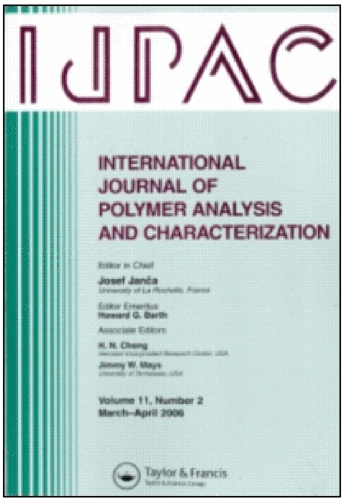

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Determination of Polymer Heterogeneity by Two-Dimensional Orthogonal Liquid Chromatography

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Liquid adsorption chromatography at critical conditions (LACCC) is a versatile method for the determination of different types of chemical heterogeneity in polymers. Coupling of LACCC in the first and size exclusion chromatography (SEC) in the second dimension is a useful tool to characterize polymers. Experimental problems of this orthogonal two-dimensional chromatographic method and applications are presented. Polyesters, polyethers and macromers are characterized according to their functionality and molar mass. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is an excellent method for identification of LACCC fractions and SEC calibration. The results of two-dimensional liquid chromatography are presented by a contour map (fingerprint of polymer).

KEY WORDS Orthogonal chromatography, size exclusion chromatography, liquid adsorption chromatography at critical conditions, matrix-assisted laser desorption ionization time-of-flight mass spectrometry, polyester, polyether

INTRODUCTION

Polymers are heterogeneous in terms of their molar mass distribution and chemical composition, that is, distribution of functional groups, distribution of different monomers in copolymers, and sequence length distribution. Other aspects of heterogeneities in polymers concern topological or structural features, such as linear, graft, cyclic, star-shaped, dendritic, and random, block and alternating distributed parts of polymers.

Nearly all high-tech or high-performance polymers are more or less heterogeneous. Their characterization is a challenge and is needed for optimizing synthesis and studying environmental effects and polymer recycling. One way for determining polymer heterogeneity is the application of liquid chromatography (*LC*). The coupling of different modes of *LC* [1–5] in a two-dimensional approach using optimized detectors provides a useful technique for determining polymer heterogeneity.

It is well known that size exclusion chromatography (SEC), without interactions with the mobile or stationary phases, separates according to hydrodynamic volume in an

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entropic-controlled mechanism. In case of liquid adsorption chromatography (LAC), the separation is caused by interaction of the solute with the stationary phase in an enthalpic-controlled process. The dependence of elution volume on molar mass is contrary in both these chromatographic modes. If the retention volume is independent of molar mass, liquid adsorption chromatography at critical conditions (LACCC) is realized. In this case the entropic difference of SEC in the separation process will be compensated by the enthalpic difference of LAC and the basic unit of the polymer chain is chromatographically "invisible". In this case the retention depends on other structural differences of polymers, LACCC has the potential of separating polymers according to the above mentioned heterogeneities.

Maximum information about the polymer system is possible using, in a two-dimensional chromatographic system (2D-HPLC) by on-line coupling of different chromatographic modes. Only by coupling orthogonal separating modes (e.g., LACCC and SEC), a "fingerprint" of the polymer will be realized. The aim of this contribution is to discuss the problems of 2D-HPLC and to apply this approach to polyesters and polyethers.

EXPERIMENTAL

LACCC System

The first dimension in the 2D-HPLC polyester characterization consisted of a Jasco pump, type 880 PU (Jasco, Groβzimmern, Germany) in which acetone/hexane (50.5 vol%:49.5 vol%) was the mobile phase for critical conditions. For analyzing polyester samples, a 7-µm Si-120 silica column (Tessek, Praha, CS) was used. A refractive index detector (model 7511, ERC, Alteglofsheim, Germany) was used for determining the concentration of fractions. In the case of polyethers, a Macherey Nagel Nucleosil 5C18 column (250 × 4mm i. d., Düren, Germany) was used. The mobile phase at critical conditions consisted of a mixture of water/acetonitrile (54 vol%:46 vol%). All LACCC columns were thermostated at 30°C. The concentration detector was an ELSD detector (Sedex 45) from ERC. The mobile phase was degassed by an ultrasonic bath.

SEC System

The SEC measurements were carried out at ambient temperature. The system consisted of an HPLC pump 64 (Knauer, Berlin, Germany), two columns (300 × 8mm i. d.), 5-µm particle size, Knauer Eurogel GP 50 Å and Knauer PL Gel 500 Å for polyesters. In the case of polyethers, SDV 3-µm 100Å (PSS, Mainz) and 5-µm Knauer PL 50Å were used. For the polyester analysis, the SEC columns acetone was used as mobile phase, polyethers were analyzed in THF/Lichrosolve (Merck, Darmstadt, Germany).

MALDI-TOF-MS

Kratos Kompact MALDI III equipment (Shimadzu, Duisburg, Germany) was used. Samples were prepared by mixing approximately 5µL of analyte in ethanol solution (1mmol/L) with 5µL of a 60 mmol/L 2,5-dihydroxybenzoic acid matrix solution in ethanol. MALDI-TOF mass spectra were obtained with 337-nm radiation from a N_2-laser as positive ion spectra in the reflectron mode. The equipment was calibrated with bovine insulin. The result of each spectrum was obtained from averaging 122 single shots. Spectra were evaluated using software provided by Kratos.

