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Characterization of low-molar-mass polymers by gradient polymer elution chromatography
III. Behaviour of crystalline polyesters under reversed-phase conditions

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Abstract

Gradient polymer elution chromatography (GPEC) can be used to characterize polyester resins according to molar mass and chemical composition. In the third part in this series, the application of GPEC on crystalline polyesters was studied. In contrast to amorphous polyesters, crystalline polyesters were found to exhibit non-reproducible chromatographic behaviour in GPEC under certain conditions. The cause of this phenomenon was found in the dominance of precipitation (crystallization) and redissolution effects in the total retention mechanism. Crystalline polyesters were found to crystallize 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 a different redissolution behaviour. From the minor effects of increasing sample load and gradient steepness, it was concluded that separation is mainly governed by thermodynamics that determine at what % solvent during the gradient, the melting point drops below the environmental temperature rather than by redissolution kinetics. 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 was 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.

Keywords: Gradient elution; Polymers; Polyesters; Bisphenol-A (dipropoxylated) polyesters; Maleic acid ploye sters; Dodecanedioic acid polyesters; Butanediol polyesters; Decanediol polyesters

1. Introduction

Complex polymer systems such as copolymers or telechelics, do not only have a distribution with respect to molar mass (chain length) but also to chemical composition and/or functionality. The relevance of these distributions in relation to polymer properties is becoming more widely recognized during recent years. This explains the increasing interest in the application of non-exclusion liquid chromatographic techniques for polymer characterization. In this respect, liquid chromatography under

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critical conditions (LCCC) can be applied for the characterization of telechelics according to functionality [1–5] and for the determination of block length distributions of block copolymers [6–9]. Chromatography in the sorption mode, which is mostly carried out with the use of gradient elution, is nowadays frequently used for the characterization of copolymers according to chemical composition distribution (CCD) [10–13] and for the detailed characterization of resins (fingerprinting) and oligomer mixtures [14–20]. In the case of gradient elution, the contribution of various processes influencing the total retention mechanism, e.g., precipitation/redissolution, sorption and exclusion, vary from case to case. Therefore, the more universal name gradient polymer elution chromatography (GPEC) has been introduced by us [21–24].

Recently, we have shown the potential of GPEC under reversed-phase conditions in the determination of differences in chemical microstructure of co-polyester resins [23]. In the second part in this series of papers, the separation of these resins was shown to be mainly determined by sorption [24], 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 the present, i.e., third, part we will focus on the application of GPEC to crystalline polyesters. It was found that under certain conditions, unexpected and highly irreproducible results, as compared to amorphous polyesters, are obtained. We will show that the cause of this phenomenon can be found in the dominance of precipitation (crystallization) and redissolution effects which, in the case of crystalline polymers, are highly dependent on experimental conditions.

The precipitation of a polymer can be represented by means of a phase diagram. Polymer solutions mostly exhibit upper critical solution temperature (UCST) behaviour [25], which means that a maximum temperature can be found above which no phase separation occurs. An example is shown in

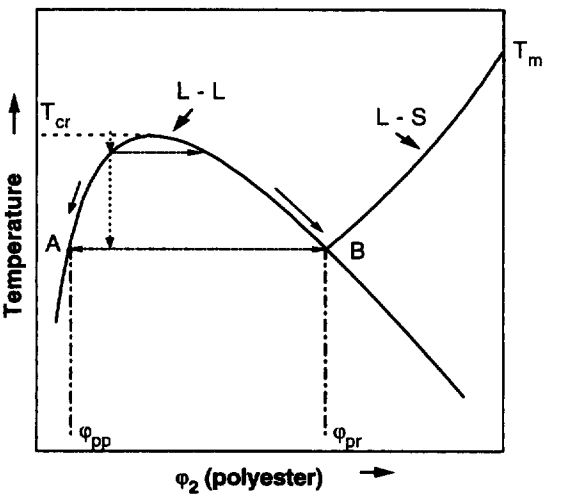

Fig. 1. Schematic representation of the interference between L–L demixing and L–S transition in a binary polymer solution.

Fig. 1. Below the critical temperature, T_{cr}, the polymer solution demixes into two co-existing phases, a swollen polymer-rich phase (φ_{pr}) and a polymer-poor phase (φ_{pp}), the composition of which is given by the L–L curve. This of course, is only valid in the case that the polymer/solvent composition lies within the L–L area [25]. Furthermore, the described situation is only valid under equilibrium conditions.

In the chromatographic case this representation is in fact too simple, since the system consists of at least four different phases, i.e., solvent, non-solvent, stationary phase and polymer. However, because the precipitation process will be mainly determined by polymer–solvent and polymer–non-solvent interactions, the influence of the stationary phase can be neglected in most cases. Furthermore, the mobile phase in which precipitation occurs, as an approximation, can be considered as one, homogeneous, phase. Therefore, the phase diagram from Fig. 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 no equilibrium is obtained.

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 Fig. 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. (1), which has been derived from the Flory–Huggins theory [25].

$$1/T_{mp} - 1/T^0_{mp} = -RV_{up}/(V_l \Delta H_p)\left[(1/m_p)\ln \varphi_p + (1/m_p - 1/m_s)\varphi_s + \chi\varphi_s^2\right] \quad (1)$$

in which 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, φ_p and φ_s are the molar fractions, χ is the polymer–solvent interaction parameter and ΔH_p is the melting heat of the pure polymer.

From this equation 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. (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 χ therefore causes the total term between the brackets to become less negative, thus giving rise to a decrease in melting point depression.

The chromatographic 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 dipropoxylated bisphenol-A, which had already been used in previous studies [23] was also taken into account in some experiments. For both types of polyesters, chromatographic results on three different columns, e.g., C_{18}, cyanopropyl 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. By means of differential scanning calorimetry (DSC) experiments, microscopy and two-dimensional GPEC–size-exclusion chromatography (SEC) experiments, further information on the behaviour of crystalline polyesters is obtained. Furthermore, the influence of several variables such as injected mass and injection volume, flow-rate, initial gradient conditions, precipitation medium and temperature is investigated and 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.

