# Mechanisms of gradient polymer elution chromatography and its application to (co)polyesters

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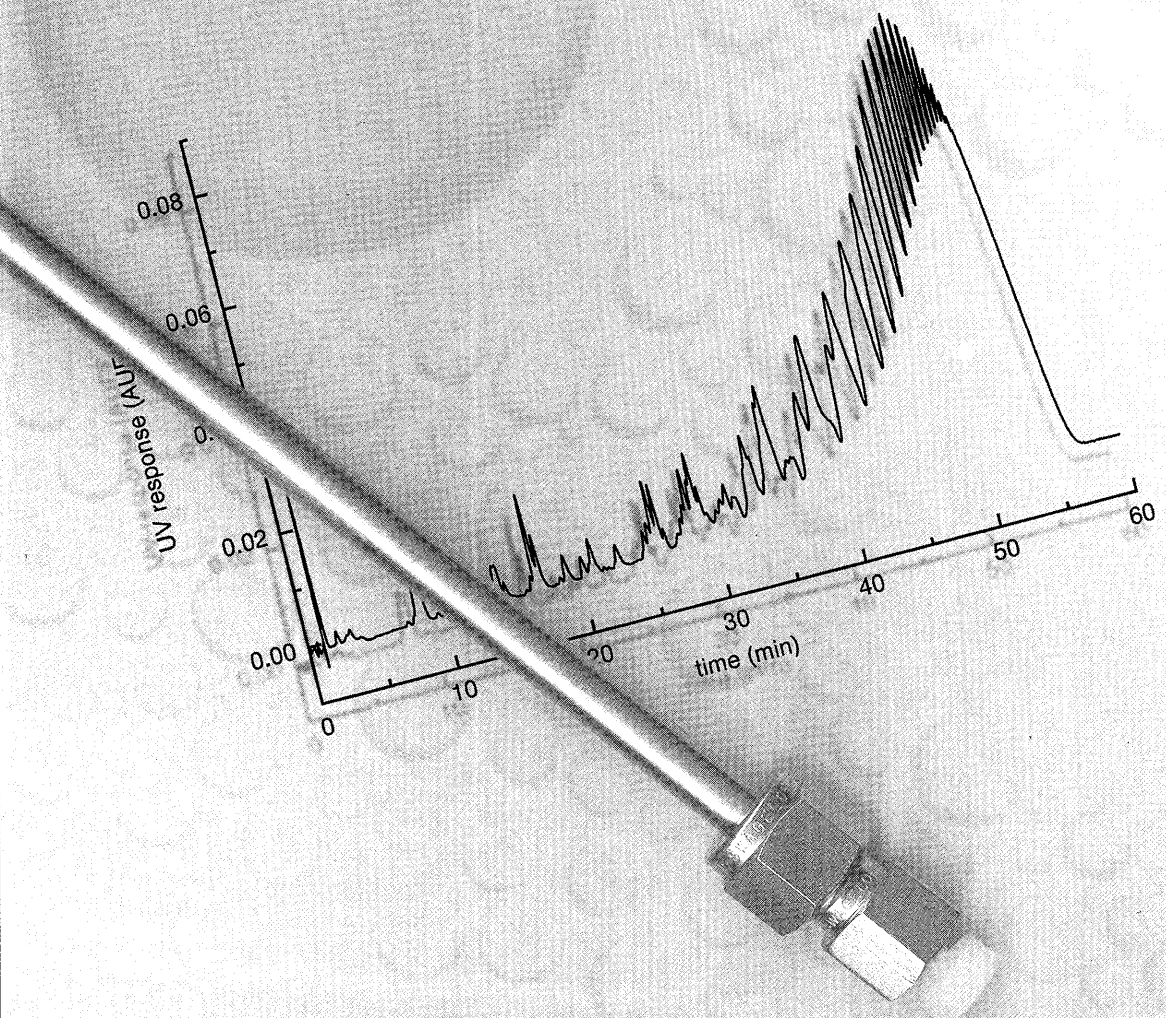


H.J.A. Philipsen

# Mechanisms of Gradient Polymer Elution Chromatography and its Application to (Co)polyesters

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# Mechanisms of Gradient Polymer Elution Chromatography and its Application to (Co)polyesters

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 dinsdag 3 november 1998 om 16.00 uur

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**Henricus Johannes Antonius Philipsen**

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prof.dr.ir. A.L. German
en
prof.dr.ir. C.A.M.G. Cramers

Copromotor:

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*Wat is je vak? Goed zijn. Waar anders berust dat op dan op algemene inzichten, enerzijds over de Universele Natuur en anderzijds over de eigen aanleg van de mens?*

Marcus Aurelius

Voor mijn ouders

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# CHAPTER 1

# General Introduction and Scope

## 1.1 INTRODUCTION

The continuous efforts being invested into research on polymer characterisation methods, both chemical and physical, are induced by the increasing complexity of macromolecules used for a growing and broad field of applications. Nowadays, the use of polymer-based materials is indispensable in, among others, industry, agriculture, life sciences and household. Increasing demands on polymer properties with respect to mechanical and chemical resistance and biodegradability, and the need for dedicated products with special visco-elastic, optical or electrical properties, necessitates the development and use of complex polymer systems such as copolymers and terpolymers, block copolymers and polymer blends.
For this purpose, new polymerisation techniques are being investigated with the objective to enable the synthesis of tailor-made polymers having dedicated properties.[1-3] The availability of characterisation methods able to unravel the molecular architecture of these products is an absolute prerequisite to enhance a thorough understanding and further development of polymerisation techniques, in turn leading to fine-tuning of polymer properties. Chemical characterisation methods for polymers include spectroscopy, separation methods, and classical, wet chemical methods.[4,5]

In the field of separation methods, which can either be chromatographic or non-chromatographic, interesting new techniques have come to development during the past two decades. Traditionally, Size Exclusion Chromatography (SEC) has been the method of choice for the determination of one of the most important molecular characteristics of a polymer, its molar mass distribution (MMD).[6,7] Although the separation selectivity of SEC is large, the efficiency is only moderate, which leads to characteristic low resolution chromatograms. For polymers with a molar mass

exceeding approximately 100,000 daltons, also Hydro-Dynamic Chromatography (HDC) can be used, next to SEC, in this respect.[8,9] By this technique, a separation according to molar mass is realised using the velocity differences existing in a narrow, capillary channel or in the interstitial volume between the particles of a packed column. This is caused by the parabolic velocity profile of laminar flow in flow channels. The separation selectivity, however, is limited, but in contrast to SEC the efficiency is very high.
More complex polymers such as copolymers and functionalised polymers, may have, next to an MMD, also distributions with respect to chemical composition, functionality, monomer sequence, branching, etc. By classical spectroscopic methods such as IR and NMR, only the average values of these characteristics can be determined. For the evaluation of their related distributions, like for the MMD, separation methods must be used, possibly even in combination with spectroscopic methods.
In this respect, Temperature Rising Elution Fractionation (TREF) is frequently applied for semi-crystalline polymers, mainly polyolefines. Hereby, polymers are separated according to differences in their melting point. Since the melting point of homopolyolefines is mainly determined by the degree of branching and for copolymers by the chemical composition, a branching distribution is obtained for homopolymers[10,11] and a chemical composition distribution (CCD) for copolymers.
In Field Flow Fractionation (FFF), like for HDC, separation is realised through velocity differences existing in a narrow channel through which a solvent flows. By applying an external field or gradient perpendicular to the flow direction, the distribution of solutes perpendicular to the solvent flow is influenced and separation selectivity is increased. Thermal Field Flow Fractionation (ThFFF) for instance, can be used for the separation of macromolecules according to molar mass but also according to chemical composition.[12,13] The main drawback of FFF is the restricted applicability to solutes with molar masses below 10,000 daltons.

Since the late seventies, liquid chromatographic techniques other than SEC are being used for the characterisation of polymers. Thin Layer Chromatography (TLC) in combination with SEC has been used for the evaluation of the CCD of copolymers.[14,15] In 1979 Teramachi was the first to use HPLC for copolymer analysis.[16] In the same period, van der Maeden *et al.* described the use of reversed phase HPLC for the separation of low molar mass polymers into a large number of oligomers.[17] Gradient elution was used in both cases, which, as will be discussed in the next Chapter, in most cases is a prerequisite for a successful analysis of oligomers and polymers under sorptive conditions.
During the eighties a limited number of workers, among others Glöckner *et al.*[18], Mori *et al.*[19,20], Snyder *et al.*[21,22], Boehm *et al.*[23,24] and Jandera *et al.*[25,26] used

gradient elution techniques for the characterisation of (co)polymers and oligomers. In many cases, relatively simple, high molar mass random copolymers made by polyaddition reactions were studied. For these products, the evaluation of the chemical composition distribution by gradient elution chromatography could easily be done without significant interference with molar mass effects. For several, relatively low molar mass polymers, successful oligomer separations were reported, although real applications often seemed to lack.

In the same period, an isocratic HPLC method, sometimes called Liquid Chromatography under Critical Conditions (LCCC) was introduced. This method, which is independent of molar mass, is useful for the separation of telechelic polymers according to functionality[27] and for the characterisation of block copolymers according to the block length distribution.[28]

From studies focussing on the mechanisms of gradient elution methods for polymers, it became obvious that in many cases traditional retention models for, for instance reversed phase and normal phase HPLC, cannot adequately describe the retention behaviour of high molar mass solutes. This is due to the fact that, next to sorption effects, which can either be adsorption or partitioning, also steric exclusion and solubility effects may significantly contribute to the overall retention process. Therefore several new names were introduced for gradient elution chromatography of polymers. Glöckner proposed the term High Performance Precipitation Liquid Chromatography (HPPLC) for those cases where the retention mechanism is dominated by solubility effects. By Mori, the term Liquid Adsorption Chromatography (LAC) is used when adsorption is the main mechanism of retention. Since the exact separation mechanism often results from a mixture of the various contributions, the more generally applicable term Gradient Polymer Elution Chromatography (GPEC) was recently introduced by Cools and Staal.[29,30]

During recent years, a still gradual although steadily increasing interest in gradient elution techniques for polymer characterisation can be observed. However, it is widely recognised that still much work has to be done in order to make these techniques more generally applicable for a broader range of polymers. For this reason, since the late eighties, liquid chromatography of polymers has been a major subject of investigation at the Laboratory of Polymer Chemistry at the Eindhoven University of Technology.[31,32] Based on their mutual interests in this respect, in 1994 a co-operation between this group and Océ Netherlands B.V. (nowadays Océ Technologies B.V., Venlo, The Netherlands) was initiated. The investigations carried out in this project provided new insights in liquid chromatography of, especially low molar mass, polymers and formed the basis of this thesis.

## 1.2 SCOPE OF THIS THESIS

As mentioned above, a thorough understanding of non-exclusion chromatographic techniques, such as GPEC, for polymers is still lacking. Therefore, a considerable part of the work described in this thesis focussed on studying mechanisms of GPEC in order to get a better insight in its fundamentals and working principles. For this purpose, various chromatographic studies, both gradient elution and isocratic, were performed, for reversed phase as well as normal phase systems. As polymers, mainly low molar mass (co)polyesters were used. These polymers intrinsically consist of a large variety of products differing in molar mass, end groups and chemical composition. This makes them suited for studying the effect of various molecular characteristics on the retention behaviour in GPEC and, vice versa, to study the effect of practical parameters in GPEC on the separation according to the various characteristics. In some cases, for a further support of the study and its conclusions, also other polymer types, such as low molar mass polystyrenes were used.
Another objective of this work was to get a better idea about possibilities and limitations of GPEC for the deformulation of complex polymer systems. Until now, applications of gradient elution techniques for polymers mainly focussed on relatively simple high molar mass copolymers obtained from chain polymerisations, such as poly(styrene-co-acrylates). Therefore, also in this respect, due to their intrinsic complexity, the study of copolyesters was very appropriate. Caused by their relatively low molar masses, retention is affected by chemical composition, end group composition *and* molar mass. This means that translation of GPEC results into polymer composition is more complex than for high molar mass polymers.
In *Chapter 2* some general aspects of step-reaction polymerisation and the existence of chemical heterogeneities in copolymers are presented. Furthermore, the chromatography of polymers, its various modes and the different separation mechanisms, *i.e.* exclusion, adsorption and solubility, are discussed and the potentials of the various types of chromatography to determine molecular characteristics and the related distributions of polymers are indicated.
*Chapter 3* deals with Reversed Phase Gradient Polymer Elution Chromatography (RP-GPEC) of amorphous polyesters. In the first part, the effects of various practical parameters on the highly detailed separations, which can be obtained by RP-GPEC, are discussed and compared with the chromatography of low molar mass solutes. In the second part, the role of solubility effects, *i.e.* precipitation and redissolution, in the overall separation mechanism of RP-GPEC of low molar mass polyesters is investigated.
In *Chapter 4*, further investigations on the retention mechanisms of low molar mass polymers in reversed phase systems are presented. These include the determination of thermodynamic parameters under isocratic conditions by van 't Hoff analysis for low

molar mass polystyrenes and polyesters. The effects of degree of polymerisation, polymer type and eluent composition on the sorption mechanisms are discussed.
In *Chapter 5*, a study of the molar mass dependence of retention of oligomers in RP-GPEC is described. The effects of temperature and the nature of the non-solvent/solvent system on molar mass dependence and differences between various oligomer series are studied. Explanations in terms of polymer-solvent Flory-Huggins interaction parameters and relative contributions of end groups and monomeric repeat units to retention, are given.
*Chapter 6* is concerned with RP-GPEC of crystalline polyesters and the anomalous, non-reproducible chromatographic behaviour as compared to amorphous polyesters, under certain conditions. A novel concept in terms of the effect of precipitated polymer morphology on the elution behaviour in GPEC, providing new insights in the chromatography of certain classes of polymers, is presented.
Normal Phase Gradient Polymer Elution Chromatography (NP-GPEC) of amorphous polyesters is dealt with in *Chapter 7*. In the first part, the effect of parameters such as the nature of the stationary and mobile phase on the end-group-dominated separations, is evaluated. In the second part, a further investigation of the retention mechanism by isocratic measurements is described. For this purpose, the retention model of Jandera is used, by which contributions of end groups and monomeric repeat units to retention can be distinguished. Furthermore, a refined adsorption model, assuming two types of adsorption sites, is presented.
Finally, in *Chapter 8*, a study of the possibilities and limitations of GPEC for the microstructural characterisation of copolyesters made by step-reactions is described. The potentials of RP-GPEC and NP-GPEC are compared, making use of a number of copolyesters varying in molar mass and chemical composition which allows a systematic study on the effects of those parameters in GPEC. New insights in the existence of microstructural differences between strongly resembling copolyesters, which until now could not be detected by any other method, are presented.

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# CHAPTER 2

# General Theoretical Aspects

## 2.1 POLYESTERIFICATION BY STEP REACTION POLYMERISATION

In general, polyesters can be defined as polymers containing repeating ester groups in the polymer backbone. Polymers containing ester groups in their side chains, such as polyacrylates, do not belong to this group. Nowadays, polyesters find wide application in fibers, films, toners for photocopiers and coatings. Polyesters can be synthesised by step-reaction polymerisation and (cationic or anionic) ring opening polymerisation from lactons.[1] Since in this study only products synthesised by step-reactions are used, the main characteristics of this polymerisation type are briefly discussed.
Polyesterification by step-reaction polymerisation is often involved with the reaction of a product containing two or more hydroxylic (alcohol) functional groups with a product containing two or more carboxylic (acid) functional groups:[1-3]

**Scheme 2.1.** Esterification

$$nHO—R_1—OH + nHOOC—R_2—COOH \Leftrightarrow -(-O—R_1—O—CO—R_2—CO-)-_n + 2nH_2O$$

This process, which is called direct polyesterification, usually is a bulk polymerisation. In the reaction above, the equilibrium is forced to the right by the removal of the by-product water at temperatures typically in excess of 200 °C. In the case of monomers containing more than two functional groups, branched products will be formed, eventually leading to gelled polymer structures (infinite networks).[3] This will not be further discussed here. Polyesters can also be formed from monomers containing both hydroxylic and carboxylic functional groups. Instead of carboxylic functionalised monomers also frequently acid chlorides are used, due to their higher reactivity.
The formed dimers can react with other dimers or with monomers to form longer chains. Since all molecules, regardless of their length, still contain the same functional groups, reaction proceeds in a stepwise manner and is therefore called step-reaction

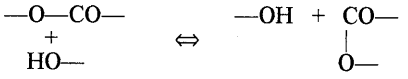
polymerisation. Due to the elimination of water, also the term polycondensation is frequently used. The stepwise proceeding of the reaction leads to a rapid decrease in monomers and a continuously increasing molar mass during the polymerisation. High molar masses can only be obtained at extremely high conversion degrees (see Eqs. (2.3-2.4)), which are in practice difficult to achieve due to the high viscosities at high conversion. This hampers the stirring and homogenisation process in the reactor and the removal of the formed water. That is why molar masses of step-reaction polymers are relatively low as compared to polymers made by polyaddition reactions (weight average molar masses, $M_w$, typically 3000 – 20.000).

A well-known side reaction of direct polyesterification is the formation of cyclic products by intramolecular reactions of hydroxylic and carboxylic functional groups. The degree of polymerisation, p, of these products mostly is in the range of 3-5.

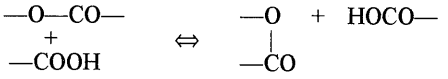
Direct polyesterification is a reaction which is self-catalysed by the carboxylic functional groups. However, since the concentration of these groups decreases during the polymerisation, often a catalyst is added. For this purpose, Lewis acids are used, such as dialkyl tinoxides.

Next to direct polyesterification, polyesters can also be formed from transesterification, *e.g.* alcoholysis, acidolysis and ester-ester interchange reactions:[4]

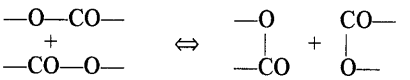
**Scheme 2.2.** Alcoholysis




**Scheme 2.3.** Acidolysis




**Scheme 2.4.** Ester-ester interchange




In practice, these reactions also occur during direct polyesterification, next to the chain growth reactions. Therefore, attempts to make block copolyesters by the coupling of two polyesters in the melt by means of their complimentary reactive end groups will always result in a certain extent of randomisation.[4] Analogous, it is often assumed that the synthesis of copolyesters by direct polyesterification will in most cases lead to fully randomised products. Although a significant difference in reactivity between the various monomers could provide blocky structures in the beginning of the reactions, ester-ester interchange reactions will lead to randomisation. It must be emphasised here, that until now characterisation methods were not sufficient to adequately characterise

copolyesters. Hence, a good knowledge of the amount of randomness or blockiness of copolyesters resulting from a wide scope of reactions, is lacking at this moment. In Chapter 8 it will be shown that Gradient Polymer Elution Chromatography offers new possibilities in this respect.

Kinetics of polyesterification reactions have been extensively studied.[3] Expressions for molar mass distributions can be derived from kinetic as well as statistical considerations in a rather simple way. This is mainly due to the fact that the reactivity of the functional groups is assumed to be independent of the chain length, which is known as Flory's principle of equal reactivity.[5] Kinetic and statistical considerations for monomers containing only one type of functional groups are identical to those for monomers containing two different types. In the former case the reaction product of a di-alcohol and a di-acid can be regarded as one monomeric unit.

It can be shown that, for the case that hydroxylic and carboxylic functionalities are present in equal concentrations, the reaction which is catalysed by the addition of an external catalyst is a second order reaction, the proceeding of which can be described by Eq. (2.1) and that in the absence of such catalyst, a third order reaction results, the proceeding of which is given by Eq. (2.2).[3]

$$\frac{1}{1-f} = k_1 c_0 t + 1 \tag{2.1}$$

$$\frac{1}{(1-f)^2} = k_2 c_0^2 t + 1 \tag{2.2}$$

Here, $c_0$ is the concentration of the carboxyl or the hydroxyl groups at time = 0, $k_1$ and $k_2$ are reactivity constants and f is the conversion which can be expressed as $f = ((c_0 - c)/c_0)$. The resulting number and weight average molar mass and the polydispersity, $D$, can be expressed according to:[3]

$$M_n = \left(\frac{1}{1-f}\right) M_0 \tag{2.3}$$

$$M_w = \left(\frac{1+f}{1-f}\right) M_0 \tag{2.4}$$

$$D = (1+f) \tag{2.5}$$

in which $M_0$ represents the molar mass of the monomeric unit. Furthermore, the weight fraction $w_p$ of molecules of degree of polymerisation p is given by:

$$w_p = p(1-f)^2 f^{(p-1)} \tag{2.6}$$

It is easily recognised from Eqs. (2.3–2.5) that polydispersity continuously increases during the polymerisation and that molar mass increases to infinity at high conversions. This can make the control of the molar mass of a polyesterification difficult. Therefore, sometimes a slight stoichiometric imbalance of the reactants is applied. The number average molar mass in such a case can be described by:[3]

$$M_n = \left( \frac{1+r}{1+r-2rf} \right) M_0 \tag{2.7}$$

where r is the stoichiometric imbalance which is given by $r = N_A/N_B$. $N_A$ and $N_B$ are the number of molecules of both reactants, where $N_B > N_A$.

## 2.2 CHEMICAL HETEROGENEITY OF (CO)POLYMERS

Next to a distribution according to molar mass, for synthetic (co)polymers several other kinds of chemical heterogeneities can be distinguished which will be briefly discussed below.

Firstly, due to side reactions during polymerisation, other products than aimed at, may be formed. An example is the formation of cyclic products during polyesterification. These products, differing in chain topology as compared to the main products, are known to influence certain polymer properties.[6]

Secondly, functionalised polymers such as telechelic polymers, may contain varying numbers and types of functional groups. In such case an additional distribution according to functional groups may exist, which may further depend on molar mass.[7,8] This is called the *Functionality Type Distribution* (FTD, see Figure 2.1)) or in the case of an additional molar mass dependence, the Molar Mass Functionality Type Distribution (MMFTD). Low molar mass polyesters may be considered as telechelic polymers.

Thirdly, during the formation of copolymers, *i.e.* polymers composed of more than one monomeric unit, several polymer heterogeneities can be generated. This is due to the fact that firstly, the formation of a copolymer is a statistical process and secondly, reactivity differences between the monomers can exist. This will be discussed here in some more detail.

The formation of heterogeneities in copolymers is especially known for *chain polymerisations*, *i.e.* copolymers formed by polyaddition reactions. Several models have been developed to describe relations between monomer reactivities and polymer heterogeneities for this type of polymerisation. In the most simple cases, these models

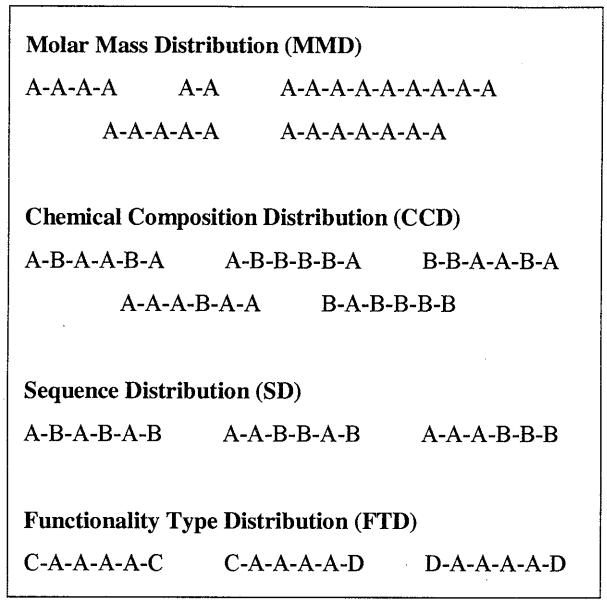



**Figure 2.1.** Schematic representation of polymer inhomogeneities. A, B: monomeric repeat units, C, D: end groups.

assume a copolymer, consisting of two monomeric units, A and B. In that case two different reactivity ratios, $r_A$ and $r_B$, can be defined, where $r_A = k_{AA}/k_{AB}$ and $r_B = k_{BB}/k_{BA}$. $k_{AA}$ represents the reactivity constant of the reaction of a growing chain with a terminal A unit with monomer A, $k_{AB}$ is de reactivity constant of a growing chain with a terminal A unit with monomer B etc.

For such copolymer, firstly, a distribution of microblocks of A and B along the polymer chain, the *Sequence Distribution* (SD, Figure 2.1), is generated. This means that even for polymer molecules which are identical in chain length and average composition, still a large variety of differently composed molecules exists. This polymer heterogeneity is also referred to as the *intra*molecular microstructure. Expressions have been derived for the number fraction distribution and the number average length of microblocks of types A and B.[9]

Secondly, a distribution of molecules differing in average chemical composition is formed, the *Chemical Composition Distribution* (CCD, Figure 2.1), together with the Molar Mass Distribution. This two-dimensional Molar Mass Chemical Composition Distribution (MMCCD) of a copolymer is also referred to as its *inter*molecular microstructure. Stockmayer developed a model describing the MMCCD formed in chain polymerisations.[10] From this model it can easily be deduced that a Gaussian distribution according to the chemical composition, y, is obtained with a standard deviation s, the *statistical* CCD, and that the broadness of this distribution is inversely proportional to the degree of polymerisation. This is in accordance with the fact that the

formation of a copolymer is primarily a stochastic process. However, when reactivity differences between the respective monomers exist, an additional chemical heterogeneity is introduced. A reactivity difference leads to depletion of the more reactive monomer in the reaction mixture during the polymerisation. This causes the incorporation of the different monomers to change during the reaction. This process is called composition drift, and the CCD caused by this process, a *conversion* CCD.

For *step-reaction polymerisations*, such as polyesterifications, as in the above described chain polymerisations, primarily the same statistical processes can occur leading to polymer inhomogeneities. Thus, both an SD and a CCD will exist in copolyesters. Especially the existence of an SD has been frequently demonstrated with help of spectroscopic methods.[11,12] A major difference between chain and step-reaction polymerisations is, however, that in chain polymerisations the time during which an individual chain grows is relatively short and that after termination no further redistribution of the monomeric units can occur. In contrast, during step-reaction polymerisations redistributions due to, for instance, transesterifications, occur throughout the whole reaction, and the growth time of the individual chains thus equals the total reaction time. Consequently, when true equilibrium is reached between chain growth and redistribution reactions in the reaction mixture, only a statistical CCD, not a conversion CCD, will be generated. In practice, such equilibrium is often assumed but no practical evidence for this assumption has been found yet. Moreover, the formation of a heterogeneity according to chemical composition can easily be demonstrated using a simple model.
When the formation of a copolyester, containing two di-acids, $Ac_1$ and $Ac_2$ and one di-alcohol, D, under stoichiometric conditions, *e.g.* $c_D = c_{Ac1} + c_{Ac2}$, is assumed, the following two chain growth reactions can occur:

**Scheme 2.5**

$$ac_1 + d \xrightarrow{k_1} c_1 + w \qquad ac_2 + d \xrightarrow{k_2} c_2 + w$$

where $ac_1$, $ac_2$ and d represent functional groups of type $Ac_1$, $Ac_2$ and D respectively, which exhibit reactivities $k_1$ and $k_2$, independent of chain length, and w represents water. Subsequently, it can be deduced that:

$$\frac{\partial c_{ac_1}}{\partial t} = k_1 c_{ac_1} c_d = k_1 c_{ac_1} \left( c_{ac_1} + c_{ac_2} \right) \tag{2.8a}$$

$$\frac{\partial c_{ac_2}}{\partial t} = k_2 c_{ac_2} c_d = k_2 c_{ac_2} \left( c_{ac_1} + c_{ac_2} \right) \tag{2.8b}$$

and thus:

$$\frac{\partial c_{ac_1}}{\partial c_{ac_2}} = \frac{k_1 c_{ac_1}}{k_2 c_{ac_2}} = C \frac{c_{ac_1}}{c_{ac_2}} \tag{2.9}$$

where C is the ratio of the individual reactivity constants. Integration over the di-acid end group concentrations reveals:

$$\frac{c_{ac_1}}{c_{ac_{1,0}}} = \left( \frac{c_{ac_2}}{c_{ac_{2,0}}} \right)^C \tag{2.10}$$

With the conversions, $f_1$ and $f_2$ being defined as $f_1 = (c_{ac1,0} - c_{ac1})/c_{ac1,0}$ and $f_2 = (c_{ac2,0} - c_{ac2})/c_{ac2,0}$, the dependence between $f_1$ and $f_2$ can be obtained:

$$f_1 = 1 - (1 - f_2)^C \tag{2.11}$$

In Figure 2.2 the dependence between $f_1$ and $f_2$ is shown for various values of C. The number average degree of polymerisation for an externally catalysed polyesterification is known to increase linearly with time.[3] Thus, from Figure 2.2 it becomes clear that for values of C that markedly exceed unity, distinct intra and intermolecular heterogeneities (a conversion CCD) will be formed. Naturally these heterogeneities will partly be suppressed by randomisation through transesterification reactions, which, unfortunately are less easy to express in a simple model. But it is qualitatively easy to imagine that especially in the case of large reactivity differences between the respective di-acids, the final degree of randomness depends on the rate of chain growth compared to that of transesterification reactions and on the time the reaction mixture is allowed to come to equilibrium. Therefore, even for step-reaction polymerisations, the possible existence of a conversion CCD cannot be ruled out a priori.

Since all described polymer heterogeneities can influence various polymer properties, it is important to have the availability of methods to determine these characteristics. Information on the chemical composition of copolymers and functional polymers is conventionally obtained by physical (*e.g.* NMR or IR) or chemical (titration, pyrolysis) methods. It must be emphasised here, that although very useful, these methods only provide information on *average* functionality, average sequence length and average chemical composition, *not* on the corresponding *distributions*. Distributions can only

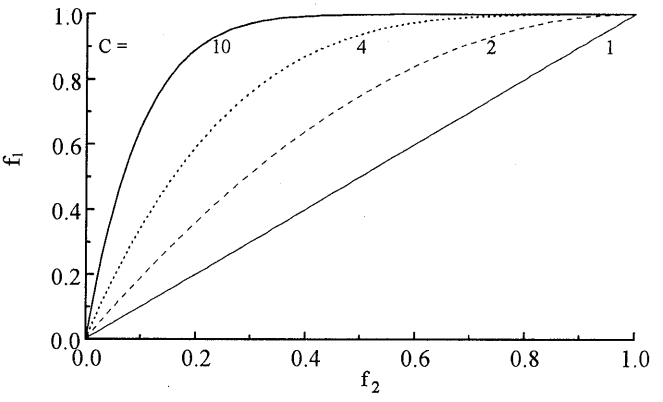



**Figure 2.2.** Dependence between $f_1$ and $f_2$ for various values of C. C values as indicated in Figure.

be obtained by separation methods, both chromatographic and non-chromatographic. The possibilities of chromatographic methods for the determination of polymer heterogeneities are further discussed in the next Section.

## 2.3 LIQUID CHROMATOGRAPHY OF POLYMERS

### 2.3.1 Separation modes in the chromatography of polymers

In liquid chromatography, generally porous column packings are used as the stationary phase. High molar mass solutes can, dependent on their size, partly penetrate into the pores of the column packing and furthermore undergo interactions with the active stationary phase, which is mainly located inside the pores. Therefore, two main processes can be distinguished in liquid chromatography of polymers: steric exclusion and enthalpic interactions which, in this Section, will further be indicated as 'adsorption'. The retention volume, $V_r$ can therefore be expressed as:[9]

$$V_r = V_i + K_{sec} V_p + K_{ads} V_s \tag{2.12}$$

In this expression, the retention volume is divided into the interstitial volume $V_i$, the pore volume $V_p$ and the stationary phase volume $V_s$. $K_{sec}$ and $K_{ads}$ represent the chromatographic distribution coefficients for steric exclusion and for adsorption, respectively. A chromatographic distribution coefficient is defined as:

$$K_D = \frac{c_s}{c_m} = \exp\left(\frac{-\Delta\mu_0}{RT}\right) \tag{2.13}$$

where $c_s$ and $c_m$ represent the concentration of a solute in the stationary and the mobile phase, respectively and $\Delta\mu_0$ is the standard chemical potential difference for solute molecules in both phases.

In the case that a thermodynamically good solvent for the polymer which also effectively suppresses enthalpic interactions with the stationary phase (a strong 'displacer') is used as the mobile phase, $K_{ads} = 0$ and retention is governed by entropic exclusion effects. In thermodynamic terms this means that $\Delta h = 0$ and $\Delta\mu = -T\Delta s > 0$ ($\Delta h$ and $\Delta s$ are the partial molar enthalpy and entropy change, respectively). This chromatographic mode is known as Size Exclusion Chromatography (SEC). $K_{sec}$ varies between 0 for large molecules which are totally excluded from the pores (total exclusion) to 1 for small molecules which can completely enter the pores (total permeation). Retention therefore decreases with increasing molar mass.

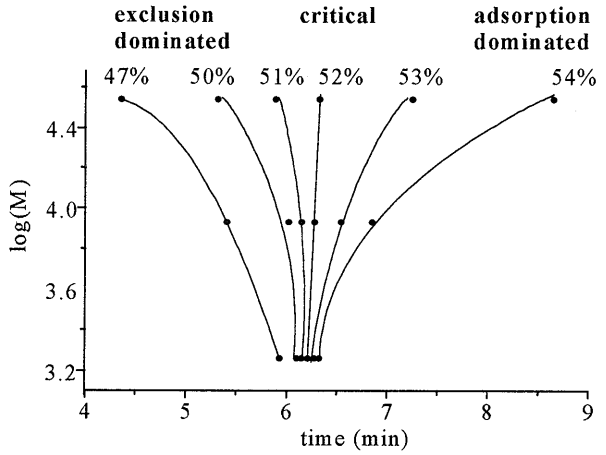



**Figure 2.3.** Separation modes in the chromatography of polymers. Data taken from ref. 14 (polystyrene on a Nucleosil $C_{18}$ column, in various compositions of ACN-DCM at 25 °C. %-ACN (v/v) as indicated in Figure).

When the thermodynamic quality of the solvent is decreased by either the addition of a poor solvent[13] or a temperature change[14], $K_{ads}$ may increase as enthalpic, adsorptive interactions start contributing to the total retention. These interactions are in most cases stronger for large than for small molecules, due to the fact that more monomeric units are available for interactions with the stationary phase. At a certain point $K_{ads}$ is

large enough such that $K_{sec}V_p + K_{ads}V_s > V_p$ and retention is dominated by adsorption. In that case $\Delta\mu = \Delta h - T\Delta s < 0$ and retention increases with increasing molar mass. The two separation modes and the transition from SEC to adsorption are demonstrated in Figure 2.3.
It is now qualitatively imaginable that under certain conditions, entropic exclusion effects and enthalpic adsorption effects are (nearly) balanced, such that $K_{sec}V_p + K_{ads}V_s = V_p$ and $\Delta\mu = 0$, and retention becomes (almost) independent of molar mass. These conditions, commonly referred to as *critical conditions*, have both been predicted theoretically[15] and found experimentally[13,14] for various polymer systems. Chromatography under these conditions will further be called Liquid Chromatography under Critical Conditions (LCCC).

### 2.3.2 Chromatography in the exclusion mode

Liquid chromatography in the exclusion mode (SEC) is the most widely applied method for the determination of molar masses and molar mass distributions of polymers. By SEC, (macro)molecules are separated according to differences in molecular size in solution or more precisely, to their hydrodynamic volume ($V_h$). In SEC, molecules having identical hydrodynamic volumes will elute at the same retention volume. $V_h$ scales with the product of molar mass and intrinsic viscosity, $[\eta]$:[9]

$$V_h \propto [\eta]M \tag{2.14}$$

The intrinsic viscosity (which can at best be regarded as the reciprocal molecular density) is related to molar mass according to the Mark-Houwink (MH) relation:[16]

$$[\eta] = K_\eta M^{a_\eta} \tag{2.15}$$

where $K_\eta$ and $a_\eta$ are the MH constants, which depend on temperature, solvent, polymer conformation and molar mass.
To obtain molar masses of polymers of interest, a SEC system has to be calibrated. This is usually done by the injection of polymer standards with a low polydispersity and a known molar mass. Herewith, the relation between retention volume and molar mass is determined. For an unknown polymer, the molar mass can subsequently be determined by the combination of Eqs. (2.14) and (2.15):

$$V_{h,x} = V_{h,cal} \qquad K_{\eta,x}M_x^{(1+a_x)} = K_{\eta,cal}M_{cal}^{(1+a_{cal})} \tag{2.16}$$

where subscripts 'x' and 'cal' refer to the unknown polymer and the polymer used for calibration, respectively. Unfortunately, in many cases the MH constants for the unknown polymer are not available. Therefore, when SEC is only equipped with a concentration detector, just molar masses relative to the polymer used for calibration, can be obtained. By the on-line coupling of SEC to a differential viscometer (DV), the intrinsic viscosity of the eluting polymer fractions is directly determined, thus providing absolute molar mass values for the unknown polymer, via Eqs. (2.14-2.16). SEC coupled to a light scattering (LS) detector, directly provides the molar mass of the eluting species without the need of calibrating the system. By both SEC coupled to DV and/or LS, additional information on polymer conformation is obtained.[17]

For copolymers or polymer blends, the determination of molar masses by SEC is often hampered by the fact that due to composition drift, the chemical composition may be a function of molar mass (see Section 2.2). Since the response of a concentration detector will in most cases be different for the various monomeric units, this means that a direct translation of the concentration signal into a (fractional) polymer concentration at each point of the SEC elution curve is not possible. In some cases, this problem can be solved by using a combination of two concentration detectors, *e.g.* differential refractive index (DRI) and ultraviolet (UV), DRI and infrared (IR) or DRI and density. In that way, the average polymer composition and therefore the real polymer concentration as function of the elution volume can be determined, thus again enabling molar mass calculations as described above.[9] Since the separation of SEC is based on hydrodynamic volume rather than molar mass, in the case of a copolymer each eluting fraction in fact represents a large number of different types of molecules, differing in chemical composition and molar mass but having identical hydrodynamic volumes. Thus the method described here, provides an approximation of the *average* molar mass as function of elution volume.

The combination of SEC with multiple detection or with infrared spectroscopy is also frequently used for the detection of polymer inhomogeneities,[18,19] since it provides an impression of *average* chemical composition as function of molar mass. It must be emphasised that, although useful, such analysis gives no information about the width of a CCD and is unable to discriminate between the difference between a polymer blend or a copolymer, which can easily lead to misinterpretations.[20,21] For the determination of CCDs other separation methods, sometimes in combination with SEC, have to be used, as will be described in the next Section.

### 2.3.3 Chromatography in the adsorption mode

#### *2.3.3.1 Adsorption of polymers*

In contrast to low molar mass products, polymers have a large number of adsorbable groups. These are all identical in the case of homopolymers but differ for copolymers. A dissolved polymer will be adsorbed from a solution onto a substrate if the overall energy gain exceeds the entropy loss. Enthalpic interactions occur between the polymer and the solvent, the polymer and the substrate and in some cases within the polymer itself by intramolecular interactions. Polymer adsorption always results in entropy loss, since the polymer coil will adapt a less probable thermodynamic conformation.[22] The achievable conformation depends on the total energy gain, which may differ when conditions such as solvent type, structure of the sorbate surface and temperature are changed. In many cases, not all the monomeric units are adsorbed simultaneously, since this would lead to a thermodynamically too unfavourable conformation. This results into unadsorbed loops and tails of polymer segments next to adsorbed trains of segments. Each segment contains a number of monomeric units, the average amount of which again depends on the experimental conditions. The simultaneous adsorption of more than one monomeric chain units is known as multi-site attachment.[9]

Polymer adsorption is also influenced by kinetic parameters since diffusion coefficients of high molar mass solutes are relatively low.[9] Thus, a slower adsorption process due to a lower energy gain or a lower temperature may result into a thermodynamically more favourable conformation since more time is available for the adsorption process.

Adsorption usually is an exothermic process. This implies less adsorption at higher temperatures. For polymers this is not always the case due to the fact that adsorption does not proceed isosterically.[22] At higher temperatures, a thermodynamically less favourable conformation may be achieved, which can result into increased instead of decreased adsorption.

In conclusion, the two main differences between adsorption of polymers and low molar mass products are the occurrence of multi-site attachment and of conformational changes.

#### *2.3.3.2 Isocratic elution*

Chromatography of polymers in the adsorption mode, where $K_{ads} > 1$ (Eq. (2.12)) can be considered as the distribution of macromolecules between the mobile and the stationary phase, dominated by enthalpic interactions. Since the adsorption enthalpy

will vary for chemically different monomeric units, it is qualitatively imaginable that chromatography in the adsorption mode will be more suitable than SEC for the characterisation of polymers according to chemical heterogeneities. In the case of low molar mass polymers, retention under equilibrium (isocratic) conditions can be described by an idealised free energy relationship. For a polymer with p repeat units of type 'y' and two chemically different end groups, 'x' and 'z', it can be written:[23]

$$\Delta\mu_{total} = \Delta\mu_x + p\Delta\mu_y + \Delta\mu_z \tag{2.17}$$

If retention is dominated by a single, adsorption, mechanism, Eq. (2.12) simplifies to:

$$V_r = V_m + K_{ads}V_s = V_m + \exp^{\left(\frac{-\Delta\mu_{total}}{RT}\right)}V_s \tag{2.18}$$

with $V_s$ being the volume of the stationary phase and $V_m$ the volume of the mobile phase. Subsequently, with the retention factor, k, being defined as $k = (V_r - V_m)/V_m$, it is obtained that:

$$\ln(k) = \left[\left(\frac{-\Delta\mu_x - \Delta\mu_z}{RT}\right) + \ln(\phi)\right] - p\left(\frac{\Delta\mu_y}{RT}\right) \tag{2.19}$$

where $\phi$ represents the phase ratio, $V_s/V_m$, of the column. The linear increase in ln(k) with p, as predicted by Eq. (2.19) is known as the Martin Equation.[24] Such dependence has been found experimentally for various oligomer systems under varying chromatographic conditions.[25,26] For high molar mass polymers, a deviation from linearity has been reported, although those experimental examples are rare.[27] This behaviour can probably be explained from entropic considerations (see previous Section). Clearly, Eq. (2.19) implies molar mass as a second molecular characteristic next to chemical composition determining retention of polymers under adsorption conditions. Eq. (2.19) has been the basis for a retention model in which the contribution of monomeric repeat units and end groups of functionalised oligomers can be distinguished.[25,28] This will be further discussed in Chapters 5 and 7. It should be mentioned here that Eq. (2.19) must mainly be considered as a first, rough approximation to express the retention behaviour of functionalised polymers. Although the equation suggests that contributions to retention due to monomeric repeat units and end groups are completely independent from each other, such behaviour will certainly not be encountered for high molar mass solutes.

Another practical implication of Eq. (2.19) is that retention under isocratic conditions increases exponentially with molar mass. Since synthetic polymers mostly have polydispersity values far exceeding unity, this means that complete elution under

isocratic conditions in most cases is virtually impossible. From statistical considerations it can further be deduced that the retention factor of a polymer can be written as:[9]

$$k_{polymer} = (k_{monomer} + 1)^p - 1 \qquad (2.20)$$

This means that a small variation in the chromatographic distribution of the monomeric units leads to large shifts in retention for the polymer. Therefore, polymers often show a sort of 'on-off' behaviour under isocratic conditions; under certain conditions a polymer elutes unretained and a subsequent small decrease in solvent strength may cause irreversible adsorption. This makes isocratic elution of high molar mass polymer virtually impossible to handle in practice. Therefore, chromatography of polymers under adsorption conditions is mostly involved with the use of gradient elution (GE). Nevertheless isocratic experiments under carefully controlled conditions can be used for mechanistic studies in order to obtain further insight in the fundamentals of polymer chromatography, as will be demonstrated in Chapters 5 and 7 of this thesis.
It must be mentioned here that enthalpic interactions which have been called adsorption until now can in fact, especially for low molar mass solutes in reversed phase systems, either be 'true' adsorption and/or partitioning. For these cases the term 'sorption', which both includes adsorption and partitioning, will be used in this thesis.

#### *2.3.3.3 Solubility of polymers*

Since the use of GE in the chromatography of polymers is often involved with the occurrence of demixing and precipitation phenomena as will be pointed out in the next Section, the principles of polymer solubility are briefly discussed here.
In general, the dissolution of a solute is associated with a decrease in the chemical potential:

$$\Delta\mu_{mix} = \Delta h_{mix} - T\Delta s_{mix} < 0 \qquad (2.21)$$

For low molar mass solutes, a considerable gain in entropy is realised due to the large increase in system disorder accompanying the dissolution. In the case of polymers, the increase in disorder and hence the entropy gain is much lower due to the large number of monomeric units remaining connected with each other. Hence, the mixing enthalpy cannot be too large and must in most cases be negative in order to ensure the chemical potential change to be negative. Actually, this leads to the property that the solubility for most polymers is restricted to a very limited number of solvents with a polarity close to that of the polymer.[29]

For a polymer being mixed with a solvent, the following approximation for the change in chemical potential was obtained from the Flory-Huggins (FH) theory:[5]

$$\Delta\mu_p = RT\left[\ln(\varphi_p)+\left(1-\frac{V_p}{V_S}\right)(1-\varphi_p)+\frac{V_p}{V_S}\chi_{p,S}(1-\varphi_p)^2\right] \tag{2.22}$$

where $\varphi_p$ is the volume fraction of the polymer, $V_S/V_p$ is the partial molar volume ratio of the solvent to the number-average value for the polymer and $\chi_{p,S}$ is the polymer-solvent FH interaction parameter. This parameter is related to the enthalpic interactions, involved with the mixing process. From its definition it follows that $\chi_{p,S}$ decreases with increasing solvent quality. For an infinitely high degree of polymerisation, $\chi_{crit}$, the value of $\chi_{p,S}$ above which the polymer becomes insoluble, equals 0.5, the so-called θ-solution (at a specific θ-temperature). Subsequently, the first two terms of Eq. (2.22) represent the entropic contributions to the mixing process (always negative) whereas the energetic contributions are expressed by the third term. $\chi_{p,S}$ depends on temperature and solvent type, thus accounting for the dependence of solubility on these parameters. The molar volume of a polymer increases with molar mass and due to the subsequent increase of the term $V_S/V_p$, solubility of a polymer decreases with molar mass. From the quadratic dependence of the chemical potential on $\varphi_p$ it can be understood that for high and positive values of $\chi_{p,S}$ two values of $\varphi_p$ exist, having an identical chemical potential. In such case, the system demixes into two coexisting phases, a polymer-rich phase and a polymer-poor phase ($\varphi_{pr}$ and $\varphi_{pp}$, Figure 2.4). A decrease in $\chi_{p,S}$ due to a higher temperature or an improved solvent quality leads to improved miscibility, eventually resulting in one homogeneous phase. In this case the favourable entropy of mixing is able to overcome the unfavourable enthalpy of mixing for all compositions. The temperature above which this occurs is commonly referred to as the critical temperature ($T_{cr}$). This type of demixing behaviour which is encountered for most polymer solutions is called Upper Critical Solution Temperature (UCST) behaviour.

$\chi_{p,S}$ is related to the Hildebrand solubility parameter, δ, according to:

$$\chi_{p,S} = (\delta_p - \delta_S)^2 \frac{V_p}{RT} \tag{2.23}$$

where δ is defined as the square root of the cohesive energy density.[30]

The solubility of polymers in binary non-solvent/solvent (NS/S) mixtures is often studied by turbidimetric titrations.[31,32] Herefrom, the following empirical relation between the volume fraction of solvent at the cloud point, $\varphi_S$, and the molar mass was found:[33]

$$\varphi_S = C_1 + C_2 M^{-0.5} \tag{2.24}$$

where $C_1$ and $C_2$ are constants, dependent on the NS/S system and on temperature. The relation between the volume fraction of the solvent and polymer concentration, c, at the cloud point is given by:[9]

$$\varphi_S = C_3 + C_4 \log(c) \tag{2.25}$$

in which $C_3$ and $C_4$ are constants, dependent on the NS/S system, the temperature and the molar mass.

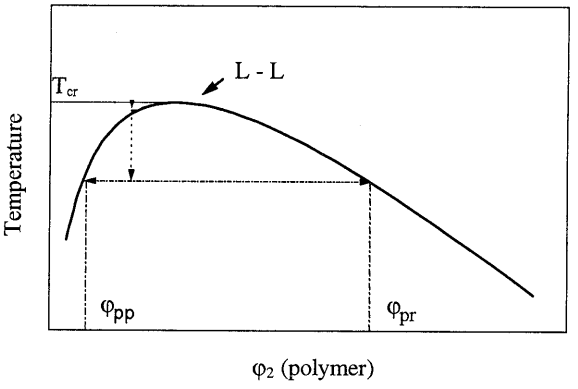



**Figure 2.4.** Schematic representation of the UCST behaviour of a binary polymer solution.

### *2.3.3.4 Gradient elution*

Gradient elution of polymers starts with the complete retention of the sample at the initial conditions followed by gradual elution during the subsequently applied gradient. Due to the limited solubility of polymers, the starting eluent is often, although not necessarily, a non-solvent for at least part of the injected polymer. During gradient elution, the thermodynamic quality of the mobile phase (eluent) increases, or to be more exact: $\chi_{p,s}$ (Eq. (2.22)), decreases, which leads to the gradual redissolution of the polymer. Subsequently, when the chromatographic strength of the eluent is large enough, meaning that $\Delta\mu_{total}$ (Eq. (2.17)) is low enough, the polymer starts migrating. Thus, retention is governed by three parameters: solubility effects, size exclusion and sorption. Because existing names for this separation type such as High Performance Precipitation Liquid Chromatography (HPPLC)[34] or Liquid Adsorption Chromatography (LAC)[35] only refer to a part of the total mechanism, the more generally applicable term Gradient Polymer Elution Chromatography (GPEC) was introduced.[36,37]
Both solubility and sorption of polymers depend on chemical composition *and* molar mass (Eqs. (2.19) and (2.22)). Thus, the final separation is determined by more than one molecular characteristic. For relatively low molar mass polymers, where the

individual oligomers can still be distinguished from each other, this needs not to be a problem. In that case, information on molar mass and chemical composition can directly be obtained from the elution pattern. In contrast, for high molar mass polymers, usually a continuous elution profile due to the large number of differing products existing in a synthetic polymer, is obtained. Thus, the translation of the elution profile into a distinct polymer characteristic such as chain length, composition of the polymer backbone or end group type, is hampered. Therefore, often a sequential combination of separation methods differing in their mechanisms is applied for the subsequent separation according to the various molecular characteristics, in order to completely unravel the polymer structure.[9]
Although a thorough understanding of several aspects of GPEC is still lacking, the technique, in some cases combined with SEC, is yet frequently applied for the determination of several polymer heterogeneities. One of the most well-known applications is the determination of CCDs of copolymers.[9] Furthermore, examples of the determination of FTDs of telechelic polymers,[7,8] the separation of polymer blends[38,39] and the fingerprinting of resins[40,41] have been reported.

#### *2.3.3.5 Normal phase and reversed phase chromatography*

For several reasons, *e.g.* the occurrence of solubility effects, special adsorption features such as multi-site attachment and conformational changes, as well as size exclusion effects, GPEC differs from gradient elution chromatography of low molar mass solutes. Nevertheless, like for conventional liquid chromatography, for GPEC in general two modes can be distinguished, *i.e.* reversed phase (RP) and normal phase (NP), which will further be referred to as RP-GPEC and NP-GPEC. The characteristics for both chromatographic modes given below mainly come from the chromatography of low molar mass solutes.

Reversed phase liquid chromatography is characterised by the use of non-polar stationary phases and eluents which are more polar than the stationary phase. Retention is driven by hydrophobic interactions with the stationary phase and polar interactions with the mobile phase. It has become clear that for low molar mass solutes, retention is mainly governed by a partitioning process, rather than by adsorption.[42] Nevertheless, detailed studies using a self-consistent field theory for adsorption showed that, although low molar mass solutes are fairly homogeneously distributed among the stationary phase, in all cases an enrichment in the boundary layer between grafted ($C_8$, $C_{18}$) chains and the solvent occurs. The enrichment was found to depend on the stationary phase and mobile phase composition and indicates that retention cannot purely be considered as partitioning.[43,44] The latter will be more

or less confirmed by the findings in Chapter 4. Furthermore, after being underestimated for a long time, it is now widely recognised that despite the predominance of (non-specific) hydrophobic interactions, the stationary phase plays an active role in the retention process of low molar mass solutes.[42]
For reversed phase systems, using a binary eluent mixture of water and an organic solvent, the dependence between the retention factor of a solute and the volume fraction, φ, of the organic solvent in the mobile phase can, in many cases, be described by:[27,45]

$$\log(k) = \log(k_w) - S'\varphi \tag{2.26}$$

Here, $k_w$ is the retention factor for water as the mobile phase and S' is the slope of the curve log(k) versus φ, which is a constant for a given solute within a restricted range of φ. S' depends on molar mass, for which it is sometimes found:[46]

$$S' = aM^b \tag{2.27}$$

For polystyrene in a water-THF system, values of $a$ = 0.22 and $b$ = 0.5 were reported.[46] Large values of S' at high molar masses are in agreement with the earlier mentioned 'on-off' behaviour of polymers under isocratic conditions. For gradient elution of oligomers and polymers, when linear gradients are applied, retention is given by:[27]

$$t_r = \left(\frac{t_0}{b}\right)\log\left[2.3k_0\left(\frac{t_{sec}}{t_0}\right)+1\right] + t_{sec} + t_s \tag{2.28}$$

where $t_0$ is the column dead time of a low molar mass solute and $t_{sec}$ is the column dead time of the injected high molar mass solute which, due to its relatively large hydrodynamic volume, undergoes steric exclusion effects from the porous column packing. $t_s$ is the system hold-up time *i.e.* the time needed for the eluent to flow from the gradient mixer in the pump to the detector in a system without a column, and $k_0$ is the value of k under isocratic conditions which equal the composition at the beginning of the gradient. b is a gradient steepness parameter given by:[27]

$$b = \Delta\varphi S'\left(\frac{t_0}{t_G}\right) = \Delta\varphi S'\left(\frac{V_m}{Ft_G}\right) \tag{2.29}$$

where Δφ is the change in φ during the gradient, $t_G$ (gradient time) is the time during which φ is varied, $V_m$ is the total volume of the mobile phase, *i.e.* $V_m = V_i + V_p$, and F is the flow rate.

Normal phase liquid chromatography (NPLC), which is in fact the oldest form of HPLC, is involved with the use of polar stationary phases and eluents less polar than the stationary phase. As the stationary phase, classical adsorbents such as silica and alumina can be used, but bonded phases are more popular nowadays. The use of varying bonded phase types, *i.e.* cyanopropyl (CN), aminopropyl ($NH_2$) and diol, can result in large differences in selectivity.[28,47] As compared to RPLC, the influence of the stationary phase is more pronounced in NPLC, thus offering an extra parameter to control selectivity.[42]

The main mechanism of NPLC is based on displacement, *i.e.* the competition of solute and solvent molecules for active sites.[48] The original model of Snyder assumes an equilibrium where 'n' solvent molecules at the surface of the stationary phase are displaced by 1 solute molecule. Retention of a solute is enhanced by its energy of adsorption (from a non-polar solvent *i.e.* n-pentane), $Q_0$, and is decreased proportionally with the polarity of the mobile phase and with the area of the adsorbed molecule of the solute on the surface of the sorbent, $A_s$ ($m^2$). This is shown in the basic Snyder equation describing retention in NPLC:[48]

$$\log(K_{ads}) = \log(V_a) + \alpha'(Q_0 - A_s\varepsilon) \tag{2.30}$$

where $K_{ads}$ is the distribution coefficient of the solute, now defined as the adsorbent amount of solute per gram adsorbent over the concentration in the solvent (thus, the dimension is $cm^3/g$), $V_a$ is the volume of the adsorbed solvent monolayer per unit weight of the adsorbent ($cm^3/g$), $\alpha'$ characterises the activity of the adsorbent and $\varepsilon$ is another parameter for the solvent strength of the mobile phase used ($m^{-2}$). $Q_0$ is the dimensionless free energy of adsorption. Based on this equation, the following relationship for the retention factor in a binary mobile phase consisting of a weak non-polar solvent (*e.g.*, heptane) and a strong, polar solvent was derived:[49]

$$\log(k) = \log(k_S) - \left(\frac{A_s}{n_b}\right)\log(N_b) \approx \log(k_S) - n\log(\varphi) \tag{2.31}$$

Here $k_S$ is the retention factor in the strong solvent alone, $n_b$ is the molecular area of the strong solvent, $N_b$ is the mole fraction of the strong solvent in the mobile phase, n is the number of solvent molecules displaced by 1 solute molecule and $\varphi$ is the volume fraction of the strong solvent in the NS/S mixture. The original model does not account for preferential adsorption of solutes and solvents onto strong sites (localisation) and secondary solvent effects (solvent-solute and solvent-sorbate interactions). Therefore, further refinements were made to include these phenomena which are nowadays known to be important parameters influencing both retention and selectivity.[42]

### 2.3.4 Chromatography under critical conditions

Liquid Chromatography under Critical Conditions (LCCC) can be considered as a special case of adsorption chromatography of polymers, where adsorption of the polymer backbone and exclusion are of the same order. Under critical conditions, for a non-functional homopolymer $\Delta\mu_y = 0$ and therefore $\Delta\mu_{total} = 0$ (Eq. (2.17)), thus no separation to molar mass will occur. However, chemically different sites in the polymer chains, such as functional (end) groups will generally exhibit an affinity towards the substrate, different from that of the repeat unit, *e.g.* $\Delta\mu_y \neq \Delta\mu_x \neq \Delta\mu_z$ (Eq. (2.17)). Therefore, under critical conditions, $\Delta\mu_{total}$ of a functional macromolecule is only determined by energetic interactions of the functionalities. This means that the distribution coefficients, $K_{ads}$, of functional and non-functional macromolecules are different, and the difference only depends on the number and type of the functionalities. As a result, a separation exclusively determined by functionality is obtained.[13,50] When combined with a second separation technique such as SEC, LCCC can provide unique information on the MMFTD of polymers.

By several workers, it has been shown that LCCC is also applicable to block copolymers.[13,51] The interaction energies of chemically different blocks will mostly differ significantly. So under critical conditions for the A block of an AB block copolymer, the presence of the B block has a similar effect as, for instance, an end group. Retention will therefore only be determined by the B block. When conditions are chosen such that critical conditions for A form exclusion conditions for B, then the elution of the block copolymer is solely determined by the block length of B. Hence, the resulting SEC curve will provide information on the block length distribution of B.

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# CHAPTER 3

# Practical Parameters and Solubility Effects in Reversed Phase Gradient Polymer Elution Chromatography of Amorphous Polyesters


## SUMMARY

*By reversed phase gradient polymer elution chromatography (RP-GPEC), using a $C_{18}$ column and a water-THF non-solvent/solvent (NS/S) system, highly detailed oligomer separations of amorphous polyesters were obtained. The separation was found to be dominated by molar mass and to a lesser extent by chemical composition. The effects of practical parameters such as gradient steepness, sample load, injection volume and temperature were investigated. In some cases distinct influences on the separation result were found, especially for the low molar mass parts of the polyesters.*

*The effect of solubility effects in RP-GPEC was investigated under chromatographic conditions, using inert column packings and low polydispersity fractions obtained by SEC. As an inert medium, non-porous glass was shown to be the best choice. By comparison of the results of the various polyester fractions on a glass and a $C_{18}$ column, it was shown that the separation on $C_{18}$ is solely determined by sorption effects. A comparison with measurements of maximum solubility under static equilibrium conditions of four different polyester fractions in various NS/S combinations revealed that concentrations of the eluting fractions on the glass column are considerably lower than maximum solubility. This can be explained by kinetic effects, influencing redissolution.*



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H.J.A. Philipsen, M.R. de Cooker, H.A. Claessens, B. Klumperman and A.L. German, *J. Chromatogr. A*, 761 (1997) 147.

## 3.1 INTRODUCTION

The applicability of reversed phase liquid chromatography for the chemical analysis of polymers has been known since a considerable time. For reasons described in Chapter 2, in most cases gradient elution is applied, which will here be referred to as Reversed Phase Gradient Polymer Elution Chromatography (RP-GPEC). RP-GPEC has mainly been used for the characterisation of copolymers according to their chemical composition distribution[1-5] and for the fingerprinting of resins.[6-12] Especially in the latter case, practical applications often seem to lack which may be due to the difficulties encountered to obtain unambiguous information on chemical composition (differences) from the complex elution patterns. This is probably at least partly caused by a lack of good understanding of possibilities and limitations of RP-GPEC and of its mechanisms.
Little information is available on the effect of various practical parameters in RP-GPEC, thus hampering the discrimination between chromatographic artefacts and real effects resulting from the polymer composition. Furthermore, the separation mechanism is frequently a matter of debate.[13,14] It is recognised that solubility effects *i.e.* precipitation and redissolution do occur in many RP-GPEC separations.[1,2] This is due to the weak sorption forces inherent to RP separations, giving rise to the need of weak displacers in order to achieve complete retention. Due to the limited solubility of polymers these displacers are mostly non-solvents, thus causing precipitation. However, the role of solubility effects on the final separation result is often not clear.
The potential applicability of RP gradient elution techniques for polyester resins has been demonstrated by several authors.[6-9,12] In this Chapter, the effect of various practical parameters and the role of solubility effects in RP-GPEC of amorphous polyesters are investigated.

Studies to the effect of solubility effects in polymer chromatography are mostly concerned with the comparison of eluent compositions at the point of elution with cloud points, determined by turbidimetric titrations.[15-18] Since, according to Eq. (2.25) the cloud point composition, $\varphi_S$, depends on the polymer concentration, care must be taken that concentrations in both chromatography and cloud point titration are comparable. Furthermore, from Eq. (2.24) it is obvious that $\varphi_S$ also depends on molar mass, the effect of which will especially be pronounced for relatively low molar mass resins. Even for polyester resins with a relatively low polydispersity (approximately 2), a single determination of the cloud point of the complete resin can clearly not be used for comparison with chromatographic data to account for the retention mechanism. Such a comparison is further hampered by the increasing concentration dependence of the cloud point with decreasing molar mass, which results in lower values of $C_4$ in Eq. (2.25).