Polyesters (PE) described in ref. [6] Were synthesized from:

$$n * \mathrm{X\,CO\,(CH_2)_4\,CO\,X} + n * \mathrm{HO\,(CH_2)_6\,OH} \longrightarrow$$
$$\mathrm{X\,[CO\,(CH_2)_4\,CO\,O\,(CH_2)_6\,O]}_n\,\mathrm{H} + (2n-1)\,\mathrm{HX}$$

where X is Cl or OH and n = degree of polymerization.

Experimental Optimization

Determining "Critical" Conditions. The realization of the "critical" point of polymer adsorption is possible by variation of mobile phase composition, temperature, and pH. For determining the "critical" point of polymer adsorption, it is necessary to investigate a number of samples with the same chemical structure, but different molar masses by variation of the composition of the binary mobile phase. Beginning with a thermodynamic good solvent in the SEC mode, one successively adds a thermodynamic poor solvent until the adsorption mode is reached. "Critical" conditions are realized when the retention volume of the main peak is independent of molar mass.

Figures 1 and 2 show the possibilities of experimentally determining "critical" point. Figure 1 presents molar mass dependence of elution volume of different hexane/acetone mixtures. Figure 2 is a plot of log k' of different molecular weight polyethers vs. volume fraction of acetonitrile in water. In the second case, it is necessary to determine t_o from the exclusion volume of the LACCC column. The intersection of curves of different molar mass is the "critical" point of adsorption.

In selecting a suitable stationary phase, it is necessary that the mean value of the pore size distribution must be greater than the hydrodynamic volume of the solvated polymer molecules in the "critical" mobile phase. It is better to work in an isocratic mode and to premix the mobile phase. Thermostating columns is necessary because the adsorption chromatographic process is highly dependent on temperature.

Two-Dimensional Liquid Chromatography (2 D-HPLC). The realization of orthogonal working chromatographic modes is possible by coupling of LACCC and SEC (Figure 3). The first dimension of this coupling method is LACCC. Reasons and advantages are that the separation is independent of molar mass (hydrodynamic volume) of polymers; high polymer concentrations (10%) are possible without considerable loss of resolution; this method is fast since column dimensions are small compared with SEC columns.

Coupling SEC in the Second Dimension. The mobile phase in SEC should be a component of the mobile phase of the LACCC, if possible. The number of columns in SEC should be small, but efficiency should be high, therefore, using of 3-μm particle size packing is attractive. The measurement time (run time) in SEC determines the sample transfer from the LACCC to the SEC mode. With two columns (300 x 8mm i. d.), 20 min are needed for a run; thus, the SEC 100-μL loop has to be filled in that time period. The pump in the LACCC mode must operate in the μL/min flow range.

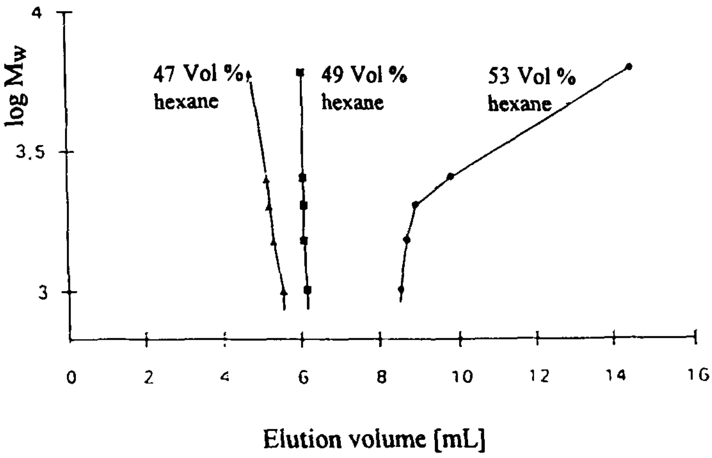


FIGURE 1 Determination of critical conditions: plot of log M_w versus elution volume of the peak of a HD-AA polyester with two OH groups.

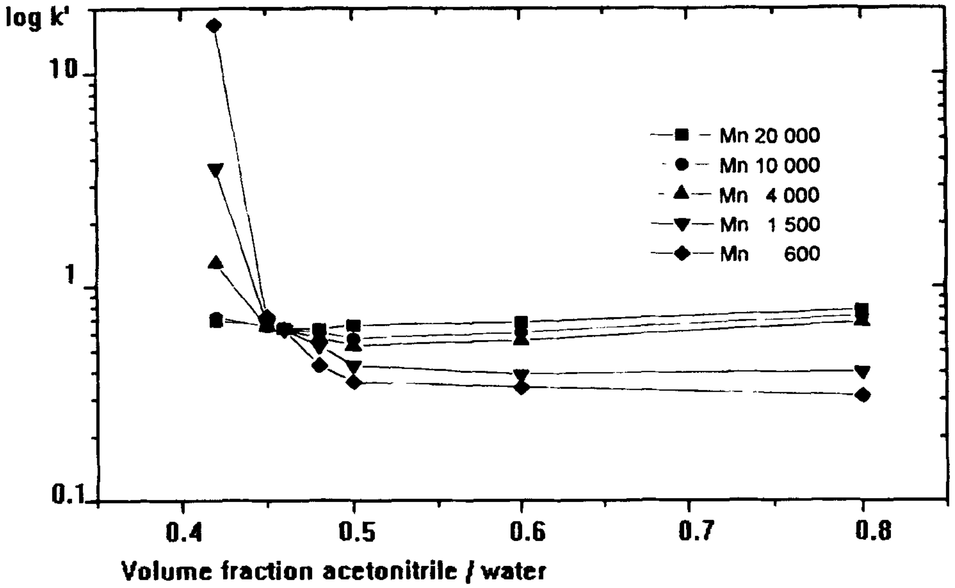


FIGURE 2 Determination of critical conditions: plot of log k' versus volume fraction of acetonitrile in ACN/H_2O for investigated polyethers ($k' = (t-t_0)/t_0$).