Crystallization 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 function of molar mass of crystalline polymers can be obtained [26]. In contrast, GPEC will be shown here to be better suited for the characterization of mixtures of amorphous and crystalline polymers, which can either be blends or copolymer systems.

2. Experimental

2.1. Polymer samples

The polyester samples used were laboratory-made polyester resins. Sample PE5 is an amorphous homopolyester consisting of maleic acid and dipropoxylated bisphenol-A which has also been used in previous studies [23]. 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 SEC, average chemical compositions measured by NMR and end group composition determined by

Table 1
Polystyrene equivalent molar masses, end group compositions and average chemical compositions of the investigated polyesters

Sample	SEC			Titrations Acid number	NMR	
	PS equivalent molar masses				Molar fractions	
	M_n	M_w	D^a	(mg KOH/g)	Diacid	Diol
PE5	3900	13 700	3.5	13	0.45	0.55
CP1	6900	16 000	2.3	<1	0.44	0.56
CP2	4400	11 400	2.6	<1	0.45	0.55

[a] Polydispersity (M_w/M_n); M_w = weight-average molar mass, M_n = number-average molar mass.

titrimetric analysis are given in Table 1. For more detailed information on the characterization of the polyester samples we refer to [23].

2.2. Solvents

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, Netherlands). To both solvents, 200 μl acetic acid, analytical-reagent quality from Merck (Darmstadt, Germany), per liter 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.

2.3. Columns

The columns used were a Novapak-C_{18} column (Waters; Milford, MA, USA, d_p=4 μm, pore size 60 Å, 150×3.9 mm), a Novapak-CN column (Waters, d_p=4 μm, pore size 60 Å, 150×3.9 mm) and a Resolve silica column (Waters, d_p=5 μm, pore size 90 Å, 150×3.9 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.

2.4. HPLC equipment

All GPEC experiments were performed, using a Waters 600E 4 solvent gradient pump and a 710 WISP from Waters. The detector was a variable wavelength detector, Waters, type 490 or type 484, which was set at 277 nm and an evaporative light scattering detection (ELSD) system. For reasons of availability, for most experiments an ELSD system, type 750/14 from Applied Chromatography Systems was used, which operated at a temperature of 80°C, using nitrogen for nebulization 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 system 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. Hereto, 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, Netherlands) equipped with a peltier element, was used. Chromatograms were recorded using the Baseline-815 system from Waters. For the isolation of eluting fractions, a fraction collector, type FC-205 from Gilson (Villiers-le-Bel, France) was used.

For the equipment and columns used for SEC, the reader is referred to [23].

2.5. Gradient elution experiments

Gradient elution was performed as follows. For most experiments a gradient was run from water–THF [both containing 0.02% (v/v) acetic acid] (70:30) to (0:100) in 23.3 min (3%/min) unless

indicated otherwise. After running each gradient, the system was reset to initial conditions in 1 min, followed by pumping 15 column volumes of the starting eluent composition to re-equilibrate the column. Prior to the analysis of the samples, two blank gradients were performed. 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 was also determined from these experiments and was found to be 3.9 ml.

After each temperature change, the system was equilibrated for at least 2 h at the starting conditions of the gradient. System equilibrium was checked by repeated injections of the PE5 sample.

2.6. Study of crystallization behaviour

To study the crystallization behaviour in the presence of solvent–non-solvent mixtures, DSC and polarization microscopy were applied. Hereto, 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 solvent–non-solvent (S–NS) 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 speed, in the case of the pure materials, the sample was re-heated again to measure the thermal behaviour without the influence of physical aging. Temperature was calibrated using an indium standard. For microscopic experiments of the mixtures of crystalline material with S–NS, a light microscope with crossed polarizers, type Universal from Zeiss was used.

3. Results and discussion

The behaviour of amorphous polyesters in GPEC under reversed-phase conditions (RP-GPEC) has been extensively studied by us [23]. It was shown that these products are separated mainly according to molar mass, whereas in the lower mass part a further separation on chemical composition occurs. This can again be observed in Fig. 2, in which the samples PE5 and CP1 are compared by SEC and GPEC, respectively.

It can easily be recognized that for PE5 the separation power of GPEC as compared to SEC is much higher. 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 [23]. Within the low-molar-mass oligomer fractions a separation according to end groups occurs. In the oligomer fraction $n=2$ (2 diol units) for instance, separate peaks for chains containing 0, 1 or 2 di-acid endgroups respectively, can be recognized (Fig. 2A).

For sample CP1, by GPEC a completely different elution pattern is obtained as compared to PE5. 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 [27]. 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 [28]. This means that the contribution of oligomer fractions may be underestimated when using ELSD. From our own experience 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 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 (Fig. 3), whereas for amorphous polyesters it has been pointed out that reproducibility in GPEC is very

