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Separation of polyesters by gradient reversed-phase high-performance liquid chromatography on a 1.5 μm non-porous column☆

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Abstract

Efficient separation of polyesters composed of a large number of individual oligomers was achieved on a 1.5 μm ''non-porous'' octadecylsilyl (ODS) silica support by gradient high-performance reversed-phase liquid chromatography (gRP-HPLC) with a mobile phase of acetonitrile, aqueous trifluoroacetic acid (0.2%) and tetrahydrofuran at ambient temperature and signal monitoring by UV absorption at 280 nm. Substantial signal splitting of oligomers in the low molecular weight (M_r) region is indicative that separation not only occurs with respect to molecular weight distribution (MWD) but also to chemical composition distribution (CCD) and functionality type distribution (FTD). Although separation according to CCD and FTD decreases with increasing number of oligomers, co-elution of species with identical number of repeat units but differing in either structure of repeat units or end-groups can be assumed from the relatively broad signals succeeding the aforementioned peaks showing at least partial resolution. Despite the observation that high M_r oligomers elute as sharp signals, the preceding observations suggest that each of these peaks presumably composes of more than one individual component. The polyester oligomers are eluted in the range of increasing M_r and therefore, either separation according to MWD or CCD/FTD was at least achieved for the low M_r sample constituents. Some principal mechanistic aspects of separation are discussed and adsorption seems to play the dominant role. The detection limit, defined as that sample amount yielding an unequivocal recognition on the base of its characteristic chromatographic fingerprint pattern was about 5,000 ppm for the pair Alftalat 3258 – Alftalat 3352 and 10,000 ppm for the pair Crylcoat 430 – Crylcoat 801.

Keywords: Gradient elution; Stationary phases, LC; Polyesters

1. Introduction

Polyesters represent an important substance class and therefore find extensive application in different fields of chemistry. Owing to the fact that in many cases more than one di-functional acid and alcohol, furthermore substantially differing in chemical properties are used as the starting components, the final product calls for a more detailed characterization according to molecular weight distribution (MWD), chemical composition distribution (CCD) and functionality type distribution (FTD). Structural as well as chemical properties of polyesters are not only determined by *''linear''* chain propagation of the starting compounds, but also markedly depend on the additional use of both tri-functional acids and alcohols yielding more or less complex three-dimensional polymer networks that exhibit pronounced influences on the resulting physico-chemical prop-

☆This paper is dedicated to Professor Gottfried Schill on the occasion of his 70th birthday.

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erties. However, when compared with the extensively investigated group of polyethers, an only relatively small number of highly efficient chromatographic procedures for polyesters are found in the literature.

Separation of individual poly-(1,6-hexanediol-adipate) oligomers was effected by gRP-HPLC on a C_8 matrix with a binary eluent of acetonitrile and water by Krüger et al. [1]. In addition, the same authors subjected polyesters prepared from adipic and phthalic acid and different alcohols (e.g., 1,6-hexanediol, 1,4-butanediol, 1,2-propanediol, 1,3-propanediol, 1,2-ethanediol, 2,2-dimethylpropanediol-1,3 (neopentylglycol), diethylene glycol, dipropylene glycol, triethylene glycol) to *"two-dimensional"* chromatography using normal-phase liquid adsorption chromatography under *"critical conditions"* (NP-LACCC) as the first step and size exclusion chromatography (SEC) as the second for determination of either CCD/FTD or MWD [2]. Matrix assisted laser desorption ionization time of flight mass spectroscopy (MALDI–TOF/MS) was used in the first dimension for monitoring of CCD as well as FTD of end-groups. Polyethylene terephthalate oligomers were investigated by Guarini et al. [3] by LC coupled to MS via a thermospray (TSP) interface, whereas atmospheric pressure chemical-ionization (APCI) MS was used by Barnes et al. [4]. Furthermore, due to suitable stereochemical conditions, substantial amounts of cyclic polyethylene terephthalate oligomers are formed [5], which are easily recognizable by means of LC–plasma spray MS, as reported by Milon [6]. A rather new technique, especially developed for polymer characterization and termed as *"gradient polymer elution chromatography"* (GPEC), providing detailed information on either MWD or CCD/FTD, was reported by Klumperman et al. [7] as well as Philipsen et al. [8–11] with gradients composed of THF and water yielding separation about 20 oligomers, although baseline separation is only achievable for a few low M_r sample constituents. More recently, Philipsen et al. [12] investigated polyesters by either gRP-HPLC with THF–water on a C_{18} material or gradient normal-phase liquid chromatography (gNP-HPLC) with heptane–THF or heptane–dichloromethane–THF on cyanopropyl (CN) and polyvinyl alcohol (PVA) stationary phases and the RP technique proved to be superior.

However, despite the growing number of new separation procedures for polyesters in the literature, there is still need of high resolution techniques, which then, in conjunction with the results obtained from $^{1}H/^{13}C$-nuclear magnetic resonance (NMR) spectroscopic measurements and LC–MS investigations, may provide further information upon CCD and FTD.

Although new non-porous ODS materials consisting of 1.5 μm particles are available since about half a decade, an only limited number of oligomer or polymer separations was reported during the course of this time period and applications are preponderantly restricted to the rapid analysis of biologically active components and pharmaceuticals. However, a more pronounced re-inspection of hitherto unpublished chromatographic data obtained with oligomers of bisphenol-A diglycidylether, polybutylene glycol and polycaprolactone performed on such non-porous 1.5 μm materials more than four years ago, prompted us to investigate their capability for separation of the same ensemble of polyesters having already been subjected to high resolution HPLC, as outlined in [13]. In this paper optimum separation of polyester oligomers up to $M_r > 10\,000$ on a conventional 125× 4.6 mm I.D. 5 μm octadecylsilyl silica (ODS) column was described.The main question was, whether non-porous stationary phases, like their conventional 5 μm analogues, could also be used for efficient separation of polyester oligomers and if, to some extent, at least partial separation according to both MWD and CCD/FTD would be possible. In addition, the higher the achievable signal resolution of the chromatographic method the better will be its suitability for hyphenation with a technique yielding precise structural information, such as a mass spectrometer, in *"on-line"* LC–MS coupling.

2. Experimental

2.1. Separation media, samples and solvents

Acetonitrile and methanol (both HPLC grade) were obtained from Biosolve (Valkenswaard, The Netherlands). Non-stabilized tetrahydrofuran (THF) and trifluoroacetic acid (TFA), all HPLC grade, were from Fluka (Buchs, Switzerland). THF was used as obtained and not further purified by, e.g., distillation.

Table 1
Gradient system I

Time (min)	% Acetonitrile	% Water	% THF	% TFA (10%)
0	10	88	0	2
25	75	23	0	2
35	93	0	5	2
60	78	0	20	2
75	78	0	20	2
76	10	88	0	2
90	10	88	0	2

Water for the use in HPLC was purified with a Milli-Q reagent water system™ from Millipore-Waters (Milford, MA, USA). The polyester samples Alftalat 3258 and Alftalat 3352 were obtained from Hoechst (Frankfurt, Germany) and Crylcoat 430 and Crylcoat 801 from UCB (Drogenbos, Belgium). For gRP-HPLC the following stationary phases were used: Micra *''non-pourous''* C_{18} (30×4.6 mm I.D., 1.5 μm particle size) from Metrohm-Bischoff (Herisau, Switzerland), Kovasil MS C14 (30×4.6 mm I.D., 1.5 μm particle size) and Kovasil-H C_{18} (30×4.6 mm I.D., 1.5 μm particle size) both from Chemie Uetikon (Uetikon, Switzerland).