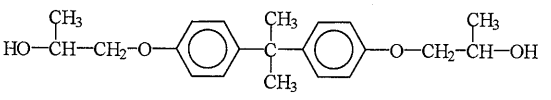
An alternative method by which sorption and solubility effects can be separately controlled, was presented by Glöckner who used so-called sudden transition (ST) gradients for this purpose.[19-21] After injection into a strong non-solvent, the eluent composition is rapidly changed by the addition of a solvent of moderate polarity, which causes the precipitated polymer to redissolve. However, the NS/S composition is changed in such a way that the sample is still retained by sorption forces. Finally, a chromatographically strong eluent ('displacer'), which is not necessarily a solvent, is added, causing the polymer to elute. By comparison of these results with a true NS/S gradient, the effect of redissolution effects can be determined. The application of ST gradients for low molar mass polymers, however, is restricted by the fact that cloud points of different molar mass fractions vary over a wide composition range. Therefore, especially under RP conditions where interaction forces with the stationary phase are weak, mostly no composition can be found that will completely redissolve the resin and, virtually, simultaneously causes complete retention.
In this Chapter, at first the effect of practical parameters such as loadability, injection volume, gradient shape and temperature in RP-GPEC of amorphous polyesters are systematically examined. This will provide more insight in the role of the individual parameters and allows to optimise the separation result for polyesters in RP-GPEC.
Second, the effect of solubility effects in the overall separation mechanism is investigated. From the considerations described above, it was concluded that solubility effects of low molar mass polymers in RP systems can at best be studied under chromatographic conditions, using inert column packings and low polydispersity polymer fractions. Therefore, various media were tested for the use as an inert column packing for polyesters. Subsequently, low polydispersity polyester fractions were subjected to RP-GPEC on these media and an RP column, and to solubility measurements under static equilibrium conditions, the results of which provide further insight in the role of solubility effects.

## 3.2 EXPERIMENTAL

### 3.2.1 Polymer samples and low polydispersity fractions

The polymer samples used were laboratory made polyester resins. Samples PE1, PE2 and PE3 are copolyesters consisting of adipic acid (A), isophtalic acid (I) and di-propoxylated bisphenol-A (D). Samples PE4 and PE7 are homopolyesters based on A and D, PE5 is a homopolyester consisting of maleic acid (Ma) and D and PE7 is a homopolyester made of I and D. The molecular structures of the monomers are given in scheme 3.1. For more detailed information on the polymer composition, see Section 3.2.2.

Low polydispersity fractions of PE1 were obtained by SEC. 200 µl of a 25 mg/ml solution was injected 200 times on the SEC system described in Section 3.2.2. The total bandwidth of about 10 minutes (see Figure 3.5) was separated into 10 fractions of 1 minute. The fractions were dried under nitrogen and the amount was determined gravimetrically. A part of each fraction was redissolved in tetrahydrofuran (THF) up to a concentration of 0.4 mg/ml and 200 µl was re-injected on the SEC system to determine the polydispersity. For gradient elution experiments where the exact injected amounts had to be known, concentrations of these solutions were carefully determined by HPLC, as described in Section 3.2.7.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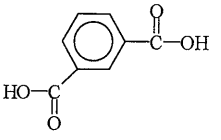
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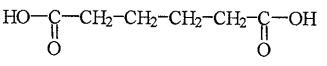
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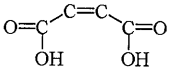
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**Scheme 3.1.** Molecular structures of the monomers. 1: dipropoxylated bisphenol-A, 2: isophtalic acid, 3: adipic acid, 4: maleic acid.

### 3.2.2 Polymer characterisation

Polystyrene equivalent molar masses of the polyesters determined by SEC, average chemical compositions measured by NMR and end group compositions determined by titrimetric analysis are given in Table 3.1.

The equipment used for SEC consisted of an isocratic pump Model 610, a WISP type 715, a column thermostat, type TCM which was set at 40 °C, and a differential refractometer type 410, all from Waters (Milford, MA, USA), a UV detector type 975 from Jasco (Tokyo, Japan) which was set at 254 nm and a Baseline-815 data system from Waters. A set of four Shodex (Showa Denko, Tokyo, Japan) KF columns (300 x 8mm) in series was used (KF804, KF803, KF802.5, KF802 and a guard column type 800P). THF

with 1% (v/v) acetic acid was used as the mobile phase at a flow rate of 1.5 ml/min. Sample size was 200 µl of 0.1% solutions in THF. Toluene was added as an internal marker. The columns were calibrated using narrow standard polystyrenes from Waters with molar masses between 418 and 450000. The reproducibility of the polystyrene equivalent molar masses was approximately ± 100 Daltons (standard deviation).

$^1$H-NMR spectra were recorded on a Bruker AC300 (300 MHz) spectrometer. The chemical shifts were determined relative to tetramethylsilane. Spectra were obtained in $CDCl_3$ at a sample concentration of 60 mg/ml and were recorded under quantitative conditions. For the determination of the molar fractions of the different monomeric units, integrals were taken between $^1$H chemical shift values: δ 7.2-6.5 ppm (D), 8.4-7.9 ppm (I), 2.6-2.0 ppm (A) and 6.3-6.1 ppm (Ma). The standard deviation was determined to be ± 0.01 (absolute). The degree of propoxylation, which is defined as the ratio of the number of propoxy groups over the number of bisphenol-A units, was calculated from the ratio of the integrals between δ 6.0-3.0 ppm and 7.2-6.5 ppm. The standard deviation was ± 0.1 (absolute).

The acid number was determined by a potentiometric titration, using a potentiometer, model AT300 from Kyoto Electronic. To this end, 300 mg of polyester was dissolved in a mixture of 70 ml THF and 30 ml methanol. The solution was titrated with 0.01 M potassium hydroxide dissolved in methanol. The relative standard deviation was approximately ± 10% KOH/g.

For sample PE1, the number average molar mass was also determined by vapour pressure osmometry, using a Mechrolab vapour pressure osmometer (see Table 8.2). Measurements were performed in chloroform. The absolute weight average molar mass was determined by SEC-LALLS, using a Chromatrix LALLS detector. For this purpose, 80 µl of 1% (w/w) solutions in chloroform were injected.

**Table 3.1.** Polystyrene equivalent molar masses, end group compositions and average chemical compositions of the investigated polyesters

| Sample | SEC | | | Titrations | NMR | | | |
|---|---|---|---|---|---|---|---|---|
| | PS-eq. molar masses | | | Acid number | Molar fractions | | | Degree of |
| | $M_n$ | $M_w$ | $D^*$ | (mg KOH/g) | A | I | D | Propoxylation |
| PE1 | 3500 | 7900 | 2.3 | 20 | 0.12 | 0.37 | 0.50 | 2.4 |
| PE2 | 3400 | 8200 | 2.4 | 24 | 0.12 | 0.38 | 0.50 | 2.2 |
| PE3 | 3300 | 7900 | 2.4 | 27 | 0.15 | 0.35 | 0.50 | 2.0 |
| PE4 | 3800 | 8700 | 2.3 | 20 | 0.51 | 0 | 0.49 | 2.2 |
| PE5 | 3900 | 13700 | 3.5 | 13 | 0.45 | (Ma)** | 0.55 | 2.0 |
| PE6 | 3000 | 6600 | 2.2 | 25 | 0 | 0.49 | 0.51 | 2.0 |
| PE7 | 3000 | 6300 | 2.1 | 23 | 0.51 | 0 | 0.49 | 2.0 |

*: polydispersity, $M_w/M_n$.
**: maleic acid.

### 3.2.3 HPLC solvents and columns

The solvents used for HPLC and solubility experiments were water, Lichrosolv quality from Merck (Darmstadt, Germany) and tetrahydrofuran (THF), HPLC grade from Rathburn (Brunschwig Chemie, Amsterdam, The Netherlands). To both solvents, 200 µl acetic acid, Pro Analysi quality from Merck, per litre was added. For HPLC the solvents were constantly sparged with helium (20 ml/min). All solvent mixtures were made by volumetric mixing by the HPLC pump, no premixes were used.
The columns used were three Novapak $C_{18}$ columns (Waters, $d_p$ = 4 µm, pore size 60 Å, 75 x 3.9 mm, 150 x 3.9 mm and 300 x 3.9 mm), a Resolve silica column (Waters, $d_p$ = 5 µm, pore size 90 Å, 150 x 3.9 mm) and a PL-gel styrene-divinylbenzene (Polymer Laboratories, Shropshire, UK, $d_p$ = 5 µm, pore size 100 Å, 600 x 7.5 mm) which was used for solubility experiments (see Section 3.2.7). Furthermore, for gradient elution experiments a column (150 x 4.6 mm) was dry-packed with non-porous glass beads, diameter 40 - 60 µm (Phase Separations, UK, part no. 750138). The performance of this column was tested by the injection of 5 µl toluene in water. The asymmetry of the resulting peak measured at 10% of the peak height was found to be 1.2. The dead volume ($V_m$) for each column was determined not only by the injection of a low molar mass solute (toluene) but also for each individual low polydispersity fraction. The thus found values were used in further calculations to correct for SEC effects (see Section 2.3.3.5, Eq. (2.28) and related discussion). For the silica and the $C_{18}$ column these values were found to vary between 0.89 - 1.36 ml and 0.78 - 1.07 ml respectively. For the glass column, all values equal 1.00 ml.
All columns were connected by a 0.02 cm (I.D.) tubing of about 50 cm to the injector, which was led through the column thermostat to ensure the sample to reach the right temperature before entering the column. For all experiments a stainless steel in-line pre-column filter (Waters, part no. 084560) was used.

### 3.2.4 HPLC equipment

All HPLC experiments described in this thesis were performed, using a Waters 600E 4 solvent gradient pump, and a 717 autosampler or a 715 WISP from Waters. The UV detectors used were a variable wavelength detector, Waters, type 484 or 486 or a Jasco (Tokyo, Japan) type 975. The column temperature was controlled using a thermostat type Mistral from Spark-Holland (Emmen, The Netherlands). Chromatograms were recorded using the Baseline-815 system from Waters. HPLC fractions were obtained using a Gilson (Villiers-le-Bel, France) model 203 fraction collector.

### 3.2.5 Gradient elution experiments

Gradient elution was performed as follows. After running each gradient, the system was reset to initial conditions in one minute, followed by pumping 15 column volumes of the starting eluent composition to re-equilibrate the column. In order to properly condition the system, prior to the analysis of the samples two blank gradients were run. All gradients were started at the moment of injection. The gradient performance of the pump (linearity and reproducibility) was checked by running gradients from methanol to methanol + 0.1% (v/v) acetone. The linearity was found to be excellent, thus allowing an easy calculation of the eluting eluent composition at each retention time. The system hold-up volume ('dwell volume') which was also determined from these experiments was taken to be equal to the first baseline deviation caused by the elution of acetone, and was found to be equal to 4.0 ml. The column dead volume was taken to be equal to the elution volume of the maximum of the system disturbance caused by the injection of 10 µl THF. This value was found to be 1.4 ml for the silica column, 1.1 ml for the $C_{18}$ column and 1.0 ml for the glass column.
For experiments on the low polydispersity fractions, a gradient was run from water-THF (both containing 0.02% (v/v) acetic acid) (100:0) to (0:100) in 33.3 min (3%/min). Initial conditions were chosen at a water content as high as possible because small parts of the low molar mass fractions were known to be soluble in water. Starting at higher amounts of THF would cause significant amounts to elute unretained on inert columns which would hamper a good comparison with elution on $C_{18}$. Although it is known that initial conditions of 100% water in RPLC can sometimes lead to bad reproducibility, no such problems were encountered during the experiments described here. Unless indicated otherwise, all these measurements were carried out at 21 °C.

### 3.2.6 Cloud point titrations

Cloud points of the unfractionated polyester (PE1) and polyester fractions were determined by visual observation at room temperature (21 °C). For the polyester fractions, 0.5 ml of a solution in THF was brought into a micro titration vial equipped with a magnetic stirrer bar.[22] Concentrations were taken such that the final concentration in the cloud point was approximately 1.5 mg/ml. By means of a 100 µl syringe, small portions of the non-solvent (water) were added until the solution became turbid. The point at which turbidity did not disappear after stirring was defined as the cloud point. %-solvent (%-S) at this point was determined gravimetrically. All cloud points were determined three times. The standard deviation of the three measurements did not exceed 0.3% Solvent, except for the very low molar mass fractions where somewhat larger values were found.

### 3.2.7 Determination of solubility

The sample preparation for the determination of the solubility as function of the NS/S ratio was as follows. From a solution of 30 mg/ml (THF) of a low polydispersity polyester fraction, 100 µl was taken with a syringe and transferred into a 4 ml vial. After drying under nitrogen, water and THF, both containing 0.02% (v/v) acetic acid were added to a volume of 1.0 ml, such that a desired NS/S ratio was obtained. All amounts were determined by weight. After thoroughly shaking for 10 minutes, the vial was put in a temperature controlled water bath at 21 °C. After equilibration for one night, the suspension was centrifuged for 6 minutes at 3000 g. From the thus obtained supernatant, 300 µl was carefully taken with a syringe and centrifuged again. Finally, a small amount of the clear solution was injected on an HPLC system, to determine the polyester concentration. This method resembles that of other workers,[23] but modifications had to be made due to the much lower molar masses of the polyesters investigated here.
It is known that determination of solubility of polymers from the dry state, especially in the case of high molar mass polymers, can provide different results from the more accurate approach of precipitation from solution.[24] The former procedure was preferred here, however, since a comparison of both methods revealed that for the low molar mass polymers used here in the latter method too high values were obtained in the low concentration range (< 0.05 mg/ml), probably due to slow precipitation.[22] For higher concentrations, identical values for both methods were obtained.
For the HPLC measurements, a PL-gel styrene-divinylbenzene column (see Section 3.2.3) was used, which was thermostated at 21 °C. The eluent was THF containing 1% (v/v) acetic acid. The injection volume was in the range 5 - 100 µl, dependent on the (estimated) polyester concentration. All solutions were at least injected twice. The system was calibrated using three independent solutions of the unfractionated polyester with known concentrations. From other experiments it was known that the extinction coefficient does not show a significant dependence on molar mass at the used detection wavelength, thus allowing for the chosen calibration procedure.

## 3.3 RESULTS AND DISCUSSION

### 3.3.1 Effect of practical parameters in RP-GPEC of amorphous polyesters

#### *3.3.1.1 Choice of the stationary and mobile phase*

In the reversed phase chromatography of low molar mass species, mostly tetrahydrofuran (THF), acetonitrile (ACN) and methanol (MeOH) in combination with water, are used to

control selectivity.[25] Due to the different nature of these organic modifiers which give rise to different types of interaction, almost any desired separation can be obtained by just using these eluents or eluent combinations. Since the high molar mass parts of the polyesters used in this study are not soluble in MeOH and ACN, only THF can be used as a strong solvent for RP-GPEC. Because the eluent strength of MeOH, being the weakest organic modifier in this case, also proved to be too strong to retain the low molar mass parts of the polyester resins on a $C_{18}$ column, water has to be used as the non-solvent. For all RP-GPEC experiments described in this Chapter, a $C_{18}$ column was used. From other experiments it appeared that the column type influences the separation of polyesters by RP-GPEC only to a minor extent.[26-28] This, together with experiments where MeOH and ACN are used as co-solvents in ternary systems, will be the subject of a separate publication.

### *3.3.1.2 Gradient steepness*

Several gradient profiles using a $C_{18}$ stationary phase and a water-THF mobile phase combination were tested. In Figure 3.1A an example is shown for sample PE1, using a linear gradient with a steepness of 1%/min. Up to 20 oligomers can be resolved in this case, whereas in the very low molar mass part of the chromatogram an additional separation according to chemical composition within each oligomer is realised. The peak assignment will be discussed later. The use of steeper gradients causes a significant decrease in the total number of oligomers that can be separated. For instance, for a gradient steepness of 3%/min, only 12 oligomers are resolved. When extremely slow gradients are applied, a slight increase in oligomer separation is observed at the cost of much longer analysis times. An example is shown in Figure 3.1B, where a steepness of 0.2%/min is applied. For linear gradients, a steepness of 1%/min proved to be an acceptable compromise between resolution and analysis time. Slightly convex shaped gradients within the same analysis time, consisting of two or three linear segments, developed during further optimisation, can provide in some cases additional resolution improvement in the high molar mass part of the chromatogram. An example can be seen in Figure 3.4. The use of continuous convex gradients, instead of these two or three-segment convex gradients, provided no further improvement of resolution, which is in accordance with the findings of Snyder *et al.*[29]

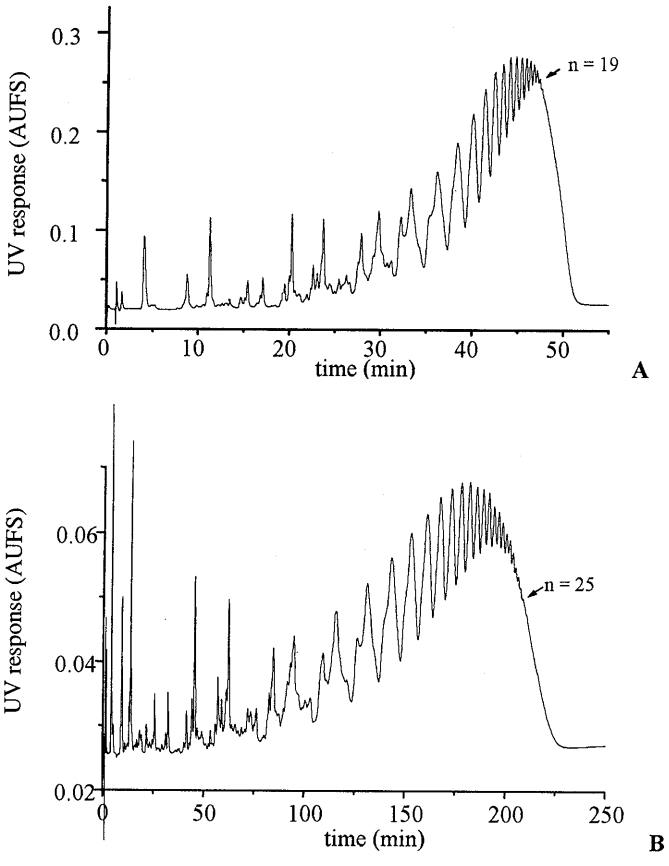


**Figure 3.1.** Effect of gradient steepness. A: 1%/min, B: 0.2%/min. Sample: PE1 (40 mg/ml), column: Novapak $C_{18}$ (150 x 3.9 mm), temperature: 35 °C, eluent: water-THF (65:35, v/v) to (15:85), flow: 1.0 ml/min, injection: 10 µl, detection: UV at 277 nm.

### *3.3.1.3 Column length*

Since the eluent at the start of the RP-GPEC analysis, 35% THF in water, is a non-solvent for the polyesters, solubility effects, next to sorption may contribute to the total separation mechanism. Therefore the effect of column length is not obvious on the beforehand.[13] In the case that solubility would influence the separation by GPEC, a longer column might cause more dispersion effects, thus giving rise to broader peaks and decreasing resolution.

In order to keep gradient elution experiments performed on columns differing in geometry, comparable, it is necessary to keep the average retention factors, k*, of the components constant, which can be done by keeping the gradient steepness parameter, b

(Eq. (2.29)), being inversely proportional to k*, constant.[25] Since S' in Eq. (2.29) is independent of column geometry and $\Delta\varphi$, which is determined by the starting and end conditions, is not changed, the term $t_G F/V_m$ has to be kept constant. The use of a column with another length therefore means that the gradient time, $t_G$, has to be changed in accordance, while keeping the flow rate constant. In Figure 3.2, the results for PE1 on a 7.5 cm, 15 cm and a 30 cm column are compared.

The increase of column length causes a significant improvement in resolution in the low molar mass part of the chromatogram. In the high molar mass part, the resolution enhancement is only minor. When using a 30 cm column instead of a 7.5 cm, 19 instead of 21 oligomers can be resolved. This agrees with the findings of Snyder *et al.*,[13,30] but the cause of it is not unambiguously clear. Since k* was kept constant, the counterbalance of the increase in plate number by a decrease in k* as argued by Snyder, provides no explanation. S' (Eq. (2.29)), however, is known to increase with molar mass (Eq. (2.27)), thus giving rise to very high values for high molar mass substances. This also leads to very low values of k* which means that during migration, the distribution into the stationary phase is minor. This might explain the rather limited effect of column length in the high molar mass part.

Furthermore, from a comparison with the low molar mass part of the chromatogram, it is obvious that the high molar mass peaks are composite peaks, consisting of several different components (see also the discussion on peak assignment). In such case, the apparent band width is the result of two effects: the width of each band for a single species and the width of the composite band, as determined by differences in retention for each species. To a first approximation, the apparent width for a high molar mass peak can be given by:

$$W^2 = W_c^2 + W_0^2 \tag{3.1}$$

where $W_c$ represents the width of an individual species and $W_o$ is the width, determined by the retention time difference between the first and last component eluting in this band. An increase of column length results in an increase in the plate number and thus resolution, which is the net result of both increasing $W_c$ and retention time, as can be observed in the low molar mass part of the chromatogram. For high molar mass bands, however, $W_c$ becomes so small that W will be mainly determined by $W_o$, thus causing no further improvement in resolution. These results indicate that sorption effects are probably dominant in the total separation mechanism.

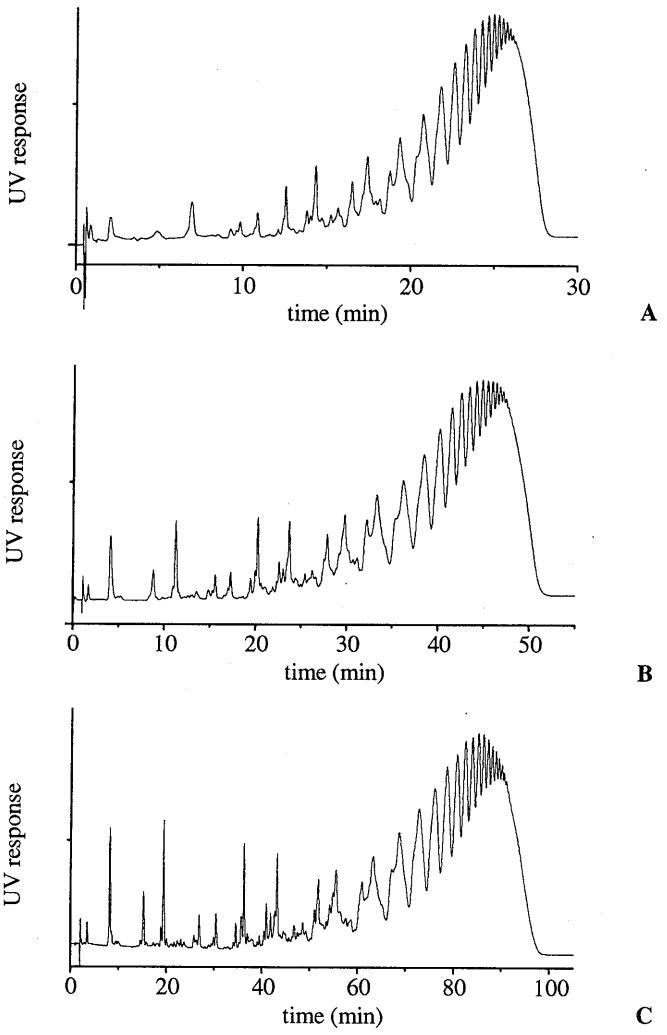


**Figure 3.2.** Effect of column length. A: 7.5 cm, B: 15 cm, C: 30 cm. Sample: PE1 (40 mg/ml), eluent: water-THF (65:35, v/v) to (15:85) (A: 2%/min, B: 1%/min, C: 0.5%/min). Further conditions: see Figure 3.1.

### *3.3.1.4 Temperature effects*

The effect of temperature in RP-GPEC of polyesters can be observed from Figure 3.3. Increasing temperature causes the oligomer distribution to shift to lower retention times. Presumably this may be due to a decrease in sorption. Furthermore, at higher temperatures, resolution increases, especially in the first part of the chromatogram. This is probably due to the increase in diffusion coefficients, giving rise to faster mass transfer and therefore a decrease in peak broadening. The rather limited resolution enhancement

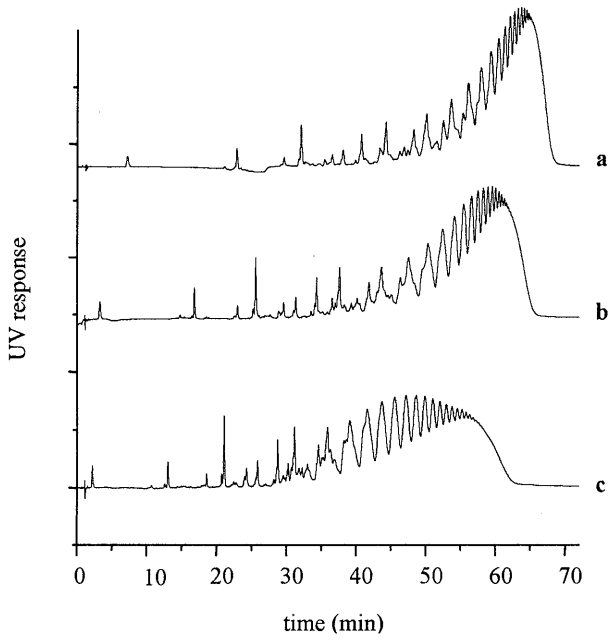


**Figure 3.3.** Effect of temperature. a: 10 °C, b: 40 °C, c: 70 °C. Eluent: water-THF (75:25) to (10:90) (0 to 65 min). Further conditions: see Figure 3.1.

in the high molar mass part can, like for the case of column length, probably also be ascribed to $W_o$ (Eq. (3.1)) dominating the resolution for the late eluting bands. The observed resolution enhancement is much less than the effects earlier reported for aliphatic polyether type oligomers on conventional porous supports[31] and for a water soluble resin on non-porous supports.[32] The cause of this difference is rather unclear and may result from various effects. The separation mechanism may be different in the respective cases. Furthermore, conformational changes caused by temperature variation which may sometimes influence the separation,[33,34] can be different for different polymer types. Therefore, further study on different polymer types under well comparable conditions is needed to get further insight in the effect of temperature on oligomer separations.

### *3.3.1.5 Sample load and injection volume*

The effect of sample load was studied by injecting different concentrations of PE1, while keeping the injection volume constant. No effects could be observed up to an injected mass of 1000 µg[27] (picture not shown here). This observation further supports the idea that sorption effects are dominant in the chromatographic process. When the separation

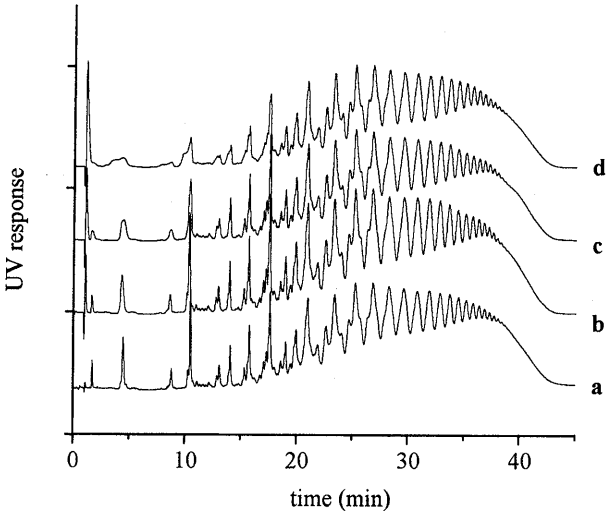


**Figure 3.4.** Effect of injection volume. a: 1 µl, b: 10 µl, c: 20 µl, d: 50 µl. Eluent: water-THF (65:35, v/v) to (40:60) (0 to 14 min), (40:60) to (32:68) (14 to 20 min), (32:68) to (15:85) (20 to 43 min). Further conditions: see Figure 3.1.

would be mainly determined by solubility effects, a clear increase of retention with increasing sample load should have been observed.[1,14] The high loadability can be contributed to the relatively low molar mass of the investigated polymer. This causes problems due to high viscosity, such as 'viscous fingering', less likely to occur. Furthermore, due to the elution of the polyester over a wide retention range, the momentary sample load will also be lowered to a great extent, thus contributing to a high allowable sample load.

The effect of injection volume was checked by injecting approximately constant amounts of 100 µg in different volumes. As can be seen from Figure 3.4, up to an injection volume of 20 µl no additional peak broadening can be observed, whereas for an injection volume of 50 µl, the lowest molar mass peaks are seriously distorted. This so-called sample-solvent effect, is well known in chromatography and can be explained by the difference in solvent strength between polymer solvent and eluent.[25] Increasing injection volumes also causes the peak at 1.0 min to increase significantly. Comparison with injections of THF revealed that this peak is solely caused by the solvent. Despite of the large injection volumes, no breakthrough occurred, an effect that is frequently observed in the chromatography of polymers,[35,36] causing parts of the sample to elute unretained due to insufficient mixing of the injection plug with the eluent. To suppress peak broadening, injection volumes should be as low as possible and, in this case, should not exceed 10 µl, which is approximately 1% of the total column volume.

### 3.3.2 Fingerprinting of polyesters by RP-GPEC

In Figures 3.5A and 3.5B RP-GPEC and SEC chromatograms for samples PE1, PE4 and PE5 are shown. Although the columns used for SEC were especially suited for relatively low molar mass polymers, it is obvious that RP-GPEC can provide much more detailed information on the composition of these resins. For sample PE1 as compared to PE4 and PE5, a larger number of peaks can be observed in the GPEC chromatogram. This is due to the fact that PE1 is a copolyester containing two different di-acids. Consequently, the number of different products that is formed during synthesis is larger.
Peak assignment for samples PE4 and PE5 is rather straightforward. In the low molar mass part of the chromatograms, repeating patterns consisting of three peaks, can be recognised. From reversed phase chromatography it is known that retention increases

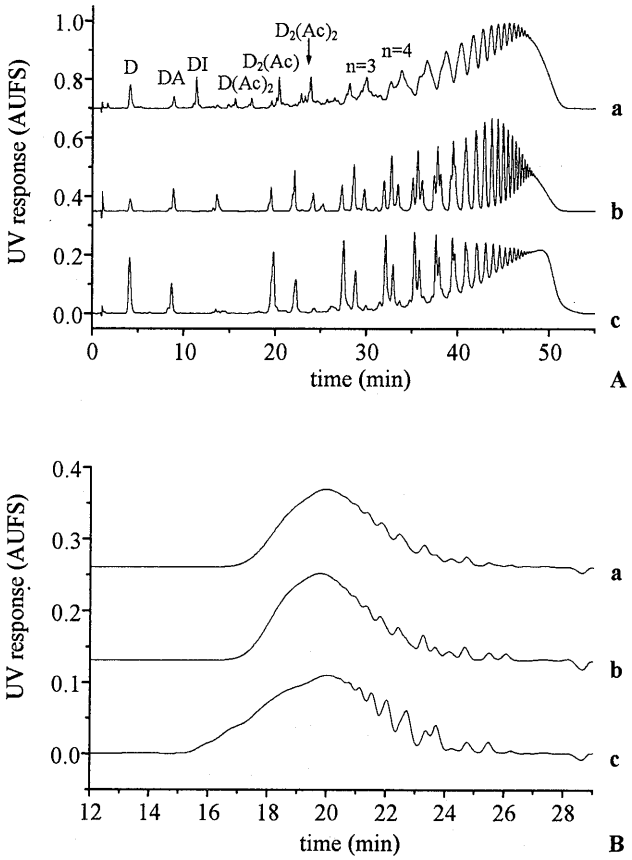


**Figure 3.5.** Comparison between GPEC (A) and SEC (B). a: PE1, b: PE4, c: PE5. GPEC conditions: see Figure 3.1A. SEC conditions: see Section 3.2.2. Ac = acid.

with increasing molecular volume.[34] For the polyesters used in this study, molecular volume is mainly determined by the number of diol units, since the molar mass of a diol monomer far exceeds that for both di-acids. It is therefore very probable that the observed repeating patterns will be caused by oligomers with a certain number of repeating units, having respectively none, one and two acidic end groups. Proof of this assumption was obtained by injection of the diol monomer and a product having mainly alcoholic end groups.[37]

Transformation of acidic end groups of PE1 into sodium salts, followed by extraction with water and injection of the water extract, proved that peaks at 9 and 11 min can be assigned to oligomers having one acidic end group. Peaks between 13 and 18 min were shown to represent oligomers having two acidic end groups. By comparison of chromatograms of PE1 and PE4 it appears that the peak at 9 min is due to the product diol - adipic acid, so the peak at 11 min is assigned to the oligomer diol - isophtalic acid.

It can be concluded that by RP-GPEC, polyesters are mainly separated according to molar mass, whereas in the low molar mass part a further separation with respect to chemical composition, such as end groups, occurs.

In order to use RP-GPEC for fingerprinting purposes, it is necessary that separation results are highly reproducible. For sample PE1, over a period of more than three years, no significant differences in the low molar mass part of the chromatogram were observed, although small retention shifts due to the use of new columns, necessitates injection of a reference sample in each analysis sequence. In the high molar mass part, however, sometimes small disturbances in the chromatograms occur, the cause of which has not been clarified until now. Duplication of the results for each sample therefore remains highly recommendable.

In Chapter 8 the applicability of RP-GPEC for the microstructural characterisation of copolyesters and for the determination of absolute molar masses, will be investigated.

### 3.3.3 Study of solubility effects in RP-GPEC of amorphous polyesters

#### *3.3.3.1 Molar mass and concentration considerations*

For the investigations of solubility effects in RP-GPEC of amorphous polyesters, low polydispersity fractions of PE1 were prepared by SEC. The polystyrene equivalent molar masses, polydispersity values and amounts of the obtained polyester fractions, are shown in Table 3.2. Most of the obtained polydispersities are close to 1.1, thus making the fractions suitable for solubility studies. Since solubility (Eq. (2.24)) and retention in GPEC both strongly depend on molar mass, polydispersities must be as low as possible to enable a meaningful comparison between solubility under equilibrium conditions and chromatographic conditions respectively. As could be

**Table 3.2.** Polystyrene equivalent molar masses, polydispersity values and amounts of low polydispersity polyester fractions obtained by SEC

| Fraction number | $M_n$ | $M_w$ | $D$ | Obtained amount (g) |
|---|---|---|---|---|
| 1 | 33300 | 38000 | 1.14 | 0.0075 |
| 2 | 21000 | 22500 | 1.07 | 0.0707 |
| 3 | 12000 | 12800 | 1.07 | 0.1506 |
| 4 | 7300 | 7800 | 1.07 | 0.2474 |
| 5 | 4200 | 4400 | 1.05 | 0.1568 |
| 6 | 2500 | 2600 | 1.04 | 0.0870 |
| 7 | 1500 | 1600 | 1.07 | 0.0564 |
| 8 | 960 | 1130 | 1.18 | 0.0202 |
| 9 | 650 | 940 | 1.45 | 0.0120 |
| 10 | 400 | 780 | 1.95 | 0.0016 |
| | | | | $\Sigma$ = 0.8102 |

expected for low molar mass polymers, the effect of concentration on the cloud point composition, $\varphi_S$, is relatively large. This is demonstrated in Figure 3.6 where $\varphi_S$ of the unfractionated polyester is plotted as a function of its concentration. The variation of $\varphi_S$ with temperature, which was also investigated for the unfractionated polyester, was found to be approximately 0.15%/°C.[22] Since the concentration and temperature dependencies increase towards lower molar masses, this situation would even be worse for the low molar mass fractions. Therefore, for comparison of $\varphi_S$ values with chromatographic results, care must be taken that concentrations in both cases are identical. This approach, which can be used for high molar mass polymers[15] would fail in the case of the polyesters, due to the extreme molar mass dependence of retention in the low molar mass range. Although the polydispersity of the fractions used here is low, they still consist of a mixture of related substances differing in molar mass and chemical composition, giving rise to different elution volumes (see for instance Figure 3.7, $C_{18}$ column). This results in a considerable chromatographic dilution, that would have to be compensated by the injection of very high concentrations which might cause redissolution problems or chromatographic overloading thus influencing the elution behaviour.

### *3.3.3.2 Gradient elution experiments on non or less adsorbing media*

From the considerations above, it seems that solubility effects in RP-GPEC of polyesters can at best be studied under non-adsorption chromatographic conditions, thus necessitating the availability of inert column packings. The use of normal phase packings, *e.g.* bare silica, under reversed phase conditions, has been suggested for this

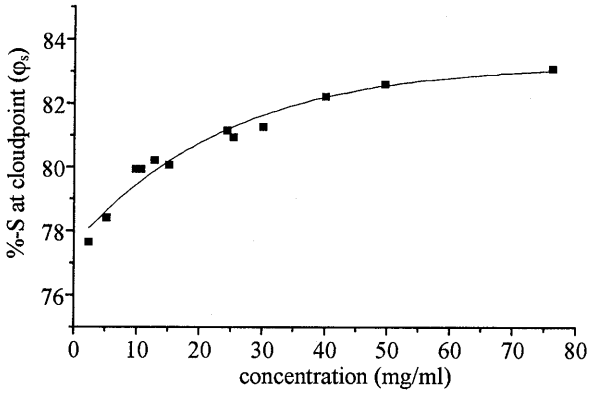


**Figure 3.6.** Effect of the polyester concentration (PE1) on %-S at the cloudpoint ($\varphi_S$). Temperature: 21 °C, NS/S: water-THF.

purpose.[18,38] Non-porous glass beads were taken into account as a possible alternative.

Although it might be expected that acidified water would strongly suppress the adsorption properties of the residual silanol groups of the stationary phase, it is known that, even under such circumstances, in certain cases silica as well as glass can be retentive.[18] Therefore, we could do no better than testing the elution behaviour of the polyester fractions in THF containing 0.02% (v/v) acetic acid and in water-THF (15:85) (v/v) + 0.02% acetic acid, which about equals the NS/S composition at which the last part of the polyester elutes. In THF, for both columns parts of the injected fractions were found to elute after the column dead volume ($V_m$), indicating adsorptive interactions. In the water-THF mixture however, all fractions completely eluted at $V_m$ in the case of non-porous glass and before $V_m$ in the case of silica, due to SEC effects. Complete elution was further confirmed by comparison of peak areas with results obtained on the $C_{18}$ column, which is known to completely elute the polyester. Since higher water contents may even better mask residual silanol groups, both glass and bare silica were assumed to be inert in the set-up of the experiments described here.

The use of columns packed with small metal particles was also considered, although it is known that even stainless steel is not completely inert in all cases.[39] Since the packing of these columns was hampered by the high specific mass of the materials,[22] in this respect only the use of a stainless steel pre-column filter was studied.

Consequently, the retention behaviour of low dispersity polyester fractions was compared using a $C_{18}$ column, a non-porous glass column, a silica column and a stainless steel pre-column filter. The injected sample amount was taken as low as possible, since it is known that in the case of solubility effects governing retention, retention times may shift to higher values with increasing sample load.[18] Due to

interference with retention differences caused by different extents of column interactions, this might hamper the comparison of results from different columns. An injected mass of 2 µg proved to be a reasonable compromise between sample load and detectability. Since gradient steepness was found to have only a slight effect on the retention characteristics (see also Figure 3.12B and related discussion in Section 3.3.3.5), for reasons of detectability and analysis time a steepness of 3%/min was chosen.

In Figure 3.7, results for the various columns are shown. Fractions were injected at least twice on each column and results were found to be highly reproducible. The retention times in all chromatograms were corrected for the system hold-up time and the column dead time, according to:

$$t_{r(\text{corrected})} = t_r - t_s - t_{sec} \tag{3.2}$$

where $t_r$ is the retention time, $t_s$ is the system hold-up time and $t_{sec}$ is the column dead time ($t_0$) for the respective fraction. The latter was found to depend on the molar mass due to SEC effects (see Section 3.2.3). This correction is necessary to calculate the exact eluent composition at the time of elution of a solute.

Due to insufficient mixing with the eluent, part of the injected sample which was dissolved in THF, eluted unretained from the glass column and the guard filter (so-called breakthrough). Since this can not be observed in the time-corrected chromatograms in Figure 3.7, an example of an uncorrected chromatogram is shown in Figure 3.8. Especially for the fractions with lowest molar masses, this effect was excessive, thus giving rise to a low signal to noise ratio, as can be observed in the chromatograms of fractions 9 and 10. By measuring peak areas of the unretained peak and the normal eluting peak, the amount of sample which eluted unretained, was calculated. For both columns the effect was found to increase upon decreasing molar masses (Table 3.3). Since these breakthrough effects were not observed for the silica and the $C_{18}$ column and were worse for the pre-column filter as compared to the glass column, they may be caused by a too low surface area, available for precipitation. Furthermore, the columns used will probably exhibit significantly different internal mixing due to the difference in particle diameter, which can also influence the elution behaviour of polymers and the occurrence of breakthrough effects.[36]

The high frequency noise in the chromatograms from the pre-column filter is probably caused by parts of the sample that were not completely redissolved and therefore eluted as small polymer particles, causing light scattering in the UV detector. The effect becomes worse when sample load is increased (result not shown) thus supporting this assumption. Since these effects are absent in results from the glass column, it seems that due to mixing effects in the column, the sample is better allowed to redissolve again.

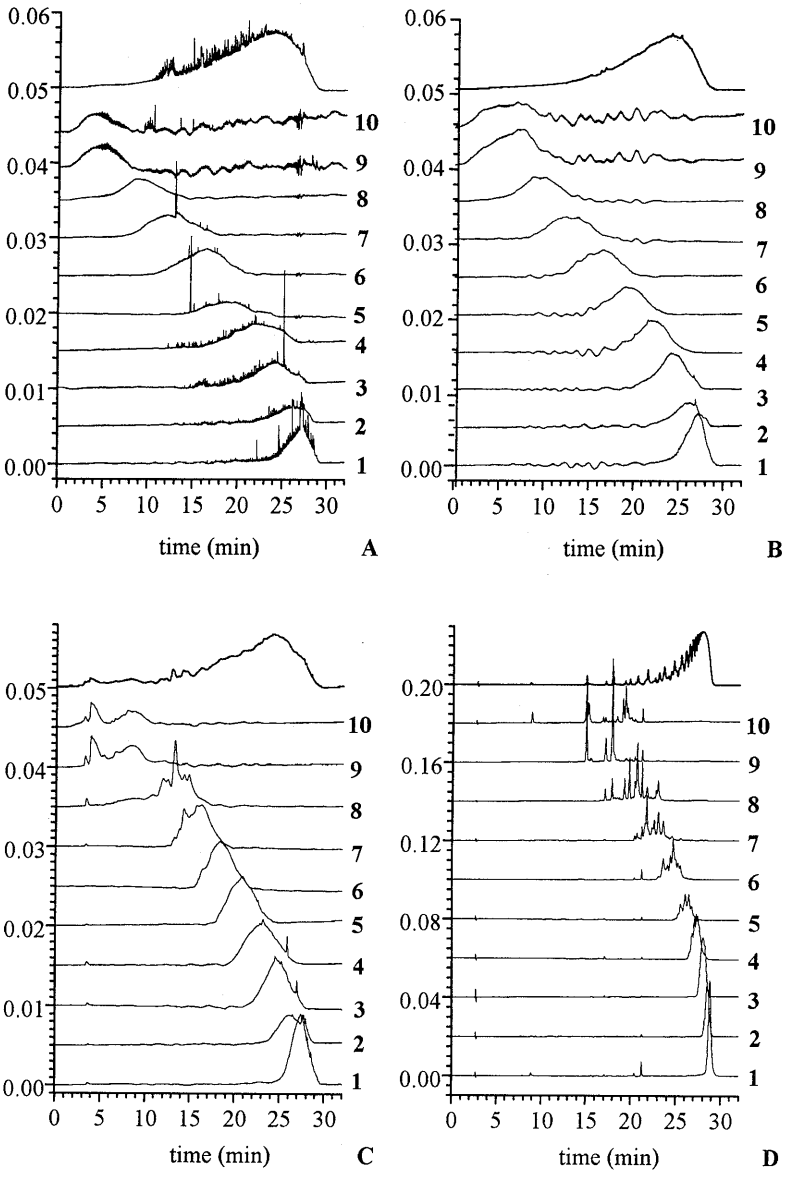


**Figure 3.7.** Elution of low polydispersity fractions (1-10) (see Table 3.2) and unfractionated polyester PE1 (upper curves) on different columns. A: pre-column filter, B: glass column, C: silica column, D: $C_{18}$ column. Conditions: sample concentration: 0.4 mg/ml in THF, temperature: 21 °C, eluent: water-THF (100:0, v/v) to (0:100) in 33.3 min, flow: 1.0 ml/min, injection: 5 µl, detection: UV at 277 nm. Chromatograms were time-corrected according to Eq. (3.2).

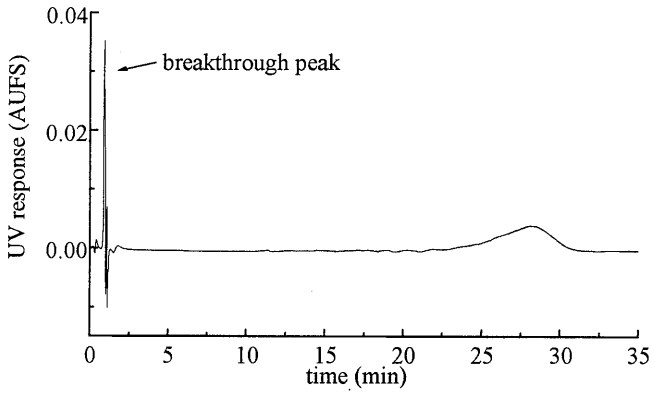


**Figure 3.8.** Blank corrected chromatogram of polyester fraction 3 on the glass column, before time-correction. GPEC conditions: see Figure 3.7.

To enable a more accurate comparison of results from the different columns, from all chromatograms, the %-solvent (%-S) at the beginning, maximum, and end of the distribution were determined, using Eq. (3.3):

$$\%-S = 100\left(\frac{t_r - t_s - t_{sec}}{t_G}\right) \tag{3.3}$$

where $t_G$ is the gradient time. All values were determined as the average value from two injections. The maximum deviation between the average and the lowest or highest value of a duplicate measurement was found to be about 1.5 %-S for peak starts and

**Table 3.3.** Fraction of the sample eluting unretained due to breakthrough on the pre-column filter and the glass column

| Fraction number | Pre-column filter | Glass column |
|---|---|---|
| 1 | 0.14 +/- 0.04* | 0.08 +/- 0.04* |
| 2 | 0.26 | 0.14 |
| 3 | 0.14 | 0.06 |
| 4 | 0.19 | 0.06 |
| 5 | 0.24 | 0.06 |
| 6 | 0.22 | 0.10 |
| 7 | 0.25 | 0.13 |
| 8 | 0.44 | 0.34 |
| 9 | 0.84 | 0.57 |
| 10 | 0.86 | 0.45 |

*: maximum deviation between average and maximum value of duplicate measurements ($dev_{max}$).

**Table 3.4.** %-S at peak start, peak top and peak end of low polydispersity polyester fractions on the investigated columns and in the cloud point

| Fraction number | %-solvent* Pre-column filter | Glass column | Silica column | $C_{18}$ column | Cloud point |
|---|---|---|---|---|---|
| *Peak start* | | | | | |
| 1 | 63.5 | 66.0 | 68.0 | 82.5 | |
| 2 | 57.5 | 64.0 | 67.0 | 82.0 | |
| 3 | 54.0 | 55.0 | 63.0 | 80.0 | |
| 4 | 47.0 | 50.0 | 55.5 | 76.5 | |
| 5 | 38.5 | 38.5 | 50.0 | 73.0 | |
| 6 | 29.0 | 28.5 | 44.0 | 63.0 | |
| 7 | 16.0 | 10.5 | 34.0 | 60.0 | |
| 8 | 4.5 | 1.5 | 11.0 | 50.5 | |
| 9 | 1.0 | 0.0 | 5.0 | 43.5 | |
| 10 | 1.0 | 1.0 | 3.5 | 25.5 | |
| *Peak top* | | | | | |
| 1 | 82.0 | 82.0 | 82.0 | 85.5 | |
| 2 | 77.0 | 80.0 | 80.5 | 85.0 | |
| 3 | 73.0 | 73.0 | 74.0 | 83.5 | |
| 4 | 66.5 | 66.0 | 70.0 | 81.5 | |
| 5 | 58.5 | 58.5 | 62.5 | more than 1 peak | |
| 6 | 49.5 | 49.0 | 55.0 | | |
| 7 | 37.5 | 38.5 | 48.5 | | |
| 8 | 27.0 | 28.0 | 39.5 | | |
| 9 | 15.5 | 22.5 | 25.4 | | |
| 10 | 13.0 | 18.5 | 24.5 | | |
| *Peak end* | | | | | |
| 1 | 88.5 | 89.0 | 89.5 | 89.5 | 80.5 |
| 2 | 86.5 | 87.0 | 87.0 | 88.0 | 79.5 |
| 3 | 85.0 | 86.0 | 85.0 | 86.5 | 75.5 |
| 4 | 81.0 | 81.5 | 83.5 | 86.5 | 72.0 |
| 5 | 74.5 | 73.5 | 76.0 | 83.5 | 66.0 |
| 6 | 65.5 | 63.5 | 67.0 | 81.5 | 60.0 |
| 7 | 55.5 | 58.5 | 60.0 | 80.0 | 51.0 |
| 8 | 45.0 | 45.5 | 55.5 | 72.5 | 43.5 |
| 9 | 26.5 | 38.5 | 39.5 | 65.5 | |
| 10 | 25.5 | 35.0 | 33.5 | 66.0 | |

*: All values are average values of two determinations. $dev_{max}$: 1.5%-S for peak starts and peak ends and 0.5%-S for peak tops and cloud points.

peak ends and 0.5 %-S for peak tops. Results are shown in Table 3.4.
From Figure 3.7 and Table 3.4 it is clear that results on the glass column and the pre-column filter are roughly comparable. Since peak start and peak ends cannot be determined unambiguously in all cases, most differences are within experimental error

of determination. Fractions 9 and 10 seem to elute somewhat later from the glass column as compared to the pre-column filter. This can probably be ascribed to the relatively high amounts of sample eluting unretained from the pre-column filter (Table 3.3). Since the fraction eluting in the gradient is significantly lower, elution is shifted to earlier retention times. Peak tops, which are more accurate to determine, coincide in the other cases, which confirms that the retention on the glass column under experimental conditions is only determined by solubility effects. This is further supported by the fact that the retention time of the peak top increases when the sample load is increased, which is illustrated in Table 3.5. When sorption effects would contribute to the separation, no such dependence or decreasing retention times due to overloading should have been observed.[1,14]

**Table 3.5.** Effect of sample load on the elution of low polydispersity polyester fractions on the glass column

| Fraction number | Injected amount (µg) | Peak start (%-S) | Peak top (%-S) | Peak end (%-S) |
|---|---|---|---|---|
| 2 | 2 | 64.4 +/- 1.5* | 69.0 +/- 0.5* | 85.5 +/- 1.5* |
| | 15 | 64.4 | 69.8 | 89.5 |
| 4 | 2 | 47.5 | 67.0 | 71.2 |
| | 15 | 47.4 | 69.1 | 79.5 |
| 6 | 2 | 32.2 | 49.0 | 59.7 |
| | 15 | 32.4 | 51.5 | 64.7 |

*: $dev_{max}$.

The use of a silica column gives rise to additional retention as compared to the pre-column filter, which is generally more manifest for the lower molar mass components (Figure 3.7). This is somewhat surprising, since no retention due to sorption effects was observed in isocratic experiments using a water-THF composition of 15:85. The additional retention at higher water contents can probably be ascribed to solvophobic effects due to minor affinity of the polyester towards the mobile phase at the point of redissolution. The different behaviour of silica as compared to non-porous glass was also observed by other workers.[18] Due to the large differences in surface area, this can probably be attributed to the differences in phase ratio ($V_s/V_m$) which, in the case of glass, will be orders of magnitude lower. Furthermore, differences in chemical composition and silanol activity of the surface may also contribute to a different retention behaviour.

From the above discussion it is obvious that a bare silica column cannot be used as an inert column packing for polyesters. The use of a non-porous glass column seems to be the best choice in this respect, since problems caused by breakthrough or insufficient

redissolution are lower as compared to the pre-column filter. Furthermore, it is worthwhile noting that the polyesters used here, are relatively extreme samples due the highly polar carboxylic end groups. Since non-porous glass with a very low phase ratio can be used even for these samples without sorption effects occurring, it is probable that this approach for studying solubility effects in polymer chromatography under RP conditions can be applied for a rather wide range of polymers.

### *3.3.3.3 Solubility effects on a $C_{18}$ column*

A comparison of results for the glass column with those for $C_{18}$ (Table 3.4), immediately reveals that separation on $C_{18}$ is dominated by sorption effects, which again is more manifest for the lower molar mass components. Especially for the low molar mass fractions a distinct separation into different peaks can be observed. This is not the case when a glass-column is used and must therefore be the result of sorption. Furthermore, from a comparison of peak starts (Table 3.4), it is obvious that elution on a $C_{18}$ column occurs at a much higher %-S of the eluent as compared to the glass column. Since the solubility capacity is high enough in such a case, redissolution effects, although present in the used system, will not dominate the final separation on a $C_{18}$ column. This is in accordance with the results on the effect of sample load where no noticeable retention difference with increasing sample load was found. Obviously, the effect of sample-load on a $C_{18}$ column is determined by the sorption capacity of the column, rather than the solubility capacity of the eluent.

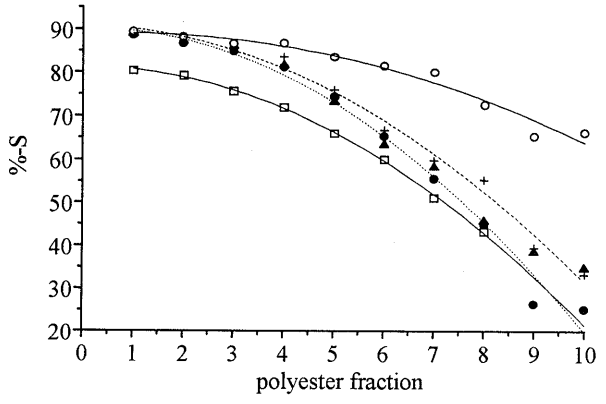


**Figure 3.9.** Cloud points and peak ends of the respective polyester fractions on different columns. □: cloud point, ●: pre-column filter, ▲: glass column, +: silica column, ○: $C_{18}$ column. GPEC conditions: see Figure 3.7.

From the present results, however, it is also clear that the retention difference for a separation which is controlled by solubility effects as compared to a separation based on sorption, decreases for increasing molar mass. This can be observed from Figure 3.9 where %-S at the peak-ends ($\varphi_e$) on different columns are compared.
This can be explained as follows. During gradient elution, the increase in %-S will both influence solubility and sorption. The average retention factor during migration, k*, which expresses the contribution of sorption, decreases with increasing molar mass, as argued in Section 3.3.1.3. Therefore, the range of the solvent composition over which polymer solutes migrate becomes very narrow, thus resulting into narrow peaks. Physically this also means, that after reaching the point at which migration starts, the sample will elute almost unretained without significant distribution into the stationary phase. Retention in RP systems is to a large extent determined by interactions between the sample and the mobile phase, which are the same interactions that determine solubility. Thus, for increasing molar masses, especially on RP systems, the retention difference for a separation dominated by solubility as compared to a separation governed by sorption, decreases.
Therefore, the finding of corresponding values of $\varphi_e$ on an inert and an adsorbing column, which can also be found for the unfractionated polyester in this study (Figure 3.7) is certainly no evidence for solubility dominating retention in the high molar mass part of the chromatogram. Experiments using low dispersity fractions as shown in this study, a careful study on the effect of sample load, as suggested by Snyder *et al.*[14] or measurements under isocratic conditions in the narrow NS/S range over which a polymer solute migrates,[13] are necessary to discriminate between solubility and sorption.

### *3.3.3.4 Comparison of gradient elution with static solubility measurements*

In Figure 3.9, also the cloud points of the individual fractions are plotted. As might be expected, due to the low molar masses the cloud points do not coincide with $\varphi_e$ values on the inert glass column. It is surprising, however, to see that %-S in the cloud points is considerably lower than $\varphi_e$, whereas concentrations under chromatographic conditions are much lower due to dilution effects. To further investigate this effect, a comparison was made between measurements of maximum solubility of four different polyester fractions in varying NS/S compositions and the concentration profiles of the eluting fractions on the glass column. In Figure 3.10, results of measurements of solubility under static conditions are shown.

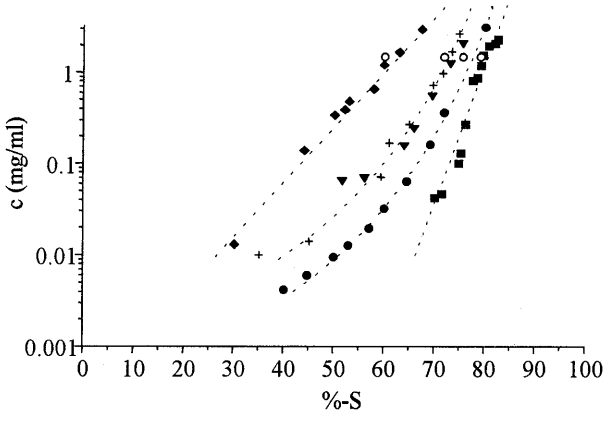


**Figure 3.10.** Maximum solubility of several polyester fractions versus NS/S compositions at 21 °C. ■: fraction 2, ●: fraction 3, +: fraction 4, ▼: fraction 4 (2), ◆: fraction 6, O: cloud point measurement. GPEC conditions: see Figure 3.7.

As could be expected from Eq. (2.25), a near linear dependence is found between log(c) and %-S in the high concentration range. At low concentrations, deviations from this dependence occur, which may be due to limitations of the method itself. Because of the relatively low molar masses of the polyester fractions, a swollen, gel-like polymer-rich phase is formed, rather than a distinct solid precipitate. Thus, for low %-S, it was difficult to obtain a non-turbid supernatant phase. Furthermore, due to low concentrations, the relative effect of the baseline disturbance due to the injection solvent in the HPLC measurements, which were carried out in the SEC-mode, on the elution profile increased, thus complicating an accurate quantification. For fraction 4, measurements were carried out twice with a time difference of 6 months, using freshly obtained fractions. As can be observed from Figure 3.10, reproducibility is satisfying.

It is also worthwhile noting that $\varphi_S$ values of the respective fractions obtained by cloud point titrations fit well in the solubility curves (Figure 3.10). This confirms the reliability of the used titration method despite of low sample amounts and visual observation of the cloud points.

For decreasing molar masses, a more gradual increase in solubility with %-S is obtained which is in accordance with observations of Glöckner.[1] The observed strong concentration dependencies explain the broad peaks obtained on a glass column, where separation is only governed by solubility effects (Figure 3.7). Furthermore, the steeper lines for higher molar mass fractions in Figure 3.10 can also be recognised in Figure 3.7, where peak width decreases with increasing molar mass.

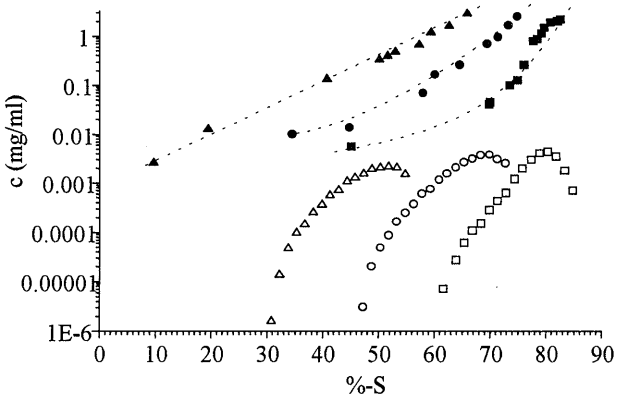


**Figure 3.11.** Comparison of solubility measurements under static conditions (solid symbols) with concentrations eluting from the glass column (open symbols) at 21 °C. ■: fraction 2, ●: fraction 4, ▲: fraction 6. GPEC conditions: see Figure 3.7.

In Figure 3.11, solubility measurements for fractions 2, 4 and 6 are compared with concentrations eluting from the glass column. Since known amounts were injected, the eluting masses at respective %-S compositions could be calculated from the fractional peak areas, after correction for breakthrough. For this purpose, slice widths of 0.5 min were taken. By dividing eluting mass by the volumetric slice width (0.5 min * F), eluting concentrations were obtained.

Clearly, concentration profiles obtained from chromatographic measurements do not coincide with the curves of maximum solubility. For the high %-S part of the distribution this is trivial, since the available mass gets exhausted, which is represented by a final decrease in concentration in the eluate. However, for the low %-S part this is remarkable, since this part represents the beginning of the gradient elution experiment, where enough polymer is available to obtain saturated solutions. The observed 'elution delay' cannot be the result of sorption effects, as has been shown earlier. Furthermore, small errors in the system hold-up volume, which is necessary to calculate the %-S at each elution time provide by no means an explanation for this phenomenon. Therefore, the eluate indeed is not saturated, indicating that no thermodynamic equilibrium was reached during redissolution, which is probably due to kinetic effects.