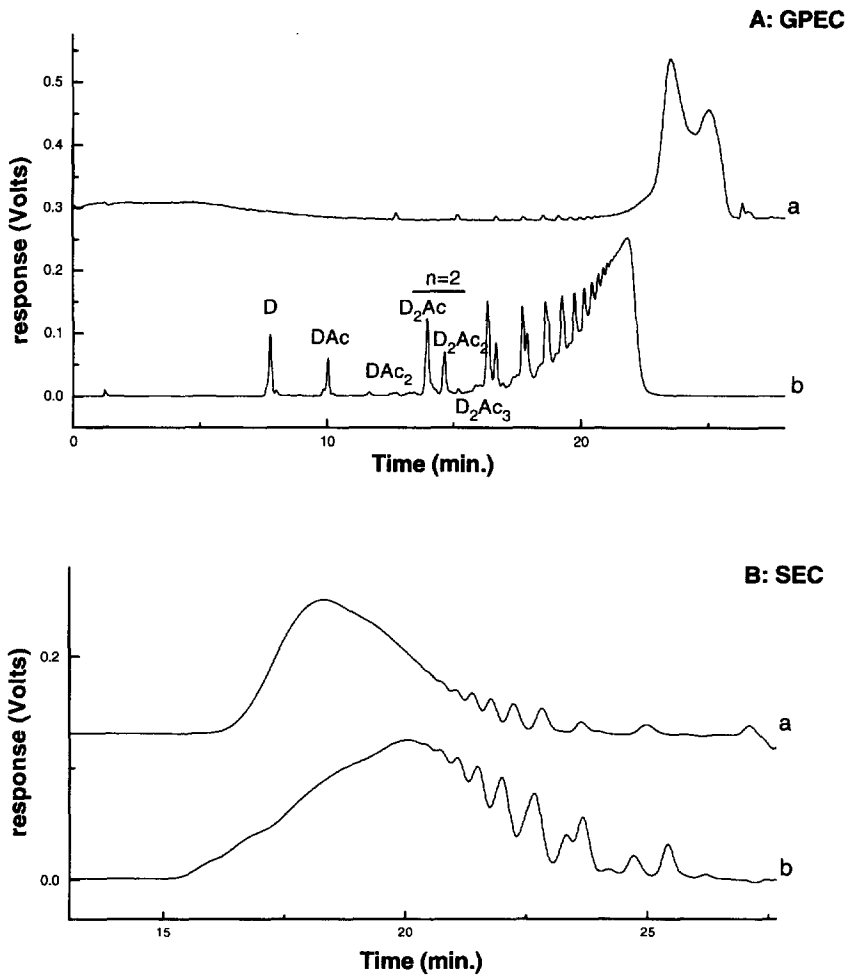

Fig. 2. Comparison between GPEC (A) and SEC (B) for CPI (upper traces) and PE5 (lower traces). GPEC: sample concentration 20 mg/ml in THF, column: Novapak C_{18} (150 mm), eluent: THF–water (30:70, v/v) to (100:0) (0 to 23.3 min), flow: 1.0 ml/min, temperature: 25°C, injection: 5 μl, detection: CP1: ELSD; PE5: UV-277 nm. D=diol, Ac=acid. SEC: concentration: 1.5 mg/ml, column: Shodex KF804 (10^4 Å), KF803 (10^3 Å), KF802.5 (500 Å), KF802 (100 Å) (in series), eluent: THF+1% acetic acid, flow: 1.5 ml/min, injection: 200 μl, detection: refractive index.

good [23]. For sample CP2, which, chemically seen, strongly resembles CP1, qualitatively the same anomalous behaviour was found (results not shown here, [29]).

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 completely different, much more time-dependent redissolution behaviour as compared to amorphous polyesters. Secondly, due to their strong aliphatic character, the polyesters may exhibit a high affinity towards the aliphatic stationary phase which possibly leads to strong partitioning effects. The resulting sorption interactions might therefore be different from that of the amorphous

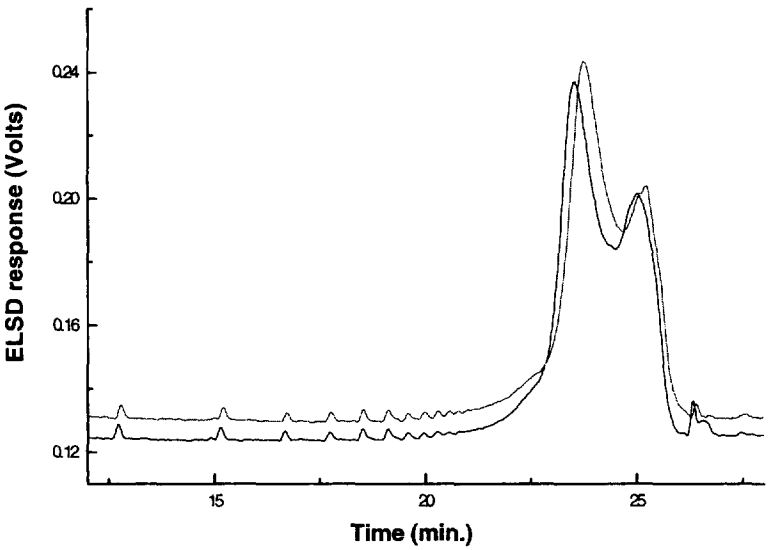

Fig. 3. Typical example of non-reproducibility of sample CP1 in RP-GPEC at 25°C. GPEC conditions as in Fig. 2A.

polyesters, thus resulting in a different chromatographic behaviour.

The validity of this second hypothesis was tested by the comparison of PE5 and CP1 on a C_{18}, a cyanopropyl (CN) and a bare silica column. In Fig. 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 [30]. Since we have shown earlier that the separation of amorphous polyesters in RP-GPEC is mainly dominated by sorption effects [23], 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 solvophobic effects, can occur, which has already been shown by us in a previous study [24]. Under such conditions almost no separation into distinct oligomers was obtained, which is in agreement with the current results.

In Fig. 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 much alike. This proves 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, indicating 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 other workers [31].

The possibility of crystallization during the precipitation step was further investigated using DSC and microscopy. Hereto, 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 THF–water ratio was 30:70 (v/v) which is the same as the starting conditions of the applied gradients. The actual concentrations under chromatographic con-