2.2. Analytical equipment

The HPLC system consisted of a P 4000 quaternary HPLC pump, an AS 3000 auto-sampler equipped with an integrated column oven and a 100 μl sample loop allowing injection of variable sample volumes, a type Spectra Focus scanning UV detector and a PC 1000 data acquisition unit, all purchased from Thermo Separation Products (San Jose, CA, USA).

2.3. Sample preparation and chromatographic separation

For gRP-HPLC solutions of the polyester samples were prepared in THF (10, 5, 2.5, 1%, w/v). Chromatography was performed with injection volumes of 5 μl at ambient temperature (approximately 22°C) and a flow-rate of 0.5 ml/min with a mobile phase composed of acetonitrile, aqueous trifluoroacetic acid (final concentration in the eluent 0.2%, v/v), THF and signal responses monitored at 280 nm. For Alftalat 3258 and Alftalat 3352 the two gradient profiles depicted in Tables 1 and 2 are used, whereas Crylcoat 430 and Crylcoat 801 were separated using the gradient profile shown in Table 3. In a *''sudden transition gradient''*-like experiment (see text) Alftalat 3352 (5% in THF, w/v) was injected into an aqueous phase containing 0.2% TFA (v/v). The concentrations of THF were suddenly raised from 0 to 10, 15, 20 and 30% three sec after sample injection. At the same moment the concentration of acetonitrile was linearly raised until a maximum amount of overall organic mobile phase of 98% (i.e., acetonitrile + THF) was reached at 35 min, so that

Table 2
Gradient system II

Time (min)	% Acetonitrile	% Water	% THF	% TFA (10%)
0	30	68	0	2
20	70	28	0	2
50	88	0	10	2
75	88	0	10	2
76	30	68	0	2
90	30	68	0	2

Table 3
Gradient system III

Time (min)	% Acetonitrile	% Water	% THF	% TFA (10%)
0	10	88	0	2
10	40	58	0	2
20	65	33	0	2
25	75	23	0	2
35	90	0	8	2
60	68	0	30	2
80	68	0	30	2
81	10	88	0	2
95	10	88	0	2

gradient conditions similar to those depicted in Table 1 were obtained. This composition of organic solvent was held for another 40 min followed by a drop to the starting conditions within 1 min and a re-equilibration period of 9 min.

3. Results

First of all it should be emphasized that satisfactory separation of polyester oligomers was only achievable on a Micra non-porous 1.5 μm support, whereas the two other tested stationary phases (i.e., Kovasil MS C14 and Kovasil-H C_{18}) showed substantially lower signal resolution, in particular for high M_r oligomers, which are poorly separated from each other and elute as a more or less broad peak envelope (results not shown). This behavior is at variance with previous findings showing much better chromatographic resolution R_s of the latter two column matrices when used for separation of oligomers of bisphenol-A digylcidylether, polybutylene glycol and polycaprolactone [14].

The sample pair Alftalat 3258 and Alftalat 3352 exhibits similar chromatographic fingerprint patterns, which is also valid for the pair Crylcoat 430 and Crylcoat 801. For this reason, only the results obtained from separation of Alftalat 3352 and Crylcoat 801 will be discussed and displayed. Furthermore, data from size exclusion chromatography (SEC) as well as $^{1}H/^{13}C$-NMR measurements have been reported elsewhere [13] and thus will also not be treated.

As can be concluded from Figs. 1a and 1b, gradient systems I and II effected extensive separation of Alftalat 3352, but no baseline resolution of oligomers was achieved for the medium-to-high M_r oligomers. Separation of low M_r oligomers with gradient system I seems to be superior to that obtained with gradient system II (Figs. 2a, 2b), whereas separation of medium M_r oligomers proved to be substantially better with gradient system II (Figs. 1a, 1b). Both gradient systems effected nearly identical chromatographic resolution R_s of sample constituents attributable to high M_r (Figs. 3a, 3b). Despite R_s of high M_r oligomers of Alftalat 3352 on a conventional Nucleosil $5C_{18}$ stationary phase (see [13]) is superior to that obtained on the 1.5 μm non-porous support, a closer inspection of the signals attributable to lower M_r sample constituents (Figs. 2a,b) revealed, that separation in this elution range is much better compared with the corresponding elution interval on the 5 μm ODS column as shown in Fig. 4 for comparison. Nevertheless, R_s of high M_r oligomers is still satisfactory (Figs. 3a, 3b) on the 1.5 μm support and therefore well-suited for on-line coupling to mass spectrometric detection (MSD). The most conspicuous feature of Figs. 2a and 2b is that low M_r oligomers show marked peak-splitting into up to five signals per oligomer, which can be interpreted with pronounced chemical heterogeneity due to the participation of a variety of starting compounds for polyester condensation [13] and/or separation with respect to different end-groups. Although none of these signals exhibits baseline separation, the overall chromatographic pattern of this elution interval indicates extensive CCD/FTD of the samples. As expected, signals responsible for CCD/FTD of sample constituents with the same mass difference between consecutive oligomers more