### *3.3.3.5 Kinetic effects in redissolution*

In order to confirm the assumption of kinetic effects occurring in RP-GPEC, several practical parameters which can influence redissolution kinetics, *e.g.* temperature and

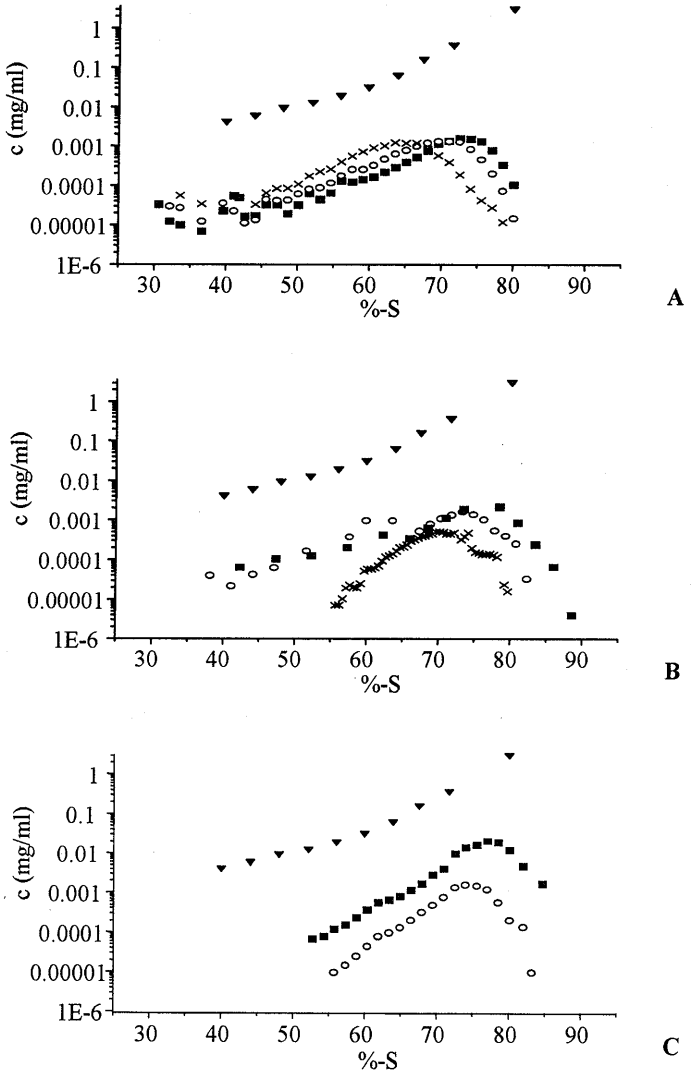


**Figure 3.12.** Effect of temperature (A), gradient steepness (B) and injected amount (C) on the eluting concentration of polyester fraction 3. A: ▼: maximum solubility at 21 °C, ■: 21 °C, O: 40 °C, x: 60 °C. B: ▼: maximum solubility, ■: 5%/min, O: 3%/min, ×: 1%/min C: ▼: maximum solubility, ■: 60 µg, O: 6 µg. GPEC conditions: see Figure 3.7, unless indicated otherwise.

gradient steepness were varied on the glass column. For reasons of available amounts, fraction 3 was used for these experiments.

From Figure 3.12A it can be observed that a temperature increase gives rise to higher concentrations of the eluting polyester, at a fixed %-S. This is due to increased solubility of the polyester in the mobile phase causing earlier elution, which is a thermodynamic

rather than a kinetic effect. The curve of maximum solubility at these temperatures, which was not measured for reasons of practical difficulties, would, however, also shift to higher concentrations. It is therefore obvious, that even at 60 °C, which is about the highest temperature that can practically be applied for the chosen NS/S system, the influence of redissolution kinetics cannot be avoided.
A decrease of gradient steepness causes the end of the distribution to elute at somewhat lower %-S (Figure 3.12B), which is an indication for the importance of redissolution kinetics. The concentration differences at low %-S, however, are within experimental error and it is obvious that a decrease of steepness to 1%/min, which is frequently used in practice, does not avoid kinetic effects.
Finally, the increase of the injected amount causes an increase of the eluting concentrations (Figure 3.12C), which is another proof that the eluate is not saturated. Furthermore, a slight shift of the concentration maximum towards higher %-S can be observed, which confirms the importance of redissolution kinetics.
Although the changes in elution behaviour, with changing experimental parameters are significant, the observed effects are rather small. This might be expected, since the dependence between kinetic effects and parameters such as temperature are generally described by exponential functions.[40] Therefore, for a further verification of redissolution kinetics, a more pronounced change in practical parameters by one or more decades, would be necessary. Since practical parameters in chromatography, *e.g.* temperature and flow rate can only be varied within small limits, this cannot be realised.
Although it is clearly shown that redissolution is influenced by kinetic effects, these effects apparently do not affect the separation on a $C_{18}$ column. Obviously, after time-dependent redissolution, adhesion forces are replaced by sorption forces, ensuring normal retention behaviour, governed by sorption effects. It is imaginable, however, that the separation on a less retaining column, for instance a silica derivatised cyanopropyl phase, could indeed be dominated by redissolution effects. Especially for the high molar mass part this might be expected since it has been pointed out in this study that the retention difference between a non-retaining column and a $C_{18}$ column is already very small. This difference would even be smaller, or non-existing when a less retaining column would be used.
A few examples of redissolution kinetics influencing separations of high molar mass polymers have been reported.[20,21,23] Furthermore, in Chapter 6 of this thesis it will be shown that RP-GPEC of crystalline polyesters is distinctly affected by solubility effects.

## 3.4 CONCLUSIONS

By RP-GPEC, highly detailed oligomer separations of amorphous polyesters can be obtained. The polyesters are mainly separated according to molar mass, whereas in the

low molar mass part, a further separation on chemical composition occurs. The separation can be adjusted by several practical parameters. A gradient steepness of 1%/min is a good compromise between resolution and analysis time. Column length only influences the separation in the low molar mass part of the chromatogram, whereas the total number of oligomers that can be resolved is hardly influenced. Temperature increase also mainly affects the separation in the low molar mass part, due to a decrease in peak broadening. Temperature effects appear to be much less pronounced compared to the results obtained by other workers for other types of polymers, the exact cause of which is still unclear. Up to a sample load of 1000 µg, no effects on the separation are observed. This already indicates that separation is probably dominated by sorption rather than solubility effects. Injection volumes exceeding 10 µl give rise to additional peak broadening for the low molar mass products, due to the sample-solvent effects. However, no breakthrough is observed under these conditions.

The effect of solubility effects in RP-GPEC of relatively low molar mass polyesters can at best be investigated under chromatographic conditions using an inert column packing and low polydispersity fractions obtained by SEC. As an inert medium, glass is the best choice, due to the absence of adsorptive interactions in the chosen system and due to the fact that breakthrough effects can be reasonably controlled. The separation on $C_{18}$ throughout the whole investigated molar mass range is solely determined by sorption effects. The observed correspondence of $\varphi_e$ on $C_{18}$ and (inert) glass for the high molar mass fractions can be explained from the low k* values giving rise to only a minor distribution in the stationary phase, after k has dropped below a certain value. The finding of this correspondence is therefore no evidence for solubility governing retention. From a comparison with measurements of maximum solubility under static equilibrium conditions of four different polyester fractions in various NS/S combinations, it can be concluded that even redissolution of low molar mass polyesters is affected by kinetic effects. The occurrence of those effects is confirmed by the effects of temperature and gradient steepness on the elution behaviour, although the observed changes are rather small. Redissolution kinetics do not affect the separation of the investigated polyesters on $C_{18}$.

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# CHAPTER 4

# Retention Behaviour of Low Molar Mass Polystyrenes and Polyesters in Reversed Phase Liquid Chromatography, Studied by the Evaluation of Thermodynamic Parameters*


## SUMMARY

*In order to get a better understanding of the retention behaviour of polymers in adsorptive systems such as Gradient Polymer Elution Chromatography, thermodynamic parameters obtained from van 't Hoff analyses on low molar mass polystyrenes (PS) and polyesters (PE) in various water-THF mixtures on a $C_{18}$ column, were evaluated. Linear van 't Hoff behaviour was observed in almost all cases. Negative values for both $\Delta h$ and $\Delta s$ which increase with increasing %-THF, were found for PS and PE oligomers. For $\Delta s$ this is explained from multi-site attachment effects. For PS, the nonlinear relations between $\Delta h$ and $\Delta s$, and degree of polymerisation (p) can possibly be explained from the nonlinear increase of the hydrodynamic volume with p. Although less clear, similar trends were found for PE. For PS evidence for penetration effects of oligomer chains into the bonded chains was obtained. Martin plots for both PS and PE were shown to be nonlinear in all investigated eluent compositions. The extent of nonlinearity is suggested to depend on the conformation of a polymer in solution. No distinct enthalpy-entropy-compensation temperature (EECT) independent of p was found for PS, thus confirming the findings of an earlier study where no exact molar mass independence was found under critical conditions. Further evaluation of EECT for PS oligomers revealed a retention mechanism independent of the binary eluent composition. This indicates that conclusions from this study can also be used for a qualitative understanding of sorption mechanisms in the gradient elution mode. Finally, for PS it was shown that $\Delta\mu$ equals zero under critical conditions, thus confirming theoretical predictions.*



* This Chapter has been published:
H.J.A. Philipsen, H.A. Claessens, H. Lind, B. Klumperman and A.L. German, *J. Chromatogr. A*, 790 (1997) 101.

## 4.1 INTRODUCTION

In Chapter 3 it has been shown that by Reversed Phase Gradient Polymer Elution Chromatography (RP-GPEC) highly detailed separations with respect to molar mass and chemical composition for low molar mass polyesters can be obtained. Furthermore it was proven that, although precipitation/redissolution effects were present, retention of amorphous polyesters throughout the whole investigated molar mass range (approximately 0 - 30,000 Daltons) was largely determined by sorption effects, which can be either adsorption, partitioning, or a combination of both effects. The same was found by Snyder *et al.*[1,2] as well as by this author[3] for polystyrenes.

A method that has frequently been used to study the (sorption governed) retention mechanism of low molar mass solutes in reversed phase liquid chromatography (RPLC) is the investigation of the temperature dependence of isocratic retention.[4-16] From the experimentally determined van 't Hoff plots (ln(k) versus 1/T, k = retention factor), thermodynamic parameters can be deduced which can be used to gain information on the effect of both the stationary and the mobile phase and of the solute itself on the retention mechanism.

In order to get a better understanding of their retention behaviour, in this Chapter thermodynamic parameters for low molar mass polystyrenes and polyesters are studied. For this purpose, retention factors for a large number of oligomers of styrene and of a copolyester that was also used in Chapter 3, were determined as a function of temperature and composition of the binary water-THF eluent combination on a $C_{18}$ column. Polystyrenes were chosen for reasons of their structural simplicity as compared to polyesters. This facilitates to study the effect of chain length more independent of differences in functionality and chemical composition of the polymer backbone. Although homologous series and oligomers have been taken into account in a few other, comparable studies,[5,8,16] the nature of the polymers studied here, is significantly different. It will be shown that different chromatographic behaviour is observed as compared to, for instance poly(ethylene oxide), especially with respect to molar mass effects. Despite the fact that measurements had to be carried out under isocratic conditions, conclusions can also be used for a qualitative understanding of the retention mechanisms in the gradient elution mode which is mostly applied for non-exclusion polymer chromatography. This is especially valid for the higher molar mass products for which it is known that chromatographic elution occurs within a very small range of eluent compositions ($\Delta\varphi$),[17] which is the same range in which under isocratic conditions, measurable retention factors can be obtained.

## 4.2 THEORY

The retention factor, k, in chromatography is defined as:

$$k = \frac{t_r - t_0}{t_0} = K_D \phi \tag{4.1}$$

where $K_D$ is the chromatographic distribution constant, $t_r$ is the retention time, $t_0$ is the column dead time and $\phi$ is the phase ratio (volume stationary phase ($V_s$)/volume mobile phase ($V_m$)). This equation can be re-written into Eq. (4.2), expressing the temperature dependence of retention in chromatography, in which $\Delta\mu^0$, $\Delta h^0$ and $\Delta s^0$ are the standard chemical potential difference, partial molar enthalpy difference and partial molar entropy difference of solute molecules in both phases.

$$\ln(k) = \frac{-\Delta\mu^0}{RT} + \ln(\phi) = \frac{-\Delta h^0}{RT} + \frac{\Delta s^0}{R} + \ln(\phi) \tag{4.2}$$

If $\Delta h^0$ and $\Delta s^0$ are invariant over the studied temperature range, these quantities can be directly determined from a plot of ln(k) versus 1/T, the van 't Hoff plot, for $\Delta s^0$ provided that $\phi$ is known. For nonlinear plots, these parameters can be approximated from the partial derivative of ln(k) to 1/T of a polynomial function.[10]
For an accurate determination of $\Delta s^0$, $\phi$ must be known. This is not straightforward, since the value $V_s$, being the effective part of the stationary phase that actually participates in the sorption process, is difficult to obtain. It strongly depends on the exact structure of the derivatised silica material *e.g.* bonding type, bonding density, pore width etc., which can be highly different between various column materials.[18]
The approximation adopted in this study was proposed by Sentell and Dorsey[19] and has also been used in other thermodynamic studies.[9,10,14] It determines the actual volume of the alkyl chains taking part in the sorption process, and is given by:

$$V_s = \frac{(\%C)(M)(W_p)}{(100)(12.011)(n_c)(\rho)} \tag{4.3}$$

where %C is the carbon load as determined from elemental analysis, M is the molar mass of the bonded alkyl ligand (g/mol), $W_p$ is the mass of the bonded packing in the column (g), 12.011 is the molar mass of carbon, $n_c$ is the number of carbons in the alkyl ligand and $\rho$ is the density (g/cm$^3$) of the bonded alkyl ligand.
For higher molar mass products that are taken into account in this study, steric exclusion can affect the accessible pore volume for a specific solute, thus influencing

$V_m$ (and $t_o$) and $V_s$. It has been shown that k for high molar mass solutes can be determined from:[1]

$$k_M = \frac{t_{r,M} - t_{sec,M}}{t_{sec,M}} \tag{4.4}$$

in which $t_{sec,M}$ is the retention time of the solute with molar mass M, under non-adsorbing conditions. The effect on $V_s$ is difficult to account for. It must be kept in mind, that this parameter only influences $\phi$ and therefore will cause a small shift of all apparent $\Delta s^0$ values in the same direction, which, however, may be slightly different for solutes that significantly differ in hydrodynamic volume. This will be further discussed in the results Section.

In earlier studies, van 't Hoff analysis has been used to study the effect of the stationary phase on the retention process. Nonlinear van 't Hoff behaviour was observed under certain conditions which could be explained from a phase transition of the $C_{18}$ column material.[9] This influences the partitioning process, which has been shown to be an important process in the overall retention mechanism on $C_{18}$ derivatised silica.[20-22] A partial insertion of the alkyl chain of a homologous series, dependent on the carbon number, was suggested from a discontinuity in the plot of $\Delta h^0$ vs. the carbon number.[8] Such behaviour, however, could not be confirmed from theoretical considerations, based on a self-consistent field theory for adsorption, the so-called Scheutjens-Fleer (SF) model, by Tijssen *et al.*[21-22] The shape of the van 't Hoff plot was shown to vary with the nature and composition of the mobile phase type which is caused by differences in the entropically driven, hydrophobic effect due to differing extents of H-bridge formation in various solvent types.[10] The existence of different conformations of (low molar mass) oligomers during the chromatographic process could also be proved from varying shapes of van 't Hoff plots.[5] Furthermore, it was shown that these plots may be used for the determination of physical and thermodynamic constants that are difficult to be obtained by other methods.[12]

To check whether retention mechanisms for various situations are comparable, Melander *et al.* have applied the concept of compensation temperature based on enthalpy-entropy-compensation.[4] This temperature, $\beta$, can be determined from the slope of a plot of ln(k) vs. $\Delta h^0$ according to Eq. (4.5):

$$\ln(k_T) = \frac{-\Delta h^0}{R}\left(\frac{1}{T} - \frac{1}{\beta}\right) - \frac{\Delta\mu_\beta}{R\beta} + \ln(\phi) \tag{4.5}$$

If the same physical-chemical process determining the energies of the retention process is operative for the various situations taken into account in the compensation plot, then a linear dependence, and therefore only one compensation temperature, will

be found. Physically this means the existence of a temperature at which retention is independent of the studied variable (eluent composition, degree of polymerisation etc.). It was also shown that for statistical reasons, k can at best be taken at the average value of the tested temperature range.[4] The concept of enthalpy-entropy-compensation has been used in several studies in order to investigate agreements in and differences between retention mechanisms.[6-8,14] Further theoretical backgrounds concerning the meaning and application of the concept were described by Boots *et al.*[23]

## 4.3 EXPERIMENTAL

### 4.3.1 Polymer samples and low polydispersity fractions

The polystyrenes used for the experiments were narrow standard polystyrenes from Waters (Milford, MA, USA). $M_p$ values (molar mass at the maximum of the molar mass distribution) as supplied by the manufacturer are: 418, 2900, 3600, 5400, 8500 and 35000. In all cases, butyllithium was used as the initiator. Polydispersity of all standards was < 1.10. Standard 2900 in which oligomers 1-30 could be separately distinguished, was used for van 't Hoff analysis. The molar mass of each respective oligomer equals: $M_p = 58 + 104\ p$.

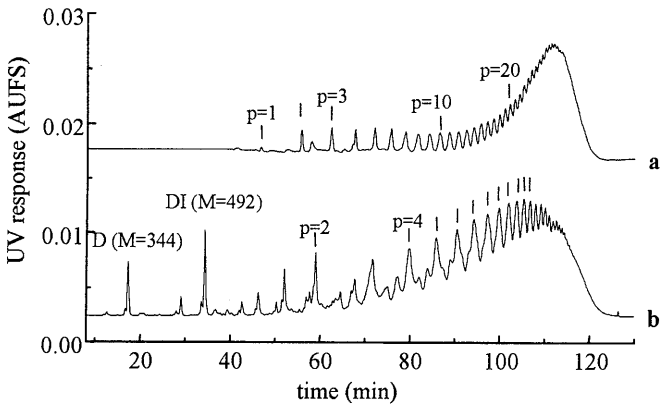


**Figure 4.1.** RP-GPEC chromatogram of PS (a) and PE (= PE1) (b). Oligomer peaks taken into account for van 't Hoff analysis: PS: all oligomers up to p = 30, PE: all indicated oligomers. Identity of PE oligomers: D: di-propoxylated bisphenol-A, DI: D + isophtalic acid, $D_pAc_p$ (Ac: acid), molar mass: (469p + 18). Column: Novapak $C_{18}$ (75 x 3.9 mm), temperature: 35 °C, eluent: water-THF (both with 200 µl acetic acid per litre added) (80:20, v/v) to (10:90) (0 to 140 min), flow: 1.0 ml/min, detection: UV at 263 nm (PS) and 277 nm (PE).

The polyester used was sample PE1, a copolyester resin consisting of adipic acid (A), isophtalic acid (I) and di-propoxylated bisphenol-A (D) (see Table 3.1). Low polydispersity fractions of the polyester were isolated by SEC. For detailed information on the preparation and the composition of these fractions it is referred to Section 3.2.1 and Table 3.2. The identity of the main components in each fraction was determined by a comparison of the RP-GPEC chromatograms of the unfractionated polyester and the respective fraction.
Oligomers and their respective molar masses that were used in the van 't Hoff analysis, are indicated in the RP-GPEC chromatograms in Figure 4.1. For PS oligomers up to p = 30 and for PE oligomers up to p = 13 were taken into account.

### 4.3.2 HPLC column, solvents and equipment

The column used was a Novapak $C_{18}$ column (Waters, $d_p$ = 4 µm, pore size 60 Å, 75 x 3.9 mm). The solvents used for HPLC were water, Lichrosolv quality from Merck (Darmstadt, Germany) and tetrahydrofuran (THF), HPLC grade from Rathburn (Brunschwig Chemie, Amsterdam, The Netherlands). To both solvents, 200 µl acetic acid, Pro Analysi quality from Merck, per litre was added. For HPLC, the solvents were constantly sparged with helium (20 ml/min). All solvent mixtures were made by volumetric mixing by the HPLC pump, no premixes were used. The HPLC equipment has been described in detail in Section 3.2.4.

### 4.3.3 Determination of the phase ratio and $t_{sec}$ values

For the determination of the phase ratio, $\phi$, of the used column, Eq. (4.3) was used to calculate $V_s$. %-C was determined in duplicate by elemental analysis using an Heraeus CHN-O Rapid apparatus and was found to be 7.3%. $W_p$ of two 7.5 cm columns was determined after thoroughly drying of the complete packing that had been quantitatively removed from the column and was found to be 0.793 g ± 1% (maximum deviation between average and maximum of duplicate measurements: $dev_{max}$). The identity of the derivates reacted onto the silica matrix were determined by $^{29}$Si-NMR using a Bruker MSL 400 spectrometer and were found to be octadecyl-dimethyl-silyl and trimethyl-silyl (endcap) respectively. The molar ratio of both derivates was determined by $^{13}$C-NMR and was found to be 1:1. Therefore, %-C due to octadecyl-dimethyl-silyl was determined to be 6.35% and %-C resulting from the endcap was 0.95%. Densities for the respective derivates have been determined by Cheng[24] and equal 0.8607 for octadecyl-dimethyl-silyl and 0.8638 for trimethyl-silyl respectively. Therefore $V_s$, being the sum of the

respective contributions of both derivates could be calculated and was found to be 0.094 ml ± 2% ($dev_{max}$).

$V_m$ was determined gravimetrically in duplicate from the differential weight of the column filled with dichloromethane and methanol respectively, a method that was also adopted by other workers,[9] and was found to be 0.52 ml ± 1% ($dev_{max}$). Hence, $\phi$ was found to be 0.18 ± 3%.

To determine $t_{sec}$ as a function of molar mass, which is necessary to calculate k (Eq. (3.4)), retention times for the narrow standard polystyrenes mentioned in Section 4.3.1, were measured in duplicate at 40 °C, which is the average temperature used for the van 't Hoff analysis, using a flow rate of 2.0 ml/min in 100% THF. This eluent composition is known to be non-retentive for polystyrene.[25] For this purpose, standards were dissolved in the eluent up to a concentration of 1 mg/ml from which 2 µl was injected. Retention times were found to vary between 0.18 min for PS-35000 and 0.26 min for PS-418 respectively. From a third order polynomial fit of log(molar mass) versus retention time, $t_{sec}$ for each individual molar mass could be determined. Retention times for low polydispersity polyester standards were measured under the same conditions and were found to vary between 0.210 min for fraction 1 and 0.235 min for fraction 9. Retention time differences due to a change of the hydrodynamic volume with varying temperatures are known to be small[25] and were therefore neglected.

### 4.3.4 Experiment strategy

For the thermodynamic evaluations, isocratic measurements were performed at various binary eluent compositions, *i.e.* 39, 44, 47, 55, 60, 64, 67 and 69% THF in water respectively, at temperatures of 10, 20, 30, 40, 55 and 70 °C, the range of which is known to be broad enough for an adequate van 't Hoff analysis.[11] To this end, 10 µl of solutions of 10 mg/ml PS-2900 in THF and 40 mg/ml of the unfractionated polyester in THF were injected in duplicate, using a flow rate of 2.0 ml/min. After 21 minutes, eluent composition was rapidly changed to 100% THF to elute the remaining, high molar mass components. Therefore, k values that could be measured approximately were in the range of 0 - 100. After 2.5 minutes, the system was rapidly returned to initial conditions and the system was re-equilibrated during 6.5 minutes. This equilibration time was found to be sufficient to obtain constant retention times. Also the injected amounts were checked to be in the range where no overloading and therefore no influence on retention time occurred. For the identification of the oligomer numbers in each chromatogram, PS-418 and two polyester standards lying in the molar mass range that was observed in the isocratic analysis under the conditions chosen, were also injected. When the identity of one or more peaks was known, the other ones could also be easily identified. System temperature was held constant until all samples were measured at all eluent

compositions. For statistical reasons, isocratic eluent compositions at one temperature and temperatures itself were changed randomly. For the determination of k values, average values of the duplicate measurement of retention times were taken.

## 4.4 RESULTS AND DISCUSSION

### 4.4.1 Enthalpy and entropy change as function of the eluent composition

Van 't Hoff plots were constructed for the oligomers indicated in Figure 4.1 at each eluent composition. Examples are shown in Figure 4.2. Especially the higher molar mass oligomers could not be measured isocratically at low %-THF and, in some cases, at low temperatures. This is due to the fact that the isocratic parameter S', which is defined as $-d\log(k)/d\varphi$ (see Section 2.3.3.5) increases with molar mass causing the range of eluent compositions in which k lies between 0 and 100, to decrease.[26]
In all cases linear plots are obtained. The coefficient of regression in most cases exceeds 0.99. Nevertheless, for low molar mass polystyrenes (PS) where 6 data points are available, it can be seen that plots tend to be slightly curved, indicating a small temperature dependence of $\Delta h$ and $\Delta s$ (see for instance Figure 4.2A). An increase in both $\Delta h$ and $\Delta s$ towards less negative values at lower temperatures, has been observed more often for low molar mass solutes.[10] It has been ascribed to the hydrophobic effect causing an increased order in the mobile phase due to the decreasing affinity of the solute for the mobile phase at lower temperatures, thus giving rise to a more entropy governed retention mechanism. An alternative explanation will be discussed later on in this Chapter. For reasons of simplicity, in most cases the observed slight curvatures were neglected and approximated as straight lines. For polyester (PE) the scattering around the linear plot in most cases is somewhat higher as compared to polystyrene (PS). This is due to the fact that peaks belonging to PE oligomers are less well defined as compared to PS. Since they are in fact composite peaks containing more than one component (see Chapter 3), they give rise to broader peaks, the retention time of which is less accurate to determine.

From the obtained van 't Hoff plots, $\Delta h$ and $\Delta s$ were calculated. For $\Delta h$ in all cases negative values were found indicating that sorption for all oligomers is an exothermic process. $\Delta h$ values increase (become less negative) with increasing %-THF which can be explained from the increased affinity of the oligomers to the mobile phase. This is in accordance with the fact that water is a non-solvent for PS and PE, whereas THF is a thermodynamically good solvent. Values for PS and PE oligomers of approximately the same molar mass at one specific eluent composition are always lower for PS, reflecting the lower affinity of PS towards the mobile phase, due to its lower polarity. For instance

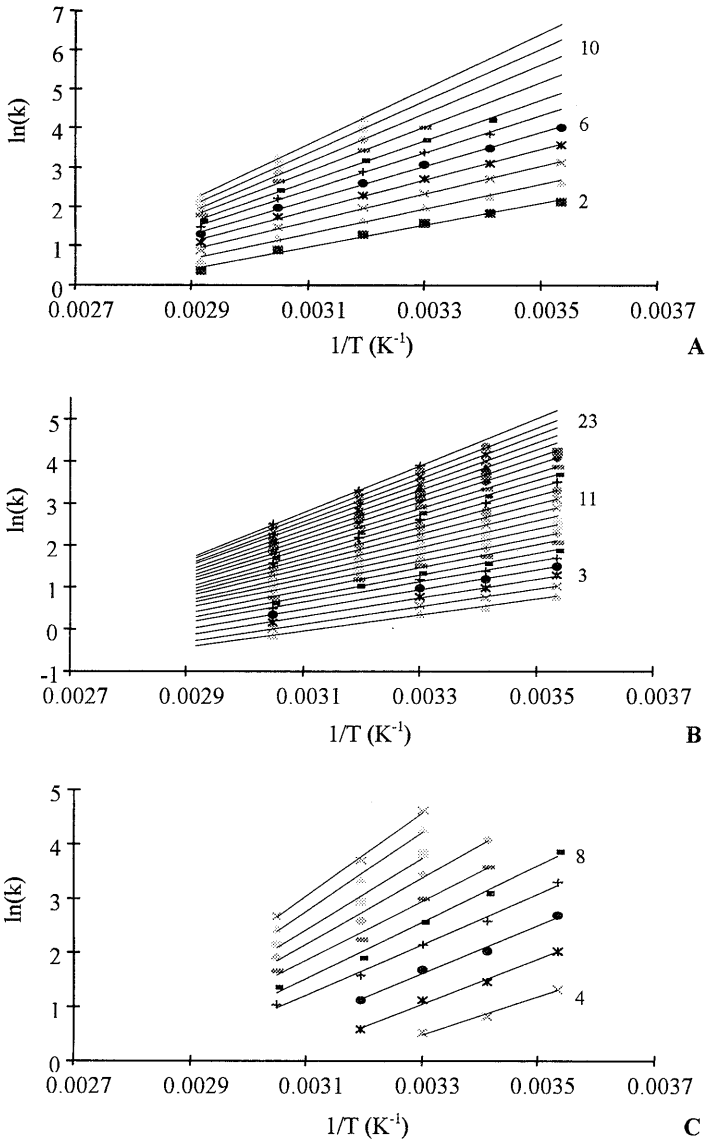


**Figure 4.2.** Van 't Hoff plots of PS oligomers at 55% (v/v) THF (A) and 67% THF (B), and PE oligomers at 67% THF (C). Oligomer numbers as indicated in Figures. Chromatographic conditions: see Section 4.3.4.

for oligomer p = 4 of PS (molar mass: 474) at 47% THF, $\Delta h$ was found to be -41.1 kJ/mol whereas for oligomer p = 2 of PE (molar mass 492) this value is -19.7 kJ/mol.
The calculated $\Delta s$ values were negative in all cases, indicating increased ordering of the system. In most cases, $\Delta s$ increases (becomes less negative) with increasing %-THF, which can be observed from Figure 4.3. At first sight this may seem strange, since at

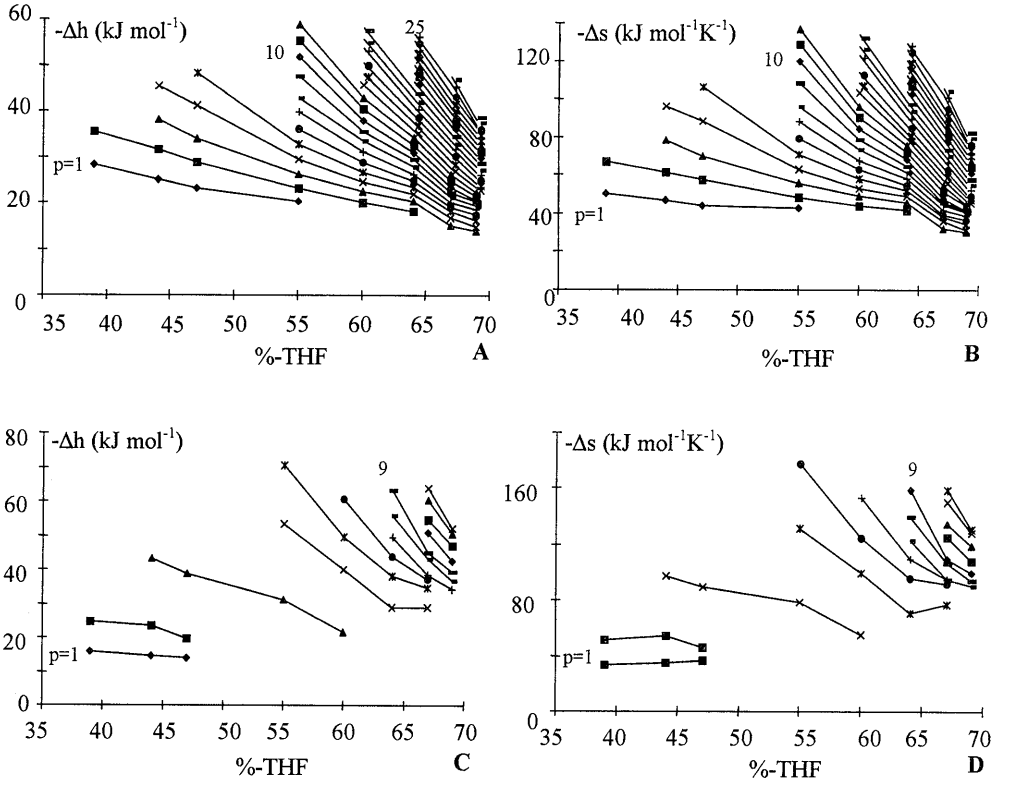


**Figure 4.3.** -Δh (A, C) and -Δs (B, D) versus %-THF (v/v) for various oligomers of PS (A, B) and PE (C, D). Oligomer numbers as indicated in Figures. Chromatographic conditions: see Section 4.3.4.

higher %-THF, less ordering of the oligomers in the mobile phase is expected due to the increasing thermodynamic quality of the solvent, which should give rise to a decrease in Δs. Decreasing Δs values with increasing %-organic modifier, especially for low molar mass solutes, were also found in other studies.[14] Obviously for the relatively high molar mass substances under study here, decreasing ordering in or at the stationary phase with increasing %-THF is more important. With increasing molar mass of the solutes, interactions with the stationary phase likely shift from a partitioning dominated type to a more adsorption like type.[21,22] Furthermore it is known that different sites of macromolecules can interact with a sorbent simultaneously, which is known as multi-site attachment.[28] This process gives rise to a sharp decrease in the degrees of freedom of the macromolecule when going from the mobile to the stationary phase. Due to increased affinity towards the mobile phase with increasing %-THF, the average number of oligomer sites that will simultaneously interact with the sorbent will decrease, giving rise to increased disorder. This effect obviously overrules the increasing disorder in the mobile phase, thus causing higher Δs values.

The fact that also for very low molar mass PS, at increasing %-THF increasing Δs values are found cannot be explained from multi-site attachment. At higher %-THF, the structure of the $C_{18}$ layer will be more 'open' and oriented towards the mobile phase, a so-called breathing surface.[18,22] This means that penetration of this layer will be easier, thus causing less ordering of solutes that will be partitioned in this layer and a net increase in Δs.

For the first two oligomers of PE, (slightly) decreasing Δs values with increasing %-THF are observed. Due to the more polar nature of these oligomers as compared to PS, ordering effects in the mobile phase due to mutual association, in order to reduce the surface area exposed to water, are more explicitly caused by the ability of hydrogen bonding. At higher %-THF, due to the increased affinity to the mobile phase, this effect will diminish, thus causing less ordering. For higher molar mass PE, such ordering effects can also occur, but, like for PS, at higher %-THF decreased ordering in the mobile phase is overruled by less ordering in the stationary phase, thus causing an increase in Δs.

For all oligomers, of both PS and PE, the magnitude of Δh is always greater (more negative) than that of TΔs. This indicates that enthalpy plays a more pronounced role in the total retention process than does entropy. For PS, for the quotient Δh/TΔs, values between 1.4 and 1.6, dependent on p and %-THF, were calculated, whereas for PE oligomers these values in most cases were approximately 1.3. From the observed trends of Δh/TΔs versus p, it could be concluded that these differences are highly significant. Obviously, retention for PS is somewhat more enthalpy driven, which may be caused by the higher affinity of the non-polar PS toward the non-polar stationary phase. Furthermore, for PS a monotonously increasing contribution of Δh was found with increasing %-THF, indicating a relatively increasing role of enthalpy. For instance, Δh/TΔs for p = 15 increased from 1.41 at 60% THF to 1.61 at 69% THF. For PE, the quotient changed in the opposite direction and changes were much smaller.

### 4.4.2 Enthalpy and entropy change as function of the degree of polymerisation

In Figure 4.4, Δh and Δs for PS oligomers are plotted as a function of p for various eluent compositions. The decrease in both parameters with p, of course, is trivial. From a critical look at the enthalpy curves, it can be observed that Δh seems to decrease slightly more than proportional with p. To check, whether this effect is real at first the standard deviation of retention time was calculated from 10 determinations at 10 °C and 70 °C respectively for both high and low molar mass oligomers. It was found to be 0.020 min for low molar mass oligomers and 0.035 min for high molar mass oligomers. Furthermore, from a comparison of the results from several polynomial fits of log(M) versus retention time, the inaccuracy in $t_{sec}$ was estimated to be 2%. Thus, inaccuracy for the retention factor of each individual oligomer could be calculated. By applying these

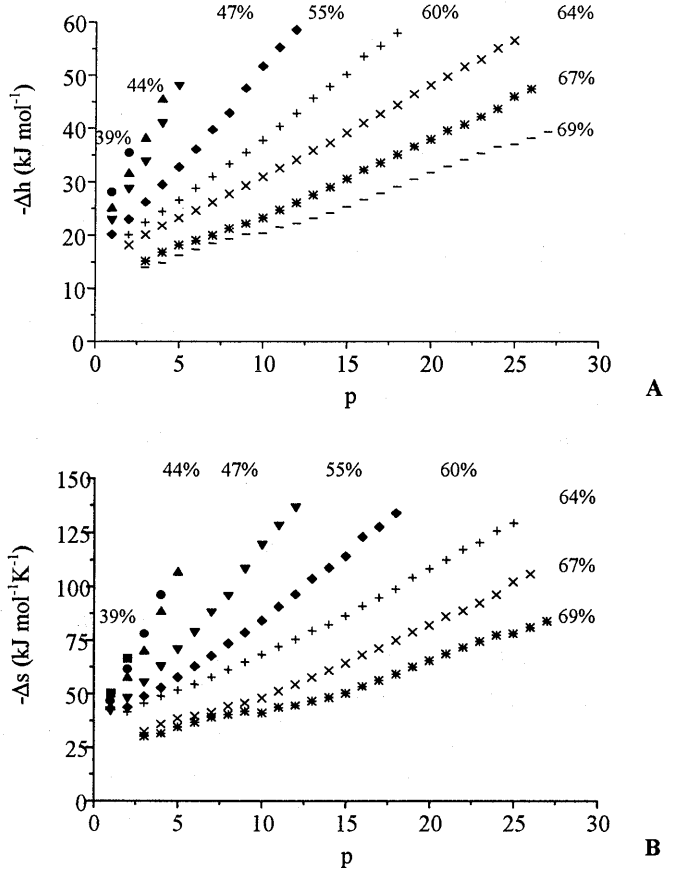


**Figure 4.4.** -Δh (A) and -Δs (B) versus p of PS at various eluent compositions. %-THF (v/v) as indicated in Figures. Chromatographic conditions: see Section 4.3.4.

results in the van 't Hoff plots, the standard deviation in Δh was found to be 4% for the lowest molar mass oligomers and 1% for the highest oligomers. After applying error bars in the Δh curve at 64% THF no linear curve could be found which included all data points (results presented elsewhere[29]). Furthermore, from a linear regression analysis, nonrandomly scattered residual values were found, indicating a nonlinear dependence between p and Δh.

The more than proportional decrease in Δh can possibly be understood from the fact that retention in reversed phase chromatography is mainly determined by molar volume.[5] Molar volume or hydrodynamic volume ($V_h$) scales with molar mass according to Eq. (2.16), which is deduced from the Mark-Houwink equation. For low molar masses, the relation between intrinsic viscosity [η] and molar mass can be better described by the equation of Stockmayer and Fixman:[30]

$$[\eta] = K_\theta M^{0.5} + K'M \tag{4.6}$$

Combination with Eq. (2.14) reveals:

$$V_h \propto K_\theta M^{1.5} + K'M^2 \tag{4.7}$$

$K_\theta$ is a constant, independent of solvent, *i.e.* it relates the intrinsic viscosity of a polymer to its molar mass under theta conditions. K' depends on the solvent composition and increases with increasing solvent quality.
Both Eq. (2.16) and Eq. (4.7) predict that $V_h$ increases more than proportional with molar mass. This may qualitatively explain the nonlinear dependencies between p and $\Delta h$.

In the enthalpy curve at 67% THF and even more so at 69% THF, an irregularity at a molar mass of about 1000 appears. A critical review of the data, revealed that this cannot be explained from errors in, for instance, $t_{sec}$.[37] As earlier mentioned, especially for the lowest molar mass oligomers where 6 data points for van 't Hoff plots are available, a slight curvature is observed. Therefore, it was considered that in these cases the determination of $\Delta h$ is not based on the same number of data points as for higher oligomers. To see whether a different data treatment can cause the observed irregularity, the enthalpy curve at 69% THF was also constructed with $\Delta h$ values determined from van 't Hoff plots in which for all oligomers only the highest three temperatures were taken into account. From the result, shown in Figure 4.5 where also error bars are depicted, it can be seen that this reveals an even more distinct discontinuity. Therefore, the effect is believed to be real and not the result of artefacts in the measurements. A discontinuity in the plot of $\Delta h$ (and $\Delta s$) versus the carbon number of several homologous series has earlier been described by Tchapla *et al.*[8] The phenomenon was explained from the fact that the lower members of the series, up to a number that was slightly less than the carbon number of the bonded chain of the stationary phase and which was called the critical carbon number ($n_c$), can penetrate into the bonded layer. This involves a closer contact between the solute and the stationary phase ligand, giving rise to a different retention mechanism. It is remarkable that the same kind of phenomenon seems to be observed for PS, since the hydrodynamic volume of PS oligomers, due to the bulky aromatic parts, is much larger as compared to members of the homologous series, which might be expected to hamper penetration in the bonded layer. The oligomer number at which the discontinuity occurs is approximately 13, which almost equals $n_c$ for several homologous series on a $C_{18}$ bonded layer, which was found to be 14.[8] It must be considered, however, that the length of the linear PS chain in such a case is (26 + 4) since each repeating unit contains two carbon atoms and each oligomer chain contains one butyl end group. In terms of the mechanism suggested by Tchapla, a change in retention mechanism at such a relatively high chain length of the oligomers might be due to a

folding of the linear oligomer chain causing insertion of both ends into the bonded layer, although this is speculative.
The fact that at lower %-THF no indications of penetration effects are observed from the enthalpy curves may be ascribed to the large values of Δh thus masking the rather minor effect. In this respect, plots of selectivity versus p are better suited, as will be pointed out later on. It must be mentioned here that the effect described above, suggesting a discontinuity in the partitioning behaviour as function of p, could not be predicted by the SF theory, as was described by Tijssen *et al.*[22]

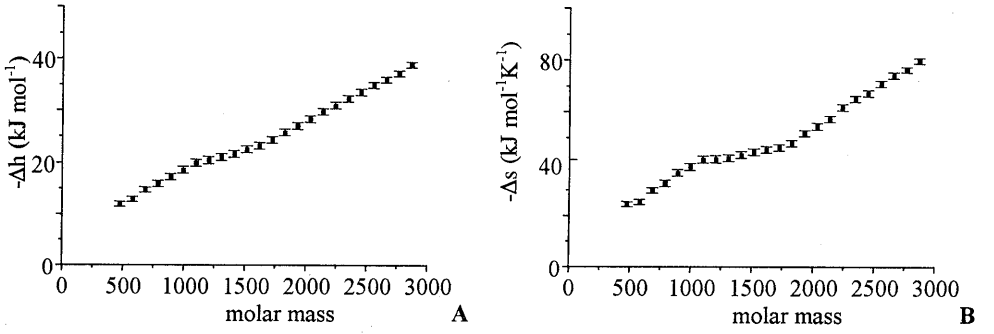


**Figure 4.5.** -Δh (A) and -Δs (B) as function of molar mass of PS at 69% (v/v) THF. Both Δh and Δs values calculated from van 't Hoff plots based on measurements at 40, 55 and 70 °C. Chromatographic conditions: see Section 4.3.4.

Penetration effects may also provide an alternative explanation for the observed curvature in the van 't Hoff plots of the low molar mass oligomers (Figure 4.2). At lower temperatures, the $C_{18}$ chains become more rigid which will especially affect the retention behaviour of solutes that (partly) penetrate into the layer, thus giving rise to curved van 't Hoff plots. This effect was already reported for other low molar mass solutes in various reversed phase systems.[31]

Similar to enthalpy, entropy decreases more than proportional with p as can be observed from Figure 4.4B. Furthermore, it can be seen that relations between Δs and p can also be described properly by Eq. (4.7), with coefficients of regression in all cases exceeding 0.999.
Like for the enthalpy curves, discontinuities are found at higher %-THF, which become more obvious when data for all oligomers are treated in the same way (Figure 4.5), thus again accounting for partitioning effects.
The accuracy of the determination of all Δs values is influenced by the phase ratio. In this study, $\phi$ is not necessarily equal for all solutes, since increasing the chain length of an

oligomer may cause the accessible stationary phase volume, as well as the mobile phase volume to decrease. The net effect on $\phi$ is hard to predict. From the SEC curve (Log(M) vs. $t_r$) that was recorded in order to determine $t_{sec}$ values, it was concluded that molar masses of the studied oligomers all lie in the linear range of the curve and no total exclusion occurs. This means that a significant decrease in $\phi$ due to a strongly decreased accessible stationary phase volume, as was found by Shaliker *et al.* for high molar mass polystyrenes[32] does not occur for the oligomers studied here. Therefore, it is assumed here, that a change in $\phi$ as function of p will be minor and will not significantly influence the observed trend of $\Delta s$ versus p.

In Figure 4.6, $\Delta h$ and $\Delta s$ of PE oligomers are plotted versus molar mass. Compared to PS, the curves are less smoothed and data points show more scatter. This is probably due to the fact that the nature of the various PE oligomers under study, differ in the kind of end group resulting in different energies of interaction. This will cause the corresponding part between brackets of Eq. (2.19) to be different, thus giving rise to a less gradual increase in $\Delta h$ and $\Delta s$ with molar mass. Although less clear, similar trends are observed for PE as compared to PS. Due to the rather different nature of both PS and PE, the observation of similar trends might indicate that the underlying mechanisms which have been attempted to account for above, are universal for non-polar and moderately polar polymers in the investigated separation system.
Due to its large repeating unit, of course for PE no transitions in the $\Delta h$ and $\Delta s$ curves can be seen, since only the first two or three oligomers might be expected to penetrate into the bonded layer. Therefore, the number of data points available in the relevant range of molar masses is too low to visualise such effects.

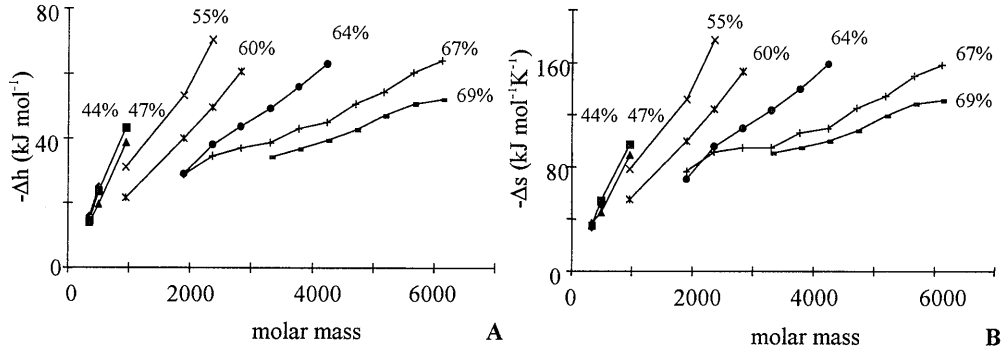


**Figure 4.6.** -$\Delta h$ (A) and -$\Delta s$ (B) as function of molar mass of PE at various eluent compositions. %-THF (v/v) as indicated in Figures. Chromatographic conditions: see Section 4.3.4.

### 4.4.3 Martin plots for polystyrene and polyester

In Figure 4.7, plots of ln(k) versus p for PS are shown. It is obvious that these Martin plots are nonlinear in all cases. Curvature in the very low p range has been observed earlier and can be ascribed to the effect of the (butyl) end group on the total free energy of transfer of the first oligomers.[27] Deviations from linearity in the higher p range for other types of oligomers and homologues have been shown to occur at the critical carbon number and have been ascribed to a change in sorption mechanism from 'dissolution' in the bonded layer to physical adsorption.[8,33] Indeed, in some of the curves in Figure 4.7, a distinct discontinuity seems to be present. This of course coincides with the value of p, for which also discontinuities in the enthalpy and entropy curves were found, which could have been anticipated from Eq. (2.19).

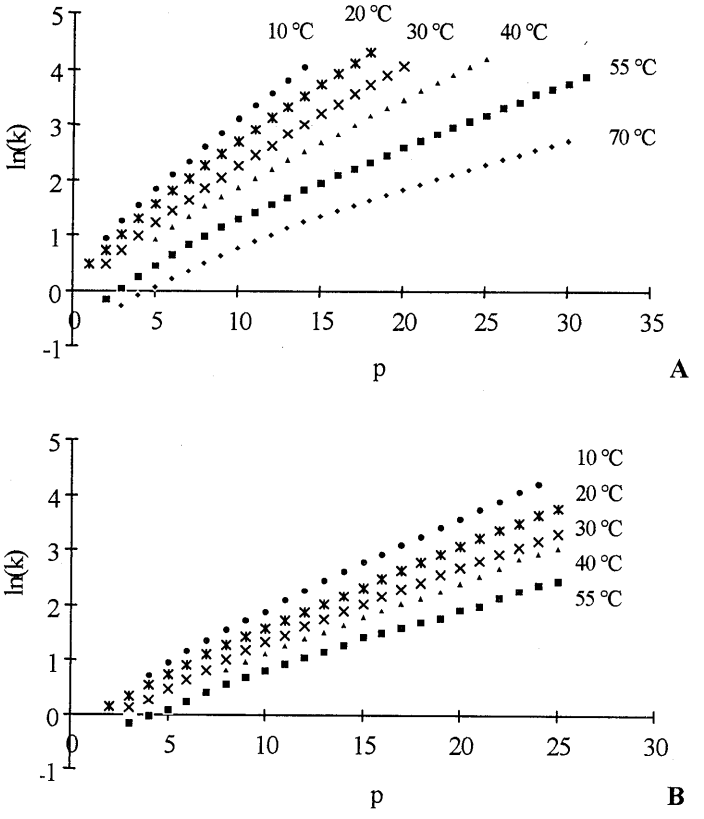


**Figure 4.7.** ln(k) versus p of PS (Martin plots) at 64% (v/v) THF (A) and 69% THF (B) at various temperatures. Temperatures as indicated in Figures. Chromatographic conditions: see Section 4.3.4.

A more detailed look upon the respective retention effects can be obtained from plots of selectivity, $\alpha$ ($k_{p+1}/k_p$) versus p. In Figure 4.8, where such plots are shown for 64% THF and 69% THF, it can be seen that two regions can be distinguished. The relatively steep decrease in $\alpha$ up to p = 14, as compared to higher values of p, again indicates a different retention mechanism, thus further accounting for partitioning effects. The distinctly decreasing values of $\alpha$ at p > 14 clearly evidence the nonlinearity of the Martin plots at higher degrees of polymerisation. Monotonously decreasing values of $\alpha$ seem logical since selectivity will reach a value of one at infinite molar mass. Obviously plots of $\alpha$ versus p are more sensitive to differences in retention behaviour as compared to plots of enthalpy and entropy, since clear evidence for partitioning effects can be obtained for 64% THF as well. This information could not be obtained from the enthalpy and entropy curves at this eluent composition (Figure 4.4).

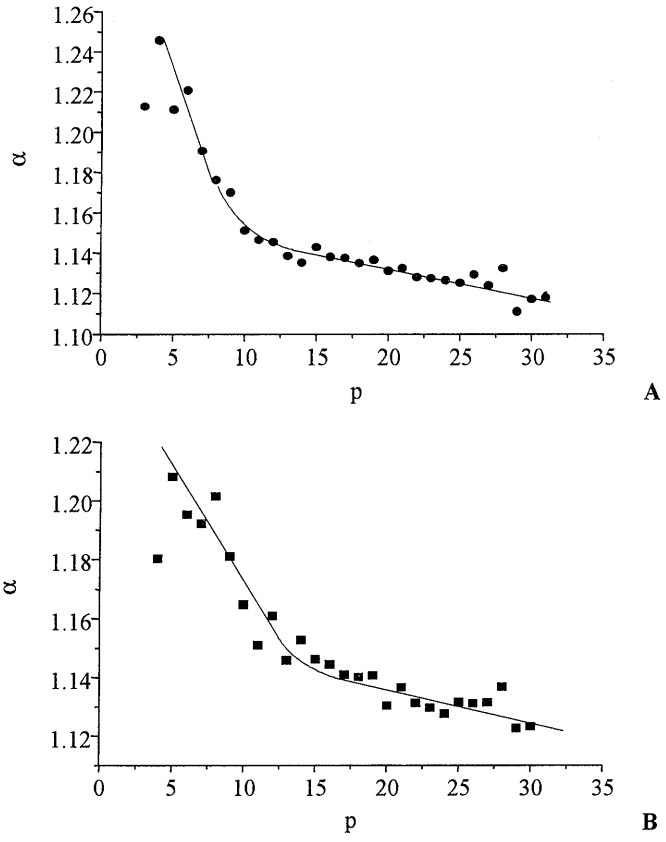


**Figure 4.8.** Selectivity, $\alpha$, ($k_{p+1}/k_p$) versus p of PS at 64% (v/v) THF and 55 °C (A) and at 69% THF and 30 °C (B). Chromatographic conditions: see Section 4.3.4.

A critical look at the data of other workers (Figure 1 of (32) and Figure 7a of (1)) reveals that slight curvature for PS at higher p seems to be found as well, in spite of the fact that linearity is claimed. Finally from Figure 4.9 it can be concluded that nonlinearity also occurs for PE, the cause of which can certainly not be ascribed to penetration effects, due to the relatively high molar masses.

From the thermodynamic data presented above, it can be concluded that the curvature can be ascribed to entropic effects. The nonlinear decrease in $\Delta h$ which would cause retention to increase more than proportional with p, obviously is overcompensated by the nonlinear decrease in $\Delta s$, affecting retention in the opposite direction. These effects found for both PS and PE which significantly differ in chemical nature, suggest that curved Martin plots are a universal phenomenon for non-polar and moderately polar polymers in the investigated separation system. These measurements confirm predictions by Larman *et al*,[1] who foresaw a bending off of the straight line plot, based on a calculation of isocratic retention data from gradient elution runs.

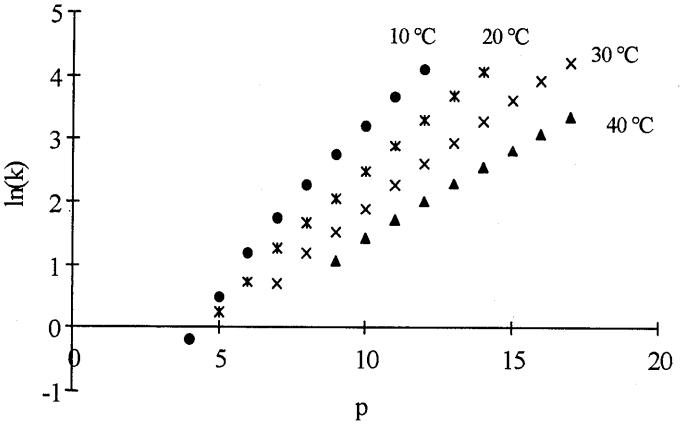


**Figure 4.9.** ln(k) versus p of PE (Martin plots) at 69% (v/v) THF at various temperatures. Temperatures as indicated in Figures. Chromatographic conditions: see Section 4.3.4.

Concurrently, very recently it was reported for polyethyleneglycol/polyethyleneoxide (PEG) that in an acetonitrile-water system both $\Delta h$ and $\Delta s$, and therefore also ln(k), increase linearly with p, up to a molar mass of at least 100,000.[16] The difference as compared to the present results can possibly be explained from a different polymer conformation and the different nature of the organic modifier. Furthermore, inherent to the different, much more polar character of PEG as compared to PS and PE, a completely different, entropy driven retention mechanism was found. The reported, positive values of $\Delta s$ indicate an increase of disorder, and therefore a completely different conformation

change for PEG when going from the mobile to the stationary phase. Presumably, the finding of linear Martin plots is inherent to molecular conformation and conformation changes due to the transition to the stationary phase and may therefore be different for various types of polymers in different solvents.
Furthermore, it is likely to expect that the exact behaviour *e.g.* the extent of nonlinearity and the molar mass at which this becomes significant, also depends on the nature of the stationary phase. The physical-chemical structure of the many available different bonded silica phases, will particularly influence molar conformation of oligomer chains, (partly) penetrated into the pores. This will alter the entropic contribution to retention and therefore also the curvature of the Martin plot.

### 4.4.4 Enthalpy-entropy-compensation

The concept of enthalpy-entropy-compensation for oligomers with varying p was tested at 40 °C which is the average value of the tested temperature range. Results are shown in Figure 4.10 where plots of ln(k) versus Δh (Eq. (4.5)) for the respective oligomers at various eluent compositions are given. For both PS and PE, like in the Martin plots, distinct curvature is found. The decreasing slope with increasing Δh in all plots indicate a monotonously decreasing enthalpy-entropy-compensation-temperature (EECT), β, according to Eq. (4.5), for higher oligomers. This again demonstrates a change in retention mechanism with molar mass. For higher oligomers, retention increases less than proportional with Δh, which may be due to the increasing dominance of entropy effects caused by multi-site adsorption. For PS at higher %-THF, again two different, almost linear parts can be observed. For these two parts, at 69% THF two different EECTs could be determined, *e.g.* 307 ± 24 °C (95% confidence intervals) for oligomers 3-15 and 147 ± 6 °C for oligomers 16-26. The fact that both values differ significantly, again indicate a difference in retention mechanism for the lower oligomers compared to the higher analogons, which has already been ascribed to insertion into the bonded layer.
The physical meaning of an EECT for oligomers with varying p is the temperature at which retention becomes independent of molar mass.[16] The conditions at which this occurs in liquid chromatography are known as critical conditions.[25,35] The fact that no distinct EECT can be found for the investigated polymers would suggest that no conditions can be established at which retention times of polymers with varying molar mass exactly match. This is in agreement with results from a recent study in which it was reported that, although conditions could be established at which retention was nearly independent of molar mass, in none of the investigated cases exact molar mass independence was found.[25] Results reported here probably support this observation.

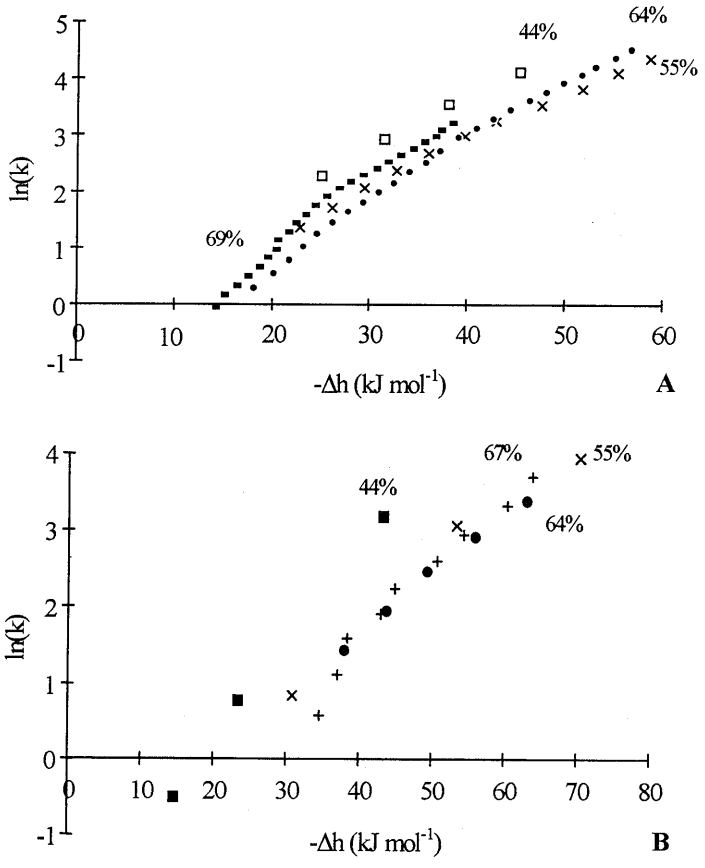


**Figure 4.10.** ln(k) versus -Δh (enthalpy-entropy-compensation plot) for PS (A) and PE (B) oligomers with varying molar mass at various eluent compositions. %-THF (v/v) as indicated in Figure. Temperature: 40 °C. Other chromatographic conditions: see Section 4.3.4.

Enthalpy-entropy-compensation was also verified for various oligomers in varying eluent compositions. For all individual PS oligomers, linear dependence with a high degree of correlation was obtained ($R^2 > 0.99$ in all cases), indicating that the retention mechanism is constant in the tested range of %-THF. The finding of an EECT in reversed phase chromatography has more often been observed for both polar and non-polar solutes.[36] Various effects, *e.g.* dominance of enthalpy effects, penetration into the bonded layer and the more than linear increase in enthalpy and entropy with p, have been shown to occur throughout the whole investigated range of eluent compositions. This, together with the observation that the retention mechanism of PS with %-THF is invariant, indicates that conclusions from this study can also be used for a qualitative understanding of the sorption mechanisms in the gradient elution mode. This is especially valid for the higher

molar mass oligomers which elute in both gradient and isocratic elution within a very small range of eluent compositions ($\Delta\varphi$).[17]
Finally, enthalpy-entropy-compensation was tested for both PE and PS oligomers at 40 °C in 64% THF. The compensation temperatures which were determined from linear regression analysis of the entire curves for both polymers are 175 ± 8 °C for PS and 120 ± 12 °C for PE. The significantly lower temperature for PE is in accordance with the more polar character of this polymer. Critical conditions in reversed phase systems for such products will be situated at an eluent composition containing less organic modifier or, as an equivalent, at a fixed %-modifier, at a lower temperature.

