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OLIGOMER SEPARATIONS BY GRADIENT ELUTION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Gradient elution high-performance liquid chromatography (HPLC) on chemically modified silica is very suitable for both the qualitative and quantitative analysis of oligomeric mixture, as has been demonstrated for low-molecular-weight resins, pre-polymers and extracts obtained from high polymers and in studying the kinetics of the early stages of polymerization and polycondensation reactions.

Examples are given of oligomer separations with epoxy resins, novolak resins, poly-(2,6-diphenyl-*p*-phenylene oxide). (Tenax), poly(ethylene terephthalate) and poly(ethylene oxide) derivatives. All of these separations easily permit quantification, as has been demonstrated for ethoxylated octylphenol, provided that strict standardization is maintained throughout the analysis. Coefficients of variation of the peak-area measurements of the 19 oligomers observed in this sample were low (1–4%); the coefficients of variation of the retention times ranged from 0.01 to 0.3%.

Using commercially available chemically modified column packing materials, the resolution in the gradient elution HPLC of oligomers is adversely affected by size exclusion and sample solubility.

INTRODUCTION

Sample retention in liquid chromatography (LC) depends strongly on the composition of the mobile phase. In many applications of LC to the separation of complex mixtures, sometimes containing widely dissimilar components, it is frequently necessary to change the composition of the mobile phase in order to elute all of the components present in the sample satisfactorily from the column. Snyder[1] has described this situation as the "general elution problem". To overcome this problem, gradient elution or solvent programming may be applied.

The successive components of an oligomeric mixture generally show only a slight difference in chromatographic behaviour; on the other hand, the number of oligomers in one sample is often considerable. Therefore, gradient elution high-performance liquid chromatography (GE-HPLC) appears to be promising for the

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analysis of such mixtures. In no other form of chromatography is there the capability of altering the relative activity of the stationary phase in such a subtle manner and to such an extent as in GE-HPLC.

Other chromatographic techniques, such as thin-layer (TLC), gas (GC) and size-exclusion chromatography (SEC), can also be used for the analysis of oligomeric mixtures but they all have severe limitations. In TLC[2,3], poor reproducibility is obtained and the quantification is cumbersome*. With GC[4] a very limited number of oligomers are usually eluted from the column, even if the volatility of the sample components is increased by derivatization[5,6]. SEC on both soft and rigid gels is suitable for oligomeric mixtures, but is considerably less selective than GE-HPLC. On the other hand, SEC on soft gels may be an excellent method for preparative separations (10–100 mg)[7,8] and SEC on rigid gels can be very rapid[9].

In this paper the application of GE-HPLC to low-molecular-weight resins, pre-polymers and extracts of high polymers and in studying the kinetics of poly-condensation reactions is demonstrated.

EXPERIMENTAL

Apparatus

Use was made of a high-performance liquid chromatograph in the gradient mode (the gradient part consisting of two Waters Model 6000 pumps and a Model 660 solvent programmer), equipped with a UV detector (Waters Model 440 for detection at 254 nm and Cecil 272 for variable wavelength), a high-pressure sampling valve (Chromatronix HPSV-20), a linear potentiometric recorder (Kipp en Zonen BD 9, two channels) and a computing integrator system (Spectra Physics SP 4000).

The columns used were a 30 cm × 3.9 mm I.D. reversed-phase μBondapak C18 column (Waters Assoc., Milford, Mass., U.S.A.; particle size 10 μm) and a 30 cm × 3.9 mm I.D. semi-polar μBondapak NH_2 column (Waters Assoc.; particle size 10 μm). Unless otherwise stated, the former column was used.

Chemicals and materials

In all experiments distilled water was used. Hexafluoroisopropanol (Uvasol grade from Merck, Darmstadt, G.F.R.) was regenerated after use by distillation from the aqueous waste. *p*-Dioxane and tetrahydrofuran (analytical-reagent grade from Baker, Deventer, The Netherlands) were freshly distilled from Fe(II) sulphate prior to use.

Polystyrene standards were purchased from Pressure Chemical Co. (Pittsburgh, Pa., U.S.A.) and Arro Laboratories (Joliet, Ill., U.S.A.). Ethoxylated octylphenol was a commercially available product (Servo, Delden, The Netherlands). Epikote DER 671 was obtained from Dow Chem. (Midland, Mich., U.S.A.). All other oligomeric samples were prepared on a laboratory scale.

Procedures

The gradient elution procedure was standardized as follows. After running

* *Editor's note:* The quantification is certainly not more cumbersome than the HPLC technique in general. See for example B. Stancher *et al.*, *J. Chromatogr.*, 111 (1975) 459. *Editor of J. Chromatogr.*

the appropriate gradient, the column was regenerated by following the reverse gradient to the starting conditions in 5 min, and subsequently re-equilibrated under these conditions for 15 min. All gradients were started at the injection point.

Baseline drift was minimized by the choice of a suitable mobile phase system and distillation of the solvents used. The baseline drift due to the change in refractive index of the mobile phase during the gradient was considerably reduced by using the Waters UV Model 440 detector with a "tapered" cell. To check for solvent de-mixing or dehomogenization of the mobile phase, a blank run was made prior to the analysis. Before use all solvents were de-gassed.

The column temperature was maintained at 20° by a water-jacket. Column overloading was checked by observing the dependence of the retention and column efficiency on sample size. At the flow-rates used, the pressure did not exceed 2000 p.s.i.