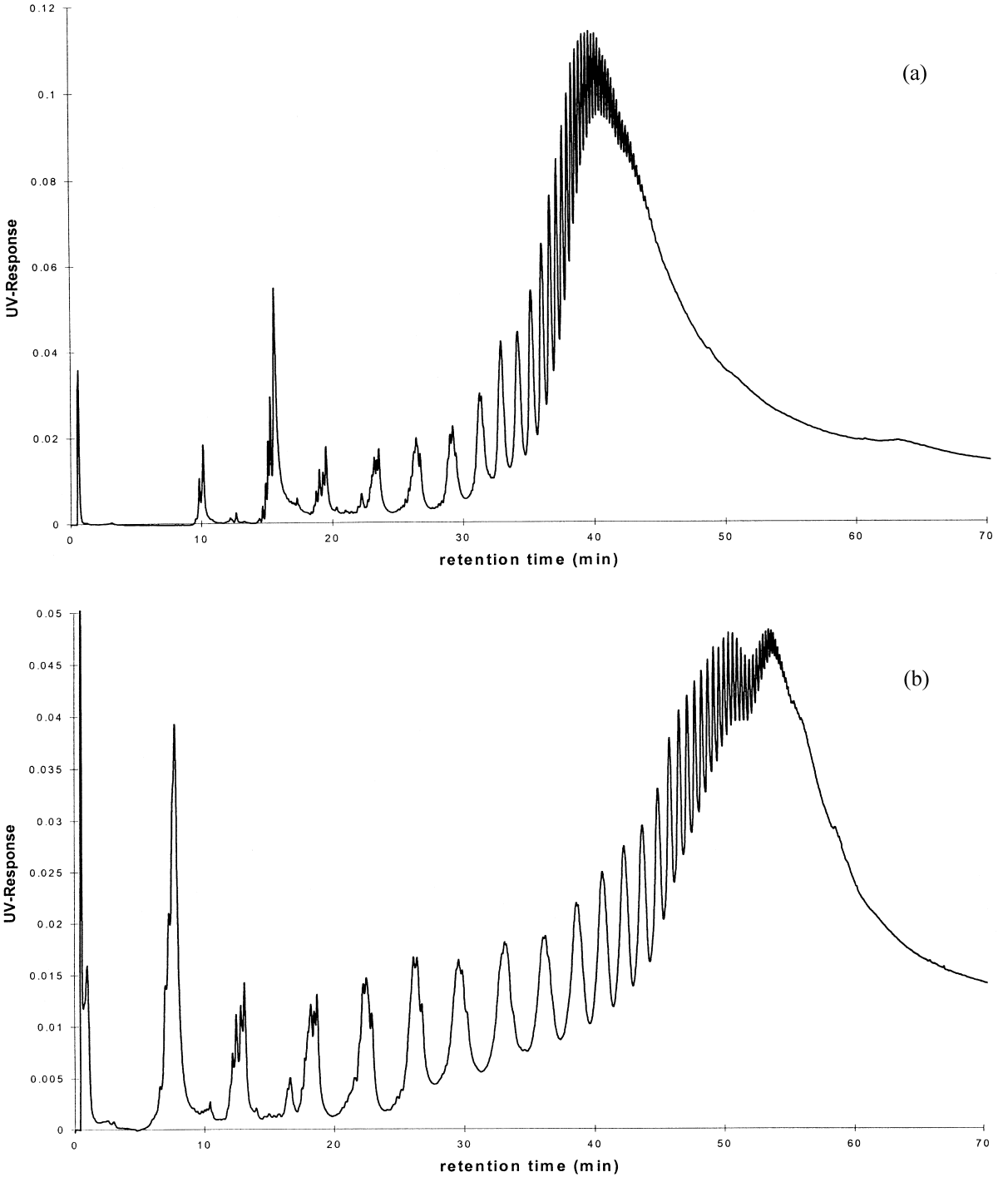

Fig. 1. Separation of Alftalat 3352 on a Micra non-porous (30×4.6 mm I.D., 1.5 μm particles) column with gradient systems I (a) and II (b).

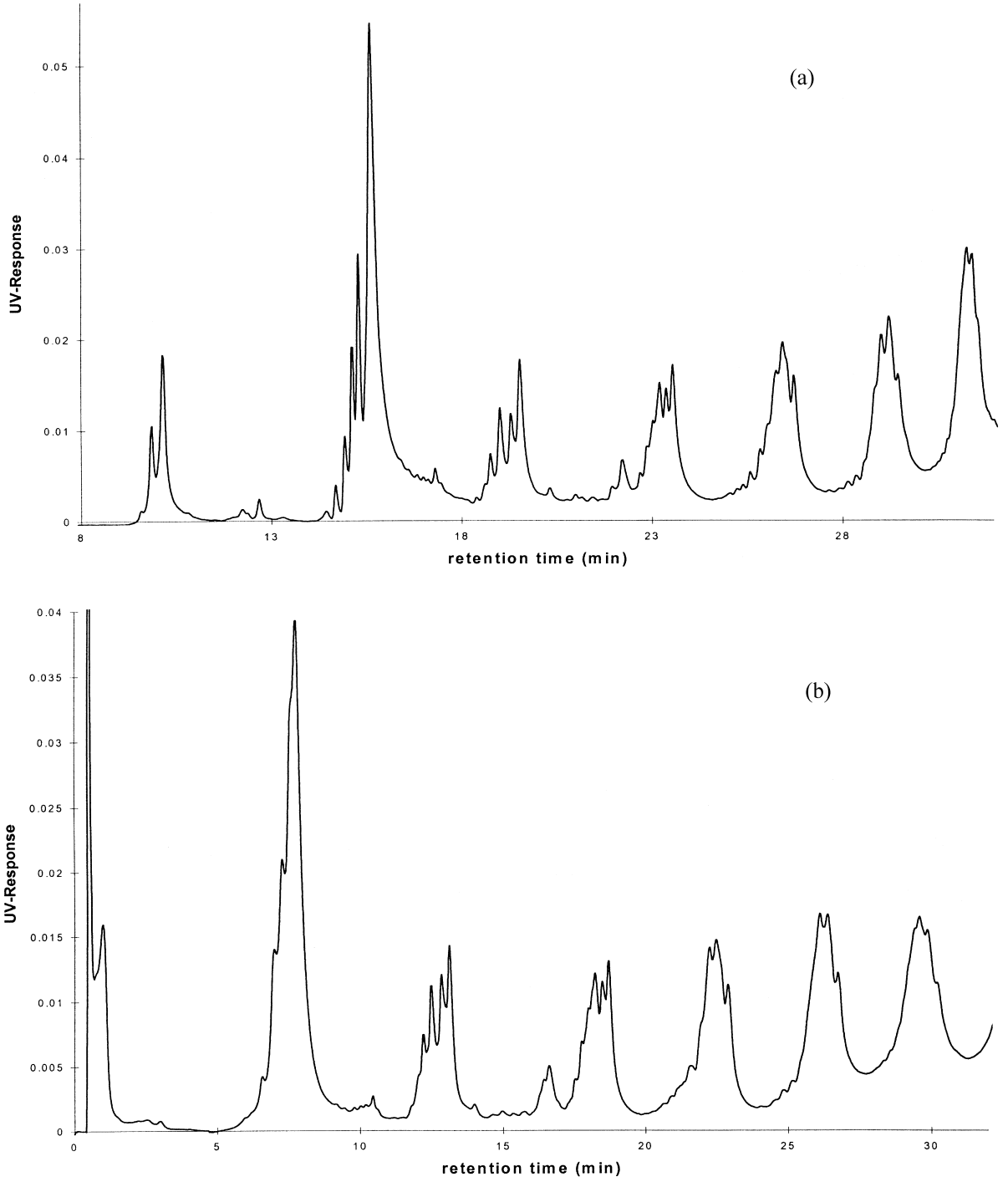

Fig. 2. Separation of Alftalat 3352 on a Micra non-porous (30×4.6 mm I.D., 1.5 μm particles) column with gradient systems I (a) and II (b): elution region of low M_r oligomers.

