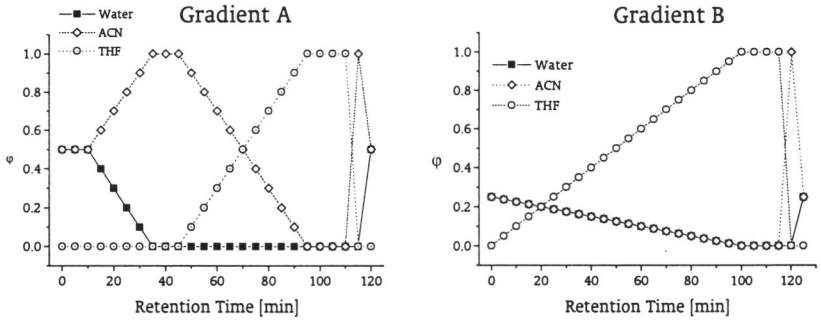


Figure 4-3, Schematic plots of the gradient A (water, ACN, THF) and B (water/ACN, THF) described in Table 4-2 and Table 4-3.

The equipment used for NP-GPEC was a Varian 9010 Solvent Delivery System, Separations UV 785A detector (275nm), a Polymer Labs PL-EMD 960 evaporative light scattering (ELS) detector (N_2-flow 5.0ml/min, evaporation temperature of 70°C), and a Separations Basic Marathon Autosampler with a fixed loop of 50μl. The solvents used were dichloro methane (DCM, HPLC grade, Merck), THF (HPLC grade, unstabilized, Merck) and 1-propanol (1-P, UV quality, Merck). The applied gradient was Gradient C (see Table 4-4). The column, successfully

applied for the NP-GPEC experiments, was an Alltech Si-diol column (100Å, 300x3.6mm). The established flow was 0.5 ml/min, and the concentration used for NP-GPEC was 10 mg/ml and the injection volume 50μl. The applied column temperature was 30°C.

Table 4-4, Gradient C used for NP-GPEC experiments.

Step	Time [min]	DCM %	THF %	1-P %
	Initial	100	0	0
1	5	99	1	0
2	10	99	1	0
3	15	0	95	5
4	20	0	100	0
5	30	0	100	0
6	35	100	0	0

The working principle of the evaporative light scattering (ELS) detector is as follows. The eluent with the sample is nebulized and heated. The eluent will evaporate and a polymer sol is formed. The analyte will then pass a light bream, and scattering of the light by the sol is detected. The detector can easily be used as a qualitative detector. The quantification with an ELS detector is still a point of discussion, especially the non-linear relation between the signal response and the concentration of the analyte. Additionally, depending on the nebulizing and evaporating technique of the ELS detector the signal depends on the molar mass, chemical composition, and eluent composition. However, the quantification with modern ELS detectors has improved over the years [17].

4.2.4 Electrospray-Ionization Mass Spectrometry

The ESI-MS experiments were performed on a Micromass (Altrincham, UK) model VG/Platform mass spectrometer, equipped with an API source, a coaxial probe for pneumatically-assisted ESI combined with a flow of sheath liquid. The drying and nebulizing gas was nitrogen. Negative ESI-MS spectra were acquired from 250 to 2000 g/mol at a scanning rate of 3 seconds per scan. The ESI-MS was calibrated in the positive-ion ESI mode using a mixture of polyethylene glycol-ammonium adducts. The source temperature was set at 85°C and the cone voltage was set (in both ionization modes) at 120 V. For more details about the conditions and the equipment of the ESI-MS experiments see reference [18].

4.2.5 Matrix-Assisted-Laser-Desorption-Ionization Mass Spectroscopy

The MALDI experiments were performed on a Bruker (Bremen, Germany) model Biflex MALDI time-of-flight mass spectrometer equipped with a 337 nm nitrogen laser, a delayed-extraction Scout ion source, a sensitive high-dynamic range detector (Himass) in the linear mode, a high resolution MCP detector in reflectron mode, and a 1 GHz digitizer. The system

was calibrated by extrapolated external multipoint calibration, based on polystyrene 7500 (g/mol). The scan range was in linear mode 1-100 kg/mol and in reflector mode 1000-8000 g/mol. The matrix used for the ionization is indoleacrylic acid (IAA) (20 mg/ml THF). For more details about the conditions and the equipment of the MALDI-MS experiments see reference [19].

4.3 Results and Discussion

During the polymerization reaction of a di-alcohol monomer (D) and a di-acid monomer (A) four different types of oligomers with the same degree of polymerization or number of repeating units (p) can be formed. The repeating unit of the oligomers is defined as AD. Oligomers with two acid end groups $(AD)_p$-A, with one acid and one hydroxy end group $(AD)_p$, and with two hydroxy end groups D-$(AD)_p$ can be formed. In addition cyclic oligomers can be formed $(AD)_p^{cyclic}$. The cyclic oligomers are thought to greatly affect the properties of the polyester. When the polyester is further processed, these oligomers will not take part in reactions involving functional end groups, *e.g.* cross-linking.

The repeating unit of the polyester is the combination of the two monomers A and D. In Table 4-5, all possible oligomers with degree of polymerization (p) from 0 to 3 are shown. The molar mass of each unit is calculated from the equations shown at the bottom of Table 4-5 (at degree of polymerization p). IA and TA are isomers and, therefore, have the same molar mass (166 g/mol). NPG has a molar mass of 104 g/mol.

Table 4-5, Oligomeric series of polyesters formed from a di-acid A and a di-alcohol D. The oligomers will have different end groups and different degrees of polymerization (p).

Degree of Polymerization	Formula	End group	Molar Mass [g/mol]
0	A	A-A	166
	D	D-D	104
1	(AD)	A-D	252
	(AD)-A	A-A	400
	D-(AD)	D-D	338
2	$(AD)_2$	A-D	487
	$(AD)_2$-A	A-A	635
	D-$(AD)_2$	D-D	573
	$(AD)_2^{cyclic}$	Cyclic	468
3	$(AD)_3$	A-D	721
	$(AD)_3$-A	A-A	869
	D-$(AD)_3$	D-D	807
	$(AD)_3^{cyclic}$	Cyclic	702
P	$(AD)_p$	A-D	$(M_A+M_B-M_{water})*p-M_{water}*(p-1)$
	$(AD)_p$-A	A-A	$(M_A+M_B-M_{water})*p+M_A-M_{water}*p$
	D-$(AD)_p$	D-D	$(M_A+M_B-M_{water})*p+M_B-M_{water}*p$
	$(AD)_p^{cyclic}$	Cyclic	$(M_A+M_B-M_{water})*p-M_{water}*p$

Polycondensation implies the formation of a low molar mass side product (water in this case). Consequently, the molar masses in Table 4-5 are calculated by the addition of the molar masses of the acid/alcohol (number of repeating units) and the subtraction of the molar mass of water. Depending on the type of end group, a di-acid monomer (A) or a di-alcohol monomer (D) is added. The statistical end group distribution of di-acid/di-ol polyesterfication are shown in Table 4-6. The cyclic oligomers are not included since the kinetics of the cyclization reaction are not known. Probably the cyclic oligomers are formed by intermolecular transesterfication ('backbiting') of the polyester chains [23].

Table 4-6, Mol fraction of the different end groups based on the probabilities with mol fraction of NPG of 0.47.

Oligomers	Calculation	Mol fraction
$D\text{-}(AD)_p$	0.47*0.47	0.22
$(AD)_p$	2*0.47*0.53	0.50
$(AD)_p\text{-}A$	0.53*0.53	0.28

The numbers are based on equal reactivities of the different end groups. Whenever the reactivities of the acid end group and the alcohol end group differ, the end group distribution calculated in Table 4-6 would not longer be correct. The mol fraction of NPG used during the synthesis is 0.47.

The oligomers, described in Table 4-5, are the oligomers to be found with MS. MS will be used to determine the molar mass of each oligomer. From the information on the molar mass, the degree of polymerization (p) and the functionality of each oligomer can be determined. With the peak position in the GPEC separation and Ultraviolet (UV) spectroscopy the ratio IA/TA of each oligomer can be determined. The most commonly used detector in HPLC is the UV absorbance detector. When the separated components have UV extinction, the components can be detected by a UV absorbance detector. The UV spectra of the homopolyesters IA and TA in hexafluoro iso-propanol (HFIP) are different (see Figure 4-4). NPG cannot be detected, since it does not have any UV extinction.

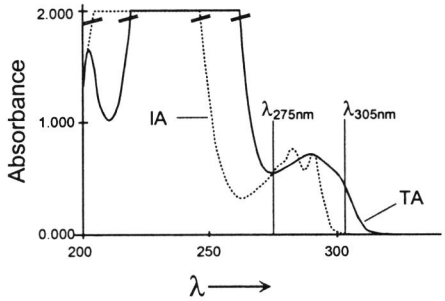


Figure 4-4, Ultraviolet spectra of homopolymers of neopentyl glycol (NPG) with isophthalic acid (IA) (···) and NPG with terephthalic acid (TA) (—) in hexafluoro iso-propanol with (0.02M KTFA).

The homopolymer TA (sample 15) has a UV extinction at λ_{305nm}, homopolymer IA (sample 14) has no UV extinction at λ_{305nm}. By comparing the UV signals at different wavelengths (λ_{275nm} and λ_{305nm}), the fraction TA can be obtained. The extinction coefficients at λ_{275nm} are equal, therefore λ_{275nm} can be used to compare the signals of the homopolymers IA and TA. The spectra obtained in THF are similar to those in HFIP shown in Figure 4-4.

4.3.1 Size Exclusion Chromatography

The MMD determination of polyesters by SEC in HFIP has already been published [20]. The UV (λ=275nm) SEC chromatograms of the homopolyester samples are shown in Figure 4-5. The UV signals are equal, except for a peak at approximately 44 minutes. The SEC data of PET show a similar peak. It is known that polyesters contain cyclic oligomers [21,22]. The peak in the PET SEC chromatogram was identified as the cyclic trimer [23,24]. The peak in the SEC chromatogram of 15 (TA polyester) can also be a cyclic oligomer. The UV chromatogram of 14 (IA polyester) does not contain such as peak. The results of the molar mass distribution determinations obtained with the conventional calibration method (based solely on the concentration detectors and on equivalent polystyrene molar masses) and with the universal calibration method (based on the viscosity signal and a polystyrene universal calibration curve) are shown in Table 4-7.

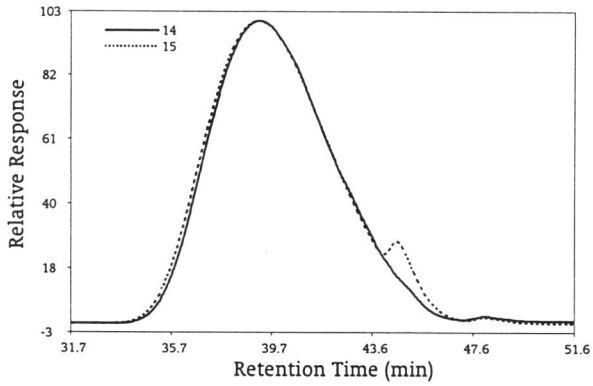


Figure 4-5, UV (λ=275nm) SEC chromatograms of samples 14 (IA polyester, –) and 15 (TA polyester···). The peak around 44 minutes is probably a cyclic oligomer.

Table 4-7, Results of the molar mass determinations by SEC in HFIP. The molar masses are determined by the conventional method (CON) and universal calibration (UC). The Mark-Houwink parameters (log K_η and a) are calculated with the UC method are also shown.

		14 (IA)	15 (TA)
CON	$\overline{M}_n$	7,560	8,490
	$\overline{M}_w$	13,700	14,900
UC	$\overline{M}_n$	2,220	2,260
	$\overline{M}_w$	4,280	4,360
	a	0.67	0.68
	log K_η	-2.80	-2.78

The average molar masses ($\overline{M}_n$ and $\overline{M}_w$) obtained with the conventional calibration method are about three times as high as obtained with the universal calibration method. These differences can be explained by the *Mark-Houwink* relation (see *Chapter* 3). The *Mark-Houwink* parameters for the polystyrene standards in THF (a_{PS}=0.72 and log K_{PS}=-4.07) differ significantly from the *Mark-Houwink* parameters for the homopolyesters of NPG/IA and NPG/TA (see Table 4-7). The difference in *Mark-Houwink* parameters between the homopolyesters, are marginal, which means that the chemical difference between IA and TA probably does not have a significant effect on the hydrodynamic volume.

4.3.2 Reversed-Phase-Gradient Polymer Elution Chromatography

Reproducibility problems occurred during the separation of the homopolyesters by RP-GPEC due to the semi-crystallinity of the homopolyesters. The specific crystallinity problems and the attempt to solve these are addressed in Chapter 5, and will not be further discussed in this chapter.

Reversed phase GPEC is applied to characterize the homopolyesters. Two different gradients, A and B (see Table 4-2 and Table 4-3) were applied with the following conditions: a Si-C18 column, column temperature of 55 °C, and HFIP as sample solvent. Gradient A was a combination of linear binary gradients and isocratic steps, from the initial conditions (50/50 water/ACN) to the final conditions (THF 100%). Gradient B was one single linear ternary gradient from the initial conditions to the final conditions. The initial conditions and the end conditions were equal for both gradients.

Gradient A, water/ACN→ACN→THF (see Table 4-2 and Figure 4-3) was used to achieve high resolution and baseline separation of the oligomers and was obtained by trial and error. Water/ACN (0.5:0.5) was used as the initial eluent (poor solvent and low eluent strength). ACN was used because of its good selectivity for the separation of isomeric compounds [25]. THF, being a good solvent and eluent for many polymers, was used as the final solvent. Beside gradient A also gradient B, water/ACN→THF (see Table 4-3 and Figure 4-3), was used. The three solvents were used simultaneously in gradient B, in contrast with gradient A, where

only combinations of two solvents were used. Gradient A is a combination of several binary gradients and isocratic steps (*see* Table 4-1, *Gradient A)*. However, when different gradient steps are used in combination with isocratic steps, multi-modal distributions can be obtained.

The chromatogram of sample 15 obtained with gradient A is shown in Figure 4-6 and can be divided into three parts.

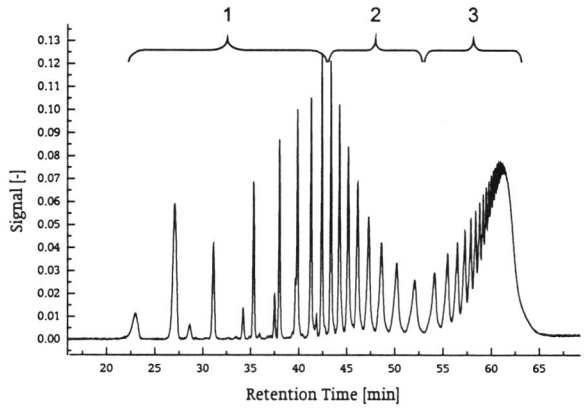


Figure 4-6, GPEC UV chromatogram of 15, homopolymer (NPG/TA), using gradient A (see Table 4-2), Symmetry SI-C18, flow=0.5ml/min, column temperature=55°C.

ESI-MS was applied to identify the peaks in the RP-GPEC chromatogram. Oligomers with different degree of polymerization and different end groups were found. As an example, the ESI mass spectra of the di-acid oligomers with different degrees of polymerization $(AD)_p$-A are shown in Figure 4-7.

In the ESI-MS spectra the di-acid oligomers ($^-$OOC~COOH) are shown. The RP-GPEC peaks were identified successfully by ESI-MS up to oligomer $(AD)_5$-A with a molar mass of 1336 g/mol. Above molar mass 1336 g/mol the oligomers could not be detected any more by ESI-MS. The reason for this is unclear [26]. To interpret the chromatograms, however, the identification of the oligomers up to p=5 was sufficient. The identification is only performed for the separations obtained with gradient A.

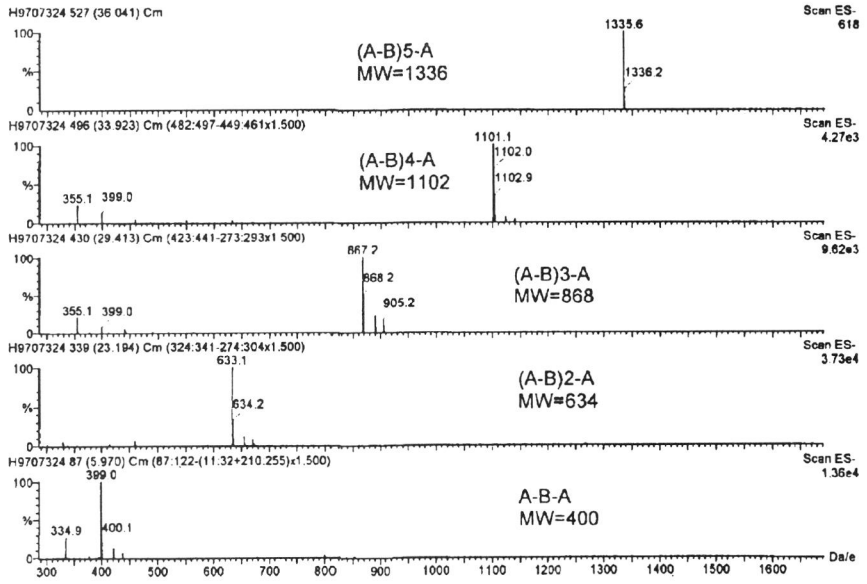


Figure 4-7, The ESI mass spectra (negative ion mode) of the di-acid oligomers with different degree of polymerization.

In order to compare the chromatogram with the gradient, the delay time between pump and the detector was determined at 7.90 minutes. Each part in the chromatogram elutes in a different step of gradient A. Part 1 elutes in the gradient step water/ACN→ACN (from 18 to 43 minutes), part 2 in the isocratic step of ACN (from 43 to 53 minutes) and part 3 elutes in the gradient step ACN→THF (from 53 to 103 minutes).

The effect of the different steps is illustrated in Figure 4-8. The degree of polymerization of the oligomers with two acid end groups $(AD)_p$-A is plotted *versus* the retention time of each peak identified as an oligomer $(AD)_p$-A. The shape of the curve in Figure 4-8 is the result of the different dependencies of the individual steps in the gradients.

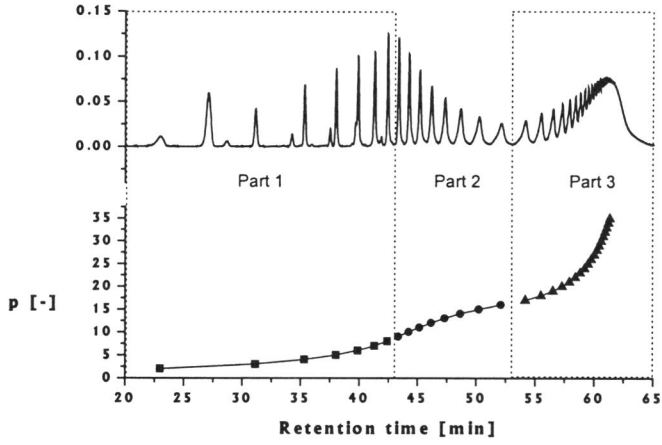


Figure 4-8, The degree of polymerization (p) of oligomers with di-acid end groups of 15 versus retention time for the different parts: part 1 water/ACN→ACN (–■–), part 2 ACN (–●–) and part 3 ACN→THF (–▲–).

The low molar mass part of the polyester (*part 1*, p<8) elutes in the H_2O/ACN gradient (see Figure 4-8, *part 1, water/ACN→ACN*, –■–). The oligomers show a molar mass dependence as found in other RP-GPEC separations (see Figure 2-10, *Chapter 2*). part 2 the oligomers (8<p<17) elute in the isocratic ACN step (see Figure 4-8, *part 2, ACN*, –●–). The elution behavior is a classical example of the isocratic molar mass dependence as can be seen in Figure 2-1 (Chapter 2). The high molar mass part (p>16) elutes in the ACN/THF gradient (see Figure 4-8, *right area ACN→THF*, –▲–) and this again shows the RP-GPEC molar mass dependency.

The labeled chromatogram of sample 15 is shown in Figure 4-9. Oligomers with different degrees of polymerization (p) were identified in the chromatogram. Besides oligomers with different p, also oligomers with different end groups were found: $(AD)_p$, $(AD)_p$-A, $(AD)_p^{cyclic}$ (see Figure 4-9). The oligomers up to p=5 were identified by ESI-MS. After p=5 the peaks could easily be labeled without identification by MS, but via recognition of the pattern.

The cyclic dimer $(AD)_2^{cyclic}$ was present in a significant concentration in sample 15 (TA) and the previously mentioned peak found in the SEC experiments is likely to be the same cyclic dimer. The oligomers D-$(AD)_p$ were not found in the chromatogram of sample 15. According to the statistic amount shown in Table 4-6, the probability of D-$(AD)_p$ in sample 15 is 0.22. However, this probability was calculated with the condition that the reactivities of the end groups (A and D) and the monomers are equal. Since the D-$(AD)_p$ oligomers are not found, the reactivities of the different monomers (A or D) with the different end groups (~A or ~D) must be different. Another possibility is that at the end of the reaction only acid monomer was left compared to NPG resulting in more $(AD)_p$-A oligomers.

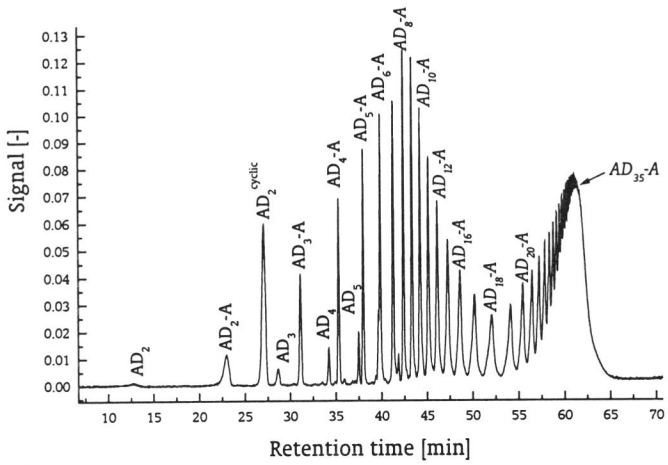


Figure 4-9, Labeled GPEC UV chromatogram of 15, homopolymer (NPG/TA), using gradient A (see Table 4-2), Symmetry SI-C18, flow=0.5ml/min, column temperature=55°C. The labeling of the peaks was performed with on-line ESI-MS.

Within a cluster of oligomers with the same p, the oligomers with different end groups could be assigned. The oligomers with different end group eluted in the following order: first the $(AD)_p$ oligomers eluted, then the $(AD)_p$-A oligomers and at the end the cyclic oligomer $(AD)_p^{cyclic}$ eluted. This sequence can partly be explained by differences in non-polar interactions. The oligomers $(AD)_p$ and oligomers $(AD)_p^{cyclic}$ can be compared directly, as they both consist of two acid monomers and two alcohol monomers, the only difference being the end groups. Since the oligomers $(AD)_p$ are more polar than the cyclic oligomers $(AD)_p^{cyclic}$, they elute before the cyclic oligomers. This is in agreement with studies found in literature [11]. The oligomers $(AD)_p$-A show different behavior. The oligomers $(AD)_p$-A should elute before the oligomers $(AD)_p$, since two acid groups are more polar than one hydroxyl group and one acid group. However, the $(AD)_p$-A oligomers have an additional acid monomer compared to the $(AD)_p$ oligomers. This additional acid monomer gives the oligomer an extra non-polar group, which will result in an extra retention (increase in molar mass). This will cause the oligomer to elute in between the oligomers $(AD)_p$ and $(AD)_p^{cyclic}$. The retention sequence might have also been influenced by the addition of HAc. The separation on end group disappeared after oligomers with p=6, the oligomers $(AD)_p$-A and $(AD)_p$ co-eluted.

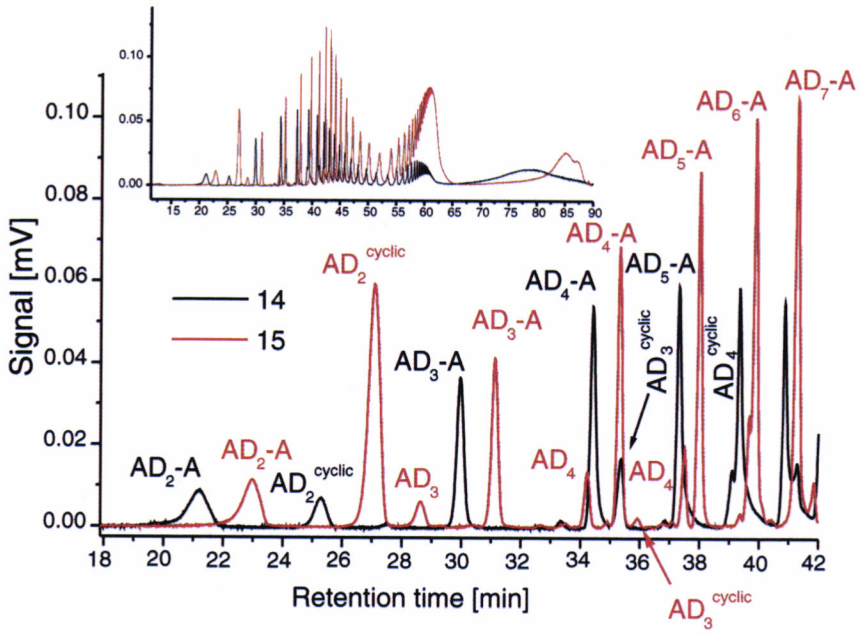


Figure 4-10, Overlay of the chromatograms and the identification of the peaks of the homopolyesters 14 (black line) and 15 (red line) obtained with gradient A. With at the top the full chromatogram and at the bottom the detailed overlay of the labeled chromatograms.

In Figure 4-10 an overlay of the chromatograms of the two homopolymers 14 and 15 obtained with Gradient A is shown. The full overlay of the chromatograms is shown at the top of the figure and the detailed overlay at the bottom. The two chromatograms at the top show crystallinity problems. The homopolyesters of NPG/IA and NPG/TA form a crystalline phase during precipitation step, which results in a poor reproducibility of the chromatogram, especially at the end of the chromatogram (around 80 minutes). The problems concerning crystallinity will be further discussed in *Chapter 5*.