The gradient profiles were tested over the full range with a solvent gradient of methanol to 0.06% (w/w) of benzene in methanol.

The distribution coefficient (K) in the molecular-weight calibration is defined as

$$K = (V_r - V_0)/(V_t - V_0)$$

where V_r is the retention volume of the solute, V_0 is the retention volume of a totally excluded species and V_t is the retention volume of a totally permeating species.

RESULTS AND DISCUSSION

Commercially available columns packed with chemically modified silica particles (10 μm) with an apolar or semi-polar character were used. These columns can be re-used in gradient work within a few minutes of returning from a previous gradient run with hardly any adverse effects on the reproducibility of retention data.

A number of excellent separations of oligomeric mixtures were achieved. The optimal gradient profiles were established experimentally[10]. In addition to the more general problems and limitations of gradient elution[10–13], some special difficulties (sample solubility and size-exclusion effect) that were encountered in this study are discussed below; the reproducibility of the quantitative analysis of oligomeric mixtures is demonstrated.

Epoxy resins

Several epoxy (bisphenol-A–epichlorohydrin) resins were characterized by GE-HPLC. Fig. 1 shows a separation of Epikote DER 671 using a reversed-phase column and a water–*p*-dioxane gradient as the mobile phase. The resin is completely fractionated up to the oligomer with $n = 16$. The identity of the oligomers with $n = 0$ and $n = 2$ was established by nuclear magnetic resonance spectroscopy after isolation of these oligomers from a 130-cm Merckogel PVA OR 2000 column (1.5 cm I.D.) in methyl ethyl ketone. The small odd oligomer peaks ($n = 1, 3, 5...$) indicate that this resin had been prepared according to the "fusion" or "advancement" process[14].

The gradient elution technique gives much more detailed information than SEC for this type of resin[9,14–16].

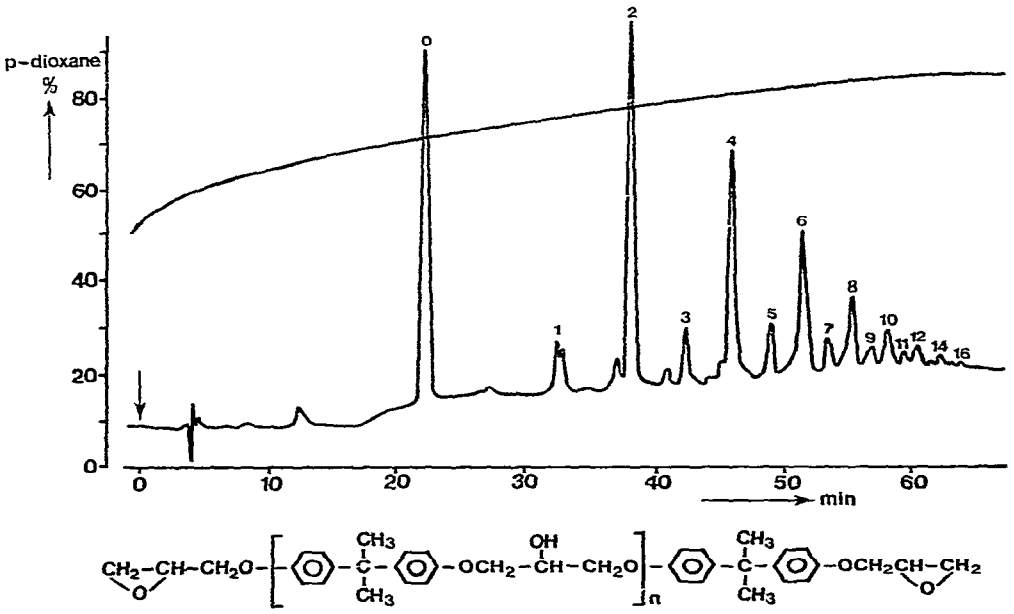

Fig. 1. GE-HPLC chromatogram of Epikote DER 671. Conditions: solvent gradient from 50% to 85% *p*-dioxane in water in 60 min; sample size, 100 μg in 20 μl of *p*-dioxane–water (80:20); flow-rate, 0.8 ml/min; UV detection at 270 nm, 0.5 a.u.f.s.

Novolak resins

Novolaks, prepared by condensation of formaldehyde and an excess of a phenol in acidic medium, are relatively low-molecular-weight resins built up of phenol groups interconnected by methylene bridges. With *p*-cresol, which possesses two equivalent reactive sites (*ortho*-positions) a distinct linear (*ortho–ortho*-coupled) polymer arises, which can be quantitatively characterized by GE-HPLC, as shown in Fig. 2. *o*-Cresol, however, has two unequivalent reaction sites (*ortho*- and *para*-positions), leading to a more complicated mixture of linear polymers, as shown by its gradient elution chromatogram in Fig. 3. The dimer of the *o*-cresol novolak already consists of three isomers (*o–o*, *o–p*- and *p–p*-coupling) that differ considerably in polarity; *e.g.*, the activity of the phenolic hydroxyl group varies as a result of steric hindrance. Hence the polarities of the *p–p*-, *o–p*- and *o–o*-dimers decrease in the order given[17]. From GC analysis, it is known that the relative amounts of the three isomers present decrease in the same order. Therefore, in the gradient elution chromatogram (Fig. 3), peak 1 relates to the *p–p*-dimer, peak 2 to the *o–p*-dimer and peak 4 probably to the *o–o*-dimer. Peak 3 represents the first isomer of the trimer.