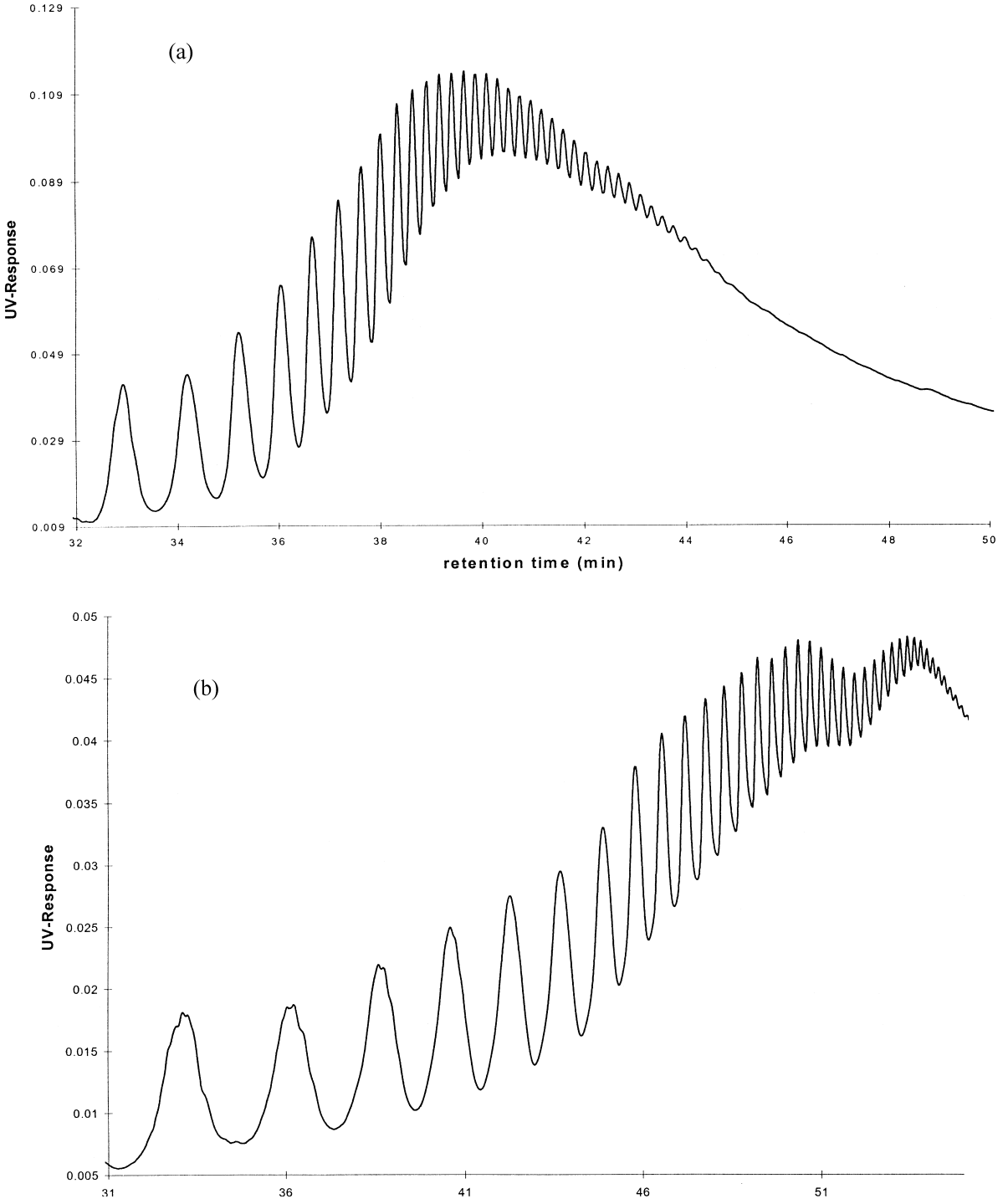

Fig. 3. Separation of Alftalat 3352 on a Micra non-porous (30×4.6 mm I.D., 1.5 μm particles) column with gradient systems I (a) and II (b): elution region of high M_r oligomers.

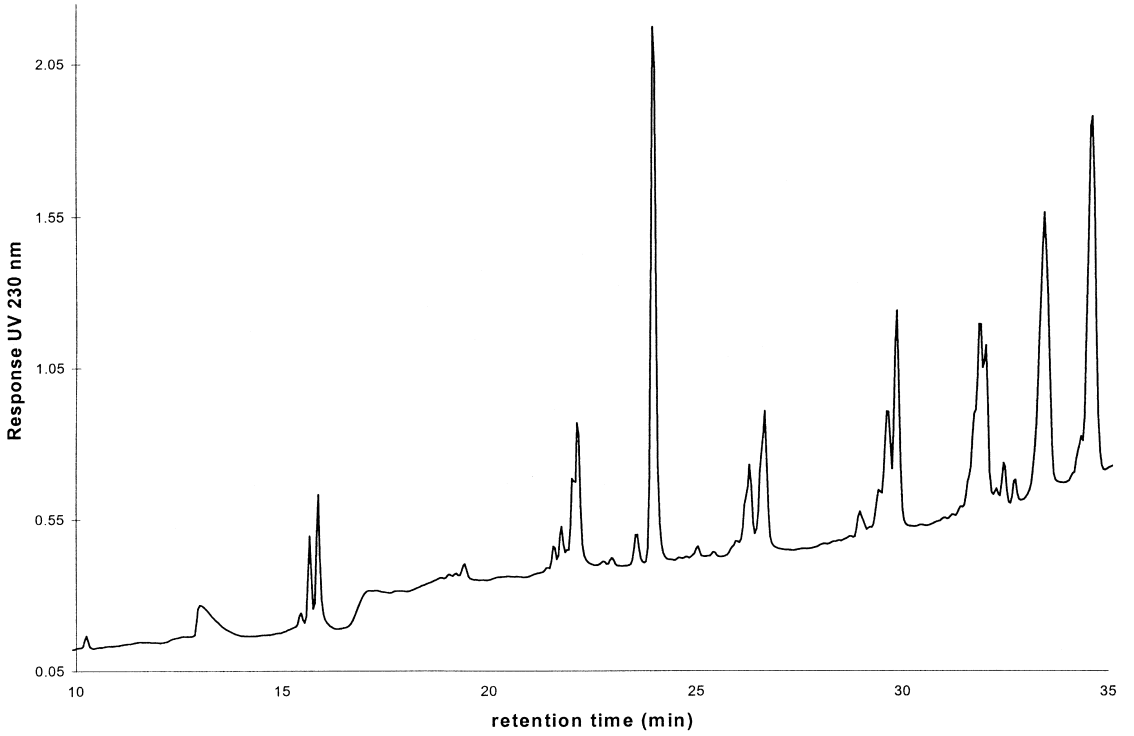

Fig. 4. Separation of Alftalat 3352 on a Nucleosil $5C_{18}$ (125×4.6 mm I.D, 5 μm particles) column (experimental conditions see [13]).

and more merge into more or less broad peaks with increasing retention time t_r and at about $t_r \cong 36$ min sharp and symmetrically-shaped signals are recognizable in the HPLC chromatogram. The decreasing R_s of oligomers with increasing number of repeat units (n) may be reasonably explained with decreasing differences in the interactive surfaces between oligomer n and oligomer $n+1$, which takes into account the continuous decrease of the term $\Delta m/M_t$ (m= mass of the repeat unit, M_t=total molecular weight) with increasing M_r. In addition, the number of possibilities attributable to variations in the ''microstructure'' dramatically increasing with increasing n contributes to progressively difficult separation. Due to the fact that low M_r oligomers show separation with respect to chemical composition and/or end-group functionality, it may be assumed that also the higher M_r sample constituents apparently showing single peaks consist of more than one component as can be inferred from the elution region at about $t_r \approx 30–45$ min (Fig. 1b), where partially resolved signals more and more merge into broad and unresolved signal envelopes.