### 4.4.5 Critical conditions

Finally, values for the chemical potential difference, which were calculated from the obtained enthalpy and entropy values for PS, were plotted versus %-THF, which is shown in Figure 4.11. It is easily recognised that, especially for the low molar mass oligomers, no linear dependence between $\Delta\mu$ and $\varphi$ is found. This is in accordance with the findings of Schoenmakers *et al.*, who showed that, when a broader range of eluent compositions is taken into account, the dependence between $\varphi$ and log(k) (and therefore also $\Delta\mu$) can at best be described, using quadratic relationships.[37,38] Therefore, at first linear regression in which only data points where (%-THF > 50%) were taken into account, was performed for all oligomers. It was found that all lines roughly have the same point of intersection at $\Delta\mu = 0$ and %-THF at which this occurs lies between 83% and 86% THF for all oligomers. This is in accordance with results from an earlier study, in which it was found that the so called Critical Solvent Composition (CSC) in a water-THF system on the same column type is 86% THF.[25] Secondly, when this CSC was taken as a fixed data point and %-THF was fitted versus $\varphi^2$, taking into account all data points, including the ones where %-THF < 50%, regression coefficients for all oligomers exceeded 0.999.
Obviously from the measurements of thermodynamic parameters under adsorption conditions, critical conditions for a polymer can be predicted within certain limits. Furthermore, strong evidence has been obtained for the theoretical prediction that under these conditions the total free energy change equals zero for non-functionalised polymers. It must be mentioned here, that strictly spoken, PS is functionalised with butyl end groups. However, since these groups strongly resemble the polymer backbone, interactions with the stationary phase will be highly similar. Therefore, under conditions where the chemical potential change of the polymer backbone equals zero, (almost) the same will be valid for the butyl end group. Furthermore, the accuracy of the analysis performed here is too low to determine whether all lines exactly intersect in the same

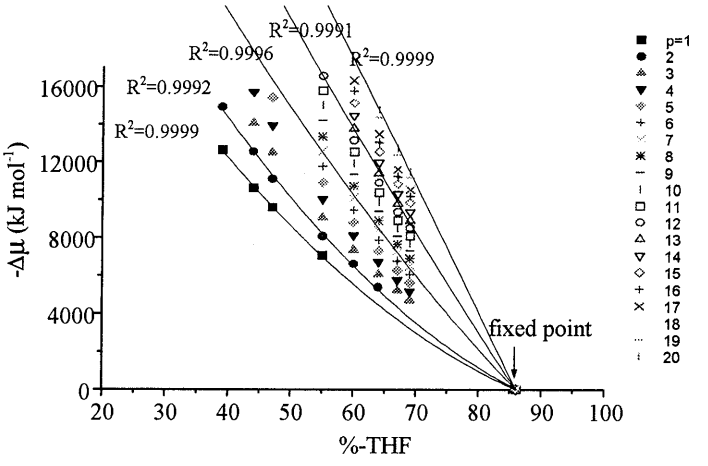


**Figure 4.11.** -Δμ versus %-THF (v/v) for PS oligomers at 35 °C. p: see legend. $R^2$ = coefficient of regression. Chromatographic conditions: see Section 4.3.4.

point or not, as was suggested above, since it was shown earlier that deviations from the point of intersection are only small.[25]

For PE, due to the limited number of different eluent compositions at which the respective oligomers could be determined isocratically, only trends can be given. Nevertheless, when linear extrapolation is performed for various oligomers, points of intersection with the x-axis lying between 74% and 80% THF are found. This indicates that CSC for PE, as could be expected, lies at a lower %-THF. As compared to PS, points of intersection for the respective oligomers show more variation. This is caused by the lower accuracy of determination of retention times. Furthermore, it must be remembered that PE can have three different end group combinations, *e.g.* 0, 1 or 2 acidic end groups. The total free energy change of these different solutes under critical conditions will be different (Eq. (2.17)), thus giving rise to different retention times and different points at which $\Delta\mu_{total}$ equals zero.

## 4.5 CONCLUSIONS

Linear van 't Hoff plots are obtained for almost all oligomers of both PS and PE. In all cases, Δh and Δs values are negative and both increase for PS as well as for PE with increasing %-THF. The increase in Δh can be ascribed to a decreasing affinity of the oligomers towards the stationary phase. The increase in Δs can be understood from the decreasing number of oligomer sites that will simultaneously interact with the sorbent. The magnitude of Δh in all cases exceeds that of TΔs, which is even more obvious for

PS as compared to PE, indicating that enthalpy plays a more pronounced role in the total retention process than does entropy. For PS, both $\Delta h$ and $\Delta s$ decrease slightly more than linearly with p. This may possibly be explained from the nonlinear increase of the hydrodynamic volume with p. Although less clear, similar trends are found for PE.

In the curves of $\Delta h$ and $\Delta s$ of PS versus p at high %-THF, discontinuities are observed at an oligomer number slightly less than the carbon number of the bonded chain of the stationary phase. This effect may be ascribed to penetration of the oligomer chains into the bonded phase. However, this discontinues behaviour is not predicted by the SF theory.

Martin plots for both PS and PE are nonlinear throughout the whole investigated range of eluent compositions, which is due to the increasing relative importance of entropic effects. This nonlinearity contradicts results of other experiments and is suggested to depend on the conformation of a polymer in solution, as described by the Stockmayer-Fixman equation. It may therefore be a universal phenomenon for non-polar and moderately polar polymers in the investigated system.

Application of the concept of enthalpy-entropy-compensation also reveals a changing retention mechanism with p for PS and PE since no distinct EECT is found independent of p. This probably explains the observation in an earlier study in which no exact molar mass independence of retention could be found under (near) critical conditions. Enthalpy-entropy-compensation is found for all PS oligomers in varying %-THF, indicating that the retention mechanism is independent of the binary eluent composition. This together with the fact that various effects mentioned earlier occur throughout the whole investigated range of eluent compositions, indicates that conclusions from this study can also be used for a qualitative understanding of the sorption mechanisms in the gradient elution mode.

Finally, from plots of $\Delta\mu$ versus %-THF, critical conditions for PS can be predicted within certain limits. The result of this experiment supports the theoretical prediction that under these conditions $\Delta\mu$ equals zero for non-functionalised polymers.

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# CHAPTER 5

# Molar Mass Effects in Reversed Phase Gradient Polymer Elution Chromatography of Oligomers


## SUMMARY

*The molar mass dependence of retention of oligomers in Reversed Phase Gradient Polymer Elution Chromatography (RP-GPEC) was investigated. For this purpose, measurements for various oligomer series, among others polystyrene (PS) and amorphous polyesters (PE), were carried out applying different non-solvent/solvent (NS/S) systems and various temperatures. In most cases, sigmoidally shaped curves were found for %-solvent (%-S) versus 1/√(molar mass (M)) but the exact shape can vary from almost linear to pronouncedly convex. Increasing temperature leads to an increase in the molar mass dependence of retention, which can be ascribed to decreasing Flory-Huggins interaction parameters. Changing the NS/S system influences the shape of the curves to a large extent but the effect is different for varying oligomer series. From results of isocratic measurements for PS and a PE, it was found that the effect of experimental conditions can be ascribed to the relative contributions of both end groups and monomeric repeat units to retention. Since these contributions are affected to different extents by the chromatographic conditions, changing these conditions also affects the shape of the curve %-S versus 1/√M.*

## 5.1 INTRODUCTION

Based on the Flory-Huggins (FH) theory, it was found by Glöckner that the volume fraction solvent, $\varphi_S$, at the cloudpoint of a polymer in a non-solvent/solvent (NS/S) system is related to the reciprocal square root of its molar mass according to Eq. (2.24).[1] In gradient elution chromatography, this relation was also found for high molar mass polymers, between the percentage solvent (%-S) at the point of elution ($\varphi_e$), and the molar mass (M).[2-5] Although this might suggest that in such a case separation is strictly based on solubility, the same relationship was established for cases where adsorption was found to affect the separation.[2-5] Recently, it was demonstrated by this author that the relation can also be found for low molar mass polyesters (oligomers) in RP-GPEC,[6] where separation is governed by sorption (adsorption and/or partitioning, see also Chapter 3). The exact shape of the curves, however, seemed to be somewhat sigmoidal rather than strictly linear. The obtained relations can be used for the determination of average molar masses of the polyester resins,[6] as will be shown in Chapter 8 of this thesis.

In this Chapter, the relation between M and $\varphi_e$ is further investigated and experiments are extended to other oligomer types, for two reasons. At first, a further understanding of this relationship and its exact form, will possibly give more insight in the underlying retention mechanism. Secondly, because the relationship also has a practical meaning, since it provides a method for the determination of molar masses and molar mass distributions of low molar mass polymers.[6]

Therefore, various, chemically different oligomer series were subjected to RP-GPEC experiments in varying NS/S combinations and at varying temperatures. Furthermore, results from isocratic experiments described in Chapter 4, were used to predict gradient elution behaviour. Explanations of the results in terms of polymer-solvent FH interactions parameters, and contributions of end groups and monomeric repeat units to retention, will be given. In a future paper, a part of the experiments described here, will be modelled using an equilibrium self-consistent field model for polymers at interfaces, which will be shown to provide qualitatively the same results.[7]

## 5.2 THEORY

From the FH theory, it can be shown that, for large values of the degree of polymerisation, p, the critical value of the polymer-solvent FH interaction parameter in an NS/S system at the cloud point of a polymer is given by:[1]

$$\chi_{cr} = 0.5 + \frac{1}{\sqrt{p}} \qquad (5.1)$$

As a first approximation, $\chi_{cr}$ can be written according to:[1]

$$\chi_{cr} = \chi_{p,S}\varphi_S + \chi_{p,NS}(1-\varphi_S) \tag{5.2}$$

where $\chi_{p,S}$ is the polymer-solvent FH interaction parameter and $\chi_{p,NS}$ is the polymer-non-solvent interaction parameter and where the interactions between solvent and non-solvent are neglected. A combination of Eq. (5.1) and Eq. (5.2) leads to:

$$\varphi_S = \frac{0.5-\chi_{p,NS}}{\chi_{p,S}-\chi_{p,NS}} + \frac{1}{(\chi_{p,S}-\chi_{p,NS})\sqrt{p}} \tag{5.3}$$

Thus, the steepness of the plot of the cloud point composition (CPC) versus $1/\sqrt{p}$ (and thus also $1/\sqrt{M}$) depends on the difference between both interaction parameters.
In a chromatographic system, according to the Martin rule (Eq. (2.19)), a repeat structural unit (a monomer unit) contributes to the logarithm of the retention factor, k, by a constant value, which will be indicated as $\log(\alpha)$ here. Thus, Eq. (2.19) can also be written as:

$$\log(k) = \log(\beta) + p\log(\alpha) \tag{5.4}$$

where $\log(\alpha)$ is the separation factor of the neighbouring oligomers representing the selectivity in a given oligomeric series and $\log(\beta)$ is the contribution of the end groups in the oligomeric series to the retention. For reversed-phase systems, the dependence between the retention factor, k, of a specific solute and the volume fraction, $\varphi$, of organic solvent in the mobile phase can, in many cases, be described by Eq. (2.26), which is also known as:[8,9]

$$\log(k) = a - m\varphi \tag{5.5}$$

Both parameters a and m depend on the organic solvent, on the stationary phase and on the polarity of the sample, which can be expressed in terms of interaction index ($I_x$, often similar to polarity index) and size, given as the molar volume ($V_x$).[10] Analogous to the Martin rule, it has been found that m (S') and a ($\log(k_w)$) linearly depend on p:[8,11]

$$a = a_0 + a_1 p \qquad\qquad m = m_0 + m_1 p \tag{5.6a,b}$$

where $a_0$, $a_1$, $m_0$, $m_1$ are constants depending on the molar volumes ($\Delta V_x$, $V_{0x}$) and on the polarities ($\Delta I_x$, $I_{0x}$) of both the repeat structural unit ($\Delta V_x$, $\Delta I_x$) and the structural residue ($V_{0x}$, $I_{0x}$) in a given series.[12] Combination of Eqs. (5.4-5.6) reveals:

$$\log(k) = \log(\beta) + p\log(\alpha) = a_0 - m_0\varphi + p(a_1 - m_1\varphi) \quad (5.7)$$

If Eq. (5.7) adequately describes the retention of an oligomeric series in a given chromatographic system, the parameters a and m are correlated:[8]

$$m = q + p'a \quad (5.8)$$

where p' and q are constants.

## 5.3 EXPERIMENTAL

### 5.3.1 Polymer samples

Several, chemically different oligomer series, in most cases commercially available low polydispersity standards, were used in this study. To assure for each oligomer type a molar mass range as broad as possible, mixtures of two or three standards were made. The following standards were used: polystyrene (PS), Polymer Laboratories (PL), (Shropshire, UK), $M_p$ values (molar mass at the maximum of the molar mass distribution as supplied by the manufacturer) 418, 1700 and 3250, all with butyllithium as initiator, polydimethylsiloxane (PDMS), Polymer Standard Service (Mainz, Germany), $M_p$ 3130, polyethyleneglycol (PEG), PL, $M_p$ 960, 1470 and 4100, polyisoprene (PIP), PL, $M_p$ 1350, 3190 and 800, polymethylmethacrylate (PMMA), PL, $M_p$ 625, 1680, 3800 and 5270. Furthermore three polyesters resins, PE1, PE4 and PE5, were used. For information regarding the composition of the polyesters it is referred to Section 3.2.2. For the isocratic experiments, low polydispersity fractions of polyester PE1 were used. For information on the preparation of these fractions, it is referred to Section 3.2.1 and Table 3.2.

### 5.3.2 HPLC column, solvents and equipment

The column used for GPEC experiments was a Novapak $C_{18}$ column from Waters (Milford, MA, USA, $d_p$ = 4 µm, pore size 60 Å, 150 x 3.9 mm). The solvents used were water, methanol (MeOH) and acetonitrile (ACN), all Lichrosolv quality from Merck (Darmstadt, Germany) and tetrahydrofuran (THF), HPLC grade from Rathburn (Brunschwig Chemie, Amsterdam, The Netherlands) To all solvents, 200 µl acetic acid, Pro Analysi quality from Merck, per litre was added, except for experiments with PEG. For HPLC, the solvents were constantly sparged with helium (20 ml/min). All solvent mixtures were made by volumetric mixing by the HPLC pump, no premixes were used.

The HPLC equipment used, was identical to that, described in Section 3.2.4. For the detection of PDMS, PEG, PIP and PMMA, instead of a UV detector an Evaporative Light Scattering Detector (ELSD) was used, type Sedex-55 (Sedère, France), which operated at 40 °C, with nitrogen as carrier gas, at an inlet pressure of 2.4 bar.

### 5.3.3 Experiment strategy

Gradient strategy was identical to that, described in Section 3.2.5. Starting and end conditions of each gradient were chosen such that all oligomer peaks eluted in the gradient part of the run. The dead volume ($V_m$) of the used column was 1.05 ml and the system hold-up volume was 4.0 ml (see Section 3.2.5).
All samples were dissolved in THF, except for PEG which was dissolved in water. The total concentration of the used mixtures of low polydispersity standards was 10 mg/ml. Unless indicated otherwise, 10 µl of these solutions were injected. From Chapter 3 it is known that for these amounts no column overloading which would influence peak position, occurs. Assignment of the oligomer peaks for the epoxy, polyester and polystyrene samples was based on previous studies.[6,13] Due to the predominance of their corresponding peaks (see Chapter 3), for polyesters PE1 and PE4, oligomers containing one alcoholic and one acidic end group were taken into account and for PE5, oligomers containing two alcoholic end groups. For PS, all oligomers contain a butyl fragment as chain end. Assignment was less straightforward for the other oligomer series, since ELSD instead of UV had to be used as detection method. Due to evaporation of the volatile, lowest molar mass oligomers, the identity of the first detectable oligomer had to be confirmed by another method. This was done by fractionation of the respective oligomer series from GPEC, followed by gas chromatography-mass spectrometry (GC-MS). For this purpose, oligomer series were injected several times each, from which the first 6 detectable oligomers were isolated. The resulting fractions were dried under nitrogen and redissolved into a small amount of THF. By re-injection on GPEC, it was confirmed that no degradation of the fractions had occurred. Subsequently, the fractions were subjected to GC, on a gas chromatograph, type HP6890 from Hewlett-Packard (HP: Avondale, PA, USA), coupled to a mass spectrometric detector (MSD), type 5971A from HP, using splitless injection. The used column was a HP1 (12.5 m x 0.2 mm I.D. x 0.33 µm) and helium was applied as the carrier gas. The temperature programme started at 40 °C and temperature was raised with 8 °C/min to 325 °C.
Isocratic measurements of PE1 and PS were the same as described in Chapter 4 of this thesis. Results from this study, measured at 40 °C at 39%, 44%, 47%, 52%, 55%, 59%, 60%, 62%, 64%, 66%, 67%, 68% and 69% THF in water were subjected here to another type of data analysis, which will be described in Section 5.4.3.

## 5.4 RESULTS AND DISCUSSION

### 5.4.1 Molar mass dependence of retention of various oligomer series

All oligomer series were initially subjected to RP-GPEC, using water-THF gradients. In all cases, a gradient steepness of 1% THF/min was applied. Examples are shown in Figure 5.1. Peak assignment for PS and PE1 was known from preceding studies.[6,13] For series without a chromophore, fractionation of several oligomers in each series, followed by GC-MS was used for identification. From these measurements, also the end group fragments and their molar masses were determined. Furthermore, it was determined by GC-MS that in all unfractionated oligomer series the lowest molar mass species is the monomer. In Table 5.1, for each series, the identity of the first detectable oligomer and the formula providing the molar masses of the oligomers, are given.

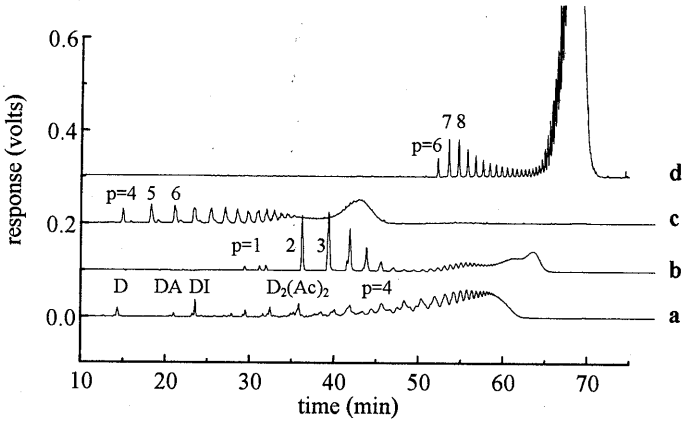


**Figure 5.1.** RP-GPEC chromatograms of various oligomer series. a: PE1, b: PS, c: PMMA, d: PDMS. GPEC conditions: sample concentration: 10 mg/ml in THF, column: Novapak $C_{18}$ (150 x 3.9 mm), temperature: 25 °C, eluent: water-THF (+ 200 µl acetic acid per litre) (75:25, v/v) to (0:100) (0 to 75 min), flow: 1.0 ml/min, injection: 10 µl, detection: PE1: UV at 277 nm, PS: UV at 254 nm, PMMA, PDMS: ELSD. D = dipropoxylated bisphenol-A, A = adipic acid, I = isophtalic acid, Ac = acid.

It is clear that the molar mass of the first oligomer that can be detected by ELSD, increases with decreasing polarity which may be due to the subsequently increasing volatility of the solutes.

Knowing their molar masses, the %-THF corresponding to the elution time of each oligomer could easily be determined from the elution time after correction for the column dead time and the system hold-up time. SEC effects were neglected in this case. In Figure 5.2, for several oligomeric series the obtained curve of %-THF versus

**Table 5.1.** First detectable oligomer, molar mass formula and end group fragment of the various oligomer series

| Polymer | Detection method | First detect. oligomer (p) | Molar mass formula | Molar mass fragment | End group |
|---|---|---|---|---|---|
| PE1 | UV | 1 | 492 | 18 + 469.p* | COOH and OH |
| PE4 | UV | 1 | 472 | 18 + 454.p* | COOH and OH |
| PE5 | UV | 1 | 344 | -80 + 424.p* | COOH and OH |
| PS | UV | 1 | 162 | 58 + 104.p | $C_4H_9$ |
| PEG | ELSD | 7 | 326 | 18 + 44.p | OH |
| PMMA | ELSD | 4 | 402 | 2 + 100.p | no end group |
| PIP | ELSD | 6 | 466 | 58 + 68.p | $C_4H_9$ |
| PDMS | ELSD | 6 | 606 | 162 + 74.p | 2 x $Si(CH_3)_2$ |

*: for the assignment of the nature of the repeating unit and its molar mass: see Chapter 3.

$1/\sqrt{M}$ is shown. All obtained curves were fitted by linear regression. The regression coefficients, being taken as a measure of the linearity of the curves here, are given in Table 5.2. In most cases, fair linearity is found, although detailed inspection of most of the curves revealed the same slight sigmoidality as was already found for polyesters:[6] see, for instance, the curve for PDMS in Figure 5.2. This is not the case for PEG, for which almost perfect linearity was observed (see also the higher regression coefficient). For PMMA, a lower coefficient was found, which was due to the presence of other products next to the main oligomers (Figure 5.1). This caused irregularities in the high molar mass part of the oligomer distribution, thus hampering the assignment

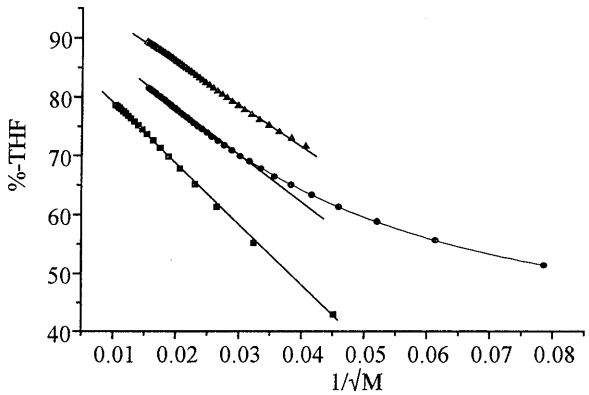


**Figure 5.2.** %-THF versus $1/\sqrt{M}$ for PE1 (■), PS (●) and PDMS (▲). GPEC conditions: sample concentrations: 10 mg/ml in THF, column: Novapak $C_{18}$ (150 x 3.9 mm), temperature: 25 °C, eluent: water-THF (+ 200 µl acetic acid per litre) 1% THF/min, flow: 1.0 ml/min, injection: 10 µl, detection: PE2: UV at 277 nm, PS: UV at 254 nm, PDMS: ELSD.

of the oligomers. The identity of these products was not further investigated, but they may be due to oligomers having a different end group. The most striking deviation from linearity was found for PS, which is obvious from Figure 5.2 and Table 5.2. Although nonlinearity for PS is much more pronounced than in the case of, for instance, the polyesters, some care must be taken by comparing these results with those for other oligomer series. In several cases, the lowest molar mass oligomers cannot be detected and especially these oligomers show the largest deviation from linearity.

**Table 5.2.** Regression coefficient of plots %-THF vs. $1/\sqrt{M}$ of the various oligomer series

| Polymer | $R^{1*}$ |
|---|---|
| PE2 | -0.9996 |
| PE4 | -0.9996 |
| PE5 | -0.9996 |
| PS | -0.9757 |
| PEG | -0.9999 |
| PMMA | -0.9944 |
| PIP | -0.9995 |
| PDMS | -0.9993 |

*: regression coefficient.

### 5.4.2 Effect of temperature and eluent system on molar mass dependence

For further gradient experiments, for reasons of its structural simplicity as compared to PE1 and due to its UV detectability, PE4 was taken as representative of the majority of cases where slightly sigmoidal curves were found. Furthermore, PS was taken as the most striking exception to the rule. At first, experiments were carried out at four different temperatures. Resulting plots of %-THF versus $1/\sqrt{M}$ are shown in Figure 5.3. Clearly, the shape of the plots is influenced by temperature. Curvature in the plots increases for both PS and PE4 with increasing temperature, which can also be observed from the decreasing regression coefficients (Table 5.3). It is furthermore interesting to note that the slope of the curves increases, especially at higher molar masses. This can be understood from Eq. (5.3). It is known that FH interaction parameters, as a first approximation, are inversely proportional with the reciprocal temperature.[14] Therefore, the term ($\chi_{p,S} - \chi_{p,NS}$) will increase (become less negative) with increasing temperature, giving rise to a steeper curve. It must be kept in mind that Eq. (5.3) was deduced for the CPC and not for a chromatographic system. Nevertheless, retention in reversed phase systems is to a large extent determined by interactions between the sample and the mobile phase, which are the same

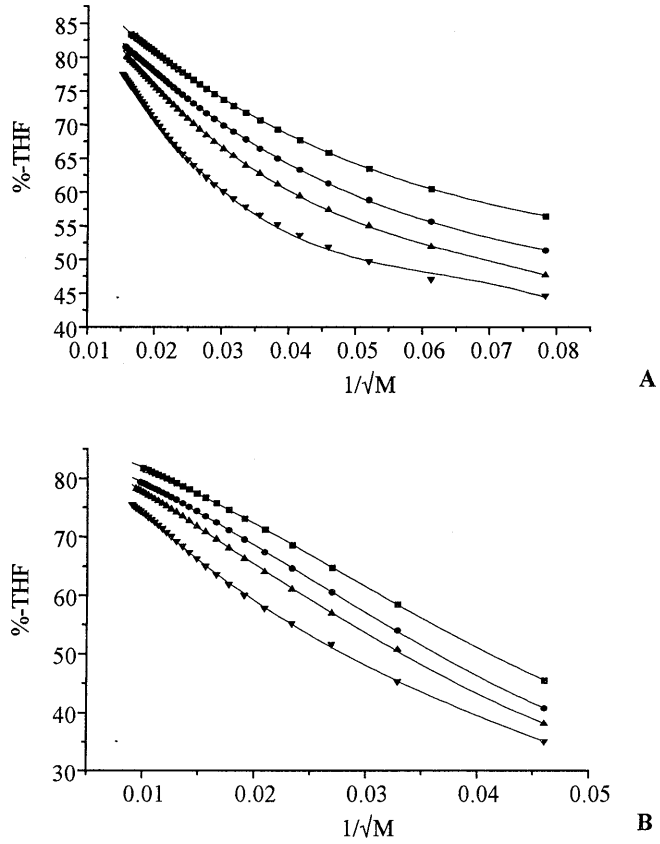


**Figure 5.3.** %-THF versus $1/\sqrt{M}$ for PS (A) and PE4 (B) at various temperatures. GPEC conditions: eluent: water-THF (75:25, v/v) to (10:90) (0 to 65 min), temperature: ■: 10 °C, ●: 25 °C, ▲: 45 °C, ▼: 65 °C. Further conditions: see Figure 5.2.

interactions that determine solubility. This allows for the use of Eq. (5.3) for the qualitative explanation of differences in retention behaviour. Apparently, molar mass dependence of retention increases with increasing temperature. This, together with the fact that diffusion coefficients also increase, accounts for the increase in the number of different oligomers that can be distinguished at higher temperatures.

Another interesting experiment would be the use of other NS/S combinations. A practical problem in this case, however, is that other frequently used eluents in RPLC, MeOH and ACN, are solvents and strong displacers for the lowest molar mass oligomers of both PS and PE4 and non-solvents for the highest molar mass oligomers. Therefore, both eluents cannot be used to replace water as a non-solvent nor to replace

**Table 5.3.** Regression coefficient and curve slope of plots %-THF vs. $1/\sqrt{M}$ at various temperatures

| Polymer | T (°C) | Slope ($\%g^{0.5}mol^{-0.5}$)* | R[1]** |
|---|---|---|---|
| PE4 | 10 | -820 ± 2%*** | -0.9997 |
| | 25 | -885 | -0.9996 |
| | 45 | -1091 | -0.9981 |
| | 65 | -1487 | -0.9882 |
| PS | 10 | -746 | -0.9793 |
| | 25 | -833 | -0.9762 |
| | 45 | -1057 | -0.9649 |
| | 65 | -1424 | -0.9347 |

*: determined at $p > 20$ for PS and at $p > 16$ for PE4.
**: regression coefficient, determined from the whole curve.
***: maximum deviation between average and maximum of duplicate measurements ($dev_{max}$).

THF as a solvent. Thus, it was chosen to use both eluents as co-solvents in ternary systems. In order to keep experimental results as comparable as possible, it was necessary to keep the solvent strength and the change in solvent strength comparable for the different gradient experiments. For RP systems, the Hildebrand solubility parameter, $\delta$, is a good measure of the eluent strength.[15] For ternary systems, $\delta$ was calculated by:

$$\delta_m = (1-\varphi_{co}-\varphi_{THF})\delta_{water} + \varphi_{co}\delta_{co} + \varphi_{THF}\delta_{THF} \qquad (5.9)$$

where $\delta_m$ is the Hildebrand solubility parameter for the ternary mixture, $\varphi_{co}$ is the volume fraction of the co-solvent and $\varphi_{THF}$ is the volume fraction of THF. The $\delta$ values used for water, MeOH, ACN and THF are 47.9, 29.7, 24.3 and 18.6 $Mpa^{0.5}$ respectively.[15] For the initial binary water-THF gradient, 75:25 (v/v) to 10:90 (0 to 65 min), $\delta_m$ is 40.6 at the start and 21.5 at the end of the gradient. Ternary gradients were chosen such, that the fraction of the co-solvent remained constant during the gradient, gradient time was kept constant and that $\delta_m$ values at the start and the end of the experiments were identical to the original binary gradient. This resulted in experiments where 13% and 26% MeOH and 15% and 30% ACN were used. Instead of %-THF, $\delta_m$ was now determined as function of $1/\sqrt{M}$.

From Figure 5.4 and Table 5.4 it can be seen that modification of the NS/S system significantly influences the molar mass dependence of retention. For PS, curvature of the plots decreases with increasing amounts of MeOH or ACN, which can also been seen from the increasing regression coefficients. For PE4, although somewhat less obvious, the opposite is observed. Furthermore, the slope of the curve in the higher molar mass part decreases with increasing amounts of co-solvent, whereas in this

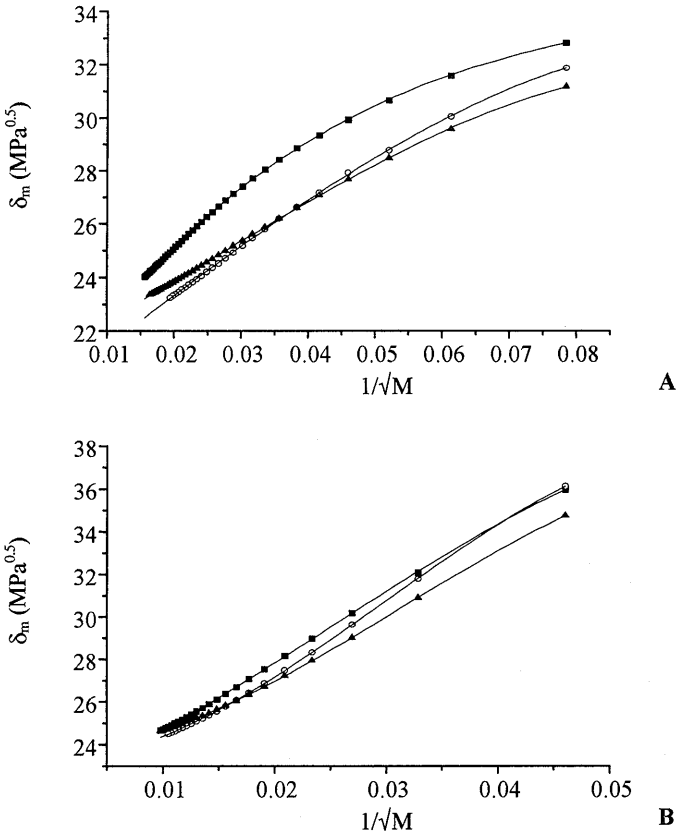


**Figure 5.4.** Solubility parameter, $\delta_m$, versus $1/\sqrt{M}$ for PS (A) and PE4 (B). GPEC conditions: eluent: ■: water-THF (75:25, v/v) to (10:90) (0 to 65 min), O: water-ACN-THF (69:30:1) to (4:30:66) (0 to 65 min), ▲: water-MeOH-THF (65:26:9) to (0:26:74) (0 to 65 min). Further conditions: see Figure 5.2.

respect the effect of MeOH is more pronounced than that of ACN. Due to the increased complexity of the system, an unambiguous explanation in terms of Eq. (5.3) is hard to give now, but apparently the difference ($\chi_{p,S}$ - $\chi_{p,NS}$) decreases (becomes more negative). This might be caused by an increase in the term $\chi_{p,NS}$ due to a decrease in the %-THF in the eluent, since THF is a stronger solvent for both PS and PE than MeOH and ACN, although this is somewhat speculative. Obviously, the separation with respect to molar mass and the number of oligomers that can be distinguished, depend on the nature of the NS/S system.

### 5.4.3 Estimation of molar mass dependence from isocratic experiments

It is clear that the appearance of the plot %-S (or $\delta_m$) versus $1/\sqrt{M}$ is not a typical characteristic of a specific oligomer series but depends on the experimental conditions such as temperature and NS/S system. Partitioning effects of the lowest molar mass oligomers in the $C_{18}$ bonded chain matrix as observed in Chapter 4, provide no explanation for deviations of the %-S versus $1/\sqrt{M}$ plots at the low molar mass side, since exactly the same shape of the plots were found on $C_8$ and $C_2$ bonded phases, for a water-THF system at 25 °C (results not shown here).[13]

**Table 5.4.** Regression coefficient and curve slope of plots $\delta_m$ vs. $1/\sqrt{M}$ at various eluent compositions

| Polymer | Eluent composition | Slope ($MPa^{0.5}g^{0.5}mol^{-0.5}$)* | $R^{1}$ ** |
|---|---|---|---|
| PE4 | water-THF | 259 ± 2%*** | 0.9996 |
| | water-THF + 15% ACN | 239 | 0.9989 |
| | water-THF + 30% ACN | 219 | 0.9985 |
| | water-THF + 13% MeOH | 219 | 0.9991 |
| | water-THF + 26% MeOH | 181 | 0.9973 |
| PS | water-THF | 244 | 0.9760 |
| | water-THF + 15% ACN | 212 | 0.9875 |
| | water-THF + 30% ACN | 176 | 0.9949 |
| | water-THF + 13% MeOH | 192 | 0.9881 |
| | water-THF + 26% MeOH | 140 | 0.9966 |

*: determined at $p > 20$ for PS and at $p > 16$ for PE4.
**: regression coefficient determined from the whole curve.
***: $dev_{max}$.

A suitable explanation may come from the contributions of both repeat structural units and end groups to the total retention. Since the contribution of end groups is relatively large for the low molar mass oligomers and since both types of contributions are affected to different extents by the chromatographic conditions, it is imaginable that the extent to which both parameters contribute to retention, determine the appearance of the plots. In order to verify this hypothesis, the isocratic measurements for PS and PE1 in water-THF of Chapter 4 were used. From these results, a and m values (Eq. (5.5)) for a large number of oligomers of both series could be obtained. In Figure 5.5, these values are plotted as a function of p, in order to determine $a_0$, $a_1$, $m_0$ and $m_1$ (Eqs. (5.6a,b)). Clearly, for PS fairly linear relations are found obeying Eqs. (5.6a,b), whereas for PE1 distinct deviations from linearity occur (non randomly scattered residual values). The finding of linear dependences for PS may seem somewhat strange, since in Chapter 4, nonlinear Martin plots (Eq. (5.4)) were found. This means that the terms $a_1$ and $m_1$ in Eq. (5.7) must at least slightly vary with p, which seems not the case when looking at

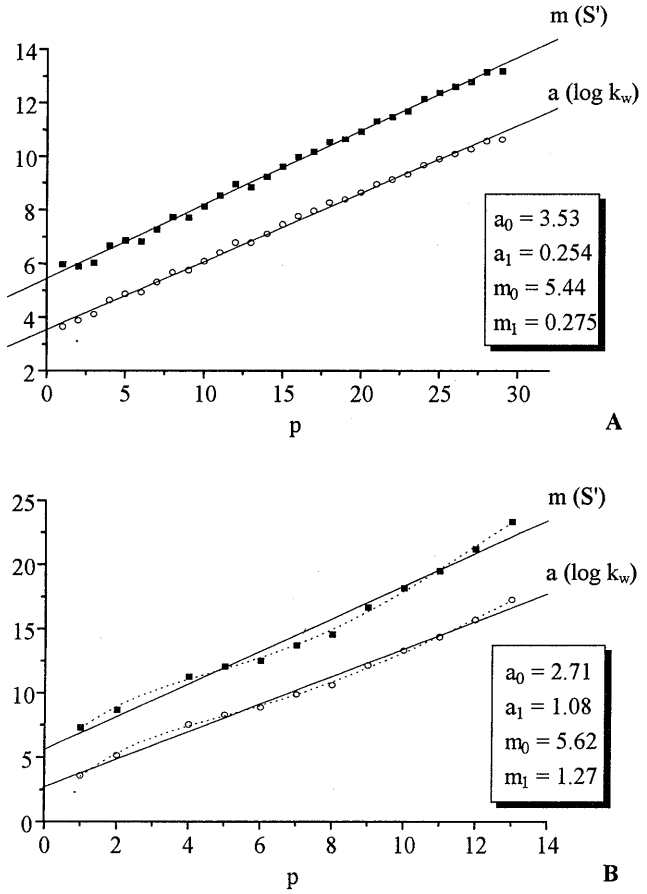


**Figure 5.5.** m (S': ■) and a (log($k_w$): O) versus p for PS (A) and PE1 (B).

Figure 5.5. The reason for this is that in order to determine a and m values, Eq. (5.5) was applied, whereas especially for the low molar mass oligomers, some deviation from linearity occurs. It is well known that for a broader range of φ, the dependence between k and φ can better be described by quadratic functions.[9,10] A variation of $a_1$ and $m_1$ with p is also implied by results of Snyder *et al.* who found for PS that for a broader range of p, the relation between m (S') and M can be described by: $m = 0.22\ M^{0.5}$.[16]

The curvature in Figure 5.5 for PE1 is possibly due to the relatively large effect of the polar end groups on retention of the lowest molar mass oligomers, thus causing differences in the contribution of additional repeat units to overall retention. From the obtained coefficients $a_0$ and $m_0$ for both oligomer series, a significant contribution of end groups to the overall retention can be concluded. The pronouncedly larger values for $a_1$ and $m_1$ for PE1 as compared to PS can be explained from the larger molar

volume of the repeat unit for PE1.[11,12] For both PS and PE1, obedience to Eq. (5.8) was found (pictures not shown).
The obtained values for a and m can be used to predict the curve %-THF versus $1/\sqrt{M}$ for an arbitrarily chosen gradient. Retention of oligomers in linear solvent strength gradients is given by Eq. (2.28). %-THF for such a gradient is given by:

$$\%-\mathrm{THF} = (t_g - t_s - t_0)\varphi' + \varphi_i \tag{5.10}$$

where $\varphi'$ is the gradient steepness ($\Delta\varphi/t_G$) and $\varphi_i$ is $\varphi$ at the gradient start. When putting: ($t_{sec}$ - $t_0 \approx 0$), combination with Eqs. (2.26, 2.28, 5.5 and 5.6) reveals:

$$\%-\mathrm{THF} = \frac{1}{m}\log[2.3m\varphi' k_0 t_{sec} + 1] + \varphi_i \tag{5.11a}$$

$$\%-\mathrm{THF} = \frac{1}{m_0 + m_1 p}\log[2.3(m_0 + m_1 p)\varphi' k_0 t_{sec} + 1] + \varphi_i \tag{5.11b}$$

$$k_0 = 10^{[\log(a) - m\varphi_i]} \tag{5.12a}$$

$$k_0 = 10^{[\log(a_0 + a_1 p) - (m_0 + m_1 p)\varphi_i]} \tag{5.12b}$$

Using Eqs. (5.11a, 5.12a), the curves %-THF versus $1/\sqrt{M}$ were calculated for a gradient with $\varphi_i$ = 0% THF and $\varphi'$ = 1%/min. For PE1, both the experimentally obtained values for a and m for each oligomer were used as well as values calculated from Eqs. (5.6a,b) using the obtained coefficients $a_0..m_1$ (see also Figure 5.5). Results are shown in Figure 5.6.
The shape of the calculated curve for PS and that for PE1, using the experimental values for a and m, are in good qualitative agreement with the experimentally obtained curves by RP-GPEC (Figure 5.2), thus justifying the adopted procedure. Notice that the isocratic measurements were carried out at 40 °C and measurements of Figure 5.2 at 25 °C, which may explain the lack of quantitative agreement. The other curve for PE1, using calculated a and m values (Eq. (5.6a,b)), distinctly deviates from the curve mentioned above. Obviously, the exact curve shape is determined (among other parameters) by deviations of Eqs. (5.6a,b) (Figure 5.5) which may be caused by the effect of end groups.

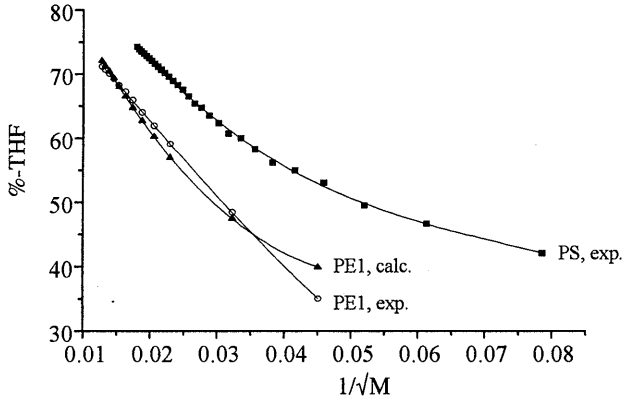


**Figure 5.6.** Calculated plots of %-THF versus $1/\sqrt{M}$ for PS (■), PE1 using experimentally obtained values of a and m (○) and PE1 using calculated values of a and m (▲). (Fictive) GPEC conditions: column: Novapak $C_{18}$ (75 x 3.9 mm), temperature: 40 °C, gradient: water-THF (100:0, v/v) to (0:100) (0 to 100 min), flow: 1.0 ml/min.

### 5.4.4 Simulation of effects of repeat unit and end group contributions

Further proof of the hypothesis of repeat unit and end group contributions to retention, determining the shape of the curves %-THF versus $1/\sqrt{M}$, was obtained by varying these contributions through adaptations of parameters $a_0..m_1$ for PS, using Eqs. (5.11b,5.12b). For this purpose, $a_0$ or $a_1$ was changed and new values for $m_0$ and $a_1$ were calculated from Eq. 5.8 (p' and q were kept constant). From Figure 5.7 it can be seen that an increased contribution to retention of the repeat unit by a ten fold increase of $a_1$ ($m_1$), causes the curve to change from (mainly) concave (■) to slightly convex (▼) (values for parameters $a_0..m_1$, p', q: see legend of Figure 5.7) . In that case the curve shape more resembles that of PE1 (Figure 5.2) which is in qualitative agreement with the also higher values of $a_1$ ($m_1$) for PE1 as compared to PS. A more pronounced convex shaped curve and an increased molar mass dependence of retention is found from a decrease in the end group contribution by a decrease in $a_0$ ($m_0$). In Figure 5.7, also a calculated curve using the values of $a_0..m_1$ from a study of Jandera for PS in a 1,4 dioxane-water system[12] is given. A sigmoidal but more linear curve as compared to the water-THF system used here, is obtained. Again, this can be attributed to an increased influence of the repeat unit as compared to the end group, which is obviously different for another NS/S system, thus confirming earlier observations in this study.

Clearly, the exact form of the molar mass dependence of retention in a given oligomer series depends on the contribution of both end groups and repeat units. Since free energy contributions of both parameters can change independently with changing temperature or NS/S system, such a change also alters the shape of the curve %-S

versus $1/\sqrt{M}$. At fixed experimental conditions, a larger repeat unit, meaning larger $a_1$ and $m_1$ values through increased contributions of the molar volume, $\Delta V_x$, gives rise to less curved dependencies.

In Chapter 8, it will be shown that linear extrapolation of the high molar mass part of the curve, in order to determine the molar masses in that part of the distribution where no oligomers can be distinguished anymore, provides correct values of average molar masses ($M_n$, $M_w$). Furthermore, it is worthwhile noting that in all cases in this study, the obtained curves can well be fitted with third order polynomials (see Figures). Coefficients of regression in most cases exceed 0.999.

In a future paper, it will be shown that from an equilibrium self-consistent field model for polymers at interfaces, qualitatively the same results concerning the effect of the repeat unit and end groups on molar mass dependence of retention can be obtained.[7]

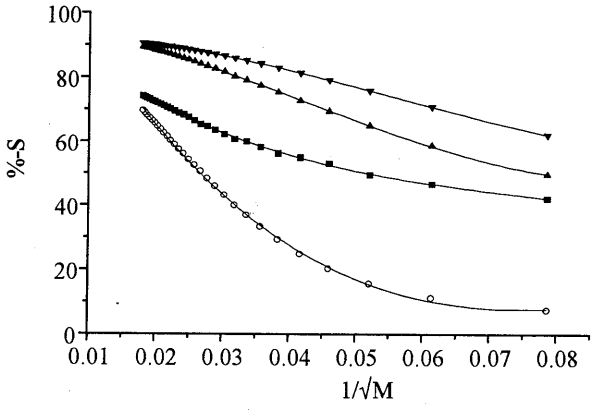


**Figure 5.7.** Calculated plots of %-solvent versus $1/\sqrt{M}$ for PS. ■: $a_0 = 3.53$, $a_1 = 0.254$, $m_0 = 5.44$, $m_1 = 0.28$, $p' = 1.08$, $q = 1.62$ (experimentally determined values), ▼: $a_0 = 3.53$, $a_1 = 2.54$, $m_0 = 5.44$, $m_1 = 2.75$, $p' = 1.08$, $q = 1.62$ (ten fold increase of $a_1$), O: $a_0 = 1.00$, $a_1 = 0.254$, $m_0 = 2.70$, $m_1 = 0.28$, $p' = 1.08$, $q = 1.62$ (decrease of $a_0$), ▲: $a_0 = 2.49$, $a_1 = 0.77$, $m_0 = 3.12$, $m_1 = 0.83$, $p' = 1.08$, $q = 0.44$ (values from Jandera[12]). (Fictive) GPEC conditions: column: Novapak $C_{18}$ (75 x 3.9 mm, gradient: water-THF (100:0, v/v) to (0:100) (0 to 100 min), flow: 1.0 ml/min.

## 5.5 CONCLUSIONS

The shape of the molar mass dependence of retention of oligomers is not a typical characteristic of a specific oligomer series. Although in most cases a slight sigmoidally shaped curve for %-S versus $1/\sqrt{M}$ is found, the exact shape, which may vary from almost linear to pronouncedly convex, depends on the experimental conditions. At higher temperatures, the molar mass dependence of retention, especially for the higher

molar mass oligomers, increases, which can be understood from the decreasing FH interaction parameters. Changing the NS/S system can influence the curve %-S versus $1/\sqrt{M}$ and its slope to a large extent but this differs for different oligomer series. The effect of experimental conditions can be ascribed to the relative contributions of both end groups and monomeric repeat units to retention. Since these contributions are affected to different extents by the chromatographic conditions, changing these conditions also influences the shape of the curve %-S versus $1/\sqrt{M}$. At fixed experimental conditions, a larger repeat unit and thus a larger effect of the molar volume, gives rise to less curved dependencies.

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# CHAPTER 6

# A Study to the Behaviour of Crystalline Polyesters in Gradient Polymer Elution Chromatography*


## SUMMARY

*The behaviour of crystalline polyesters in Gradient Polymer Elution Chromatography was studied, using a reversed phase system. In contrast to amorphous polyesters, crystalline polyesters were found to exhibit non-reproducible chromatographic behaviour under certain conditions. The cause of this phenomenon was found in the dominance of precipitation (crystallisation) and redissolution effects in the total retention mechanism. Crystalline polyesters were found to crystallise on the column after precipitation, in contrast to amorphous polyesters, where no real solid phase is formed. Varying injection volume, flow rate or precipitation medium affect the morphology of the precipitate, giving rise to different redissolution behaviour. From the minor effects of increasing sample load and gradient steepness, it was concluded that separation is mainly governed by thermodynamics rather than by redissolution kinetics. The former determine at what %-solvent during the gradient, the melting point drops below the environmental temperature. Raising the system temperature above the depressed melting point of the polyester yields highly reproducible, normal elution behaviour governed by sorption, since the formation of a crystalline phase is prevented. The difference in redissolution behaviour between amorphous and crystalline resins was used to separate blends of both types of resins by combined eluent and temperature programming.*



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## 6.1 INTRODUCTION

In Chapter 3, it was shown that Reversed Phase Gradient Polymer Elution Chromatography (RP-GPEC) can provide detailed separations of amorphous polyesters, from which information on molar mass and chemical composition can be deduced. All results were found to be highly reproducible. The separation mechanism on a $C_{18}$ derivatised silica column was shown to be dominated by sorption (which can either be adsorption and/or partitioning), although redissolution effects were present in the applied separation systems. Furthermore, redissolution was proven to be influenced by time-dependent, kinetic effects. Although this was apparently not the case on the applied separation system, redissolution effects may influence the separation of the investigated polyesters on less retaining columns. For other kinds of polymers, kinetic effects may even be important on $C_{18}$ columns.

In this Chapter, the chromatographic behaviour of crystalline polyesters in RP-GPEC is investigated. In contrast to amorphous polyesters, it was found that under certain conditions, unexpected and irreproducible results are obtained. It will be shown that the cause of this phenomenon can be found in the dominance of precipitation (crystallisation) and redissolution effects which, in the case of crystalline polymers, are highly dependent on experimental conditions. The concept of the effect of precipitated polymer morphology on the elution profile in GPEC which will be presented, is novel and provides new insights in the chromatographic behaviour of certain classes of polymers.

The behaviour of crystalline polyesters in GPEC was investigated using two resins based on dodecanedioic acid with butanediol and dodecanedioic acid with decanediol. To compare results with those of amorphous polyesters, a product based on maleic acid and di-propoxylated bisphenol-A, which was already used in Chapter 3, was also taken into account in some experiments. For both types of polyesters, chromatographic results on three different columns, *e.g.* octadecyl ($C_{18}$), cyanopropyl (CN) and bare silica under hydro-organic conditions are compared to check whether the anomalous behaviour of crystalline polyesters in GPEC could be the result of specific column interactions. From Differential Scanning Calorimetry (DSC) experiments, microscopy and two dimensional GPEC/SEC experiments, further information on the behaviour of crystalline polyesters is obtained. Furthermore, the effect of several variables such as injected mass and injection volume, flow rate, initial gradient conditions, precipitation medium and temperature was investigated and this appears to differ to a large extent from that of amorphous polyesters. Qualitative explanations will be given for the anomalous behaviour of crystalline polyesters, derived from thermodynamics. Finally the separation of amorphous and crystalline polyesters based on combined temperature and eluent programming will be demonstrated.

Crystallisation and redissolution effects are also known to dominate the separation mechanism in Temperature Rising Elution Fractionation (TREF). From this method, especially when coupled to SEC, important information on branching as a function of molar mass of crystalline polymers can be obtained.[1] In contrast, GPEC will be shown here to be better suited for the characterisation of mixtures of amorphous and crystalline polymers.

## 6.2 THEORY

The precipitation of a polymer can be represented by means of a phase diagram. As was described in Section 2.3.3.3, polymer solutions commonly exhibit Upper Critical Solution Temperature (UCST) behaviour,[2] which means that a maximum temperature can be found above which no phase separation occurs. An example is shown in Figure 6.1. Below the critical temperature, $T_{cr}$, the polymer solution demixes into two co-existing phases, a swollen polymer-rich phase ($\varphi_{pr}$) and a polymer-poor phase ($\varphi_{pp}$), the composition of which is given by the L-L curve. This of course, only occurs in the case that the polymer/solvent composition lies within the L-L area. Furthermore, the described situation is only valid under equilibrium conditions.

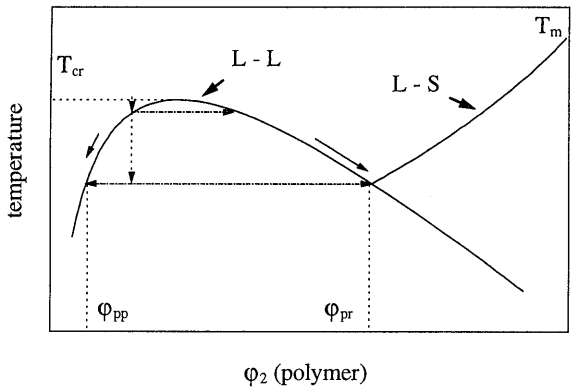


**Figure 6.1.** Schematic representation of the interference between L-L demixing and L-S transition in a binary polymer solution.

In the case of chromatography this representation is in fact too simple, since the system consists of three phases, *i.e.* the polymer-rich phase, the polymer poor phase (equal to the mobile phase) and the stationary phase. However, because the precipitation process is mainly determined by polymer/solvent and polymer/non-solvent interactions, the effect of the stationary phase can be neglected in most cases.

The mobile phase in which precipitation occurs, as an approximation, can be considered as one, homogeneous, phase. Therefore, the phase diagram from Figure 6.1 can also be used to understand the precipitation process in a chromatographic system in qualitative terms, although it must be kept in mind that under chromatographic conditions in fact equilibrium is continuously disturbed by the flowing medium.
A polymer will undergo a liquid-solid transition when the temperature drops below the melting temperature ($T_m$) in the case of a crystalline polymer or will undergo vitrification when the temperature drops below the glass transition temperature ($T_g$) in the case of an amorphous polymer. Both $T_g$ and $T_m$ decrease in the presence of solvent, which is represented by the L-S line in Figure 6.1. At a certain temperature the L-S transition (or vitrification) intersects with the L-L demixing. Below this point a solid phase (precipitate) is formed. The morphology of the solid phase highly depends on temperature and solvent composition, indicating that the precipitation is also influenced by kinetic effects. The melting point depression can be expressed mathematically by Eq. (6.1), which has been derived from the Flory-Huggins theory.[2]

$$\frac{1}{T_{mp}} - \frac{1}{T^0_{mp}} = \frac{-RV_{up}}{V_l \Delta H_p}\left[\frac{1}{m_p}\ln\varphi_p + \left(\frac{1}{m_p} - \frac{1}{m_S}\right)\varphi_S + \chi_{p,S}\varphi_S^2\right] \qquad (6.1)$$

where $T_{mp}$ and $T^0_{mp}$ represent $T_m$ in and without the presence of solvent, $V_{up}$ and $V_l$ are the molar volumes of the repeating unit and a lattice site respectively, $m_p$ and $m_S$ are the chain lengths of the polymer and the solvent expressed in lattice units, $\varphi_p$ and $\varphi_S$ are the molar fractions, $\chi_{p,S}$ is the polymer-solvent interaction parameter and $\Delta H_p$ is the melting heat of the pure polymer. From Eq. (6.1) it follows that the melting point depression decreases with increasing molar mass of the polymer. Decreasing affinity of the polymer towards the solvent, meaning a higher value of the interaction parameter, results in a decrease in the depression. Although the latter effect does not follow from Eq. (6.1) at first sight, it must be kept in mind that the first two terms between the square brackets are both negative. An increasing value of $\chi_{p,S}$ therefore causes the total term between the brackets to become less negative, thus giving rise to a decrease in melting point depression.

## 6.3 EXPERIMENTAL

### 6.3.1 Polymer samples

The polyester samples used were laboratory-made polyester resins. Sample PE5 is an amorphous homopolyester consisting of maleic acid and di-propoxylated bisphenol-A.

Samples CP1 and CP2 are crystalline polyesters based on dodecanedioic acid with butanediol and dodecanedioic acid with decanediol, respectively. In order to obtain fully alcohol-terminated resins, excess amounts of 20% diol were used during synthesis of the crystalline resins. Polystyrene equivalent molar masses as determined by Size Exclusion Chromatography (SEC), average chemical compositions measured by NMR and end group composition determined by titrimetric analysis are given in Table 6.1. For more detailed information on the characterisation of the polyester samples, it is referred to Section 3.2.2.

**Table 6.1.** Polystyrene equivalent molar masses, end group compositions and average chemical compositions of the investigated polyesters

| Sample | SEC | | | Titrations | NMR | |
|---|---|---|---|---|---|---|
| | PS-equivalent molar masses | | | Acid number | Molar fractions | |
| | $M_n$ | $M_w$ | $D^*$ | (mg KOH/g) | Diacid | Diol |
| PE5 | 3900 | 13700 | 3.5 | 13 | 0.45 | 0.55 |
| CP1 | 6900 | 16000 | 2.3 | < 1 | 0.44 | 0.56 |
| CP2 | 4400 | 11400 | 2.6 | < 1 | 0.45 | 0.55 |

*: polydispersity, $M_w/M_n$.

### 6.3.2 Chromatography experiments

The solvents used for most HPLC experiments were water, Milli-Q quality from Millipore (Bedford, MA, USA) and tetrahydrofuran (THF), HPLC grade from Rathburn (Brunschwig Chemie, Amsterdam, The Netherlands). To both solvents, 200 µl acetic acid, Pro Analysi quality from Merck (Darmstadt, Germany), per litre was added. For HPLC, the solvents were constantly sparged with helium (20 ml/min). All solvent mixtures were made instantly through volumetric mixing by means of the HPLC pump, no premixes were used.

The columns used were a Novapak $C_{18}$ column (Waters, Milford, MA, USA, $d_p$ = 4 µm, pore size 60 Å, 150 x 3.9 mm), a Novapak CN column (Waters, $d_p$ = 4 µm, pore size 60 Å, 150 x 3.9 mm) and a Resolve silica column (Waters, $d_p$ = 5 µm, pore size 90 Å, 150 x 3.9 mm). For most experiments a stainless steel in-line pre-column filter (Waters, part no. 084560) was used, unless indicated otherwise. For some experiments a guard column, guard pack module (Waters) with Novapak $C_{18}$ inserts was used.

The HPLC equipment used for GPEC was identical to that, described in Section 3.2.4, unless indicated otherwise. For the detection of the crystalline polyesters, an Evaporative Light Scattering Detector (ELSD) was applied. For reasons of availability, for most experiments an ELSD, type 750/14 from Applied Chromatography Systems was used, which operated at a temperature of 80 °C, using nitrogen for nebulisation at an inlet

pressure of 3 bar. This instrument exhibits a rather moderate sensitivity for the relatively low molar mass aliphatic, crystalline resins, which sometimes gave rise to somewhat 'noisy' chromatograms. This, however, did not influence the results of the experiments. For some experiments a more sensitive ELSD was used, type Sedex-55 (Sedère, France), which operated at 40 °C at an inlet pressure of 2.4 bar. For most experiments, the column temperature was controlled using a cryo bath, type TK-30D from Messgeräte Werk Lauda. For this purpose, the column was placed into a water jacket from Alltech Associates. For a few experiments a programmable column thermostat, type Mistral from Spark-Holland (Emmen, The Netherlands) equipped with a Peltier element, was used. After each temperature change, the system was equilibrated for at least two hours at the starting conditions of the gradient. System equilibrium was checked by repeated injections of the PE5 sample.
For the equipment and columns used for SEC, it is referred to Section 3.2.2. The gradient elution strategy has been described in Section 3.2.5.

### 6.3.3 Study of crystallisation behaviour

To study the crystallisation behaviour in the presence of non-solvent/solvent mixtures, Differential Scanning Calorimetry (DSC) and polarisation microscopy were applied. For this purpose, a differential scanning calorimeter, type 7500 - DSC-7 from Perkin Elmer (Norwalk, CT, USA) was used. About 7 mg of the pure crystalline polyester resins or 30 mg of a mixture of crystalline material and non-solvent/solvent (NS/S) was brought into a 25 µl cup, which was heated from -10 °C to 100 °C at 10 °C/min. After cooling down with the same rate, in the case of the pure materials, the sample was re-heated again to measure the thermal behaviour without the influence of physical ageing. Temperature was calibrated using an indium standard. For microscopic experiments of the mixtures of crystalline material with NS/S, a light microscope with crossed polarisers, type Universal from Zeiss was used.

## 6.4 RESULTS AND DISCUSSION

### 6.4.1 Behaviour of crystalline versus amorphous polyesters in GPEC

In Figure 6.2, the samples PE5 and CP1 are compared by SEC and GPEC, respectively. The used gradient steepness was chosen for reasons of analysis time and does not represent an optimal situation with respect to the resolution of oligomers (Chapter 3). As was already shown in Chapter 3, for amorphous polyesters such as PE5, the separation

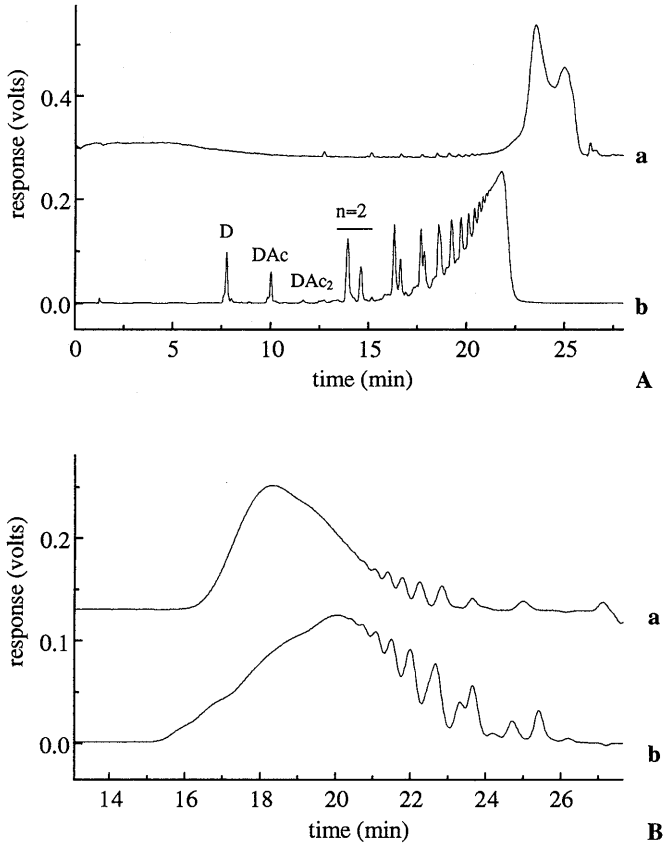


**Figure 6.2.** Comparison between GPEC (A) and SEC (B) for CP1 (a) and PE5 (b). GPEC: sample concentration: 20 mg/ml in THF, column: Novapak $C_{18}$ (150 x 3.9 mm), temperature: 25 °C, eluent: water-THF (70:30, v/v) to (0:100) (0 to 23.3 min), flow: 1.0 ml/min, injection: 5 µl, detection: CP1: ELSD, PE5: UV at 277 nm. D = diol, Ac = acid. SEC: concentration: 1.5 mg/ml, column: Shodex KF804, KF803, KF802.5, KF802 (in series), temperature: 40 °C, eluent: THF + 1% (v/v) acetic acid, flow: 1.5 ml/min, injection: 200 µl, detection: RI.

power of GPEC as compared to SEC is much higher. The identity of the various peaks as indicated in Figure 6.2 has also been explained in that Chapter.