The two chromatograms show many differences. First of all, all the oligomers AD_p-A, AD_p and $(AD)_p^{cyclic}$ with equal p were separated according to the nature of the acids. The oligomers containing IA eluted before the oligomers containing TA, irrespective of their end groups. Consequently, the TA group shows more retention. The separation on the isomer content IA/TA resembles the separation of polystyrene isomers described by *Lewis et al.* [25], who also used gradients with ACN as the poor solvent. Consequently, this separation on chemical composition can be used to separate copolyesters of IA and TA.

The cyclic oligomers $(AD)_p^{cyclic}$ are more pronounced in sample 14 (IA polyester) than in sample 15 (TA polyester). More $(AD)_p^{cyclic}$ oligomers are found (up to $(AD)_5^{cyclic}$) in sample 14,

however, the cyclic oligomer $(AD)_2^{cyclic}$ is prominently present in sample 15 and not in sample 14. With TA the formation of the dimer was preferential to the formation of the other cyclic oligomers, since the other cyclic oligomers were found only in small amounts. This can be explained by the fact that the formation of cyclic oligomers with higher p is less probable than that of the cyclic dimer. In addition, the formation of the cyclic oligomer $(AD)_1^{cyclic}$ is unlikely because of steric hindrance. In sample 14 a distribution of cyclic oligomers was found, and no cyclic oligomer was preferentially present. The cyclic oligomers are probably formed by 'backbiting' of the polyester chains [23].

In Figure 4-11 the degree of polymerization (p) of the di-acid oligomers AD_p-A of the homopolyesters 14 (IA) and 15 (TA) is plotted versus the retention time.

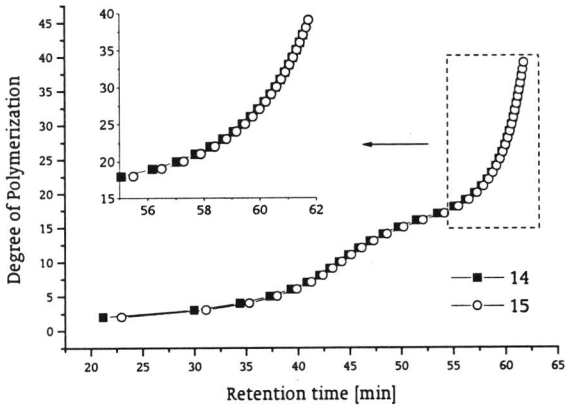


Figure 4-11, Plot of degree of polymerization versus retention time [min] of peaks of the di-acid oligomers $(AD)_p$-A of homopolyesters 14 (IA,—■—) and 15 (TA,···o···) using the binary gradient A.

When gradient B is applied, a different chromatogram is obtained. The chromatogram shows one continuous distribution (see Figure 4-13), unlike the chromatogram obtained with gradient A, and shows also a higher resolution. In Figure 4-12 the comparison is shown of RP-GPEC UV chromatograms of homopolyester 15 (TA) obtained by applying the binary gradient A and the ternary gradient B.

The chromatograms of the homopolymers obtained by the gradients A and B are comparable, especially during the first 40 minutes. With the identification of the chromatogram obtained with the binary gradient (gradient A), the chromatogram obtained with the ternary gradient can easily be interpreted.

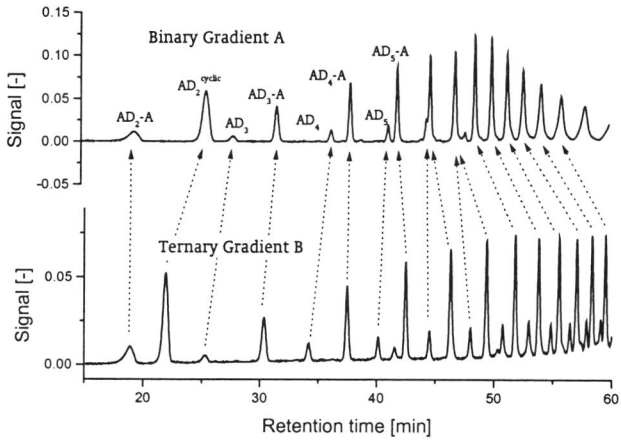


Figure 4-12, Comparison of RP-GPEC UV chromatograms of homopolyester 15 (TA) obtained by binary gradient A and ternary gradient B.

The similarities are obvious. All the oligomers present in the chromatogram obtained with gradient A can also be seen in the chromatogram obtained with the ternary gradient B. Additionally, more oligomers appeared with gradient B. The additional oligomers are likely to be the oligomers $(AD)_p$ and $(AD)_p^{cyclic}$, although the identity was not verified. Thus, oligomers with different end groups can effectively be separated by using the more resolving gradient B. The labeled chromatogram of sample 15 obtained with ternary gradient B is shown in Figure 4-13.

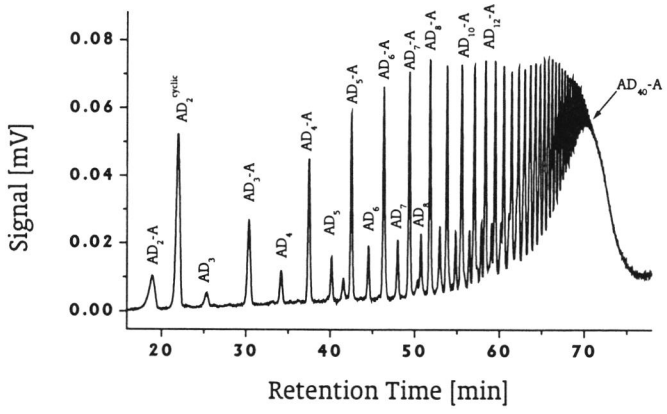


Figure 4-13, Labeled UV RP-GPEC chromatogram of sample 15 obtained with the ternary gradient B.

In Figure 4-14 the degree of polymerization (p) of the di-acid oligomers *n* of the homopolyesters 14 and 15 is plotted versus the peak retention time (ternary gradient B).

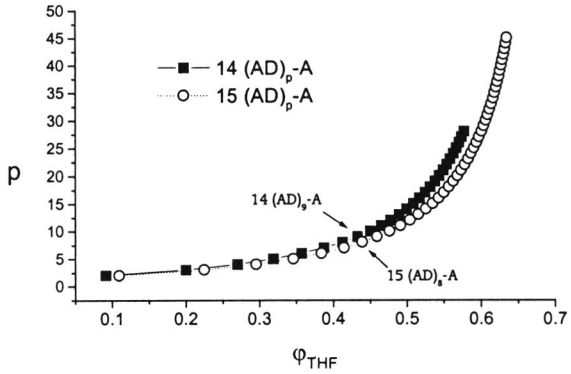


Figure 4-14, Plot of degree of polymerization versus retention time [min] of peaks of the di-acid oligomers {n} of homopolyesters 14 (–■–) and 15 (···○···) using the ternary gradient B.

Figure 4-14 shows that the retention sequence of the oligomers $(AD)_p$-A of the homopolyesters changes around p=8. The oligomers $(AD)_p$-A of sample 14 (IA) passed the oligomers $(AD)_{p-1}$-A of sample 15 (TA) with lower p (see Figure 4-14, 14 $(AD)_9$-A overtook (in φ_{THF}) 15 $(AD)_8$-A). The phenomena mentioned above resulted in a complex chromatogram. When gradient A was used, the oligomers $(AD)_p$-A of sample 14 (TA) did not overtake the oligomers $(AD)_{p-1}$-A of sample 15 (TA). With gradient B, the oligomers $(AD)_p$ and $(AD)_p$-A of sample 15 were separated until p=13.

The peak areas of each oligomer were calculated. The fractions of the oligomers $(AD)_p$-A of sample 15 (based on the peak areas: excluding the cyclic oligomers) from p=3 to p=13 are shown in Table 4-8 (the fraction $(AD)_p$-A oligomers (area $(AD)_p$-A divided by the total area $(AD)_p$+$(AD)_p$-A) for different p). In neither of the samples D-$(AD)_p$ oligomers were found, which implies, as mentioned before, that the acid end groups are preferential.

Since the response of the UV signal is based on the amount of acid monomers (NPG cannot be detected by UV) the areas of the oligomers $(AD)_p$ and $(AD)_p$-A cannot be compared one to one. The $(AD)_p$-A oligomers have an additional A in comparison with the $(AD)_p$ oligomers in the same cluster, which will give an additional response, therefore the integrated areas were corrected. The correction factor decreases with increasing degree of polymerization (see Table 4-8).

Table 4-8, The fraction oligomer AD_p-A based on the corrected integrated areas of the peaks of the oligomers with equal degree of polymerization p (from 2 to 13) of sample 15 (TA). The areas of the cyclic oligomers AD_p^{cyclic} are excluded from the total area. The correction factor for the UV signal is used to correct the areas for the additional acid monomer present in the AD_p-A oligomers.

P	Mol fraction AD_p-A	Correction Factor UV signal	P	Mol fraction AD_p-A	Correction Factor UV signal
2	0.57	0.67	8	0.70	0.89
3	0.63	0.75	9	0.67	0.90
4	0.63	0.80	10	0.75	0.91
5	0.67	0.83	11	0.74	0.92
6	0.69	0.86	12	0.76	0.92
7	0.70	0.88	13	0.79	0.93

The relation of the mol fraction AD_p-A ($\varphi_{AD\text{-}A}$) versus the degree of polymerization (p) is shown in Figure 4-15.

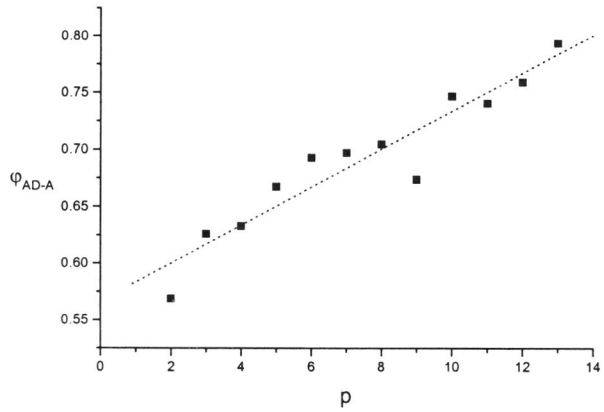


Figure 4-15, The dependency of the fraction oligomer ($\varphi_{AD\text{-}A}$) on the degree of polymerization (p).

Figure 4-15 clearly shows that $\varphi_{AD\text{-}A}$ is dependent on the degree of polymerization of the oligomers. The fact that the end group can differ with increasing degree of polymerization was recently established with NP-GPEC by *Philipsen* [11]. However, the dependency has never been shown with RP-GPEC so far. The dependency of the $\varphi_{AD\text{-}A}$ on p appears to be approximately linear. However, only a small range of p is plotted, therefore the dependency might also be curved. Nevertheless, the dependency of p on $\varphi_{AD\text{-}A}$ shows unmistakable that the reactivities of the end groups/monomers are not equal. This dissimilarity in reactivities was also noticed when the AD_p-A fractions obtained with GPEC were compared with the statistically calculated fractions. For the samples 14 (IA) and 15 (TA) the statistically expected $(AD)_p$-A fraction is 0.50 (see Table 4-6). The fractions $\varphi_{AD\text{-}A}$ obtained by RP-GPEC are all higher

than the fraction based on statistical considerations. The oligomers $(AD)_p$ were not found in sample 14 (IA), except for some low p oligomers (p=3,4), consequently the fraction $\varphi_{AD\text{-}A}$ for sample 14 is approximately equal to 1. As mentioned before, the statistical data are based on the assumption that the reactivities of the end groups for the esterfication are equal. The lower fraction obtained by GPEC compared to the statistically expected fractions can be explained by the differences in the reactivities of the end groups. The reactivities of the hydroxy end groups of the oligomers can be much higher than the reactivities of the acid end groups. However, a more likely explanation is that the reactivities of the acid monomers (IA and TA) are much higher than the reactivity of the glycol monomer (NPG). Also, the reactivities of the two acid monomers are thought to be different. IA is more reactive than TA, since the mol fraction of $(AD)_p$-A of sample 14 (IA) is higher than the mole fraction of $(AD)_p$-A of sample 15 (TA).

4.3.3 Gradient Elution Mechanism of RP-GPEC

The empirical relation for the cloud point (volume fraction of solvent, φ_s) introduced by Glöckner [27] will be used to discuss the RP-GPEC separation mechanism. This equation (see also Equation 2-8 described in *Chapter 2)* is originally used for binary solvent systems. However, the empirical relation can also be used for ternary solvent systems.

$$\varphi_s = C_1 + \frac{C_2}{\sqrt{M}}$$

Equation 4-1, The empirical relation of the cloud point (in volume fraction solvent) versus the molar mass (M), where, C_1 and C_2 are constants, and M is the molar mass.

This equation can also be used to describe the elution composition of RP-GPEC versus $M^{-½}$ [28]. Since the binary gradient shows discontinuities in the distribution, only the experiments obtained with ternary gradient will be discussed with respect to Equation 4-1. Philipsen [11] studied the relation of Equation 4-1 for different types of oligomers. For the different types of polymers (polystyrene, polyesters, and polydimethyl siloxane) curves with different shapes and slopes were obtained. The slope and intercept depended on the experimental conditions (temperature, solvent/non-solvent).

The elution compositions (φ_{THF}) of the oligomers AD_p-A of the samples 14 (IA) and 15 (TA) are plotted versus $M^{-½}$ (see Figure 4-16). The plots show linear curves (with some deviation at higher molar mass) described by Equation 4-1.

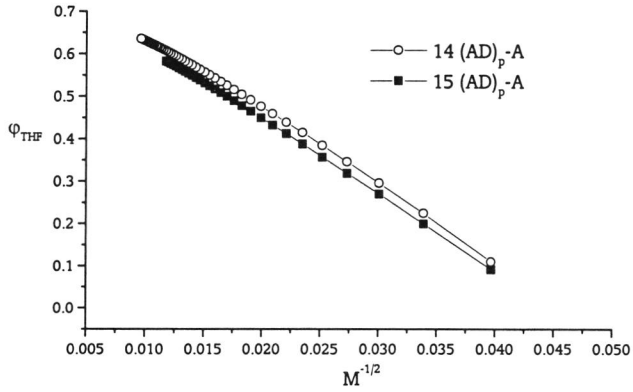


Figure 4-16, Plot of elution composition (φ_{THF}) versus $M^{-1/2}$ of the oligomers AD_p-A of the samples 14 (—○—) and 15 (—■—).

It is remarkable that the slopes of the curves for $(AD)_p$-A oligomers of the different homopolymers are similar. The only difference is found in the intercept of the curves. Since the only difference is the (geometric) structure of the acid monomer, IA is 1,3-benzene dicarboxylic acid and TA is 1,4-benzene dicarboxylic acid, the difference in configuration is thought to be the cause of the difference between the intercepts. This implies that Equation 4-1 is applicable to describe the separation of the polyesters and that the parameters C_1 and C_2 described in Equation 4-1 could be related to a physical effect.

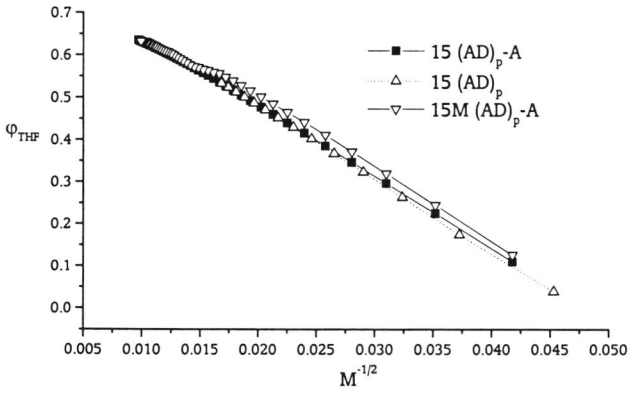


Figure 4-17, Plot of φ_{THF} versus $M^{-1/2}$ for the di-acid oligomers $(AD)_p$-A of sample 15 (–■–), di-methyl ester oligomers $(AD)_p$-A of sample 15m (alkylated) (–▽–), and mono-acid oligomers $(AD)_p$ of sample 15 (–△–).

Sample 15M is the alkylated product of sample 15. The acid end groups of sample 15 were alkylated and changed into methyl ester groups by diazomethane [16]. In Figure 4-17 the plots of φ_{THF} versus $M^{-½}$ for samples 15 and 15M of AD_p-A are shown. Also, the data of the mono- acid oligomers $(AD)_p$ of sample 15 are included.

The retention of the alkylated sample (15M) shows an irregular behavior. At intermediate molar mass, the curve shifts and continues with a similar slope. This phenomenon is thought to be the disappearance of the end group contribution on the separation. However, another explanation could be that the alkylation reaction was not completed and that the higher molar masses still contain acid end groups (not checked). Nevertheless, besides these small deviations, the slopes of the curves are similar.

The retention behavior of the polyesters of NPG with IA or TA as well as the retention behavior of the polyesters described by *Philipsen* [11] could be described by Equation 4-1.

4.3.4 Reversed Phase Chromatogram with Hexafluoro Isopropanol

Hexafluoro isopropanol (HFIP) can be applied as a good solvent for the GPEC separation of polyesters. The application of HFIP in gradient elution HPLC for poly(ethylene terephthalate) has already been published by Van de Maeden *et al.* [1]. HFIP is a good solvent for PET. It can dissolve PET at ambient temperatures (see chapter 3). The chromatogram of sample 15 obtained with binary gradient A with HFIP instead of THF can be seen in Figure 4-18.

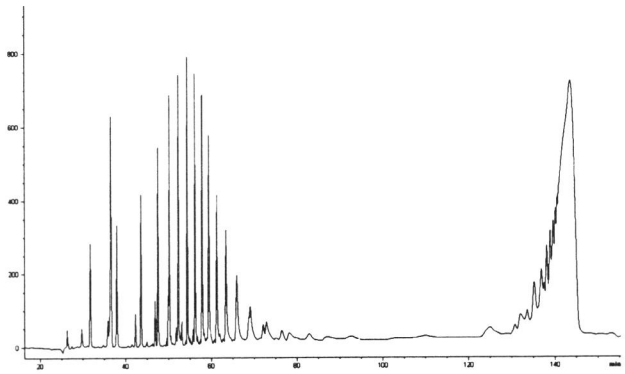


Figure 4-18, RP-GPEC chromatogram of sample 15 obtained with the binary gradient A with HFIP (gradient steepness 1% per minute). The detection was performed with the HP 1010 DAD (λ275nm).

The chromatograms obtained with THF and HFIP are comparable (see **Error! Reference source not found.** and Figure 4-18), and the bi-modality (discussed earlier in this chapter) is also present. The results with HFIP show that HFIP is a poorer eluent compared to THF, since more HFIP is necessary to elute the highest molar mass polyesters than THF. The eluent strength of a solvent can be described by the Hansen solubility parameters [29]. However, the Hansen solubility parameter of HFIP has never been determined. The explanation for the

different eluent properties of THF and HFIP is probably the difference in polarity. HFIP is more polar than THF and therefore in RP-GPEC a poorer eluent. Apart from the poorer eluent properties, the first 40 minutes of the HFIP gradient were similar to the THF gradient, since the same conditions were used. Since no additional information was obtained by using HFIP as eluent, the gradient A with THF was used to characterize the copolyesters. The characterization of the copolyesters is described in *Chapter 6*.

4.3.5 Separation According to Functionality by NP-GPEC

MALDI-TOF MS has been applied to identify the peaks obtained with NP-GPEC. The analysis with MALDI-TOF MS of the polyester used for this study has already been optimized by Nielen *et al.* [19]. The authors describe the analysis of the polyester sample 15, as well as other types of polymers. The polymers are separated by SEC and fractionated according to hydrodynamic volume. The ideal combination is a separation technique coupled with MALDI-TOF-MS. MALDI-TOF-MS can determine the absolute molar mass of the SEC fractions.

The application of NP-GPEC to different polyesters was already described by Philipsen [11]. In NP-GPEC the polymer molecules dissolve in the initial conditions (100 %DCM, see Table 4-4). Consequently, the molecules will be solely separated by polar column interactions. For NP-GPEC a polar column was used, Silica modified with diol. A gradient from 100% DCM to 100% THF was applied. 1-propanol was used to desorb the $(AD)_p$-A oligomers, since they did not elute when pure THF was used. Polar interactions can be very strong, or even irreversible. In some cases, very polar eluents have to be used in order to desorb the molecules.

The NP-GPEC gradients were performed with two different detectors, a UV detector and an evaporative light scattering (ELS) detector. As indicated before, the ELS detector can detect polymers without chromophores. However the response of the detector is dependent on the eluent composition, which makes the quantification difficult [30]. The NP-GPEC separations were performed for both homopolymers. The UV and ELSD chromatograms of sample 15 are shown in Figure 4-19.

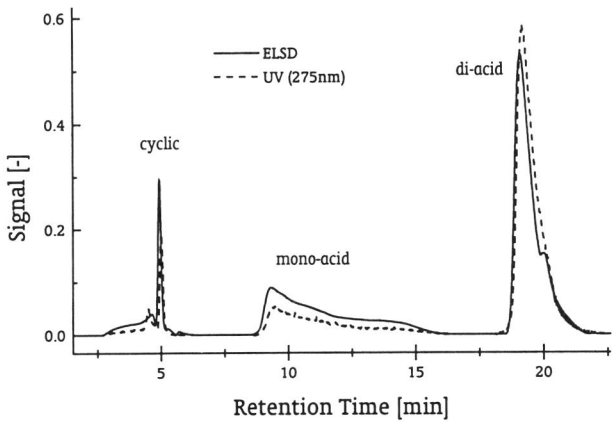


Figure 4-19, NP-GPEC chromatograms of the ELSD signal (—) and the λ_{275} (···) UV signal of sample 15 (TA) obtained with gradient C.

The chromatogram can be divided into three fractions. The first fraction was identified by MALDI-TOF MS as the cyclic oligomers $(AD)_p^{cyclic}$. However, in this fraction also oligomers with acid end groups ($(AD)_p$ and $(AD)_p$-A) were found. This is due to breakthrough of the sample (see *Chapter 2*). The sample solvent used was THF, and the initial conditions were DCM. The THF elutes at the beginning of the chromatogram and part of the oligomers will elute together with the THF. Fraction 2 was identified as the mono-acid oligomers. The peak was very broad, and at the start of the peak a very small amount of diol oligomers D-AD_p was found. The third peak was identified as the di-acid peak $(AD)_p$-A. Part of the MALDI-TOF spectrum of fraction 3 is shown in Figure 4-20.

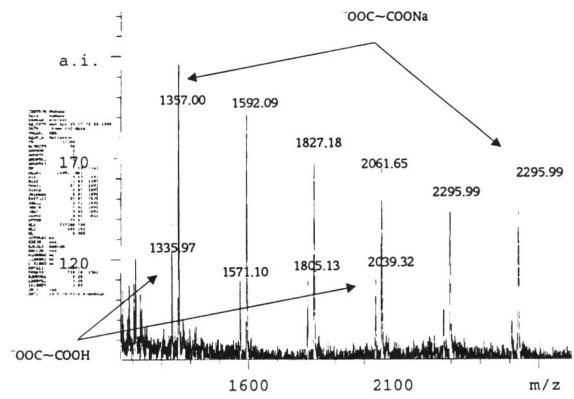


Figure 4-20, MALDI-TOF spectrum of the di-acid oligomer fraction.

In the MALDI-TOF spectrum de-protonated oligomers $(AD)_p$-A (p=5 to p-10) can be noticed. Also adducts with Na^+ were found

Recently, Nielen *et al.* have applied a gradient similar to gradient C and have analyzed sample 15 with on-line ESI-TOF [31]. The on-line analysis shows that low p mono-acid $(AD)_p$ oligomers co-elute at the beginning of the peak (around 20 minutes) of the di-acid $(AD)_p$-A oligomers. Consequently, the NP-GPEC separation is mainly based on differences in functionality. However, still a molar mass dependency exists, especially for low molar mass mono-acid $(AD)_p$ oligomers. Another problem was the strong adsorption of the acid groups. Although 1-propanol was used to desorb the $(AD)_p$-A oligomers, part of the $(AD)_p$-A oligomers were still adsorbed on the column, and eluted in a subsequent blank run after the sample chromatogram. This makes the quantification of the separation highly questionable.

Sample 14 was also analyzed by NP-GPEC. The chromatograms showed only two peaks, the cyclic oligomers $(AD)_p^{cyclic}$ and the oligomers $(AD)_p$-A. The mono-acid oligomers $(AD)_p$ were not found. This is in agreement with the results form the RP-GPEC experiments described before in this thesis (see Figure 4-10) and MALDI-MS experiments performed in early studies [32].

The peaks of sample 15 obtained with NP-GPEC (UV and ELS) have been integrated and the results are shown in Table 4-9. The ratios of the end groups obtained with NP-GPEC can be compared to the fractions obtained with RP-GPEC (see Table 4-8).

Table 4-9, Relative peak areas of the NP-GPEC and the RP-GPEC separation of sample 15 of oligomers with different end groups.

Sample 15		Fraction			
		AD_p^{cyclic}	D-$(AD)_p$	$(AD)_p$	$(AD)_p$
NP-GPEC	UV	0.11	-	0.29	0.60
	ELS	0.07	-	0.20	0.73
	Statistical	-	0.22	0.50	0.28
RP-GPEC	UV	-	-	0.25 ±0.05	0.75 ±0.05

The fractions found for sample 15 for the different oligomers obtained with NP-GPEC do not agree with the fractions obtained statistically, however, they are comparable to the fractions obtained with RP-GPEC. On the other hand, the RP-GPEC separation provides fractions for each degree of polymerization until p=13 and the NP-GPEC separation only an overall fraction. The fractions obtained with UV detection and NP-GPEC are comparable to the fractions obtained for the oligomers with low degrees of polymerization (see Table 4-8) and the fractions obtained with the ELS detector and NP-GPEC are comparable to the fractions obtained for the oligomers with higher degrees of polymerization (p<13).

Nevertheless, the quantification of the NP-GPEC separations should be interpreted with great care. The response of the ELS detector depends on the eluent composition. Additionally, the earlier mentioned adsorption problem of the acid groups $(AD)_p$-A on the diol column also affects the results.

The NP-GPEC separations were promising. However, the sorption of the di-acid oligomers and the overlap of the oligomers with different end groups makes the application of NP-GPEC still questionable. In addition, NP-GPEC is more sensitive to the purity of the solvent [28], which makes it less robust than RP-GPEC. Probably with a good optimization of the conditions, NP-GPEC can be a very useful technique to separate polymers according to end groups and chemical composition [11].