In contrast to the situation of the two Alftalat samples, oligomer resolution is much lower with Crylcoat 430 and Crylcoat 801, as shown in Fig. 5a for the latter sample. This primarily concerns the high M_r elution region (Fig. 5b), whereas the low M_r oligomers exhibit comparable separation with the two Alftalates according to CCD/FTD (Fig. 5c). In this context it is worthy to note, that for exhaustive elution from the stationary phase both Crylcoat samples required much more *''good''* solvent THF as the *''solubility enhancing''* organic modifier (gradient profile III) than the Alftalates (gradient profiles I and II). This phenomenon is somewhat surprising because, as reported in [13], Table 4, the M_n, M_w, and M_w/M_n data obtained by calibration with polystyrene standards yielded values in a comparable range and thus, at least influences of M_r should not play a substantial role.

Furthermore, Alftalat 3352 was used as the model component to evaluate the feasibility of a method, termed as *''sudden transition gradient''* technique, for polyester separation. It has been developed about a decade ago by Glöckner et al. [15–21] and targeted

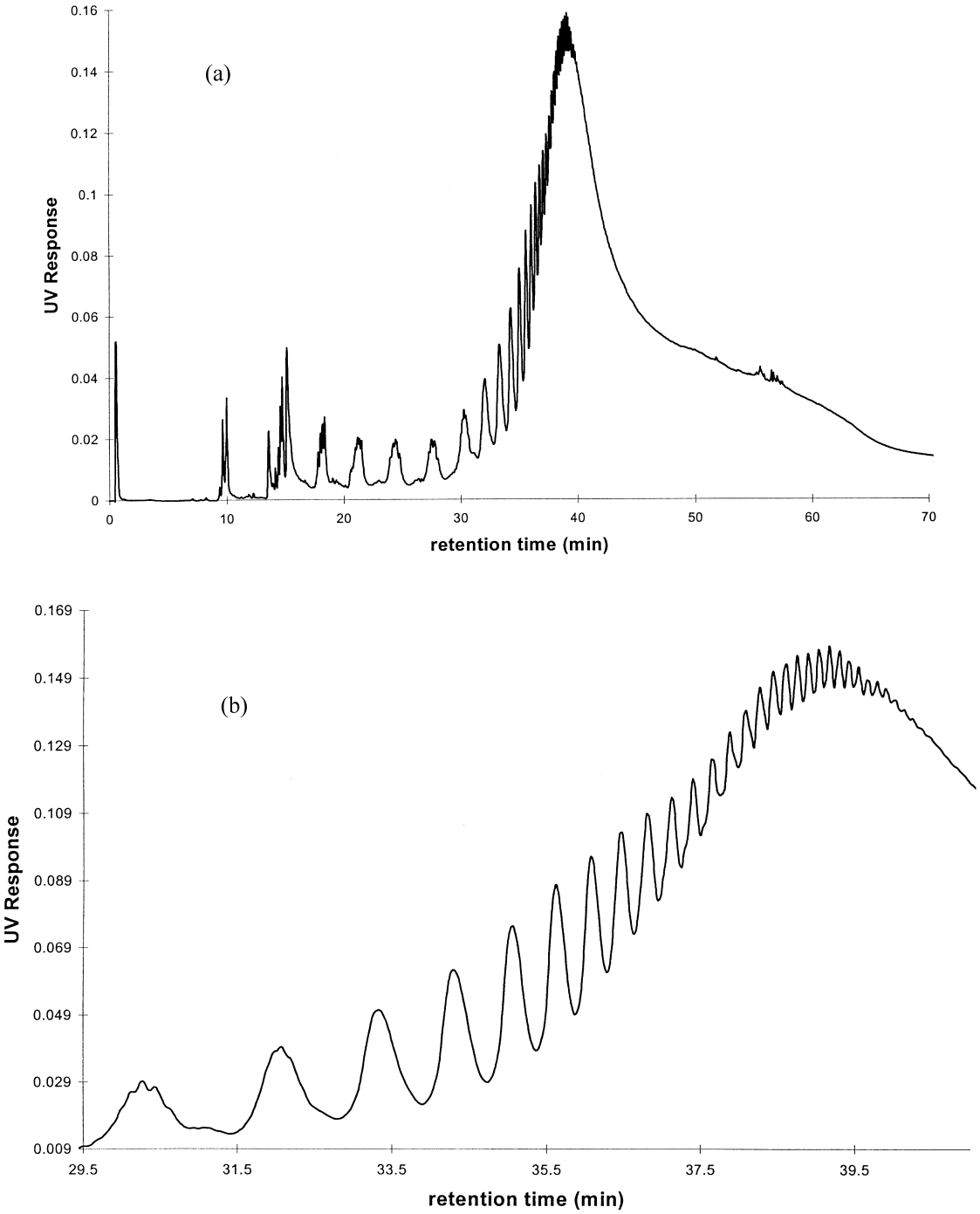

Fig. 5. Separation of Crylcoat 801 on a Micra non-porous (30×4.6 mm I.D., 1.5 μm particles) column with gradient system III: total chromatogram (a), elution region of high (b) and low (c) M_r sample constituents.

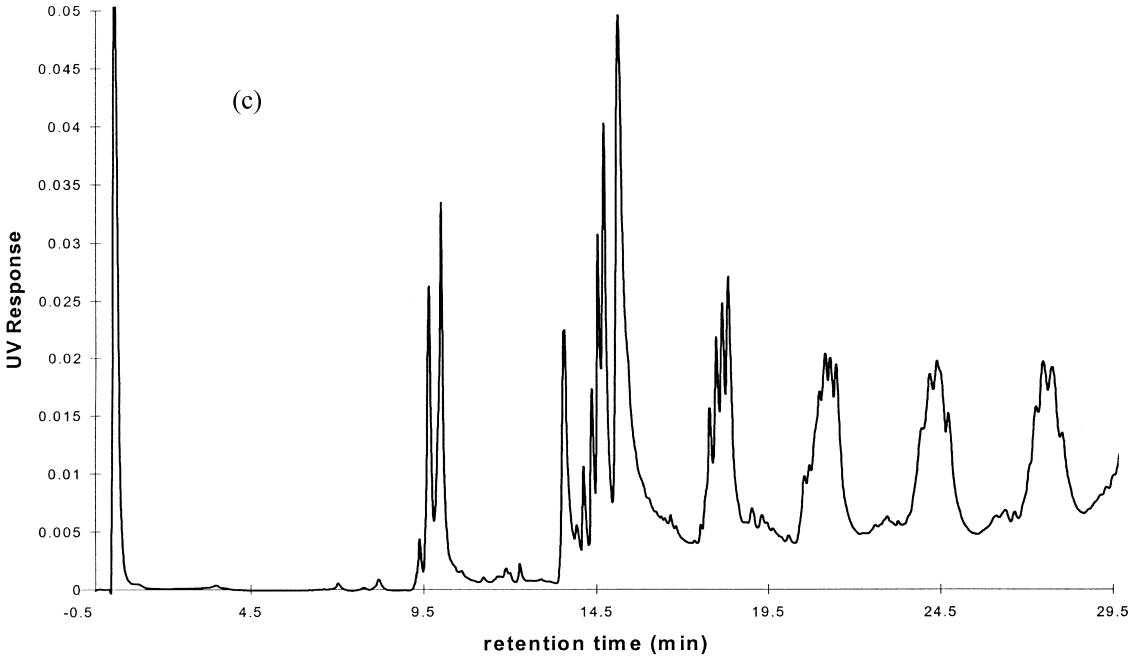

Fig. 5 (*continued*).