These examples indicate that GE-HPLC is very suitable for obtaining either a "fingerprint" or a complete quantitative picture of novolak resins.

Oligomers of poly-(2,6-diphenyl-p-phenylene oxide)

Fig. 4 shows the separation of an oligomeric mixture arising from the oxidative coupling of 2,6-diphenylphenol (Tenax preparation). All oligomers present are baseline separated and can be easily quantified. In addition to the main oligomer

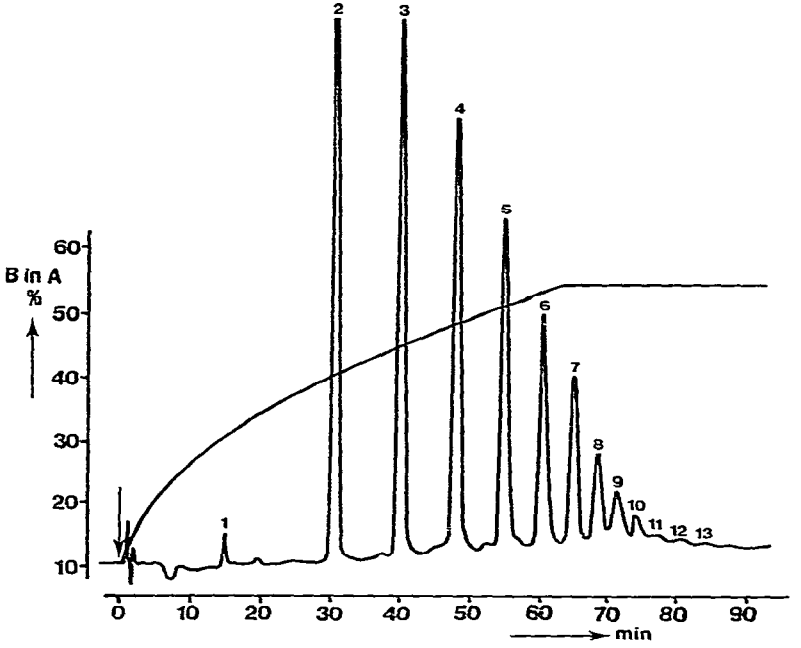

Fig. 2. GE-HPLC chromatogram of a *p*-cresol novolak. Peaks: 1 = *p*-cresol monomer; 2 = dimer; 3 = trimer; etc. Conditions: solvent gradient from 10% to 55% of solvent B in A in 60 min.; solvent A is 10% tetrahydrofuran (THF) in water; solvent B is 55% THF in water; sample size, 50 μg in 20 μl of THF–water (80:20); flow-rate, 1.0 ml/min; UV detection at 287 nm, 0.5 a.u.f.s.

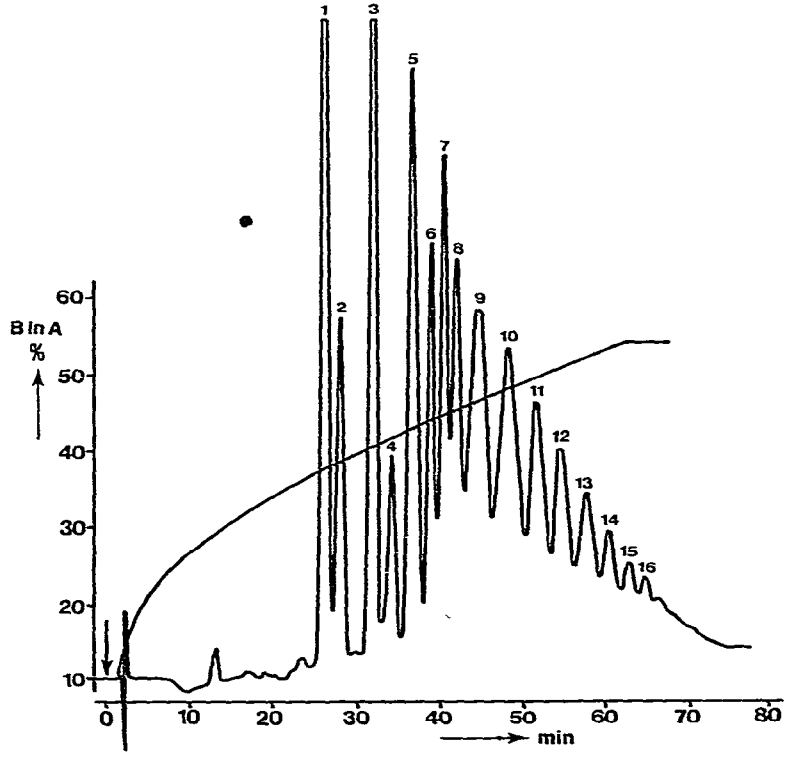

Fig. 3. GE-HPLC chromatogram of an *o*-cresol novolak. Conditions as in Fig. 2; sample size, 100 μg.