4.4 CONCLUSIONS

With SEC/viscosity the homopolyesters of NPG/IA and NPG/TA showed similar MMDs and no significant differences were found in the *Mark-Houwink* parameters of the different polyesters.

Reversed phase GPEC and normal phase GPEC were successfully applied to separate the homopolymers. Differences in retention behavior between the homopolymers were found, which offers the possibility to separate according to chemical composition. This will be used for the characterization of copolyesters of NPG, IA and TA that will be discussed in Chapter 6.

Different types of gradients were applied to separate the polyesters: gradient A (water/ACN→ACN→THF) and gradient B (water/ACN→THF). Also HFIP was used as good solvent for the homopolyesters. Due to the lower eluent strength of HFIP, and similarity of the applied gradient, the chromatograms did not contain additional information compared to the two THF gradients. With the THF gradients, separation of the homopolyesters according to chemical composition (IA or TA), molar mass (repeating unit) and end groups (acid end group or hydroxy end group) was achieved. The results showed that the end group distribution varies with the degree of polymerization, and the obtained distribution deviates from the statistically calculated end group distribution. This deviation is thought to arise from a difference in reactivity of the monomers and the hydroxy end groups.

The mechanism of RP-GPEC of polyesters was studied. For this study well-defined polyesters were synthesized and MS was applied to identify the separated oligomers. The separation of the homopolyesters of NPG/IA and NPG/TA was dominated by the molar mass (repeating unit) of the oligomers. Within the peak clusters with a given degree of polymerization the oligomers were separated according to end groups. The oligomers with different end groups were separated according to differences in chemical composition (IA/TA). However, the resolution between the oligomers with different chemical composition was lost after polymerization degrees (p) exceeding 6. The resolution between the oligomers with different end groups was lost above $p=13$, and finally the resolution between oligomers with different p vanished above $p=35$. It was found that the molar mass dependence of the RP-GPEC separation could well be described by an empirical equation introduced by Glöckner [33].

Besides RP-GPEC, also NP-GPEC was applied to separate the homopolyesters according to end groups. Three peaks were found with NP-GPEC. The three fractions are oligomers with

different end groups: cyclic oligomers, acid/hydroxy oligomers and di-acid oligomers. The three different fractions were identified with MALDI-TOF MS. The sorption of the di-acid oligomers was difficult to control. Consequently, additional research has to be done to optimize the NP-GPEC separation.

The combination of mass spectrometry with GPEC proved to be highly effective for the characterization of the polyesters. MALDI-TOF MS and ESI-MS were successfully applied for the identification of the GPEC fractions. On the other hand, GPEC was successfully applied to pre-separate polymer samples for the analysis by MALDI-TOF MS or ESI-MS.

4.5 REFERENCES

1. F.P.B. van der Maeden, M.E.F. Biemond and P.C.G.M. Janssen, *J. Chromatogr.*, **149**, *539-552 (1978).*
2. G. Wick and H. Zeitler, *Angew. Makromol. Chem.*, **112**, *59-94 (1983).*
3. M. Szesztay, ZS. László-Hedvig and F. Tüdös, *J. Appl. Polym. Sci.: Appl. Polym. Symp.*, **48**, *227-232 (1991).*
4. A.M. Robertson, D. Farnan, D. Littlejohn, M. Brown, C.J. Dowle and E. Goodwin, *Anal. Proc.*, **30**, *268-271 (1993).*
5. Š. Podzimek and J. Heršl, J. Appl. Polym. Sci., 53, 1351-1356 (1994).
6. R.P. Krüger, H. Much and G. Schultz, *J. Liq. Chromatogr.*, **17 (14&15)**, *3069-3090 (1994).*
7. H.J.A. Philipsen, B. Klumperman and A.L. German, *J. Chromatogr. A*, **746**, *211-224 (1997).*
8. H.J.A. Philipsen, M. Oestreich, B. Klumperman and A.L. German, *J. Chromatogr. A*, **775**, *157-177 (1997).*
9. H.J.A. Philipsen, M.R. de Cooker, H.A. Claessens, B. Klumperman and A.L. German, *J. Chromatogr. A*, **761**, *147-162 (1997).*
10. K. Rissler, *J. Chromatogr. A*, **786**, *85-98 (1997).*
11. Philipsen H.J.A., "Mechanisms of Gradient Polymer Elution Chromatography and its Application to (Co)Polyesters", Ph.D. thesis, Eindhoven University of Technology, Eindhoven, *1998.*
12. S. Teramachi, A. Hasegawa, Y. Shima, M. Akatsuka and M. Nakajima, *Macromolecules*, **12 (5)**, *992-996 (1979).*
13. S. Teramachi, A. Hasegawa, Y. Shigekuni and S. Natunaga, *Polymer J.*, **21 (10)**, *803-808 (1989).*
14. S. Teramachi, A. Hasegawa and K. Motoyama, *Polymer J.*, **22 (6)**, *489-496 (1990).*
15. P.J.C.H. Cools, F. Maesen, B. Klumperman, A.M. van Herk and A.L. German, *J. Chromatogr. A*, **736**, *125-130 (1996).*
16. Akzo Nobel internal sample.
17. T. Willems, *"Evaporative Light Scattering Detectors"*. Internal report, Eindhoven University of Technology, *1997.*
18. M.W.F. Nielen, *Rapid Commun. Mass Spectron.*, **10**, *1652-1660 (1996).*
19. M.W.F. Nielen and S. Malucha, *Rapid Commun. Mass Spectrom.*, **11**, *1194-1204 (1997).*
20. F.A. Buijtenhuijs and A.M.C. van de Riet, *to be published.*
21. L.H. Peebles, JR., M. W. Huffman and C.T. Ablett, *J. Polym. Sci.: part A-1*, **7**, *479-496 (1969).*
22. G. Wick and H. Zeitler, *Angew. Makromol. Chem.*, **112**, *59-94 (1983).*
23. I. Goodman and B.F. Nesbitt, *J. Polym. Sci.*, **48**, *423-433 (1960).*
24. F.A. Buijtenhuijs, *AKZO NOBEL Internal report.*
25. J.J. Lewis, L.B. Rogers and R.E. Pauls, *J. Chromatogr.*, **264**, *339-356 (1983).*
26. H.J.M van de Ven, *AKZO NOBEL internal report* (1996).
27. G. Glöckner, *Z. Phys. Chem.*, **229**, *98 (1965).*
28. G. Glöckner, *"Gradient HPLC of Copolymers and Chromatographic Cross Fractionation"*, Springer-Verlag Berlin Heidelberg, *1991.*
29. P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen and L. De Galan, *J. Chromatogr.*, **149**, *519-537 (1978).*

30. T. Willems, *"Evaporative Light Scattering Detectors"*. Internal report, Eindhoven University of Technology, *1997*.
31. M.W.F. Nielen and F.A. Buijtenhuijs, *Anal. Chem.*, in press.
32. M.W.F. Nielen, *Akzo Nobel internal report* (1996).
33. G. Glöckner, *Z. Phys. Chem.*, **229**, *98 (1965)*.

Chapter 5

Gradient Polymer Elution Chromatography of Crystalline Polyesters

The gradient elution behavior of the polyesters of neopentyl glycol and isophthalic acid/terephthalic acid is not reproducible for different solvent/non-solvent systems. These problems are probably caused by the semi-crystallinity of the polyesters. In this chapter the crystallinity of polyesters is investigated and the influence of the crystallinity on the gradient elution behavior of these polymers is discussed.

5.1 INTRODUCTION

The application of HPLC in the field of crystalline (or semi-crystalline) polymers is very restricted. The problem of crystalline polymers is their low solubility into the frequently used eluents, such as water, methanol, acetonitrile, tetrahydrofuran, and chloroform. Therefore, the determination of the molar mass distribution (MMD) of these polymers with size exclusion chromatography (SEC) usually requires 'exotic' conditions. The best know example is the MMD determination of polyolefins, *e.g.* the MMD of polyethene is determined by SEC in trichloro benzene at high temperatures (T>100°C) [1]. However, not always exotic conditions have to be used. In some cases the crystalline polymer can be dissolved in the exotic solvent and can be diluted with eluent of choice without the precipitation of a crystalline phase [2]. The crystallinity of polymers can also be used for separation according to chemical composition. For instance, temperature rising elution fractionation (TREF) can be applied to separate polyolefins according to molar mass and chain conformation based on differences in melting point (T_m) [3].

Philipsen [4] studied the GPEC retention behavior of crystalline polyesters. The polyesters described by this author showed irregular retention behavior at a column temperature of 25°C. The elution behavior changes gradually with increasing column temperature, and at 35°C, which is above the T_m (melting trajectory) of the polyester, the elution behavior becomes normal. During the experiments described in Chapter 4, similar problems concerning the reproducibility were observed, which will be described in this Chapter.

5.2 CRYSTALLINITY

The Gibbs free energy of a binary polymer/solvent system is described by the Flory-Huggins relation [5].

$$\frac{\Delta G_{mix}}{RTn_{\varphi}} = \varphi_S \ln \varphi_S + \frac{\varphi_P}{m_P} \ln \varphi_P + \chi_{P/S} \varphi_S \varphi_P$$

Equation 5-1, Flory-Huggins relation, where ΔG_{mix} [J] is the Gibbs free energy of mixing, R [J/mol·K] is the gas constant, n_φ is the total number of lattice places to be filled [mol], φ_S is the volume fraction of solvent [-], φ_P [-] is the volume fraction of polymer, m_P [-] is the relative chain length, and $\chi_{P/S}$ [-] is the Flory-Huggins interaction parameter (see Equation 2-6) [5].

When the polymer can crystallize, the Gibbs free energy for the crystalline/amorphous phase transition can be defined as follows.

$$\frac{\Delta G}{RT_m} = \frac{\Delta H_m}{R}\left(\frac{1}{T_m^0} - \frac{1}{T_m}\right)$$

Equation 5-2, Equilibrium condition for the crystalline/amorphous phase transition, where R [J/mol·K] is the gas constant, T_m^0 [K] is the melting point of the pure polymer, T_m [K] is the melting point at equilibrium, and ΔH_m [J/mol] is the enthalpy of melting.

After some thermodynamic and algebraic manipulations Equation 5-1 and Equation 5-2 can be combined to give the so-called melting point depression equation.

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \frac{-RV_P}{V_{Sol}\Delta H_m}\left[\frac{\ln\varphi_P}{m_P} + \left(\frac{1}{m_P} - \frac{1}{m_S}\right)\varphi_P + \chi_{P/S}\varphi_P^2\right]$$

Equation 5-3, Melting point depression equation, where R [J/mol·K] is the gas constant, φ_P [-] is the volume fraction of polymer, φ_S [-] is the volume fraction of solvent, m_{sol} and m_{pol} [-] are the relative amounts of respectively solvent molecules and polymer segments, $\chi_{P/S}$ [-] is the Flory-Huggins interaction parameter, T_m [K] is the melting point at equilibrium, T_m^0 [K] is the melting point of the pure polymer, V_{Sol} and V_P [ml] are the molar volumes of the solvent and the polymer, and ΔH_m [J/mol] is the melting enthalpy.

The melting point depression (MPD) describes the decrease of the melting point of a polymer in the presence of a solvent, which depends on the number of repeating segments (m_P), the volume fraction of polymer (φ_P) and the interaction parameter $\chi_{P/S}$. In a fast approximation, the interaction parameter can by described by the Hildebrand solubility parameters [6].

$$\chi \approx 0.34 + \frac{V_P}{RT}(\delta_p - \delta_s)^2$$

Equation 5-4, Interaction parameter equation, where V_p is the molar volume of the polymer, R is the gas constant, T is the temperature, and δ_p and δ_s are the Hildebrand solubility parameters of the polymer and the solvent.

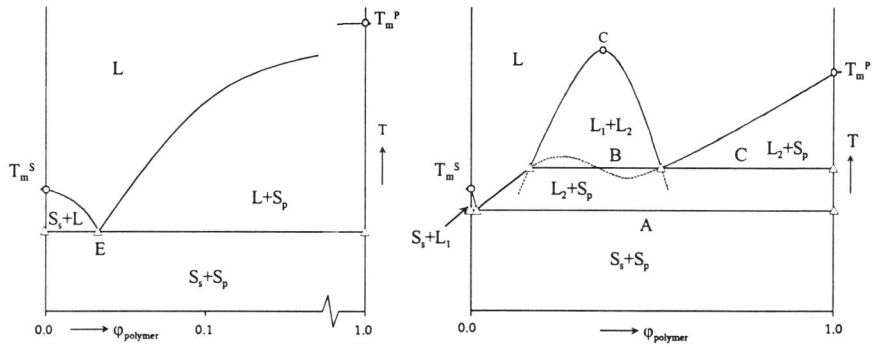


Figure 5-1, Phase diagrams of a binary polymer/solvent system with crystallinity. Left: no de-mixing occurs. Right: de-mixing of polymer and solvent in liquid phase occurs. Where L, L_1 and L_2 are liquid phases, S_s (solvent) and S_p (polymer) are the solid states, and T_m^S and T_m^P are the melting points of the solvent and polymer.

The T versus φ_{pol} phase diagrams of two binary systems are shown schematically in Figure 5-1. On the left side of Figure 5-1 is the T *vs.* φ_{pol} phase diagram of a binary system that shows no de-mixing behavior in the liquid phase. The T *vs.* φ_{pol} phase diagram of a system where de-mixing occurs in the liquid phase is depicted on the right. In the left T vs. φ_{pol} phase diagram four regions can be distinguished: one 'one phase' systems (L), two 'three phase' systems (S_S+L and S_P+L) and one 'three phase' (S_S+S_P). A eutectic point (E) can be seen where all three phases L, S_S and S_P co-exist. The melting points of the polymer and the solvent, T_m^P and T_m^S, normally differ to a large extend (and, to be correct, a gas phase of the solvent should be added to the phase diagram). The line from T_m^P to E, the eutectic point, describes the melting point depression (see Equation 5-3).

In the T vs. φ_{pol} phase diagram of a L-L de-mixing solvent/polymer system an additional area exists where two liquid phases are present one solvent rich phase L_1 and a polymer rich phase L_2). Three 'three phase' lines can be seen where three phases exist simultaneously (A, B and C). Also a L-L critical point (C) is found, with a critical temperature (T_C) and volume fraction ($\varphi_{c,pol}$).

The phase diagrams shown in Figure 5-1 are for binary systems. In GPEC ternary systems and even quaternary systems are applied. The theoretical approach of ternary systems is possible, but difficult [7].

5.3 EXPERIMENTAL

5.3.1 POLYESTER SAMPLES

The homopolyesters of NPG with IA (sample 14) and NGP with TA (sample 15) were used to investigate the crystallization behavior. The synthesis of the samples is described in Chapter 4. The samples 14 and 15 were the reaction products of bulk polymerizations in the molten

state. In order to investigate the precipitates of the polyesters in water/THF, the samples were precipitated as follows. The samples 14 and 15 were dissolved in tetrahydrofuran (THF, Fluka, HPLC-grade, Germany) with a concentration of 30 mg/ml at ambient temperature. Sample 14 precipitated after a certain amount of time, although initially the sample was dissolved in 100% THF. After sedimentation the precipitate of sample 14 was partly separated (sample 14A). To the remaining solution, water was added (ratio THF/water 1:1) which resulted in a further precipitation. This second precipitate was named 14B. Sample 15B was obtained by adding water to the initial polymer solution in THF (ratio THF/water 1:1).

Table 5-1, Samples used for the crystallinity study.

Sample	Ratio IA:TA	Remarks
14	1.0	*Direct reaction product from melt*
14A	1:0	*Sample 14 precipitated in THF*
14B	1:0	*Sample 14A precipitated in water/THF (1:1)*
15	0:1	*Direct reaction product from melt*
15B	0:1	*Sample 15 precipitated in water/THF (1:1)*

5.3.2 Determination of Crystalline Phase

In order to prove the formation of a crystalline phase during precipitation differential scanning calorimetry (DSC, Perkin-Elmer TEA systems, sample weight 4 mg, scanning rate 20°C/min. Light microscopy with crossed polarizers (Universal, Zeiss) with a 'hot stage' heating capability was also used. In addition, X-ray-measurements (A Philips X'Pert SR 5068 instrument operating with CuKa radiation and a 5°/min scan rate were used for X-ray reflection (XRD) analyses (2q=0.5 - 30 °)) were carried out.

5.3.3 Gradient Polymer Elution Chromatography

The following equipment is used: a Waters 616 Gradient Pump, a Waters 490 multiple wavelength UV detector (λ=275nm), a Hewlett Packard 1010 photodiode array detector (DAD, λ=275nm), a Gaston GT-103 on-line degassing device, a Separations Mistral column oven and a Waters 717 WISP Autosampler with variable injection volume. Different solvent/non-solvent (S/NS) systems were studied. The solvents were THF (HPLC-grade Fluka, Germany) and hexafluoro isopropanol HIFP (UV quality, Matco). When THF gradients were used acetic acid (1 vol%) was added. Trifluoro acetate (0.002 M) was used in the case of the HFIP gradients. Acetic acid and trifluoro acetate were added to avoid strong polar interactions. The non-solvents were water (MilliQ, Millipore), acetonitrile (ACN), methanol and isopropanol (UV quality, Merck). The column, applied for the RP-GPEC experiments, was a Symmetry Si-C18. The flow rate was 0.5 ml/min. The column temperature was established at 55 °C. The injection volume was varied between 10 and 100μl. The samples were all dissolved in HFIP (concentration 10 mg/ml) in order to prevent crystallization in the sample solution

(see next paragraph). The applied gradients were all from 100% non-solvent to 100% solvent with a gradient steepness of 2%/min. Most of the experiments were performed using the Waters 490 multiple UV detector. When the experiments were performed with the HP 1010 DAD, this is explicitly mentioned.

5.4 Results and Discussion

Crystallization of polymers can occur from the melt or from solution [8]. Cystallalization is dependent on the mobility and the concentration of the polymer molecules, low concentrations and high mobilites advances cystallization. In the melt, the mobility of the molecules is lower than in solution. Additonationally, the concentration in the melt is higher than in solution. Due to the higher mobility and the lowere concentration, crystallization of the polymer molecules is easier from solution than from the melt [8]. Samples 14 (IA) and 15 (TA) were obtained from the melt, whereas the samples 14A, 14B, and 15B are obtained from solution.

In order to study the crystallinity, the first heating run DSC-results were used. The DSC-plots of the precipitated samples (14A, 14B and 15 B) were difficult to interpret due to a decrease in weight of the sample as determined by thermogravimetric analysis (TGA). Although the samples 14A, 14B and 15B were dried, they still contained a significant amount of solvent (water and/or THF), especially the samples precipitated in water. The evaporation of the solvents caused a decrease in weight and a disturbance in the DSC signal, which was more pronounced for sample 15B. Normally, the solvents evaporate during the first DSC run (open pan), and the thermal properties of the polymer can be measured in a second run without disturbance of the evaporation of the solvent.

The first run DSC-plots of the samples 14, 14A and 14B are shown in Figure 5-2. Sample 14 showed a glass transition, which also showed the relaxation peak [9], no melt transition was observed for this sample. Sample 14A showed a clear melt transition at T_m=150°C. In the case of sample 14B two melt transitions were observed, a melt transition corresponding to that of sample 14A at Tm=150°C and a second melt peak at a lower T_m of 130°C. The difference between the two melt transitions of the precipitate in THF and in water/THF could be the molar mass dependency, a different crystal structure or a difference in perfection of the crystalls.

Sample 15 (not shown) showed similar behavior as sample 14, only a glass transition and a relaxation peak could be observed. Additionally, sample 15A showed a melt transition. However the DSC plots were difficult to interpret due to the decrease in weight of the sample already discussed before.

When second DSC runs were performed, the melt transitions were not noticed for all samples, only a glass transition at 75°C was observed. Apparently, the polyester did not form a crystalline phase from the melt.

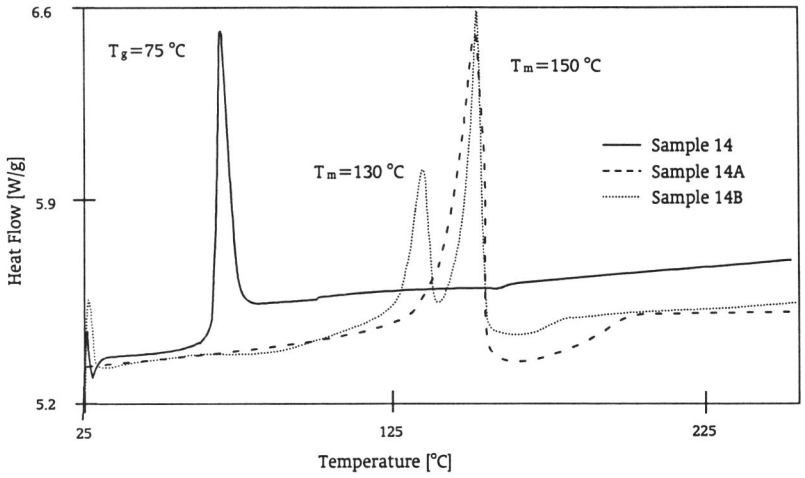


Figure 5-2, The DSC plots of sample 14 (—, from melt), sample 14A (--, precipitate from THF) and sample 14B(···, precipitation from water/THF).

With X-ray diffraction ordered structures can be distinguished from amorphous structures. Furthermore, X-ray diffraction can differentiate between different crystal lattices [10]. Sample 14 was found to be amorphous, which is in agreement with the DSC-data. The diagrams of the samples 14A and 14B showed ordering. No significant differences were found between samples 14A and 14B, which suggests that the different first order transitions are not caused by different crystal structures. Probably, the different first order transitions observed with DSC found for the precipitates in THF and water/THF were caused by different molar masses or by difference in perfection of the crystals in the precipitates. It is thought that a high molar mass fraction precipitated in THF, whereas lower molar mass fractions precipitated in water/THF. However, this was not verified, and in order to fully explain the different melting points, additional research should be performed.

With polarized light microscopy, crystallites can be detected. The samples 14A and 14B showed crystallites. A photograph of the crystallites of sample 14B is shown on the cover of this thesis. The colors in the photograph indicate the diffraction of the polarized light. The crystallites melted when the temperature was increased up to 170°C (above the melting point). After cooling down, the samples did not crystallize anymore. This again proved that the homopolyesters do not form crystallites from the a melt.

The homopolymer of NPG and IA (sample 14A) is a special case. The homopolyester dissolved in THF, but formed a crystalline phase over time. The solution was stored in a closed flask, so the evaporation of THF and the hygroscopic properties of THF cannot be the cause of the precipitation. This precipitation can be explained by looking at the phase diagram of a binary polymer/solvent mixture (see Figure 5-1, *left*). The equilibrium conditions (ambient temperature and a concentration of 30 mg/ml) are probably in the $L+S_p$ area.

Since the precipitate was proven to be crystalline, the reproducibility problems encountered in GPEC are likely due to the kinetics of the redissolution of a crystalline phase [4]. In GPEC separations, the precipitation step and the redissolution step are crucial. Thus, the redissolution must be reproducible. At the precipitation stage, an equilibrium according to the phase diagram will be established. When the solvent and non-solvent are miscible, the phase diagram of the polymer/solvent/non-solvent system will look like the one described in Figure 5-1 on the right. Depending on the system polymer/solvent/non-solvent two situations can exist. In the first situation the system separates in two liquid phases (area L_1 and L_2). In the second situation the polymer will form a crystalline phase in combination with one liquid phase. The phase of the solute at redissolution is assumed to be the determining factor in GPEC separation. If the solute is in a (semi-) crystalline solid state, the redissolution will become highly irregular, resulting in poorly reproducible GPEC separations (*see next paragraphs*). If the solute is in a liquid phase (separated from the solvent phase) the redissolution becomes reproducible and so does the GPEC separation. Therefore, the formation of a crystalline phase is not desirable when GPEC is applied.

5.4.1 GRADIENT POLYMER ELUTION CHROMATOGRAPHY

Tetrahydrofuran (THF), and hexafluoro iso-propanol (HFIP) are both good solvents for the polyesters 14 and 15. Size exclusion chromatography can be performed using both solvents. Water, methanol, acetonitrile, and iso-propanol can be used as non-solvent for the gradient separations. Water is a non-solvent, the others are poor solvents (the polyester dissolves partly). Since sample 14 (IA homopolyester) precipitates from a solution in THF after a certain time, the sample solvent for *all* the GPEC experiments was HFIP. The initial conditions of the gradient play an important role in the GPEC retention behavior of homopolyesters, especially when they are crystalline. The GPEC retention behavior of the polyesters in different combinations of the above solvents will be discussed in this section.

5.4.1.1 WATER AND TETRAHYDROFURAN

The chromatograms of samples 14 and 15 for the water/THF gradient are shown in Figure 5-3 and Figure 5-4. The chromatograms can be divided into two regions. The first region shows the low molar mass oligomers (region I: 10-40 minutes) and the second region displays huge broad peaks which are due to crystallinity effects. Philipsen has found similar chromatograms [4].

The chromatograms are not reproducible, especially in the second region. In addition, they show a strong dependence of the injection volume on the separation. The dependence of the injection volume implies that the initial conditions are crucial for the separation.

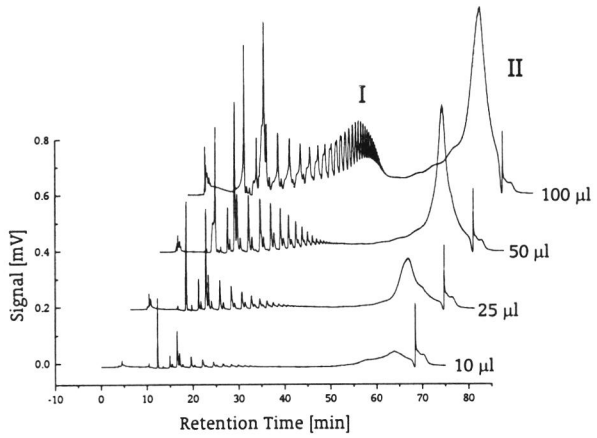


Figure 5-3, Chromatograms of sample 14 with different injection volumes (concentration 10 mg/ml) with a water/THF gradient (2%/min) on a Symmetry C18 column (55°C). The injection volumes were 100µl, 50µl, 25µl and 10µl. I and II refer to the 'oligomer region' and the 'crystallinity effects region', see text.