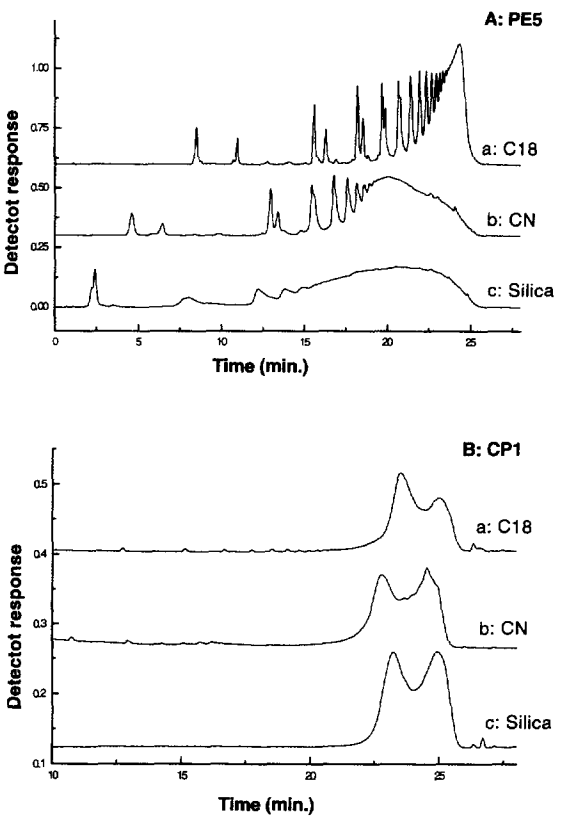

Fig. 4. Retention behaviour of PE5 (A) and CP1 (B) in RP-GPEC on different columns. (a) Novapak C_{18} (150 mm); (b) Novapak CN (150 mm); (c) Resolve silica (150 mm). Detection: PE5: UV-277 nm; CP1: ELSD. Other GPEC conditions as in Fig. 2A.

ditions 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 polarization microscopy, a clear crystalline phase could be observed in both precipitates, which is illustrated in Fig. 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 Fig. 6. For the precipitate, the transition is seriously broadened towards a temperature of about 30°C (Fig. 6B), whereas for the pure polyester melting starts at about 55°C (Fig. 6A). The maximum of the melting curve of the precipitate is hardly affected and, just like 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.

From both microscopy and DSC experiments it can clearly be concluded that crystallization 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 crystallization, due to intersection with the L–S transition (Fig. 1). For amorphous polyesters, this does not occur, since the glass transition temperature (T_g) is much more affected by the presence of solvent–non-solvent than T_m. Only small amounts of solvent can dramatically lower T_g [32]. Therefore, the intersection of T_g with L–L demixing occurs at temperatures far below the temperature of operation, thus preventing the formation of a true solid-phase. At present it is not completely clear, whether the anomalous chromatographic results for crystalline polyesters are due to the formation of a solid-phase a priori or to the fact that this solid-phase is crystalline.

It is interesting to further study the elution behaviour of sample CP1, to see whether, for instance, only a part of the sample crystallizes, 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 Fig. 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 Fig. 3. In Fig. 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

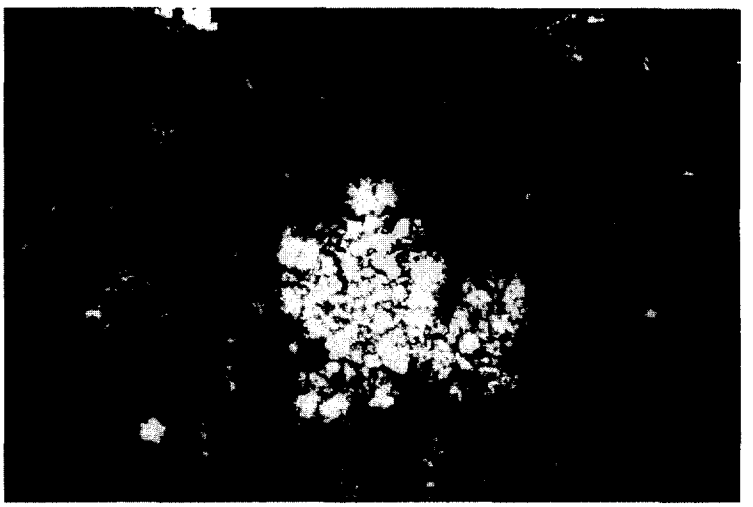

Fig. 5. Polarization microscopy picture of sample CP1 after precipitation in THF–water (30:70, v/v). Final (average) concentration: 5 mg/ml.

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 surface [33,34]. This was also observed for amorphous polyesters in a previous study [24] and can again be seen from Fig. 2. 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 fact that, for instance, 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 crystallize during the precipitation. The exact 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. (1)). Therefore, with increasing thermodynamic solvent quality, resulting in a further increase of the melting point depression (Eq. (1)), the point at which the melting points drop 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 Fig. 1. It is well known for polydisperse polymers that a precipitation is accompanied by fractionation [35]. Therefore, the polymer-poor phase will be highly enriched by low-molar-mass fragments, for which the melting point depression is more pronounced (Eq. (1)). Presumably, for this phase, no crystallization 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 crystallization, these parts will 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 en-