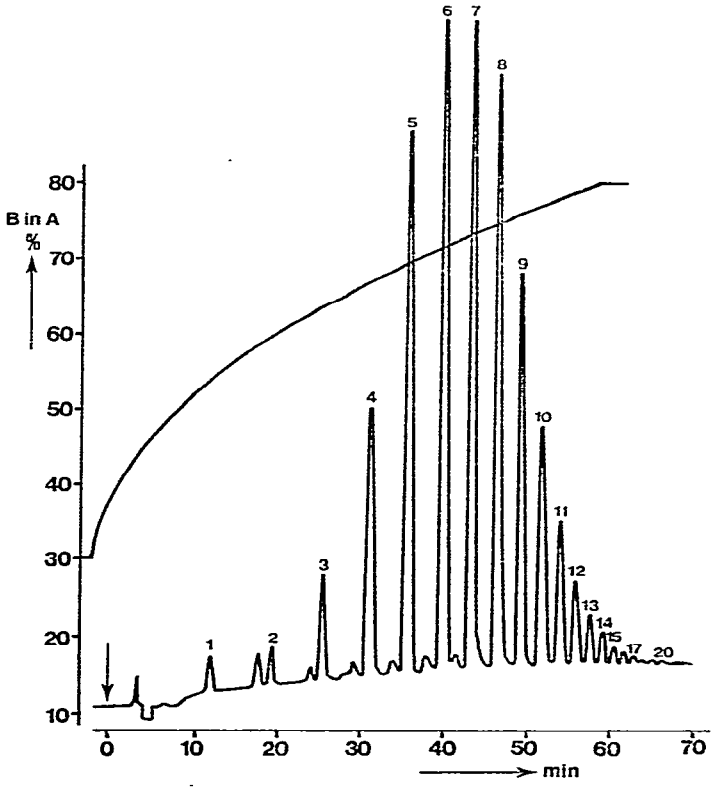

Fig. 4. GE-HPLC chromatogram of poly-(2,6-diphenyl-*p*-phenylene oxide) oligomers dissolved in *p*-dioxane–water (80:20). Peaks: 1 = monomer; 2 = dimer; etc. Conditions: solvent gradient from 30% to 80% of solvent B in A in 60 min; solvent A is 50% *p*-dioxane in water; solvent B is 1% water in *p*-dioxane; sample size, 25 μg in 50 μl of *p*-dioxane–water (80:20); flow-rate, 0.8 ml/min; UV detection at 254 mm, 0.5 a.u.f.s.

distribution, the gradient elution chromatogram shows the presence of a secondary distribution in this sample.

In this instance a separation up to a molecular weight of about 5000 (about the 20-mer) can be achieved.

Poly(ethylene terephthalate) oligomers

There have been several partly successful attempts to analyse poly(ethylene terephthalate) oligomer mixtures[5,18–20]. With a water–hexafluoroisopropanol mobile phase on a reversed-phase column, we succeeded in obtaining complete separations of poly(ethylene terephthalate) oligomers up to the 20-mer. An example of such a separation is shown in Fig. 5A. The minor peaks represent by-products such as cyclic oligomers.

A detailed report of this analysis method and its application to the kinetics of poly(ethylene terephthalate) polycondensation will be given elsewhere[21].

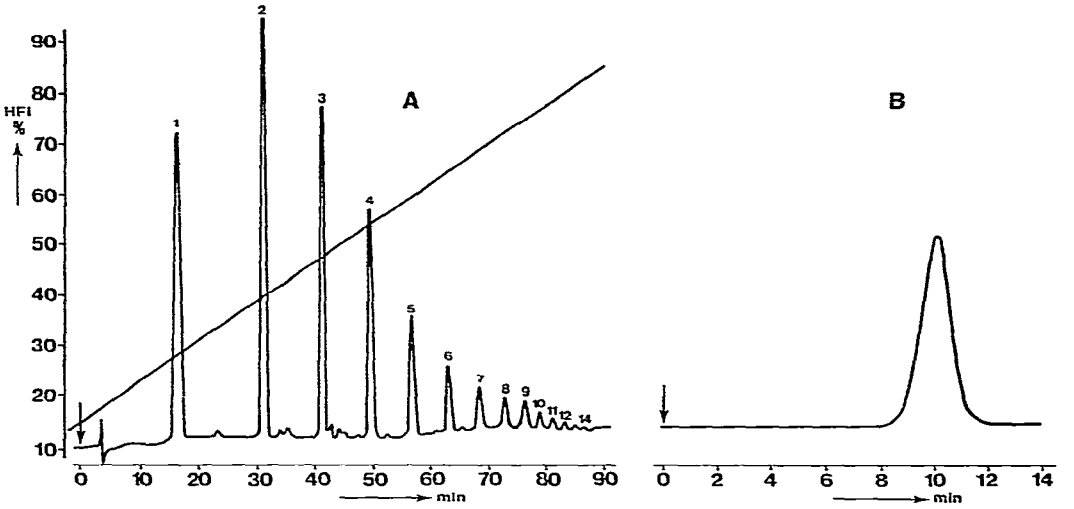

Fig. 5. (A) GE-HPLC chromatogram of poly(ethylene terephthalate)oligomers in hexafluoroisopropanol (HFI)–water. Peaks: 1 = monomeric bis-(2-hydroxyethyl)terephthalate; 2 = dimer; etc. Conditions: solvent gradient from 15% to 100% HFI in water in 105 min; sample size, 100 μg in HFI; flow-rate, 0.8 ml/min; UV detection at 270 nm, 0.5 a.u.f.s. (B) Size-exclusion chromatogram of the same sample. Conditions: mobile phase, HFI; sample size, 20 μg in HFI; pressure, 1000 p.s.i.; flow-rate, 0.3 ml/min; UV detection at 270 nm, 0.5 a.u.f.s.