For sample CP1, by GPEC a completely different elution pattern as compared to PE5, is obtained. From a comparison of both SEC curves, it can be observed that the $M_w$ of CP1 is higher. The SEC curve of CP1, however, does not show any irregularities that may be indicative of the formation of side products. It is therefore remarkable that by GPEC a bimodally-shaped curve is found that may not be expected for a polycondensation reaction that seems to have proceeded normally.[3] The contribution of the oligomer fractions to the total distribution is also remarkably low as compared to SEC. Of course,

the higher molar mass as compared to PE5 will lower the oligomer signals. Furthermore, it must be kept in mind that ELSD was used for the detection of this polyester. For this type of detector it is known that, at least up to a certain value, the response increases exponentially with injected mass.[4] This means that the contribution of oligomer fractions may be underestimated when using ELSD. From the experience of this author with polymers with a comparable molar mass, however, it is not likely, that this can fully explain the observed low intensity of the oligomer peaks. The relatively low number of different oligomers as compared to PE5 can be understood from the fact that sample CP1 is fully alcohol terminated. Therefore, no additional separation according to end groups occurs.

Furthermore, it was observed that the exact shape and retention of the maximum of the elution pattern of CP1 can vary from injection to injection (Figure 6.3), whereas for amorphous polyesters it has been pointed out that reproducibility in GPEC is very good (Section 3.3.2). For sample CP2, which, chemically seen, strongly resembles CP1, qualitatively the same, anomalous behaviour was found.[5]

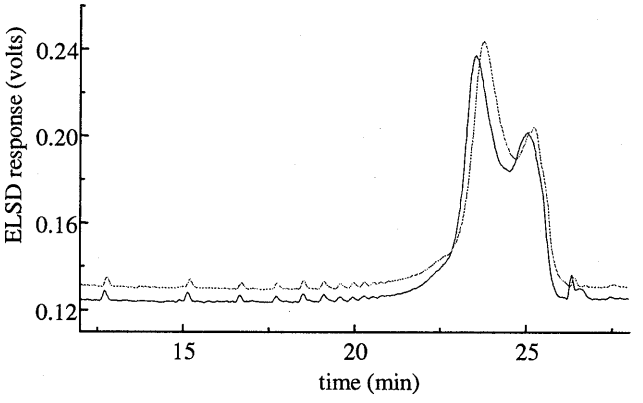


**Figure 6.3.** Typical example of non-reproducibility of sample CP1 in RP-GPEC at 25°C. GPEC conditions: see Figure 6.2.

All these observations lead to the conclusion that the chromatographic behaviour of the crystalline polyester under the chosen conditions for GPEC, is rather uncontrolled and obviously different from that of amorphous resins. No relevant information on polymer composition can be obtained from such results.

Two possible explanations for this phenomenon were considered. Firstly, the observed results may be due to the formation of a crystalline phase at the top of the column, giving rise to a different, much more time-dependent redissolution behaviour as compared to

amorphous polyesters. Secondly, due to their strong aliphatic character, the polyesters may exhibit a higher affinity towards the aliphatic, octadecyl ($C_{18}$) stationary phase which possibly leads to stronger partitioning effects. The resulting adsorptive interactions might therefore be different from that of the amorphous polyesters, thus resulting in a different chromatographic behaviour.

### 6.4.2 Chromatographic behaviour on various column types

The validity of the hypothesis of stronger adsorptive interactions between the aliphatic crystalline polyesters and the $C_{18}$ stationary phase was tested by the comparison of PE5 and CP1 on a $C_{18}$, a cyanopropyl (CN) and a bare silica column. In Figure 6.4A, the results for PE5 are shown. It can be seen that the use of a CN column results in a shift towards lower retention times. It is generally known that for separations performed in the reversed phase mode, a CN column is much less retentive as compared to $C_{18}$, due to its lower hydrophobicity.[6] Since it was shown in Chapter 3 that the separation of amorphous polyesters in RP-GPEC is mainly dominated by sorption effects, the observed shift may not be surprising. The use of a silica column leads to a complete distortion of the oligomer separation. Under the conditions chosen, the active silanol groups will be masked to a large extent by water and acetic acid. Therefore, only minor retention, probably caused by residual sorption effects, can occur, which was already shown in Chapter 3. Under such conditions almost no separation into distinct oligomers was obtained, which is in agreement with the current results.
In Figure 6.4B, the results of CP1 are shown. Although only few oligomers can be observed, it seems obvious that for the low molar mass region the same conclusions as for PE5, are valid. On the CN column, the oligomers shift to lower retention times, whereas for the silica column no oligomers can be observed. The high retention time part of the chromatograms, however, is roughly comparable for all three columns. Although the exact elution patterns differ to some extent, the changes as compared to PE5 are much less, indicating that in all three cases, the retention mechanisms are roughly alike. This indicates that the observed effects are not caused by strong interactions with, for instance, the $C_{18}$ chains. Furthermore, sorption effects obviously play a minor role in the separation of crystalline polyesters under the conditions chosen, suggesting that the separation mechanism is governed by redissolution effects. The correspondence of elution patterns on columns differing widely in polarity, providing evidence of a separation mechanism governed by redissolution effects, has been reported earlier by Glöckner.[7]

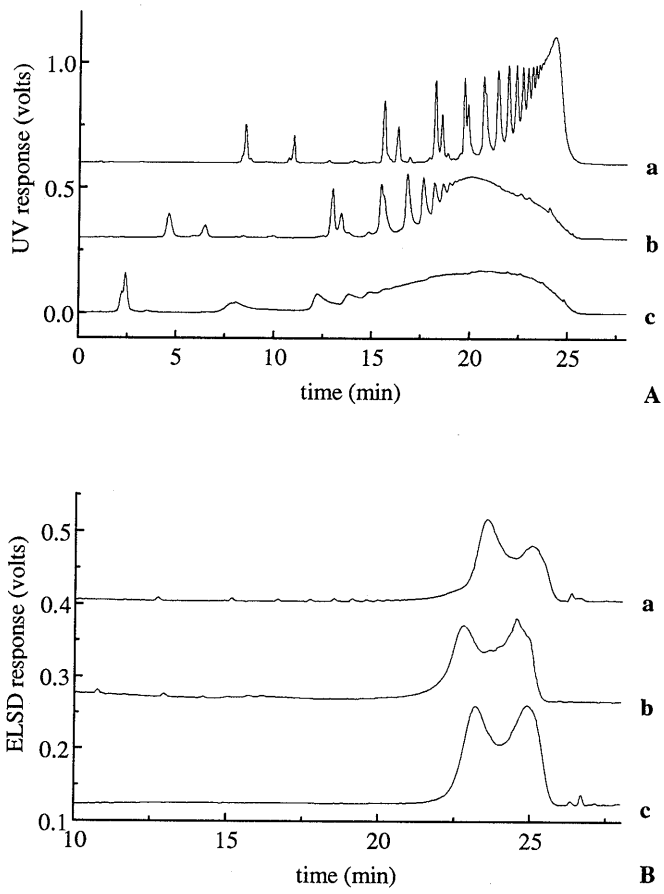


**Figure 6.4.** Retention behaviour of PE5 (A) and CP1 (B) in RP-GPEC on different columns. a: Novapak $C_{18}$, b: Novapak CN, c: Resolve silica, detection: PE5: UV at 277 nm, CP1: ELSD. Other GPEC conditions: see Figure 6.2.

### 6.4.3 Study of the crystallisation behaviour by DSC and microscopy

The possibility of crystallisation during the precipitation step was further investigated using DSC and microscopy. To this end, several precipitates of sample CP1 were prepared. After dissolving a certain amount in THF at room temperature, water was subsequently added such that the final water-THF ratio was 70:30 (v/v) which is the same as the starting conditions of the applied gradients. The actual concentrations under chromatographic conditions after precipitation at the top of the column, are unknown. Although some dissolution as compared to the initial concentration will occur, the actual concentration of a polymer precipitate may be quite high. Therefore, the amounts of the polyester were taken such that after precipitation the average concentration was 1 or 5

mg/ml. After filtration of the solution over a glass filter, parts of the residues were subjected to DSC and microscopy experiments.
By polarisation microscopy, a clear crystalline phase could be observed in both precipitates, which is illustrated in Figure 6.5. From DSC thermograms of both the pure polyester and the precipitates, distinct endothermic transitions due to melting of the polyester were found as can be seen from Figure 6.6. For the precipitate, the transition is seriously broadened towards a temperature of about 30 °C (6B), whereas for the pure polyester melting starts at about 55 °C (6A). The maximum of the melting curve of the precipitate is hardly affected and, just as for the pure polyester, is found to be at approximately 70 °C. The broadening of the melting curve is caused by melting point depression due to the presence of water and THF.[8]

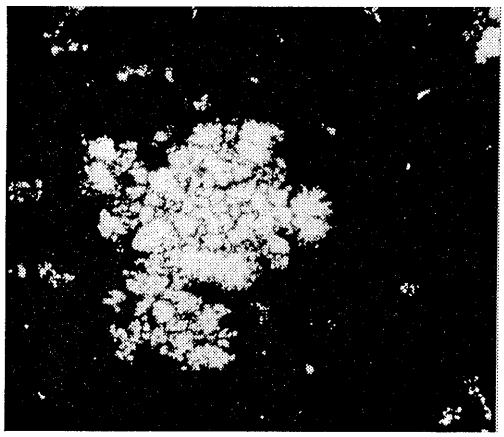

**Figure 6.5.** Polarisation microscopy picture of CP1 after precipitation in water-THF (30:70, v/v). Final (average) concentration: 5 mg/ml.

From both microscopy and DSC experiments it can clearly be concluded that crystallisation of the investigated polyester in the presence of a water-THF mixture can occur. From this point of view, the differences in chromatographic behaviour between PE5 and the crystalline polyesters can probably be explained. In the case of crystalline polyesters, the L-L demixing, caused by the injection of the sample into a non-solvent rich environment is followed by crystallisation, due to intersection with the L-S transition (Figure 6.1). For amorphous polyesters, this does not occur, since the glass transition temperature ($T_g$) is much more affected by the presence of non-solvent/solvent than $T_m$. Already small amounts of solvent can dramatically lower $T_g$.[9] Therefore, the intersection of $T_g$ with L-L demixing occurs at temperatures far below the temperature of operation, thus preventing the formation of true solid phase. At present it is not

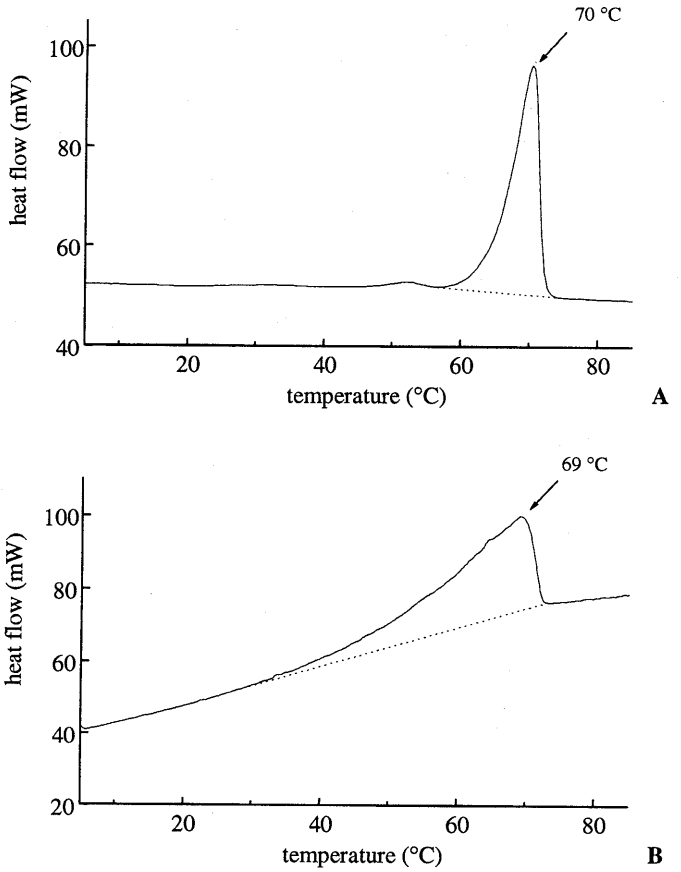


**Figure 6.6.** DSC thermograms of pure polyester CP1 (A) and the precipitate (B). DSC conditions: see Section 6.3.3.

completely clear, whether the anomalous chromatographic results for crystalline polyesters are due to the formation of a solid phase as such or to the fact that this solid phase is crystalline. The concept presented here, *i.e.* the effect of precipitated polymer morphology in GPEC, is new and will later on in this Chapter be shown to be useful to explain the effects of various practical parameters on the retention behaviour of crystalline polyesters.

### 6.4.4 Investigation of GPEC fractions by SEC

It is interesting to further study the elution behaviour of sample CP1, to see whether, for instance, only a part of the sample crystallises, thus giving rise to an extra peak causing

bimodality for the total elution pattern. Therefore, the polyester was separated into ten, equally spaced, fractions between 21.5 and 25.75 min according to the GPEC separation shown in Figure 6.2. The resulting amounts from 10 injections were dried under nitrogen and redissolved into 300 µl THF, from which 100 µl was injected on a SEC system. Reproducibility between the 10 GPEC runs was of the same order as shown in Figure 6.3. In Figure 6.7, for a few fractions the SEC chromatograms are shown. An increasing fraction number corresponds to an increasing retention time in GPEC.
It can be seen that the average molar mass gradually increases with increasing fraction number. However, no strict separation into low dispersity fractions that only slightly overlap each other, occurs. This would have been expected in the case of sorption governing the separation, since it is well known that in the case of reversed phase chromatography of oligomeric series, the separation is mainly governed by molar volume.[10,11] This can also be seen from the GPEC chromatogram of PE5 in Figure 6.2.

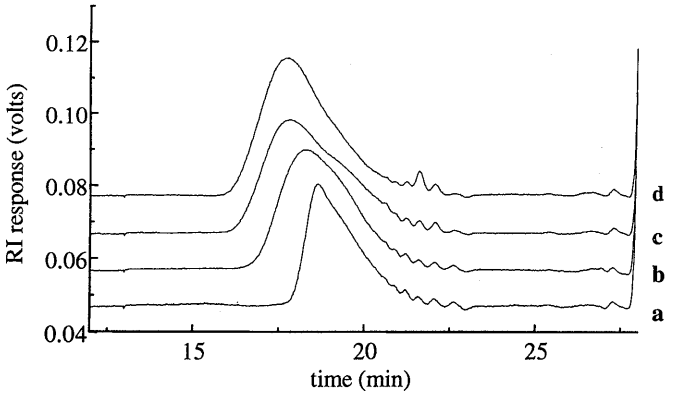


**Figure 6.7.** SEC chromatograms of several fractions of sample CP1, obtained by GPEC. Fractionation conditions: see text. Fractions and corresponding retention times in GPEC (Figure 3): a: fraction-2: 21.93-22.35 min, b: fraction-4: 22.78-23.20 min, c: fraction-7: 24.05-24.48 min, d: fraction-10: 25.33-25.75 min. SEC conditions: see Figure 6.2B.

Furthermore, in all fractions the appearance of oligomers can be observed, although the oligomer part of the GPEC chromatogram (13-21 min) was not collected during the fractionation. From these results, it can be concluded, that elution over the entire (investigated) part of the chromatogram is governed by redissolution effects rather than sorption effects. No evidence is found for the assumption that the first of the two main peaks of the bimodal distribution is caused by the elution of that part of the sample that did not crystallise during the precipitation. The explanation for the bimodally shaped elution pattern therefore remains rather vague. The observed, slight molar mass

dependence of the elution can probably be understood from the fact that the melting point depression is more pronounced for the low molar mass parts of the polyester (Eq. (6.1)). Therefore, with increasing thermodynamic solvent quality, resulting in a further increase of the melting point depression (Eq. (6.1)), the point at which the melting point drops below the analysis temperature is reached sooner for low molar masses. These fractions will start eluting earlier during the analysis, thus resulting into a relative enrichment of low molar mass fragments in the early eluting fractions. The fact that oligomer fractions are present in the later eluting fractions as well as in the early part of the chromatogram where distinct oligomers can be observed, can be explained as follows. During precipitation, a L-L demixing into a polymer-rich and a polymer-poor phase occurs, according to Figure 6.1. It is well known for polydisperse polymers that a precipitation is accompanied by fractionation.[12] Therefore, the polymer-poor phase will be highly enriched by low molar mass fragments, for which the melting point depression is more pronounced (Eq. (6.1)). Presumably, for this phase, no crystallisation takes place and normal retention due to sorption can occur thus resulting in normal elution, which is represented by the oligomer part of the chromatogram. The polymer rich phase, however, also contains low molar mass parts. Due to crystallisation, these parts will probably be encapsulated in the crystalline phase. Consequently, these oligomers are not accessible for the mobile phase anymore and will only start eluting when the solvent quality of the eluent has increased sufficiently to lower the melting point of the high molar mass part below the environmental (column) temperature. The encapsulation effect is very sample size dependent, as will be pointed out later on.

### 6.4.5 Study of the elution behaviour by variation of chromatographic parameters

From the results described above, it is clear that for crystalline polyesters redissolution effects are dominant in the separation under the conditions studied. Therefore, it is worthwhile checking the chromatographic parameters that can affect precipitation or redissolution, in order to find out whether a normal and reproducible behaviour under GPEC conditions can be obtained. Furthermore, by studying the effect of changing chromatographic parameters, chromatography can be used to study its own underlying separation mechanisms. For these experiments, again sample CP1 was used, although the conclusions were found to be valid for CP2 as well.[5]

#### *6.4.5.1 Injection volume and sample load*

At first, the effects of varying the injection volume and the injected mass were tested. An increase in the injection volume from 1 to 15 µl, while keeping the injected mass of CP1

constant to 100 µg, gives rise to increasing peak heights for the oligomers, whereas the average of the distribution shifts to lower retention times (Figure 6.8). The end of the distribution, however, only slightly shifts to a lower value of %-solvent (%-S), as is shown in Table 6.2, where the peak end for CP1 as function of various system parameters is given. All peak ends were determined from the point of intersection of the tangent to the chromatogram with the base line. Furthermore, no unimodal distribution is obtained in any case. The observed differences all exceed variations that might be due to the relatively non-reproducible chromatographic behaviour, which was confirmed by duplicate injections. The results indicate that redissolution effects remain dominant in the

**Table 6.2.** Effect of various chromatographic parameters on %-solvent at the peak end of sample CP1

| Parameter | Magnitude | %-S at peak end* |
|---|---|---|
| Injection volume (µl) | 1 | 91.7 ± 0.2** |
| | 5 | 91.5 |
| | 10 | 91.1 |
| | 15 | 90.5 |
| Injected mass (µg) | 25 | 92.1 |
| | 50 | 92.0 |
| | 100 | 92.8 |
| | 200 | 93.1 |
| | 400 | 93.6 |
| Gradient steepness (%/min) | 1.5 | 90.7 |
| | 3.0 | 90.5 |
| | 6.0 | 91.5 |
| Gradient steepness (%/min)/ flow-rate (ml/min) | 0.9/0.3 | 88.6 |
| | 1.5/0.5 | 90.0 |
| | 3.0/1.0 | 92.7 |
| | 4.5/1.5 | 93.1 |
| Gradient steepness (%/min)/ flow-rate (ml/min) after injection at 1.0 ml/min | 0.9/0.3 | 93.0 |
| | 1.5/0.5 | 93.7 |
| | 3.0/1.0 | 94.6 |
| Precipitation medium | Analytical column | 93.8 |
| | Guard column | 91.9 |
| | Pre-column filter + guard | 91.8 |
| Initial conditions NS/S | 90:10 | 92.1 |
| | 70:30 | 92.2 |
| | 50:50 | 92.8 |

*: peak ends determined by tangent method.
**: maximum deviation between average and maximum value of duplicate measurements ($dev_{max}$).

behaviour of, at least, the fractions eluting later. The injection of the same amount of mass into a smaller volume probably leads to the formation of a more compact crystalline phase for which time dependency of redissolution is more pronounced. This could explain the shift of the distribution average towards higher elution times.
Due to the increasing concentrations at lower injection volumes, the amount of the crystalline, polymer-rich phase as compared to the polymer-poor phase, which in a phase diagram is determined by the lever rule, increases. This explains the lower intensity of the oligomer peaks. In the case of amorphous polyesters, the increase in injection volumes in the studied range, hardly influences the separation (Section 3.3.1.5). A further increase would lead to peak broadening due to sample-solvent effects, since THF as the polymer solvent, is much stronger than the initial eluent composition. Therefore, although it seems that larger injection volumes favour the separation of crystalline polyesters, within the range of injection volumes that can be practically applied it is not possible to obtain normal chromatographic elution behaviour governed by sorption.

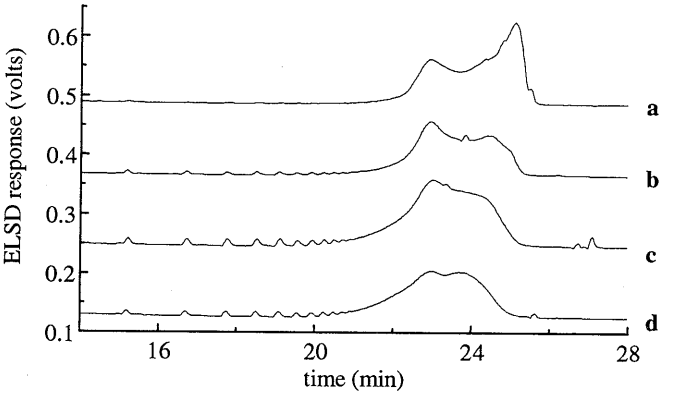


**Figure 6.8.** Effect of injection volume of CP1 on the elution behaviour. a: 1µl, 100 mg/ml, b: 5 µl, 20 mg/ml, c: 10 µl, 10 mg/ml, d: 15 µl, 6.67 mg/ml. GPEC conditions: see Figure 6.2A.

Decreasing the injected mass from 400 µg to 25 µg while keeping the injection volume constant at 5 µl causes the ratio of both peaks of the bimodal distribution to shift in favour of the early eluting peak, which is shown in Figure 6.9. Obviously, the elution behaviour is influenced by the sample load, whereas in the case of amorphous resins, for the investigated range no effect was observed (Section 3.3.1.5). As in the case for varying injection volumes, the end of the distribution only slightly shifts, which can also be seen in Table 6.2 and bimodal distributions were found in all cases. Therefore, it appears that

by lowering the injected mass, no normal elution behaviour can be achieved although a lower sample load seems to favour the final separation result slightly.
The effect of the injected mass differs from results of other workers, who investigated this case for amorphous polymers where separation was also dominated by precipitation/redissolution.[13,14] From their experiments, a gradual increase in the distribution maximum to higher retention times with increasing sample load was observed, which was due to the limited solubility capacity of the mobile phase. In the case of crystalline polyesters, however, the elution pattern seems to be determined by the melting point of the respective molar mass fractions which gradually drops below the environmental temperature due to the increasing thermodynamic quality of the eluent, rather than by the solubility in the NS/S mixture. Therefore, a less pronounced dependence between injected mass and elution time is found, although a slight shift of the end of the distribution is observed, which may be due to time-dependency of redissolution effects.

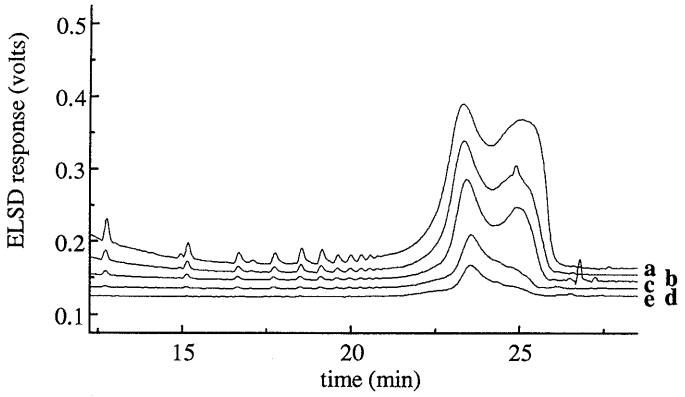


**Figure 6.9.** Effect of injected mass of sample CP1 on the elution behaviour. a: 400 µg, b: 200 µg, c: 100 µg, d: 50 µg, e: 25 µg. Samples dissolved in THF, injection volume: 5 µl. GPEC conditions: see Figure 6.2A

### *6.4.5.2 Gradient steepness and flow rate*

Since redissolution of crystalline material may exhibit a pronounced time-dependency thus possibly causing anomalous chromatographic results, it is interesting to check the effect of gradient steepness on the elution behaviour. Therefore, gradients of 1.5, 3 and 6%/min were applied at a constant flow rate of 1.0 ml/min. From these experiments, only a very slight shift of the end of the distribution towards lower %-S for decreasing gradient steepness was found, which can be seen from Table 6.2. Furthermore, the ratio

of both main peaks moderately changes in favour of the early eluting peak.[5] The effect of gradient steepness is therefore rather limited and seems different from that of amorphous resins. The change in peak ratio may indicate time dependent, kinetic effects, influencing redissolution. For amorphous resins, however, a more pronounced shift in peak end, expressed in %-S, is found with decreasing gradient steepness both in the case of sorption dominating the separation (see for instance Figure 3.1) as well as a separation governed by redissolution (Figure 3.12). Thus it seems that, although kinetic effects slightly influence the total separation, the redissolution behaviour of crystalline polyesters is much more governed by thermodynamics, determining at which %-S the melting point, due to increasing depression, drops below the environmental temperature. Only this can explain the relative independence of gradient steepness. This hypothetical conclusion would also confirm the results of above described experiments where a relative independence of elution time with varying injected mass was found.
A simultaneous decrease in both flow rate and gradient steepness results in an increase in the ratio in favour of the second peak (picture not shown[5]) and in a relatively large decrease in %-S at the end of the distribution (Table 6.2). These results may seem to contradict the findings described above, but it was considered that a decreasing flow rate may also affect the morphology of the precipitate, which has already been shown to influence the chromatographic behaviour. Thus, experiments were carried out in which the sample was injected at a constant flow rate of 1.0 ml/min. After 0.5 min, the flow was rapidly changed to the desired value and the gradient was started. By applying this experimental set-up, the effect of a simultaneous decrease in flow rate and gradient steepness on redissolution effects could be studied separately from the altered morphology of the precipitate due to a changed flow rate, which will also influence redissolution. The results of these experiments were qualitatively comparable to those, in which only gradient steepness was varied (picture not shown[5]), which can also be observed from the relative invariance of the peak end, as illustrated in Table 6.2. This again emphasises the relative independence of the results of experimental variables, probably caused by the dominance of thermodynamic effects. On the other hand, the morphology of the precipitate, which can obviously be influenced by the flow rate at the moment of injection, also affects the elution behaviour. This again indicates that the redissolution process is also influenced by kinetic effects, although to a lesser extent than by thermodynamic effects.

#### *6.4.5.3 Precipitation medium*

According to these results, it would also be interesting to test whether the chromatographic result could be affected by changing the precipitation medium, in order to influence the precipitation process. It has been claimed that precipitation in GPEC can

be affected by using a guard column with a special flow distributor,[15] which causes the precipitate to be distributed over a large area at the top of the column. In relation, it has also been shown that by influencing the mixing process between the injection solvent and the eluent, the chromatographic behaviour can be seriously affected.[16] Therefore, experiments were performed (1) with only a column, (2) with this type of guard column added to the system, and (3) with both a pre-column filter and a guard column added. Results are shown in Figure 6.10.

In all three cases the elution of the oligomers is hardly influenced which could be expected since this part of the chromatogram represents normal elution behaviour, as has been shown earlier. In contrast, the main distribution is seriously affected. Especially when no guard column or filter is used, the elution pattern completely changes and is broadened towards higher %-S, which can also be observed from Table 6.2. By adding a pre-column filter to the column plus guard column, the ratio of both main peaks changes and the distribution further shifts to lower %-S, the cause of which is not completely clear. From these experiments, it is again shown that by altering the precipitation medium, and therefore also the total precipitation process, the chromatographic result of crystalline polyesters can be influenced, although still no normal elution behaviour is obtained. For amorphous polyesters, no effect of the precipitation medium was found.[5] Therefore, it is obvious that precipitation and redissolution in RP-GPEC for both types of polyesters is different.

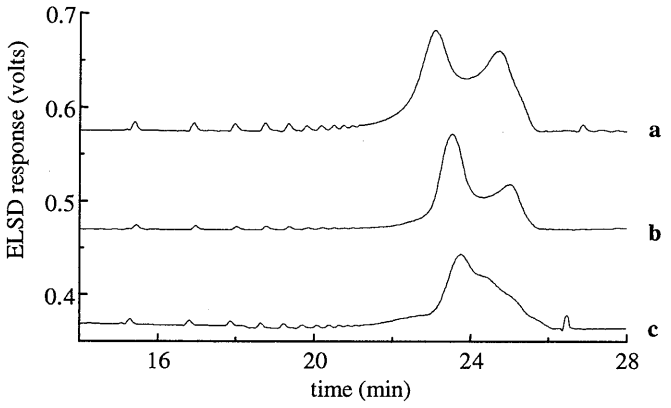


**Figure 6.10.** Effect of precipitation medium on the elution behaviour of sample CP1. a: pre-column filter + guard column + analytical column, b: guard column + analytical column, c: only analytical column. GPEC conditions: see Figure 6.2A.

### *6.4.5.4 Starting conditions of the gradient*

From the previous results it has become clear that the chromatographic behaviour and especially the precipitation and redissolution process of crystalline polyesters is influenced by both thermodynamic and kinetic effects. By affecting the latter effects, the separation result can only slightly be modified. Therefore, it was tried to influence the separation in a thermodynamic way. From Eq. (6.1), it appears that melting point depression increases with increasing quality of the solvent. Therefore, experiments were performed with different initial NS/S compositions of the gradient, while keeping gradient steepness constant. Results can be found in Figure 6.11. With decreasing initial NS/S ratio, the intensity of the oligomer peaks increases, just like the total peak area. The former observation can be understood from the shift in the phase diagram (Figure 6.1) towards lower temperatures at a lower NS/S ratio. This causes the volume fraction of the polymer poor phase, which is formed at the precipitation step, to increase as compared to the polymer rich phase. Since the former phase is enriched with oligomer fractions, the total amount of these fractions, which were already shown to exhibit normal elution behaviour, will also increase. The increase in the total peak area indicates that at higher NS/S ratio probably parts of the sample remain on the column. This can only be explained by time-dependency of redissolution which will be different at varying initial conditions due to the formation of another morphology of the precipitate. This observation was confirmed by blank runs after a sample injection, which in some cases indeed showed peaks of eluting polyester.

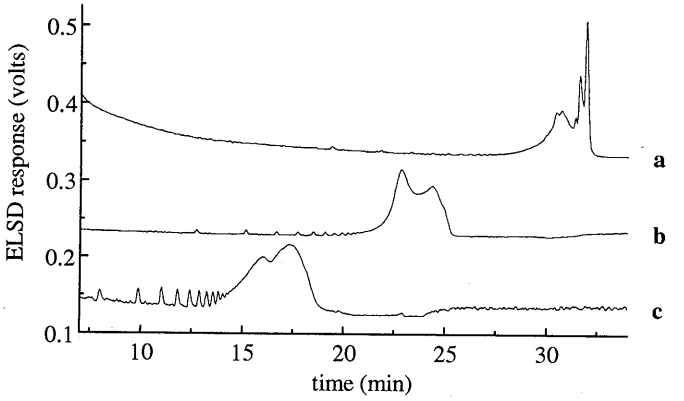


**Figure 6.11.** Elution behaviour of sample CP1 at various initial gradient conditions. Initial conditions water-THF: a: (90:10, v/v), b: (70:30), c: (50:50), gradient steepness: 3%/min to 100% THF. Other GPEC conditions: see Figure 6.2A.

Furthermore, at a lower initial NS/S ratio the distribution broadens towards lower %-S, indicating a gradual shift towards a normal elution behaviour. Due to the increasing melting point depression at a lower NS/S ratio, the melting point of a larger fraction of the polyester will be higher than the system temperature, giving rise to normal elution behaviour governed by sorption effects. To this end, it must be remembered that melting point depression depends on molar mass, according to Eq. (6.1).
The distribution end is hardly influenced by changing the NS/S ratio (Table 6.2). Furthermore, the bimodality of the distribution does not completely disappear, even at initial conditions of 50/50 NS/S. Therefore, it is obvious that also under these conditions, elution of at least part of the polyester is determined by redissolution effects caused by crystallisation after precipitation. Nevertheless, the elution of crystalline polyesters is clearly favoured by taking the solvent quality at the initial conditions thermodynamically seen as good as possible. Furthermore, it is obvious from the present results, that influencing thermodynamics of the total chromatographic process has a much more pronounced effect as compared to changing kinetic parameters.
For amorphous polyesters, elution is hardly influenced by changing initial gradient conditions.[5] Only when conditions are chosen such that initial retention factors of the early eluting species are low, changes in resolution or selectivity can occur, which is in agreement with predictions from reversed phase theories.[17]

#### *6.4.5.5 Temperature effects*

Based on these results, the next step is to check the effect of column temperature on elution behaviour. Results for sample CP1 are shown in Figure 6.12, whereas this evaluation already has been performed for amorphous polyesters (Section 3.3.1.4).
Temperature dramatically influences the elution behaviour of the crystalline polyesters. Especially between 25 °C and 35 °C, the elution pattern gradually changes to a normal, unimodal distribution (see also Figure 6.13). Obviously, the depressed melting points of the respective polyester fractions lie in this range. This is roughly in agreement with DSC experiments, from which it was shown that the melting curve of CP1 in a water-THF mixture starts at about 30 °C (Figure 6.6). It must be mentioned that DSC measurements were carried out under incomparable conditions. Therefore, an exact comparison of results from DSC and GPEC, is difficult.
At higher temperatures, no crystallisation takes place anymore, thus giving rise to normal elution behaviour, most probably governed by sorption, just as it has been found for amorphous polyesters. This effect becomes even more clear by comparing the %-S at the elution time of one specific oligomer and at the distribution ends for both samples CP1 and PE5 as a function of temperature. (Figure 6.13).

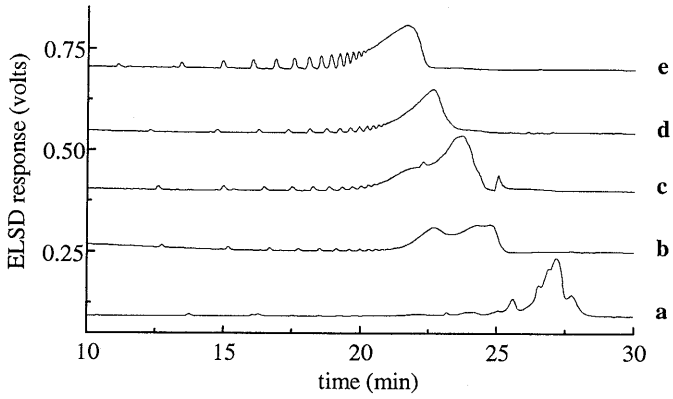


**Figure 6.12.** Effect of temperature on the elution behaviour of sample CP1. a: 16°C, b: 27°C, c: 29°C, d: 31°C, e: 50°C. GPEC conditions: see Figure 6.2A.

As can be seen, for the two oligomers originating from CP1 and PE5, retention time gradually decreases with increasing temperature, which can be explained from decreasing sorption effects. This further indicates that the oligomer part of the chromatogram of CP1 represents normal elution behaviour at all temperatures. In contrast the distribution end of CP1 up to a temperature of about 35 °C is much more affected by a raise in temperature than that of PE5. For temperatures exceeding 35 °C, qualitatively the same gradual decrease in retention time is found. This again shows the anomalous elution behaviour due to crystallisation effects up to a temperature of 35 °C. At higher temperatures, obviously no additional redissolution effects influence the retention behaviour anymore.

For sample CP2, qualitatively the same results were found, although the temperature above which no crystallisation effects were observed anymore, is approximately 5 - 10 °C higher. This is in accordance with the fact that the melting curve of pure CP2 as compared to CP1 also lies about 10 °C higher. Furthermore, for a sample with the same chemical composition as CP1 but a polystyrene equivalent molar mass of 3200 instead of 16000, a distinctly lower temperature was found above which no crystallisation effects were observed. This can be attributed to the lower melting temperature of the pure polyester and of the increased melting point depression due to its lower molar mass (Eq. (6.1)).

Consequently, to ensure normal elution behaviour of crystalline polyesters in GPEC, system temperature has to be higher than the depressed melting temperature at the initial conditions of the gradient. It has to be mentioned here, that during later experiments, especially when using a more sensitive ELSD, sometimes small peaks eluting after the main distribution of crystalline polyesters were observed, even at high temperatures.

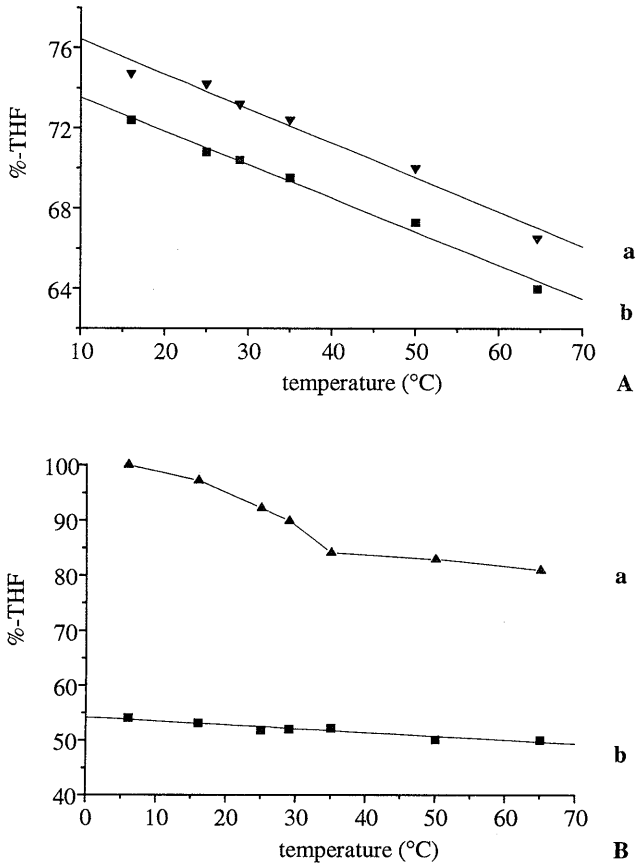


**Figure 6.13.** Dependence between %-THF at the elution of oligomers (A) and distribution ends (B), respectively, and temperature. A: oligomers: a: CP1, molar mass = 2078, b: PE5, molar mass = 2050, B: distribution ends: a: CP1, b: PE5. GPEC conditions: see Figure 6.2A.

Presumably, these peaks are caused by crystallisation of small parts of the sample in the (non-thermostated) autosampler and in the tubing from injector to the column. Therefore, it is probably best to use a system with a thermostated injector.

Although the retention mechanism of polyesters on normal phase systems is different from that on reversed phase systems, as will be shown in the next Chapter, similar differences between amorphous and crystalline polyesters occur in NP-GPEC. In a heptane/THF system, the elution behaviour of CP1 was found to be highly irreproducible and strongly dependent on experimental conditions at temperatures below 35 °C, whereas this is not the case for PE5. Above this temperature, normal elution patterns were observed. Obviously, this typical behaviour of crystalline polyesters can also be found in other phase systems, as might be anticipated from its underlying mechanism.

### 6.4.6 Separation of polyester blends

It was considered that the difference in redissolution behaviour between amorphous and crystalline polyesters may be used to separate blends of both types of resins. At temperatures exceeding 40 °C, both distributions would completely overlap in RP-GPEC. Therefore no information on, for instance, the respective molar mass distributions could be obtained. If the analysis would be run, however, at a low temperature, the amorphous resin would be completely eluted, whereas the crystalline resins would remain on the column. The latter product then could be eluted later, at a higher temperature. This concept was tested for mixtures of PE5 and CP1, and PE5 and CP2 respectively. After running the first gradient at a low temperature, the system was returned to initial conditions and temperature was programmed to 40 °C, followed by equilibration and a subsequent second gradient. Temperatures, lower than 6 °C could not be applied due to excessive back pressure. It was found, that both lowering the injection volume and %-S at initial gradient conditions favour the separation of the blend, since the formation of the crystalline phase in the first step is enhanced. This is in accordance with results discussed above, where, in contrast, it was tried to suppress the formation of a crystalline phase. Although only an injection volume of 1 µl was applied and starting conditions were taken at 10% solvent, the separation of PE5 and CP1 was not completely reproducible and part of the crystalline resin eluted in the first gradient, which is shown in Figure 6.14A. Corresponding gradient conditions are given in Table 6.3. Nevertheless, separation is fairly good, as compared to SEC or RP-GPEC at higher temperatures. The separation of PE5 and CP2 was even better and except for a very small oligomer fraction, almost no crystalline material was found to elute in the first gradient, as can be seen from

**Table 6.3.** HPLC conditions of combined eluent and temperature programming experiments

| Time (min) | %-THF | Temperature (°C) | Flow (ml/min) | |
|---|---|---|---|---|
| 0 | 10 | 6 | 1 | |
| 30 | 100 | 6 | 1 | First run analysis |
| 40 | 100 | 6 | 1 | |
| 41 | 10 | 6 | 1 | |
| 55 | 10 | 6 | 1 | |
| 55.1 | 10 | 6 | 0.1 | Heating |
| 70 | 10 | 40 | 1 | |
| 100 | 100 | 40 | 1 | Second run analysis |
| 110 | 100 | 40 | 1 | |
| 111 | 10 | 40 | 1 | |
| 125 | 10 | 6 | 1 | |
| 125.1 | 10 | 6 | 0.1 | Cooling down |
| 190 | 10 | 6 | 0.1 | |

Figure 6.14B. The difference between CP1 and CP2 can probably be explained from the somewhat higher melting point of CP2, giving rise to a higher thermodynamic driving force causing crystallisation.
Thus, it is clearly shown that differences in redissolution behaviour between resins can be used for the separation of blends by combined temperature and eluent programming. This can not be performed in a single run at higher temperatures when elution is almost solely governed by sorption, at least not for reversed phase systems.

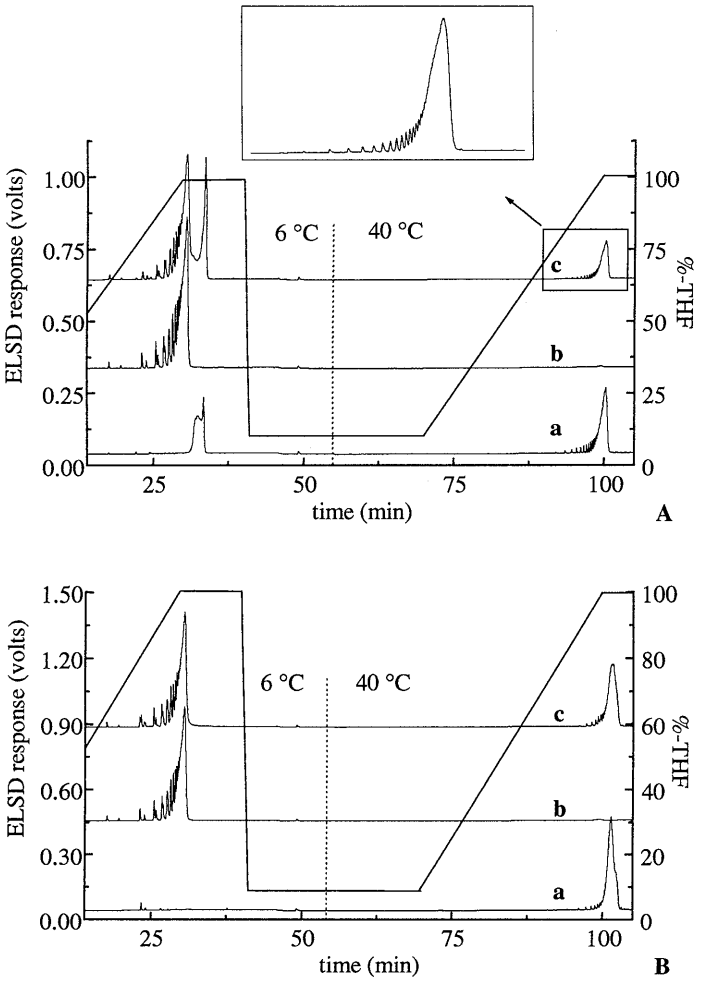


**Figure 6.14.** Separation of blends of amorphous and crystalline polyesters by combined temperature and eluent programming. A: CP1/PE5: a: CP1, b: PE5, c: blend (50:50, w/w), B: CP2/PE5: a:CP2, b: PE5, c: blend (50:50, w/w). GPEC conditions: sample concentration: 50 mg/ml (total concentration) in THF, column: Novapak $C_{18}$, injection: 1µl, detection: ELSD. Flow, gradient conditions and temperature: see Table 6.3.

## 6.5 CONCLUSIONS

In contrast to amorphous polyesters, crystalline polyesters exhibit non-reproducible chromatographic behaviour in GPEC below a certain temperature. In such case, chromatographic behaviour is governed by precipitation (crystallisation)/redissolution effects, rather than by sorption. This is due to the formation of a solid, crystalline phase after injection, which can be shown by DSC and microscopy experiments. This contrasts the situation for amorphous polymers where a swollen, polymer rich phase is formed, rather than a real solid phase.

By studying the effect of various chromatographic parameters, further information on the separation mechanism can be obtained. Varying the injection volume while keeping the injected mass constant, the flow rate at the moment of injection or the type of precipitation medium mainly affects the morphology of the precipitate. This gives rise to a different redissolution behaviour, which indicates that kinetic effects influence the separation result.

The effect of increasing sample load is more pronounced as compared to amorphous polyesters, but much less as compared to other polymers for which separation is also dominated by precipitation/redissolution. This, together with the fact that the effect of gradient steepness is also minor, leads to the conclusion that the redissolution behaviour of crystalline polyesters is mainly governed by thermodynamics. This determines at what %-S during the gradient, the melting point due to increasing melting point depression drops below the environmental temperature. Kinetic effects influence the separation to a lesser extent and by varying the parameters mentioned above, no significant improvement towards normal elution behaviour can be achieved.

A more pronounced effect is observed when influencing the thermodynamic conditions during the separation. Changing the initial gradient conditions towards a thermodynamically better eluent, significantly influences the elution behaviour due to increased melting point depression. Raising the temperature above a certain value leads to highly reproducible, normal elution behaviour, governed by sorption. Therefore, to ensure normal elution behaviour of crystalline polyesters in GPEC, system temperature has to be higher than the depressed melting temperature at the initial conditions of the gradient to prevent the formation of a solid, crystalline phase. The concept of precipitated polymer morphology influencing the elution profile in GPEC is new and provides new insights in the chromatographic behaviour of certain classes of polymers.

Finally, the difference in redissolution behaviour between amorphous and crystalline polyester resins can be used to separate blends of both types of resins by combined eluent and temperature programming. This kind of separation cannot be performed by SEC or by GPEC at a single temperature.

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# CHAPTER 7

# Normal Phase Gradient Polymer Elution Chromatography of Amorphous Polyesters. A Study to the Chromatographic Behaviour, Supported by Isocratic Experiments


## SUMMARY

*The retention behaviour of (co)polyesters by Normal Phase Gradient Polymer Elution Chromatography (NP-GPEC) was investigated on various stationary phase – mobile phase combinations. The separation was found to be dominated by end groups in most cases and to a lesser extent by molar mass and composition of the backbone. A distinct influence of both the stationary and the mobile phase on the separation with respect to end groups and molar mass was observed. Practical conditions such as temperature and gradient steepness were found to only moderately affect end group selectivity. The separation mechanism in all cases is governed by adsorption rather than by re-dissolution effects. Next to end group separations, NP-GPEC can be used for the characterisation of polyesters according to the composition of the backbone, independent of end groups.*

*Isocratic normal phase behaviour of polyesters can, in most cases be satisfactorily described by using the approach of Jandera et al. Results were shown to be useful for a further understanding of the retention behaviour in NP-GPEC. A refined adsorption model assuming two different types of adsorption sites yielded improved description of the experimental retention data.*



* Parts of this Chapter will be published:
H.J.A. Philipsen, H.A. Claessens, M. Bosman, B. Klumperman and A.L. German, *Chromatographia*, accepted.

## 7.1 INTRODUCTION

The principles of Normal Phase Liquid Chromatography (NPLC) on both classical adsorbents (silica, alumina) and bonded phases, including localisation effects (both site-competition and restricted access) and secondary solvent effects have been well documented over the years.[1-10] Due to the popularity of RPLC caused by the high separation power for a wide range of products and its practical versatility, NPLC has been somewhat out of scope during the last decade. Nevertheless, a distinct advantage of NPLC is that the selectivity can be modified by both the stationary and the mobile phase, whereas in RPLC the stationary phase only moderately affects selectivity.[11]
In polymer analysis, normal phase gradient elution chromatography, *e.g.* NP-GPEC, has mainly been used for the characterisation of copolymers according to chemical composition.[12-15] For this purpose, NP-GPEC is more widely applied than RP-GPEC, where, due to weak sorption forces, strong non-solvents have to be used in gradients. Due to the limited solubility of polymers, this causes precipitation effects and consequently an increased dependence of retention on molar mass.[16] Relatively little work has been done so far on the characterisation of low molar mass polymers by NP-GPEC.[17-23] A few papers demonstrated the possibilities of the technique to obtain a separation with respect to functionality.[19,21] A considerable part of this work mainly focussed on applications rather than mechanisms of polymer separations.
Jandera developed a model in which contributions of polymer chain segments and polymer end groups to *isocratic* retention can be distinguished and quantified and related to physical properties.[20-27] Using this model, observations regarding functionality separations under both isocratic *and* gradient conditions were explained. Although the model has been applied in both RP[22,24-27] and NP[20-23] systems, the use in NPLC has been limited to mainly ethoxylated alkylphenols until now.
In this Chapter, the possibilities of NP-GPEC for the characterisation of amorphous polyesters are investigated. For this purpose, two polyester samples were characterised under several conditions, using various mobile and stationary phases. The potentials and limitations of the technique to separate polyesters according to functionality and to characterise copolyesters according to their chemical composition distribution will be demonstrated. Furthermore, isocratic experiments were carried out for a homopolyester, the results of which were subjected to the retention model of Jandera. The conclusions from this part of the work are used for a better understanding of the NP-GPEC mechanisms.

## 7.2 THEORY

As was shown in Chapter 2, based on the Snyder model of adsorption[1] it was found that, within certain limits, the logarithm of the retention factor in NPLC linearly decreases with the logarithm of the fraction of the polar solvent, B (Eq. (2.30)), which will here be written as:

$$\log(k) = \log(k_s) - n\log(\varphi) = a - m\log(\varphi) \tag{7.1}$$

where a and m are constants that frequently have been found to linearly depend on the degree of polymerisation:

$$a = a_0 + a_1 p \qquad\qquad m = m_0 + m_1 p \tag{7.2a,b}$$

$a_0$, $a_1$, $m_0$, $m_1$ are constants depending on the structure of the oligomers, on the adsorbent (stationary phase) and on the solvents used in the mobile phase.[23] Combination of the Martin equation expressed as Eq. (5.4) with Eqs. (7.1-7.2) reveals:

$$\log(k) = \log(\beta) + p\log(\alpha) = a_0 - m_0\log(\varphi) + p\left(a_1 - m_1\log(\varphi)\right) \tag{7.3}$$

By Jandera *et al.* it was assumed that, to a first approximation, both the energy of adsorption, $Q_0$, and the adsorbed area, $A_S$, of the Snyder equation (Eq. (2.30)) increase linearly with p. Thus it was shown that the constants of Eq. (7.3) are related to the contributions to the retention factor in pure solvent A by the repeat unit, $\log(k_{a1})$, and by the end groups, $\log(k_{a0})$, and to the areas of the adsorbent occupied by a molecule of the polar solvent, $n_b$, by a repeat unit, $A_1$, and by the end groups, $A_0$:[22,23]

$$a_0 = \log(k_{a0}) - \alpha' A_0(\varepsilon_b - \varepsilon_a) \tag{7.4a}$$
$$a_1 = \log(k_{a1}) - \alpha' A_1(\varepsilon_b - \varepsilon_a) \tag{7.4b}$$
$$m_0 = A_0/n_b \tag{7.4c}$$
$$m_1 = A_1/n_b \tag{7.4d}$$

$A_0$ = adsorbed area of an end group ($m^2$)
$A_1$ = adsorbed area of a repeat unit ($m^2$)
$n_b$ = adsorbed area of a polar solvent molecule ($m^2$)
$k_{a0}$ = retention factor of an end group in pure, less polar solvent, A
$k_{a1}$ = retention factor of a repeat unit in pure, less polar solvent, A
$\alpha'$ = activity of the adsorbent (-)
$\varepsilon_a$ = solvent strength of the less polar solvent, A ($m^{-2}$)
$\varepsilon_b$ = solvent strength of the polar solvent, B ($m^{-2}$)

Here, $\varepsilon_b$ and $\varepsilon_a$ are the solvent strengths (polarities) of the more polar (B) and of the less polar (A) solvent, respectively, and $\alpha'$ is the activity of the adsorbent. $\log(k_{a0})$ and $\log(k_{a1})$ are proportional to the adsorption energies of the end groups and of the oligomer units, respectively, in the solvent A.

Hence, the retention in binary mobile phases increases with an increasing adsorption energy of the repeat units and of the end groups in pure solvent A, but it decreases if the adsorbed areas occupied by these groups increase. This is the case if the molecule contains bulky non-polar parts with a low affinity to the adsorbent, that can shield the access of other solute molecules to the neighbouring adsorption sites. This means that the retention in an oligomeric series increases with increasing degree of polymerisation ($\log(\alpha) > 0$) if the repeat structural units contribute significantly to the energy of adsorption. It can also decrease ($\log(\alpha) < 0$) if the repeat unit is large and has a low or moderate polarity, so that the effect of increasing molecular size in the series prevails over the weak contribution to the energy of adsorption of the repeat unit.[22] Thus, with an increasing amount of the polar solvent B in the mobile phase, the retention of all oligomers decreases, but the retention order within one series may invert causing decreasing retention with increasing p.

Therefore, it is likely that at a certain composition of the mobile phase the effect of the energy of adsorption and of the size of the repeat unit are exactly counterbalanced and all oligomers are coeluted. In such case a common intersection point for a series of plots of log(k) versus log(φ) for different oligomers will be found. The co-ordinates of this intersection point, as predicted by the Jandera model are:

$$\log(\varphi_{0,i}) = a_1/m_1 \tag{7.5}$$

$$\log(k_i) = a_0 - a_1 m_0/m_1 = a_0 - m_0 \log(\varphi_0) \tag{7.6}$$

Coelution is observed if $\varphi_0 < 1$ ($\log(\varphi_0) < 0$) and $a_1/m_1 < 0$.

As is described in Chapter 2, coelution of oligomers is often attributed to the counterbalance of size exclusion and adsorption and the conditions under which this occurs are called critical conditions.[28,29] Obviously, as shown above, coelution can also be predicted by adsorption phenomena alone. Although the definitions of coelution, following from both theories show qualitative resemblance, *i.e.* the counterbalance of steric and enthalpic effects, model discrimination has never been properly carried out yet.

The above described simple adsorption model fails if the increase of the adsorbed area with increasing size of the polymer molecules is nonlinear and the plots of log(k) versus p are not linear in such a case. Such nonlinear behaviour was found for polystyrene and polyester on reversed phase systems, as is described in Chapter 4 of this thesis. Nonlinear dependence of log(k) on p could also be observed if either size-

exclusion effects significantly affect the retention or if there is more than one single type of adsorption sites on the surface of the column packing.

## 7.3 EXPERIMENTAL

### 7.3.1 Polymer samples and low polydispersity fractions

The polymer samples used for GPEC and isocratic experiments, PE2, PE6 and PE7, were laboratory-made polyester resins. For detailed information on the characterisation and the composition of the polymer samples, it is referred to Section 3.2.2.
For isocratic experiments, low polydispersity fractions of homopolyester PE7 with known degree of polymerisation and known end group composition, were obtained by RP-GPEC. 14 µl of a 100 mg/ml solution was injected 20 times on an RP-GPEC system, using a Novapak $C_{18}$ column (150 x 3.9 mm) (Waters, Milford, MA, USA), at a temperature of 35 °C and a water-THF gradient from (65:35) (v/v) to (15:85) in 50 minutes. Oligomers that were fractionated and used for the isocratic measurements are indicated in Figure 7.1. Peak assignment in the RP-GPEC chromatogram as indicated has been described in detail in Chapter 3.

### 7.3.2 HPLC solvents, columns and equipment

The solvents used for HPLC were heptane (HEP), dichloromethane (DCM), chloroform ($CHCl_3$) ethylacetate (ETAC), methyl-*tert.*-butyl-ether (MTBE), acetonitrile (ACN), isopropanol (IPA) and methanol (MeOH), all Lichrosolv quality from Merck (Darmstadt, Germany) and tetrahydrofuran (THF), HPLC grade from Rathburn (Brunschwig Chemie, Amsterdam, The Netherlands). All solvents were dried overnight on molecular sieve, 0.3 nm (Merck) and filtered by vacuum filtration, prior to use. During use, solvents were constantly sparged with helium (20 ml/min). All solvent mixtures were made by volumetric mixing by the HPLC pump, no premixes were used.
The columns used were a Nucleosil-100-5 ('silica') column ($d_p$ = 5 µm, pore size 100 Å, 200 x 4.0 mm), a Nucleosil-100-5-$NH_2$ ('$NH_2$') column ($d_p$ = 5 µm, pore size 100 Å, 200 x 4.0 mm), a Nucleosil-100-5-$N(CH_3)_2$ ('DMA') column ($d_p$ = 5 µm, pore size 100 Å, 200 x 4.0 mm), a Nucleosil-100-5-CN ('CN') column ($d_p$ = 5 µm, pore size 100 Å, 200 x 4.0 mm), a Nucleosil-100-7-OH ('diol') column ($d_p$ = 7 µm, pore size 100 Å, 250 x 4.0 mm), all from Machery Nagel (Düren, Germany), a Jordi Gel divinylbenzene Polyamine ('PA') column ($d_p$ = 5 µm, pore size 500 Å 250 x 4.6 mm) from Jordi (Bellingham, MA, USA) of which several different columns were used for the experiments and a polyvinyl alcohol coated silica ('PVA') column ($d_p$ = 5 µm, pore size 120 Å, 250 x 4.6 mm) from

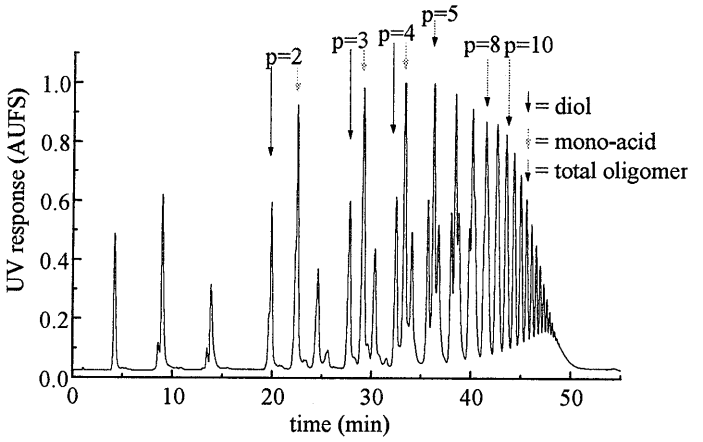


**Figure 7.1.** RP-GPEC fractionation of polyester PE7. Fractions were taken as indicated in Figure. Conditions: sample concentration: 100 mg/ml in THF, column: Novapak $C_{18}$ (150 x 3.9 mm), temperature: 35 °C, eluent: water-THF (both with 200 µl acetic acid per litre added) (65:35, v/v) to (15:85) in 50 minutes, flow: 1.0 ml/min, injection: 14 µl, detection: UV at 277 nm.

YMC (Kyoto, Japan). For all experiments a stainless steel in-line pre-column filter (Waters) was used.
HPLC equipment was identical to that, described in Section 3.2.4.

### 7.3.3 Experiment strategies

Gradient elution in NP-GPEC was performed as follows. After running each gradient, which mostly consisted of at least two steps, the system was stepwise reset to initial conditions. For instance, in the case that a gradient was run from solvent A, via solvent B and C to solvent D, the system was reset from D to A via C and B. All reset steps took one minute, followed by pumping 2 column volumes of the intermediate solvent. After the last step, 15 column volumes of the starting eluent composition were pumped to re-equilibrate the column. For the exact gradient conditions for each experiment, it is referred to the Figures and Table 7.1. For comparative experiments on various columns, the same batches of the different solvents were used, to eliminate variations caused by small differences in mobile phase composition such as water content. Prior to the analysis of the samples, two blank gradients were run. All gradients were started at the moment of injection. The system hold-up volume was 4.0 ml (see Section 3.2.5). A relative measure for the dead volume of each column was determined from the refractive index disturbance caused by the injection of 5 µl heptane in DCM as mobile phase. The

thus found values were used for the calculation of gradient elution programs to correct for differences in column dimensions (see Table 7.1). Unless indicated otherwise, all gradient elution measurements were carried out at 35 °C, using UV detection at 277 nm. Samples of 10 mg/ml in DCM were used, of which 10 µl was injected.
Prior to the isocratic experiments, the range in which measurable retention factors could be obtained was estimated from a gradient elution experiment in which the whole, unfractionated polyester was injected. Isocratic measurements were performed for two columns using three binary eluent combinations. For all experiments using a specific binary eluent combination, the same batch of solvents was used. For all isocratic measurements, 10 µl of solutions of 20 µg/ml in DCM of the low polydispersity fractions were injected in duplicate, using a flow rate of 2.5 ml/min. System equilibration was checked by repeated injection of a sample. After finishing measurements at one specific binary eluent composition, the column was flushed with 100% MeOH to ensure elution of the most polar fractions. For statistical reasons, isocratic eluent compositions and injected low polydispersity fraction were changed randomly. The column dead volume used for the determination of retention factors was 2.31 ml for the silica column and 2.67 ml for the PA column. Calculations of fits of log(k) versus Log(φ) and log(p) respectively, were performed using matrix least squares regression. For the calculation of fits of Eq. (7.7) (Section 7.4.6.3), nonlinear regression was applied.