Detection. Evaporative light scattering detector (ELSD) is recommended in SEC because the second component of the mobile phase does not disturb the separation; it is not necessary to know response factors of different species for the determination of mass content.

Calibration. SEC calibration is necessary for every peak separated by LACCC. Model polymers of the same molar mass range, and the first species of every homologous series have to be synthesized. For oligomers, it is possible to carry out the calibration by MALDI-TOF-MS. By assigning the main peak from the MALDI-TOF spectrum to the

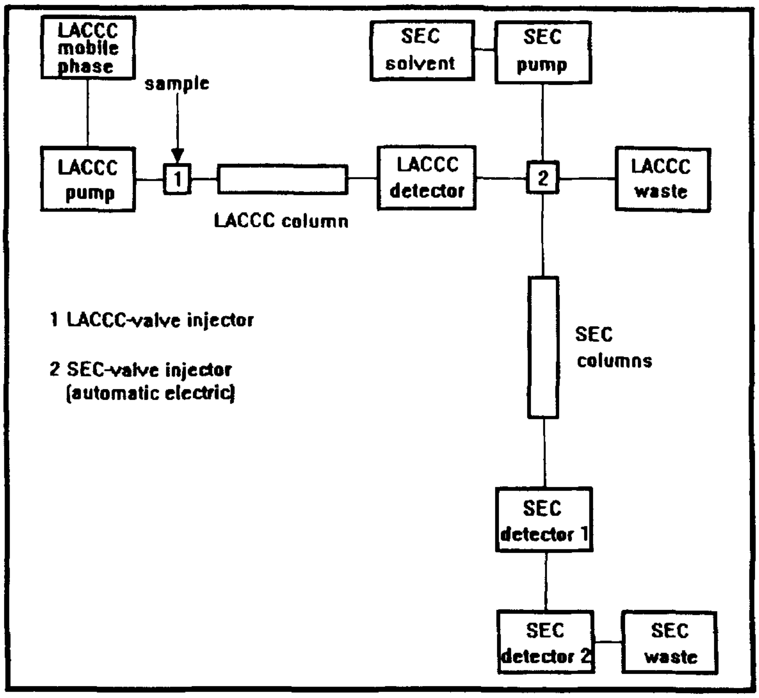


FIGURE 3 Experimental scheme of two-dimensional chromatography (coupling LACCC-SEC).

peak maximum of highly resolved SEC chromatogram and counting of the other peaks, one can obtain a relation between elution volume and molar mass.

Data Acquisition. Data acquisition was done by using 2D-HPLC software (Polymer Standards Service, Mainz, Germany). It is possible to present the results in a three-dimensional contour plot. Contour maps can be read like geographical maps. The *y*-axis corresponds to chemical heterogeneity and the *x*-axis corresponds to SEC elution volume or by calibration with molar mass; the relative concentrations of the components are indicated by colors.

RESULTS AND DISCUSSION

Two-dimensional HPLC of aliphatic polyesters (PE)

The aims of these investigations were the quantitative determination of cyclic species, the determination of molar mass distribution of different fractions, and the two-dimensional presentation of molar mass and functionality type distribution of polyesters, to study polycondensation kinetics and to differentiate the following different functionalities:

Alk-PE-COOH	HO-PE-COOH	HOOC-PE-COOH
Alk-PE-Alk	HO-PE-OH	Alk-PE-OH

where Alk is an alkyl or alkylene group.

The following secondary reactions by synthesis of polyesters are possible:

Dehydration

$$\cdots-CH_2-CH_2-OH \longrightarrow \cdots-CH=CH_2+H_2O$$

Decarboxylation

$$\cdots-CH_2-CH_2-COOH \longrightarrow \cdots-CH_2-CH_3+CO_2$$

Hydrolysis

$$\cdots-CH_2-COO-CH_2-+H_2O \longrightarrow \cdots-CH_2-COOH+\cdots-CH_2-OH$$

Dehydration

$$\cdots-OH+HO- \longrightarrow \cdots-O-\cdots+H_2O$$

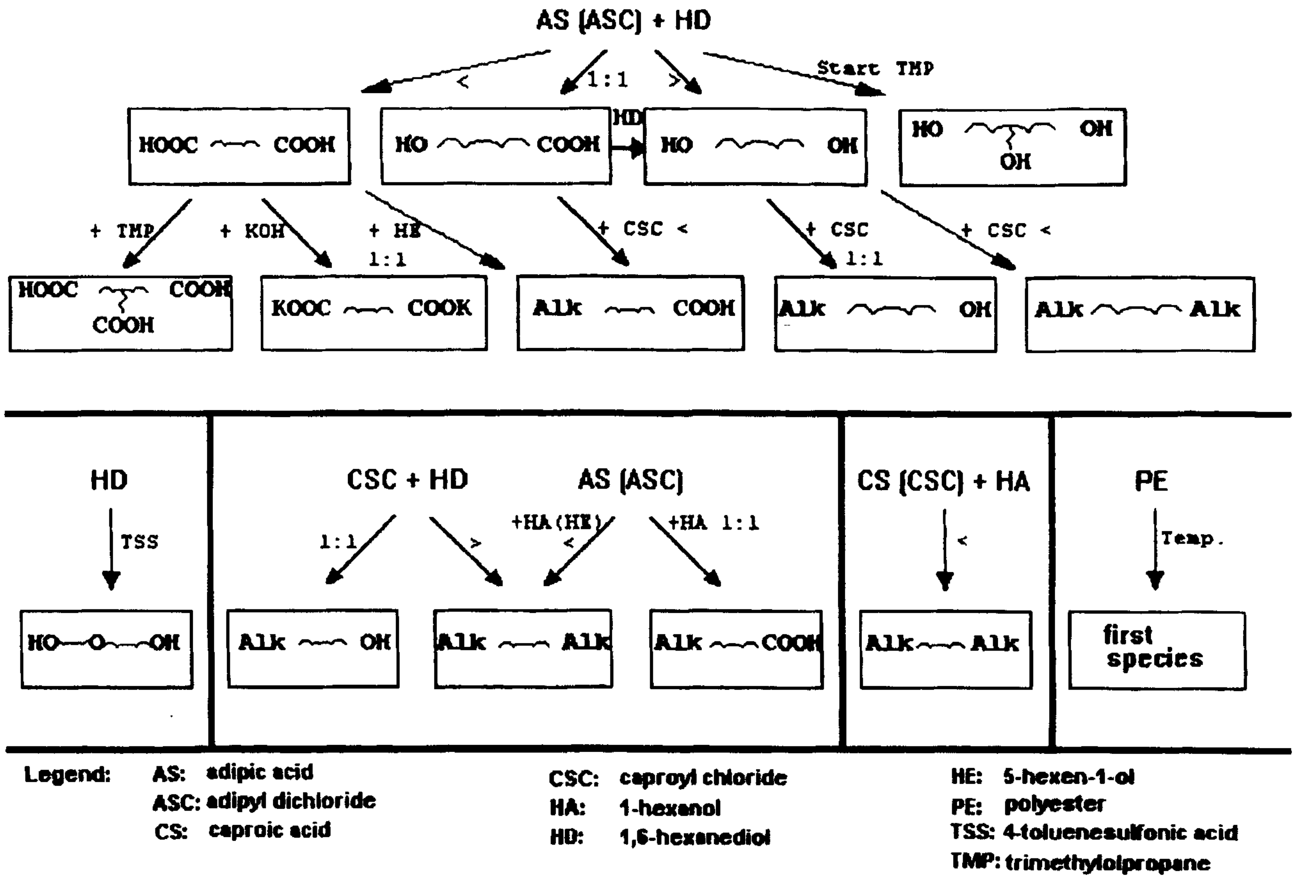
Telechelic aliphatic polyesters were produced according to Scheme A by polycondensation, based on adipic acid and hexanediol (AA-HD) in various stoichiometric amounts to synthesize polyesters of different end group functionality. Figure 4 presents the LACCC of AA-HD polyester and of model polyesters with defined end groups. The separation of isolated diols after alkaline saponification in Figure 5 shows that these ether structures are incorporated into all polyesters, thus making identification of all species in the AA-HD polyester possible. In Table I quantitative analysis of fractions are presented, measured by ELSD in the LACCC mode. These results are dependent on the molar mass. Without ether structures all side reaction products grow with increasing molar mass of polyesters. Ether structures results from hexanediol and can not grow during polycondensation process because the diol will be consumed.

After preparative separation, all fractions and the first species of the homologous series were measured by SEC. The resulting calibration curves of all species are shown in Figure 6. The hydrodynamic volumes of species with different end groups are significantly different. The SEC runs of all fractions are presented in Figure 7. The 3D-contour plot, Figure 8, shows the “fingerprint” of the AA-HD polyester after calibration of every homologous series, in which the different shades represent concentrations. (In reality this contour plot is colored.)

Two-dimensional HPLC of Polyethers

The optimization of the synthesis of macromers on the basis of ethylene oxide (EO) demand the characterization of the products according to chemical heterogeneity and molar mass. Scheme B shows the reaction scheme of this macromer. Components 1–4 were characterized by LACCC and SEC. A reversed-phase column was used because of the more hydrophobic properties of the end groups. The critical point for EO, the main chain unit, was realized by a mixture of 43 vol% acetonitrile and 57 vol% water (Figure 2).

The LACCC chromatograms of the mixture and of the isolated substances are shown in Figure 9. To fingerprint the polymer mixture, the SEC system was calibrated for all compo-



SCHEME A Synthesis scheme of model polyesters.