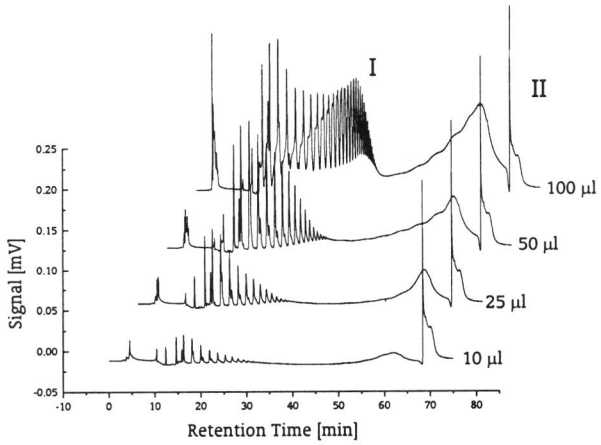


Figure 5-4, Chromatograms of sample 15 with different injection volumes (concentration 10 mg/ml) with a water/THF gradient (2%/min) on a Symmetry C18 column (55°C). The injection volumes were 100µl, 50µl, 25µl and 10µl. I and II refer to the 'oligomer region' and the 'crystallinity effects region', see text.

When low injection volumes are applied, the low molar mass oligomers formed a crystalline phase or were included into the crystalline phase of high molar mass oligomers, the latter seems more likely. As a consequence, the low molar mass oligomers will elute partially in the last part of the chromatogram although they should elute completely at the beginning. At higher injection volumes, the oligomers are incorporated in the crystalline phase to a lesser extent, explaining the presence of the higher molar mass oligomers in region I (around 40 minutes).

In order to identify the peaks in region II, a fraction was collected from 40 minutes to 65 minutes. The fractions were found to be cloudy, apparently because the dissolved polyesters precipitated as soon as the eluent was collected ($T_{column} > T_{ambient}$). The fractions were analyzed by matrix-assisted-laser-desorption-ionization mass spectrometry (The conditions for the MALDI-MS analyses of the polyesters are described in *Chapter 4*). The analysis of the fractions was problematic, possibly due to the crystallinity of the polyesters, which hampered their mixing with the matrix. The MALDI-MS analyses showed that the peaks in region II correspond to polymers with molar masses from 3000 g/mol to 4000 g/mol.

In order to obtain normal and reproducible GPEC behavior, the polymer molecules must not be present in a crystalline state at the point of redissolution. In that case strong kinetic effects will dominate the separation [4]. Two factors can achieve circumvention of the poorly reproducible separation results. First the temperature of the column can be increased to a value above the melting point of the polymer solution, *i.e.* the melting point of the crystalline polymer in the presence of a solvent. *Philipsen* [4] indeed solved the separation problems by raising the column temperature to 50°C, which is above the apparent melting point depression ($T_m < T_m^0$, see Equation 5-3, $T_m^0 = 70°C$). In this study the temperature was varied from 35°C to 55°C, but the reproducibility of the separation did not improve much. (a slight improvement in reproducibility was found at higher temperatures). Unlike the results published by Philipsen [4], the separation problems could not be solved by increasing the temperature. Apparently, the melting point depression caused by dissolution of the high-melting polyesters of NGP/IA/TA ($T_m \approx 150°C$) does not go below 55°C. Furthermore, the boiling point of THF ($T_b = 65°C$ at atmospheric pressure) and its high vapor pressure do not allow the application of higher column temperatures. Consequently, changing the temperature does not solve the poorly reproducible separations caused by the crystallinity of the samples. The second way to improve separation is to change the solvent strength (χ) of the eluent. The melting point depression (see Equation 5-3) will be higher when better solvent conditions are applied. To change the solvent conditions a different solvent (HFIP) and/or a different non-solvent (ACN) were used.

5.4.1.2 Water and Hexafluoro Isopropanol

The chromatogram of sample 14 obtained with the water/HFIP gradient is shown in Figure 5-5. The sample was dissolved in HFIP (concentration 10 mg/ml) and injected in 100% water (injection volume 25 µl).

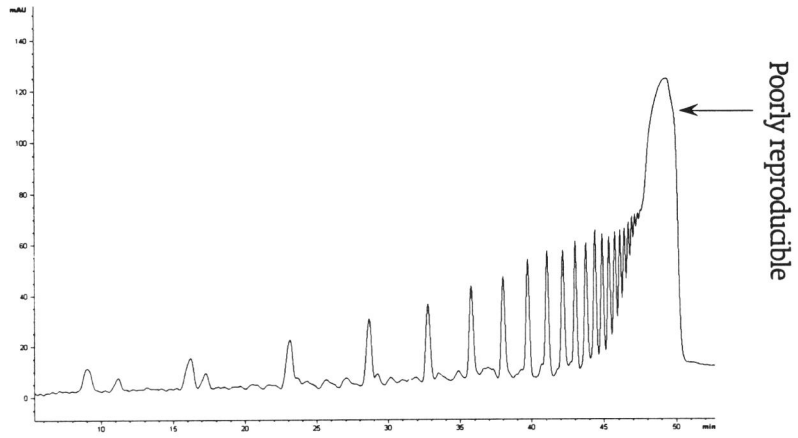


Figure 5-5, Chromatogram of sample 14 obtained with the water/HFIP gradient (2%/min) on a symmetry C18 column (55°C), injection volume 25µL.The HP 1010 DAD was applied (λ 275nm).

The chromatograms obtained with the water/HFIP gradient were found to be fairly reproducible. However, the high molar mass peak showed again reproducibility problems, although the reproducibility had improved when compared to that observed for the water/THF gradients. The application of HFIP as solvent solved the crystallinity problems for the low molar mass polymers, but did not solve the problems over the whole range of the molar mass. HFIP was not only used as eluent but also as sample solvent. Consequently, at injection, the phase separation occurred in the presence of HFIP, which was thought to prevent the incorporation of the low molar mass oligomers, resulting in a better reproducibility.

5.4.1.3 Acetonitrile and Tetrahydrofuran

The chromatogram of sample 14 obtained with the ACN/THF gradient is shown in Figure 5-6. The sample was dissolved in THF (concentration 10 mg/ml) and injected in 100% ACN (injection volume 25µl).

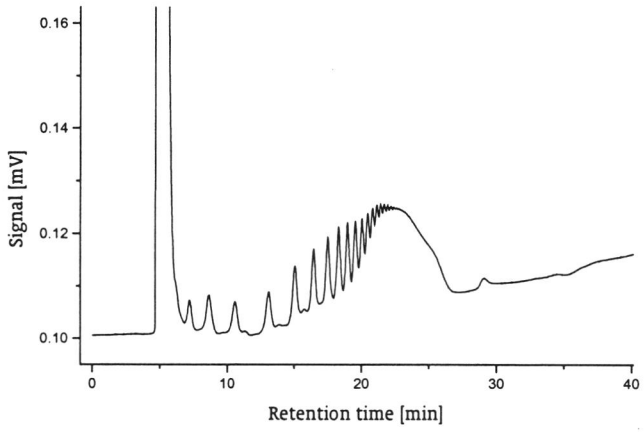


Figure 5-6, Chromatogram of sample 14 obtained with the ACN/THF gradient (2%/min) on a symmetry C18 column (55°C), injection volume 25μL.

The chromatogram showed that the oligomers dissolve partially in ACN. The eluent properties of ACN are better than that of water, therefore part of the oligomers showed no retention and eluted at the beginning of the chromatogram. The reproducibility of the separation was better than that obtained with the water/THF gradient and comparable to that obtained with the water/HFIP gradient. However, still problems occurred in the high molar mass part, which implies that still a crystalline phase was formed upon injection.

The application of ACN as non-solvent improves the reproducibility of the separation significantly. ACN will have an influence on the precipitation step and will probably lower the melting point due to an improved interaction with the oligomers, which will result in an improved reproducibility.

5.4.1.4 Acetonitrile and Hexafluoro Isopropanol

The chromatogram of sample 14 obtained with the ACN/HFIP gradient is shown in Figure 5-7. The sample was dissolved in HFIP (concentration 10 mg/ml) and injected in 100% ACN (injection volume 25μl).

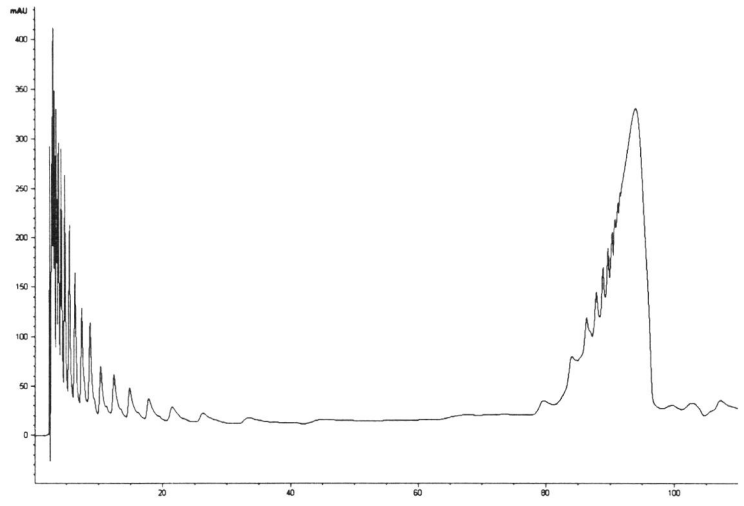


Figure 5-7, Chromatogram of sample 14 obtained with the ACN/HFIP gradient (2%/min) on a symmetry C18 column (55°C), injection volume 25μL HP 1010 DAD (λ275nm).

The reproducibility is excellent in the low molar mass part, as well as in the high molar mass part. Consequently, when ACN/HFIP was applied no problems related to the crystallinity of the polyesters were observed. This can either mean that no crystalline phase will form in 100% ACN or upon redissolution no crystalline phase is present anymore. The first option is in contradiction with the problems encountered when the ACN/THF gradient was used (the formation of a crystalline phase was formed during precipitation). The same sample solvent was applied (HFIP) and the initial conditions of the ACN/THF and ACN/HFIP gradients was ACN, the conditions at injection are equal. As a consequence, it can be stated that the existence of a crystalline phase at the initial conditions is not the determining factor, but the existence of a crystalline phase at the point of redissolution is crucial for occurrence of poorly reproducible separations.

The chromatogram of the ACN/HFIP (see Figure 5-7) can be divided into two parts: Part 1 from 0 to 40 minutes and part 2 from 60 to 100 minutes. The first part are low molar mass oligomers that dissolve and (pre-)elute in ACN (see also Figure 5-6 the ACN/THF gradient). Due to the low eluent strength of HFIP, a higher fraction of HFIP is necessary to desorb the higher molar mass oligomers (part 2) than compared to THF (see Figure 5-6).

5.4.1.5 Water, Acetonitrile and Tetrahydrofuran Gradient

Other non-solvents, like methanol and isopropanol were also applied and these results were similar to the results obtained with the ACN/THF gradient and the ACN/HFIP gradient. However, the best conditions to avoid crystallinity problems were found to be the ACN/HFIP gradient. Despite the good reproducibility the separation obtained with the ACN/HFIP

gradient was not suitable to characterize the copolymers adequately. The selectivity in the ACN/HFIP gradient was not sufficient to obtain a separation. In contrast, the application of water as non-solvent gave a high selectivity (see Figure 5-3). With this observation, water/ACN non-solvent was tested and found to be the best solution for the separation of the copolyesters. In addition, the random copolyesters did not show any poor reproducibility behavior as can be seen in Figure 5-8, where the chromatograms of the samples 14 , 15 and the 1:1 ratio IA/TA copolyesters obtained with gradient from 50:50% water/ACN to 100% THF are shown.

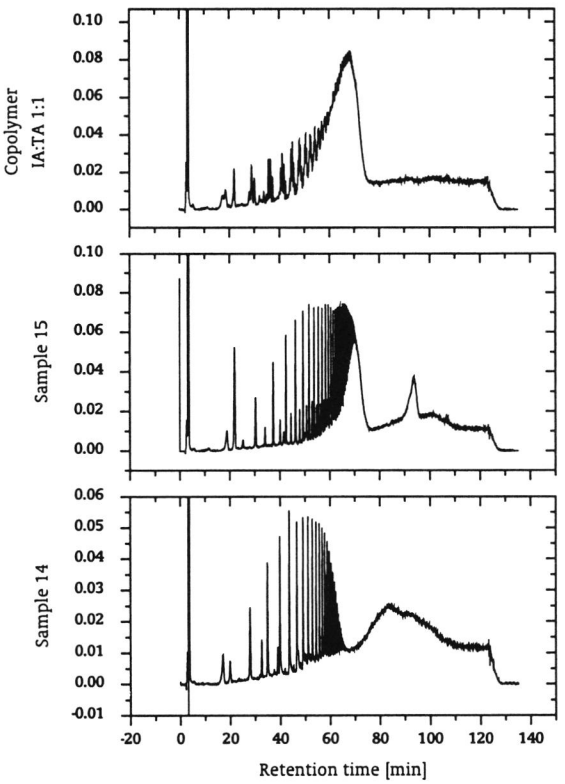


Figure 5-8, Chromatograms of sample 14, 15 and the copolymer IA:TA 1:1 obtained with a gradient from water/ACN (50:50) to THF on a Symmetry C18 column (55°), injection volume 25µl, concentration 10 mg/ml.

Therefore THF as eluent could be used as eluent to obtain reproducible chromatograms for the copolyesters. In order to avoid crystallization (see sample 14A), HFIP was used as the sample solvent for the separation of the homopolymers and the copolymers. The characterization of the copolymers is described in Chapter 6.

5.5 Conclusions

When GPEC was applied to the polyesters containing NPG/IA and NPG/TA, reproducibility problems were observed. These problems were caused by the formation of a crystalline phase during precipitation. DSC, X-ray and polarized light microscopy experiments showed that the homopolyesters of NPG with IA or TA formed a crystalline phase during precipitation, although, from the melt the polyesters did not crystallize.

Increasing the column temperature could not solve the problems related to the crystallinity of the polyesters samples. A solution was found by applying the right solvent/non-solvent combination. In the water/THF gradient, the reproducibility was very poor. Depending on the injection volume and concentration of the injected polyesters, different peak proportions were obtained. Water appears to be a strong non-solvent, and THF a weak solvent. In the water/HFIP gradient, the reproducibility of the oligomers was good, however, that of the high molar mass part was poor. When an ACN/THF gradient was applied, the reproducibility improved, especially for the low molar mass oligomers. The ACN/HFIP gradient yield the best reproducibility for both the low molar mass oligomers as well as for those of high molar mass. Apparently, the initial conditions and the end conditions are both very important for the separation and the reproducibility. The best conditions for a reproducible redissolution are obtained when the polymer dissolves from a melt phase or a swollen phase (gel). In such a phase, the solvent molecules can easily penetrate. In contrast, when the polymer phase is crystalline, the diffusion of the solvent molecules is difficult. Redissolution, especially from a crystalline phase is highly irregular [4], and the separation becomes irreproducible.

The results can be explained qualitatively with the melting point depression equation (Equation 5-3). The interaction parameters of the polyesters with water are very high, water is a strong non-solvent. When water is used in combination with THF, the interaction parameter of THF is too high to prevent crystallization of the polyester. The better reproducibility of the chromatograms with HFIP could be explained by difference between the interaction parameters of THF and HFIP ($\chi_{p/HFI} < \chi_{p/HF}$). In addition, the poor reproducibility could also be explained by the inclusion of the low molar mass oligomers by the high molar mass molecules in their crystalline phase. For the water/THF gradient, this could be the cause for the poor reproducibility of the peak areas of the oligomers.

Although the gradient ACN/HFIP is found to be the best system to prevent reproducibility problems, the separation obtained with the ACN/HFIP gradient is not sufficient. The combination of a water/ACN as non-solvent and THF as solvent appeared to be the best gradient for the separation of the copolyesters. Although the homopolyesters still showed the some reproducibility problems at the end of the chromatogram, the separation obtained with the water/ACN/THF gradient could be successfully applied for the characterization of the copolyesters according to chemical composition.

5.6 REFERENCES

1. W.W. Yau, J.J. Kirkland and D.D. Bly, *"Modern Size Exclusion Liquid Chromatography"*, Wiley-Interscience, New York, *1979*.
2. F.A. Buijtenhuijs, *Personal conversation*.
3. G. Glöckner, G. Glöckner, *"Gradient HPLC of Copolymers and Chromatographic Cross Fractionation"*, Springer-Verlag Berlin Heidelberg, *1991*.
4. Philipsen H.J.A., *"Mechanisms of Gradient Polymer Elution Chromatography and its Application to (Co)Polyesters"*, *Ph.D. Thesis*, Eindhoven University of Technology, Eindhoven, The Netherlands, *1998*.
5. P. Flory, *"Principles of Polymer Chemistry"*, Cornell University Press, Ithaca, New York, *1953*.
6. D.W. van Krevelen, *"Properties of Polymers"*, 3rd Edition, Elsevier, Amsterdam, *1990*.
7. W.J. Staal, *"Gradient Polymer Elution Chromatography"*, *Ph.D. Thesis*, Eindhoven University of Technology, Eindhoven, The Netherlands, *1996*.
8. B. Wunderlich, *"Macromolecular Physics"*, **Vol. 3** *"Crystal Melting"*, Academic Press, New York, *1980*.
9. S.M. Vleeshouwers, *"Modeling and Predicting Properties of Polymers in the Amorphous Glassy state"*, *Ph.D. Thesis*, Eindhoven University of Technology, Eindhoven, The Netherlands, *1993*.
10. Hans-Georg Elias, Makromoleküle, Bd. 1, Grundlagen, 5.Auflage, Hüthig & Wepf Verlag, Basel *1990*.

Chapter 6

The Microstructural Analysis of Copolyesters

For the characterization of copolyesters of neopentyl glycol with isophthalic acid (IA) and terephthalic acid (TA) copolymers with different ratio IA/TA were synthesized. The separation conditions described in Chapter 4, were used to separate the copolymers on chemical composition. The chemical composition distributions (CCDs) of the copolymers with different overall ratio of IA/TA were calculated and compared to a CCD based on statistics. Besides random copolyesters also a transesterfication sample of the two homopolymers and commercial copolyesters were characterized. By applying GPEC in combination with ESI-MS and UV spectroscopy differences in microstructure (molar mass, chemical composition, end groups) among the commercial samples could be revealed.

6.1 INTRODUCTION

The properties of the copolymers of neopentyl glycol (NPG), isophthalic acid (IA) and terephthalic acid (TA) depend on microstructure (molar mass, chemical composition and end groups), especially on the functional end groups. The acid mol fraction of TA affects the flexibility of the polymer matrix, and the acid mol fraction IA directly affects the chemical and physical resistance. Consequently the information on the chemical composition distribution is essential for the understanding of these properties. The copolymers will be separated by reversed-phase gradient-polymer-elution-chromatography GPEC (RP-GPEC). The cyclic oligomers present in the polyesters play an important role for the properties, since they do not take part in the cross-linking reaction during curing of the polymer matrix. Additionally the cyclic dimer of TA and NPG does not dissolve in the polymer matrix, resulting in phase separation of polymer matrix and the cyclic dimer. The cyclic dimer will migrate to the surface causing imperfections in the gloss properties of the coating. These imperfections are also called 'the blooming effect' and, consequently, the cyclic dimer is called the 'blooming' molecule [1].

In order to study the GPEC retention behavior of the copolyesters, random copolyesters with different acid mol fraction of TA were synthesized. With these well-defined copolymers, the separation according to chemical composition was studied. A transesterfication copolymer was synthesized from the initial homopolymer samples with a TA mol fraction of 0.50. This transesterfication copolyester could be compared with the random copolyester with similar chemical composition. Besides the copolyester samples, commercial copolyester samples (one of the components of powder coatings) were characterized. The experimental conditions of the analysis were similar to the analysis of the homopolyesters described in *Chapter 4*.

6.2 EXPERIMENTAL

6.2.1 SYNTHESIS OF COPOLYESTERS

Copolyester samples of neopentyl glycol (NPG), isophthalic acid (IA) and terephthalic acid (TA) with different ratios IA/TA were synthesized. The two acid monomers are not miscible, consequently the copolymerizations were performed in two steps. In the first step TA was polymerized with NPG, and in step 2, IA was added to the reaction mixture. Apart from the second step the synthesis of the copolyesters is comparable to the synthesis of the homopolyesters described in *Chapter 4*. The synthesis of the copolymer with an acid mol fraction TA of 0.50 will be described in detail.

Initially, 50g of water and 500g NPG (=4.8 mol) were mixed in a two liter reactor and heated to a temperature of 80°C (water was added to melt the NPG at a lower temperature).

The reactor was purged with nitrogen. In addition, 1.5g dibutyl tin oxide was added as catalyst for the esterfication reaction. When the NPG was melted, 450 g of TA (2.7 mol), was added to the reactor, and the reactor was slowly heated to a temperature of 240°C in order to melt the acid monomer. The water formed during the esterfication was distilled off. After an acid value (AV), determined by titration, of approx. 5 [mg KOH/g] was reached, the reactor was cooled down to 180°C. Then 450 g of IA (2.7) was added to the reactor and the temperature was increased to 240°C. The reaction was stopped when an AV of 35 [mg KOH/g] was reached. The copolymer samples with different IA/TA ratio are listed in Table 6-1. Copolymers with different acid mol fraction of TA are obtained by changing the ratio of acid monomers. Depending on the acid mol fraction of TA different reaction times were used. The reactions were stopped when an acid value of approximately 35 [mg KOH/g].

In addition, a transesterfication of the homopolymer samples 14 (IA) and 15 (TA) was performed. The two homopolymers were mixed one to one in the reactor and no additional catalyst was used. The transesterfication was performed at 240°C and the reaction was stopped after 5 hours. The trans-esterfication reaction is sample 24.

Except from the acid mol fraction TA of 0.75 and the incorporation of a small fraction of tri-functional alcohol (trimethylol propane (TMP)), detailed information about the reaction conditions of the commercial samples is not known. TMP is a tri-functional monomer and is added to obtain multi-functional oligomers instead of bi-functional as obtained with NPG. Presumably, the amount of TMP added is low (less than 1%vol). The four different commercial samples are called C1, C2, C3 and C4.

Table 6-1, The random copolyester samples with different chemical composition.

Sample *Code*	Acid mol fraction *IA*	Acid mol fraction *TA*	Addition of IA at *Hours*	Total Reaction Time *Hours*
16	0.50	0.50	5	10
17	0.20	0.75	10	20
18	0.75	0.25	4	12
24	0.50	0.50	-	5

6.2.2 Analytical Techniques

The applied SEC conditions and equipment were similar to those for the homopolymers. Also the RP-GPEC conditions and the conditions used for the ESI-MS identifications were comparable to the conditions used for the homopolyesters. The description of the conditions and equipment used for the analysis of the homopolyesters can be found in *Chapter 4*.

6.3 Results and Discussion

6.3.1 Size Exclusion Chromatography

The results of the SEC experiments with the viscosity detector are shown in Table 6-2. Besides the data of the copolyesters also the data of the homopolyesters is included.

Table 6-2, Results of the molar mass determinations by SEC in HFIP calculated with the conventional method (CON) and the universal calibration method (UC). The number average molar mass and the weight average molar were calculated.

		14 (IA)	15 (TA)	16 (φ_{TA}=0.50)	17 (φ_{TA}=0.75)	18 (φ_{TA}=0.25)	24 (φ_{TA}=0.50)
CON	$\overline{M}_n$	7,560	8,490	7,380	7,060	7,450	7,730
	$\overline{M}_w$	13,700	14,900	11,500	11,500	11,600	13,200
UC	$\overline{M}_n$	2,220	2,260	2,060	1,600	2,060	1,880
	$\overline{M}_w$	4,280	4,360	3,550	3,090	3,630	3,840
	a	0.67	0.68	0.66	0.70	0.66	0.67
	Log K_n	-2.80	-2.78	-2.65	-2.74	-2.70	-2.75

The $\overline{M}_w$s of the copolyesters are slightly lower than the $\overline{M}_w$s of the homopolymers. The $\overline{M}_w$s of the copolyesters are similar, and so are the *Mark-Houwink* parameters. As already mentioned in *Chapter 4*, the molar masses obtained with the conventional method are about four times higher than those obtained with the universal calibration method. The data obtained with the universal calibration method are the more accurate molar masses.

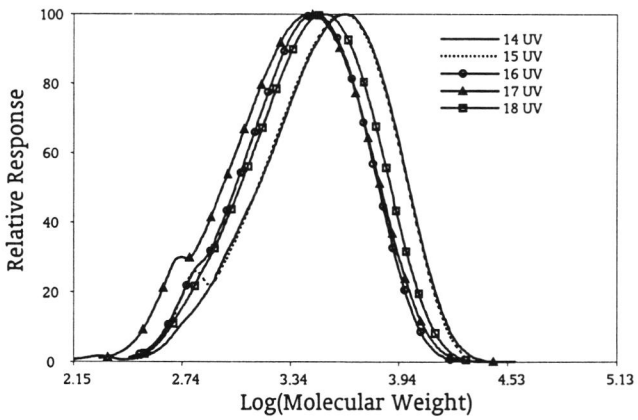


Figure 6-1, Molar mass distributions of sample 14 (—), sample 15 (···), sample 16 (–○–), sample 17 (–△–) and sample 18 (–□–).

The MMDs of the commercial samples obtained with the universal calibration method are shown in Figure 6-2. The average mol masses are summarized in Table 6-3.

Table 6-3, Results of the molar mass determinations by SEC in HFIP.

		C1	C2	C3	C4
UNIV	$\overline{M}_n$	1,640	1,520	1,800	2,100
	$\overline{M}_w$	3,010	3,130	3,550	3,790
	A	0.63	0.69	0.66	0.67
	Log K_η	-2.51	-2.74	-2.62	-2.69

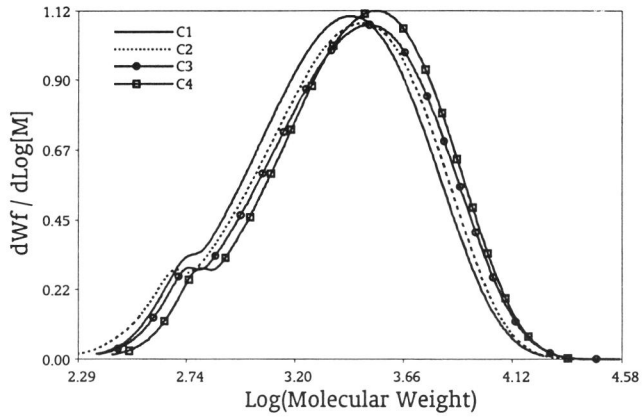


Figure 6-2, Molar mass distributions of commercial samples C1 (—), sample C2 (···), sample C3 (–○–) and sample C4 (–□–) obtained with the universal calibration method.