## 7.4 RESULTS AND DISCUSSION

### 7.4.1 Separation of polyesters by NP-GPEC

In NPLC, separation can be modified by both the stationary and the mobile phase. Mobile phase types for NPLC can be divided into non-localising (for instance DCM, $CHCl_3$), basic localising (THF, MTBE), non-basic localising (Etac) and proton donor solvents (MeOH, IPA).[30] Each solvent type can provide its own typical type of interactions with solutes and sorbents, giving rise to different selectivities. The polyesters used in this study are only completely soluble in moderately polar solvents, such as THF, DCM, $CHCl_3$ and ETAC, whereas HEP, ACN, IPA and MeOH can be considered as non-solvents, except for the lowest molar mass oligomers.
The separation of polyesters PE2 and PE7 by NP-GPEC was investigated on various columns, using different eluent types as is shown in Figures 7.2 and 7.3. From these results it is clear that in NP-GPEC the separation largely depends on both stationary and mobile phase. This is in contrast with RP-GPEC, where column and eluent type only moderately influence the separation of polyesters.[31] In none of the cases of NP-GPEC, the high resolution of RP-GPEC is obtained (see Chapter 3). Nevertheless, NP-GPEC offers specific advantages over RP-GPEC as will become clear, later on.

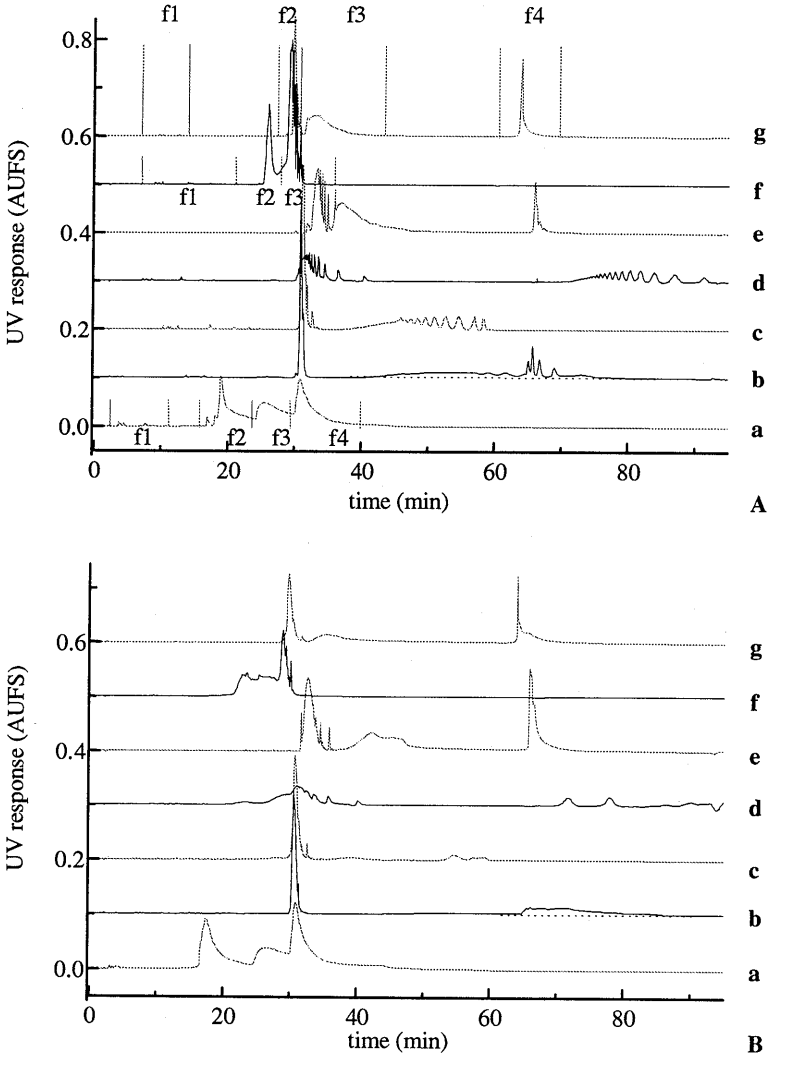


**Figure 7.2.** Normalised NP-GPEC chromatograms of samples PE7 (A) and PE2 (B) on various columns. a: CN, b: diol, c: $NH_2$, d: DMA, e: silica, f: PVA, g: PA. Gradient conditions and flow: see Table 7.1. Further conditions: see Section 7.3.3.

In most cases, the polyesters are separated into two or three main fractions. In order to verify their identity, sample PE7 was separated into its main fractions on a CN, a PVA and a PA column, as indicated in Figure 7.2, followed by RP-GPEC analysis of the resulting samples. In Figure 7.4, the results for the PA column are shown. The injection of the second fraction (f2, first 'main' fraction) mainly gives rise to the appearance of the first peaks of the triplets in RP-GPEC which are due to oligomers containing two alcoholic end groups (Chapter 3). Analogously, it can be concluded that fractions f3 and f4 belong to oligomers with one and two acidic end groups.

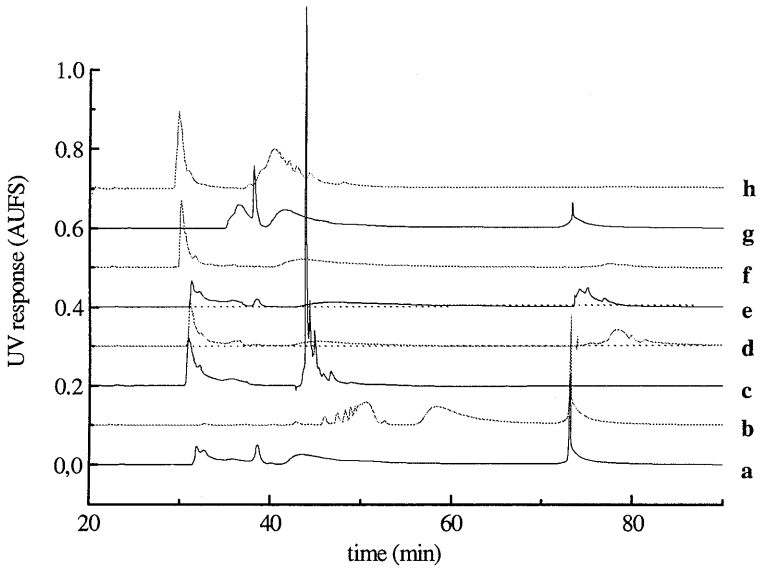


**Figure 7.3.** NP-GPEC separations of sample PE7 on a PA column, using various eluent combinations. a: HEP-DCM-THF-MeOH (100:0:0:0, v/v) to (0:100:0:0) (0 to 33.3 min), (0:100:0:0) to (0:0:100:0) (33.3 to 66.6 min), (0:0:100:0) to (0:0:0:100) (66.6 to 100 min), b: HEP-THF-MeOH (100:0:0) to (0:100:0) (0 to 66.6 min), (0:100:0) to (0:0:100) (66.6 to 100 min), c: HEP-DCM-MeOH: (100:0:0) to (0:100:0) (0 to 33.3 min), (0:100:0) to (0:0:100) (33.3 to 100 min), d: as a, THF replaced by MTBE, e: as a, THF replaced by ETAC, f: as a, THF replaced by ACN, g: as a, DCM replaced by $CHCl_3$, h: as a, MeOH replaced by IPA. Flow: 1.32 ml/min. Further conditions: see Section 7.3.3.

Qualitatively the same results were observed for the CN column. For the PVA column it was found that fraction f2 represents chains containing two alcoholic end groups, whereas in fraction f3 both parts containing one and two acidic end groups are eluted. The observed elution order is in accordance with the polarity, which increases for fractions containing more acidic end groups. The first, small, fraction from all three columns, f1, mainly consists of low molar mass oligomers (Figure 7.4). These fractions most likely represent cyclic and therefore relatively non-polar, products

which are formed due to a well known side reaction for polyesters, and which mainly have a low degree of polymerisation.[32]

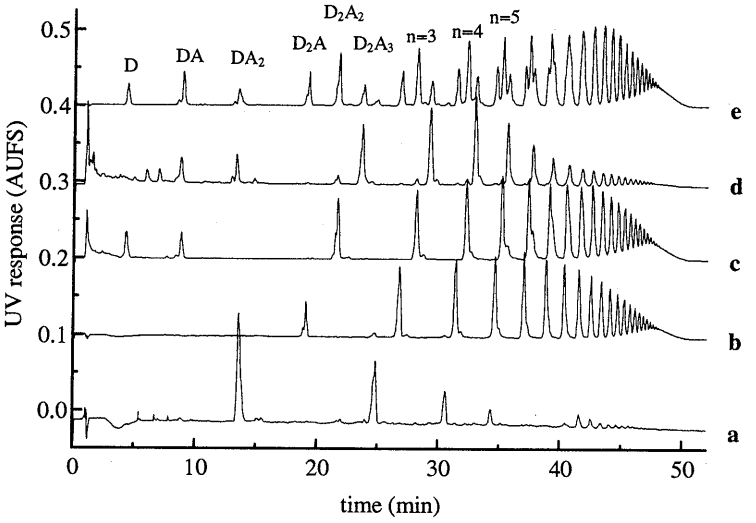


**Figure 7.4.** RP-GPEC chromatograms of fractions of sample PE7 taken from NP-GPEC on a PA column (see Figure 7.2). a: 1st fraction (f1, cyclics), b: 2nd fraction (f2, 1st main fraction, diol), c: 3rd fraction (f3, 2nd main fraction, mono-acid), d: 4th fraction (f4, 3rd main fraction, di-acid), e: unfractionated polyester. RP-GPEC conditions: see Figure 7.1. D = diol, A = adipic acid.

The various end group fractions are not completely separated as can be seen from Figures 7.2-7.4. From a comparison of curves d and e of Figure 7.4 for instance, it can be observed that the fraction with two acidic end groups also contains low molar mass products of the fraction with one acidic end group. Therefore, next to end groups, NP-GPEC separations are affected by p, which is also obvious from the oligomer peaks that can be distinguished in Figures 7.2 and 7.3.

These results clearly contrast the situation for RP-GPEC. Where in this mode separation is dominated by molar mass and to a lesser extent by chemical composition (Chapter 3), for NP-GPEC the situation is reversed. Separation of polyesters is clearly governed by end groups, whereas molar mass plays a less important role.

### 7.4.2 Column influences in NP-GPEC

To further elucidate the role of the stationary phase in NP-GPEC of polyesters, various column types were compared for samples PE2 and PE7. To this end a gradient from HEP to THF via DCM as intermediate solvent was used, since various experiments showed that this provides the best separations with respect to end groups. Furthermore a gradient from THF to MeOH was added to ensure complete elution of all polar substances. Since the investigated columns differ in geometry, adaptations in flow and gradient times had to be made, in order to ensure a good comparability of the results. For this purpose the procedure, to keep average retention factors, k*, during gradient elution constant, which is frequently used in RPLC, was adopted (see Chapter 3). For the Nucleosil columns the flow and gradient steepness were arbitrarily chosen as 1.0 ml/min and 3%/min respectively. In Table 7.1, column dead volume ($V_m$) and the resulting flow and gradient times ($t_G$: $t_1$, $t_2$, $t_3$) for the various columns are given. Results are shown in Figure 7.2. To facilitate a direct comparison with other results, retention times of chromatograms which had been recorded with $t_G$ values differing from the ones for the 20 cm Nucleosil columns, were normalised by multiplication with a factor $t_{G,column}/t_{G,nucleosil}$.

**Table 7.1.** Column dead volume, flow and gradient times used for the various columns

| Column | $V_m$ (ml) | Flow (ml/min) | t1* (min) | t2 (min) | t3 (min) |
|---|---|---|---|---|---|
| CN | 2.11 | 1.00 | 23.3 | 56.6 | 90.0 |
| Diol | 2.46 | 1.00 | 27.2 | 66.0 | 104.8 |
| $NH_2$ | 2.13 | 1.00 | 23.3 | 56.6 | 90.0 |
| DMA | 2.11 | 1.00 | 23.3 | 56.6 | 90.0 |
| Silica | 2.11 | 1.00 | 23.3 | 56.6 | 90.0 |
| PVA | 3.47 | 1.32 | 29.2 | 71.0 | 112.8 |
| PA | 3.48 | 1.32 | 29.2 | 71.0 | 112.8 |

*: applied gradient: HEP-DCM-THF-MeOH (70:30:0:0, v/v) to (0:100:0:0) (0 to t1 min), (0:100:0:0) to (0:0:100:0) (t1 to t2 min), (0:0:100:0) to (0:0:0:100) (t2 to t3 min).

Significant differences with respect to both overall retention and selectivity between the various columns are obtained. The *CN column* is the least retentive column, which is in accordance with its rather moderate polarity as compared to the other columns.[6,7,20] The position of the third fraction corresponds to the retention time at which THF breaks through as could be concluded from a comparison with a blank gradient. Therefore this peak is mainly caused by acceleration of the elution of the more polar fractions rather than to a 'normal' retention process. It will be highly enriched of chains containing two acidic end groups. The total peak area and the ratio of peak areas of the various fractions are given in Table 7.2.

As compared to the CN column, the *diol column* is much more polar which is due to its H-bond donor properties. A separation into oligomers can be observed, especially for PE7. Nevertheless, no distinct separation between the mono- and di-acid fractions is obtained. From Table 7.2 it can be seen that peak area for PE2 is significantly lower than for the CN column, indicating irreversible adsorption.

For both the *$NH_2$ and the DMA column* distinct oligomer separations are obtained for PE7, which is less detailed for PE2. This difference can be attributed to the fact that PE7 is a homopolyester and PE2 is copolyesters. The latter contains a larger number of different oligomers which will result in more complex elution patterns. Both columns provide a high selectivity with respect to the various end group fractions as can especially be seen for PE7. For this product the di-acid fraction does not elute at all, whereas for PE2 also large parts of the mono-acid fractions are irreversibly retained as can also be concluded from peak areas in Table 7.2. A change to an even more polar mobile phase containing 50% water, in order to elute these polar substances, proved to make no sense due to the eluent which becomes a non-solvent for the polyesters in that case. The highly retentive properties of $NH_2$ and DMA toward fractions containing acidic end groups is caused by the basic character of these columns,[6] which obviously is most pronounced for DMA.

On the *silica column*, for both polyesters a separation into three main fractions is obtained, whereas the diol fraction is further separated into oligomers. The third fractions coincide with the point at which MeOH breaks through and, like on the CN column, this peak is not due to a normal retention process. Nevertheless, selectivity towards the various end group fractions is much better than on CN. Retention of the mono and di-acid fractions on silica is much less than on $NH_2$ and DMA, which is in accordance with the more acidic character of the silica column.[6] From Table 7.2 it can be seen that hardly any irreversible adsorption occurs. The difference compared to the diol column, where fractions containing acidic end groups are only partly eluted can presumably be explained from the more basic character of the alcoholic OH groups in the diol column as compared to the silanolic OH groups of silica. It is also interesting to note that on both $NH_2$ and DMA, and silica, relatively regular elution patterns for the diol oligomers of PE7 are obtained, which clearly contrasts the results for both the CN and diol columns. The same difference was found by Jandera *et al.* for ethoxylated alkylphenols that also contain polar OH end groups, in both isocratic and gradient elution mode.[20] Perhaps a change in oligomer conformation depending on their chain length gives rise to a changing mechanism on the diol and CN column, thus causing irregular elution patterns, as was suggested by Jandera. The fact that such a conformation change can depend on the nature of the active sites may explain the different elution behaviour for the various columns.

The exact chemical nature of the less commonly used PVA and PA columns is not supplied by the manufacturer. Therefore, explanations for differences as compared to the

**Table 7.2.** Total peak area and peak area percentages for the end group fractions under various conditions

| Parameter | Sample | Total peak area (counts*$10^{-7}$) | Diol (%) | Mono-acid (%) | Di-acid (%) | Mono+ di-acid (%) |
|---|---|---|---|---|---|---|
| *Column* | | | | | | |
| CN | PE2 | 4.6 | 32 | 22 | 46 | 68 |
| | PE7 | 4.3 | 35 | 35 | 30 | 65 |
| Diol | PE2 | 2.7 | | | | |
| | PE7 | 4.3 | 32 | --- | --- | 68 |
| $NH_2$ | PE2 | 1.5 | | | | |
| | PE7 | 3.1 | | | | |
| DMA | PE2 | 2.0 | | | | |
| | PE7 | 2.2 | | | | |
| Silica | PE2 | 4.6 | 31 | 40 | 29 | 69 |
| | PE7 | 4.1 | 30 | 58 | 12 | 70 |
| PVA | PE2 | 4.5 | 32 | --- | --- | 68 |
| | PE7 | 4.0 | 28 | --- | --- | 72 |
| PA | PE2 | 4.6 | 33 | 37 | 30 | 67 |
| | PE7 | 4.1 | 29 | 49 | 22 | 71 |
| RP-GPEC* | PE2 | 4.6 | | | | |
| | PE7 | 4.3 | | | | |
| *Gradient steepness (%/min)* | | | | | | |
| 3 | PE7 | 5.1 | 34 | 35 | 31 | 66 |
| 5 | | 5.1 | 35 | 24 | 41 | 65 |
| 7 | | 5.0 | 36 | 16 | 48 | 64 |
| 10 | | 5.0 | 37 | 10 | 53 | 63 |
| *Temperature (°C)* | | | | | | |
| 10 | PE7 | 4.8 | 29 | 61 | 10 | 71 |
| 35 | | 4.8 | 29 | 65 | 6 | 71 |
| 60 | | 4.8 | 29 | 65 | 6 | 71 |
| *Sample load (µg): CN column* | | | | | | |
| 50 | PE2 | 2.0 | 33 | 33 | 34 | 67 |
| 100 | | 4.8 | 29 | 42 | 29 | 71 |
| 200 | | 8.4 | 27 | 59 | 14 | 73 |
| 500 | | 20.1 | 26 | 65 | 9 | 74 |
| 1000 | | out of range | | | | |
| *Sample load (µg): PA column* | | | | | | |
| 50 | PE2 | 2.1 | 24 | 34 | 42 | 76 |
| 100 | | 3.5 | 25 | 40 | 35 | 75 |
| 200 | | 6.4 | 26 | 43 | 31 | 74 |
| 500 | | 16.6 | 25 | 53 | 22 | 75 |
| 1000 | | out of range | | | | |

*: this measurement was performed to check whether elution under NP conditions was complete or not. For RP-GPEC it is known that complete elution occurs.

other columns can only be speculated on. Overall retention on the *PVA column* is considerably less than for most other columns, which can either be the result of an effective shielding of the silica matrix by the PVA coating, or of a relatively small number of active OH sites. No complete baseline separation of the diol fraction and the acidic end groups containing fractions, is realised. As was already found, mono- and di-acid fractions are completely co-eluted. This qualitatively resembles results for the diol column. Obviously, alcoholic OH groups provide a relatively low selectivity towards components containing a differing number of carboxyl groups.
The *PA column* provides a fairly good separation into three main fractions. In contrast with the $NH_2$ and DMA columns, all fractions are completely eluted. This difference can probably be explained from the less basic character of the $NH_2$ groups of PA due to their aromatic nature. Like for silica, the third peak coincides with the breakthrough point of MeOH.
Together with silica, the PA column provides the best separation with respect to functionality. Nevertheless it must be mentioned, that a detailed inspection of the chromatograms revealed that even for these columns no strict baseline separation between the mono and di-acid fraction is obtained. This can also be concluded from Table 7.2 where varying amounts for both fractions on the two columns are found. Since silica is known to provide poorly reproducible results due to its sensitivity to low amounts of moisture,[30] PA was chosen for further research.

### 7.4.3 Mobile phase effects in NP-GPEC

On the PA column, various eluent combinations were investigated for sample PE7, the results of which are shown in Figure 7.3. The *initial gradient* consisted of the quaternary combination HEP-DCM-THF-MeOH. A gradient steepness of 3%/min was applied for all steps (Figure 7.3a). The resulting elution pattern, especially that of the diol fraction, differs from that in Figure 7.2. This is due to the fact that experiments were carried out on another PA column. Variations from column to column as well as ageing effects on one column were found to affect results to a certain extent. Nevertheless, a comparison of results within one experiment series can well be made, since care was taken to use the same column and eluent batches in such series.
*Removing the DCM step* while keeping the other conditions constant, which means that gradient steepness in the first step was decreased to 1.5 %/min, considerably affects the separation (Figure 7.3b). For the diol fraction, reversed retention order with respect to p as compared to Figure 7.3a is found and the selectivity between the diol fraction and the mono-acid fraction is slightly improved. The systems of Figure 7.3a and 7.3b are essentially different. In the case of Figure 7.3a, a large part of the first (diol) fraction is eluted in the DCM-THF part of the gradient program. DCM is a good solvent for the

polyesters, but apparently a weak displacer. For Figure 7.3b, a real non-solvent/solvent gradient is applied, since HEP is a non-solvent for polyesters. Nevertheless, the last part of the diol fraction is eluted at approximately 75 % THF, whereas the cloud point lies as 55 % THF. This indicates that, although precipitation and re-dissolution effects are present in this system, retention, like in 7.3a, is dominated by adsorption. Selectivity differences with respect to p must therefore be explained from adsorption phenomena, as will be further discussed in Section 7.4.6.

*Removing the THF step* as compared to Figure 7.3a leads to a complete detoriation of the separation between the mono and di-acid fraction, as can be seen from Figure 7.3c. On another PA column, where adsorption was slightly stronger thus causing complete elution of the diol fraction in the DCM-THF part in a situation like in Figure 7.3a, removing the THF step completely detoriated the end group separation (result not shown here). The introduction of small amounts of the strong proton donor MeOH in DCM leads to a instantaneous desorption of the complete polyester. Therefore, MeOH must only be used for the displacement of the strongest adsorbing polyester components.

Replacement of the basic localising solvent THF by the weaker, also basic localising *MTBE* causes a larger part of the mono-acid fraction to be eluted in the MeOH step as can be seen in Figure 7.3d. Furthermore overall retention of both the mono-acid and di-acid fraction is delayed due to the lower polarity of MTBE compared to THF. No significant improvement in resolution between diol and mono-acid is realised. The same effect, although to a lesser extent, is observed when THF is replaced by the essentially different non-basic localising *ETAC* (Figure 7.3e). Obviously, resolution of the end group separation is hardly affected by using another type of eluent. The use of *ACN* instead of THF causes the total amount of polyester that is eluted, to decrease which can be seen from Figure 7.3f. This is due to the fact that ACN is a non-solvent, causing the solubility of the polyester to decrease, thus preventing elution although polarity increases. Therefore, ACN can not be used in this part of the solvent program.

*Replacement of DCM by $CHCl_3$* increases retention of the diol fraction due to the lower polarity of the latter solvent, but again, resolution between the end group fractions is hardly influenced. Finally, *replacement of MeOH by IPA* causes the di-acid fraction to completely co-elute with the mono-acid fraction. This is surprising, since elution occurs in the DCM-THF step, where no MeOH or IPA should affect elution. Since these results were found to be reproducible it was speculated that IPA is not completely removed when returning the system to initial conditions. This might be caused by the relatively high viscosity of IPA, thus preventing sufficient mixing with other solvents.

Hence, it can be concluded that the end group separation can hardly be improved as compared to the situation of Figure 7.3a, by using other eluent combinations. The use of a localising solvent, is necessary to obtain a separation between mono- and di-acid fractions. The use of a non-localising, intermediate solvent can slightly influence

resolution between the diol and mono-acid fraction and MeOH is needed to displace and elute the most polar fractions.
In none of the cases described above a complete separation between the mono- and di-acid fraction was obtained. Since, in another study, the addition of small amounts of acetic acid to the mobile phase proved to favour the separation of polyester end groups under critical conditions,[31] it was tried whether this could also work for NP-GPEC. Therefore, for the eluent combination HEP-DCM-THF-MeOH, an amount of 0.02% (v/v) acetic acid was added to all eluents. Unfortunately this lead to a complete detoriation of the end group separation and the elution of the complete polyester in one fraction on the PA column (result not shown here). This is presumably due to the occupation of the most active sites of the stationary phase by acetic acid. Obviously, the use of organic acids in the mobile phase cannot be used in this case to improve end group separation. Therefore, it is worthwhile to further investigate the possibilities of chromatography under critical conditions to obtain a better separation between the respective end group fractions.
From the differences between cloud point compositions and %-solvent at the point of elution that were observed for the various non-solvent/solvent systems, it is obvious that even in those cases where a real non-solvent/solvent system is used, separation is dominated by adsorption rather than by precipitation/re-dissolution effects.

### 7.4.4 Effect of various practical parameters in NP-GPEC

Short term reproducibility of NP-GPEC, for instance between two batches of eluent, was essentially worse as compared to RP-GPEC. This is a well known phenomenon for NP separations which is often ascribed to subtle variations in water content of the mobile phase.[30] Furthermore PA as well as $NH_2$ columns were found to change as a function of time, presumably due to irreversible adsorption of (small) parts of the polyester samples. For PA, flushing the column with polar solvents containing small amounts of acetic acid was found to restore the original separation to some extent, but not completely. Finally, PA columns were found to vary from batch to batch, thus giving rise to column dependent elution results, as was already observed from the comparison of Figures 7.2g and 7.3a.

The use of steeper gradients hardly affects the resolution between the diol- and the mono-acid-fraction. In addition, a larger part of the latter fraction is co-eluted with the di-acid fraction in the THF-MeOH step (Table 7.2). Therefore, in those cases where, due to a lack of complete separation between the mono-acid and di-acid fraction, the amounts of both fractions must be taken together and compared with the diol fraction, a higher gradient steepness can be used without loss of information.

From Figure 7.5 the minor effect of temperature increase on the resolution between the diol- and mono-acid fraction can be seen. A larger part of the latter fraction is eluted in the DCM-THF step, which is explained from the apparent exothermic character of the separation, leading to decreased adsorption at higher temperatures (Table 7.2). Although not clear from the shown chromatograms, the elution order of the oligomers may reverse with increasing temperature as was observed for a CN column (chromatogram not shown). This can be explained from the fact that the interaction of the end groups ($\Delta\mu_x$, Eq. (2.19)) and the repeat units ($\Delta\mu_y$) are affected in a different way by a temperature change, which can result in a reversal of the retention order.

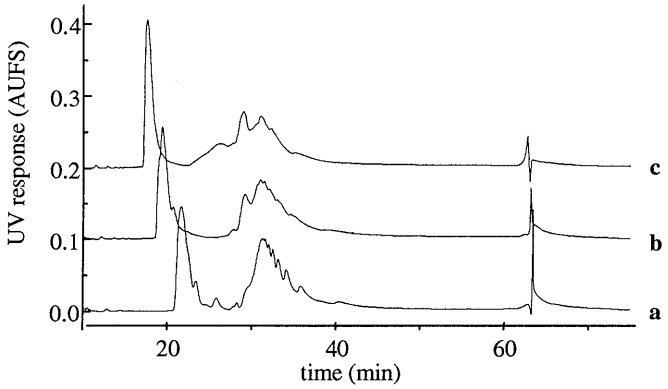


**Figure 7.5.** Effect of temperature for sample PE7 on a PA column. a: 10 °C, b: 35 °C, c: 60 °C. Conditions: eluent: HEP-DCM-THF-MeOH (70:30:0:0, v/v) to (0:100:0:0) (0 to 23.3 min), (0:100:0:0) to (0:0:100:0) (23.3 to 56.6 min), (0:0:100:0) to (0:0:0:100) (56.6 to 90 min), flow: 1.32 ml/min. Further conditions: see Section 7.3.3.

Like for RP-GPEC, the effect of sample load was studied by injecting different concentrations of sample PE2 while keeping the injection volume constant. For this purpose, the PA and the CN column were chosen. Increasing the sample load up to 1000 µg on the PA column causes a decreasing fraction to elute in the THF-MeOH step, which is obviously due to overloading (Table 7.2). Furthermore, resolution between the diol and the mono-acid fraction decreases, although this hardly influences the calculated percentage for the former fraction. For the CN column, the effect of sample loading is much stronger (Figure 7.6). Thus, the maximum allowable sample load depends on column type and the related adsorption isotherm. Furthermore, the effect is much more pronounced as compared to RP-GPEC, where no effect at all was observed up to 1000 µg (Chapter 3). This difference can probably also be attributed to the relatively low

instantaneous sample load in RP-GPEC due to the elution of the polyester over a wide retention range.

Finally, the effect of injection volume was checked by injecting approximately constant amounts of 100 µg in different volumes for sample PE2 on the same two columns. No effect at all was observed on the PA column, for injection volumes up to 50 µl. In contrast, injection volumes exceeding 10 µl caused significant peak broadening and increasing sample breakthrough for the CN column. This difference proves that the sample-solvent effect[30] is not solely caused by the difference between the eluent and the sample solvent, which is identical for both columns. Obviously, the adsorption strength of the stationary phase under conditions of injection also influences the extent of the zone over which the sample is adsorbed.

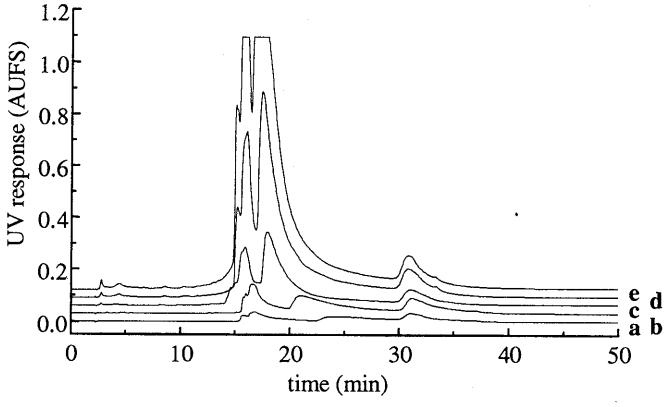


**Figure 7.6.** Effect of injected mass of sample PE2 on the elution behaviour on a CN column. a: 50 µg, b: 100 µg, c: 200 µg, d: 500 µg, e: 1000 µg, each dissolved in 10 µl DCM. Conditions: eluent: HEP-DCM-THF (70:30:0) to (0:100:0) (0 to 23.3 min), (0:100:0, v/v) to (0:0:100) (23.3 to 56.6 min), flow: 1.0 ml/min. Further conditions: see Section 7.3.3.

### 7.4.5 Separation of copolyesters according to the backbone composition

A detailed comparison of the chromatograms of polyesters PE2, PE6 and PE7 on a PA column reveals that there are slight but significant retention differences between both homopolyesters PE6 and PE7. The diol fraction of PE6 elutes somewhat earlier than that of PE7, whereas the elution maximum of PE2 lies in between both homopolyesters (Figure 7.7A). Using an $NH_2$ column instead of PA, the retention differences between the diol fractions are even significantly larger, especially when gradient steepness and temperature are further optimised (Figure 7.7B), and a baseline separation between the

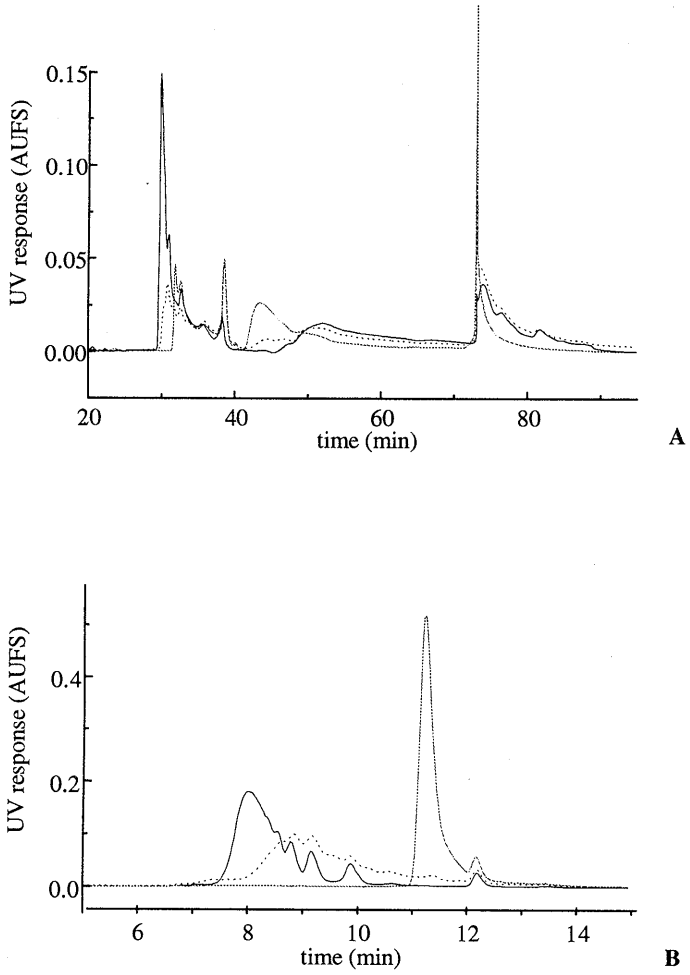


**Figure 7.7.** Separation of homopolyesters PE6 and PE7 and copolyester PE2 on PA (A) and $NH_2$ (B). In (B) only elution of the diol fractions is shown. Black line: PE6, grey line: PE7, dotted line: PE2. A: temperature: 35 °C, eluent: HEP-DCM-THF-MeOH (100:0:0:0, v/v) to (0:100:0:0) (0 to 33.3 min), (0:100:0:0) to (0:0:100:0) (33.3 to 66.6 min), (0:0:100:0) to (0:0:0:100) (66.6 to 100 min), flow: 1.32 ml/min. B: temperature: 45 °C, eluent: DCM-THF (100:0) to (94:6) (0 to 15 min), flow: 1.5 ml/min. Further conditions: see Section 7.3.3.

diol and the mono-acid fraction is obtained. This clearly implies that NP-GPEC can be used for the characterisation of copolyesters according to the chemical composition distribution (CCD) of the backbone independent of end groups, which cannot be done by any other technique at this moment. In Chapter 8, the use of NP-GPEC for the determination of the chemical composition distribution and the functionality type distribution of copolyesters, will be described.

It is interesting to note that in contrast to the diol fraction, the mono-acid fraction of PE6 is more strongly retained than that of PE7 (Figure 7.7A). Obviously, the polarity of an isophtalic end group as compared to an adipic acid end group significantly differs from the polarity of the (esterified) isophtalic and adipic acid chain units. This is probably due to the significantly lower $pK_a$ value of isophtalic acid as compared to adipic acid (3.5 and 4.4 respectively) giving rise to stronger interactions of the former end group type with the basic column packing.

### 7.4.6 Investigations on the mechanisms of NP-GPEC by isocratic measurements

In order to get further understanding of the retention behaviour of polyesters in NP-GPEC, isocratic measurements were performed on various systems. For this purpose, the silica and the PA column were chosen in combination with three binary eluent combinations, *i.e.* HEP-THF, DCM-THF and HEP-ETAC at at least five different compositions for each combination. The corresponding NP-GPEC measurements for each system are shown in Figure 7.8. Since no MeOH was used in the gradients, oligomers with two acidic end groups are not eluted. For the HEP-THF gradient on the silica column, only one fraction was obtained, with the mono-acid oligomers eluting at the high retention side of the distribution. This order of elution was confirmed by the injection of the various low polydispersity standards obtained by RP-GPEC (see Section 7.3.3).

#### *7.4.6.1 Isocratic measurements on a silica column*

For the silica column, reasonable fit of Eqs. (7.1) and (7.2) was found in the binary mobile phases tested (Figures 7.9 and 7.10). Experimental values of the parameters $a_0$, $a_1$, $m_0$, $m_1$, $\log(\alpha)$ and $\log(\beta)$ are given in Tables 7.3. and 7.4.
Values of $m_1$ are significantly lower than $m_0$, indicating a lower adsorbed area of the repeat unit as compared to the adsorbed area of the end groups (Eqs. 7.5c,d: $m_1$ is the quotient of the adsorbent area occupied by a repeat unit and by a polar solvent molecule, $m_0$ is the quotient of the adsorbent area occupied by an end group and by a polar solvent molecule). This may possibly be explained from the orientation of the adsorbed molecules towards the surface of the stationary phase, and emphasises the apparent importance of end groups in the total retention behaviour. The parameters $a_1$ and $m_1$, which are related to the repeat units, should not depend on the end groups and indeed, rather similar values of these constants are found for the diol and the mono-acid series in both HEP-THF and DCM-THF on the silica column.

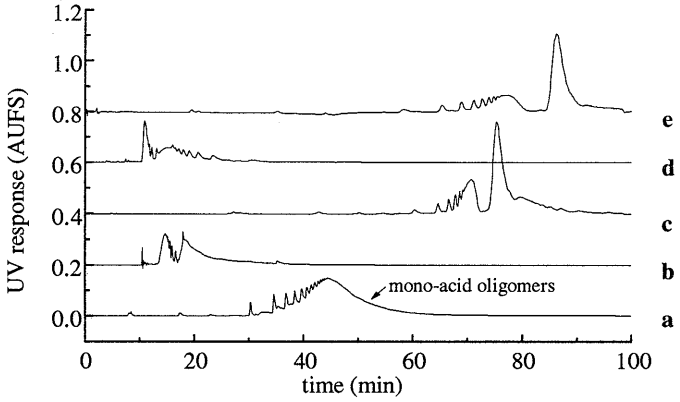


**Figure 7.8.** NP-GPEC separations of polyester PE7 under various conditions. a: column: silica, eluent: HEP-THF (100:0, v/v) to (0:100) (0 to 100 min), flow: 1.0 ml/min, b: column: silica, eluent: DCM-THF (100:0) to (0:100) (0 to 100 min), flow: 1.0 ml/min, c: column: PA, eluent: HEP-THF (100:0) to (0:100) (0 to 100 min), flow: 1.32 ml/min, d: column: PA, eluent: DCM-THF (100:0) to (0:100) (0 to 100 min), flow: 1.32 ml/min, e: column: PA, eluent: HEP-ETAC (100:0) to (0:100) (0 to 100 min), flow: 1.32 ml/min. Further conditions: see Section 7.3.3.

Coelution of oligomers differing in p *i.e.* critical solvent composition (CSC), can be predicted from Eq. (7.5) to occur at 4.4% THF in DCM and at 50% THF in HEP for both the diol and mono-acid series (Figure 7.10). In agreement with Eq. (7.5), $\varphi_0$ does not depend on the end group type. The finding of these coelution points is in agreement with the character of the polyester. The repeat unit is bulky and contains both a polar and a non polar part, but is significantly less polar than the end group. Thus, it will take a mobile phase with a moderate eluent strength to suppress the polar interactions of the repeat units. Therefore, it can be expected that $\varphi_0$ will be found at moderately polar eluent combinations, which is indeed the case.

The energy of adsorption of the oligomers is higher in pure HEP than in pure DCM due to the higher polarity of DCM. Consequently, due to a higher value of $\varepsilon_a$ for DCM, the values of the parameters $a_0$ and $a_1$ are lower in DCM-THF than in HEP-THF mobile phases. The differences between the parameters $m_0$ and $m_1$ in the two types of mobile phases can possibly be attributed to the effects of solvation of the adsorbed molecules, causing a decrease of the adsorbed area. A practical consequence is that the CSC lies at a higher concentration of THF in HEP-THF than in DCM-THF mobile phases. The retention factors of the diol oligomers at the CSC 50% THF in HEP are approximately the same as for the mono-acid oligomers (k = 0.8 and 1.3, respectively), which explains the coelution observed in Figure 7.8a. In the gradient elution run shown in Figure 7.8a, the diol oligomers are eluted before the CSC of THF in HEP is achieved, causing

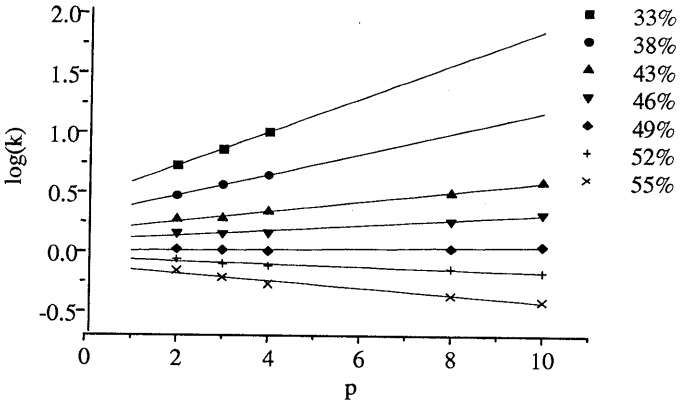


**Figure 7.9.** Log(k) versus p for diol oligomers on the silica column in HEP-THF. Fraction THF: see legend.

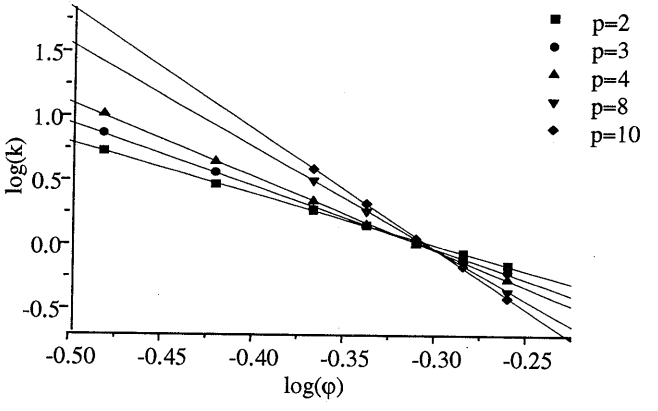


**Figure 7.10.** Log(k) versus log(φ) for diol oligomers on the silica column in HEP-THF. Degree of polymerisation: see legend.

increasing retention with increasing p (Figure 7.10). Due to the low retention factor of the di-acid oligomers at the CSC, in NP-GPEC these products elute just after this concentration has been reached, causing coelution with the diol fraction. On the other hand, in the separation shown in Figure 7.8b, the polymers are eluted at a concentration of THF in DCM higher than the CSC. Obviously, at this CSC the interactions of the alcoholic end groups with the stationary phase are less effectively suppressed than at the CSC of THF in HEP. Thus, the oligomers are eluted in the order of decreasing p (compare the elution pattern of the first groups of peaks in Figures 7.8a and 7.8b).

Although strongly dependent on the eluent composition, the difference in overall retention, log(β), between the diol and monoacid fraction is larger in DCM-THF than in HEP-THF, which reflects the larger end group selectivity in DCM-THF as compared to

**Table 7.3.** Experimental selectivity (log(α)), overall retention (log(β)) and constants of Eq. (7.3) for diol and mono-acid polyester oligomers on the silica column in HEP-THF

| **Diol oligomers** | | | | **Mono-acid oligomers** | | | |
|---|---|---|---|---|---|---|---|
| φ | log(β) | log(α) | $R^1$ | φ | log(β) | log(α) | $R^1$ |
| 0.43 | 0.165 | 0.042 | 0.9941 | 0.40 | 0.319 | 0.063 | 0.9979 |
| 0.46 | 0.079 | 0.024 | 0.9999 | 0.45 | | | |
| 0.49 | 0.050 | -0.017 | 0.9904 | 0.48 | -0.100 | 0.061 | 0.9932 |
| 0.52 | 0.028 | -0.053 | 0.9636 | 0.50 | | | |
| 0.55 | -0.016 | -0.079 | 0.9904 | 0.52 | -0.0187 | -0.0192 | 0.9978 |
| $a_0 = -0.8 \pm 0.1$ | | $a_1 = 0.198 \pm 0.001$ | | $a_0 = -1.0 \pm 0.1$ | | $a_1 = -0.214 \pm 0.001$ | |
| $m_0 = 3 \pm 1$ | | $m_1 = 0.65 \pm 0.01$ | | $m_0 = 3 \pm 1$ | | $m_1 = 0.70 \pm 0.01$ | |
| p = 2-10 | | $\varphi_0 = 0.50$ | | p = 3-10 | | $\varphi_0 = 0.50$ | |

**Table 7.4.** Experimental selectivity (log(α)), overall retention (log(β)) and constants of Eq. (7.3) for diol and mono-acid polyester oligomers on the silica column in DCM-THF

| **Diol oligomers** | | | | **Mono-acid oligomers** | | | |
|---|---|---|---|---|---|---|---|
| φ | log(β) | log(α) | $R^1$ | φ | log(β) | log(α) | $R^1$ |
| | | | | 0.06 | 1.147 | -0.169 | 0.9607 |
| 0.05 | 0.789 | -0.081 | 0.9828 | 0.07 | 0.697 | -0.054 | -0.9809 |
| 0.06 | 0.655 | -0.116 | 0.9957 | 0.08 | 0.575 | -0.068 | -0.9793 |
| 0.07 | 0.498 | -0.075 | -0.9915 | 0.09 | 0.555 | -0.091 | -0.9984 |
| 0.08 | 0.411 | -0.095 | -0.9958 | 0.10 | 0.345 | -0.097 | -0.9964 |
| $a_0 = -0.9 \pm 0.1$ | | $a_1 = -0.480 \pm 0.01$ | | $a_0 = -2.0 \pm 0.5$ | | $a_1 = -0.35 \pm 0.01$ | |
| $m_0 = 1.2 \pm 1$ | | $m_1 = 0.353 \pm 0.003$ | | $m_0 = 2 \pm 0.4$ | | $m_1 = 0.26 \pm 0.01$ | |
| p = 2-10 | | $\varphi_0 = 0.04$ | | p = 3-10 | | $\varphi_0 = 0.045$ | |

**Table 7.5.** Experimental selectivity (log(α)), overall retention (log(β)) and constants of Eq. (7.3) for diol and mono-acid polyester oligomers on the PA column in HEP-THF

| **Diol oligomers** | | | | **Mono-acid oligomers** | | | |
|---|---|---|---|---|---|---|---|
| φ | log(β) | log(α) | $R^1$ | φ | log(β) | log(α) | $R^1$ |
| 0.62 | 0.585 | 0.074 | 0.9992 | 0.70 | 1.155 | -0.022 | 0.7215 |
| 0.64 | 0.536 | 0.054 | 0.9934 | 0.72 | 1.057 | -0.024 | 0.9778 |
| 0.66 | 0.508 | 0.031 | 0.9969 | 0.74 | 1.103 | -0.083 | 0.9998 |
| 0.68 | 0.479 | 0.003 | 0.9989 | 0.76 | 1.014 | -0.087 | 0.9996 |
| 0.70 | 0.497 | -0.048 | 0.81 | 0.78 | 0.952 | -1.011 | 0.9968 |
| $a_0 = -0.11 \pm 0.02$ | | $a_1 = -0.226 \pm 0.001$ | | $a_0 = 0.17 \pm 0.03$ | | $a_1 = -0.162 \pm 0.001$ | |
| $m_0 = 3 \pm 1$ | | $m_1 = 1.44 \pm 0.02$ | | $m_0 = 6 \pm 2$ | | $m_1 = 1.04 \pm 0.04$ | |
| p = 2-10 | | $\varphi_0 = 0.70$ | | p = 3-10 | | $\varphi_0 = 0.70$ | |

**Table 7.6.** Experimental selectivity (log(α)), overall retention (log(β)) and constants of Eq. (7.3) for diol and mono-acid polyester oligomers on the PA column in DCM-THF

| Diol oligomers | | | | Mono-acid oligomers | | | |
|---|---|---|---|---|---|---|---|
| φ | log(β) | log(α) | $R^1$ | φ | log(β) | log(α) | $R^1$ |
| | | | | 0.10 | 1.548 | -0.221 | 0.9992 |
| 0.04 | 0.857 | -0.138 | 0.9853 | 0.11 | 1.416 | -0.190 | 0.9996 |
| 0.05 | 0.774 | -0.181 | 0.9978 | 0.12 | 1.425 | -0.230 | 0.9996 |
| 0.06 | 0.744 | -0.210 | 0.9980 | 0.13 | 1.299 | -0.206 | 0.9995 |
| 0.07 | 0.657 | -0.208 | 0.9978 | 0.14 | 1.289 | -0.237 | 0.9995 |
| $a_0 = -0.21 \pm 0.02$ | | $a_1 = -0.440 \pm 0.001$ | | $a_0 = -0.71 \pm 0.03$ | | $a_1 = -0.157 \pm 0.001$ | |
| $m_0 = 0.54 \pm 0.01$ | | $m_1 = 0.27 \pm 0.02$ | | $m_0 = 1.95 \pm 0.02$ | | $m_1 = 0.05 \pm 0.02$ | |
| p = 2-10 | | $\varphi_0 = 0.02$ | | p = 3-10 | | $\varphi_0 = 0.001$ | |

HEP-THF (Figure 7.8). This means that a stronger solvent A in a binary mobile phase enhances the end group selectivity for the studied polyesters.

### *7.4.6.2 Isocratic measurements on a polyamine column*

For the PA column, reasonable fit of Eqs. (7.1) and (7.2) is found for HEP-THF (Figure 7.11) and DCM-THF mobile phases. The parameters of Eq. (7.3) for these systems are given in Tables 7.5 and 7.6. For HEP-ETAC mobile phases, the plots of log k versus p are curved, which means that Eqs. (7.1) and (7.2) do not properly describe retention. Obviously, ETAC being a non-basic localising solvent, gives rise to another separation mechanism than THF, a basic localising solvent.

In HEP-THF, large differences between the parameters $a_0$ and $m_0$ of the diol and mono-acid series are found, in contrast to the results for the silica column. This suggests that the acidic end groups occupy a much larger area on the adsorbent surface than the alcoholic end groups, possibly because of stronger proton donor-acceptor interactions with the amino groups of the adsorbent. Consequently, the end group selectivity in HEP-THF is much better for the PA than for the silica column, but the retention is significantly higher (Figure 7.8c). The calculated CSC for the PA column is higher than for the silica gel column, 70% THF in HEP, for both diol and mono-acid oligomers, which reflects the stronger interaction of the repeat unit with the PA stationary phase. Hence, the diol oligomers are eluted in order of increasing p in mobile phases with a relatively low concentration of THF in NP-GPEC (Figure 7.8c), but the mono-acid polymer fraction is eluted without significant separation of the individual oligomers close to the CSC.

In DCM-THF mobile phases, the oligomer selectivity on the PA column is significantly higher than on the silica column (higher values of $a_1$, lower values of $m_1$) and the CSC is very low (2% THF in DCM), which means that the oligomers are eluted in order of

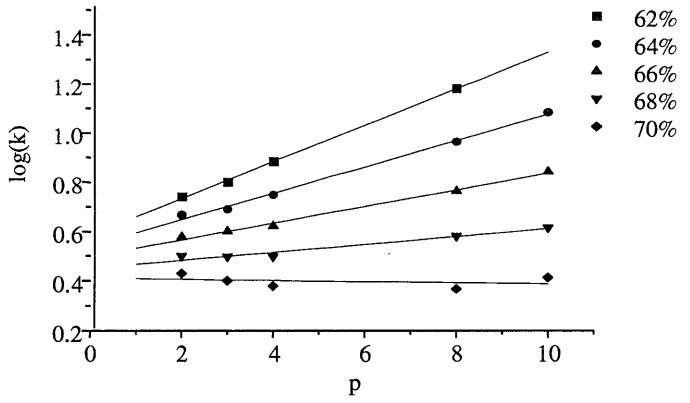


**Figure 7.11.** Log(k) versus p for diol oligomers on the PA column in HEP-THF. Fraction THF: see legend.

decreasing p over the whole concentration range of DCM-THF mobile phases (Figure 7.8d).

On the PA column, the $m_1$ and $a_1$ values are not independent of the end group type. For the mono-acid series, $m_1$ values are significantly lower than for the diol series, indicating a lower adsorbed area of the repeat unit. Possibly, the orientation of the repeat unit is affected by adsorption of the end group. Due to strong interactions of the acidic end groups causing a large adsorption enthalpy, the sterical hindrance of certain orientations may more easily be compensated, giving rise to a changed orientation of the repeat unit towards the stationary phase.

### *7.4.6.3 Two sites adsorption model for the polyamine column*

On the PA column, for HEP-ETAC mobile phases, the retention cannot be described adequately by Eqs. (7.1) and (7.2). A possible explanation may be that the PA column, which contains both an aromatic polymer backbone and amino groups, behaves as an adsorbent with two different types of adsorption sites (1 and 2) with respect to the polyester. Thus, the retention behaviour can be complex, with retention resulting from the additive effects of π-π interactions of the hydrocarbon moieties in the repeat units with the aromatic matrix of the adsorbent, and of polar proton donor-acceptor interactions of the ester moieties in the repeat units and the end groups, with the amino groups of the adsorbent. Therefore, two distribution constants $K_{D1}$, $K_{D2}$ and two retention factors $k_1$, $k_2$ can be defined, each representing adsorption on one of the two types of adsorption sites. Assuming that the adsorption on each adsorption site can be

described by Eqs. (7.1) and (7.2), the resulting retention factor of a solute in a two-site adsorption system is given as:

$$k = k_1 + k_2 = \phi_1 K_{D1} + \phi_2 K_{D2} = \beta_1 \alpha_1^p + \beta_2 \alpha_2^p = \frac{k_{s1}}{\varphi^{m1}} + \frac{k_{s2}}{\varphi^{m2}} \quad (7.7)$$

where $\phi_1$ and $\phi_2$ are the partial phase ratios given by the fractions of the adsorbent surface occupied by each type of the adsorption sites. $\alpha_1$ and $\alpha_2$ represent the

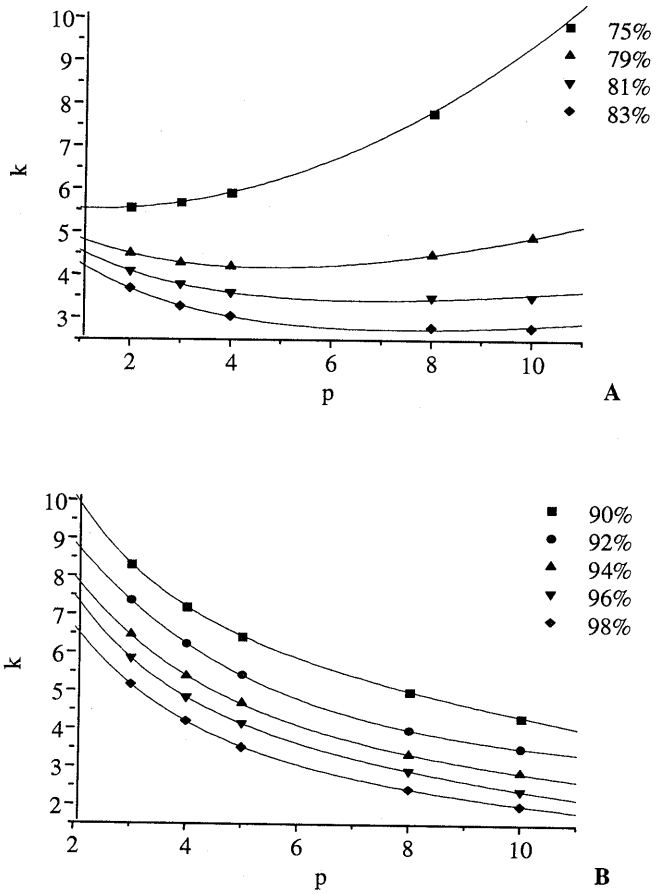


**Figure 7.12.** k versus p for diol (A) and mono-acid (B) oligomers on the PA column in HEP-ETAC. The points are experimental data and the full lines are best-fit dependencies for Eq. (7.7). Fraction ETAC: see legend.

contribution of the repeat unit to the retention, *i.e.* the oligomeric selectivity on the two different adsorption sites, and $\beta_1 = k_{e1}$, $\beta_2 = k_{e2}$ are partial retention factors characterising the retention of the end groups on the two types of adsorption sites. $k_{s1}$ and $k_{s2}$ relate to the retention factors on the adsorption sites in pure polar solvent as the mobile phase.

Very good fit of Eq. (7.7) to the experimental data for the HEP-ETAC system is found, as can be seen in Figure 7.12. Furthermore, the retention in HEP-THF and DCM-THF can also be well described by this equation (pictures not shown). The parameters of Eq. (7.7) for all binary mobile phases tested on the PA column are given in Table 7.7.

Clearly, adsorption on two different types of adsorption sites may possibly explain the experimental behaviour observed on the PA column where, especially in the case of HEP-ETAC, even minima on the log(k) versus p plots are observed (Figure 7.12). Furthermore, possibly the two-site adsorption mechanism plays a more significant role in this mobile phase than in the mobile phases with more polar THF as the B solvent.

Due to the availability of relatively little data, not all the results as shown in Table 7.7

**Table 7.7.** Experimental parameters of Eq. (7.7) on the PA column

| Series | Mobile phase | $\varphi$ | $\beta_1$ | $\alpha_1$ | $\beta_2$ | $\alpha_2$ | $\beta_1/\beta_2$ | $R^1$ |
|---|---|---|---|---|---|---|---|---|
| diol | HEP-ETAC | 0.75 | 3.24 | 1.109 | 2.41 | 0.807 | 1.34 | 0.9994 |
| | | 0.79 | 2.59 | 1.062 | 2.77 | 0.754 | 0.94 | 0.9660 |
| | | 0.81 | 2.58 | 1.030 | 2.68 | 0.709 | 0.93 | 0.9883 |
| | | 0.83 | 1.86 | 1.038 | 3.23 | 0.719 | 0.58 | 0.9988 |
| mono-acid | | 0.90 | 8.57 | 0.934 | 10.13 | 0.509 | 0.85 | 0.9998 |
| | | 0.92 | 3.29 | 0.992 | 10.34 | 0.737 | 0.32 | 0.9999 |
| | | 0.94 | 5.07 | 0.941 | 8.47 | 0.640 | 0.60 | 0.9998 |
| | | 0.96 | 5.92 | 0.913 | 8.90 | 0.533 | 0.67 | 0.9999 |
| | | 0.98 | 4.38 | 0.923 | 8.33 | 0.591 | 0.52 | 0.9999 |
| diol | HEP-THF | 0.66 | 2.68 | 1.099 | 1.04 | 0.729 | 2.58 | 0.9880 |
| | | 0.68 | 2.61 | 1.047 | 2.05 | 0.377 | 1.27 | 0.9790 |
| | | 0.70 | 1.87 | 1.034 | 2.83 | 0.503 | 0.66 | 0.7864 |
| mono-acid | | 0.70 | 10.91 | 1.016 | 7.52 | 0.469 | 1.45 | 0.8603 |
| | | 0.72 | 9.63 | 0.977 | 12.69 | 0.251 | 0.76 | 0.9788 |
| | | 0.74 | 4.69 | 1.004 | 6.71 | 0.673 | 0.70 | 0.9957 |
| | | 0.76 | 5.00 | 0.961 | 6.80 | 0.492 | 0.74 | 0.9949 |
| | | 0.78 | 4.91 | 0.922 | 14.14 | 0.236 | 0.35 | 0.9973 |
| diol | DCM-THF | 0.03 | 0.32 | 1.189 | 6.47 | 0.749 | 0.05 | 0.9723 |
| | | 0.04 | 1.87 | 0.914 | 7.54 | 0.425 | 0.25 | 0.9988 |
| | | 0.05 | 2.02 | 0.853 | 10.60 | 0.296 | 0.19 | 0.9993 |
| mono-acid | | 0.10 | 8.80 | 0.852 | 51.64 | 0.430 | 0.17 | 0.9995 |
| | | 0.11 | 2.94 | 0.913 | 25.32 | 0.613 | 0.12 | 0.9996 |
| | | 0.12 | 5.11 | 0.856 | 26.01 | 0.505 | 0.21 | 0.9990 |
| | | 0.13 | 5.57 | 0.836 | 26.99 | 0.455 | 0.21 | 0.9996 |
| | | 0.14 | 1.36 | 0.935 | 17.50 | 0.587 | 0.08 | 0.9994 |

can be interpreted unambiguously. For both the diol and mono-acid series, the ratio $\beta_1$ : $\beta_2$ is significantly lower than unity in most cases. This behaviour can probably be explained by stronger adsorption of the hydroxy and the carboxylic acid end groups on the $NH_2$ adsorption sites than on the aromatic rings of the column packing material. Taking into mind the physical meaning of the present model, this means that $\beta_1$ and thus also $\alpha_1$ relate to the interaction with the benzene rings and $\beta_2$ and $\alpha_2$ are related to interactions with the amino groups.
In many cases, decreasing values for $\beta_1$, $\beta_2$, $\alpha_1$, and $\alpha_2$ with increasing concentration of the polar solvent B are found, which is characteristic behaviour for a normal-phase retention mechanism. The reason for an opposite trend which is observed, for instance, for diol oligomers in HEP-THF, can only be speculated on at this moment. Increasing $\beta_2$ values with increasing concentration B might result from decreasing polar interactions with the aromatic backbone as demonstrated by decreasing $\beta_1$ values, thus enhancing adsorption on the amino sites.
Retention minima can be found for an oligomeric series if the retention as a function of p increases on one type of adsorption sites ($\alpha > 1$) while it decreases on the other type ($\alpha < 1$). This is the case for the diol series in HEP-ETAC, where the repeat unit contributes to the retention on the '1' sites, but decreases the retention on the '2' sites of the adsorbent (Figure 7.12A, Table 7.7).
In HEP-ETAC mobile phases, the retention of the mono-acid oligomers decreases with increasing p and the separation selectivity for the oligomers slightly increases in mobile phases with higher concentrations of ETAC. On the other hand, the diol oligomers generally are eluted in the order of increasing p in mobile phases containing less than 80% ETAC, but in the opposite elution order in mobile phases more rich in ETAC. The oligomeric selectivity increases with decreasing concentration of ETAC. Consequently, the elution behaviour in NP-GPEC with increasing concentration of ETAC in HEP (Figure 7.8e) is similar as in the elution with THF in HEP (Figure 7.8c), but the separation of the individual diol oligomers is better and the elution times are longer in the first case.
From the results described above, it is obvious that isocratic measurements can be used for a further understanding of the chromatographic behaviour of polyesters in NP-GPEC.