for separate control of solubility and adsorption of copolymers of styrene with acrylonitrile, methacrylates, ethylmethacrylates and methylmethacrylates. For this reason, the sample was injected into aqueous TFA (0.2%, v/v) and THF immediately added at concentrations of 30, 20, 15 and 10% (v/v). However, only in the case of sudden addition of 10% THF as the solubility modifier, separation shows substantial similarity (Figs. 6a–c) with Figs. 1a, 2a and 3a. In contrast, the chromatograms obtained by sudden addition of 30, 20 and 15% THF yielded much lower R_s with respect to either low M_r or high M_r oligomers (results not shown) than the chromatogram obtained when the concentration of both acetonitrile and THF was gradually changed (gradient profile I) as depicted in Figs. 1a, 2a and 3a.

All polyester oligomers are eluted in the range of increasing M_r and for this reason, at least for the low M_r sample constituents, separation according to both MWD and CCD/FTD was accomplished.

Unlike the situation reported in [13], measurement of the responses from evaporative light scattering detection (ELSD) was not feasible in the present investigations and although good R_s of oligomers was obtained by UV detection at either 230 or 280 nm, ELSD provided broad and more and more merging signals. This unexpected phenomenon markedly contrasts with separation of the same polyester samples on a conventional 5 μm ODS column, where band broadening invoked by ELSD connected to the UV detector in series proved to be negligible. At present, no reasonable explanation for this observation is available. UV detection at 280 nm was finally chosen, because the baseline mismatch invoked by admixture of THF due to the solvent's inherent absorption was tremendously lower compared with 230 nm applied in [13]. Taking into account that the limit of detection (LOD) considers unequivocal recognition of an oligomeric sample on the basis of its typical chromatographic fingerprint pattern, as proposed in [22], LOD's are approximately 5,000 ppm for Alftalat 3258 – Alftalat 3352 and 1,000 ppm for Crylcoat 430 – Crylcoat 801.

4. Discussion

In 1995 Bullock [23] reported his findings on

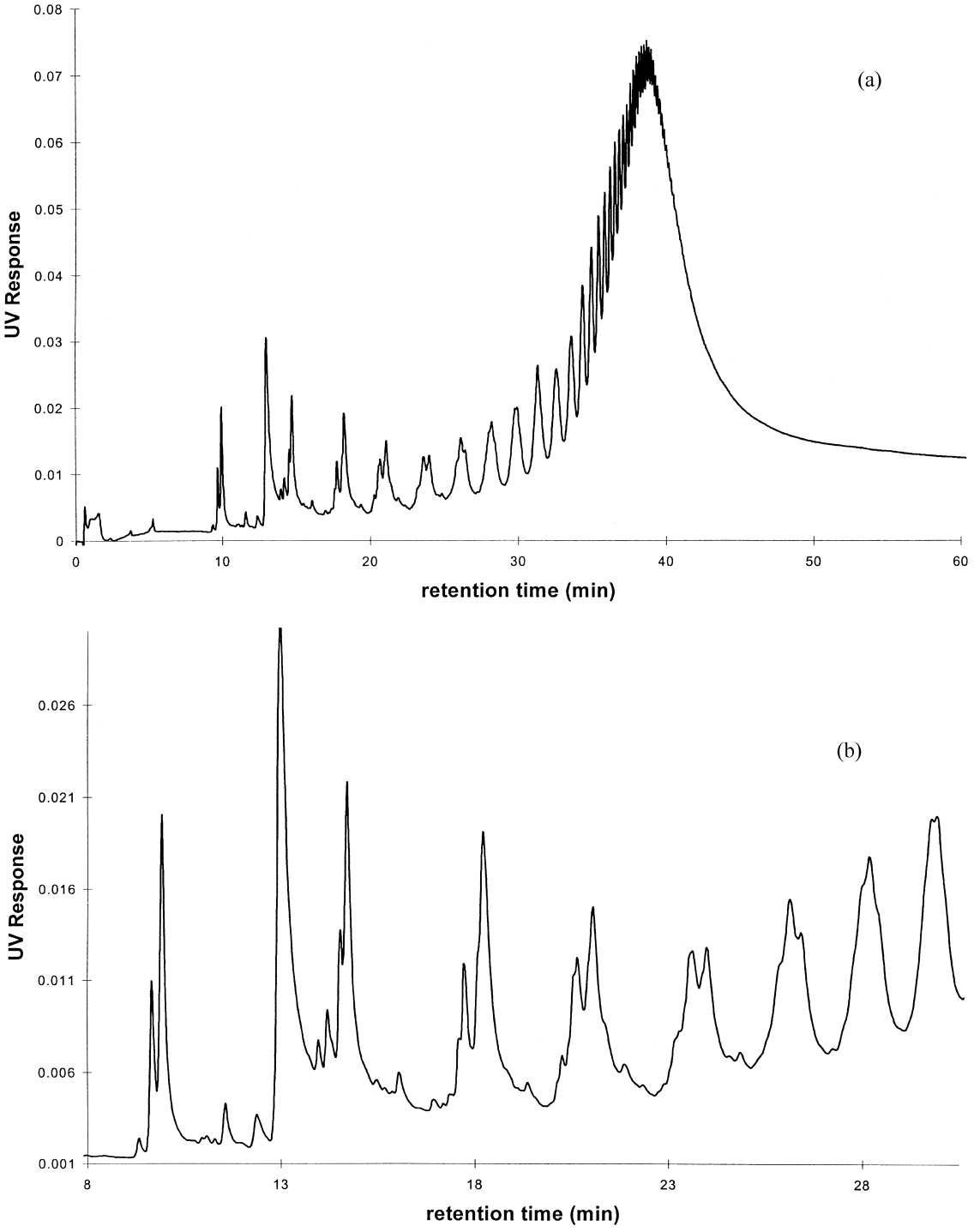

Fig. 6. Separation of Alftalat 3352 on a Micra non-porous (30×4.6 mm I.D., 1.5 μm particles) column with a *"sudden transition gradient"* by addition of 10% THF immediately after sample injection into an aqueous phase containing a final concentration of 0.2% (v/v) TFA: total chromatogram (a), elution region of low (b) and high (c) M_r sample constituents (for detailed elution conditions see Experimental).