The MMDs of the commercial samples are different, however these differences are not expected to cause different properties of the commercial samples. The *Mark-Houwink* parameters of the commercial samples did not differ significantly.

6.3.2 Separation of Copolyesters by GPEC

In order to interpret the GPEC chromatograms of the copolymers, the peaks of sample 16 (acid mol fraction of TA $\varphi_{TA}=0.50$) were analyzed by ESI-MS. Besides oligomers with different end groups, also oligomers with different acid mol fraction of TA (φ_{TA}) may exist. The possible oligomers $(AD)_p$-A with different acid mol fraction TA and degree of polymerization (p) are described in Table 6-4. Since the $(AD)_p$-A are dominantly present (see *Chapter 4*), the separation of these oligomers will be used to discuss the separation on chemical composition.

Since the two acid monomers have the same mass, the oligomers with different chemical composition with respect to the ratio IA/TA will have the same molar mass. Therefore, MS will not be able to determine the chemical composition (ratio of IA/TA) of each oligomer. The ratio IA/TA can be determined in combination with GPEC.

Two different gradients were applied to separate the copolyesters. The two gradients were already applied to separate the homopolyesters. Gradient B was found to be the optimal gradient for the homopolyesters (see *Chapter 4*), however, since the copolyester are more complex in microstructure, also oligomers with different chemical composition will be present. The chromatograms of sample 16 (φ_{TA}=0.50) obtained with gradient A and gradient B can be seen in Figure 6-3.

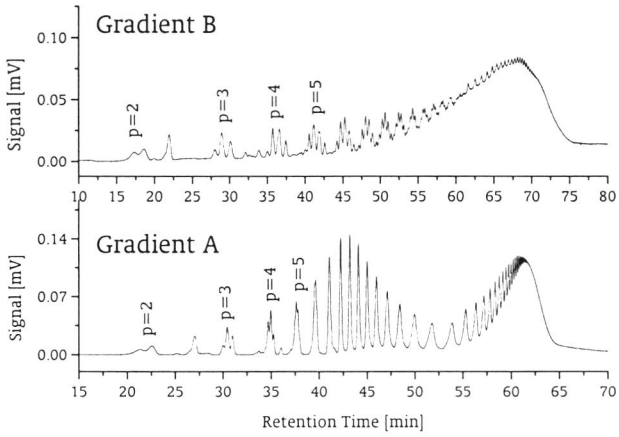


Figure 6-3, Comparison of the chromatograms of sample 16(φ_{TA}=0.50) obtained with gradient A (bottom) and gradient B (top). For more detailed information about gradients A and B see Chapter 4.

Gradient B shows more resolution within a cluster with equal p. However, the peaks start to overlap early in the chromatogram ($(AD)_4$-A), resulting in a baseline drift and a loss of quantitative information. Gradient A shows a better resolution between the cluster with different p. Although gradient B showed the highest density of information, the separation in the chromatogram obtained with gradient A the peaks can be quantified better due to the baseline separation. Consequently, gradient A will be used to characterize the copolymers.

Table 6-4, The oligomers $(AD)_p$-A with different acid mol fraction TA (φ_{TA}) with different degree of polymerization (p). The probabilities of the different oligomers are calculated with the number of acid groups (#IA and #TA) in the oligomer and the number of possible species with the same acid mol fraction TA but different sequence of TA (#). The probabilities depend on the overall acid mol fraction TA (φ_{TA}).

Oligomer	Composition				Probability	Probability	Probability
					Sample 18	Sample 16	Sample 17
	φ_{TA}	#IA	#TA	#	φ_{TA}0.25	φ_{TA}=0.50	φ_{TA}=0.75
P=2	0.00	3	0	1	0.4219	0.1250	0.0156
$(AD)_2$-A	0.33	2	1	3	0.4219	0.3750	0.1406
	0.60	1	2	3	0.1406	0.3750	0.4219
	1.00	0	3	1	0.0156	0.1250	0.4219
P=3	0.00	4	0	1	0.3164	0.0625	0.0039
$(AD)_3$-A	0.25	3	1	4	0.4219	0.2500	0.0469
	0.50	2	2	6	0.2109	0.3750	0.2109
	0.75	1	3	4	0.0469	0.2500	0.4219
	1.00	0	4	1	0.0039	0.0625	0.3164
P=4	0.00	5	0	1	0.2373	0.0313	0.0010
$(AD)_4$-A	0.20	4	1	5	0.3955	0.1563	0.0146
	0.40	3	2	10	0.2637	0.3125	0.0879
	0.60	2	3	10	0.0879	0.3125	0.2637
	0.80	1	4	5	0.0146	0.1563	0.3955
	1.00	0	5	1	0.0010	0.0313	0.2373
p=5	0.00	6	0	1	0.1780	0.0156	0.0002
$(AD)_5$-A	0.17	5	1	6	0.3560	0.0938	0.0044
	0.33	4	2	15	0.2966	0.2344	0.0330
	0.50	3	3	20	0.1318	0.3125	0.1318
	0.66	2	4	15	0.0330	0.2344	0.2966
	0.83	1	5	6	0.0044	0.0938	0.3560
	1.00	0	6	1	0.0002	0.0156	0.1780

The numbers in Table 6-4 are calculated with the assumption of equal reactivities of TA and IA. From Table 6-4 the chemical composition distributions (CCD) for oligomers AD_p-A with different degree of polymerization (p) can be calculated based on statistics. These statistically calculated CCDs will be discussed in more detail further on in this chapter.

From the overlay of the complete chromatograms no differences can be noticed, however, when the first part of the chromatograms are studied in more detail, small differences are found. The first part of the chromatograms (first 40 minutes) of the copolyester samples (16, 17 and 18) and the homopolyesters (14 and 15) obtained with gradient A are shown in Figure 6-4. The oligomers with equal degree of polymerization (p) of the copolymers elute in between the oligomers with equal p of the homopolyesters, hence, separation on chemical composition was obtained. Nevertheless, besides separation on chemical composition also separation of the oligomers with equal p based on different acid end groups can occur. This hypothesis can be checked by looking at the number of peaks present in a cluster of oligomers with equal p and the number of possible oligomers described in Table 6-4.

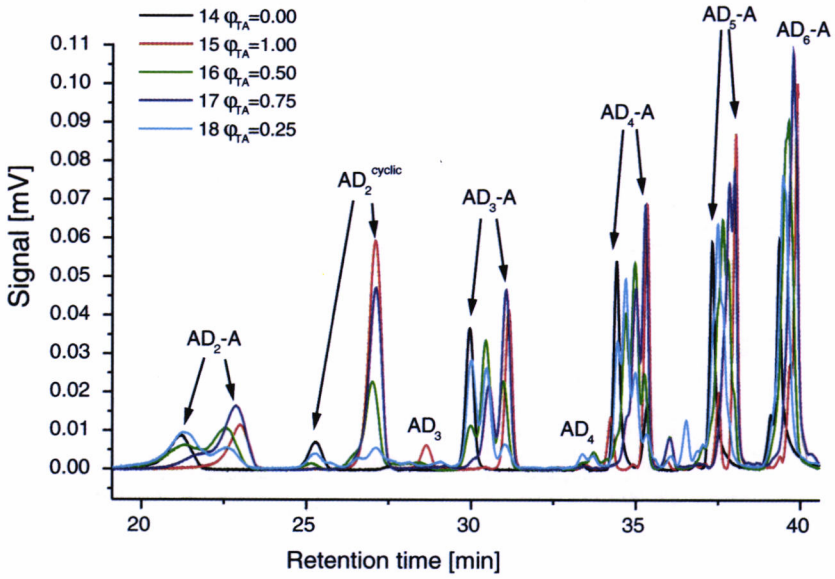


Figure 6-4, UV Chromatograms (λ_{275}) and identification of the samples 14 (φ_{TA}= 0.00, black line), 15 (φ_{TA}= 1.00, red line), 16 (φ_{TA}= 0.50, green line), 17 (φ_{TA}= 0.75, blue line), and 18 (φ_{TA}= 0.25, cyan line) using gradient A.

In Figure 6-4 it can be seen that with increasing degree of polymerization an additional oligomer was obtained. When the separation would be based on different end groups TA or IA, only three different oligomers are possible: IA~IA, IA~TA and TA~TA. As a consequence only 3 peaks would be observed. However, more oligomers (than 3) were found in each cluster. Therefore the separation was not based on different end groups. In addition to the separations according to acid end groups and to acid mol fraction of TA, separation on sequence of acid monomers can occur. However, this is unlikely to occur, since in that case more oligomers should be present in the chromatogram (see Table 6-4). Consequently, the separation within an oligomeric cluster with similar p the separation is based on mol fraction TA (φ_{TA}).

The UV chromatograms discussed so far are the signals with wavelength of 275nm (λ_{275}). At 275nm both monomer units are detected with equal detector response. By looking at the signal at 305nm (λ_{305}) additional information about the chemical composition of the oligomers is obtained, since only TA can be detected.

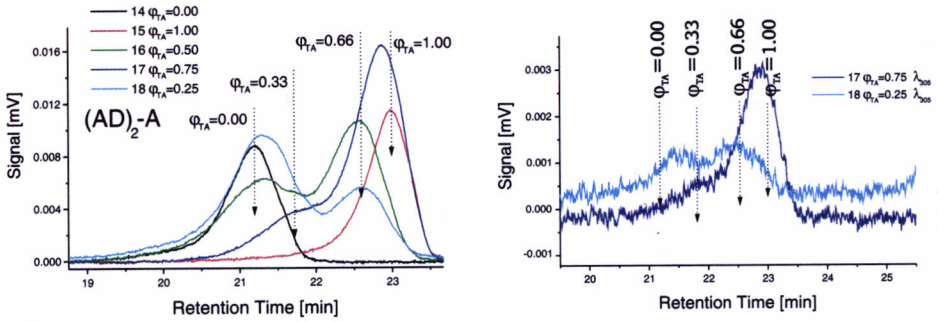


Figure 6-5, Detailed UV RP-GPEC chromatogram of the oligomeric cluster with degree of polymerization (p) of 2. Left: λ_{275} samples 14 (φ_{TA}=0.00, black), 15 (φ_{TA}=1.00, red), 16 (φ_{TA}=0.50, green), 17 (φ_{TA}=0.75. blue), and 18(φ_{TA}=0.25, cyan). Right: comparison of sample 17 (φ_{TA}=0.75, λ_{305}, blue) and sample 18 (φ_{TA}=0.25, λ_{305}, cyan). The arrows indicate the oligomers with different acid mol fraction (φ_{TA}) described in Table 6-4.

The first oligomeric cluster that can be seen is $(AD)_2$-A around 22 minutes. Four oligomers $(AD)_2$-A were found with different acid mol fraction of TA (φ_{TA}) as can be seen in Figure 6-5. This was in agreement with the amount of oligomers described in Table 6-4. Two oligomers eluted simultaneously with the oligomers of the homopolyesters, and two oligomers eluted in between the two homopolymers.

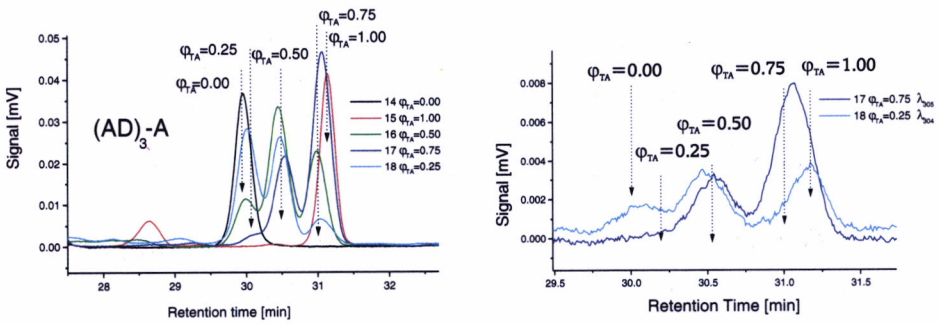


Figure 6-6, Detailed UV RP-GPEC chromatogram of the oligomeric cluster with degree of polymerization (p) of 3. Left: λ_{275} samples 14 (φ_{TA}=0.00, black), 15 (φ_{TA}=1.00, red), 16 (φ_{TA}=0.50, green), 17 (φ_{TA}=0.75. blue), and 18(φ_{TA}=0.25, cyan). Right: comparison of sample 17 (φ_{TA}=0.75, λ_{305}, blue) and sample 18 (φ_{TA}=0.25, λ_{305}, cyan). The arrows indicate the oligomers with different acid mol fraction (φ_{TA}) described in Table 6-4.

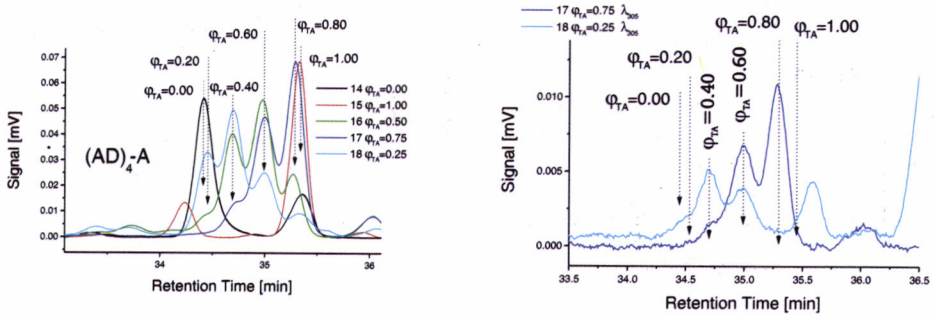


Figure 6-7, Detailed UV RP-GPEC chromatogram of the oligomeric cluster with degree of polymerization (p) of 4. Left: λ_{275} samples 14 (φ_{TA}=0.00, black), 15 (φ_{TA}=1.00, red), 16 (φ_{TA}=0.50, green), 17 (φ_{TA}=0.75. blue), and 18(φ_{TA}=0.25, cyan). Right: comparison of sample 17 (φ_{TA}=0.75, λ_{305}, blue) and sample 18 (φ_{TA}=0.25, λ_{305}, cyan). The arrows indicate the oligomers with different acid mol fraction (φ_{TA}) described in Table 6-4.

From Table 6-4, the oligomers (retention times approx. 21 minutes and 23 minutes) are assigned to respectively the oligomers φ_{TA}=0.00 and φ_{TA}=1.00. The other two oligomers (retention times approx. 21.5 and 22.5 minutes) are assigned to the oligomers φ_{TA}=0.33 and φ_{TA}=0.66.

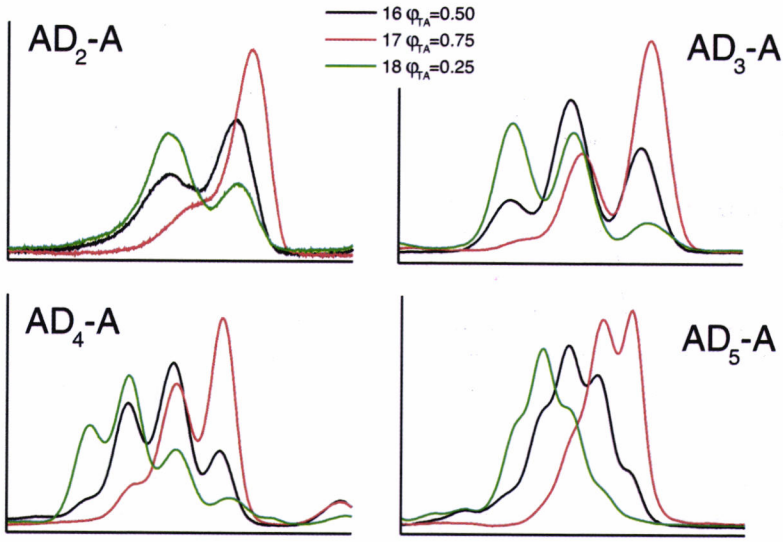


Figure 6-8, Peak distributions of the copolymer samples 16 (φ_{TA}=0.50, black lines), 17 (φ_{TA}=0.75, red lines) and 18(φ_{TA}=0.25) for the oligomer clusters $(AD)_2$-A, $(AD)_3$-A, $(AD)_4$-A, and $(AD)_5$-A; retention time [min] versus signal λ_{275}.

In Figure 6-5 (*right*) the chromatograms of sample 17 (φ_{TA}=0.50, λ_{305}) and sample 18 (φ_{TA}=0.25, λ_{305}) are shown. The other three oligomers (φ_{TA}=0.33, φ_{TA}=0.66 and φ_{TA}=1.00) show an increase in the λ_{305} signal, consequently the oligomers must contain at least one TA group. Therefore, the λ_{305} signals of the samples 17 (φ_{TA}=0.50) and sample 18 (φ_{TA}=0.25) were used to elucidate φ_{TA} of each oligomer.

In Figure 6-6 the oligomers $(AD)_3$-A with different φ_{TA} are shown. The oligomer cluster $(AD)_3$-A must contain an additional oligomer (total of 5) compared to cluster $(AD)_2$-A as can be seen in Table 6-4. In the oligomer cluster $(AD)_3$-A 5 peaks were found (see Figure 6-6) which could be labeled φ_{TA}=0.00, φ_{TA}=0.25, φ_{TA}=0.50, φ_{TA}=0.75 and φ_{TA}=1.00. The λ_{305} signal of sample 17 clearly showed the different oligomers containing TA.

The oligomer cluster $(AD)_4$-A, see Figure 6-7, has also a distribution of oligomers as described in Table 6-4. The oligomers with low TA and low IA content were difficult to observe, since they co-eluted. However, by looking at chromatograms obtained at λ_{305}, the oligomers with different acid mol fraction of TA could be labeled (see on the right). In the λ_{275} signal the peak was noticed and in λ_{305} signal the peak was absent, therefore the peak must contain solely IA (see also *Chapter 4*). Similar results are found for the oligomer cluster AD_5-A (not shown). Again the oligomers with low TA content were difficult to be observed.

The overlays of all the oligomer clusters AD_p-A with different p of the different copolyesters are shown in Figure 6-8. The distributions of the oligomers clearly follow patterns from which CCDs can be translated.

For the calculation of the CCDs the dependency of the retention time (at top of the peak) of the oligomers $(AD)_p$-A with different acid mol fraction TA on the chemical composition (φ_{TA}) of each peak was used (see Figure 6-9).

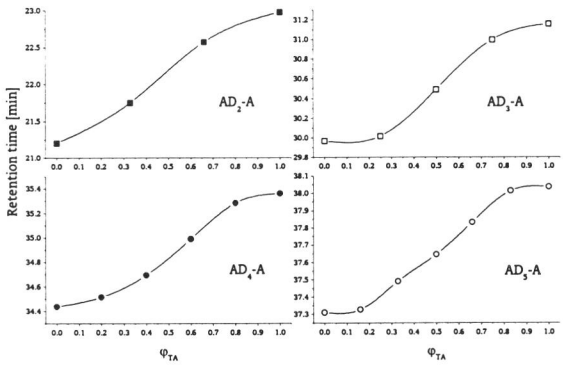


Figure 6-9, The chemical composition dependency of the retention composition of the oligomers AD_p-A with different degree of polymerization (p) on the fraction of TA of the RP-GPEC separation obtained with gradient A (see Chapter 4).

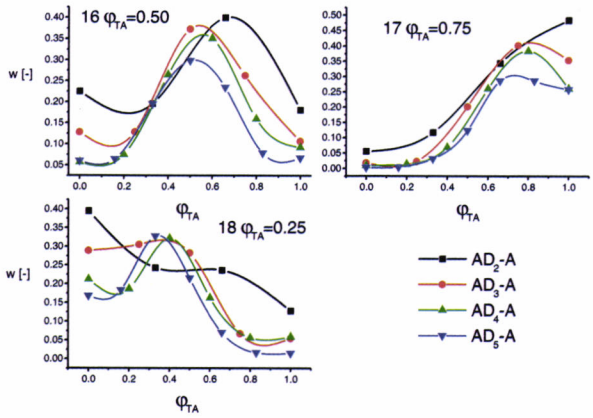


Figure 6-10, Chemical composition distributions of copolyesters 16 (φ_{TA}=0.50), 17 (φ_{TA}=0.75), and 18 (φ_{TA}=0.25) for the oligomer clusters $(AD)_2$-A (black lines), $(AD)_3$-A (red lines), $(AD)_4$-A (green lines) and $(AD)_5$-A (blue lines).

The quantification of the CCD calculation was based on the peak heights (λ_{275}). Also the peak areas could have been used, however, de-convolution of the peaks should have been applied, which is not preferable.

The chemical composition dependencies show non-linear curves, especially at the extreme fractions ($\varphi_{TA}\approx0.00$ and $\varphi_{TA}\approx1.00$). However, the chemical composition distributions of the different oligomers with different degree of polymerization could still be calculated. For the calculation of the CCD, the peak heights of the oligomers were used.

The CCD's obtained for different degrees of polymerizations are similar for a given copolymer (see Figure 6-10). The CCD of oligomer cluster $(AD)_2$-A is slightly different from the others. This is due to the limited number of individual species in the case of $(AD)_2$-A. With increasing degree of polymerization (p) the number of oligomers increases, consequently more detailed CCDs are obtained. This can be noticed by studying the statistically determined CCDs (φ_{TA}=0.50) in Figure 6-11 obtained from Table 6-4. The top of each distribution is in agreement with the overall composition of the specific copolymer.

Although only the CCDs of oligomers with low degree of polymerization were calculated, these CCDs are thought to be representative for the whole sample. In Figure 6-12 the experimentally determined CCD's of the copolyesters are compared with the statistical CCDs obtained for the oligomer cluster AD_5-A.

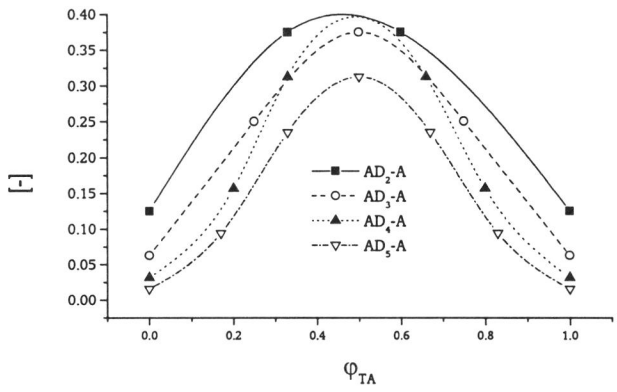


Figure 6-11, The theoretical chemical composition distributions of the oligomers with different degree of polymerization (p) for φ_{TA}=0.50 obtained from Table 6-4.

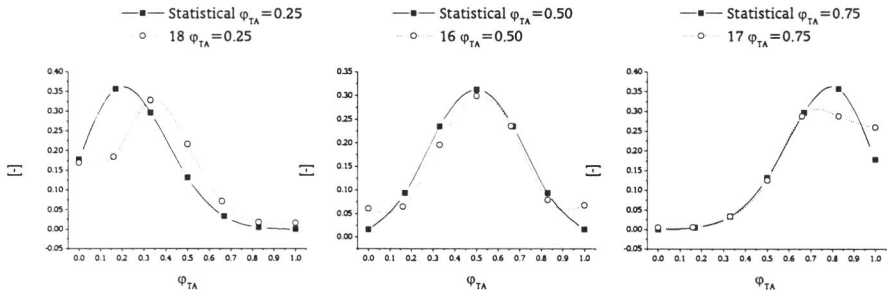


Figure 6-12, The comparison between the experimental chemical composition distribution (CCD) of the oligomers $(AD)_5$-A of the different copolymers 18 (left, φ_{TA}=0.25), 16 (middle, φ_{TA}=0.50) and 17 (right, φ_{TA}=0.25) (—○—) and the theoretical chemical composition distribution obtained from statistics ().

The comparison in Figure 6-12 shows that the CCDs of copolymers deviate from the statistically determined CCDs based on similar reactivities of the two acids. This deviation can again be explained by different reactivities of the two acids IA and TA, but more likely by different reaction times. The experimental obtained CCDs of the copolyesters 16 (φ_{TA}=0.50) and 17 (φ_{TA}=0.75) showed good agreement with the statistical determined CCDs. The experimental CCD of the copolyesters 18 (φ_{TA}=0.25) was different from the statistically calculated CCD. This deviation is thought to be caused by the low reaction time of sample 18 (φ_{TA}=0.25). The reaction time of the second step (addition of IA) of sample 18 (φ_{TA}=0.25) is much shorter (with respect to the amount of IA added in the second step) compared to the

reaction time of the other copolyesters (see Table 6-1). Probably, this results in an incomplete reaction of IA monomer in the second step. As already mentioned before, the copolyesters reactions were stopped when a certain acid value was reached (35 [mg KOH/g]). The stop condition based on the acid value appears to be incorrect. However, this point needs further investigation.

6.3.3 *Trans-Esterfication of Homopolyesters*

In Figure 6-14 the first part of the RP-GPEC UV chromatograms of the samples 16 (φ_{TA}=0.5), and the transesterfication product 24 (φ_{TA}=0.50) are shown. Figure 6-14 shows that the trans-esterfication sample 24 is not similar to the sample 16 (φ_{TA}=0.50) copolyester.

The overall compositions are equal, and if the transesterfication reaction would have been completed, the CCDs should be equal. The CCDs calculated from the chromatograms are shown in Figure 6-13. The CCDs are completely different. Sample 24 is best described as a polyester blend of copolymer and the two homopolymers, in other words the trans-esterfication reaction was not complete. No additional catalyst was added to the transesterfication reaction, this is likely to be the cause of the incomplete transesterfication.

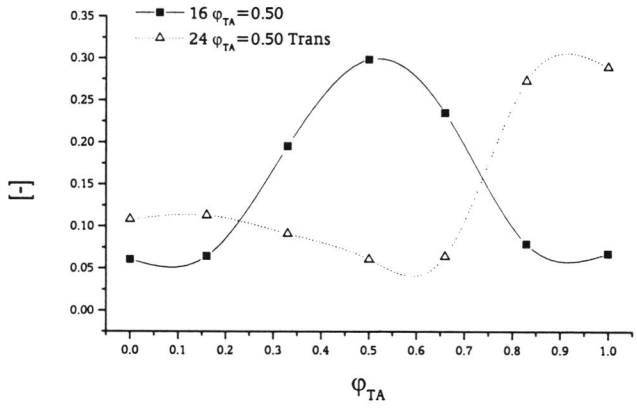


Figure 6-13, The chemical composition distributions of the samples 16 random copolyester (φ_{TA}=0.50).