Ethoxylated surfactants

Rapid chromatographic separation methods, such as GE-HPLC and capillary column GC, can be used for the chemical characterization of the often very complex technical ethoxylated products. The potentialities of capillary column GC in this field have already been described[6]. In earlier work on the HPLC separation of polyethylene oxide derivatives[22–25] mainly qualitative information could be obtained owing to inadequate separations.

We succeeded in obtaining a baseline separation of an ethoxylated octylphenol up to the 20-mer using a semi-polar NH_2-bonded column, permitting an accurate quantification (Fig. 6). Separations up to the 30-mer are possible. The separation of non-aromatic surfactants, *e.g.*, ethoxylated alcohols, is still being investigated[6].

Sample solubility

A serious problem in the application of GE-HPLC to oligomer analysis is the sample solubility. As the number of oligomers in one sample is often considerable and the detector attenuation should be such that excessive drifting of the baseline is avoided, sample loading of the column should be rather high. In reversed-phase chromatography, most samples dissolve easily in the apolar component of the gradient system and to only a limited extent in the polar solvent mixture at the start of the gradient elution. Injection of the sample, dissolved in the apolar, organic component of the gradient system, however, may cause a temporary disturbance of the phase system, leading to inferior separation.

For the separation of poly-(2,6-diphenyl-*p*-phenylene oxide) oligomers this is

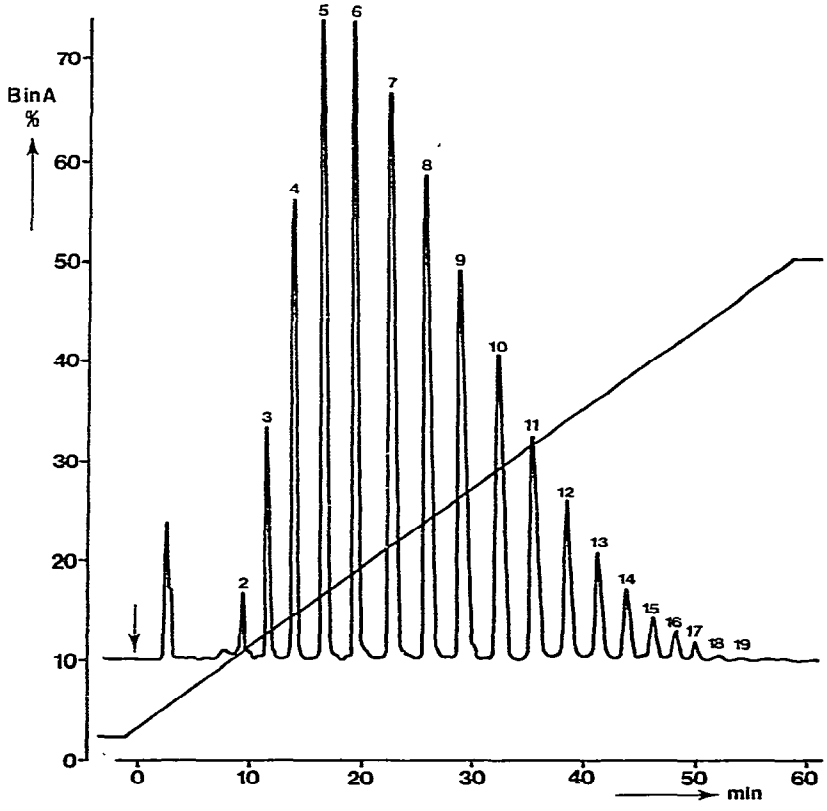

Fig. 6. GE-HPLC chromatogram of an ethoxylated octylphenol on a μBondapak NH_2 column. Peaks: 2 = oligomer with 2 EO units; 3 = 3 EO units; etc. Conditions: solvent gradient from 2% to 50% of solvent B in A in 60 min; solvent A is 20% THF in *n*-hexane; solvent B is 10% water in isopropanol; sample size, 200 μg in 20 μl of solvent A; flow-rate, 1 ml/min; UV detection at 280 nm, 0.5 a.u.f.s.

shown in Fig. 7 (sample solvent *p*-dioxane) in comparison with Fig. 4 (sample solvent, 80% *p*-dioxane in water); a suitable amount of water in the sample solvent eliminates the "sample solvent effect". The percentage of water, however, is limited by the decrease in solubility of the sample. Further, too large an injection volume leads to extra band broadening and also to a stronger "sample solvent effect". Hence in many GE-HPLC separations of oligomeric mixtures, a compromise has to be found between sample loading, injection volume and compatibility of the sample solvent and the initial phase system. Therefore, the gradient elution technique is somewhat limited and complicated.

An example of this limitation is the impossibility of obtaining a high-performance separation of the poly(ethylene terephthalate) oligomers in a mobile phase system other than in the rather unusual hexafluoroisopropanol–water. Fig. 8 shows the best separation achieved with *p*-dioxane–water; *p*-dioxane is one of the most common and water-miscible solvents for poly(ethylene terephthalate) oligomers. Leading peaks occur in the first part of the chromatogram ("sample solvent effect");