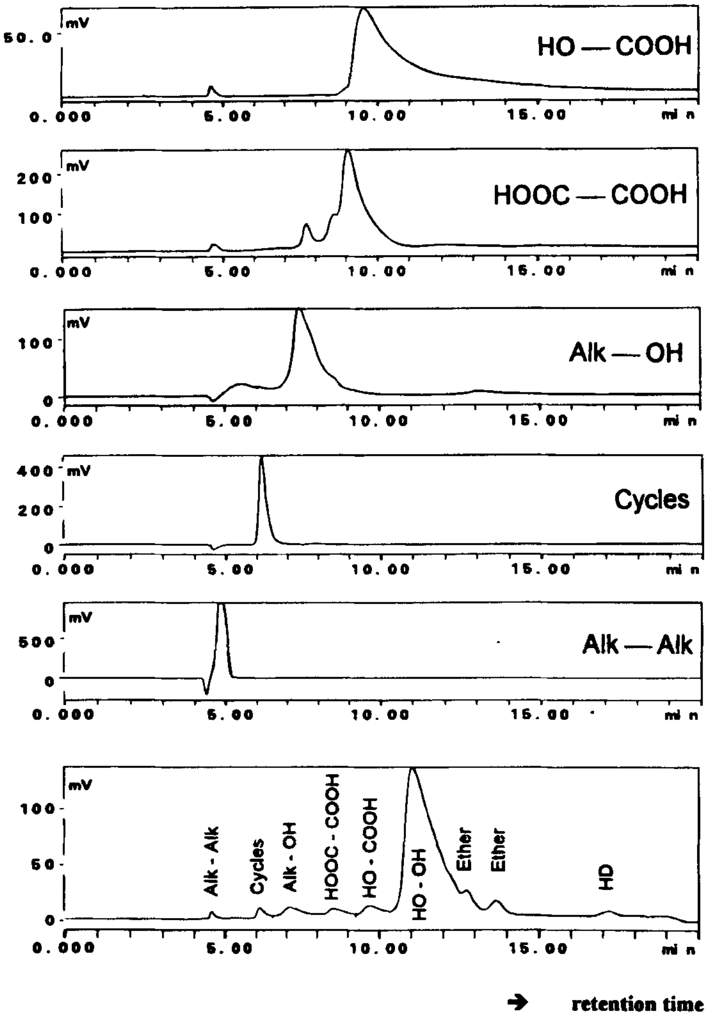


FIGURE 4 LACCC chromatograms of AA-HD-polyester and of model polyesters with defined end groups.

nents. The influence of the different end groups on the calibration curves was not negligible. The calibration was done using MALDI-TOF-MS. Typical MALDI-TOF-MS of these four components are shown in Figure 10. The calculated data from this mass spectra are presented in Table II. With MALDI-TOF-MS, SEC calibration is simple. By comparison of mass spectra with well-resolved size exclusion chromatograms the main peak of the chromatogram can be assigned the corresponding molar mass from the mass spectrum. For lower molar masses, there are enough points to complete the calibration curve. The two-dimensional HPLC contour plot of the polyether mixture after SEC calibration is shown in Figure 11.

From this contour map it is possible to obtain information about the number of species, the relative concentrations and the molar mass distribution of all components.

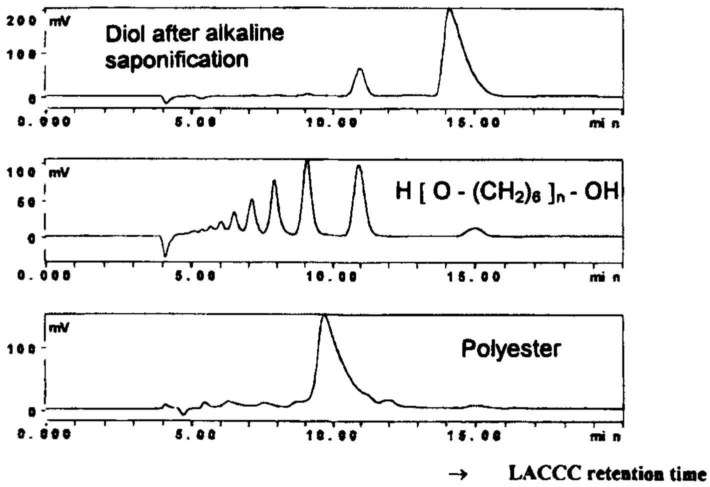


FIGURE 5 Identification by decomposition reaction.

TABLE I

Mass content of different species of AA-HD polyester as a function of molar mass.

molar mass of polyesters	% Alk–Alk	% Cycles	% COOH–COOH	% COOH–OH	% OH–OH	a% —O—
1,000	0.22	0.54	1.21	1.33	76.80	16.95
1,500	1.28	1.64	5.14	7.70	76.32	11.84
2,000	1.15	1.81	4.89	6.07	76.44	10.25
3,500	2.11	3.22	10.13	11.53	59.64	9.57

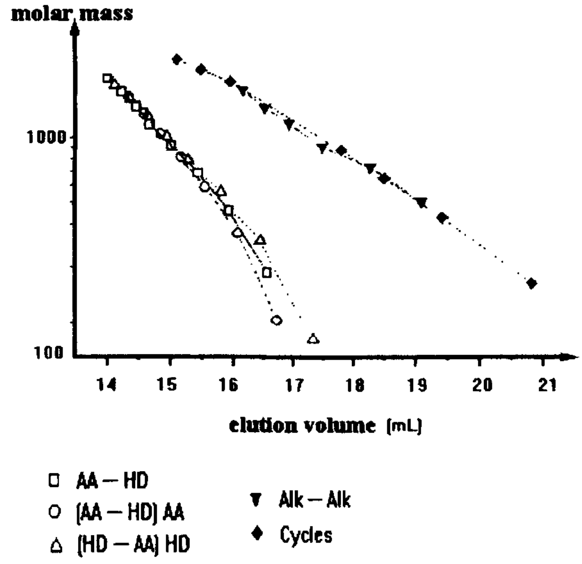


FIGURE 6 SEC calibration curves of polyester fractions.

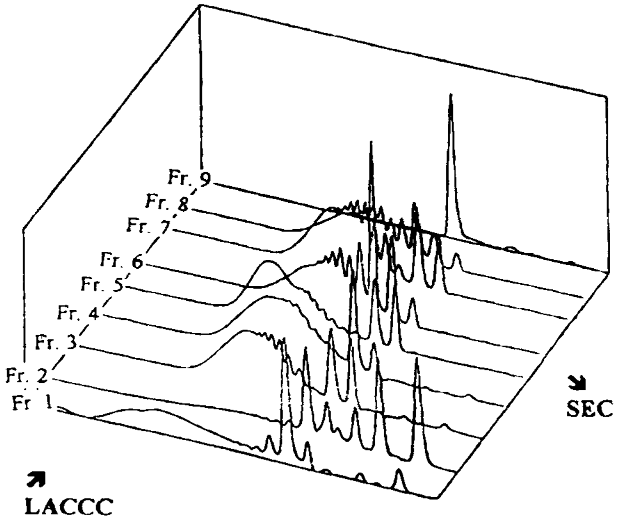


FIGURE 7 SEC runs of all fractions, separated by LACCC.