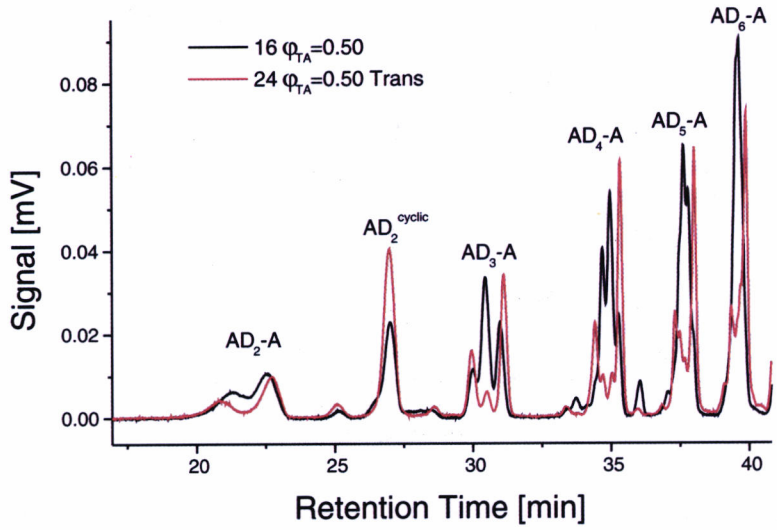


Figure 6-14, Detailed part of the RP-GPEC UV chromatograms of the 16 (copolyester φ_{TA}=0.50, black line), and 24 (transesterfication polyester φ_{TA}=0.50, red line).

6.3.4 Commercial Samples

The commercial samples were not identified by ESI-MS. Since the chromatograms of the copolyester samples are assigned using model compounds, the chromatograms of the commercial copolyesters could be interpreted. The commercial polyesters all consisted of NPG, IA and TA. The overall acid mol fraction of TA was in all cases 0.75. This means that the commercial copolyesters should be comparable to copolyester sample 17. The only difference between sample 17 and the commercial polyesters was the addition of trimethylol propane (TMP) as an additional alcohol functional monomer. TMP was added to increase the functionality of the polyesters. By using NPG, oligomers with only two functional end groups will be obtained, whereas when TMP is used oligomers with more than two functional end groups will be obtained. The functional end groups of these copolyesters are very important, since a cross-linking reaction with the functional end groups will occur during curing.

Since the detailed information can be found in the first part of the chromatogram, the first part of the RP-GPEC UV chromatograms of the commercial polyesters and sample 17 are shown in Figure 6-15.

Differences were found in the mono-acid oligomer clusters $(AD)_p$. When different amounts of TMP are used, different mono-acid peaks will be found. Some of the commercial polyester lack AD_p.

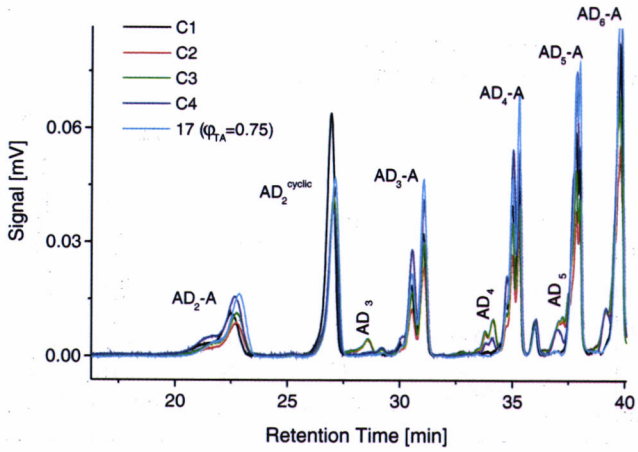


Figure 6-15, The RP-GPEC UV chromatograms of the commercial polyesters and sample 17 using gradient B (see Chapter 4).

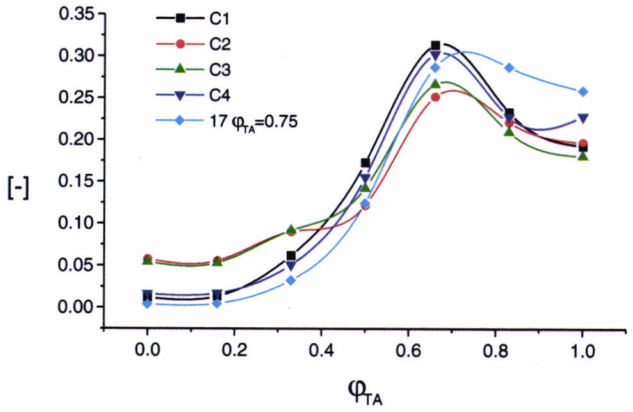


Figure 6-16, The chemical composition distributions of the commercial samples C1 (black line), C2 (red line), C3 (green line) and C4 (blue line) and the copolymer sample 17 (φ_{TA}=0.75, cyan line) obtained with gradient B.

The differences with respect to the AD_p oligomers might be due to a different overall ratio acid/alcohol monomer or the monomer addition method. As already mentioned, the two acid monomers are not miscible and therefore, the acid monomers are added separately at different reaction times.

The CCDs obtained for the different commercial samples show differences. Although the overall compositions were the same, the experimentally obtained CCDs were somewhat

different. These differences in the CCD might cause different properties. However, this was never studied for these polyesters and therefore no real conclusions can be drawn for the relationship between chemical composition and properties.

For sample 17 a mol fraction of NPG of 0.47 was used and the NPG was added in total in the first step (see experimental). Different fractions of NPG could have been used for the commercial copolyesters and, in addition, NPG could have been partially added in second step of the synthesis (instead of in total in the first step). This will influence the microstructure of the polyesters and might result in an increase in $(AD)_p$ oligomers.

6.4 *Conclusions*

The copolyesters of NPG, IA and TA were successfully analyzed. RP-GPEC was used to separate the copolyesters according to molar mass, chemical composition, and functional end groups. Two different gradients were applied: gradient A and gradient B. The ternary gradient B showed a superior resolution in the low molar mass region, but the resolution decreases rapidly with increasing molar mass. On the other hand, the binary gradient A showed a very high resolution with respect to molar mass.

A lot of information about the chemical composition and the functionalities was found in the beginning of the chromatogram. The chemical composition distribution of the copolyester samples for different oligomeric clusters could be determined. Differences between commercial copolyesters with similar overall compositions (φ_{TA}=0.75) and comparable MMDs were found. These differences are expected to be caused by different monomer addition methods.

6.5 *References*

1. F. Martinazzo, G. Brocca, R. Cavalieri, and A. Martina, *Pitture e Vernici Europe*, **9**, 5-9 *(1994)*.

Chapter 7

Characterization of Block Copolymers by Gradient Polymer Elution Chromatography (GPEC)

The characterization of different types of block copolymers is described in this chapter. The block copolymers studied were obtained by anionic polymerization and by two step free radical polymerization. Gradient polymer elution chromatography (GPEC) was applied to characterize the block copolymers. Two gradients were applied, a reversed phase gradient and a normal phase gradient. By using the two gradients, quantitative information on the amount of homopolymer present in the block copolymer sample was obtained.

7.1 Introduction to Block Copolymers

Block copolymers can consist of two or more blocks, each consisting of a different type of monomer. The block copolymers described in this chapter, all consist of two monomers. They are synthesized by multi step copolymerizations: 1) the synthesis of functional precursor of monomer A molecules, and 2) the formation of a block copolymer A-B [1]. In the first step, a precursor molecule is formed consisting only of monomer A. This precursor molecule has functional groups, on which, in the second synthesis step, the other block can grow. The precursor molecule can have one or more functional groups depending on the desired type of block copolymer.

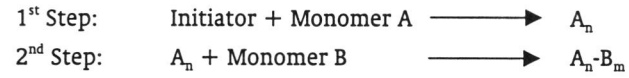


Figure 7-1, Reaction scheme of a two step block copolymerization.

In the second step the functional groups can be 'initiated' in different ways depending on the functional group(s). The functional groups have to be activated by thermal initiation or initiation by UV light in order to obtain chemically bonded blocks. By adding monomer B, the block copolymer A-B is formed.

The functional group can also be a 'living' species. For instance, in anionic polymerization it might be the anionic initiator [2,3], which is still active, in atom transfer radical polymerization (ATRP) the 'functional' group is a halide which can be reversibly homolytically split of the chain via metal catalysis [4,5,6], and with controlled radical polymerization (CRP) the polymer molecules are reversibly trapped with nitroxides [7,8,9]. The latter polymerization techniques (anionic, ATRP, and CRP) are called living polymerization techniques, which means that the polymer molecules are 'alive' and all the polymer molecules are active during the polymerization. The monomers are added gradual in time, and the polymer molecules will grow further until the monomer is consumed completely or until the living species are deactivated. When living polymerization techniques are used, the second step starts when monomer A is consumed completely. The block copolymer is formed by adding monomer B.

Depending on the functional group of the precursor, different types of block copolymers occur (see Figure 7-2): di-block, tri-block, branched, star, etc. The block copolymers studied in this chapter are of the types di-block and tri-block.

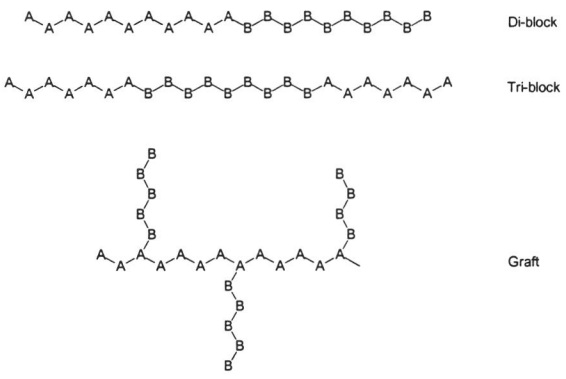


Figure 7-2, Different types of Block Copolymers consisting of block A and block B . The precursor is block A.

Side reactions may lead to chemically heterogeneous copolymers. Due to imperfections in the first step, the synthesis of the precursor, polymer molecules with no functionality (dead polymers) may be formed. In the second step, the precursor may not be activated. Both phenomena result in a homopolymer A. In the second step, secondary initiation of the monomer B may occur, which results in homopolymer B. Consequently, the final product can be a blend of two homopolymers and a block copolymer.

7.2 Characterization of Block Copolymers

Different analytical techniques can be used to characterize block copolymers. NMR, IR spectroscopy, and SEC are the most common techniques. Techniques as NMR and IR can give information about the overall chemical composition of a copolymer sample. However, the overall chemical composition cannot distinguish between a polymer blend and a copolymer with the same overall chemical composition. As mentioned in the previous paragraph, a block copolymer sample can be a blend of block copolymer molecules and homopolymer molecules. Consequently, the overall chemical composition is not a key parameter for a block copolymer sample.

With SEC separation according to hydrodynamic volume occurs. The hydrodynamic volume of a polymer molecule depends on the applied solvent, the molar mass, molecular configuration (branching) and the chemical type of the molecule. By applying SEC with triple detection (UV spectroscopy, refractive index, and viscosity), accurate molar masses can be determined for homopolymers. However, when copolymer samples are analyzed, SEC can be used to compare different samples. Due to the chemical differences of the polymer only the $\overline{M}_n$ of copolymer samples can be determined [10]. Since the samples are a blend of homopolymers and block copolymer, 'absolute' molar masses cannot be determined. SEC with triple detection will be used to get an indication of the chemical structure (blocks, blend). SEC can give an indication of the existence of block copolymer by measuring an increase of the hydrodynamic volume of the copolymer versus the hydrodynamic volume of

the precursor. When the second monomer is polymerized on the precursor, the hydrodynamic volume of the precursor molecules will increase, and therefore the hydrodynamic volume of the final product will increase. This increase must be visible in the SEC chromatograms. However, also homopolymer B can be formed in the second step (see Figure 7-1). This additional homopolymer can also cause an increase in hydrodynamic volume of the final product. SEC can, therefore, not be used to give proof for the existence of block copolymer. By using two concentration detectors, such as a UV detector and a differential refractive index detector, a better indication can be obtained. As an example, in Figure 7-3, the SEC chromatograms are shown of a polystyrene/polymethyl methacrylate block copolymer and its polystyrene precursor [11]. The block copolymer was synthesized via a two step free radical copolymerization with di(methoxy-xanthogen) disulfide [12].

In the RI signal the contribution of the two segments, polystyrene and polymethyl methacrylate, can be seen. Homopolymer methyl methacrylate (PMMA) is only detected by the RI detector (3, blue line). The polymer blend (4, black line) shows two peaks in the RI signal and only one in the UV, since PMMA is not detectable by UV. The comparison of the UV chromatograms of the precursor and the block copolymer shows that a shoulder appears on the precursor peak (compare 1 and 2). The UV signal only shows the styrene segments. So, if a shoulder appears in the UV chromatogram, this shoulder must contain styrene units.

Hence, the shoulder in Figure 7-3 (2) must be block copolymer. Since no styrene monomer was present any longer, block copolymer molecules must be formed. The shoulder can also be seen in the RI chromatograms of the block copolymer. In this case, SEC can be used to obtain an verification of the presence of block copolymer. But when solely non-chromophoric components are applied, SEC is not applicable as reliable characterization method.

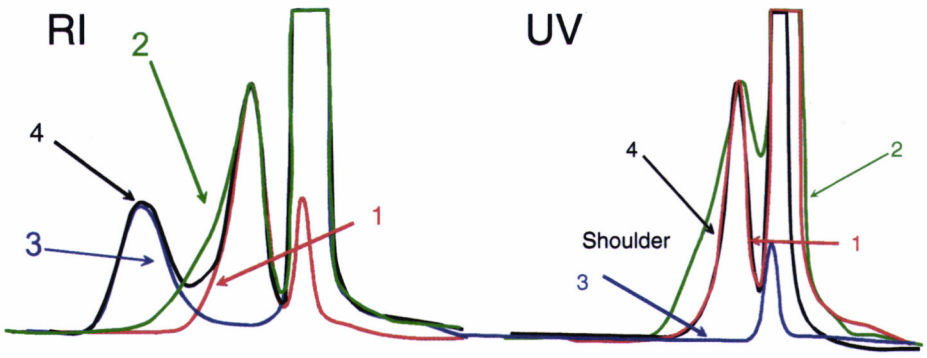


Figure 7-3, UV (λ_{254}) and RI SEC chromatograms of precursor polystyrene (1, red lines), block copolymer styrene/methyl methacrylate (2, green lines), homopolymer methyl methacrylate (3, blue lines), and a polymer blend of the precursor polystyrene and the homopolymer methyl methacrylate (4, black lines) [11].

7.3 Characterization of Block Copolymers by GPEC

In the literature the characterization of block copolymers by gradient elution HPLC is seldom described. *Glöckner et al.* [13,14] compared the elution behavior of random copolymers and block copolymers. *Augenstein et al.* [15] described the separation of decyl methacrylate/ methyl methacrylate block copolymers obtained with group transfer polymerization. The experiments described in this chapter are based on the experiments described by *Augenstein et al.*.

With GPEC, polymers can be separated according to chemical composition. Reversed phase (polar solvent/non-polar column) and normal phase (non-polar solvent/ polar column) GPEC can be applied to obtain two independent separations according to polarity/chemical composition.

For the characterization of polymers by HPLC, well-defined standards have to be used to describe, verify, and validate the separation. Other analytical techniques, such as mass spectroscopy, can be used to identify the separated components. However, no other analytical method than GPEC can distinguish between a blend of two homopolymers and a block copolymer with similar overall chemical composition.

The definition of a standard is very difficult, since no other techniques can prove existence of block copolymer or verify the value of the standard. However, the block copolymer samples obtained by 'controlled' block copolymerizations could be considered as standards. Polymerization methods, such as anionic polymerization [3] and atom transfer radical polymerization (ATRP) [6] thus could be applied to obtain block copolymers suitable for standardization. Anionic polymerization and ATRP are 'living' techniques, which can be applied to synthesize polymers with a specific molar mass. By using controlled polymerization techniques, homopolymers with a narrow MMD [4], and copolymers with narrow MMD and narrow CCD can be obtained.

7.4 Strategy

Based on the fact that block copolymers can be separated from the respective homopolymers [15], the following strategy has been developed in order to characterize block copolymers. Two separate gradients will be used to separate the homopolymers from the block copolymer. The separation of homopolymer and block copolymer is shown schematically in Figure 7-4.

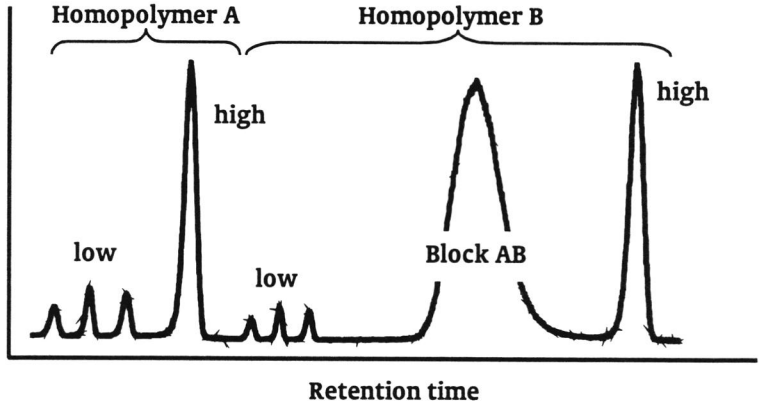


Figure 7-4, Schematic plot of the separation of two homopolymers A and B and a block copolymer.

In Figure 7-4 the molar mass areas of the homopolymers (area where the homopolymers elute from low molar mass to high molar mass) are shown. The optimal situation exists when the two areas *do not* overlap. The copolymer will elute in between the two homopolymer areas or in the elution area of the homopolymer which elutes last. The optimal conditions can also be represented as in Figure 7-5.

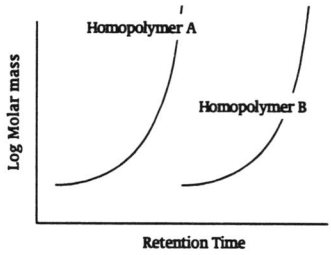


Figure 7-5, The molar mass dependencies areas of two homopolymers.

If the homopolymers have separate molar mass dependency areas, the block copolymer can be separated from homopolymer A and B. Different gradients are necessary when elution of copolymer and one of the homopolymers occur. By using a different gradients, the elution sequence can be reversed (homopolymer B elutes first, homopolymer A elutes last), and the block copolymer can then be separated from homopolymer B.

The strategy is thus to find two gradients that show the behavior described in Figure 7-4 and Figure 7-5. Two gradients will be applied, a reversed phase gradient and a normal phase gradient. In the reversed phase mode (polar solvents and non-polar column type) the separation will be depending on the non-polar segments or functionalities of the polymer chains. In the normal phase (polar column type and non-polar solvents) gradient the polar segments and functionalities of the polymer chain will govern the separation.

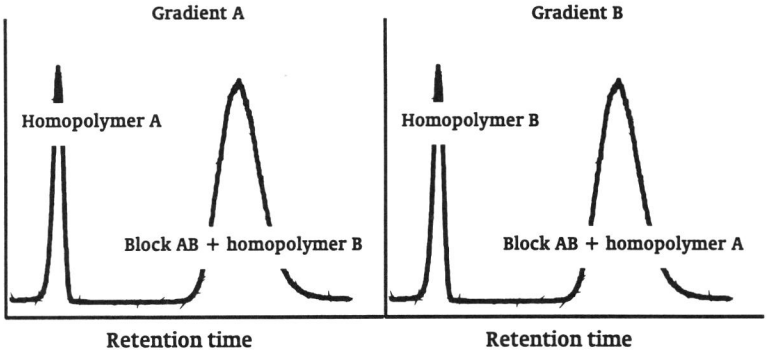


Figure 7-6, Schematic plot of the separation of block copolymer and homopolymer.

The separations of the two gradients are shown schematically in Figure 7-6. In gradient A, homopolymer A is separated from homopolymer B and the block copolymer. In gradient B, homopolymer B is separated from homopolymer A and the block copolymer. Consequently, quantitative information about the ratio homopolymer A/B and block copolymer can be obtained by applying the two gradients.

7.5 EXPERIMENTAL

7.5.1 BLOCK COPOLYMER SAMPLES

Different block copolymer samples were used: block copolymers synthesized by a two step anionic polymerization, and block copolymers produced by a two step radical polymerization. In the first step of the block copolymerizations, a precursor polymer A was synthesized, and in the second step, a new block B was formed. In order to find the optimal conditions, and to validate the separation, the homopolymers of the block copolymers were used. The homopolymers were SEC standards, and were well defined.

7.5.2 FREE-RADICALLY PREPARED BLOCK COPOLYMERS

The samples used in this study can be found in Table 7-1.

Table 7-1 Styrene-Isoprene Samples Synthesized by a two step free radical polymerization. Conv stands for the conversion, Prec stands for the precursor and block Copol is the block copolymer.

Sample	Type	w% Styrene	1st Step pre	Conv. %	Prec M_w* [kg/mol]	2nd Step block	Conv. %	Block Copol M_w* [kg/mol]
A	P(iP/S)	25	Isoprene	86	135	Styrene	97	168
B	P(S/iP)	57	Styrene	72	13	Isoprene	78	35

* Molar masses determined by conventional method in THF according to polystyrene standards

The two samples were polymerized in a two step radical copolymerization. The precursor of sample A was PiP and PS was the precursor of sample B. The conversion was determined by gas chromatography, and was defined as the amount of monomer (%) that was consumed. The MMDs were determined by conventional SEC in THF. The weight percentage styrene in sample A and B are, respectively 25 w% and 57 w% styrene.

7.5.3 Anionically Prepared Block Copolymers

Different types of KRATON block copolymers were used as reference material (see Table 7-2).

Table 7-2 KRATON Block Copolymers, synthesized by SHELL.

Name	Type	Sample Code	w% Styrene	Remarks
G1701	Styrene/ethylene,propylene	P(S/E,P)	37	linear diblock
D1107	Styrene/isoprene/styrene	P(S/PiP/S)	15	linear triblock
TR1101	Styrene/butadiene/styrene	P(S/B/S)	31	linear triblock
G1652	Styrene/ethylene,butylene/styrene	P(S/E,B/S)	30	linear triblock

The KRATON polymers were synthesized by anionic polymerization. Detailed information about the synthesis is not known. The P(S/E,P) and the P(S/E,B/S) block copolymers were probably obtained from the P(S/iP/S) and P(S/B/S) block copolymers via a hydrogenation reaction.

7.5.4 Homopolymer standards

The homopolymer standards of PS, PMMA, and PiP were used to describe the separation and to find the optimal conditions. Standards with different molar mass (Polymer labs, SEC calibration kits) were used to determine the molar mass dependencies of the homopolymers in the specific gradients.

7.5.5 Size Exclusion Chromatography

The SEC experiments were performed with a Kratos Spectroflow 400n solvent delivery system, a Separations UV detector (254nm), a Waters 410 DRI detector, a Viscotek Model 100 DP detector, and a Separation Marathon autosampler. The flow was 0.8 ml/min of tetrahydrofuran (THF) with 0.02 v% acetic acid (HAc). The column used was a Polymer Labs mixed-D 50 cm (temperature 30°C). The polymer samples were dissolved in THF + 0.02 v% HAc at different concentrations (± 1 mg/ml). The system was calibrated by polystyrene SEC standards of Polymer Labs (SEC calibration kit).

7.5.6 Reversed Phase GPEC

The gradient commonly used for RP-GPEC is shown in Table 7-3. The initial conditions were water/ACN (50: 50 vol%) pre-mixed in order to prevent mixing problems of the water and ACN in the pump.

Table 7-3 RP-GPEC Gradient.

Step	Time	% water/ACN (1:1)	% ACN	% THF	Flow ml/min
1	Initial	100	0	0	0.5
2	5	0	100	0	0.5
3	10	0	70	30	0.5
4	80	0	0	100	0.5
5	83	0	0	100	0.5
6	85	100	0	0	0.5

In the different experiments the steps 2 and 3 of the gradient might vary in time and solvent compositions. However, the gradient speed was kept constant in step 4 (at 1 % per minute). Also the initial conditions, the end conditions and the condition steps (2, 3 and 5) were not varied. The additional conditions for RP-GPEC were a Symmetry C18 15cm (Waters) column at 30°C, THF (not stabilized, Merck, HPLC grade), ACN (Merck), Water (MilliQ, Millipore), Varian 9010 solvent delivery system, UV 785A Separations detector (260nm), ELSD (Polymer Labs PL-EMD 960, N_2-flow 5.0ml/min, temp 70°C), autosampler (Separations, Basic Marathon).

7.5.7 Normal Phase GPEC

In Table 7-4 the gradient used for NP-GPEC is shown.

Table 7-4 NP-GPEC Gradient.

Time	% heptane	% THF	Flow ml/min
Initial	100	0	0.5
40	40	60	0.5
45	0	100	0.5
50	0	100	0.5
55	0	100	1.0
60	100	0	0.5

The additional conditions for NP-GPEC were a Sperisorb Silica 5μ 15cm (Alltech) column at 30°C, THF (not stabilized, Merck, HPLC grade), heptane (Merck), Varian 9010 solvent delivery system, UV 785A Separations detector (260nm), ELSD (Polymer Labs PL-EMD 960, N_2-flow 5.0ml/min, temp 70°C), autosampler (Separations, Basic Marathon).

7.6 Results and Discussion

7.6.1 Size Exclusion Chromatography

The SEC separation is dependent on differences in the hydrodynamic volume. Consequently, the retention volume is not only a measure of the molar mass, but also of the chemical composition of the polymer molecule.

7.6.1.1 Free-Radically Prepared Block Copolymers

The free-radically prepared block copolymers contain styrene and isoprene. The SEC chromatograms of a block copolymer can give an indication for the existence of chemically bonded blocks. Since PiP is not detectable by UV at 254 nm, the UV signal will only detect PS.

The Mark-Houwink parameters log K and a of PS and PiP are respectively -4.17 and 0.78, and -4.07 and 0.72. This difference is significant and will result in dependency of the retention volume on the chemical composition. This means that an increase in retention volume does not necessarily imply an decrease of molar mass. 'Absolute' molar masses are not determined, since the different Mark-Houwink parameters of PS and PiP will result in 'apparent' molar masses. The RI, UV, and DP chromatograms of the sample A and B are shown in Figure 7-7 and Figure 7-8, respectively. The difference between the RI chromatograms did not give proof for the existence of block copolymer molecules. However, on the 'high molar mass' part at lower retention volumes in the chromatogram, disturbances in all signals could be noticed. This might be an indication for the existence of chemically bonded blocks. PS could be noticed over the complete distribution, which might also be an indication for the presence of blocks.