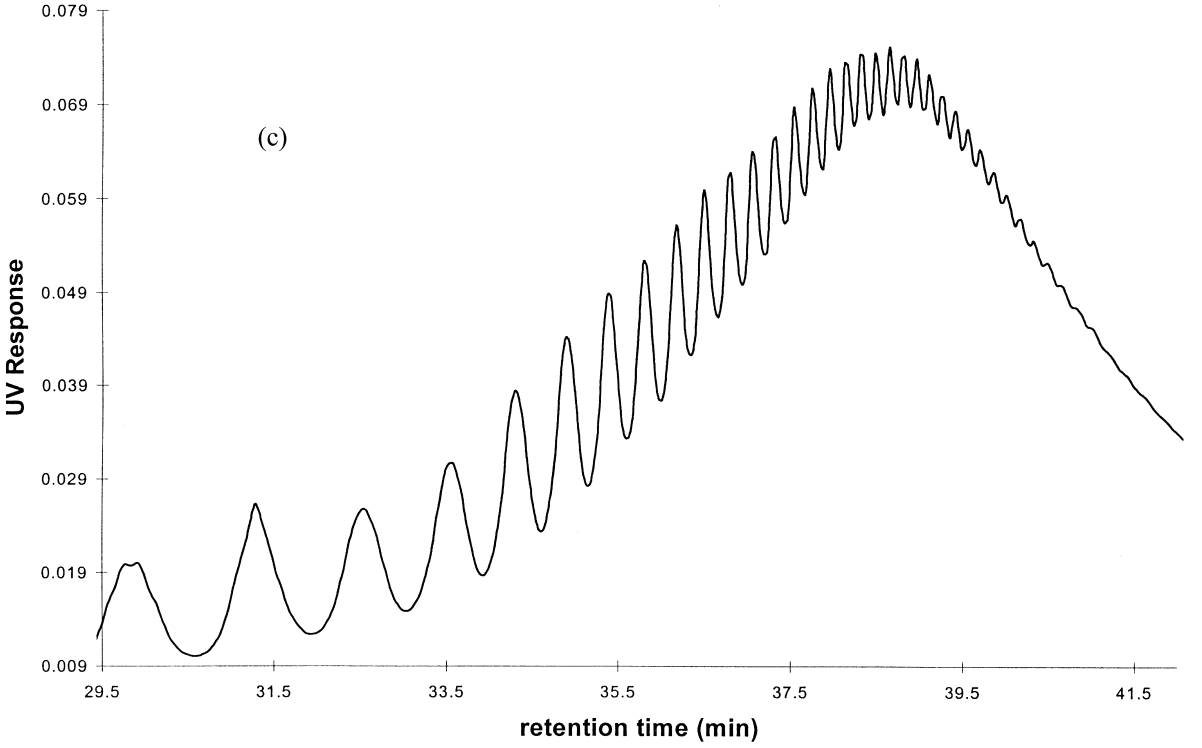

Fig. 6 (*continued*).

efficient separation of polymers used as magnetic resonance imaging (MRI) contrast agents in the M_r range of 10 000 up to over 30 000 on chromatographic supports composed of 2 μm diameter non-porous ODS particles. He applied binary gradients composed of acetonitrile and aqueous sodium perchlorate and observed markedly better separation compared with conventional macro-porous and traditional-porous stationary phases. The author's encouraging results, in common with hitherto unpublished data on HPLC of bisphenol-A diglycidylether resins, polybutylene glycols and polycaprolactones from our laboratory [14], was the incentive to evaluate the separation potential of a 1.5 μm non-porous ODS stationary phase with respect to some polyester samples recently having been subjected to gRP-HPLC on a 5 μm ODS column with a pore diameter of 100 Å [13]. We found excellent separation of polyester oligomers up to $M_r > 10\,000$ on the 1.5 μm support, making this column type a generally useful tool for chromatographic analysis of polymeric systems. However in contrast to Bullock [23], who observed increasing R_s with increasing column temperature (i.e., no separation into individual oligomers at a column temperature of 25°C and excellent separation at 100°C), this alternative was not considered, because signal resolution proved to be satisfactory at room temperature.

Elution of polyester oligomers with respect to increasing M_r is in accordance with the findings from a variety of authors [7–13]. Separation of high M_r sample constituents is substantially better on a conventional 5 μm ODS support (see Figs. in [13]) compared with the non-porous 1.5 μm analogue. Nevertheless, substantial splitting of signals with identical number of repeat units on the latter column gives rise to some reflections. Non-porous chromatographic supports have been originally developed for rapid separations of low M_r drugs (in most cases with $M_r < 1000$) in biological samples and thus are excellently suited for *"on-line"* coupling to mass spectroscopy. The virtual absence of a porous structure, which in general, is an indispensable prerequisite for efficient separation, was at least partially

counterbalanced by an overall increase in the outer particle surface as a consequence of their small diameter. In a first approximation, due to the obvious lack of a porous structure, the diffusion term, playing an important role by use of traditional stationary phases, can be neglected and adsorption–desorption equilibria are much more rapidly adjusted. Therefore, diffusion-mediated band broadening should be markedly reduced and R_s improved for the low M_r oligomers compared with a 5 μm ODS support. The superiority of the latter stationary phase with respect to the high M_r sample constituents can be explained by its higher overall interactive surface due to either increased column length of 125 mm versus 30 mm of the non-porous analogue or the porous structure. As a logical consequence, decreased discrimination between oligomer n and oligomer $n+1$ above a *"critical"* M_r barrier was achieved for the 1.5 μm non-porous ODS support.

When taking into account a distinction of different polyesters based on their individual fingerprint patterns [15], detection sensitivity seems to be unacceptably high at first glance. However, it should be kept in mind that signal spreading into a large amount of either completely or at least partially resolved peaks occurs, by which detection sensitivity of the whole sample is markedly decreased. Nevertheless, when merged into one unresolved peak envelope, as, e.g., encountered in a solvent of THF and water (90:10, v/v), LOD is improved at about two orders of magnitude (results not shown).