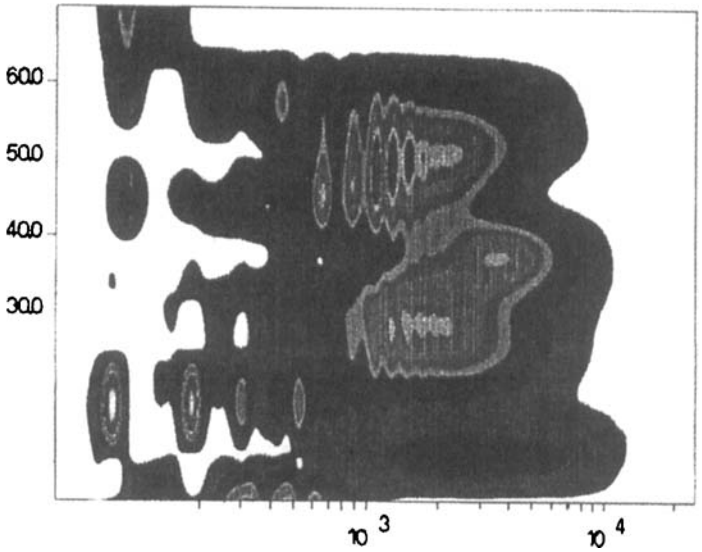
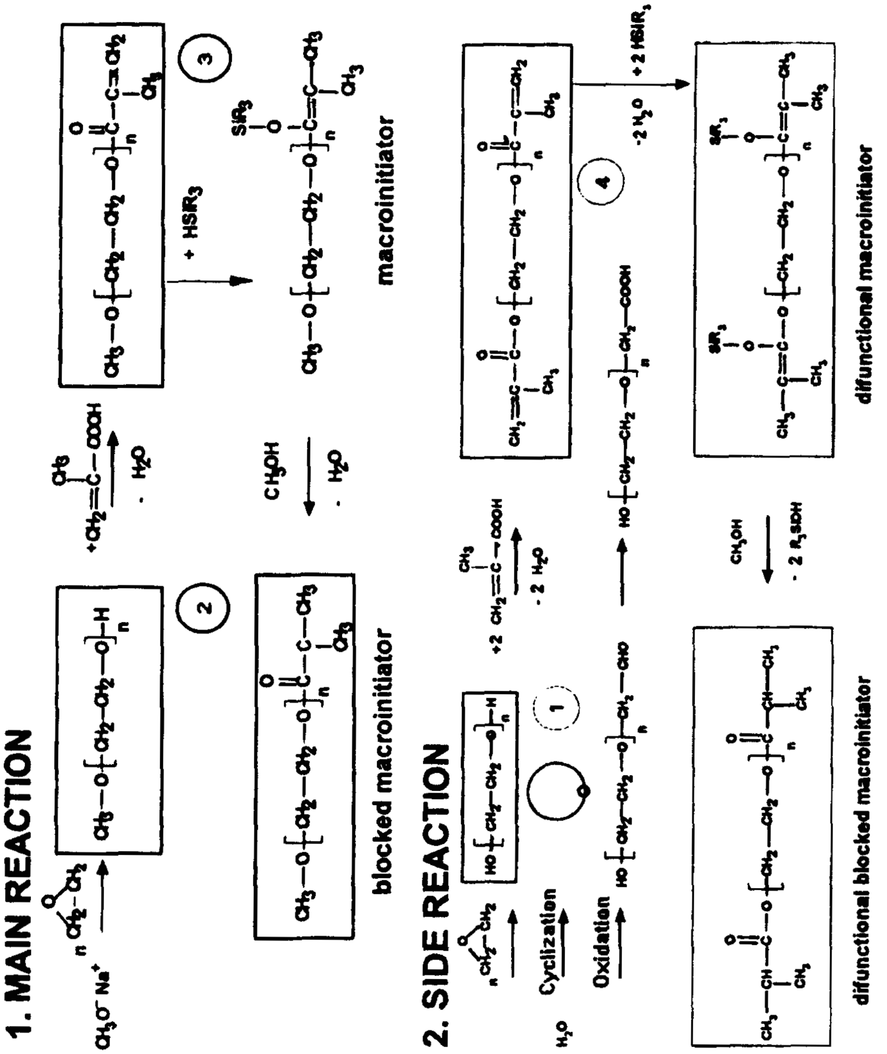


FIGURE 8 2D-HPLC contour plot of AA-HD polyester. The y-axis is functionality from LACCC and the x-axis is molar mass from SEC.



SCHEME B Reaction scheme for synthesis of poly(ethylene oxide)-methacrylic acid macromers.