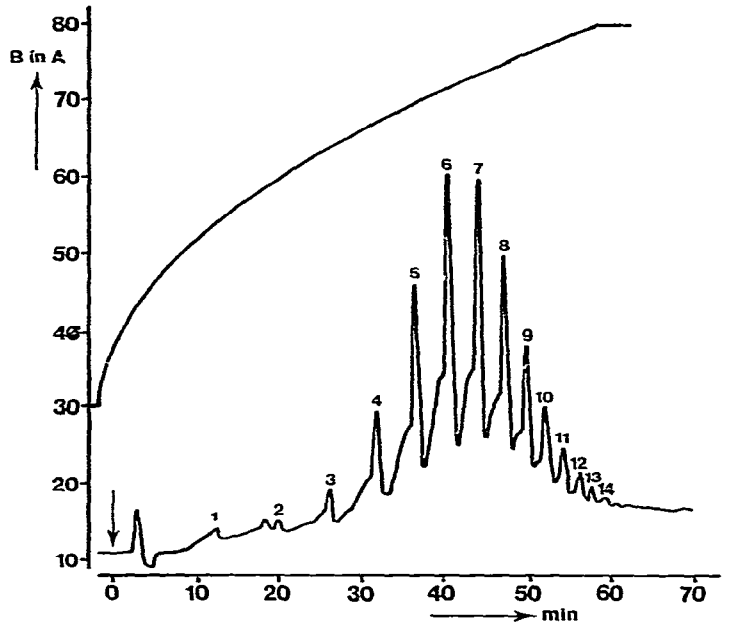

Fig. 7. GE-HPLC chromatogram of poly-(2,6-diphenyl-*p*-phenylene oxide) oligomers dissolved in *p*-dioxane. Conditions as in Fig. 4; sample size, 25 μg in 50 μl of *p*-dioxane.

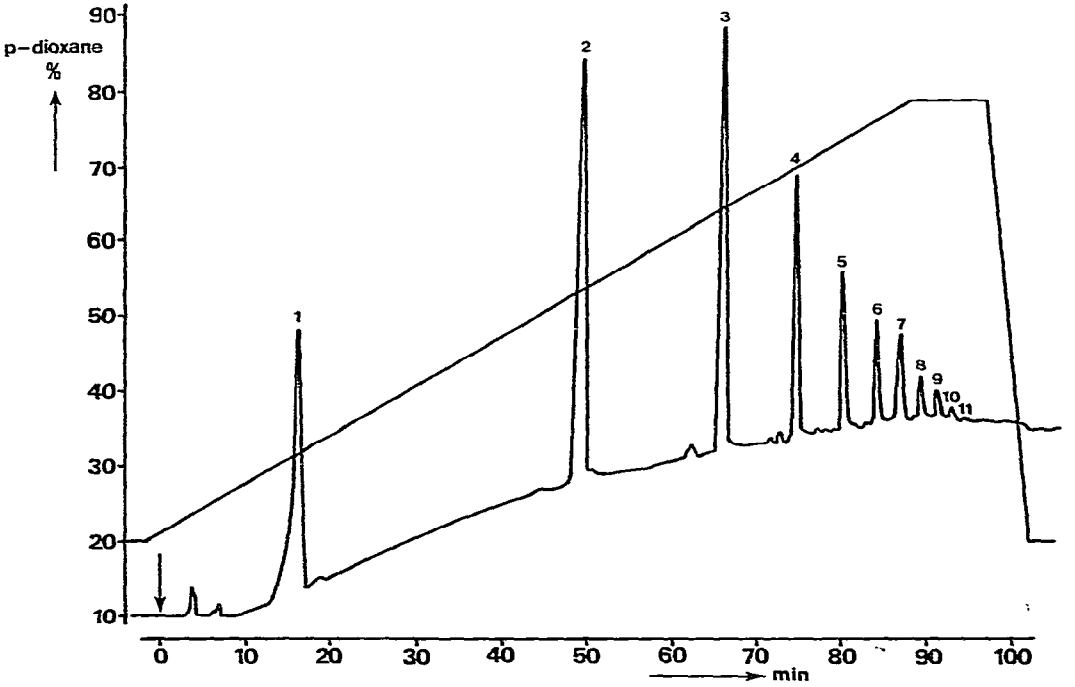

Fig. 8. GE-HPLC chromatogram of poly(ethylene terephthalate) oligomers in *p*-dioxane–water. Peaks: 1 = monomer; 2 = dimer; etc. Conditions: solvent gradient from 20% to 80% *p*-dioxane in water in 90 min; sample size, 20 μg in 20 μl of *p*-dioxane–water (90:10); flow-rate, 0.8 ml/min; UV detection at 270 nm, 0.2 a.u.f.s.

a relatively large baseline drift (sample loading too low and hence detector sensitivity too high) and a strongly decreasing distance between the successive oligomers (overall compatibility of the phase system insufficient) can be seen.

Preliminary experiments indicated that elevated column temperatures can effect some improvement in these inferior separations.

Size-exclusion effect

A second problem is the size-exclusion effect. This effect is shown in Fig. 9 for the separation of poly(ethylene therephthalate) oligomers. In the 10- to 20-mer region, the resolution between the successive oligomers decreases considerably and it appears to be impossible to improve the separation, even with an optimal gradient profile for this region.

The following causes of this poor resolution for the higher molecular weight oligomers can be mentioned.

(i) It may be partly due to the continuously decreasing relative differences between successive oligomers with increasing molecular weights.