When the four polyester samples are subjected to gRP-HPLC at markedly different concentrations, i.e., ranging from 1–10% (w/v), neither increase nor decrease of retention times of the individual oligomers was observed, although precipitation at the column head might actually occur when such high sample amounts are injected into a thermodynamically *"poor"* solvent at the starting conditions of gradient elution, as will be the case in a mixture of 10% acetonitrile in water (gradient system I). However, at first sight, these results are in accordance with a mechanism based on liquid adsorption chromatography (LAC) and not, or at least only to a minor extent attributable to precipitation, as valid in high-performance precipitation liquid chromatography (HPPLC) [24–33]. Nevertheless, a mixed separation mechanism, based on either adsorption or precipitation, as discussed by Glöckner and van den Berg [29] cannot be ruled out. Typical separations by means of HPPLC are characterized by increased retention times with increasing sample concentration and thus markedly contrast to the situation in LAC, where retention decreases with increasing amount of sample. Furthermore, when separation is governed by a precipitation process, retention of polymer samples is extensively independent of the chemical composition of the stationary phase, as shown with copolymers of styrene and acrylonitrile [34]. This does not seem to occur in the present investigations, because different elution patterns are observed on different stationary phases [13]. In this respect, HPPLC, which can be explained by a process of continuous precipitation–dissolution during the sample's passage across the column [30] will not preponderantly contribute to chromatographic separation. However, a mechanism like this will substantially participate in separation of components far exceeding the M_r range of the comparatively low M_r polyester samples. In this respect, it is worthy to mention that typical macromolecular behaviour is only observable at about $M_r \cong 30\,000$ [35]. This will be true for high M_r samples of styrene, acrylonitrile and (meth)acrylate polymers of different chain length as well as their corresponding copolymers, furthermore markedly differing in composition, all obtained by radical polymerization. It should be emphasized however, that an exhaustive and satisfactory proof whether separation is dominated by either precipitation or adsorption will only be achievable in a satisfactory manner with *"narrow range"* polyester samples obtained by pre-fractionation according to M_r using SEC. In this context it is worthy to note, that gradient polymer elution chromatography (GPEC), mentioned above [7–12], exploits precipitation of the sample at the column head and its subsequent re-dissolution after the solvent front of *"good"* solvent has reached the sample for efficient oligomer separation. Nevertheless, as can be concluded from the previous discussion of results, adsorption seems to be the dominant retention mechanism in the present investigation.

As already reported elsewhere [13], a binary gradient of acetonitrile and aqueous acetic acid only affords elution of a few low M_r oligomers making admixture of THF a fundamental prerequisite for

either extensive sample elution or satisfactory oligomer separation. Substitution of acetonitrile in the binary gradient by THF, similar to GPEC [7–12], yet effected complete sample elution, but in contrast to the examples treated in [7–12], neither low nor high M_r oligomers are sufficiently separated (results not shown). Nevertheless, THF, although being inefficient for separation into individual oligomers, acts as an indispensable *"fine-tuning"* modifier, which is presumably attributable to its properties as a thermodynamically *"good"* solvent. This assumption is in agreement with the observation that neither acetonitrile nor methanol as the only organic modifiers, both being thermodynamically *"poor"* solvents or even precipitants for polyesters, are able to effect their elution, except some low M_r oligomers [13].

When acetonitrile as the polar organic modifier was replaced by methanol, while leaving all other parameters constant (see gradient profiles I–II), either incomplete sample elution or both, decreased signal resolution in the low as well as high M_r region was observed (results not shown). This finding is somewhat surprising for the following two reasons: (i) either concentration of the *"solubility-enhancing"* modifier THF or the gradient profile remained fully unchanged and (ii) both acetonitrile and methanol are precipitants for the polyester samples. Despite some differences in elution potency (acetonitrile in general being the better eluent in RP-HPLC), the observed elution characteristics are unexpected, at least to this extent[2].

In contrast to [13], where a final concentration of 0.5% (v/v) of acetic acid was used to suppress dissociation of free carboxyl groups[3], 0.2% (v/v) TFA was chosen in this case. This was done, because as found recently with the same polyesters on a conventional 5 μm ODS matrix, admixture of a final concentration of 0.2% TFA either permits chromatography at room temperature or effected better R_s [36].

Due to its capability in the independent control of either solubility or adsorption, the *"sudden transition gradient"* technique of Glöckner et al. [16–22] was tested as an attractive alternative for polyester separation. In this procedure, directly after sample injection into a thermo-dynamically *"poor"* solvent, a *"good"* solvent with moderate polarity, such as THF[4] was added, rising the solubility of the sample to a level where only adsorption is responsible for remaining retention. In this respect rapid re-dissolution of the precipitated sample is achieved, but the elution potency is still too low for sample elution in the absence of a more polar organic modifier, such as, e.g., methanol and/or acetonitrile. The concentration of the latter is then changed at a constant level of solubility and analytes are primarily separated according to their interactions with the chromatographic support. The range of THF added to effect *"sudden transition gradient"* conditions for Alftalat 3352 with acetonitrile as the polar organic modifier was restricted to 10–30%, because too high concentrations of THF would not only influence solubility but also be a rather good eluent in particular for the low M_r oligomers. Therefore, it is expected, that these sample constituents will be swept more or less unretained out of the column. When the chromatograms obtained after the sudden addition of 10, 15, 20 and 30% THF were compared with those obtained from the same sample by use of gradient profile I (Table 1), neither separation in the low nor the high M_r region reveals any advantages and only the chromatogram obtained from the sudden increase to 10% THF (Figs. 6a–c) shows marked similarity with Figs. 1a, 2a and 3a. These results provide proof that highly efficient separation of either low M_r or high M_r oligomers is only effected when the amounts of both, acetonitrile and THF are simultaneously changed during gRP-HPLC. Completely unsatisfactory results were obtained with *"sudden transition gradients"* and methanol as the modifier, which fits well with the observations reported before.

[2]Even when elution at a final concentration of 78% methanol and 20% THF (see Table 1) was extended for another 15 min, the "release" of oligomers from the column was incomplete when compared with acetonitrile as the organic modifier.

[3]Determination of the free acid content, attributable to carboxyl-terminated species revealed that about 10% of the acids used for esterification contribute to the acid number.

[4]The amount of "good" solvent, necessary for re-dissolution and subsequent elution of a precipitated sample with known composition can be easily determined by "cloud point" titration experiments [27,28].

Chromatographic separation of a large number of oligomers offers a wide range of perspectives for the combination with mass spectroscopy for exhaustive characterization according to either CCD and FTD. As reported recently [13], atmospheric pressure chemical ionization (APCI) has a limited application range of only about $M_r \approx 2{,}500$, but MALDI–TOF MS should provide more detailed data up to M_r of about 10 000 [7]. Unfortunately, as seen in recent investigations with resins based on bisphenol-A diglycidylether, structural information strongly decreases even at $M_r > 8000$ Da [37]. Nevertheless, it is expected that liquid chromatography coupled to the electrospray ionization time of flight mass spectroscopy (ESI–TOF/MS) in the *"on-line"* mode, as experienced by Prokai and Simonsick Jr. [38] as well as Nielen [39] in SEC of terephthalic acid neopentylglycol polyesters, will offer a powerful tool for future applications in the field of oligomer as well as polymer characterization.

5. Conclusions

Although at least at present small-dimensioned columns filled with non-porous 1.5 μm particles are only scarcely used as separation media for polymeric components, they proved as efficient chromatographic media for polyesters, composed of different acids and alcohols as the starting monomers showing $M_r >$ 10 000. Acceptable separation of a wide range of individual oligomers was achieved not only with respect to molecular weight but also to chemical composition and functionality type distribution, the latter two aspects preponderantly being valid at least in the case of low M_r sample constituents. Separation seems to be mainly governed by a mechanism based on liquid adsorption chromatography. Although the overall analysis time was not markedly different from that required with a conventional ODS matrix [13], columns containing small-sized particles allow substantial reduction of mobile phase consumption and thus efficiently contribute to a decrease in environmental hazards. Last but not least, due to its high separation power, the method is well-suited for coupling to an appropriate mass-specific detection system, as encountered in LC–MS, which then provides detailed structural information of the target components.

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