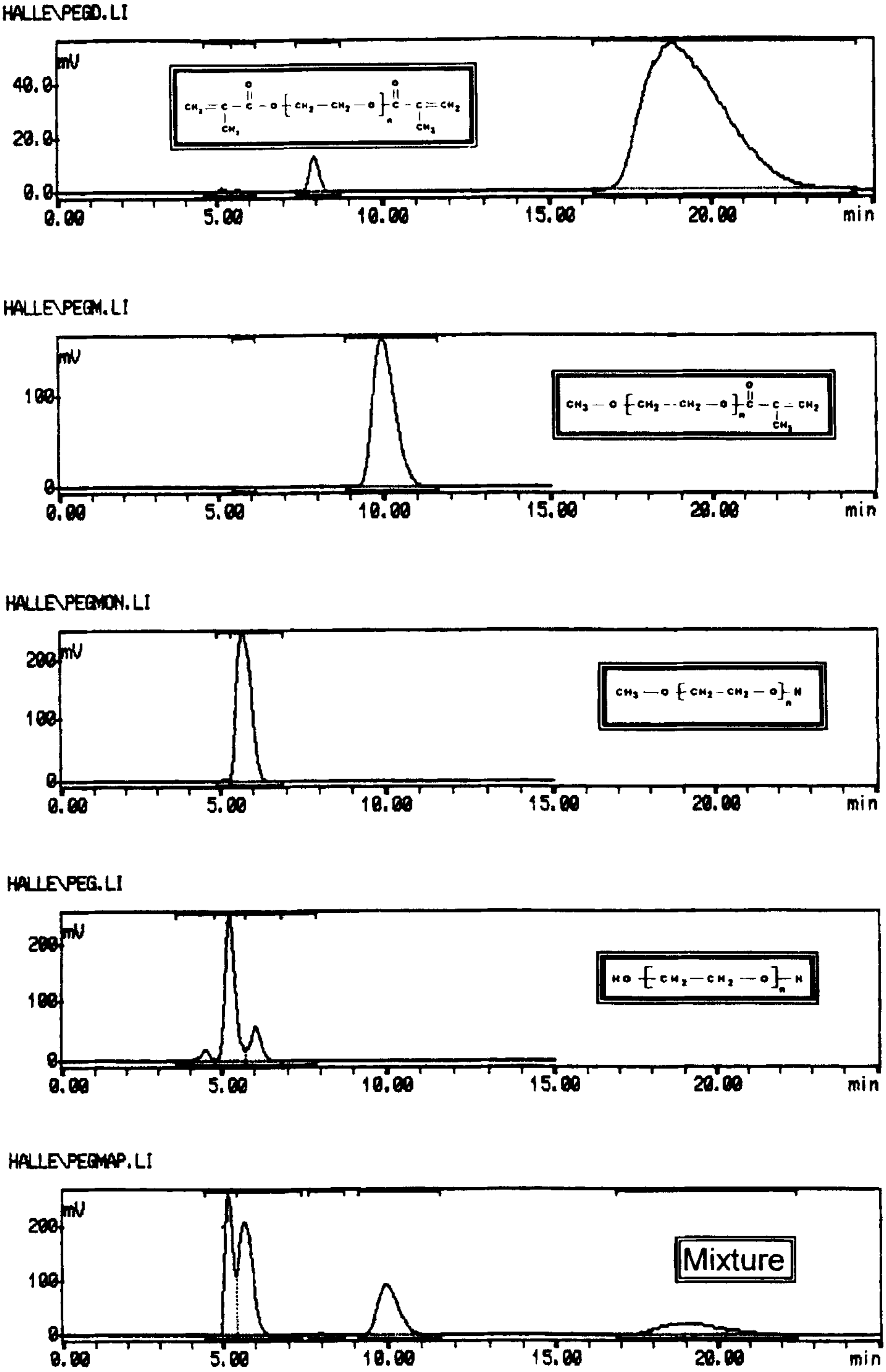


FIGURE 9 LACCC of investigated polyethers.