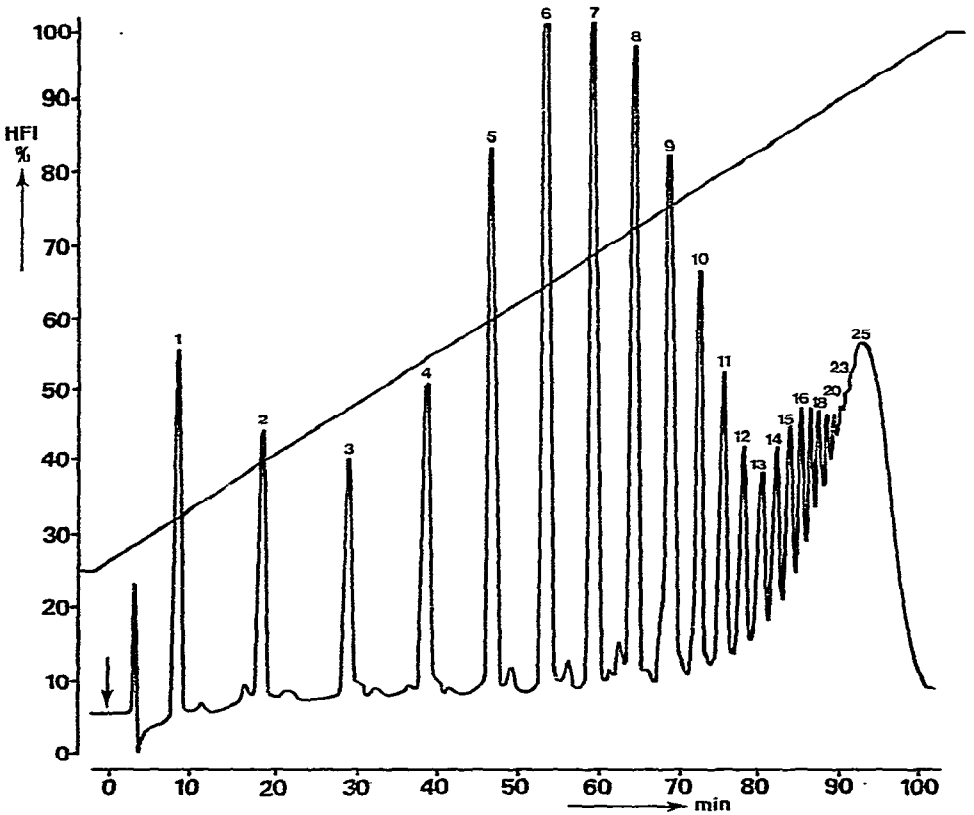

Fig. 9. GE-HPLC chromatogram of poly(ethylene terephthalate) oligomers of high average molecular weight. Conditions: solvent gradient from 25% to 98% HFI in water in 105 min; sample size, 200 μg in HFI; flow-rate, 0.8 ml/min; UV detection at 270 nm, 0.5 a.u.f.s.

(ii) Another cause may be the precipitation of the sample at the head of the column, owing to incompatibility of the sample with the initial mobile phase, followed by gradual re-dissolution. This process may be accompanied by additional band broadening. Owing to the gradient, however, this broadening will be largely neutralized during passage through the column. A further study is being made of this precipitation–re-dissolution process.

(iii) In our opinion, the main cause is the decreasing accessibility to the silica pores of the higher molecular weight oligomers (size exclusion), leading to less retention owing to (a) a decrease in the active reversed-phase surface area available (main effect) and (b) a decrease in the total pore volume that is passed through by the molecules (minor effect).

The extent of the size-exclusion effect is clearly demonstrated in Figs. 10 and 11. Fig. 10 shows the elution behaviour of a number of polystyrene standards on the μBondapak C18 column in tetrahydrofuran. All of the standards, except the monomer, are eluted before the unretarted position owing to size exclusion.

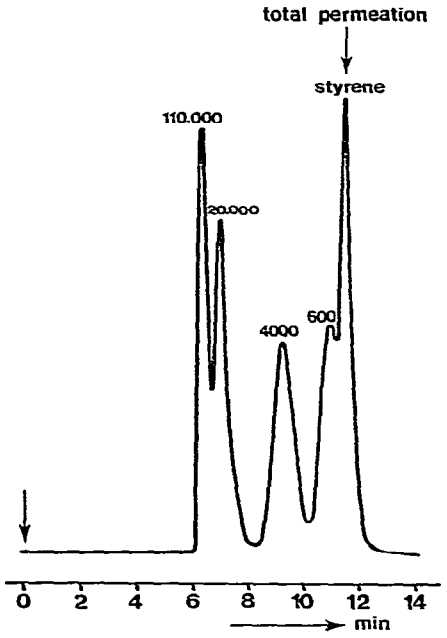

Fig. 10. Size-exclusion chromatogram of polystyrene standards on μBondapak C18. Conditions: mobile phase, THF; pressure, 800 p.s.i.; flow-rate, 0.25 ml/min; UV detection at 254 nm, 0.5 a.u.f.s.

In Fig. 11, the logarithm of the molecular weight of the standards is plotted against the distribution coefficient, *K*. According to Scott and Kucera[26], it has been calculated from this curve that for polystyrene standards on μBondapak C18 the size-exclusion effects are already present at a molecular weight of *ca.* 200. At a molecular weight of *ca.* 17,000, total size exclusion occurs.