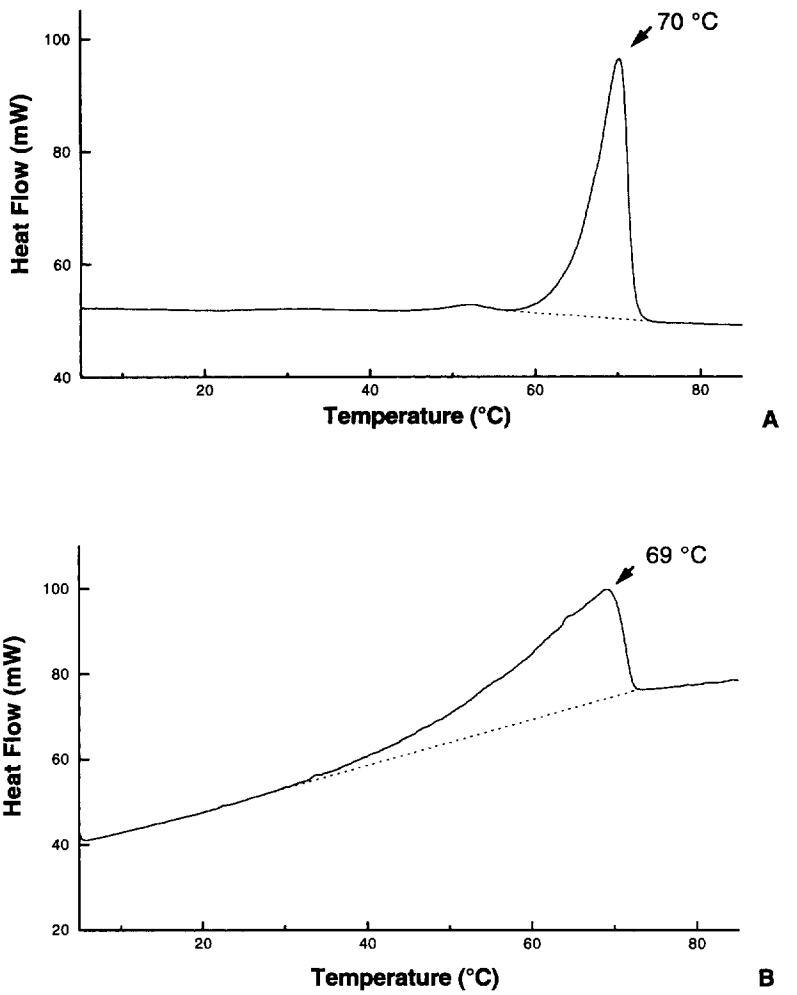

Fig. 6. DSC thermograms of the pure polyester (A) and the prcipitate (B). DSC conditions as in Section 2.6.

vironmental (column) temperature. The encapsulation effect is very sample size dependent, as will be pointed out later on.

In a previous study, the occurrence of time-dependent, kinetic effects in the redissolution of amorphous polyesters in RP-GPEC was shown [24]. Due to additional sorption, these effects did not influence the elution behaviour on a C_{18} column. From the results described above, however, it is obvious 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 underlying separation mechanisms! For these experiments, again sample CP1 was used, although the conclusions were found to be valid for CP2 as well.

At first, the influences of varying the injection volume and the injected amount were tested. An increase in the injection volume from 1 to 15 μl, while keeping the injected amount 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, as can be seen in

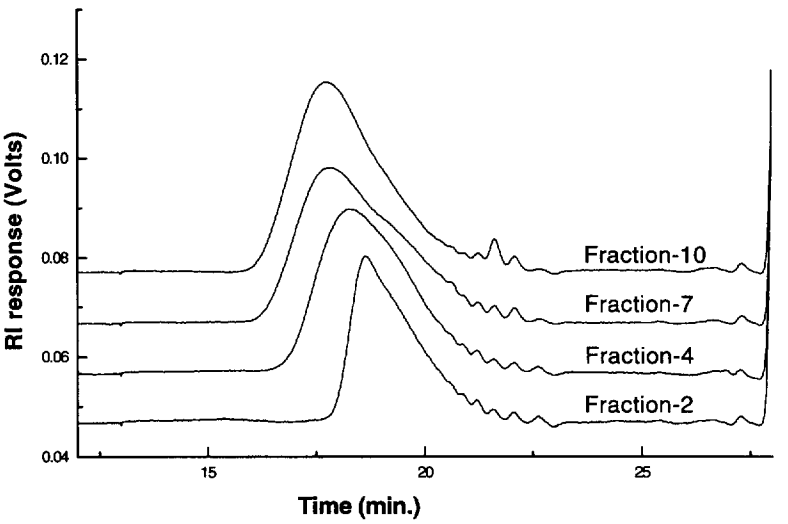

Fig. 7. SEC chromatograms of several fractions of sample CP1, obtained by GPEC. Fractionation conditions: see Section 3. Corresponding retention times of the respective fractions in GPEC (Fig. 3): fraction-2: 21.93–22.35 min, fraction-4: 22.78–23.20 min, fraction-7: 24.05–24.48 min, fraction-10: 25.33–25.75 min. SEC conditions as in Fig. 2A.

Fig. 8. The end of the distribution, however, only slightly shifts to a lower value of % solvent (%-S), as is shown in Table 2, where 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 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

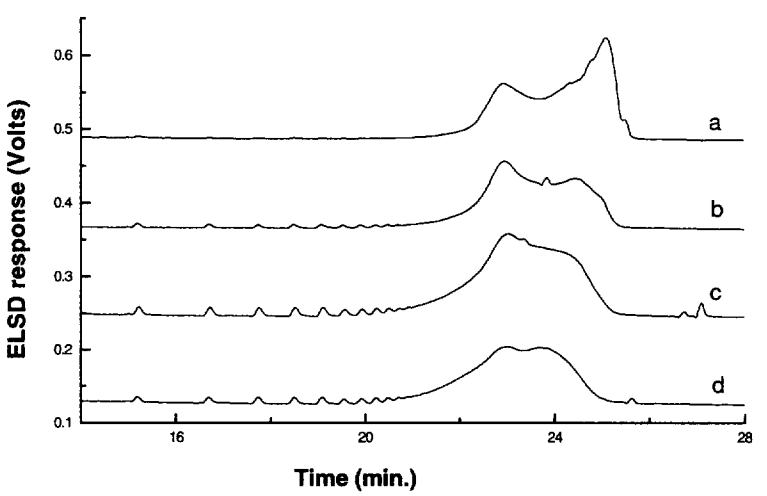

Fig. 8. Effect of injection volumne. (a) 1 μl, 100 mg/ml; (b) 5 μl, 20 mg/ml; (c) 10 μl, 10 mg/ml; (d) 15 μl, 6.67 mg/ml. Sample: CP1. GPEC conditions as in Fig. 2A.

Table 2
Effect of various chromatographic parameters on % solvent at the peak end of sample CP1

Parameter	Magnitude	% Solvent at peak end[a]
Injection volume (μl)	1	91.7±0.2[b]
	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 S–NS	10:90	92.1
	30:70	92.2
	50:50	92.8

[a] Peak ends determined by tangent method.
[b] Maximum deviation between duplicate measurements.

dependency of redissolution is more pronounced. This explains 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 [23]. 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. Due to insufficient mixing of the injection plug onto the top of the column, a micro gradient is generated, causing parts of the sample to migrate much faster through a part of the column before experiencing the actual eluent composition, thus causing peak broadening. Therefore, although it seems that larger injection volumes favor 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.