The UV chromatogram of sample B revealed most of the information. When the two chromatograms of the precursor PS and the block copolymer sample are identical, no chemically bonded block exists. If chemically bonded blocks of PS and PiP exist, two different chromatograms should be obtained. The UV chromatograms showed that at lower retention (larger hydrodynamic volume) PS eluted. Since the precursor cannot react any further with styrene, this indicates the presence of block copolymer. The shift, which can be seen in the UV chromatograms, can also be noticed in the other chromatograms (DP and RI). However, Figure 7-8 is more informative than Figure 7-7.. The precursor PS was still present in the block copolymer. Apparently, not all of the precursor molecules have reacted. With GPEC the presence of free PS can be confirmed. The calculated data from the SEC measurements are shown in Table 7-5. The data are calculated via the universal calibration method (UC).

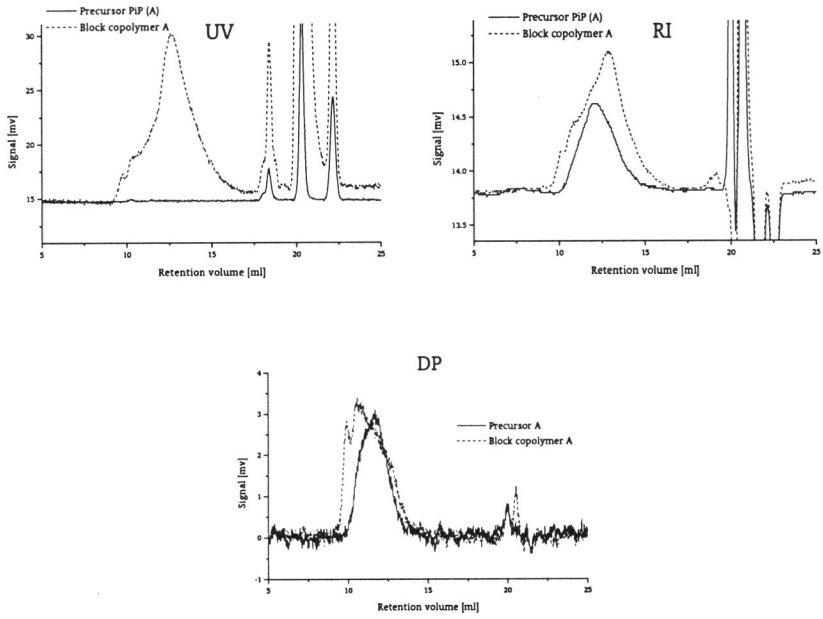


Figure 7-7 SEC chromatograms (UV, RI, and DP) of sample A precursor PiP (—) and block copolymer A PiP/PS(—).

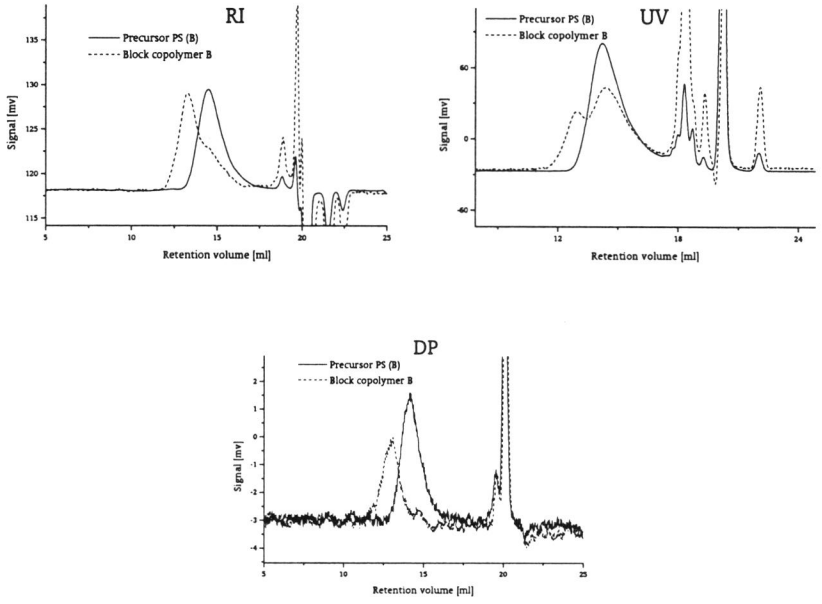


Figure 7-8 SEC chromatograms (UV, RI, and DP) of sample B precursor PS (—) and block copolymer PS/PiP (—).

Table 7-5, SEC UC data of the free-radically prepared block copolymers.

Sample	Molar mass	Precursor A	Block Copolymer A	Precursor B	Block Copolymer B
UV	$\overline{M}_n$	-	88,200	3,980	13,600
	$\overline{M}_w$	-	237,700	8,510	23,900
RI	$\overline{M}_n$	283,900	118,400	5,910	27,200
	$\overline{M}_w$	600,500	467,200	12,700	60,500

As mentioned before the Mark-Houwink parameters differ and therefore the separation on hydrodynamic volume will not yield equal results for PS and PiP polymers of identical molecular masses. Only for sample B the UV data gives reliable molar masses. Since the UV only detects the PS, the increase in molar mass can only be due to formation of block copolymer.

7.6.1.2 Anionically Prepared Block Copolymers

The UV chromatograms of the anionically prepared samples are shown in Figure 7-9. The SEC chromatograms of the KRATON block copolymers D1107 and TR1101 show nice narrow peaks, which indicates that the synthesis of the block copolymers was successful.

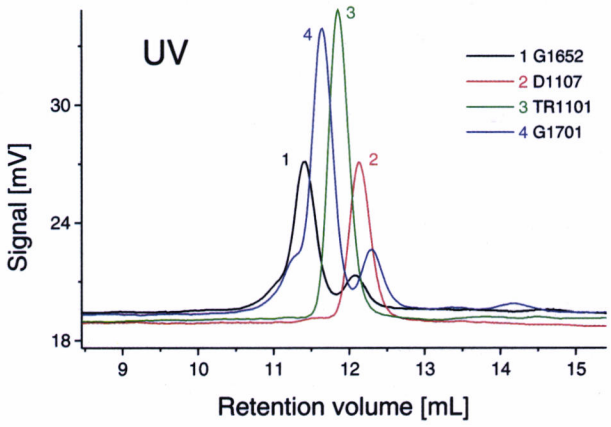


Figure 7-9, The UV SEC chromatograms of the anionically prepared block copolymers, G1652 (1, black line), G1701 (1, red line), TR1101 (3, green line) and D1107 (4, blue line)

The KRATON block copolymers G1652 and G1701 showed shoulders, which indicate that the synthesis did not work out that well. Since these block copolymers are hydrogenated, this is thought to be the cause of the shoulders. However, the peak widths were still comparable to the widths of the polystyrene standard peaks used for calibration, indicating a very low polydispersity. The molar mass data, calculated by the universal calibration method, are depicted in Table 7-6.

Table 7-6, SEC UC data of the anionically prepared block copolymers.

Sample	Molar mass	TR1101 P(S/B/S)	G1652 P(S/E,B/S)	G1701 P(S/E,P)	D1107 P(S/iP/S)
UV	$\overline{M}_n$	156,800	302,900	184,500	150,000
	$\overline{M}_w$	157,900	327,400	200,600	151,100
RI	$\overline{M}_n$	137,200	306,400	189,800	126,900
	$\overline{M}_w$	146,000	308,300	190,900	139,600

The data in Table 7-6 show that the anionically prepared block copolymers all have a very narrow molar mass distribution, comparable to that of homopolymer SEC standards (also synthesized by anionic polymerization).

7.6.2 Reversed Phase GPEC

The gradient used for all RP-GPEC separations is described in Table 7-3. The initial conditions were 1:1 water/ACN. The composition ACN increases up to 100%. The composition of THF was increased after 100% ACN was reached. As already mentioned, some of the

experiments were carried out with different gradient steps. As a consequence the chromatograms cannot be compared directly, therefore, the retention times of the chromatograms were all converted to percentage THF at the time of elution. For the calculation from retention time to volume fraction of THF (φ_{THF} in percent) Equation 2-11 (*Chapter 2*) was used.

7.6.2.1 Free-Radically Prepared Block Copolymers

With RP-GPEC the homopolymer PS can be separated from the homopolymer PiP and the block copolymer. The molar mass dependencies of PS and PiP in the RP-GPEC gradient are shown in Figure 7-10. The symbols represent the elution composition of the peaks of different PS SEC standards.

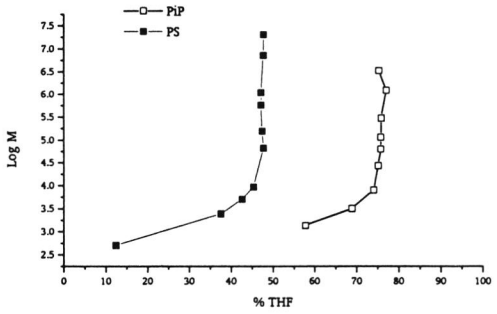


Figure 7-10, Molar mass dependency of PS (-■-) and PiP(-□-) for the RP-GPEC separation in water/ACN/THF gradient and symmetry C18 column.

From Figure 7-10, it can be seen that the molar mass dependencies of PS and PiP standards did not overlap in the RP-GPEC system. Nevertheless, the oligomers of PiP ($\overline{M}_w$<1,000 g/mol) eluted simultaneously with the high molar masses of PS. Since the block copolymers P(S/iP) have higher molar masses, this overlap will not cause problems (see Table 7-5). The RP-gradient (see Table 7-3) is suitable for the separation of PS/PiP copolymers. An elution difference of THF of about 25% was obtained, which is appropriate to characterize copolymers. After 50% THF no PS molecules will elute anymore, unless the segments are chemically bonded, like in a copolymer. Copolymer molecules elute in between the two elution limits of the homopolymers.

With the RP-GPEC gradient the block copolymer will be separated from the homopolymer PS, however, the block copolymer will elute simultaneously with the PiP homopolymer, if present. Styrene segments are detected by the UV detector (λ=254nm) and the ELSD. Isoprene segments will only be detected by the ELSD. Consequently, if the UV signal shows

polymer after the elution limit of PS, the polymer molecules must be copolymer. In Figure 7-11 the RP-GPEC chromatograms of sample A (precursor and block) are shown.

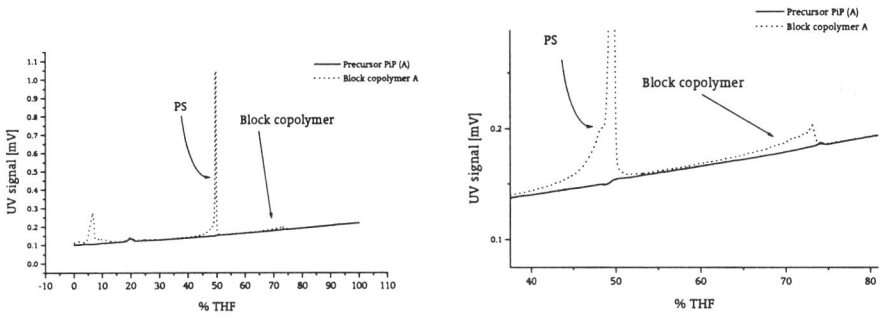


Figure 7-11 RP-GPEC UV (baseline corrected) chromatograms of the precursor PiP (–) and the block copolymer (···) of sample A.

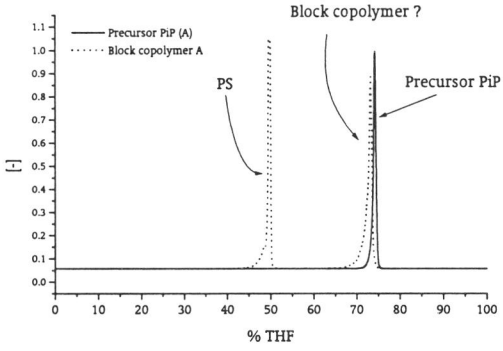


Figure 7-12, RP-GPEC ELSD chromatograms of the precursor PiP (–) and the block copolymer (···) of sample A.

The ELSD chromatogram of the block copolymer (Figure 7-12) shows two peaks: a PS peak and a block copolymer/PiP peak. The second peak PiP is shifted in % THF to less THF, compared to the PiP of the precursor. This shift is probably due to chemically attached styrene. The shift of the peak depends on the amount of styrene attached to the PiP precursor. The shift was about 1% THF, and the whole PiP peak had shifted which implies that most the PiP molecules had styrene molecules attached.

In the UV chromatogram (Figure 7-11) due to the block copolymer a clear UV-signal can be seen. The UV chromatogram shows that polymer with UV absorption elutes after the elution point of the homopolymer PS (50% THF). Since the UV detector does not detect PiP, the peak

can only be styrene units attached to PiP. This together with the shift of the PiP peak gives the proof of the existence of block copolymer.

Although block copolymer molecules exist in sample A, a large amount of free PS can be observed. The UV area of the PS peak is 90% and the UV area of the bounded styrene in block copolymer is 10%. Consequently, in the second step (first step precursor PiP) 90% of the reacted styrene is present as free homopolymer PS and only 10 % of the consumed styrene is attached to the precursor PiP.

In Figure 7-13 the RP-GPEC UV (baseline corrected) and ELSD chromatograms of sample B are shown. The chromatograms show two peaks: a PS peak from the precursor and a block copolymer/PiP peak. The chromatograms of the precursor are not shown, since they show the PS peak (same position but different height) as can be seen in the block copolymer.

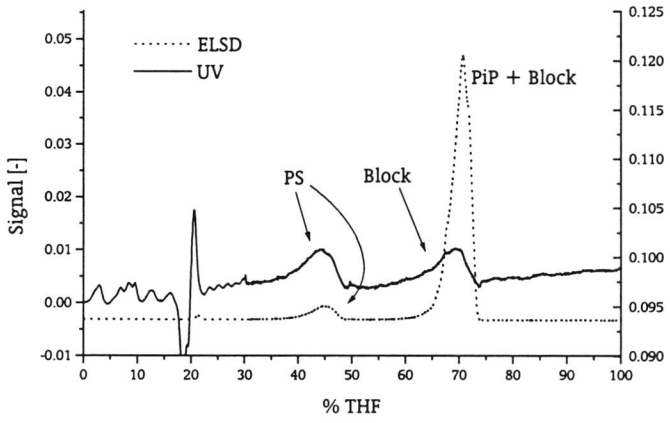


Figure 7-13, RP-GPEC UV (baseline corrected) and ELSD chromatogram of sample B P(S/iP).

The UV chromatogram shows that isoprene had reacted with the PS precursor. The ratio of the PS peak and the block copolymer peak in the UV signal are approximately equal. This implies, that approximately 50% of the precursor reacted with isoprene. The ELSD chromatogram showed a small PS peak and a large block copolymer/PiP peak. Although the response of the ELSD was are not equal for PS, PiP, and the block copolymer, the areas of the peaks gave some indication of the amounts. From Figure 7-13, no reliable information can be obtained about the amount of PiP that has been formed in the second step. NP-GPEC had to be applied in order to give proof of homopolymer PiP in the block copolymer. The peak areas of sample A and B are shown in Table 7-7.

Table 7-7, Peak areas of the RP-GPEC UV and ELSD chromatograms of sample A and B.

Sample	UV (% area)		ELSD (% area)	
	PS	Block	PS	Block/PiP
A	90	10	50	50
B	50	50	5	95

The area percentage of the ELS detector signal has to be treated with care. The ELS detector response is not equal for different types of polymers [16]. Also the molar mass and the eluent composition can have a large influence on the ELS detector response and the signal does not vary linearly with the analyte concentration. Consequently, the ELS detector signals and areas cannot be compared accurately. However, the trend of the areas obtained with ELS detector can give an indication. The quality and selectivity of modern ELS detectors have improved significantly. In spite of the poor quantification, the ELS detector areas are still used for the characterization, since the ELS detector is the only detector that can detect PiP (polyolefins) in GPEC.

In Figure 7-14 the three RP-GPEC ELSD chromatograms of sample A and B and KRATON D1107 are shown.

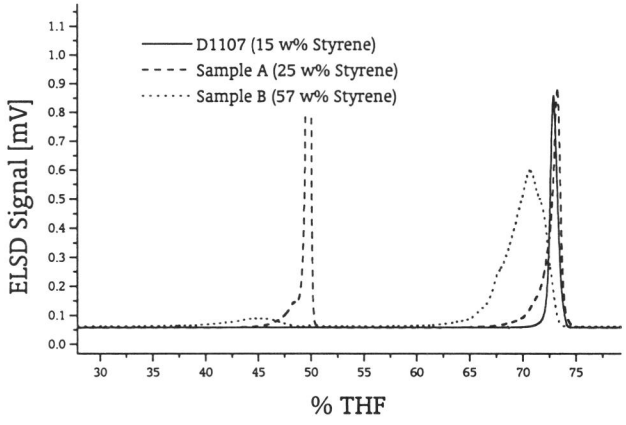


Figure 7-14 RP-GPEC ELSD chromatograms of sample A (– –), sample B (···), and KRATON D1107 (–).

The three samples are PS/PiP block copolymers with different ratio Styrene/isoprene. The peak of the KRATON D1107 was narrower in comparison to the peaks of samples A and B. This implies that the KRATON D1107 had a much narrower chemical composition distribution, *i.e.* a narrower block length distribution. In samples A and B (see Figure 7-14) PS homopolymer is present. In KRATON D1107 no PS homopolymer could be noticed. PiP might be present in the block copolymer since PiP co-elutes with the block copolymer. NP-GPEC will provide additional information on the presence of PiP.

7.6.2.2 Anionically Prepared Block Copolymers

The ELSD chromatograms of the anionically prepared block copolymers are shown in Figure 7-15.

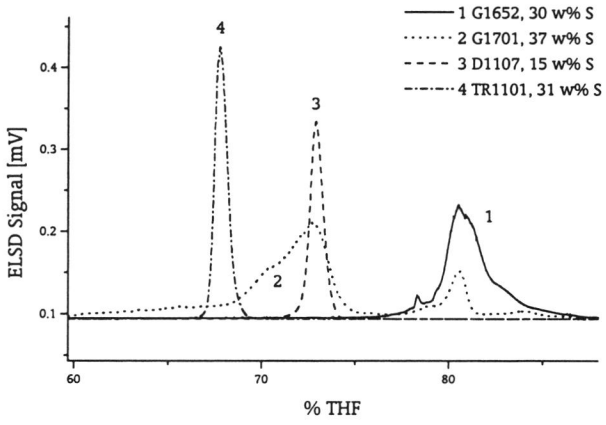


Figure 7-15 RP-GPEC ELSD Chromatograms of the anionically prepared block copolymers, G1652 P(S/E,B/S —), D1107 P(S/iP/S – –), G1701 hydrogenated P(S/E,P ···), and TR1101 P(S/B/S –·–·).

There is a big difference between the hydrogenated block copolymers G1652 and G1701, and the block copolymers D1107 and TR1101. D1107 and TR1101 show only one small peak, and the hydrogenated block copolymers show a broad peaks. The broadening and the separation into multiple peaks are caused by chemical inhomogeneities. The hydrogenation step (incomplete hydrogenation) probably caused these chemical differences.

The separation according to chemical composition (w%S) is significant. The elution behavior of polybutadiene (PB) is comparable to the elution behavior of polyisoprene (PiP), see Figure 7-16.

TR1101 has a higher styrene content (31 w%) than D1107 (15 w%). TR1101 elutes at a lower % THF than D1107 due to the difference in styrene content. The copolymer with the highest styrene content will elute at lower % THF.

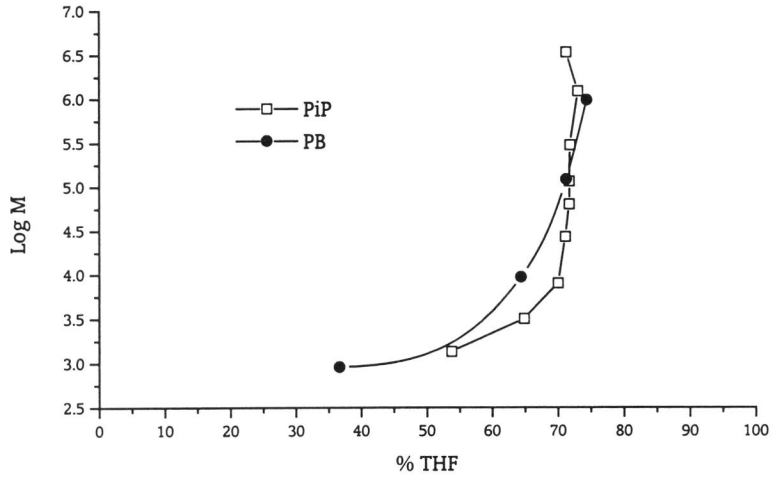


Figure 7-16, Molar mass dependencies of PiP (-●-) and PB (-□-) in the RP-GPEC gradient (see Table 7-3).

7.6.3 Normal Phase GPEC

7.6.3.1 Free-Radically Prepared Block Copolymers

The NP-GPEC chromatograms were all performed with the gradient in Table 7-4. The molar mass dependency intervals of the two homopolymers can be seen in Figure 7-17.

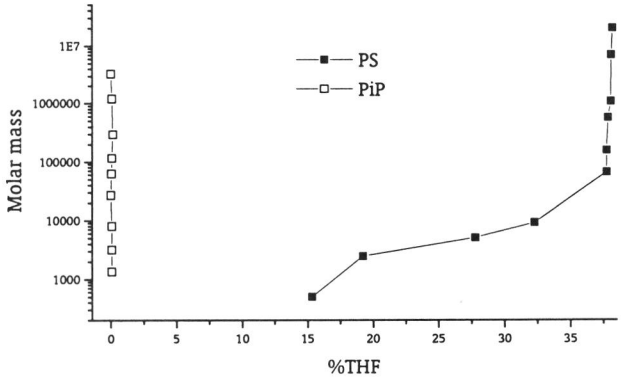


Figure 7-17, Molar mass dependency of PS (■) and PiP (□) in the system heptane/THF on a silica column (35°C).

As can be observed in Figure 7-17, the NP-PGEC gradient can be used to separate the block copolymers. The NP-GPEC chromatograms of samples A and B are shown in Figure 7-18.

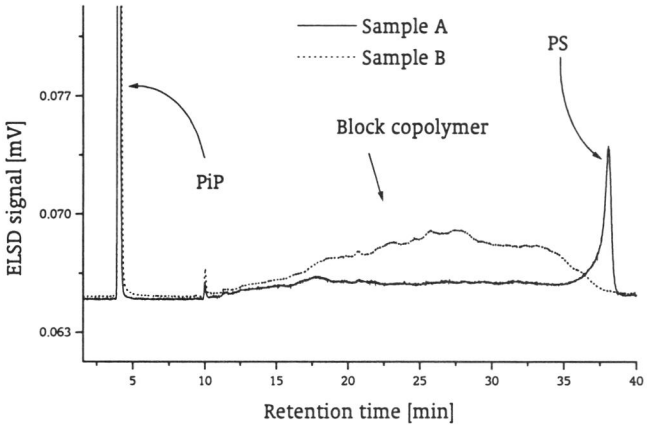


Figure 7-18, NP-GPEC ELSD chromatograms of sample A (–) and B (···).

The chromatograms show broad irregular peaks, representing polymer eluted between the homopolymers (PS and PiP). The peaks were fractionated, and pyrolysis gas-chromatography mass spectrometry (pyrolysis GC-MS) was applied to identify the fractions. In the first peak and the broad peak styrene and isoprene were found, indicating that the NP-GPEC separation is not optimal. This pre-elution of styrene can be explained by breakthrough [17]. The polymer molecules co-elute with the injected THF.

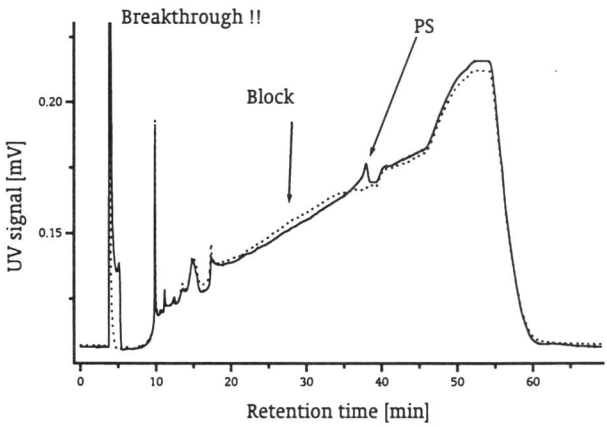


Figure 7-19, NP-GPEC UV chromatograms of sample A (–) and B (···).

The NP-GPEC UV chromatograms showed a slight increase of the UV signal. The UV signals of the NP-GPEC separations were not satisfying. The response was very low. This low response was probably due to the broadening of the peaks caused by the molar mass dependency (see Figure 7-17).

7.6.3.2 Anionically Prepared Block Copolymers

The NP-GPEC chromatograms of the KRATONS are shown in Figure 7-20.

The anionically prepared block copolymers show highly reproducible chromatographic peaks in comparison with the chromatograms obtained from the free-radically prepared block copolymers (see Figure 7-18). G1652 (hydrogenated block copolymer) overlaps with D1107 (styrene/isoprene/styrene tri-block copolymer). The peaks of G1701 were fractionated and also identified with pyrolysis GC-MS. The first peak (±2.5 minutes) was identified as PS and is caused by breakthrough (described in *Chapter 2*). In the second peak (±4 minutes) no polymer was found and is regarded as the solvent peak. Consequently, no PiP homopolymer was present in the sample G1701. Since the solvent peak of other KRATON block copolymers are of comparable heights, the KRATON are thought to have no PiP present.