In accordance with Kirkland[27], it can be concluded that in the GE-HPLC of higher molecular weight oligomers the efficiency of wide-pore reversed-phase materials

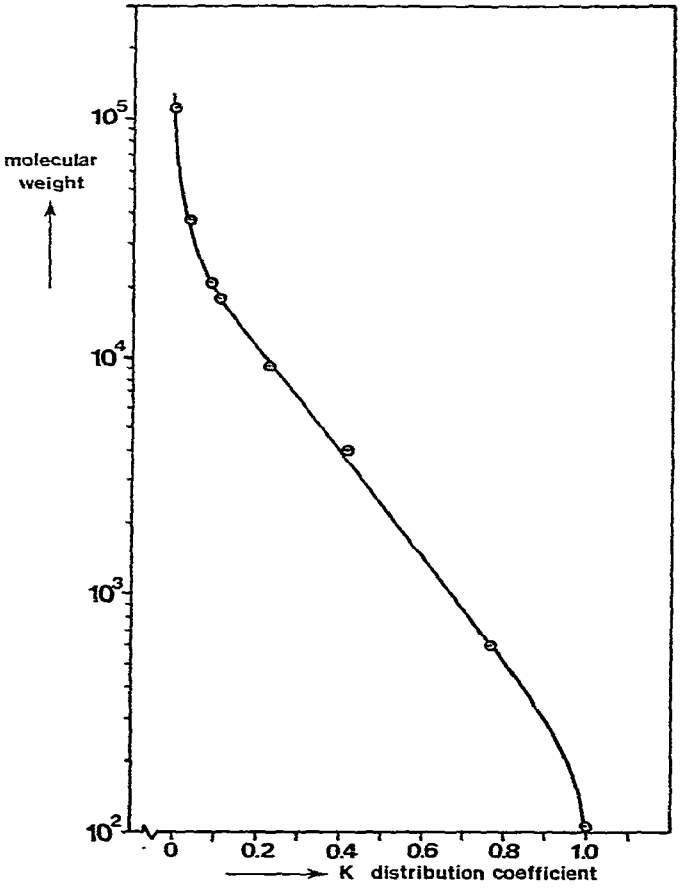

Fig. 11. Molecular-weight calibration plot of polystyrene standards for a μBondapak C18 column in THF.

(100–300 Å) may be equivalent to or even better than that of small-pore materials owing to the size-exclusion effect.

In principle, the size-exclusion effect enables us also to achieve molecular weight distributions by SEC on the same reversed-phase column. This is demonstrated in the Figs. 5A and 5B for poly(ethylene terephthalate) oligomers.

The selectivity of a μBondapak C18 column as a size-exclusion support, however, is low owing to its relatively small total pore volume[9].

Quantitative analysis

GE-HPLC is not often considered for quantitative analysis because of severe experimental difficulties[10]. Nevertheless, for oligomeric mixtures we found that a complete quantitative analysis can be carried out very well by the gradient elution technique, provided that the experimental conditions are carefully standardized (see Experimental).

The reproducibility attainable in the quantitative analysis of oligomeric

samples has been demonstrated for an ethoxylated octylphenol (Fig. 6). In Table I, the average retention times and the coefficients of variation ($n = 6$) are given for the 19 oligomers examined. Table I also gives the composition of the sample (100% analysis) and the coefficients of variation of the peak areas (in integrator units) for the six measurements.

TABLE I

QUANTITATIVE ANALYSIS OF AN ETHOXYLATED OCTYLPHENOL

Oligomer No.	*Average retention time (sec)*	*Coefficient of variation (n=6) of the retention time*	*Average amount present (mole-%)*	*Coefficient of variation (n=6) of peak area (i.u.)*
2	579	0.28	0.73	3.8
3	717	0.23	3.21	1.5
4	863	0.01	7.01	1.4
5	1022	0.01	10.51	1.3
6	1193	0.18	12.51	1.4
7	1381	0.12	13.37	1.3
8	1567	0.10	12.95	1.6
9	1758	0.14	11.38	1.4
10	1948	0.14	9.18	1.4
11	2139	0.08	6.76	1.2
12	2323	0.12	4.65	2.6
13	2493	0.07	2.96	1.4
14	2645	0.01	1.84	4.1
15	2780	0.01	1.13	2.5
16	2901	0.06	0.77	2.7
17	3007	0.04	0.47	5.9
18	3188	0.10	0.25	2.7
19	3279	0.10	0.18	3.3
20	3389	0.12	0.12	3.8

The identities of oligomers 2–6 were established by nuclear magnetic resonance spectroscopy after their isolation by gel permeation chromatography on Sephadex LH-20 in methanol (three columns, each 140 × 1.5 cm I.D.)[8].

The results in Table I show that the coefficients of variation of the peak areas (integrator units) obtained by repeated injections are fairly low (*ca.* 1–4%). Similarly, we found coefficients of variation of the same order of magnitude in the quantitative analysis of poly(ethylene terephthalate) oligomers[21]. The coefficients of variation of the retention times (0.01–0.3%) illustrate the accuracy that can be obtained with the gradient apparatus used and the total gradient elution procedure applied.

CONCLUSIONS

GE-HPLC has been shown to be an important and accurate method for both quantitative and qualitative characterization of a wide variety of oligomeric mixtures. For an accurate analysis the gradient elution procedure has to be strictly standardized.

An advantage of GE-HPLC over high-performance size-exclusion chromatography is its greater selectivity, and hence its better resolution. A drawback of the

gradient technique is that it is more time consuming than SEC. The use of either separation technique depends on the nature of the sample and the information desired.

With the chemically modified column packing materials available at present, the application of GE-HPLC to oligomer separations is mainly limited by size exclusion, and the sample solubility also has a limiting effect on the gradient technique.

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