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 favor of the early eluting peak, which is shown in Fig. 9. Obviously, the elution

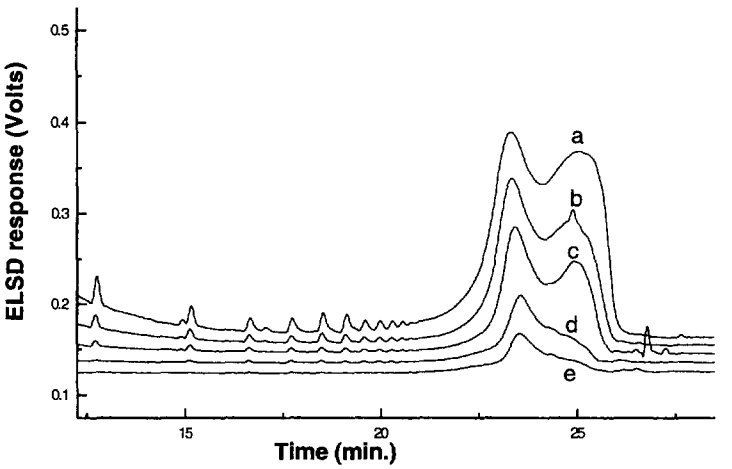

Fig. 9. Influence of injected mass of sample CP1 on the elution behaviour. (a) 400 μg; (b) 200 μg; (c) 100 μg; (d) 50 μg; (e) 25 μg (each dissolved in 5 μl THF). GPEC conditions as in Fig. 2A.

behaviour is influenced by the sample load, whereas in the case of amorphous resins, for the investigated range no effect was observed in an earlier study [23]. Like for varying injection volumes, the end of the distribution only slightly shifts, which can again be seen in Table 2 and bimodal distributions were found in all cases. Therefore, it is obvious that by lowering the injected mass, no normal elution behaviour can be achieved although a lower sample load seems to favor the final separation result slightly. The influence 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 [36,37]. From their experiments, a gradual increase of 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 gradually dropping below the environmental temperature, due to the increasing thermodynamic quality of the eluent, rather than the solubility capacity of the S–NS 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.

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 2. Furthermore, the ratio of both main peaks moderately changes in favor of the early eluting peak (picture not shown here, [29]). The influence 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 1 of [23]) as well as a separation governed by redissolution (Figure 7b of [24]). 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 conclusion also explains and confirms the results of above described experiments where a relative independence of elution time with varying injected mass was found.

A simultaneous decrease of both flow-rate and gradient steepness results in an increase in the ratio in favor of the second peak (picture not shown here, [29]) and in a relatively large decrease of %-S at the end of the distribution (Table 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 setup, the effect of a simultaneous decrease of 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 here, [29]), which can also be observed from the relative invariance of the peak end, as illustrated in Table 2. This again emphasizes 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.

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 influenced by using a guard column with a special flow distributor [38], 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 [39]. 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 Fig. 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

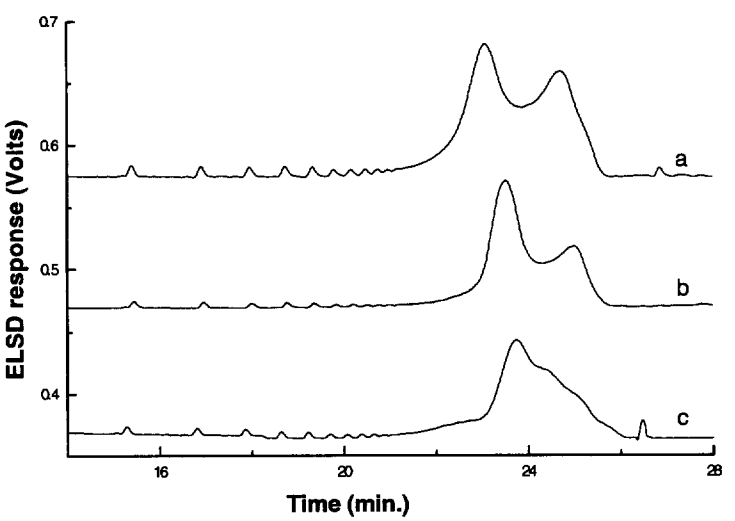

Fig. 10. Influence of precipitation medium on the elution behaviour of sample CP1. (a) Pre-column filter + guard column + analytical column. (b) Guard column + analytical column. (c) Analytical column only. GPEC conditions as in Fig. 2A.

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 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 exact 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 can be obtained. For amorphous polyesters, no influence of the precipitation medium was found [29]. Therefore, it is obvious that precipitation and redissolution in RP-GPEC for both types of polyesters is completely different.

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 influencing the latter effects, the separation result can only slightly be modified. Therefore, it was tried to influence the separation in a more thermodynamic way. From Eq. (1), it appears that melting point depression increases with increasing quality of the solvent. Therefore, experiments were performed with different initial S–NS compositions of the gradient, while keeping gradient steepness constant. Results can be found in Fig. 11. With increasing initial S–NS 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 (Fig. 1) towards lower temperatures at a higher S–NS 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 lower S–NS 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.

Furthermore, at a higher initial S–NS 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

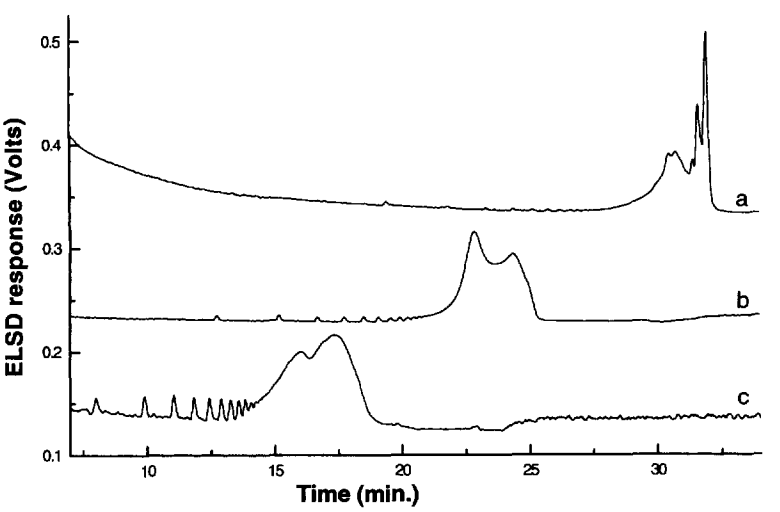

Fig. 11. Elution behaviour of sample CP1 at various initial gradient conditions. (a) Initial conditions THF–water (10:90, v/v); (b) THF–water (30:70); (c) THF–water (50:50). Gradient steepness: 3%/min to 100% THF. Other GPEC conditions as in Fig. 2A.

higher S–NS 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. Hereto it must be remembered that melting point depression depends on molar mass, according to Eq. (1).