## 7.5 CONCLUSIONS

The separation of polyesters by NP-GPEC is dominated by end groups in most cases and to a lesser extent by molar mass and composition of the backbone. A distinct effect of both the stationary and the mobile phase on the separation is found. Best end group separations for the investigated polyesters are observed on the silica and the PA column, although no complete separation between the mono-acid and di-acid fraction

can be achieved. Dependent on the stationary phase – mobile phase combination, retention can either increase or decrease with increasing p. The separation mechanism in all cases is governed by adsorption rather than by re-dissolution effects. Reproducibility is much worse as encountered in RP-GPEC. Gradient steepness and temperature only moderately affect the end group separation. The effect of sample-load is more pronounced as compared to RP-GPEC. Both the effect of sample-load and injection volume depend on the column type. Next to end group separations, NP-GPEC can be used for the characterisation of polyesters according to the composition of the backbone, independent of end groups.

The separation of polyesters in isocratic normal phase chromatography can, with exception of the eluent combination HEP-ETAC on a PA column, be satisfactorily described by using the approach of Jandera *et al.* Results are useful for a further understanding of the retention behaviour in NP-GPEC. Due to its large adsorbed area, the bulky repeat unit of the polyesters largely influences retention behaviour which is demonstrated by the finding of a CSC for all investigated stationary phase – mobile phase combinations. The occurrence of critical conditions explains differences in retention order with respect to p for the various NP-GPEC systems. Differences in end group separation between a silica and a PA column can be attributed to especially strong proton donor-acceptor interactions of the acidic end groups with the amino groups of the PA column. The adsorption model assuming two different types of adsorption sites yields improved description of the experimental retention data of oligomers on the PA column, especially for the HEP-ETAC mobile phases. This model can possibly explain the experimental retention minima in log(k) versus p.

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# CHAPTER 8

# Microstructural Characterisation of Copolyesters made by Step-reactions, by Gradient Polymer Elution Chromatography


## SUMMARY

*The potentials of Gradient Polymer Elution Chromatography (GPEC) in both the reversed phase (RP) and normal phase (NP) mode, for the characterisation of aromatic copolyesters made by step-reactions, according to their molar mass and chemical microstructure, were studied. To this end, a number of copolyesters, varying in molar mass and chemical composition (CC) was synthesised, which allowed a systematic study on the effects of those parameters in GPEC. The highly detailed RP-GPEC separations for copolyesters were used for the evaluation of average molar masses and oligomer distributions, which were found to be in good agreement with theoretical values. Especially for the low molar masses, additional information on chemical composition differences was obtained. Qualitative evidence for differences in the chemical microstructure of two strongly resembling copolyesters was found. Nevertheless, it appeared difficult to unambiguously assign observed differences in the high molar mass parts of RP-GPEC chromatograms. Therefore, RP-GPEC must mainly be considered as a versatile, qualitative fingerprinting tool. In contrast, NP-GPEC provides more and quantitative information on microstructural differences. By a combination of SEC and NP-GPEC the Molar-Mass-Functionality-Type-Distribution (MMFTD) of the (co)polyesters and the Molar-Mass-Chemical-Composition-Distribution (MMCCD) of the fraction containing two alcoholic end groups, of the copolyesters could be studied. Significant differences between strongly resembling copolyesters were found which, as far as the MMCCDs are concerned, can*



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*only be the cause of the different reaction kinetic behaviour in step-reaction copolymers. This makes the assumption that a predictable, theoretical statistically determined CCD is formed in all cases, questionable.*

## 8.1 INTRODUCTION

Until now, most work on the microstructural characterisation of copolymers has been focused on polymers, often styrene containing, made by chain polymerisation. For the determination of the chemical composition distribution (CCD), especially gradient HPLC, which is called gradient polymer elution chromatography (GPEC) in this context, has been shown to be a versatile technique. After first being applied by Teramachi[1] the technique has been used by a (still rather limited) number of workers for the structural investigation of statistical copolymers[1-20] and, to a lesser extent, of block copolymers[21,22] and graft copolymers.[23-29] In this respect, little attention has been paid so far to polymers of relatively low molar mass, synthesised by step-reactions, such as copolyesters. The microstructural characterisation of these products has been focused on the determination of the *intra*molecular microstructure, *e.g.* the *average* sequence distribution (SD) by spectroscopic methods.[30-31]
Due to the occurrence of transesterification reactions next to chain growth (see Chapter 2), it is often assumed that, in the case of copolyesters, complete randomisation will occur. In such a case, with respect to the *inter*molecular microstructure, a statistical CCD will be obtained which only depends on the initial molar ratios of the monomers. No such phenomenon like a conversion dependent CCD due to reactivity differences of the respective monomers causing composition drift, would be expected to occur. In contrast with this, it is sometimes found that, although the *average* composition of step-reaction copolymers is kept constant, the final thermo-mechanical properties depend on the applied reaction scheme or reaction properties[31] which presumably must be ascribed to differences in *inter*molecular microstructure. No methods are available yet to experimentally determine the CCD of copolyesters, which is due to the intrinsic complexity of these products. Caused by their low molar masses, both molar mass and end group effects will seriously interfere with effects from the chemical composition of the polyester backbone in any separation. This hampers the evaluation of the CCD.
In previous Chapters of this thesis, the (qualitative) possibilities of GPEC for the separation of (co)polyesters according to their molar mass, end group composition and composition of the polymer backbone were shown. However, most attention was paid to the investigation of the underlying separation mechanisms. In this Chapter, the potentials of both RP-GPEC and NP-GPEC for the quantitative characterisation of amorphous copolyesters according to their molar mass and chemical microstructure,

are further investigated. For this purpose, a number of copolyesters, varying in molar mass, average chemical composition and CCD were synthesised and used for a systematic study on the effects of those parameters in GPEC. A separation system has been developed which allows a qualitative and quantitative evaluation of CCDs. Relatively large microstructural differences were found between strongly resembling copolyesters. This makes the assumption that a predictable, statistically determined CCD is formed in all cases, questionable. To the author's knowledge, this is the first example in which the existence of a CCD in polyesters made by step-reactions, has been proven experimentally.

## 8.2 EXPERIMENTAL

### 8.2.1 Polymer samples, synthesis and characterisation

All polymer samples used were copolyesters consisting of adipic acid (A), isophtalic acid (I) and di-propoxylated bisphenol-A (D), and their respective homopolyesters. Two well characterised samples, PE2 and PE3, which were synthesised on a large scale, were also used here, for comparative characterisation. Polystyrene equivalent molar masses as determined by SEC, average chemical composition as measured by $^{1}$H-NMR and end group compositions as determined by titrimetric analysis, are given in Table 8.1. For detailed information on the characterisation of the polyesters by NMR and titrimetric measurements, it is referred to Section 3.2.2.

To study the effect of average chemical composition on the chromatographic behaviour, polyesters with varying ratio A:I were synthesised.[32] Equimolar portions of the monomers D and (A + I) (both PA grade from Merck, Darmstadt, Germany) were carefully weighed into a 1 litre glass reactor such that the total amount was about 250 g. As catalyst, 0.25% (m/m) dibutyltinoxide (PA grade, Merck) was added. The mixture was heated up to a temperature of 220 °C, which was reached in 20 minutes, under a nitrogen flow (0.2 l/min) and constant stirring at 150 rpm. During polycondensation, water was constantly removed by the nitrogen flow and was condensed in a Liebig cooler. The progress of the reaction was monitored by SEC and the reaction was stopped at a polystyrene equivalent molar mass of about 7500. To this end, the hot reaction mixture was poured on a cold metal plate to quench the reaction. See products DA, DI, DAI31 - DAI13 in Table 8.1.

To study the effect of the variation in SD and CCD on the chromatographic behaviour, a copolymerisation by transesterification was carried out. For this purpose, portions of the two homopolyesters, DA and DI (Table 8.1) were carefully weighed in a 250 ml glass reactor such that the molar ratio A:I was 1:3 (comparable to samples PE2 and PE3) and the total amount was about 60 g. The reaction mixture was heated up to 204 °C. Other

**Table 8.1.** Polystyrene equivalent molar masses, end group compositions and average chemical compositions of the investigated polyesters

| | | SEC | | | Titrations | NMR | | |
|---|---|---|---|---|---|---|---|---|
| Sample | Reaction time | PS-eq. molar masses $M_n$ | $M_w$ | $D^*$ | Acid number (mg KOH/g) | Molar fractions A | I | D |
| PE2 | | 3400 | 8200 | 2.4 | 24 | 0.12 | 0.38 | 0.50 |
| PE3 | | 3300 | 7900 | 2.4 | 27 | 0.15 | 0.35 | 0.50 |
| DA | 4.5 hours | 3800 | 8300 | 2.2 | 19 | 0.45 | 0 | 0.55 |
| DAI31 | 8.8 | 3300 | 7600 | 2.3 | 17 | 0.37 | 0.13 | 0.50 |
| DAI21 | 10.0 | 3400 | 7900 | 2.3 | 17 | 0.33 | 0.16 | 0.51 |
| DAI11 | 12.0 | 3200 | 6800 | 2.1 | 19 | 0.26 | 0.24 | 0.50 |
| DAI12 | 14.8 | 3600 | 7800 | 2.2 | 18 | 0.16 | 0.33 | 0.51 |
| DAI13 | 18.5 | 3200 | 6900 | 2.2 | 19 | 0.12 | 0.37 | 0.51 |
| DI | 16.8 | 3000 | 6600 | 2.2 | 25 | 0 | 0.49 | 0.51 |
| DAI13-2 | 1.0 hours | 510 | | | | | | |
| | 2.0 | 640 | | | | | | |
| | 3.6 | 770 | | | | | | |
| | 4.0 | 780 | | | | | | |
| | 5.0 | 1180 | | | | | | |
| | 6.0 | 1300 | | | | | | |
| | 6.5 | 1320 | | | | | | |
| | 11.3 | 3200 | | | | | | |
| | 12.3 | 3640 | | | | | | |
| | 13.3 | 4180 | | | | | | |
| | 20.5 | 4100 | 9170 | 2.2 | 14 | 0.13 | 0.37 | 0.50 |
| trans— S2 | 20 min | 3000 | 6900 | 2.3 | | | | |
| S3 | 35 | 3100 | 6900 | 2.2 | | | | |
| S4 | 50 | 3200 | 7100 | 2.2 | | | | |
| S5 | 65 | 3300 | 7200 | 2.2 | | | | |
| S6 | 80 | 3400 | 7400 | 2.2 | | | | |
| S14 | 204 | 4900 | 11500 | 2.3 | | | | |
| endpr. | 305 | 6500 | 16400 | 2.5 | | 0.12 | 0.37 | 0.51 |

*: polydispersity, $M_w/M_n$.

conditions were the same as described above. During reaction, samples of about 500 mg were taken (samples 'trans' in Table 8.1) and the reaction was stopped after 5 hours.

### 8.2.2 Chromatography experiments

The HPLC equipment used was identical to that described in Section 3.2.4. SEC analysis was the same as that described in Section 3.2.2, except that a set of four Shodex (Showa Denko, Tokyo, Japan) KF columns (300 x 8 mm) consisting of KF805, KF804, KF803,

KF802 and a guard column, 800P, was used. This SEC system was also used for fractionation experiments in order to obtain low polydispersity fractions.
For RP-GPEC, the column was a Novapak $C_{18}$ ('$C_{18}$') column ($d_p$ = 4 µm, pore size 60 Å, 150 x 3.9 mm) from Waters (Milford, MA, USA), and the solvents were water (Lichrosolv quality from Merck) and THF (Rathburn, Brunschwig Chemie, Amsterdam, the Netherlands) to both of which 200 µl acetic acid per litre was added.
End group analysis by NP-GPEC was based on results described in Chapter 7. For this purpose, a Jordi Gel DVB Polyamine column ($d_p$ = 5 µm, pore size 500 Å, 250 x 4.6 mm) from Jordi (Bellingham, MA, USA) was used at a temperature of 35 °C and a flow rate of 1.32 ml/min. The applied gradient was heptane (HEP) : dichloromethane (DCM) : THF : methanol (MeOH) (70:30:0:0) (v/v) to (0:100:0:0) (0 to 23.3 min), (0:100:0:0) to (0:0:100:0) (23.3 to 56.6 min), (0:0:100:0) to (0:0:0:100) (56.6 to 80 min), followed by an equilibration procedure as described in Section 7.3.3 (HEP, DCM and MeOH, all Lichrosolv quality from Merck).
For the determination of CCDs by NP-GPEC, a Nucleosil-100-5-$NH_2$ ('$NH_2$') column ($d_p$ = 5 µm, pore size 100 Å, 200 x 4.0 mm) from Machery Nagel (Düren, Germany) was used and the solvents were DCM and THF which were dried overnight on molecular sieve, 0.3 nm (Merck), prior to use.
All solvents were constantly sparged with helium (20 ml/min). All solvent mixtures were made by volumetric mixing by the HPLC pump, no premixes were used. Unless indicated otherwise, for RP-GPEC samples were dissolved in THF and for NP-GPEC in DCM to a concentration of 10 mg/ml of which 10 µl was injected. For gradient strategy, it is referred to Sections 3.2.5 and 7.3.3.

### 8.2.3 Chromatographic fractionation

Chromatographic fractionation according to functionality by NP-GPEC, in order to determine the distribution of the functional end groups over the molar mass distribution was performed using the NP-GPEC method for end group analysis, described in the previous Section. For this purpose, the resulting amounts of 5 injections, each 10 µl and 50 mg/ml, were dried under nitrogen and redissolved into 200 µl THF, from which 50 µl was injected on a SEC system, together with an unfractionated polyester sample.
Fractionation by SEC in order to determine the distribution of the functional end groups over the molar mass distribution was done using the method for SEC analysis, described in the previous Section. Fractions were taken every 0.75 min from 18.5 min to 29 min. The resulting amounts of 4 injections, each 200 µl of 20 mg/ml were dried under nitrogen and redissolved up to a concentration of 3 mg/ml in DCM, from which 30 µl was injected on a PA column.

SEC fractionation for the determination of the CCD as function of molar mass was carried out using the method for SEC analysis, described in the previous Section. To this end, 200 µl of solutions of 20 mg/ml of each sample were injected twice on a SEC system and fractions were taken every 0.75 min between 18.5 and 29 min. SEC fractions were dried under nitrogen and redissolved up to a concentration of 2.0 mg/ml in DCM, from which 10 µl was injected on an $NH_2$ column.
Fractionation by NP-GPEC with the purpose to determine the chemical composition as function of elution time was performed using the NP-GPEC method for CCDs, indicated in the previous Section. Hereto, 20 µl of a solution of 2 mg/ml of the SEC fraction was injected twenty times and ten fractions were taken every 0.7 min between 6.5 and 13.5 min. The obtained NP-GPEC fractions were dried under nitrogen and redissolved in 50 µl deuterated chloroform. NP-GPEC fractions 3-8 were measured by $^1$H-NMR, using a 2.5 mm micro capillary probe.

## 8.3 RESULTS AND DISCUSSION

### 8.3.1 Characterisation of copolyesters according to molar mass, by RP-GPEC

As was shown in Chapter 3, by RP-GPEC highly detailed oligomer separations for (co)polyesters can be obtained. Such separations can be used for the calculation of absolute, average molar masses, provided that a complete separation into oligomers is obtained. Furthermore, detector response factors of the individual oligomers must be known or must approximately be similar. Molar masses of the polyesters used in this study, however, are too high to obtain complete resolution of all oligomers (see for instance Figure 8.1). In Chapter 5 it was shown that for polyesters under certain conditions an almost linear dependence between %-THF at the point of elution ($\varphi_e$) and $1/\sqrt{M}$ is obtained. For sample PE1 (see Table 3.1), it was tried whether this dependence could be useful in this case. This sample was chosen since the average molar masses were known from absolute methods (see Section 3.2.2).
Therefore, for a separation carried out at 25 °C, $\varphi_e$ versus $1/\sqrt{M}$ was described by a first order polynomial, starting at oligomer number 3, to cancel out the deviation in the low molar mass part (see Figure 5.2). From this fit, for which a coefficient of regression, $R^2$, of 0.9992 was found, retention times of the oligomers in the high molar mass range of the chromatogram, up to p = 50 were calculated. Using these retention times, the chromatogram was divided into slices, where each slice represents the weight fraction of the corresponding (known) molar mass. Finally, $M_n$ and $M_w$ values could be calculated using their respective definitions. Results are shown in Table 8.2, together with the molar masses from absolute methods. $M_n$ and $M_w$ from GPEC are average values from nine determinations.

**Table 8.2.** Average molar masses for sample PE1

| | Absolute* | GPEC |
|---|---|---|
| $M_n$ | 3090 ± 5%** | 2630 ± 0.4% |
| $M_w$ | 4900 ± 10% | 4790 ± 0.6% |

*: vapour pressure osmometry ($M_n$) and light scattering ($M_w$).
**: relative standard deviation.

Clearly, $M_w$ calculated from GPEC is in good agreement with the absolute value, whereas the deviation in $M_n$ is somewhat larger. This is probably caused by the fact that in the lower molar mass area, detector response will not be independent of molar mass, which mainly influences $M_n$. In the high molar mass part this dependence is much smaller, since products slightly differing in chemical structure, such as end groups, are taken together into one 'oligomer number', having roughly the same average chemical composition. Furthermore, the detection wavelength, 277 nm, was chosen at the absorption maximum of the diol used. The UV absorption is therefore mainly caused by the diol parts of the oligomers, thus reducing the influence of the di-acid type.
The high reproducibility of the molar mass determinations can be explained from the fact that, unlike in for instance SEC measurements, no external calibration with narrow standard polymers is needed. Inaccuracies due to temperature or flow variations from run to run do not influence the results. Therefore, the method presented here can be considered as a new alternative for the determination of molar masses of relatively low molar mass polymers. A condition for the successful application of this method is of course certain knowledge of chemical composition which is required for the proper assignment of the oligomer peaks. In some cases it will be necessary to use a polynomial fit of higher order to accurately describe the dependence between $\varphi_e$ versus $1/\sqrt{M}$. In this respect, a third order polynomial was found to provide a good description of the data ($R^2 > 0.999$) for all cases, shown in Chapter 5.

The detailed oligomer separations for polyesters obtained by GPEC, can also be of help for kinetic studies. Expressions for molar masses and molar mass distribution of polyesters were discussed in Section 2.1. Since $M_w$ and $M_n$ were already determined by GPEC, the conversion, f, could be calculated from either Eq. (2.3) or Eq. (2.4). In both cases, a value of 0.821 was found. Knowing f, the theoretical oligomer distribution was determined from Eq. (2.6). Since the response of a concentration sensitive detector, such as a UV detector, is directly related to the weight fraction, the oligomer distribution can also be determined from the GPEC chromatogram. To this end, peaks having (x-1), x and (x+1) di-acid units were taken together. The results for the theoretical and practically determined oligomer distribution of sample PE1 are shown in Table 8.3.

**Table 8.3.** Theoretical and experimental oligomer distribution of PE1

| p | Percentage by weight | |
|---|---|---|
| | GPEC | Theoretical |
| 1 | 3.2 ± 0.1* | 3.2 |
| 2 | 5.4 | 5.3 |
| 3 | 6.4 | 6.5 |
| 4 | 7.2 | 7.1 |
| 5 | 7.2 | 7.3 |
| 6 | 7.1 | 7.2 |
| 7 | 6.7 | 6.9 |
| 8 | 6.1 | 6.4 |
| 9 | 5.7 | 6.0 |
| 10 | 5.1 | 5.4 |
| 11 | 4.7 | 4.9 |
| 12 | 4.2 | 4.4 |
| 13 | 3.8 | 3.9 |
| 14 | 3.4 | 3.4 |
| 15 | 3.0 | 3.0 |
| 16 | 2.6 | 2.7 |
| 17 | 2.3 | 2.3 |
| 18 | 2.1 | 2.0 |
| 19 | 1.8 | 1.7 |

*: standard deviation.

The theoretical distribution is in excellent agreement with results found from GPEC. For sample PE1 it can therefore be concluded that the polyesterification proceeded in a regular way, without the occurrence of many side reactions caused by, for instance, anhydride formation. This also corresponds to the fact that the oligomer patterns found in GPEC are very regularly shaped. No unusual peak patterns due to side products can be observed.

### 8.3.2 Characterisation of copolyesters according to chemical composition, by RP-GPEC

In Figure 8.1, the RP-GPEC chromatograms of copolyester PE2 and the homopolyesters, DA and DI, are compared. In the chromatogram of PE2 extra peaks and shoulders as compared to the homopolyesters are present which must be due to copolymer products. This indicates that RP-GPEC can be used to detect the formation of certain products during and after the copolymerisation. As an example, the formation of dimers consisting of two diol units and one di-acid unit, $D_2A$ and $D_2I$ respectively (see also Figure 8.1), was followed during the synthesis of DAI13-2. In Figure 8.2 the logarithm of the peak-area-ratio $D_2A$:$D_2I$ is plotted versus the reaction time. It can be seen that during the first

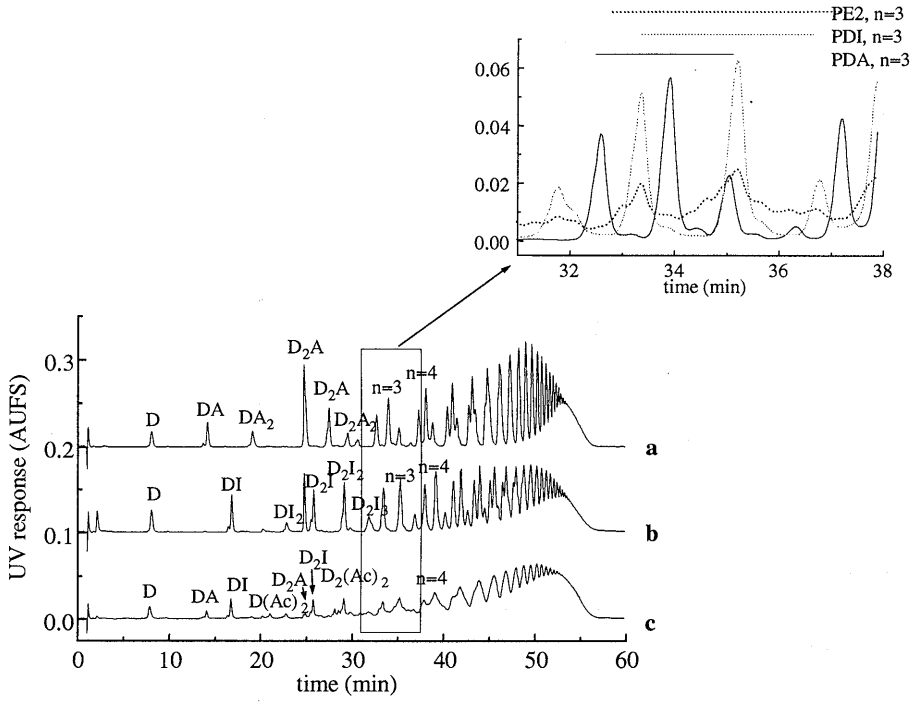


**Figure 8.1.** RP-GPEC chromatograms for homopolyesters PDA (a) and PDI (b) and copolyester PE2 (c). Conditions: sample concentrations: 10 mg/ml, column: Novapak $C_{18}$ (150 x 3.9 mm), temperature: 35 °C, eluent: water-THF (+ 200 µl acetic acid per litre) (70:30, v/v) to (10:90) (0 to 60 min), flow: 1.0 ml/min, injection: 10 µl, detection: UV at 277 nm. D = diol, A = adipic acid, I = isophtalic acid, Ac = acid.

stage of the polycondensation mainly the product $D_2A$ was formed in spite of the fact that the initial amount of the monomer A was much less than the amount of monomer I.
During the reaction, the peak-area ratio $D_2A$:$D_2I$ gradually changes in favour of the latter product. These observations indicate large reactivity differences between both di-acids. Based on SEC measurements, the reactivity ratio $k_A/k_I$ is estimated to be approximately 4.[32] This is in accordance with theory from which it would be expected that due to sterical hindrance, the reactivity of I would be considerably smaller than that of A.[33] Even after a reaction time of 21 hours, when a weight average molar mass of 9200 was reached (Table 8.1), the peak area ratio has not reached a stable value, indicating that the chemical composition in this part of the molar mass distribution is still changing. Qualitatively the same results were obtained for peak ratios of oligomers with a higher degree of polymerisation (p).[32] This indicates that the expectation of the formation of a purely statistically determined CCD, based on the assumption of a thermodynamic equilibrium, is not justified in this case. The (initial) formation of copolyesters with a non-homogeneous distribution of both di-acids over

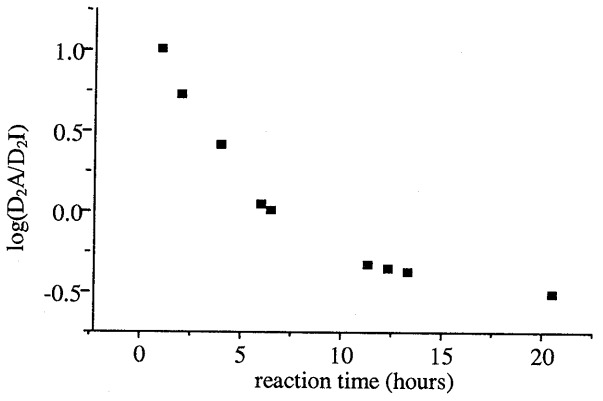


**Figure 8.2.** Logarithm of the peak area ratio of peaks $D_2A$ : $D_2I$ of product DAI13-2 measured by RP-GPEC as function of reaction time. RP-GPEC conditions: see Figure 8.1.

the molar mass distribution due to the existence of reactivity differences, is in accordance with the theoretical considerations as described in Section 2.2.
The formation of oligomers having 0, 1 or 2 acidic end groups can also be followed from RP-GPEC. An example for the oligomer p = 2 of sample DAI13-2 is shown in Figure 8.3, but qualitatively the same observations were made for other oligomers and other copolyesters with a different average chemical composition. During the first stage of the reaction, preferentially oligomers with two alcoholic end groups are formed, whereas the formation of oligomers containing one or two acidic end groups, increases as the reaction proceeds. Nevertheless, even after 21 hours no statistical distribution of 1:2:1 is reached

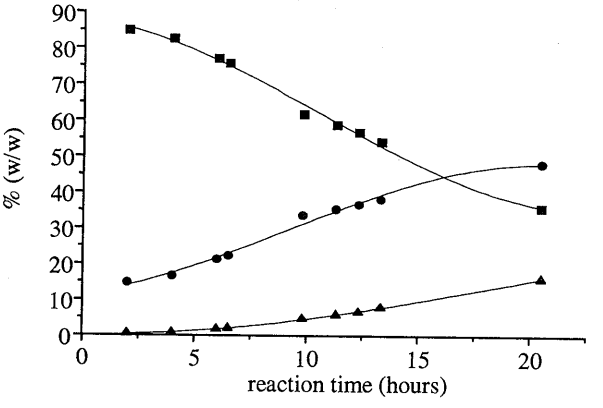


**Figure 8.3.** Relative amounts (%, w/w) of oligomers with p = 2, having different end groups, as function of reaction time, measured by RP-GPEC. ■: 0 acid end groups, ●: 1 acid end group, ▲: 2 acid end groups. RP-GPEC conditions: see Figure 8.1.

for oligomers containing 0, 1 and 2 acidic end groups, which would be expected from the molar ratios of the monomers. This indicates that also the formation of end groups does not develop randomly.

In Figure 8.4, the copolyesters made at a large scale, PE2 and PE3, which are similar in overall chemical composition as was confirmed by SEC and NMR (Table 8.1), are compared by RP-GPEC. Both samples appear to exhibit somewhat different mechanical properties. Several chemical differences between both products can be indicated from the low molar mass part of the chromatograms. At first, from the different peak shape of the diol peaks, it can be concluded that the purity of this monomer is different in both cases, which was confirmed by NMR analysis. Furthermore, a large difference between the peak area ratio DA:DI (Figure 8.4) is found: 0.29 ± 0.01 for PE2 versus 0.62 ± 0.01 for PE3. Although this indicates that PE3 contains more adipic acid, these differences are much larger than the differences in molar ratios A:I found by NMR: 0.32 ± 0.02 for PE2 and 0.43 ± 0.02 for PE3. Since NMR provides information on the bulk composition and GPEC, in this case, on the composition of a low molar mass part, it is obvious that for PE3 the amount of adipic acid in the low molar mass part of the sample compared to the average is much higher than for PE2. This indicates that the distribution of A and I over the molar mass

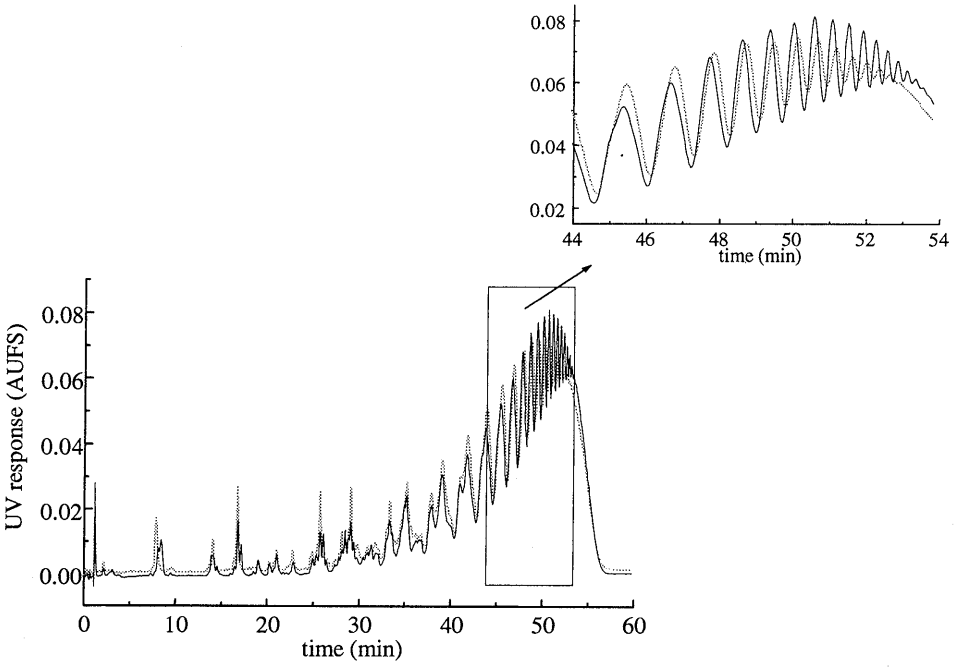


**Figure 8.4.** Comparison of PE2 (grey line) and PE3 (black line) by RP-GPEC. RP-GPEC conditions: see Figure 8.1.

distribution, at least in the low molar mass part, is not completely homogeneous and clearly different for PE2 and PE3. The existence of such intermolecular microstructural differences cannot be detected by a conventional method such as NMR, alone.

### 8.3.3 Effect of chemical composition on elution characteristics in RP-GPEC

From Figure 8.4, several other differences between the elution patterns of PE2 and PE3 can be observed. Retention times of the higher molar mass oligomers are somewhat higher for PE2 than for PE3. Furthermore, peak heights of oligomers 10-20 are significantly larger for PE3 than for PE2. All differences were found to be highly reproducible and must therefore be the result of chemical differences between both copolyesters. Hence, the effect of several parameters, *e.g.* average chemical composition, CCD and end group composition, on the peak retention times and peak widths was further investigated. It was hoped that this would provide more insight in the nature of the observed differences between both copolyesters.

#### *8.3.3.1 Effect of average chemical composition on oligomer retention*

For the investigation of the influence of average chemical composition on oligomer retention, retention times for all oligomers of the polyesters DA, DI and DAI31-DAI13 were determined. A characteristic example is shown in Figure 8.5, from which it can be seen that for oligomer p = 12 retention time decreases with increasing amount of A. Qualitatively the same trends were found for all other oligomers. Subsequently, for all oligomers with varying p, retention times were fitted versus chemical composition. The used software (TableCurve) provided several curve types, both polynomial and logarithmic, which reasonably described the obtained trends for the respective oligomers. For each oligomer with a specific p, the five best curves showing a monotonous decrease as function of chemical composition, were taken into account for further calculations. Finally, for the two copolyesters of interest, PE2 and PE3, the chemical composition for each oligomer was determined from the respective calibration curves of retention time versus chemical composition. For these determinations, the average retention time error (0.02 min) which was determined from duplicate measurements, was taken into account as well as the various experimentally obtained functions which resulted from the fitting procedure (TableCurve). Thus, it was possible to obtain an average chemical composition for each oligomer peak together with a reasonable estimate of the experimental error of the method of determination. Results for both copolyesters are shown in Figure 8.6 from which it is easily seen that large differences are found.

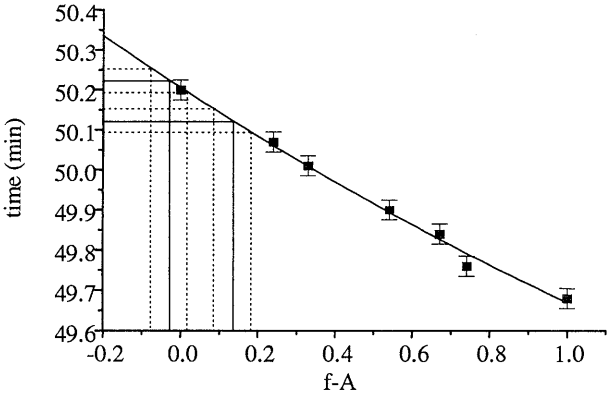


**Figure 8.5.** Retention time of oligomer p = 12 in RP-GPEC as function of the average chemical composition of the copolyesters. RP-GPEC conditions: see Figure 8.1.

For PE3, no significant dependence of the (average) chemical composition on p is found. (Note that the chemical composition is expressed in the fraction of A, f-A, where the sum of both fractions, f-A and f-I is taken unity. This differs from the molar fractions as given in Table 8.1). Furthermore, although calculated f-A values seem to be somewhat lower than f-A found by NMR ($0.30 \pm 0.02$), in most cases no significant difference is found when the experimental error is taken into account. For PE2, lower values of f-A as compared to PE3 are found, which is in qualitative agreement with NMR (Table 8.1). However, in contrast with PE3, large variations of f-A with p are observed even resulting in negative values which have, of course, no physical

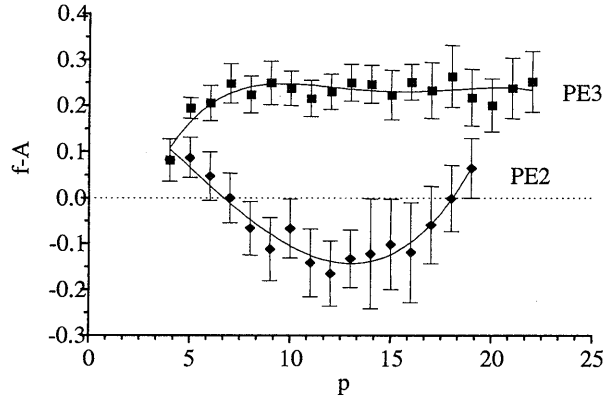


**Figure 8.6.** Calculated fractions adipic acid (f-A) from RP-GPEC for the various oligomers of PE2 and PE3. RP-GPEC conditions: see Figure 8.1.

relevance.
These findings contrast with results of SEC in combination with UV and DRI detection. In that method, chromatograms from both detectors exactly coincide, except for the low molar mass oligomers. This clearly suggests no large dependence between the *average* chemical composition and p for the higher molar masses.[32] Presumably, peak position is also influenced by other parameters than average chemical composition, thus giving rise to negative f-A values (see next Sections). Nevertheless from Figure 8.6 it can again be concluded that significant chemical differences between both copolyesters are present.

### *8.3.3.2 Effect of the average end group composition on oligomer retention*

As a next step, the effect of end group composition on the oligomer peak position was investigated. For the higher molar mass oligomers, peaks are in fact composite peaks, consisting of fractions containing 0, 1 and 2 acidic end groups. Retention time within an oligomer fraction with a certain degree of polymerisation, p, increases with increasing number of acidic end groups (see Chapter 3). Therefore, the average end group composition of the various copolyesters was determined by NP-GPEC (see experimental Section), to elucidate whether the dispersity in peak positions (Figure 8.4) could possibly be explained from different end group compositions. Results are shown in Table 8.4.

**Table 8.4.** Peak area percentages from NP-GPEC for the end group fractions of various (co)polyesters

| Sample | 2 alcoholic end groups (%, w/w) | 1 or 2 acidic end groups (%, w/w) |
|---|---|---|
| PE2 | 0.30 ± 0.01* | 0.70 ± 0.01 |
| PE3 | 0.40 | 0.60 |
| DA | 0.33 | 0.67 |
| DAI31 | 0.36 | 0.64 |
| DAI21 | 0.40 | 0.60 |
| DAI11 | 0.39 | 0.61 |
| DAI12 | 0.38 | 0.62 |
| DAI13 | 0.41 | 0.59 |
| DI | 0.39 | 0.61 |

*: standard deviation.

Clearly, PE2 contains more acidic end groups than PE3 and the other, 'model' copolyesters. This will result in somewhat longer retention times of the respective oligomers and therefore to apparent f-A values which are too low. It is furthermore worthwhile noting that the end group composition of PE3 is roughly the same as that of most model copolyesters, whereas for PE2 a significant difference is found. Thus, end groups differences may account for the observed retention time differences between

oligomers from PE2 and PE3 and therefore also for differences as observed in Figure 8.6. Nevertheless, when looking at Figure 8.4, it is felt that this explanation can not completely account for the differences in the oligomer patterns, since retention time shifts for especially the higher molar mass oligomers are relatively large as compared to the total peak width.

### *8.3.3.3 Effect of chemical microstructure on oligomer retention*

Another possible explanation for retention time differences is a difference in blockiness between the copolyesters. From comparative studies on block and random copolymers[21] it is known that retention time differences between both types of polymers will occur, due to the fact that block copolymers behave more like homopolymers of one kind. To check whether such intramolecular structural differences also influence retention behaviour of copolyesters, the elution behaviour of samples 'trans' which were taken during the transesterification reaction, was studied. In these products, the blockiness will decrease with time, resulting in a more randomised product. In Figure 8.7, only the retention time of oligomer p = 12 is plotted as a function of the transesterification time, but exactly the same trends were found for the other oligomers. Clearly, retention decreases with increasing reaction time. This is not caused by a changing end group composition during transesterification, since NP-GPEC measurements showed no significant differences between the respective products. Therefore, observed retention differences may be attributed to the fact that in the beginning of the reaction, products will behave more like a homopolymer with isophtalic acid. Later on, more randomised

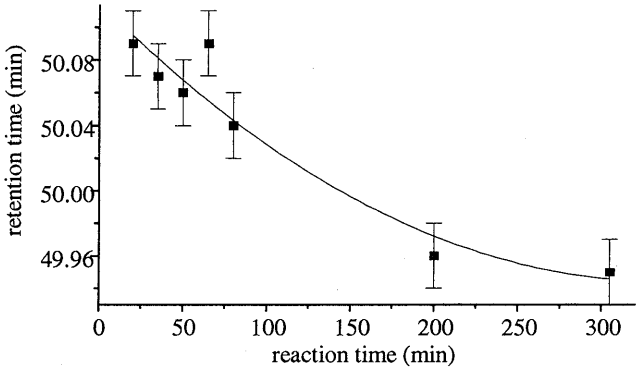


**Figure 8.7.** Retention time of oligomer p = 12 of the copolyester made by transesterification, in RP-GPEC as function of transesterification time. RP-GPEC conditions: see Figure 8.1.

copolymers are formed, thus giving rise to a decrease in retention. This, however, is somewhat speculative, since opposite trends, *e.g.* increasing oligomer retention times, were found for products taken during another transesterification, where the molar ratio of A:I was 1:1.[32] In any case, oligomer retention is clearly influenced by its intramolecular microstructure.

### *8.3.3.4 Effect of chemical microstructure on oligomer resolution*

Next to differences in oligomer retention times, also differences in resolution for the higher molar mass oligomers between PE2 and PE3 are found (Figure 8.4). Peak widths of the various oligomers are certainly related to the broadness of the distributions according to chemical composition, oligomer sequence and end groups. This is evidenced from the comparison of the chromatographic behaviour of products trans-S2-trans-S6 (Figure 8.8). Due to the transesterification, a broadening of SD and CCD occurs which obviously results in an increasing peak width for the respective oligomers. The increasing peak widths cannot be ascribed to end groups, since, from NP-GPEC, the end group composition was found to remain constant during transesterification (result not shown).

Thus, indeed differences in SD and/or CCD *might* be the cause of the difference in oligomer resolution between PE2 and PE3. Nevertheless, these may also be caused by the observed differences in end group composition (Table 8.4), or FTD. From RP-GPEC alone, it is not possible to discriminate between the various phenomena.

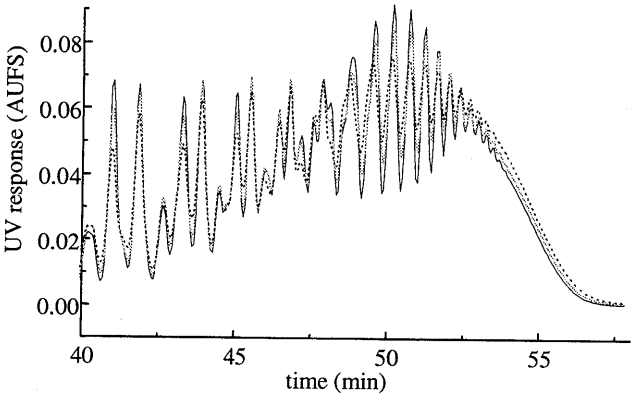


**Figure 8.8.** Effect of transesterification time on the peak width in the high molar mass part of the RP-GPEC chromatograms. Black line: trans-S2 (20 min), grey line: trans-S4 (50 min), dotted line: trans-S6 (80 min). RP-GPEC conditions: see Figure 8.1.

During the transesterification reaction in which products 'trans' were formed, the molar mass changed only to a minor extent (Table 8.1) during the first 80 minutes, and no differences at all could be observed from NMR[32] in contrast to RP-GPEC (Figure 8.8). After this time, molar mass started to increase which could be observed from both SEC and RP-GPEC. These results suggest that in the beginning mainly transesterification reactions occur, and after a certain time, chain growth becomes more important. Thus, RP-GPEC provides more information on the proceeding of copolymerisation by transesterification than conventional methods do. Nevertheless, due to the relatively low resolution within especially the higher molar mass oligomers, it cannot unambiguously be seen, whether the transesterification after 80 minutes has led to a completely random product.

### *8.3.3.5 Conclusions on the use of RP-GPEC for microstructural characterisation*

It has been shown that both peak retention and resolution of oligomer peaks of copolyesters with nearly equal molar mass in RP-GPEC, are influenced by various parameters. RP-GPEC seems to be very sensitive to microstructural differences between copolyesters. This is again demonstrated in Figure 8.9, where three copolyesters are compared, polymerised at a large scale, at laboratory scale, and by transesterification respectively. Although SEC and NMR measurements suggested the products to resemble closely (Table 8.1), clear differences are observed from RP-GPEC. Nevertheless, from RP-GPEC alone it is difficult to assign those variations, especially for the higher molar mass oligomers, to either end group, chemical composition or sequence differences.

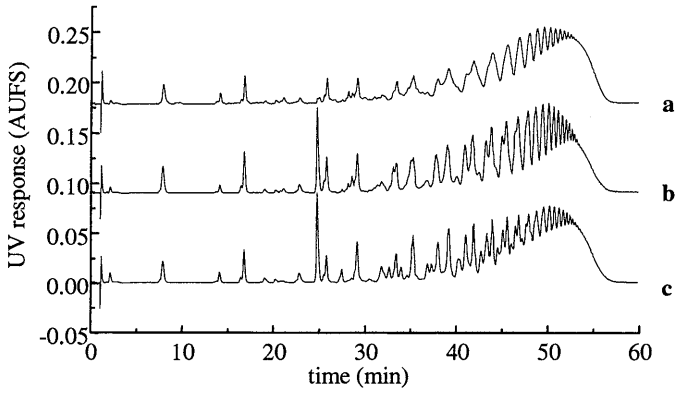


**Figure 8.9.** Comparison of PE2 (a, copolyester made at a large scale), DAI13 (b, copolyester made at laboratory scale) and trans-S6 (c, copolyester from transesterification) by RP-GPEC. RP-GPEC conditions: see Figure 8.1.

This is mainly due to the fact that separation is dominated by molar mass, and resolution with respect to chemical composition differences is relatively low. Therefore, RP-GPEC for low molar mass copolyesters must mainly be considered as a qualitative fingerprinting tool, rather than a method by which structural differences can be quantitatively detected.

### 8.3.4 Determination of the MMFTD of copolyesters by NP-GPEC/SEC

In Chapter 7 it was shown that in NP-GPEC, separation of (co)polyesters is dominated by their chemical composition, especially the end group composition, whereas molar mass plays a less important role as compared to RP-GPEC. From a separation, comparable to that shown in Figure 7.7, the amounts of the various end group fractions, *e.g.* fractions containing respectively 0, 1 or 2 acidic end groups, for the two copolyesters, PE2 and PE3, were obtained. The weight fraction containing two alcoholic end groups was found to be 0.30 ± 0.01 for PE2 and 0.40 for PE3. This significant difference in average end group composition was not reflected in the acid numbers from titration analysis, which revealed 24 ± 10% mg KOH/g for PE2 and 27 for PE3 (Table 1). Obviously, results from NP-GPEC are much more sensitive to deviations in end group composition than titration analysis.
Furthermore, it is interesting to determine the molar mass distributions (MMD) of the respective end group fractions, to obtain information about the distribution of the end groups over the MMD. For this purpose, samples PE2, PE3 and PE7 were separated into three fractions on the PA column, *e.g.* cyclics, diol and mono-acid + di-acid. The latter two fractions were taken together, since no baseline separation could be achieved in NP-GPEC (see Chapter 7). See Section 8.2.3 for the fractionation conditions. The thus obtained fractions were characterised by SEC, together with an unfractionated polyester sample. The resulting chromatograms for PE2 and PE7 are shown in Figure 8.10 and the corresponding polystyrene equivalent weight average molar masses in Table 8.5. It can be seen that the MMDs of the respective end group fractions are not identical to the MMDs of the unfractionated polyesters. The molar masses of the cyclic products are very low, as is expected from theory.[34] Molar masses of all diol fractions are shifted towards lower molar mass as compared to the unfractionated polyesters, whereas a shift towards higher molar masses is found for the mono-acid + di-acid fractions. Shifts are comparable for both PE2 and PE3 (not shown), but significantly lower for PE7. These results are in qualitative agreement with results from other workers.[27,35] Shifts in molar masses most probably cannot be attributed to differences

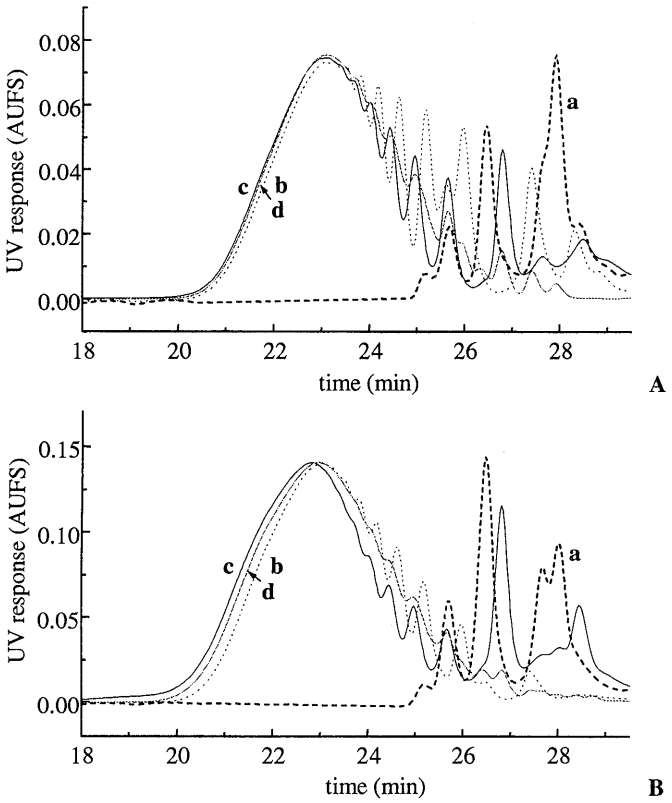


**Figure 8.10.** SEC chromatograms of NP-GPEC fractions of PE7 (A) and PE2 (B). a (dash): cyclics, b (dot): diol, c (black): mono + di-acid, d (grey): unfractionated polyester. NP-GPEC conditions: see text. SEC conditions: sample concentrations: see text, columns: Shodex KF805, KF804, KF803, KF802, KF800p (guard columns) (in series), temperature: 40 °C, eluent: THF + 1% (v/v) acetic acid, flow: 1.5 ml/min, injection: 200 µl, detection: UV at 254 nm.

in hydrodynamic volume due to differing end groups, since it was shown for other polyester types that above a molar mass of about 700, such differences no longer affect hydrodynamic volumes.[35]

To further confirm these results, the reversed analysis was carried out. For this purpose all three polyesters were separated into 14 fractions on SEC, which were subsequently injected on a PA column. Fractionation conditions: see Section 8.2.3. End group compositions were determined for each SEC fraction, the results of which are shown in Figure 8.11. These measurements qualitatively confirm the NP-GPEC/SEC results. For PE7 only slight changes of end group composition as function of molar mass are observed, whereas changes for both PE2 and PE3 are much more pronounced. In contrast to the analysis described above, also differences between PE2 and PE3 are found. This is

**Table 8.5.** Weight average molar masses ($M_w$) of the end group fractions, obtained by NP-GPEC

| Sample | $M_w$ | | | |
|---|---|---|---|---|
| | Unfractionated | Cyclics | Diol | Mono + di-acid |
| PE2 | 8200 | 1200 | 7600 | 9500 |
| PE3 | 7900 | 1200 | 7500 | 8800 |
| PE7 | 6800 | 1200 | 6300 | 6900 |

presumably due to the higher number of fractions taken from SEC in this analysis as compared to the NP-GPEC fractionation described above, thus providing more detailed information. Obviously, by combining SEC and NP-GPEC, differences in FTMMD between closely resembling polyesters can be determined. The explanation for the non-homogenous distribution of end groups over the MMD remains unclear and does not follow straightforwardly from theories on kinetics of polyesterification.[34]

## 8.3.5 Determination of the MMCCD of copolyesters by SEC/NP-GPEC

### *8.3.5.1 Separation according to the chemical composition of the polyester backbone*

In Section 7.4.5 it was shown that within a specific end group fraction, a further separation according to the chemical composition of the polyester backbone can be obtained. Hence, NP-GPEC in potential can be used to study the CCD of copolyesters without interference with the end group composition (Figure 7.7B). For the kind of copolyesters in this study, this can only be done for the diol fractions. In the mono-acid and di-acid fractions, end groups can be either isophtalic acid or adipic acid. Since the di-acid type also influences the separation, for these fractions no method can be obtained which is independent of end group composition.

In order to use NP-GPEC for the determination of CCDs, at first the separation was optimised. In Chapter 7, it was found that DCM-THF is a good combination of a chromatographically weak and a strong displacer for this purpose and not much improvement in separation can be achieved by choosing other eluents. The separation was improved by decreasing the gradient steepness. A steepness of 0.4 % (v/v) THF/min was found to be a good compromise between resolution and analysis time.[36] Temperature especially influences resolution with respect to molar mass (oligomers). In Figure 7.7B, it can be seen from oligomer patterns that molar mass separation within one polyester end group fraction, also occurs. This, of course, is unwanted when a separation to solely chemical composition is desired. At a temperature of 45 °C, molar mass

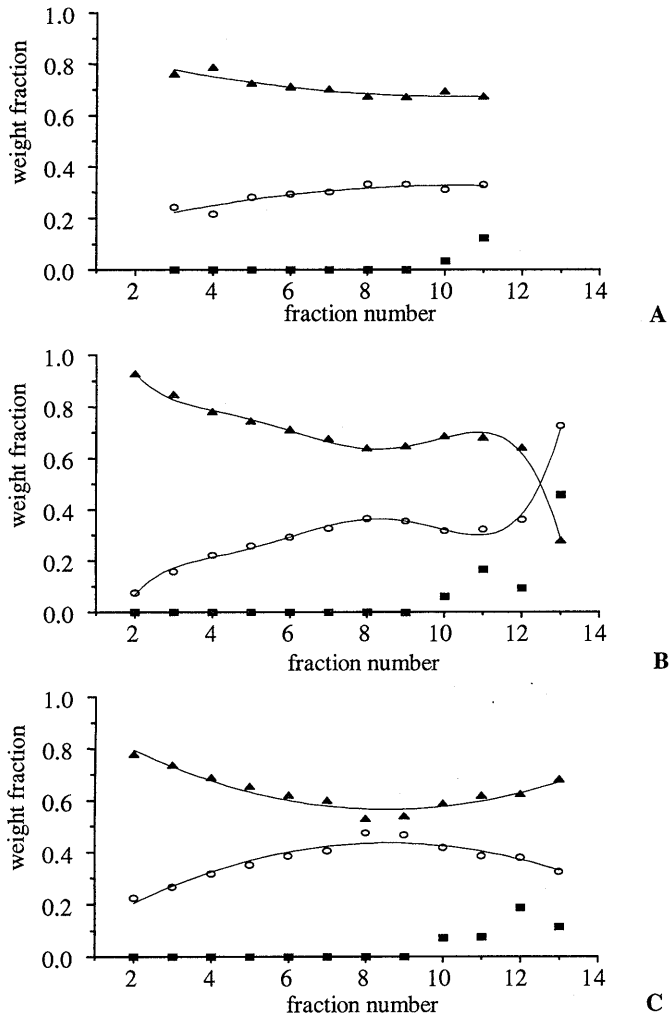


**Figure 8.11.** Amounts of the respective end group fractions versus SEC-fraction number. A: PE7, B: PE2, C: PE3. ■: cyclics, O: diol terminated chains, ▲: mono and di-acid terminated chains. SEC and NP-GPEC conditions: see Section 8.2.2.

resolution is minimised and the homopolyester PDA almost elutes as one peak. Figure 7.7B shows the optimal separation result.

Since molar mass influences cannot be completely suppressed in NP-GPEC, an additional step is necessary in order to obtain a separation which is only governed by the chemical composition of the polyester backbone. Therefore, as a first step, (co)polyesters were fractionated by SEC (see Section 8.2.3). Thus, for each (co)polyester, low dispersity fractions with equal hydrodynamic volume and therefore approximately equal molar masses, were obtained. Polystyrene equivalent molar masses and polydispersity values,

obtained by re-injection of the fractions on SEC, are given in Table 8.6. For the subsequent analysis by NP-GPEC, SEC fractions were redissolved up to a concentration of 2.0 mg/ml in DCM. Care was taken that the final concentrations of 2.0 mg/ml were made accurately, since concentration variations have been found to influence retention time.[36] This of course is unfavourable here, since retention is used for the estimation of chemical composition. Thus, by combined SEC/NP-GPEC in fact a three dimensional separation *e.g.* subsequent separation on molar mass, end groups and chemical composition of the polyester backbone, is obtained, in which the latter two separation steps are brought about in one chromatographic run.

**Table 8.6.** Polystyrene equivalent molar masses of low polydispersity fractions of (co)polyesters obtained by SEC

| Fraction number | $M_n$ | $M_w$ | $D^*$ |
|---|---|---|---|
| 3 | 25500 | 27600 | 1.08 |
| 4 | 16800 | 18200 | 1.07 |
| 5 | 11600 | 12400 | 1.05 |
| 6 | 8000 | 8600 | 1.08 |
| 7 | 5400 | 5700 | 1.06 |
| 9 | 2400 | 2500 | 1.07 |

*: polydispersity ($M_w/M_n$).

In Figure 8.12, NP-GPEC chromatograms of SEC fraction 5 of the homopolyesters and copolyesters DAI31-DAI13 are shown. For both homopolyesters, relatively narrow peaks are obtained. The retention of the copolyesters steadily increases with increasing f-A which is in accordance with expectations based on the chromatographic behaviour of the homopolyesters. It is interesting to note that the observed retention time dependence is opposite to what was found in RP-GPEC (Figure 8.5) which would also be anticipated from polarity rules. The peak width for the copolyesters is significantly larger than that for the homopolyesters. This must be due to the fact that the chemical composition of the former polymers is less homogeneous thus proving that copolyesters indeed have a CCD. To the author's knowledge, this is the first example of an experimental verification of the occurrence of a CCD in a copolyester made by step-reaction.

In order to further confirm the separation as shown in Figure 8.12, SEC fraction 3 of sample PE2 was further separated into fractions by NP-GPEC (Section 8.2.3). The obtained fractions were measured by $^1$H-NMR, spectra are shown in Figure 8.13. The different signal to noise ratio for the respective spectra is due to a varying number of pulses. Signals at 8.2 and 8.6 ppm are due to isophtalic acid and the signal at 2.3 ppm is due to adipic acid. It is easily recognised that the relative intensity of the signal at 2.3 ppm increases with increasing fraction number, indicating an increasing amount of adipic acid. This confirms earlier observations that the NP-GPEC separation is indeed

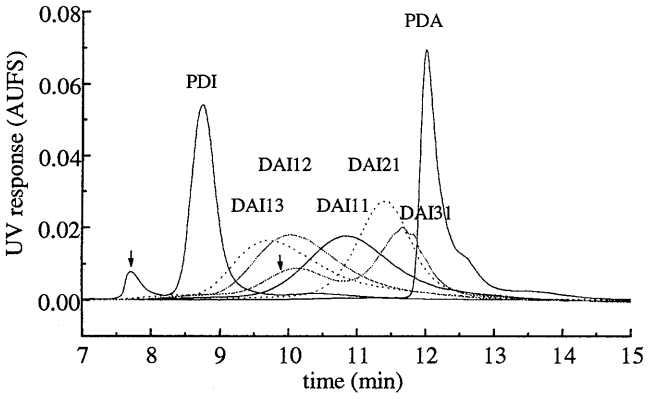


**Figure 8.12.** NP-GPEC chromatograms of SEC fraction 5 of homopolyesters PDA and PDI and copolyesters DAI31 - DAI13. Contaminations indicated with arrows (DAI31 is probably contaminated with DAI12). Sample concentration: 2 mg/ml in DCM, column: Jordi gel DVB polyamine (250 x 4.6 mm), temperature: 45 °C, eluent: DCM-THF (100:0, v/v) to (94:6) (0 to 15 min), flow: 1.5 ml/min, injection: 10 µl, detection: UV at 277 nm.

based on the chemical composition of the polyester backbone.

### *8.3.5.2 Evaluation of the CCD of copolyesters*

In order to be able to calculate CCDs of copolyesters, the NP-GPEC system has to be calibrated. This was done by fitting the retention times of the distribution maxima of SEC fractions 3-7, versus chemical composition (f-A). Thus, for each SEC fraction, *i.e.* molar mass, a calibration curve was obtained. Although, like for RP-GPEC, again several curve types providing a reasonable fit of the data points were obtained, for further calculations only one curve type showing a monotonously increasing function, was taken into account.[36] Since the peak maximum does not necessarily represent the average composition, the method used here must mainly be considered as a rough, first approximation of the quantitative calculations of polyester CCDs. Improvements could be made by using an iterative procedure as proposed by Teramachi *et al.* to calculate the retention time corresponding to the average chemical composition.[23] Fractions 1 and 2 were not taken into account due to the low amounts of sample available. This was also the case for fractions 8 and higher, since separations into oligomers were obtained, thus hindering unambiguous chemical composition calibration.

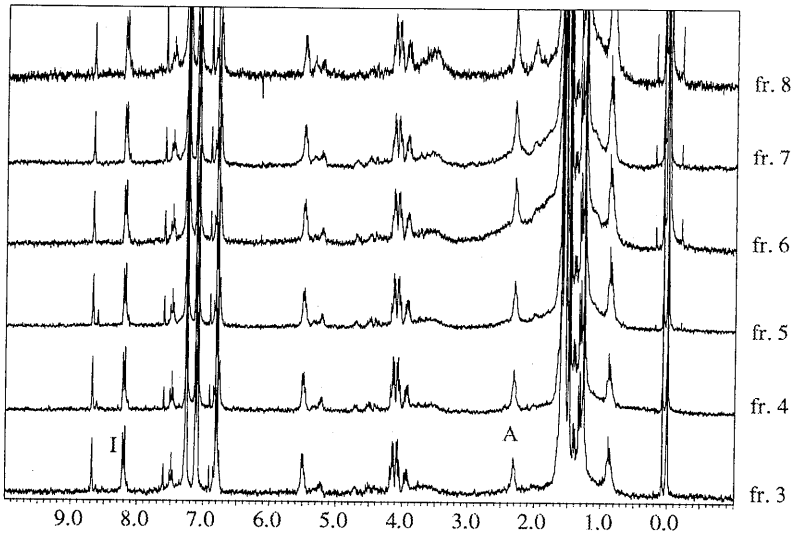


**Figure 8.13.** $^1$H-NMR spectra of NP-GPEC fractions of SEC fraction 3 of PE2. NP-GPEC fractions as indicated in Figure. NP-GPEC fractionation conditions: see text. NMR conditions: 2.5 mm micro capillary probe, solvent: $CDCl_3$. A and I indicate signals due to adipic acid and isophtalic acid, respectively.