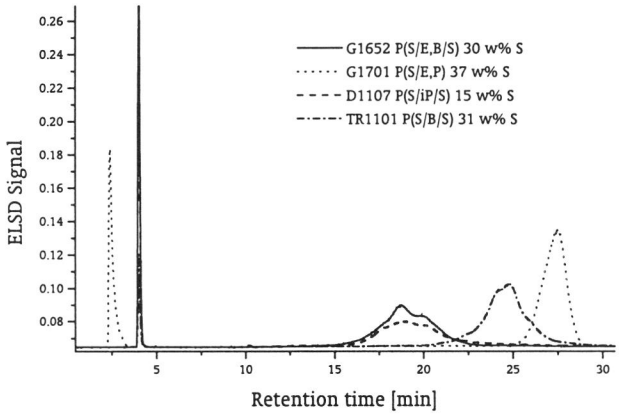


Figure 7-20, NP-GPEC ELSD Chromatogram of KRATONS G1652 P(S/E,B/S —), D1107 P(S/iP/S – –), G1701 hydrogenated P(S/E,P ···), and TR1101 P(S/B/S –·–·).

The NP-GPEC separation on a silica column appears to have a lot of possibilities. The results of the KRATON block copolymers are useful and different information is obtained in comparison with the results of the RP-GPEC. Nevertheless, the results obtained for the free-radically prepared block copolymers were not suitable. The NP-GPEC measurements were not optimal and additional research is necessary.

7.7 CONCLUSIONS

In Table 7-8 a summary of the results discussed in the former paragraph are shown.

Table 7-8 The results of the characterization of the block copolymers. The presence (+) of homopolymer and copolymer and the percentage of homopolymer and copolymers.

Sample	PS	PiP	Block	%Block
A	+	+	+	<10
B	+	+	+	<10
D1107	-	-	+	100
TR1101	-	-	+	100
G1652	-	-	+	?
G1701	-	-	+	?

The samples obtained with free radical polymerization are all polymer blends of the homopolymers and block copolymer.

The KRATON samples all show nice distributions with SEC as well as with GPEC. However, the hydrogenated KRATON block copolymers showed multiple peaks in SEC and GPEC. Probably the multiple peaks are caused by an incomplete or uncontrolled hydrogenation reaction.

The overall conclusion is that the strategy described in this chapter was successful to characterize block copolymers. RP-GPEC, as well as NP-GPEC can be applied to investigate block copolymers. Although NP-GPEC shows similar results to RP-GPEC, NP-GPEC has to be studied in more detail in order to draw reliable conclusions.

7.8 REFERENCES

1. G. Odian, *"Principles of Polymerization"*, second edition, John Wiley & Sons, New York, *1970*.
2. M. Szwarc, M. Levy and R.J. Milkovich, *J. Am. Chem. Soc.*, **78**, *2556 (1956)*.
3. M. Szwarc, *Nature*, **178**, *1168 (1956)*.
4. D.M. Haddleton and A.J. Shooter, *Polym. Prep.*, **38 (1)**, *738 (1997)*.
5. T. Matyjaszewski, T.E. Patten and J. Xia, *J. Am. Chem. Soc.*, **119**, *674 (1997)*.
6. T.E. Patten, T. Abernathy and K. Matyjaszewski, *Science*, **272**, *866 (1996)*.
7. D.H. Solomon, E. Rizzardo and P. Caciolo, **P. Eur. Pat. Appl. 135280 (1985)**, *Chem. Abst.*, **102**, *221335q (1985)*.
8. C.H.J. Johnson, G. Maud, D.H. Solomon, T.H. Spurling and D.J. Vearing, *Aust. J. Chem.*, **43**, *1215 (1990)*.
9. S. Bon, *"Debut"*, Ph.D. thesis, Eindhoven University of Technology, Eindhoven, *1998*.
10. J.M. Goldwasser, *Proc. Int. Gel Permeation Chromatography Symposium 1989*, Newton, MA, *150 (1990)*.
11. Paul van Haeren, Internal report, AKZO NOBEL *(1995)*.
12. K. Wendler, A.K. Balyan, Th. Dittrich and M. Fedtke, *Plaste u. Kautschuk.*, **8**, *446-448 (1982)*.
13. G. Glöckner and A.H.E. Müller, *J. Appl. Polym. Sci.*, **38**, *1761-1774 (1989)*.
14. G. Glöckner and J.H.M. van den Berg, *J. Chromatogr.*, **550**, *629-638 (1991)*.
15. M. Augenstein and M.A. Müller, *Makromol. Chem.*, **191**, *2151-2172 (1990)*.

16. T. Willems, *"Evaporative Light Scattering Detectors"*. Internal report, Eindhoven University of Technology, *1997*.
17. T.L.J. Willems, M.Sc. Thesis, Eindhoven University of Technology, Eindhoven, The Netherlands (1993).

Epilogue

As new polymer architectures with novel properties will be explored and investigated, their microstructure becomes more complex. With increasing complexity of the microstructure, the need for separation techniques, such as HPLC, is an absolute necessity. However, when a new polymer is developed, the (complete) microstructural analysis is seldom taken into account. Unfortunately, this phenomenon is frequently observed in the polymer industry, and the copolyesters described in this thesis are a good example. Although they have been applied for many years in coatings, they have never been studied to the same extent as described in this thesis.

GPEC can be applied to characterize the copolyesters in detail. However, various peaks could never have been assigned without the identification by mass spectrometry (MS). The combination HPLC-MS was shown to be a powerful tool.

Whether the strategy or (less likely) the conditions described in this thesis can be applied to characterize other copolyester systems remains to be proven. The relevant copolyester system seems to be among the most difficult polymer structures to separate, since isophthalic acid and terephthalic acid are isomers. However, by using well-defined homopolyester and copolyester standards, the separation of copolyesters could be achieved.

Unfortunately, GPEC is not universally applicable. For each type of polymer, different optimal conditions have to be determined which can make GPEC a time-consuming method. As long as the polarity difference between the polymers to be separated is large enough to obtain selectively, separation between the specific polymers can be achieved. However, if the polarity difference is very small, separation of the specific polymers might be impossible with the equipment available nowadays.

For proper characterization of polymers, an understanding of the physical-chemical characteristics of polymers is needed for the correct interpretation of the data. Nevertheless, it will still be very difficult to obtain a relationship between the microstructure of the polymer and the (mechanical) properties, which is beyond the scope of this thesis.

Despite all the difficulties encountered when applying HPLC and MS to polymers, the need for separation techniques will never become obsolete.

List of Symbols and Abbreviations

A	di-acid monomer
a	Mark-Houwink parameter [-]
ACN	acetonitrile
$(AD)_p$	oligomers with an acid end group and a hydroxy end with degree of polymerization p
$(AD)_p^{cylic}$	cyclic oligomers with degree of polymerization p
$(AD)_p$-A	oligomers with two acid end groups with degree of polymerization p
c	concentration [mg/ml]
CCD	chemical composition distribution
CSC	critical solvent composition
D	di-alcohol monomer
DRI	differential refractive index
DSC	differential scanning calorimetry
DP	differential pressure (viscosity detector)
ELS	evaporative light scattering
ESI	electrospray ionization
FTD	functional type distribution
GPEC	gradient polymer elution chromatography
HAc	acetic acid
HFIP	hexafluoro isopropanol
HPLC	high performance liquid chromatography
IA	isophthalic acid
IR	infrared
K_η	Mark-Houwink parameter [dl·mol/g^2]
K_{enth}	distribution coefficient based on enthalpy effects [-]
K_{entr}	distribution coefficient based on entropy effects [-]
k_{funct}	capacity factor of a functional group of a polymer [-]
k_{pol}	capacity factor of a polymer [-]
k_{segm}	capacity factor of a segment of a polymer [-]
KTFA	potassium trifluoro acetate
LC	liquid chromatography
LCCC	liquid chromatography under critical conditions
LCST	lower critical solvent temperature
M	molar mass [g/mol]
m_p	relative chain length of the polymer molecule [-]
m_s	relative chain length of the solvent molecule [-]
MALDI	matrix assisted laser desorption ionization
MMCCD	molar mass chemical composition distribution
MMFTD	molar mass functional type distribution
MMD	molar mass distribution
MS	mass spectrometry
n_φ	total number of lattice places to be filled by the solvent and polymer [mol]
NMR	nuclear magnetic resonance
NP	normal phase
NPG	neopentyl glycol
NS	non solvent
p	degree of polymerization [-]
PS	polystyrene
PiP	polyisoprene
R	gas constant [J/mol·K]

RI	refractive index
RP	reversed phase
S	solvent
SEC	size exclusion chromatography
T	temperature [K]
T_{cr}	critical temperature [K]
T_m	melting point of a polymer [K]
T^0_m	melting point of the pure polymer [K]
t_r	retention time [min]
t^0_{grad}	dead time of the gradient [min]
t^0_{col}	dead time of the column [min]
TA	terephthalic acid
TGIC	temperature gradient interaction chromatography
THF	tetrahydrofuran
TLC	thin layer chromatography
TOF	time of flight
UCST	upper critical solvent temperature
UV	ultraviolet
V_{excl}	exclusion limit expressed in volume [ml]
V_h	hydrodynamic volume [l/mol]
V_i	interstitial volume [ml]
V_p	pore volume [ml]
V_{perm}	permeation limit expressed in volume [ml]
V_{ret}	retention volume [ml]
V_s	volume of stationary phase [ml]
V_{Sol}	volume of the solvent [ml]
$\chi_{P/NS}$	Flory-Huggins interaction parameter of the non solvent and the polymer [-]
$\chi_{P/S}$	Flory-Huggins interaction parameter of the solvent and the polymer [-]
χ_{cr}	critical interaction parameter [-]
ΔG	change in Gibbs free energy [J/mol]
Δg_{funct}	change in Gibbs free energy of the functional groups [J/mol]
ΔG_{mix}	change in Gibbs free energy on mixing of a solvent and a polymer [J/mol]
Δg_{segm}	change in Gibbs free energy of a segment of the polymer [J/mol]
ΔH_{back}	change in enthalpy of the polymer backbone [J/mol]
ΔH_{funct}	change in enthalpy of the functional groups [J/mol]
ΔH_m	change in enthalpy on melting a polymer [J/mol]
ΔH_{mix}	change in enthalpy on mixing of a solvent and a polymer [J/mol]
Δp	pressure difference [bar]
ΔS_{back}	change in entropy of the polymer backbone [J/mol·K]
ΔS_{funct}	change in entropy of the functional groups [J/mol·K]
ΔS_{mix}	change in entropy on mixing of a solvent and a polymer [J/mol·K]
$[\eta]$	intrinsic viscosity [dl/g]
η_{sp}	specific viscosity [-]
$\varphi_{program}$	programmed gradient speed [-/min]
$\varphi_{initial}$	initial solvent fraction of the gradient [-]
φ_S	volume fraction of solvent [-]
φ_P	volume fraction of polymer [-]
φ_{TA}	mole fraction of terephthalic acid based on the total amount of acid (TA + IA) [-]
λ	wavelength [nm]

Summary

The objective of this study was to investigate the broad applicability of gradient polymer elution chromatography (GPEC) to various types of copolymers.

Copolyesters consisting of neopentyl glycol (NPG), isophthalic acid (IA) and terephthalic acid (TA) have been characterized according to molar mass, chemical composition, and functional end groups. In order to characterize the copolyesters, the GPEC retention behavior of the homopolyesters of NPG/IA and NPG/TA was first studied. During the optimization of the separation of the homopolyesters, problems concerning the reproducibility of the separation were encountered. These problems were attributed to the crystallization of the homopolyesters from solution during the GPEC analysis. Using differential scanning calorimetry (DSC) it was shown that although the homopolyester samples did not form a crystalline phase from the melt, they did from solution.

The optimal gradient for the separation of the homopolyesters was found to be that starting from water/acetonitrile (50:50 vol%) and proceeding via acetonitrile (100%) to tetrahydrofuran (100%) on a C18 modified silica column. Approximately 100 peaks could be observed in the GPEC chromatograms and the identification of the molar mass of the peaks was carried out using on-line electrospray-ionization mass spectrometry (ESI-MS). After identification, the structure of the homopolyesters could be completely elucidated.

Copolyesters of NPG, IA and TA were separated using the same optimal gradient used for the homopolymers. The functional end groups, the chemical composition and the molar mass were elucidated by the combination of GPEC with ESI-MS. With this knowledge it became possible to characterize commercial copolyester samples.

Block copolymers consisting of styrene/isoprene and styrene/butadiene were also investigated by GPEC. A method was developed to determine the amount of free polystyrene and polyisoprene present in the block copolymers, and consequently valuable information on the block copolymerization process was obtained. Similarly, various commercial block copolymers were characterized by GPEC.

The work described in this thesis shows the broad and powerful applicability of GPEC to the characterization of copolymers. In combination with MS-detection, unique information about the microstructure of polymers is obtained, which cannot be achieved with any other technique.

Samenvatting

In dit proefschrift wordt de toepassing van gradiënt-polymeer-elutie-chromatografie (GPEC) voor de karakterisering van verschillende typen co-polymeren beschreven. Het algemene doel van de studie was het onderzoeken van de brede toepasbaarheid van GPEC

Voor een goede karakterisering van de co-polyesters bestaande uit neopentylglycol (NPG), isophtaalzuur (IA), en terephtaalzuur werd het GPEC retentiegedrag van de homopolyesters bestudeerd. Tijdens het onderzoek bleek de reproduceerbaarheid van de scheiding van de homopolyesters niet voldoende te zijn. Deze problemen werden veroorzaakt door de kristallizatie van de homopolyesters vanuit oplossing tijdens de GPEC analyse. Nadere bestudering met differential scanning calorimetry (DSC) wees uit dat vanuit de smelt géén, maar vanuit de oplossing wèl een semi-kristallijne structuur gevormd wordt.

De optimale condities voor de scheiding van de homopolyesters bestonden uit een gradiënt die begint bij water/acetonitril (50:50 vol%) en verloopt via acetonitril (100%) naar tetrahydrofuran (100%) op een C18 gemodificeerde silicakolom. In de GPEC chromatogrammen van de homopolyesters kunnen meer dan 100 pieken worden onderscheiden. De gescheiden pieken werden vervolgens geïdentificeerd met on-line electrospray-ionisatie massa spectrometrie (ESI-MS) en zodoende kon de structuur van de homopolyesters volledig worden ontrafeld.

Co-polyesters van NPG, IA en TA werden onder de geoptimaliseerde scheidingscondities gekarakteriseerd. De eindgroepen, chemische samenstelling en molecuulmassa konden worden bepaald (met behulp van de combinatie GPEC en ESI-MS). Op grond van deze kennis was het mogelijk commerciële co-polyesters te karakteriseren.

Naast co-polyesters werden ook blok co-polymeren bestaande uit styreen/isopreen en styreen/butadieen bestudeerd met behulp van GPEC. Er werd een methode ontwikkeld voor het bepalen van vrij polystyreen en polyisopreen in blok co-polymeren en daarmee kon waardevolle informatie over het blok co-polymerisatie proces verkregen worden. Op analoge wijze werden verschillende typen (commerciële) blok co-polymeren gekarakteriseerd.

Uit dit onderzoek blijkt dat GPEC een krachtige en breed toepasbare methode is om co-polymeren (mengsels) te karakteriseren. In combinatie met on-line MS-detectie wordt unieke informatie over de microstructuur van polymeren verkregen die met geen enkele andere techniek gevonden kan worden.

Dankwoord

Bij het tot stand komen van dit proefschrift zijn velen betrokken geweest en bij deze wil ik ieder die op de een of andere manier een bijdrage heeft geleverd bedanken.

Ten eerste wil ik de directie van de voormalige Central Research van Akzo Nobel bedanken voor het sponsoren van het project en Cees Groenenboom voor zijn ondersteuning van het project binnen de organisatie. In het bijzonder wil ik mijn eerste promotor Ton German bedanken voor de mogelijkheid om te promoveren en zijn vertrouwen. Ik wil mijn tweede promotor Peter Schoenmakers bedanken voor de nuttige discussies en het grondig nakijken van mijn proefschrift. Tevens wil ik mijn copromotor Bert Klumperman bedanken voor zijn begeleiding en zijn nuttige weerwoord. Mijn dank gaat uit naar de leden van de leescommissie, Eric Nies en Carel Cramers voor het doorlezen van mijn proefschrift en hun waardevol commentaar.

Speciale dank gaat uit naar Ab Buijtenhuijs. Ab jouw bezielende begeleiding, maar vooral de discussies over uiteenlopende onderwerpen waren zeer nuttig, interessant, leerzaam en aangenaam, en ik hoop dan ook dat we de discussies de komende jaren kunnen blijven voortzetten. Ab's kamer- en lotgenoot, Michel Nielen, wil ik danken voor de leuke en vruchtbare samenwerking op het gebied van MS. Henk, Amaia, Tonnie en Harry wil ik graag bedanken voor het doorlezen van de verschillende versies van mijn proefschrift, voor het geven van nuttig commentaar en het voeren van wetenschappelijke discussies.

Het grootste gedeelte van het praktisch werk heb ik verricht op het analytische lab van de toenmalige Central Research van Akzo Nobel in Arnhem en ik wil daar de volgende (ex-) medewerkers bedanken: Mathijs, Bert, Evelien, Paul, Mieke, Henk, Monique, Peter en Remie en alle anderen die ik vergeten ben te noemen. In het bijzonder wil ik Leo, Luc, Andre en Marco bedanken voor hun inhoudelijke bijdrage in de vorm van onderwerpen, discussies en monsters.

Verder wil ik alle AIO's waar ik de afgelopen jaren met samengewerkt heb in de groep 'Polymeerchemie' op de TUE (teveel om op te noemen) bedanken voor een fijne tijd. Mijn speciale dank en medeleven gaat uit naar mijn ex-kamergenoten Eugene, John (de K), Henno, Harry, Tijs, Hans, Mike, Wilfred en Tonnie. Wieb en Alfons wil ik van harte bedanken voor de plezierige samenwerking en Wieb bedankt voor het gewillige oor. Greg, bedankt voor de vele bakjes koffie tijdens het roddelen en voor de sportieve afleiding. De discussies met Piet Piet zal ik nooit meer vergeten, en Helly, Carolien, Steven (voor het rekruteren van interessante post-docs), Alex, Peter, en Ruud wil ik bedanken voor alles wat ze voor mij gedaan hebben. I

would like to thank Michael for reading and correcting some parts of my thesis and Joseph for the scanner. Ook wil ik Joost bedanken voor zijn MS-inbreng. Ik heb tijdens mijn AIO-schap verschillende afstudeerders en/of stagiaires begeleid: Mike, Frans, Franc, Mark en Tasmara bedankt voor jullie bijdrage. Anne en Pauline wil ik bedanken voor het aanleveren van zeer mooie foto's en mijn ex-huisgenoten Jan en Henk wil ik bedanken voor hun emotionele bijdrage.

Mijn speciale dank gaat uit naar Wim. Jouw enthousiasme is met geen pen te beschrijven en ik ben blij dat je me zo'n 10 jaar geleden met het HPLC virus hebt geïnfecteerd. Ik wil mijn huidige collega's bij TNO bedanken voor het geduld en de verkregen ruimte tijdens het afronden van mijn proefschrift.

Ik wil mijn ouders bedanken voor hun steun en vertrouwen and last but not least I want to thank Amaia for the love and support over the last (two) crucial years.....ez zaitez kezkatu, ez bait zaitut utziko.....

Paul

Maart 1999

Curriculum Vitae

Paul Cools is geboren op 9 april 1968 te Tilburg. In 1986 behaalde hij het Atheneum B diploma aan het Odulphus Lyceum te Tilburg en begon hij de studie scheikundige technologie aan de Technische Universiteit Eindhoven. In juni 1992 studeerde hij af op de Technische Universiteit Eindhoven in de vakgroep Polymeerchemie en Kunststoftechnologie op het onderwerp "Isocratic Retention Behavior of Homopolymers", waarna hij in juli 1992 startte met de twee jarige ontwerpersopleiding Proces- en Productontwerp. De ontwerpopdracht "On-line Monitoring of an Emulsion Polymerization" werd eveneens uitgevoerd in de vakgroep Polymeerchemie en Kunststoftechnologie. Na het behalen van het ontwerpersdiploma begon hij in juli 1995 zijn promotieonderzoek in dezelfde vakgroep (huidige naam Capaciteitsgroep Polymeerchemie en Coatingstechnologie) onder leiding van prof.dr.ir. A.L. German. Het promotieonderzoek werd volledig gesponsord door Central Research van Akzo Nobel te Arnhem. In februari 1999 is hij in dienst getreden van de afdeling verpakking van TNO Voeding in Zeist.

Stellingen

behorende bij het proefschrift

Characterization of Copolymers by Gradient Polymer Elution Chromatography

van

Paul Cools

1. "*Normal* phase chromatography" van polymeren is niet zo *normaal* als de naam wel doet vermoeden.
   Hoofdstukken 4 en 7 van dit proefschrift

2. Het feit dat polymeermoleculen geëxcludeerd worden uit de poriën van een gepakte kolom en dat daardoor het bereikbare actieve oppervlak vermindert neemt niet weg dat de polymeermoleculen aan sterke adsorptieverschijnselen onderhevig kunnen zijn, dit in tegenstelling tot de bewering van *Schultz et al.*
   *Schultz R and Engelhardt H., Chromatographia, **29 (5/6)**, 205-213 (1990).*

3. Vloeistofchromatografie onder kritische condities (liquid chromatography at critical conditions LCCC) is kritischer dan *super*kritische vloeistofchromatografie (*super*critical fluid chromatography SFC).
   Met dank aan Peter Schoenmakers

4. In gradiënt elutie HPLC van polymeren wordt *exclusie* zelden of nooit in acht genomen, ook niet in dit proefschrift.
   Dit proefschrift

5. Er kan een duidelijke bimodaliteit geobserveerd worden tijdens het "*baywatchen*".

6. Veel chemici en vooral polymeerchemici gaan voorbij aan het feit dat *chemie* in het Nederlands ook wel bekend staat als *scheikunde*.

7. Op de suggestie dat door "*on-line*" koppeling *LC-NMR* op eenvoudige en goedkope wijze betrouwbare informatie kan worden verkregen is het volgende citaat van *Dolly Parton* van toepassing: "*It takes an awful lot of money to make this person look so cheap.*"
Citaat: http://www.gold-eagle.com/asian_corner_98/dines013098.html

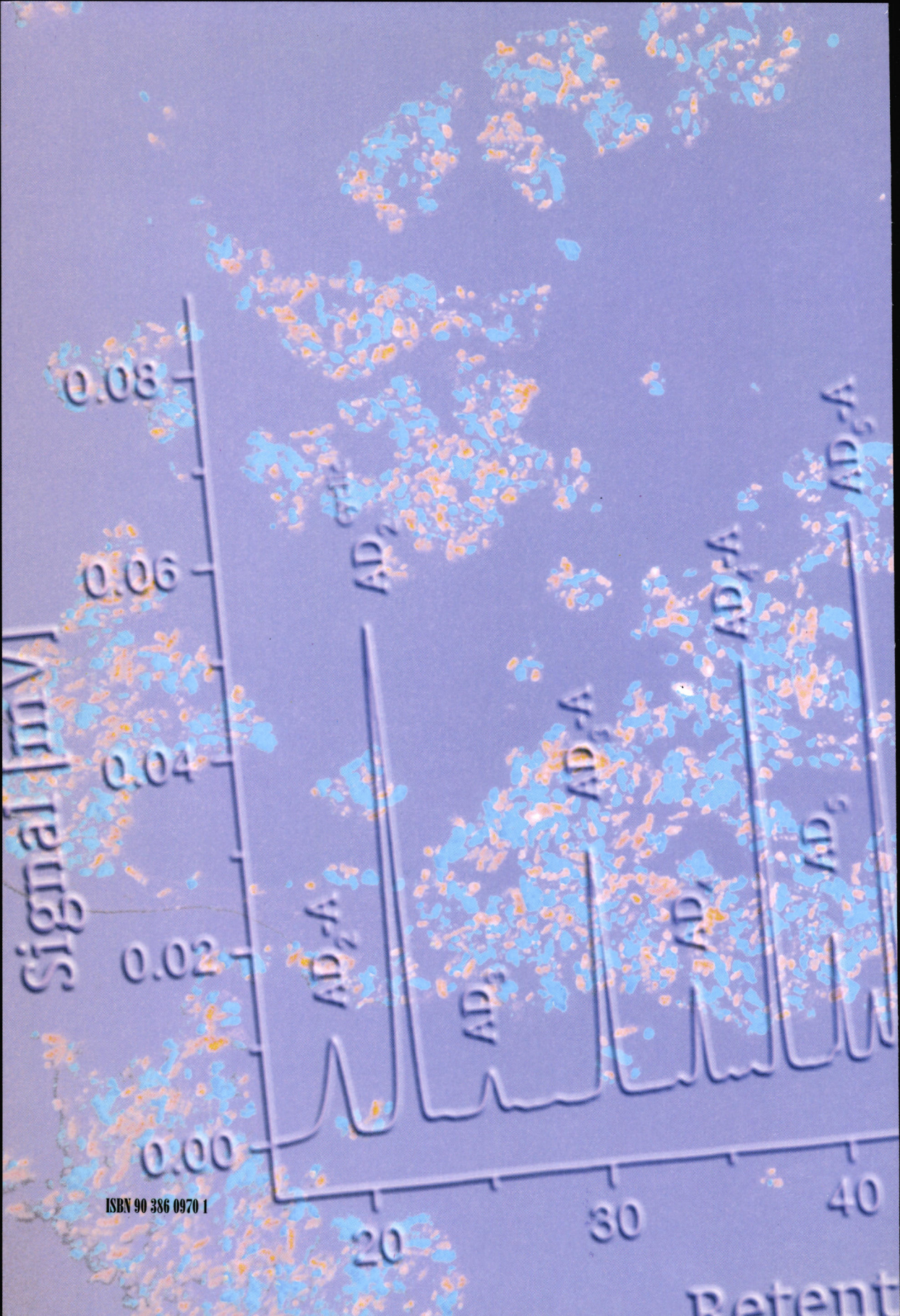
8. De hoogte van salarissen in de topsport zijn veelal niet in overeenstemming met de geleverde prestaties.

9. Ondanks de vele winstpartijen en behaalde punten laat Feijenoord ook dit jaar (1999) toch maar weer eens blijken de kampioen van de armoede te zijn.
Harold Schoonbrood "Emulsion Co- and Terpolymerization", proefschrift Eindhoven 1994, Stelling 15.

10. "Gerrari ohitu zintezke, baina gerra egon badago."
"Je kunt wel wennen aan oorlog, maar daarmee gaat de oorlog nog niet voorbij."
Vrij vertaalde Baskische uitspraak van de groep Hertzainak

11. Minds are like parachutes, they only function when opened.
Auto sticker

Eindhoven, 27 mei 1999



ISBN 90 386 0970 1