The distribution end is hardly influenced by changing the S–NS ratio (Table 2). Furthermore, bimodality of the distribution does not completely disappear, even at initial conditions of 50:50 S–NS. Therefore, it is obvious that also under these conditions, elution of at least part of the polyester is determined by redissolution effects caused by crystallization after precipitation. Nevertheless, the elution of crystalline polyesters is clearly favored 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 [29]. 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 [40].

Based on these results, the next step is to check the influence of column temperature on elution behaviour. Results for sample CP1 are shown in Fig. 12, whereas this evaluation already has been performed for amorphous polyesters [23].

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. 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 (Fig. 6). It must be mentioned that DSC measurements were carried out under dynamic conditions. Therefore, an exact comparison of results from DSC and GPEC, is difficult.

At higher temperatures, no crystallization takes place anymore, thus giving rise to normal elution behaviour, most probably governed by sorption, just as it has been found for amorphous polyesters [23]. 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. (Fig. 13).

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

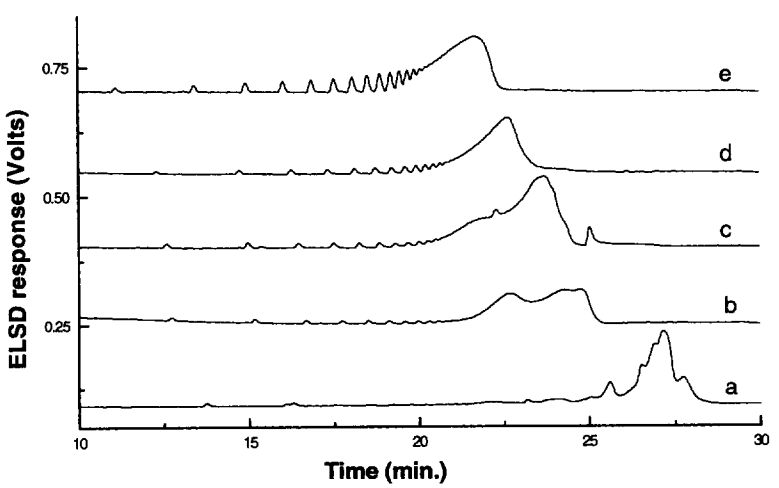

Fig. 12. Influence of temperature on the elution of sample CP1. (a) 16°C; (b) 27°C; (c) 29°C; (d) 31°C; (e) 50°C. GPEC conditions as in Fig. 2A.

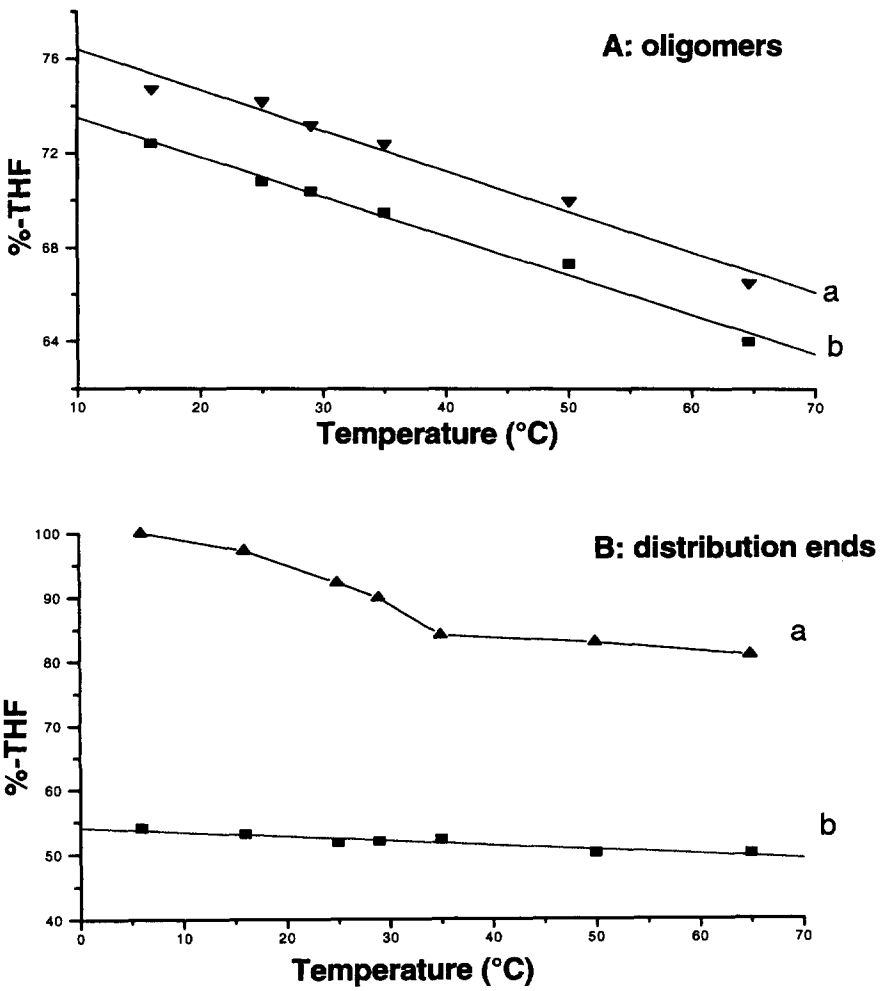

Fig. 13. Dependence of %-THF at the elution of oligomers (A) and distribution ends (B), respectively, on temperature. (A) Oligomers: (a) CP1, molar mass = 2078; (b) PE5, molar mass = 2050. (B) Distribution ends: (a) CP1; (b) PE5. GPEC conditions as in Fig. 2A.