The calibration procedure for the calculation of CCDs, as described above, is very time consuming, since for each molar mass fraction several standards (in this example: 7) have to be measured. Furthermore, due to the relatively poor reproducibility of NP-GPEC (see Chapter 7), the calibration has to be repeated each time when fresh eluent or another column is used. Therefore, it was tested whether repeated calibrations could be restricted to the analysis of only the homopolyester fractions. For this purpose, retention differences between two calibration procedures for the homopolyesters were used to calculate, from linear interpolation, the retention shifts for the respective copolyesters. From these shifts, retention times in the new analysis for the copolyesters could be calculated. The values thus obtained were found to differ only 1% at maximum from the experimentally obtained retention times, thus validating the proposed simplification of the calibration procedure.

In Figure 8.14, the calculated CCDs of fractions 4-6 of both PE2 and PE3 are shown. Distinct differences between both copolyesters are found. For fractions 4-6, it is easily recognised that the average f-A of PE2 is lower than that of PE3. This is in qualitative

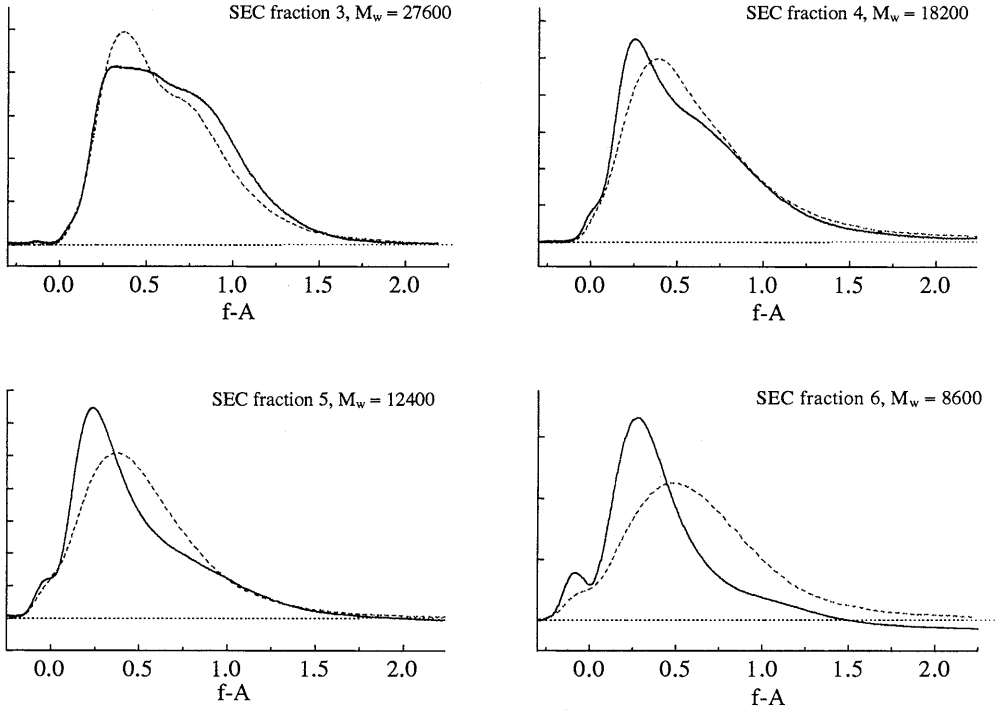


**Figure 8.14.** Comparison of calculated CCDs of SEC fractions 4-6 of PE2 (straight line) and PE3 (dashed line).

accordance with results from NMR (Table 8.1), although the found differences by NP-GPEC seem to be relatively large compared to the differences found by NMR. In contrast, for fraction 3, f-A for PE3 is slightly lower than that for PE2, which indicates differences in the distribution of both di-acids over the molar mass distribution. The relatively high f-A for PE3 in the low molar mass fractions qualitatively confirms RP-GPEC results where peak ratios were compared with NMR results (Section 8.3.2). For PE2 a slight trend towards a lower f-A is observed with decreasing molar mass. This is in accordance with the lower reactivity of isophtalic acid as compared to adipic acid (reactivity ratio, C, approximately 4 (Eq. 2.9)), as was argued in Section 2.2 (see Figure 2.2). Some care must be taken at this point, however, since NMR measurements of the various fractions could not unambiguously confirm this observation.

Since the ratio A:I for PE3 deviates somewhat less from unity than that for PE2, it might be expected that the CCDs of the former copolyester are somewhat broader. This is indeed the case for fractions 5-7. Nevertheless, the differences in broadness for fractions 5 and 6 seem to be larger than what might be expected from the small difference in average composition. This is confirmed by a comparison of the chromatograms of those

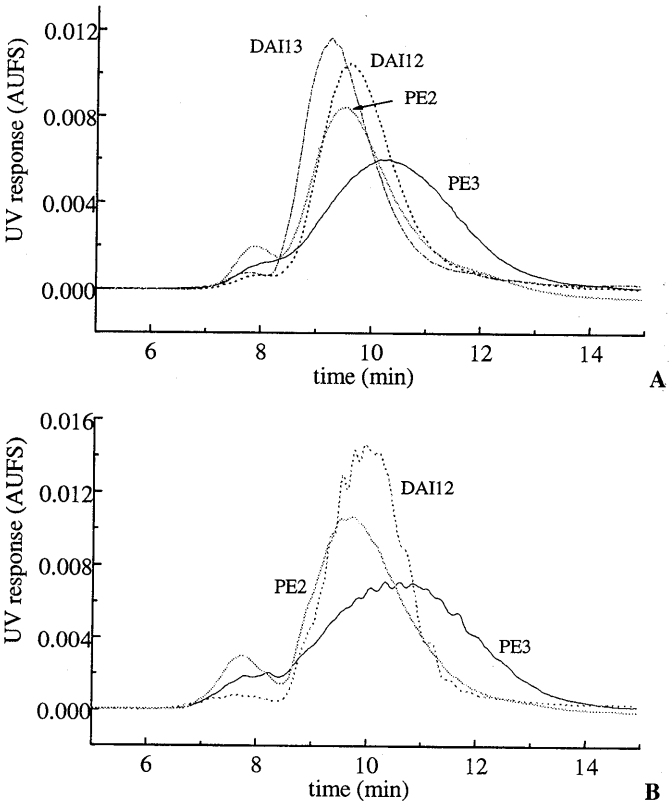


**Figure 8.15.** Comparison of peak width in NP-GPEC of SEC fraction 5 (A) and 6 (B) of PE2, PE3, DAI12 and DAI13. NP-GPEC conditions: see Figure 8.12.

fractions with the chromatograms of corresponding fractions of copolyesters DAI13 and DAI12, see Figure 8.15. Although f-A for PE3 lies in between that of DAI13 and DAI12 (Table 8.1), the distribution for PE3 is significantly broader. In contrast, the broadness of the distribution of PE2 is comparable to that of DAI13 and DAI12. It seems that in terms of CCD, PE2 much more resembles the model copolyesters than PE3 does, whereas with respect to the end group composition the opposite was found (Table 8.4). Furthermore, the somewhat broader CCD of PE2 as compared to PE3 for fraction 3 is also unexpected.

For statistical reasons, the broadness of the CCD is expected to decrease with increasing molar mass, since the probability of the formation of long chains with a chemical composition largely differing from the average composition is lower than that of the formation of short chains.[37] Especially for PE2, the opposite trend is observed, which indicates that the CCDs of the respective molar mass fractions cannot be purely described from statistics based on the assumption of thermodynamic equilibrium conditions.

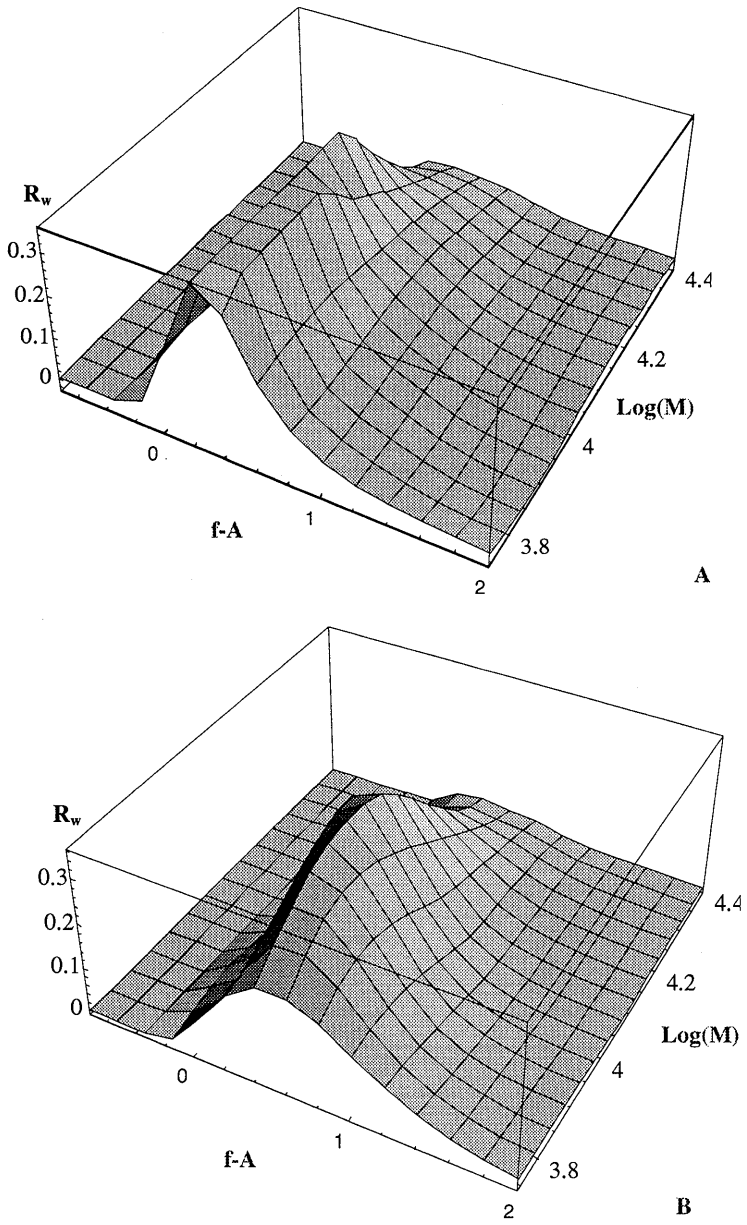


**Figure 8.16.** Comparison of (a part of) the MMCCDs of the diol fractions of PE2 (A) and PE3 (B). SEC conditions: see text. NP-GPEC conditions: see Figure 8.12. $R_w$: relative amounts.

Obviously, microstructural differences found between both copolyesters can qualitatively only be explained from the different reaction kinetic behaviour, determining the rates of transesterification versus chain growth reactions and thus also the final microstructure (see Section 2.2).
It must be kept in mind that the calculated CCDs in Figure 8.14 have not been corrected for chromatographic broadening, which explains f-A values exceeding unity. This effect influences the total peak width to a significant extent as can be concluded from a comparison of the homopolyesters and the copolyesters in Figure 8.12. Therefore, the differences between the two copolyesters are certainly masked by the chromatographic broadening, indicating that the relative differences due to chemical composition variations are even larger than would be concluded from Figure 8.14. Unfortunately, a model for the chromatographic broadening correction is not available for polymers in adsorption chromatography under non-equilibrium, gradient elution conditions. A final comparison between PE2 and PE3 is made in Figure 8.16, where the MMCCD plots (which, for reasons mentioned earlier, cover only a part of the total molar mass distribution) for the diol fractions are given. From this Figure and from the discussions above, it is clear that the chemical microstructure of both products is significantly different, an insight not revealed by the slightly different average composition.

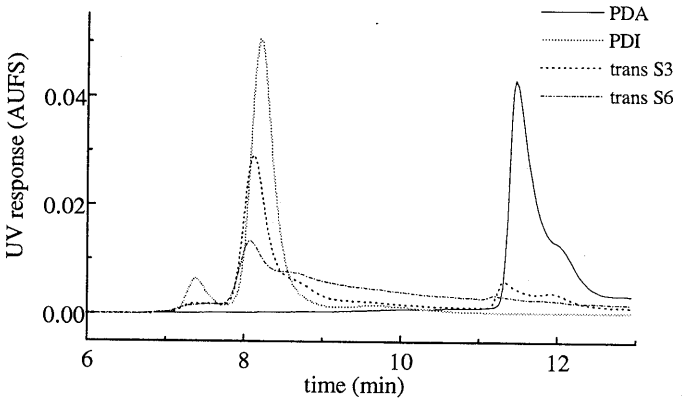


**Figure 8.17.** NP-GPEC chromatograms of SEC fraction 5 of homopolyesters PDA (black) and PDI (grey) and transesterification products trans-S3 (dot) and trans-S6 (dash). NP-GPEC conditions: see Figure 8.12.

In Figure 8.17, a comparison is made between chromatograms of SEC fraction 5 of both homopolyesters and products trans-S3 and trans-S6 taken during the transesterification reaction. It is observed that in the copolyesters relatively large fractions of the homopolyesters are present and that the CCDs are far from a statistical distribution. This

indicates that the transesterification reaction after 80 minutes (trans-S6) has not led to a product with a degree of randomisation comparable to the other copolyesters in this study. This conclusion could not unambiguously be drawn from RP-GPEC where the observations on oligomer peak width in the high molar mass part even might suggest that randomisation of this product is complete (see Section 8.3.3.4).
It is obvious that NP-GPEC provides more insight in the chemical microstructure of copolyesters than RP-GPEC. Whereas RP-GPEC is mainly a fingerprinting tool which can be used as a relatively simple and versatile method to detect differences between samples, by NP-GPEC it is possible to determine, both qualitatively and quantitatively, the origin of these differences, *e.g.* either end group distribution or the CCD of the backbone. In future, the coupling of NP-GPEC results with practical behaviour of polyesters will provide more insight in relations between chemical microstructure and properties of copolyesters.

## 8.4 CONCLUSIONS

The highly detailed RP-GPEC separations of copolyesters can be used for the evaluation of the average molar masses and the oligomer distribution. The molar masses found are in good agreement with values from absolute methods and the oligomer distribution perfectly matches with the theoretical distribution. Especially for the low molar mass oligomers, additional information upon chemical composition (CC) differences between copolyesters can be obtained. Even after a polyesterification time of 21 hours, the chemical microstructure of the investigated copolyesters still changes. Furthermore, for two strongly resembling copolyester samples qualitative evidence for differences in their respective microstructures is found. This makes the assumption questionable that a predictable, statistically determined CCD is formed in all cases.
It is impossible to unambiguously assign differences in the high molar mass parts of RP-GPEC chromatograms, which is due to the fact that separation is dominated by molar mass and resolution with respect to CC differences is relatively low. The peak position of the oligomers depends on the average CC of the copolyester, the end group composition and the SD and/or CCD of each oligomer fraction. The width of the oligomer peaks is also influenced by SD and/or CCD and can furthermore be expected to depend on the FTD. Therefore, although RP-GPEC provides more information on structural differences than conventional methods such as SEC and NMR do, the technique must mainly be considered as a versatile, qualitative fingerprinting tool, rather than a method by which these differences can be quantitatively verified.
NP-GPEC, in combination with SEC, provides more information on microstructural differences, *i.e.* differences in MMFTD and MMCCD than RP-GPEC. This is due to

the fact that the separation in NP-GPEC is more strongly based on CC. For the investigated copolyesters, end groups are non-homogeneously distributed over the molar mass distribution. Furthermore, the FTMMD between two strongly resembling copolyesters is different.

By NP-GPEC, experimental verification of the existence of a CCD in copolyesters made by step-reactions can be obtained, which was not possible until now. Significant differences between the MMCCDs of the two strongly resembling copolyesters are found. In contrast to RP-GPEC, information on CCDs can be quantified and can also be obtained for the high molar masses. The observed differences can only be explained by the relative importance of reaction kinetics in step-reaction copolymers, which obviously has been underestimated until now.

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# Epilogue

In this Chapter, the main achievements of the investigations described in this thesis, will be reviewed and placed in a coherent context. Furthermore, suggestions and recommendations for future research from the author's point of view, will be given.
The objectives of the investigations were to get more insight in the separation mechanisms of Gradient Polymer Elution Chromatography (RP-GPEC) and to get a better idea about possibilities and limitations of GPEC for the microstructural characterisation of complex polymer systems. For these purposes, low molar mass copolyesters were used.
GPEC under normal phase conditions (NP-GPEC) was shown to be better suited to provide quantitative information on the chemical microstructure of copolyesters than GPEC under reversed phase conditions (RP-GPEC). To this end, NP-GPEC has to be coupled to Size Exclusion Chromatography (SEC). For NP-GPEC, selectivity with respect to chemical composition can be modified by choosing other column or eluent types. In contrast, for RP-GPEC selectivity is hardly influenced by those or other experimental parameters (part of these results are not shown in this thesis and will be published elsewhere). Nevertheless, RP-GPEC for low molar mass polymers can be used as a versatile fingerprinting tool and the application as such should be stimulated. Since NP-GPEC is more difficult to handle than RP-GPEC and due to the fact that the method is time consuming since it must be coupled to SEC, for copolyesters NP-GPEC should only be used for research purposes, not for routine applications.
By NP-GPEC it could be experimentally proven for the first time that even the formation of copolyesters by step-reactions can result into products with a different chemical microstructure. This can only be caused by a kinetically controlled reaction. Further research in this area can provide new insights in relations between the conditions of step-reaction polymerisations, the microstructure that will be formed and the final properties of copolyesters.
Solubility effects were found not to influence the final separation of amorphous copolyesters in RP-GPEC. However, it was also proven that those effects are kinetically determined. Therefore, it can be foreseen that for other amorphous polymers in other, less sorptive, separation systems, problems may arise. For crystalline polyesters, the non-reproducible retention behaviour was shown to be caused by the formation of a crystalline phase after injection. This should be avoided by choosing a higher column temperature, if possible. Probably, redissolution kinetics and/or the formation of a crystalline phase, even for polymers which are amorphous in the melt, may partly account for the irregularly shaped polymer distributions from

GPEC that are sometimes reported in literature. Further research on this topic, especially for high molar mass polymers, seems very worthwhile.
Further insights in the molecular mechanisms of GPEC were obtained from isocratic measurements on polystyrenes and polyesters, using the so-called van 't Hoff analysis (RP-GPEC) and the retention model of Jandera (RP-GPEC and NP-GPEC). In some cases, results from the van 't Hoff analysis contradict the theoretical findings from other workers, which were based on a self-consistent field theory for adsorption, the so-called Scheutjens-Fleer (SF) model. In other cases, results predicted from the Jandera model could be supported by the SF model (results will be presented in a separate publication). To the author's opinion, a further exploration of the SF model, in combination with chromatographic experiments (which should not necessarily be the laborious, iscoractic, van 't Hoff experiments) will undoubtedly lead to an increasing depth of understanding of GPEC and related techniques, and is therefore highly recommended.
Due to the increasing complexity of polymer systems, ongoing development of chemical characterisation methods for polymers is required. In this respect, multidimensional methods will become increasingly important. This has already been shown in this thesis, by the off-line coupling of SEC and GPEC. Especially, the coupling of liquid chromatographic (SEC and GPEC) with mass spectrometric (MS) methods, *i.e.* electronspray MS (ES) and Matrix Assisted Laser Desorption Ionisation (MALDI) MS, is of very high importance and should certainly be incorporated in future research. The main advantage of MS methods is that they, next to identification, in fact provide an additional separation step, which may be of extra help in the unravelling of polymer structures. Also coupling with Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectrometry are of interest, although the latter is still hampered by a low sensitivity. Investigations should initially focus on off-line coupling in order to investigate the potentials of the various techniques, rather than immediately going into on-line coupling with its specific interfacing problems.
Other, related topics that deserve further attention are:

- alternative non-exclusion liquid chromatographic techniques for polymers, such as Liquid Chromatography under Critical Conditions (LCCC);
- the development of new, non-silica based, polar column packings for GPEC and related techniques;
- the application of SEC coupled to viscometry and light scattering detection to copolymers.

In conclusion, it is the author's opinion that further research into GPEC and related methods, and especially the combination with other, analytical methods, should be encouraged. This will allow for better insights in the composition of complex polymers and relations with synthesis and properties, and will thus enhance a further development of new polymer products.

# Glossary of Symbols and Abbreviations

Some minor symbols which are used only once are not included.

| | |
|---|---|
| A | adipic acid |
| $A_s$ | area occupied by an adsorbed solute molecule on the sorbent surface ($m^2$) |
| $A_0$ | area occupied by an adsorbed end group on the sorbent surface ($m^2$) |
| $A_1$ | area occupied by an adsorbed repeat unit on the sorbent surface ($m^2$) |
| a | abscise in linear relation between log(k) and log(φ) in NPLC, log(k) = a - m log(φ), identical to log($k_s$), *or* abscise in linear relation between log(k) and φ in RPLC, log(k) = a - m φ, identical to log($k_w$) (-) |
| *a* | pre-exponential constant in the dependence between S' and M, $S' = a\ M^b$ ($mol.g^{-1}$) |
| $a_\eta$ | exponent in Mark-Houwink relation, $[\eta] = K_\eta M^{a\eta}$ (-) |
| $a_0$ | abscise, end group contribution in linear relation between a and p, $a = a_0 + a_1 p$ (-) |
| $a_1$ | slope, contribution of repeat unit in linear relation between a and p, $a = a_0 + a_1 p$(-) |
| Ac, $Ac_1$, $Ac_2$ | (di)acid, diacid-1, diacid-2 |
| $ac_1$, $ac_2$ | functional group of type $Ac_1$, $Ac_2$ |
| ACN | acetonitrile |
| b | gradient steepness parameter, $b = \Delta\varphi\ S'\ t_0/t_G$ (-) |
| *b* | exponent in the dependence between S' and M, $S' = a\ M^b$ (-) |
| C | ratio of reaction constants, $C = k_1/k_2$ (-) |
| c | concentration ($mol.l^{-1}$) |
| $c_s$ | concentration of a solute in the stationary phase ($mol.l^{-1}$) |
| $c_m$ | concentration of a solute in the stationary phase ($mol.l^{-1}$) |
| $c_0$ | concentration at time = 0 ($mol.l^{-1}$) |
| CC | chemical composition |
| CCD | chemical composition distribution |
| CSC | critical solvent composition |
| CPC | cloud point composition |
| D | dipropoxylated bisphenol-A |
| *D* | polydispersity, $D = M_w/M_n$ (-) |
| $d_p$ | particle size (μm) |
| DCM | dichloromethane |
| $dev_{max}$ | maximum deviation between average and maximum of duplicate measurements |
| DMA | dimethylamine |
| DRI | differential refractive index |
| DSC | differential scanning calorimetry |
| DV | differential viscometry |
| EECT | enthalpy-entropy-compensation temperature (K) |
| ELSD | evaporative light scattering detection |
| ETAC | ethylacetate |
| F | flow rate ($ml.min^{-1}$) |
| f, $f_1$, $f_2$ | extent of reaction (conversion), $f = (c_0\text{-}c)/c_0$ (-) |
| f-A, f-I | fraction adipic acid, isophtalic acid (-) |
| FH | Flory Huggins (interaction parameters etc.) |
| FTD | functionality type distribution |
| GC | gas chromatography |

| | |
|---|---|
| GE | gradient elution |
| GPEC | gradient polymer elution chromatography |
| $\Delta h$ | partial molar enthalpy change ($kJ.mol^{-1}$) |
| $\Delta H_p$ | melting heat of a pure polymer ($kJ.mol^{-1}$) |
| HEP | heptane |
| HPLC | high performance liquid chromatography |
| HPPLC | high performance precipitation liquid chromatography |
| HV | hydrodynamic volume, HV = M [η] ($l.mol^{-1}$) |
| I | isophtalic acid |
| $I_x$ | interaction index (a.u.) |
| $I_{0x}$ | interaction index of a structural residue (end group) (a.u.) |
| $\Delta I_x$ | interaction index of a monomeric repeat unit (a.u.) |
| I.D. | internal diameter of a column (mm) |
| IPA | isopropanol |
| IR | infrared spectroscopy |
| $K_{ads}$ | chromatographic distribution constant for adsorption (-) |
| $K_D$ | overall chromatographic distribution constant (-) |
| $K_{sec}$ | chromatographic distribution constant for size exclusion (-) |
| $K_\eta$ | pre-exponential constant in Mark-Houwink relation, $[\eta] = K_\eta M^{an}$ ($dl.mol.g^{-2}$) |
| $K_\theta$ | proportionality constant in the Stockmayer-Fixman equation, $[\eta] = K_\theta M^{0.5} + K'M$ ($dl.mol^{0.5}.g^{-1.5}$) |
| K' | proportionality constant in the Stockmayer-Fixman equation ($dl.mol.g^{-2}$) |
| k | retention factor (-) |
| $k_{AA}$, $k_{AB}$, $k_{BB}$ | reactivity constant of monomer A with A, monomer A with B, monomer B with B ($mol^{-1}.l.s^{-1}$) |
| $k_{a0}$ | retention factor (NPLC) of an end group in 100% less polar solvent, A (-) |
| $k_{a1}$ | retention factor (NPLC) of a repeat unit in 100% less polar solvent, A (-) |
| $k_S$ | retention factor (NPLC) in 100% strong solvent, B (-) |
| $k_w$ | retention factor (RPLC) in 100% water (-) |
| $k_0$ | retention factor (RPLC) at isocratic conditions equal to the starting conditions of a gradient (-) |
| k* | average retention factor (RPLC) in gradient elution (proportional to 1/b) (-) |
| $k_1$, $k_2$ | reactivity constants ($mol^{-1}.l.s^{-1}$) |
| MH | Mark-Houwink (equation, constants) |
| LAC | liquid adsorption chromatography |
| LALLS | low angle laser light scattering (detection) |
| LCCC | liquid chromatography under critical conditions |
| LS | light scattering |
| M | molar mass ($g.mol^{-1}$) |
| $M_0$ | molar mass of the monomeric repeat unit ($g.mol^{-1}$) |
| $M_n$ | number average molar mass ($g.mol^{-1}$) |
| $M_p$ | molar mass at the maximum of the molar mass distribution ($g.mol^{-1}$) |
| $M_w$ | weight average molar mass ($g.mol^{-1}$) |
| $M_z$ | z-average molar mass ($g.mol^{-1}$) |
| m | slope in linear relation between log(k) and log(φ) in NPLC, log(k) = a - m log(φ), identical to n, *or* slope in linear relation between log(k) and φ in RPLC, log(k) = a - m φ, identical to S' (-) |
| $m_p$ | chain length of a polymer in lattice units (-) |
| $m_S$ | chain length of the solvent in lattice units (-) |
| $m_0$ | abscise, end group contribution in linear relation between m and p, $m = m_0 + m_1 p$ (-) |
| $m_1$ | slope, contribution of repeat unit in linear relation between m and p, $m = m_0 + m_1 p$ (-) |

| | |
|---|---|
| Ma | maleic acid |
| MeOH | methanol |
| MMCCD | molar mass chemical composition distribution |
| MMD | molar mass distribution |
| MS | mass spectrometry |
| MTBE | methyl-*tert.*-butylether |
| MMFTD | molar mass functionality type distribution |
| $N_A$, $N_B$ | number of molecules of reactant A, B |
| $N_b$ | mole fraction of the strong solvent in the mobile phase (NPLC) (-) |
| n | number of solvent molecules at the surface of a sorbent, displaced by 1 solute molecule (NPLC) |
| $n_b$ | molecular area of the strong solvent, b, in a binary eluent mixture ($m^2$) |
| $n_c$ | number of carbon atoms in the bonded alkyl ligand |
| NMR | nuclear magnetic resonance spectroscopy |
| NP-GPEC | normal phase gradient polymer elution chromatography |
| NPLC | normal phase liquid chromatography |
| NS | non-solvent |
| p | degree of polymerisation (-) |
| $p_n$ | number average degree of polymerisation (-) |
| p' | slope in linear relation between m and a, $m = q + p' a$ (-) |
| PA | polyamine |
| PDMS | polydimethylsiloxane |
| PE | polyester |
| PEG | polyethyleneglycol |
| PIP | polyisoprene |
| PMMA | polymethylmethacrylate |
| PS | polystyrene |
| PVA | polyvinyl alcohol |
| $Q_0$ | dimensionless free energy of adsorption of the solute from a non-polar solvent (n-hexane, n-pentane) (-) |
| q | abscise in linear relation between m and a, $m = q + p' a$ (-) |
| R | molar gas constant = 8.3144 ($J.mol^{-1}.K^{-1}$) |
| $R^1$ | coefficient of regression |
| $R^2$ | quadratic coefficient of regression |
| r | stoichiometric imbalance, $r = N_A/N_B$ (-) |
| $r_A$, $r_B$ | reactivity ratio, $r_A = k_{AA}/k_{AB}$, $r_B = k_{BB}/k_{BA}$ (-) |
| RP-GPEC | reversed phase gradient polymer elution chromatography |
| RPLC | reversed phase liquid chromatography |
| S | solvent |
| S' | isocratic parameter in RPLC, $S' = -d\log(k) / d\varphi$ (-) |
| %-S | volume percentage of solvent (%) |
| $\Delta s$ | partial molar entropy change ($J.mol^{-1}.K^{-1}$) |
| SD | sequence distribution |
| SF | Scheutjens-Fleer (model, theory) |
| SEC | size exclusion chromatography |
| ST | sudden transition (gradients) |
| T | absolute temperature (K) |
| $T_{cr}$ | critical temperature (K) |
| $T_g$ | glass transition temperature (K) |
| $T_m$ | melting temperature (K) |
| $T_{mp}$ | melting temperature of a polymer in the presence of solvent (K) |

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| $T^0_{mp}$ | melting temperature of a pure polymer (K) |
| $t_G$ | gradient time, time during which the eluent composition is changed (min) |
| $t_r$ | retention time (min) |
| $t_s$ | system hold-up time (min) |
| $t_{sec}$ | column dead time of a high molar mass solute exhibiting SEC effects (min) |
| $t_0$ | column dead time (min) |
| THF | tetrahydrofuran |
| TREF | temperature rising elution fractionation |
| UCST | upper critical solution temperature (K) |
| UV | ultraviolet |
| $V_a$ | volume of the adsorbed solvent monolayer per unit weight of the adsorbent ($cm^3.g^{-1}$) |
| $V_h$ | hydrodynamic volume, $V_h = M [\eta]$ ($l.mol^{-1}$) |
| $V_i$ | interstitial volume (ml) |
| $V_l$ | molar volume of a lattice site ($l.mol^{-1}$) |
| $V_m$ | volume of the mobile phase (column dead volume) (ml) |
| $V_p$ | pore volume (ml) |
| $V_r$ | retention volume (ml) |
| $V_s$ | volume of the stationary phase (ml) |
| $V_{up}$ | molar volume of the monomeric repeat unit ($l.mol^{-1}$) |
| $V_x$ | molar volume ($l.mol^{-1}$) |
| $V_S$ | molar volume of a solvent ($l.mol^{-1}$) |
| $V_p$ | number average molar volume of a polymer ($l.mol^{-1}$) |
| $V_{0x}$ | molar volume of a structural residue (end group) ($l.mol^{-1}$) |
| $\Delta V_x$ | molar volume of a monomeric repeat unit ($l.mol^{-1}$) |
| W | peak width |
| $W_c$ | peak width of an individual species in a composite peak |
| $W_o$ | peak width determined by the retention time difference between the first and the last eluting component in a composite peak |
| $W_p$ | mass of the bonded packing in the column (g) |
| $w_p$ | weight fraction of molecules with degree of polymerisation p |
| $x_A, x_B$ | molar fraction of A, B (-) |
| $\alpha$ | chromatographic selectivity (-) |
| $\alpha'$ | activity of the adsorbent (-) |
| $\log(\alpha)$ | slope, contribution of the repeat unit in the Martin equation, $\log(k) = \log(\beta) + p \log(\alpha)$ (-) |
| $\beta$ | compensation temperature (K) |
| $\log(\beta)$ | abscise, end group contribution in the Martin equation, $\log(k) = \log(\beta) + p \log(\alpha)$ (-) |
| $\chi_{cr}$ | critical value of the Flory-Huggins interaction parameter between polymer and solvent (-) |
| $\chi_{p,NS}$ | Flory-Huggins interaction parameter between polymer and non-solvent (-) |
| $\chi_{p,S}$ | Flory-Huggins interaction parameter between polymer and solvent (-) |
| $\delta$ | Hildebrand solubility parameter ($MPa^{0.5}$) |
| $\delta_m$ | Hildebrand solubility parameter of a mixture of solvents ($MPa^{0.5}$) |
| $\varepsilon$ | solvent strength of the mobile phase (NPLC) ($m^{-2}$) |
| $\varepsilon_a, \varepsilon_b$ | solvent strength of the less polar solvent (A), polar solvent (B) ($m^{-2}$) |
| $\phi$ | phase ratio of a chromatographic column, $\phi = V_s/V_m$ (-) |
| $\phi^*$ | volume fraction solvent at the cloud point (-) |
| $[\eta]$ | intrinsic viscosity ($dl.g^{-1}$) |
| $\varphi$ | volume fraction of strong solvent in a binary eluent mixture (-) |
| $\varphi_e$ | volume fraction of strong solvent in a binary eluent mixture at the point of elution (-) |
| $\varphi_i$ | volume fraction of strong solvent at the start of a gradient elution experiment (-) |
| $\varphi_p$ | volume fraction of polymer in a binary polymer-solvent system (-) |
| $\varphi_S$ | volume fraction of solvent in a binary polymer/solvent system or a ternary |

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|---|---|
| | polymer/non-solvent/solvent system (-) |
| $\varphi_0$ | volume fraction of strong solvent B at critical conditions (CSC) (-) |
| $\varphi'$ | gradient steepness, $\Delta\varphi/t_G$ (%.min$^{-1}$) |
| $\varphi_{pp}$ | volume fraction of the polymer-poor phase in a demixed polymer-solvent system (-) |
| $\varphi_{pr}$ | volume fraction of the polymer-rich phase in a demixed polymer-solvent system (-) |
| $\mu_s$ | standard chemical potential of a solvent (kJ.mol$^{-1}$) |
| $\Delta\mu$ | partial molar free energy change (kJ.mol$^{-1}$) |
| $\rho$ | density of the bonded alkyl ligand (g.cm$^{-3}$) |

# Summary

In this thesis, a study on the mechanisms of Gradient Polymer Elution Chromatography (GPEC) and the application of GPEC to the microstructural characterisation of (co)polyesters, is described. GPEC is a liquid chromatographic method by which polymers are separated according to differences in molar mass and chemical composition *e.g.* functional groups, polymer backbone and chain topology. Therefore, the technique can be used for the characterisation of complex polymer systems such as the fingerprinting of resins, characterisation of copolymers according to their chemical composition distribution (CCD) and the separation of polymer blends. The separation principle of GPEC is based on a combination of precipitation/redissolution effects, sorption (adsorption and/or partitioning) and steric exclusion. Nevertheless, until now, a thorough understanding of the mechanisms of GPEC is lacking. Furthermore, the application of GPEC has mainly been restricted to, from a chromatographic point of view, relatively simple, high molar mass polymers made by chain polymerisation. The objectives of the work described in this thesis were to get a better insight in the fundamentals and working principles of GPEC, and to get a better idea about possibilities and limitations of GPEC for the deformulation of complex polymer systems. For this purpose, in this study, relatively low molar mass copolyesters ($M_w \approx 5000$) made by step-reactions were used.

By GPEC under reversed phase conditions (RP-GPEC) it was possible to separate copolyesters into a large number of oligomers (Chapter 3). The resolution in RP-GPEC was shown to be largely determined by gradient steepness up to a certain limit, and, in contrast to the chromatography of low molar mass solutes, much less by temperature, column length and sample load. The influence of solubility effects, which are present in the chosen separation system, was investigated under chromatographic conditions on several adsorbing and less or non-adsorbing media, using low polydispersity fractions obtained by Size Exclusion Chromatography (SEC). It was found that as an inert medium, a column packed with glass beads can at best be used. Solubility effects were shown not to contribute to the final separation, which is fully governed by sorption. By a comparison with measurements of maximum solubility under static equilibrium conditions, the redissolution behaviour in GPEC, even of low molar mass polyesters, was proven to be kinetically determined. Although redissolution kinetics do not influence the separation of the investigated polyesters, for other polymers or in other separation systems, problems may arise due to those effects. Sorption effects in RP-GPEC were further studied by the evaluation of thermodynamic parameters obtained from isocratic measurements on polyesters and polystyrenes ('van

't Hoff analysis', Chapter 4). Penetration of oligomers into the $C_{18}$ bonded phase, up to high molar masses was found. Both enthalpy and entropy changes caused by the separation process, were found to decrease more than linearly with the degree of polymerisation (p). This may possibly be explained from the more than proportional increase of hydrodynamic volume with p. Entropic effects were found responsible for nonlinear Martin plots, the exact shape of which was suggested to depend on the conformation of the polymer in solution. Enthalpy-entropy-compensation analysis also revealed a changing retention mechanism with p, which can possibly account for the fact that no complete molar mass independent retention can be found for polystyrene in the same separation system, under near critical conditions. The retention mechanism was shown to be independent of the %-THF in the eluent, thus justifying the conclusions of this study on a further understanding of GPEC. Critical conditions of polystyrene could be predicted from the results of the van 't Hoff analysis, within certain limits.

The dependence between the reciprocal square root of molar mass and the %-solvent at the point of elution in GPEC was studied for various oligomer series (Chapter 5). The shape of this dependence was found to be not a typical characteristic of a specific series but to depend on experimental conditions such as temperature and non-solvent/solvent system. From results of isocratic measurements for polystyrene and a polyester, it was demonstrated that the effects of experimental conditions can be ascribed to the relative contributions of both end groups and monomeric repeat units to retention.

A novel concept on the effect of precipitated polymer morphology on the elution profile in GPEC was presented in Chapter 6, where the non-reproducible retention behaviour of crystalline polyesters in GPEC was investigated. The cause of this behaviour was proven to be the formation of a crystalline phase at the top of the column, after injection. The morphology of the precipitate depends on injection volume, flow rate and precipitation medium thus giving rise to variations in redissolution behaviour. Nevertheless, separation was argumented to be mainly governed by thermodynamics rather than by redissolution kinetics. The former determines at what %-solvent during the gradient, the melting point drops below the environmental temperature. Raising the system temperature above the depressed melting point of the polyester was shown to give rise to highly reproducible, normal elution behaviour governed by sorption, since the formation of a crystalline phase is prevented.

In Chapter 7, the possibilities and limitations of GPEC under normal phase conditions (NP-GPEC) for copolyesters were further investigated. For NP-GPEC, the separation was found to be dominated by the (polar) end groups in most cases and to a lesser extent by molar mass and chemical composition of the polyester backbone. In contrast to RP-GPEC, a distinct influence of both the stationary and the mobile phase was observed. Practical parameters such as temperature and gradient steepness were shown

to only moderately affect end group selectivity. Next to end group separations, NP-GPEC can be used for the characterisation of copolyesters according to the composition of the backbone, independent of end groups. The retention behaviour in NP-GPEC was further studied by isocratic measurements, using the approach of Jandera *et al.* Results were shown to be useful for a further understanding of the mechanisms of NP-GPEC. A refined adsorption model assuming two different types of adsorption sites was introduced, which yielded improved description of the experimental, isocratic retention data.

Finally, the potentials of both RP-GPEC and NP-GPEC for the microstructural characterisation of copolyesters, were studied (Chapter 8). To this end, a number of copolyesters, varying in molar mass and chemical composition (CC) were synthesised. RP-GPEC could be used for the evaluation of average molar masses and oligomer distributions of copolyesters, which were found to be in good agreement with theoretical values. Furthermore, RP-GPEC was shown to be a versatile, qualitative fingerprinting tool, by which differences between strongly resembling copolyesters can easily be detected. However, it appeared impossible to unambiguously assign observed differences in the high molar mass parts of RP-GPEC chromatograms to physical differences between the polymers. For this purpose, NP-GPEC was demonstrated to be better suited, since it provides more and quantitative information on microstructural differences. By a combination of SEC and NP-GPEC, the Molar-Mass-Functionality-Type-Distribution of the (co)polyesters and the Molar-Mass-Chemical-Composition-Distribution (MMCCD) of the fraction containing two alcoholic end groups, of the copolyesters were studied. Significant differences between strongly resembling copolyesters were found which, as far as the MMCCDs are concerned, can only be caused by a kinetically controlled reaction. This makes the assumption that a predictable, statistically determined CCD is formed for copolyesters in all cases, questionable.

In conclusion, this study provided a further understanding of the working principles of GPEC and clearly demonstrated that the technique can be used for the deformulation of complex polymers such as copolyesters.

# Samenvatting

In dit proefschrift wordt een onderzoek beschreven naar de mechanismen van Gradiënt Polymeer Elutie Chromatografie (GPEC) en de toepassing van GPEC op de microstructurele karakterisering van (co)polyesters. GPEC is een vloeistofchromatografische methode met behulp waarvan polymeren gescheiden worden naar verschillen in molecuulmassa en chemische samenstelling, zoals functionele groepen, polymere hoofdketen en ketentopologie. De techniek kan derhalve gebruikt worden voor de karakterisering van complexe polymeersystemen zoals het fingerprinten van harsen, de karakterisering van copolymeren naar hun chemische samenstellingsverdeling en de scheiding van polymere blends. Het scheidingsprincipe van GPEC is gebaseerd op een combinatie van precipitatie/heroplossings-effecten, sorptie (adsorptie en/of verdeling) en sterische exclusie. Een goed begrip omtrent mechanismen van GPEC ontbreekt tot op heden echter. Bovendien is de toepassing van GPEC tot nog toe beperkt gebleven tot, naar chromatografische begrippen, relatief eenvoudige, hoogmoleculaire polymeren, gemaakt via ketenpolymerisatie. Het doel van het werk, beschreven in dit proefschrift was het verkrijgen van een beter inzicht in de werkingsprincipes van GPEC en van een duidelijker idee van mogelijkheden en beperkingen van GPEC voor de deformulering van complexe polymeer systemen. Hiertoe werden in deze studie relatief laagmoleculaire copolyesters ($M_w \approx 5000$), gemaakt via stapreacties, gebruikt.

Via GPEC onder reversed phase condities (RP-GPEC) was het mogelijk om copolyesters te scheiden in een groot aantal oligomeren (Hoofdstuk 3). Aangetoond werd dat de resolutie bij RP-GPEC, tot een bepaalde bovengrens, grotendeels bepaald wordt door de gradiëntsteilheid en, in tegenstelling tot de chromatografie van laagmoleculaire producten, veel minder door temperatuur, kolomlengte en monsterbelading. De invloed van oplosbaarheidseffecten, die een rol spelen in het gekozen scheidingssysteem, werd onderzocht onder chromatografische omstandigheden, op verschillende adsorberende en minder of niet adsorberende media, gebruik makend van fracties met een lage polydispersiteit, verkregen via Sterische Exclusie Chromatografie (SEC). Als inert medium bleek een kolom gepakt met glasparels het beste te voldoen. Aangetoond werd dat oplosbaarheidseffecten niet bijdragen aan de uiteindelijke scheiding, die dan ook volledig bepaald wordt door sorptie. Uit een vergelijking met metingen van maximale oplosbaarheden onder statische evenwichtscondities kon bewezen worden dat het heroplosgedrag bij GPEC, zelfs van laagmoleculaire polyesters, sterk kinetisch bepaald is. En hoewel deze heroploskinetiek de uiteindelijke scheiding van de onderzochte polyesters niet

beïnvloedt, kunnen er voor andere polymeren en/of in andere scheidingssystemen hierdoor mogelijk problemen ontstaan.
Via de evaluatie van thermodynamische parameters verkregen uit isocratische metingen aan polyesters en polystyrenen ('van 't Hoff analyse') werden sorptie effecten bij RP-GPEC verder bestudeerd (Hoofdstuk 4). Gevonden werd dat oligomeren tot een hoge molmassa kunnen penetreren in de $C_{18}$ gebonden fase. Zowel de enthalpie als de entropieverandering die het gevolg is van het scheidingsproces blijken meer dan evenredig af te nemen met de polymerisatiegraad (p). Dit kan mogelijk verklaard worden door de meer dan evenredige toename van het hydrodynamisch volume met de molecuulmassa. Het bleek dat entropie-effecten verantwoordelijk zijn voor niet-lineaire Martin plots. Gesuggereerd werd dat de exacte vorm van deze plots afhangt van de conformatie van een polymeer in oplossing. Ook via enthalpie-entropie-compensatie analyse bleek dat het retentiemechanisme verandert met p, hetgeen mogelijk een verklaring vormt voor het feit dat geen volledige molmassa onafhankelijke retentie gevonden kan worden in hetzelfde scheidingssysteem voor polystyreen, rond de kritische condities. Omdat aangetoond kon worden dat het scheidingsmechanisme onafhankelijk is van het %-THF in het eluens, is het gebruik van de conclusies van deze studie voor een verdere begripsvorming van GPEC, gerechtvaardigd. De kritische condities van polystyreen konden, binnen bepaalde grenzen, voorspeld worden uit de resultaten van de van 't Hoff analyse.
In Hoofdstuk 5 werd voor verschillende oligomeerseries de afhankelijkheid tussen de reciproke wortel uit de molmassa en het %-solvent op het punt van elutie in GPEC, bestudeerd. De vorm van deze afhankelijkheid bleek geen typisch kenmerk te zijn van een specifieke oligomeerserie, maar af te hangen van experimentele condities, zoals temperatuur en non-solvent/solvent systeem. Met behulp van de resultaten van isocratische metingen voor polystyreen en een polyester kon worden aangetoond dat de invloeden van experimentele condities toegeschreven kunnen worden aan de relatieve bijdrages van zowel eindgroepen als repeterende eenheden aan de retentie.
Een nieuw concept ten aanzien van de invloed van de morfologie van geprecipiteerd polymeer op het elutieprofiel bij GPEC werd gepresenteerd in Hoofdstuk 6, waar het niet-reproduceerbaar gedrag van kristallijne polyesters in GPEC onderzocht werd. Bewezen werd dat de oorzaak van dit gedrag ligt in de vorming van een kristallijne fase, na injectie, op de top van de kolom. De morfologie van het precipitaat hangt af van het injectievolume, de flow en het precipitatiemedium, hetgeen aanleiding geeft tot verschillen in heroplosgedrag. Niettemin kon aannemelijk gemaakt worden dat de scheiding vooral gedomineerd wordt door thermodynamische effecten en niet zozeer door heroploskinetiek. De eerste bepalen bij welk %-solvent tijdens de gradiënt het smeltpunt beneden omgevingstemperatuur komt. Aangetoond werd dat het verhogen van de temperatuur tot boven het verlaagde smeltpunt van de polyester aanleiding

geeft tot zeer reproduceerbaar, normaal elutiegedrag, gedomineerd door sorptie, omdat de vorming van een kristallijne fase vermeden wordt.
In Hoofdstuk 7 werden de mogelijkheden en beperkingen van GPEC onder normal phase condities (NP-GPEC) voor copolyesters verder onderzocht. Gevonden werd dat de scheiding in NP-GPEC in de meeste gevallen gedomineerd wordt door de (polaire) eindgroepen en in mindere mate door molmassa en chemische samenstelling van de hoofdketen van de polyester. In tegenstelling tot de resultaten voor RP-GPEC, werd een duidelijke invloed van zowel stationaire als mobiele fase waargenomen. Praktische parameters zoals temperatuur en gradiëntsteilheid bleken de eindgroepselectiviteit slechts in geringe mate te beïnvloeden. Behalve voor eindgroepscheidingen kan NP-GPEC ook gebruikt worden voor de karakterisering van copolyesters naar de samenstelling van de hoofdketen, onafhankelijk van eindgroepen. Het retentiegedrag bij NP-GPEC werd verder bestudeerd via isocratische metingen, waarbij gebruik gemaakt werd van de benadering van Jandera *et al.* De resultaten hieruit bleken nuttig te zijn voor een verdere begripsvorming ten aanzien van mechanismen van NP-GPEC. Een verfijnd adsorptiemodel dat uitgaat van twee verschillende typen adsorptiesites werd geïntroduceerd. Hiermee werd een verbeterde beschrijving van de experimentele, isocratische retentiedata verkregen.
Tenslotte werden de mogelijkheden van zowel RP-GPEC als NP-GPEC voor de microstructurele karakterisering van copolyesters onderzocht (Hoofdstuk 8). Hiertoe werden een aantal copolyesters, variërend in molmassa en chemische samenstelling, gesynthetiseerd. RP-GPEC kon gebruikt worden voor de berekening van gemiddelde molmassa's en oligomeerverdelingen van copolyesters, die in goede overeenstemming bleken te zijn met theoretische waardes. Verder werd aangetoond dat RP-GPEC een goed hanteerbare methode voor kwalitatieve fingerprinting is, met behulp waarvan verschillen tussen sterk gelijkende copolyesters eenvoudig opgespoord kunnen worden. Het bleek echter niet mogelijk te zijn om waargenomen verschillen in het hoogmoleculaire gedeelte van de RP-GPEC chromatogrammen eenduidig toe te kennen aan fysische verschillen tussen de polymeren. Aangetoond werd dat NP-GPEC beter geschikt is voor dit doel, omdat deze techniek meer en kwantitatieve informatie levert ten aanzien van microstructurele verschillen. Via een combinatie van SEC en NP-GPEC konden de molmassa-functionaliteits-verdeling van de (co)polyesters en de molmassa-chemische-samenstellingsverdeling (MMCCD) van de fractie met twee alcoholische eindgroepen bestudeerd worden. Tussen sterk gelijkende copolyesters werden significante verschillen aangetoond die, met name voor de MMCCD, alleen het gevolg kunnen zijn van een kinetisch gecontroleerde reactie. Hieruit blijkt dat de aanname, dat voor copolyesters in alle gevallen een voorspelbare, statistisch bepaalde CCD gevormd wordt, betwijfeld moet worden.

Concluderend heeft dit onderzoek een beter begrip over de werkingsprincipes van GPEC opgeleverd en is duidelijk aangetoond dat de techniek gebruikt kan worden voor de deformulering van complexe polymeren, zoals copolyesters.

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Harry

# Curriculum Vitae

Harry Philipsen, auteur van dit proefschrift, werd geboren op 28 augustus 1965 te Boxmeer. Hij volgde zijn middelbare opleiding aan het Elzendaal College te Boxmeer, waar hij in 1983 het VWO diploma behaalde. In datzelfde jaar begon hij aan de Hogere Laboratorium Opleiding (HLO) te Venlo, waaraan hij in 1987 afstudeerde in de analytisch-chemische richting. In 1990 volgde hij de Top Opleiding Polymeren bij de stichting Polymeer Technologie Nederland, waarvan hij in 1991 het diploma behaalde.

Vanaf 1987 is hij werkzaam bij Océ Nederland B.V., tegenwoordig Océ Technologies B.V., te Venlo in de sector Research and Development. Hier vervulde hij als researchmedewerker verschillende functies in de groep chemische analyse van de afdeling Analytical research and Measurements. Vanaf 1990 werd hij belast met de verantwoordelijkheid voor het stuk vloeistofchromatografie in deze groep, in welke functie hij in aanraking kwam met de groep van prof.dr.ir. A.L. German aan de Technische Universiteit Eindhoven. Vanaf 1996 is hij groepscoach van de groep chemische analyse.

In 1994 begon hij zijn promotieonderzoek in de vakgroep Polymeerchemie en Kunststoftechnologie (TPK), tegenwoordig de capaciteitsgroep Polymeerchemie en Coatingstechnologie (SPC), onder leiding van prof.dr.ir. A.L. German. Dit onderzoek naar mechanismen en toepassingen van non-exclusie vloeistofchromatografische methodes voor polymeren, werd opgezet en uitgevoerd in een samenwerkingsverband tussen Océ Technologies en de vakgroep TPK. Een deel van de resultaten hiervan is vastgelegd in dit proefschrift. Verder leverde hij bijdragen in de vorm van publicaties in diverse internationale tijdschriften en lezingen tijdens verschillende symposia.

Sinds 1994 is hij bestuurslid en sinds 1997 voorzitter van de discussiegroep Gel Permeatie Chromatografie. In die hoedanigheid is hij sinds 1998 tevens bestuurslid van de werkgroep scheidingsmethoden die, net als voornoemde discussiegroep, deel uitmaakt van de Sectie Analytische Chemie (SAC) van de Koninklijke Nederlandse Chemie Vereniging (KNCV).

# List of Publications

1. H.J.A. Philipsen, *'Océ copieert gradient polymeer elutie chromatografie'*, Laboratoriumpraktijk, augustus 1993, 228-230.

2. H.J.A. Philipsen, B. Klumperman, A.M. van Herk and A.L. German, *'Critical retention behaviour of polymers. A study on the influence of some practical parameters'*, J. Chromatogr. A, 727 (1996) 13-25.

3. B. Klumperman, P. Cools, H. Philipsen and W. Staal, *'A qualitative study to the influence of molar mass on retention in gradient polymer elution chromatography'*, Macromol. Symp., 110 (1996) 1-13.

4. H.J.A. Philipsen, B. Klumperman and A.L. German, *'Characterization of low-molar-mass polymers by gradient polymer elution chromatography. I. Practical parameters and applications of the analysis of polyester resins under reversed phase conditions'*, J. Chromatogr. A, 746 (1996) 211-224.

5. H.J.A. Philipsen, M.R. de Cooker, H.A. Claessens, B. Klumperman and A.L. German, *'Characterization of low-molar-mass polymers by gradient polymer elution chromatography. II. Solubility effects in the analysis of polyester resins under reversed phase conditions'*, J. Chromatogr. A, 761 (1997) 147-162.

6. H.J.A. Philipsen, B. Klumperman and A.L. German, *'Characterization of polyester resins by gradient polymer elution chromatography under reversed phase conditions'*, Proc. Int. GPC Symp., San Diego, September 1996, 368-376.

7. H.J.A. Philipsen, M. Oestreich, B. Klumperman and A.L. German, *'Characterization of low-molar-mass polymers by gradient polymer elution chromatography. III. Behaviour of crystalline polyesters under reversed phase conditions'*, J. Chromatogr. A, 775 (1997) 157-177.

8. H.J.A. Philipsen, H.A. Claessens, H. Lind, B. Klumperman and A.L. German, *'A study on the retention behaviour of low molar mass polystyrenes and polyesters in reversed phase liquid chromatography by evaluation of thermodynamic parameters'*, J. Chromatogr. A, 790 (1997) 101-116.

9. B. Klumperman and H.J.A. Philipsen, *'Gradient polymer elution chromatography as a versatile tool in polymer characterization'*, LC-GC, (1998), 18-25.

10. H.J.A. Philipsen, H.A. Claessens, M. Bosman, B. Klumperman and A.L. German, *'Normal Phase Gradient Polymer Elution Chromatography of Polyester Resins'*, Chromatographia, accepted.

11. H.J.A. Philipsen, F.P.C. Wubbe, B. Klumperman and A.L. German, *'Microstructural characterization of aromatic copolyesters made by step reactions by gradient polymer elution chromatography'*, J. Appl. Polym. Sci., accepted.

12. H.J.A. Philipsen, H.A. Claessens, P. Jandera, M. Bosman and A.L. German, *'Chromatographic behaviour of low molar mass polyesters in normal-phase high performance liquid chromatography'*, Chromatographia, submitted.

13. H.J.A. Philipsen, B. Klumperman, F.A.M. Leermakers, F.P.C. Wubbe and A.L. German, *Molar mass effects in reversed phase gradient polymer elution chromatography of polymers*, in preparation.

14. F.A.M. Leermakers, H.J.A. Philipsen and B. Klumperman, *Molecular modeling of chain end effects in separating oligomers by reversed phase gradient gradient polymer elution chromatography. The adsorption transition as revealed by a lattice-based self-consistent-field theory for polymer adsorption*, in preparation.

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Journal of Chromatography A, 746, H.J.A. Philipsen, B. Klumperman and A.L. German, *'Characterization of low-molar-mass polymers by gradient polymer elution chromatography. I. Practical parameters and applications of the analysis of polyester resins under reversed phase conditions'*, 211-224, Copyright 1996, with permission from Elsevier Science
and
Journal of Chromatography A, H.J.A. Philipsen, M.R. de Cooker, H.A. Claessens, B. Klumperman and A.L. German, *'Characterization of low-molar-mass polymers by gradient polymer elution chromatography. II. Solubility effects in the analysis of polyester resins under reversed phase conditions'*, 147-162, Copyright 1997, with permission from Elsevier Science.

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**STELLINGEN**

behorende bij het proefschrift

# Mechanisms of Gradient Polymer Elution Chromatography and its Application to (Co)polyesters

van

Harry Philipsen

1. Extreme piekverbreding voor hoogmoleculaire polymeren in chromatografie onder kritische condities is een tot nog toe door vrijwel alle onderzoekers niet opgemerkt of genegeerd fenomeen, dat de toepasbaarheid van deze techniek echter drastisch kan beperken.

   H.J.A. Philipsen, B. Klumperman, A.M. van Herk and A.L. German, *J. Chromatogr. A*, 727 (1996) 13.

2. Hoewel uit hoofdstuk 8 van dit proefschrift blijkt dat met 'normal phase gradient polymer elution chromatography' (NP-GPEC) meer en eenduidiger informatie verkregen kan worden over de microstructuur van copolyesters dan met 'reversed phase' GPEC (RP-GPEC), blijkt in de praktijk dat de betrekkelijke leek op dit gebied meer waardering heeft voor de hoge resolutie chromatogrammen zoals verkregen kunnen worden met laatstgenoemde techniek. Naast een (beperkte) praktische waarde is RP-GPEC daarom met name geschikt voor het propageren en verbreiden van de techniek GPEC.

   Hoofdstuk 8 van dit proefschrift.

3. De door Krüger, Much en Schulz beschreven retentievolgorde van polyesterfracties onder kritische condities is principieel in strijd met de polariteitsregels, niet reproduceerbaar en dus onmiskenbaar fout.

   R.P. Krüger, H. Much and G. Schulz, *J. Liq. Chrom.*, 17 (1994) 3069.

4. Het recente werk van Pasch, waarin het gebruik van telkens weer andere, gekoppelde analysetechnieken ten behoeve van de karakterisering van een beperkte set laagmoleculaire polymeren beschreven wordt en waarin de conclusie telkens dezelfde is, namelijk dat gekoppelde technieken nieuwe en interessante mogelijkheden bieden, voegt door zijn oppervlakkigheid niets toe aan een beter begrip ten aanzien van de gebruikte scheidings- en detectietechnieken.

H.Pasch and K. Rode, *J. Chromatogr. A*, 699 (1995) 21.
K.E. Esser, H. Pasch and P. Montag, *GIT Spezial Chromatographie*, 2 (1996) 68.
H. Pasch and K. Rode, *Macromol. Chem. Phys.*, 197 (1996) 2691.
H. Pasch and W. Hiller, *Macromolecules*, 29 (1996) 6556.

5. Uit hoofdstuk 6 van dit proefschrift zou terecht geconcludeerd mogen worden dat GPEC tevens geschikt zou zijn voor de (grove) bepaling van smeltpunten van polymeren in aanwezigheid van oplosmiddelen. De werkelijke relevantie van GPEC in dit opzicht is echter vergelijkbaar met die van kernspinresonantie (NMR) voor de bepaling van glasovergangstemperaturen en kan dus betwijfeld worden.

Hoofdstuk 6 van dit proefschrift.
L. Mandelkern, *Pure Appl. Chem.* 54 (1982) 611.

6. Het toenemend gebruik van viscositeits- en lichtverstrooiingsdetectoren bij sterische exclusie chromatografie (SEC) waarmee het mogelijk is om absolute molecuulmassa's te bepalen, doet steeds duidelijker beseffen dat absoluut ook maar relatief is.

7. De salarisontwikkeling binnen een bedrijf met een platte organisatie is minder makkelijk te duiden aan spectroscopisten dan aan chromatografisten omdat eerstgenoemde groep gewend is te denken in termen van gemiddelden in plaats van in, vaak meer relevante, verdelingen.

8. Polymeerchemie en chromatografie vormen een zichzelf versterkend koppel. Immers, de ontwikkeling van nieuwe polymeren en polymerisatiemethoden stimuleert de ontwikkeling van nieuwe kolommaterialen, en de beschikbaarheid daarvan kan weer een impuls vormen voor verdere polymeerontwikkeling.

M. Petro, F. Svec, J.M.J. Fréchet, S.A. Haque and H.C. Wang, *J. Polym. Sci. A: Polym. Chem.*, 35 (1997) 1173.
A. Schellen, *Chemisch Weekblad*, 21 februari 1998, p.1.

9. De soms gehoorde stelling onder analytisch chemici dat de ene analysetechniek complexer is dan de andere, wijst in het algemeen op een gebrek aan inzicht in de analytische chemie.

10. De door de historica van der Zee geponeerde stelling dat de vestiging van een civiel bestuur in Nederland door de Duitse bezetters, waarvoor de juridische basis gevormd werd door de vlucht van koningin en kabinet op 13 mei 1940 naar Engeland, van grote betekenis is geweest voor de positie van het Joodse volksdeel onder de Duitse bezetting, kan, mede gelet op het door haarzelf aangehaalde argument dat de liquidatie van de Joden het primaire en belangrijkste oorlogsdoel was van Hitler, in twijfel getrokken worden.

    N. van der Zee, *Om Erger te Voorkomen*, Meulenhoff, Amsterdam, 1997.

11. De nieuwste versie van het 'Groene boekje' en de daarmee gepaard gaande commotie rondom het gebrek aan logica in de nieuwe spellingsregels, maakt andermaal duidelijk dat deze regels niet door taalkundigen maar door bètawetenschappers opgesteld zouden moeten worden.

    Nederlandse Taalunie, *Woordenlijst Nederlandse taal*, Sdu Uitgevers, Den Haag, 1997.

12. De recente rage onder kinderen rondom de Tamagotchi is een illustratie van toenemende geestelijke armoede bij kinderen en hun ouders.

    W. Thijssen, *De Volkskrant*, 4 oktober 1997, p.3.

13. Zomer in Nederland is een korte periode waarin er, ten opzichte van de rest van het jaar, een verminderde kans op herfst bestaat.

14. De in kranten en programmabladen frequent aangetroffen term 'Amerikaanse komedie' is een contradictio in terminis.