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 temperature raise than that of PE5. For temperatures exceeding 35°C, qualitatively the same gradual decrease of retention time is found. This again shows the anomalous elution behaviour due to crystallization effects up to a temperature of 35°C. At higher temperatures, obviously no additional, time-dependent redissolution effects influence the retention behaviour anymore.

For sample CP2, qualitatively the same results were found, although the temperature above which no crystallization effects were observed anymore, is approximately 5–10°C higher. This can easily be explained from 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 16 000, a distinctly lower temperature was found above which no crystallization effects were observed. This can be explained from the lower melting temperature of the pure polyester and of the increased melting point depression due to its lower molar mass (Eq. (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 detector, sometimes small peaks eluting after the main distribution of crystalline polyesters were observed, even at high temperatures. Presumably, these peaks are caused by crystallization 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.

In a future publication we will show that similar differences between amorphous and crystalline polyesters occur in normal-phase GPEC experiments.

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 favor 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 Fig. 14A. Corresponding gradient conditions are given in Table 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 Fig. 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 crystallization.

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 [23], at least not for reversed-phase systems. In a future study, however, we will show that GPEC under normal-phase conditions is better suited for the separation of polyester blends under sorption conditions.

4. Conclusions

In contrast to amorphous polyesters, crystalline polyesters were found to exhibit non-reproducible chromatographic behaviour in GPEC below a certain temperature. From a comparison of the elution behaviour on various columns, it was shown that under the investigated conditions, chromatographic behaviour was governed by precipitation (crystallization)/redissolution effects, rather than by sorption. This is due to the formation of a solid, crystalline phase after precipitation which was 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 was 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.

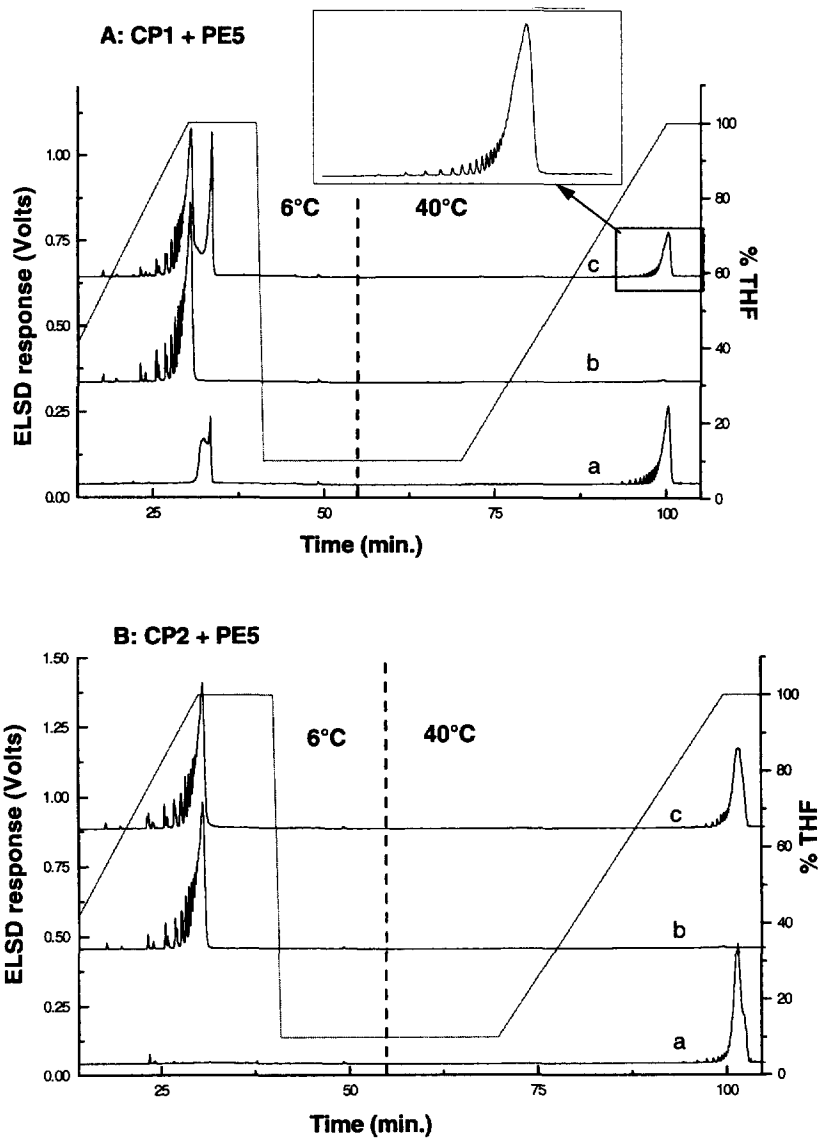

Fig. 14. Separation of blends of amphorous and crystalline polyesters by combined temperaure and eluent programming. (A) CP1/PE5: (a) CP1; (b) PE5; (c) blend (50:50, w/w). (B) CP2/PE5: (a) CP2; (b) PE5; (c) blend (50:50, w/w). GPEC conditions: sample concentration: 50 mg/ml (total concentration) in THF; column: Novapak C_{18} (150×3.9 mm), injection: 1 μl, detection: ELSD (Sedex-55), flow, gradient conditions and temperature: see Table 3.

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 influence of gradient steepness is also minor led to the conclusion that the redissolution behaviour of crystalline polyesters is mainly governed by thermodynamics by which it is determined 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 improve-

Table 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 period
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 period
190	10	6	0.1	

ment towards normal elution behaviour could be achieved.

A more pronounced effect was 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, finally led 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.

Finally, it was shown that 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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