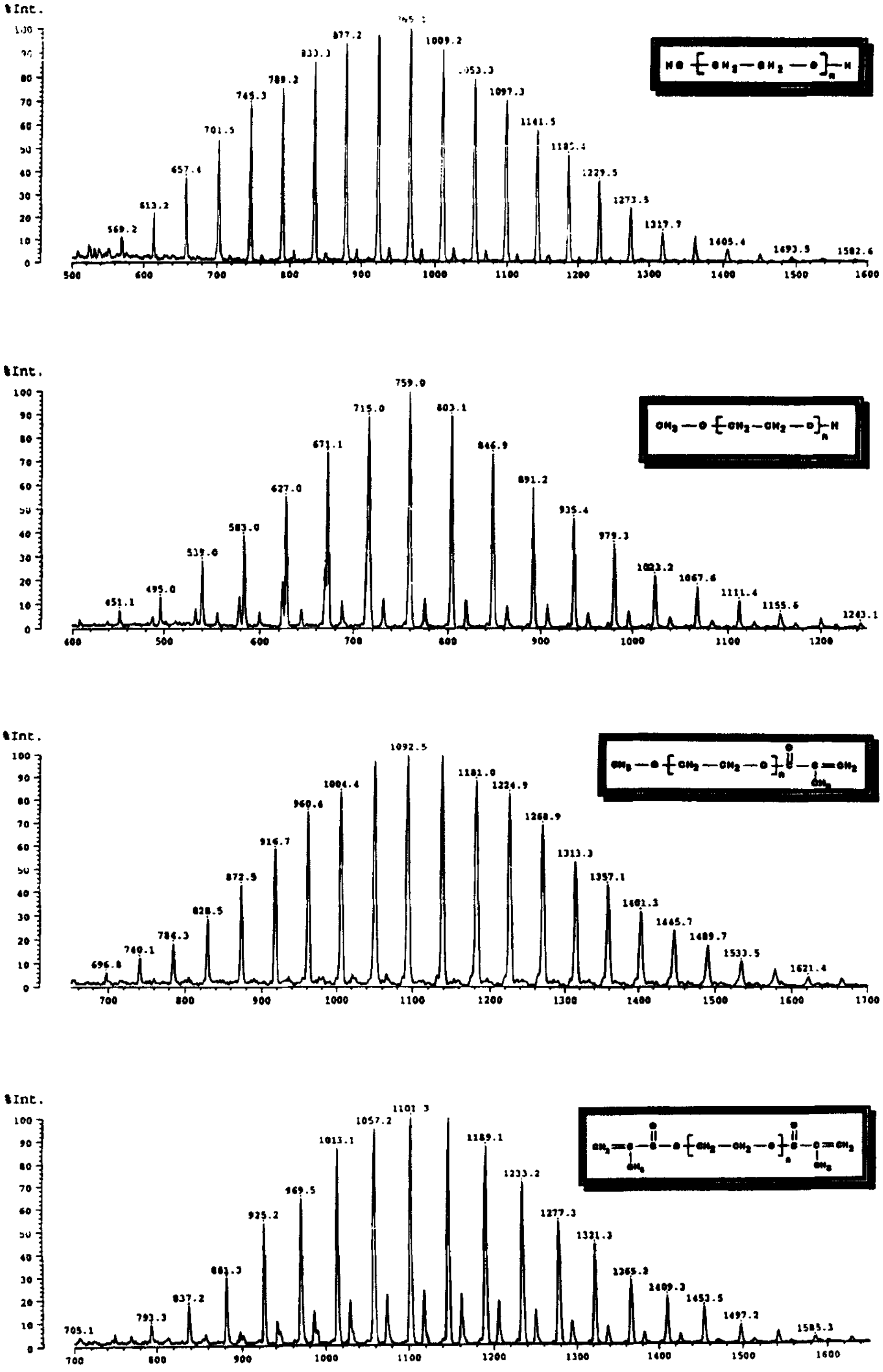


FIGURE 10 MALDI-TOF-MS of end group modified PEG's.

TABLE II

Calculated data from MALDI-TOF mass spectra.

	HO—OH	CH_3—OH	CH_3—Ma	Ma—Ma
Basic statistics				
Monomer mass	44	44	44	43
Residual mass	18	32	12	22
Peaks	22	19	27	21
Weight averages				
M_n	917	756	1120	1097
M_w	955	787	1157	1122
M_z	990	817	1194	1148
Polydispersity	1.04	1.04	1.03	1.02

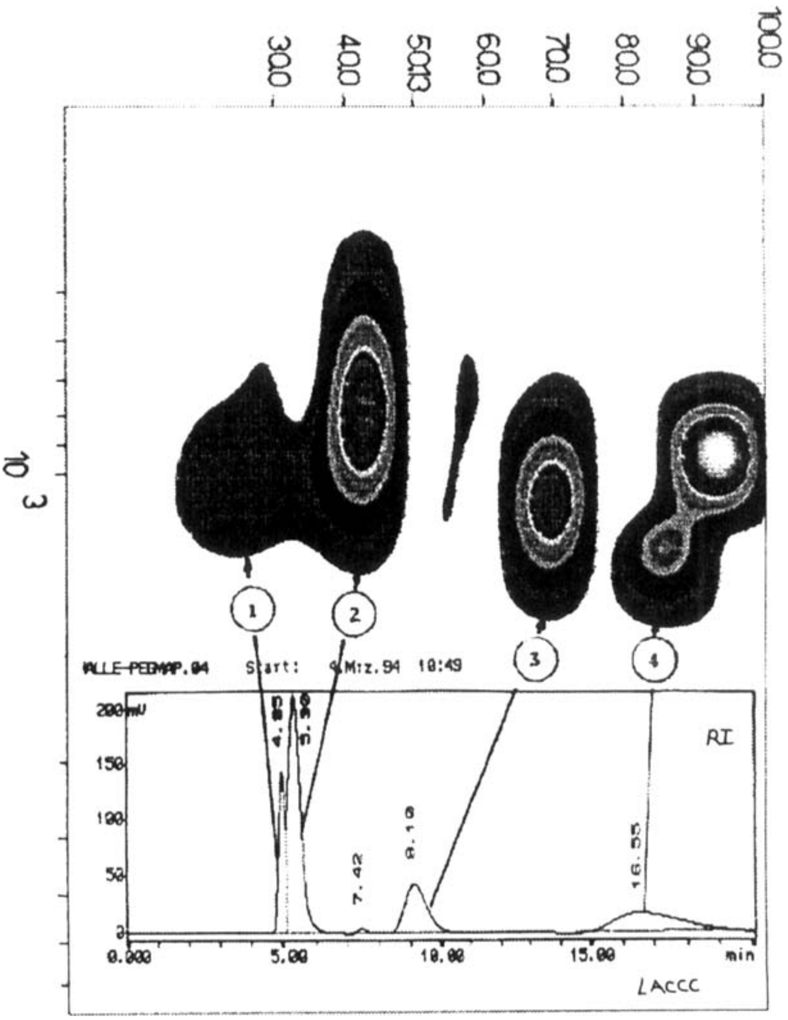


FIGURE 11 2D-HPLC plot of endgroup-functionalized polyethers. The y-axis is functionality from LACCC and the x-axis is molar mass from SEC.

CONCLUSIONS

To determine polymer heterogeneity, different species can be measured in the LACCC mode with ELSD as the detector. The knowledge of response factors of different structures is not required. If molar mass distribution of the peaks separated by LACCC is needed MALDI-TOF-MS can be applied to collected peak fractions. To determine the molar mass distribution of the whole polymer and the concentration of all species, it is necessary to use 2-D-HPLC, that is LACCC in the first dimension and SEC in the second dimension with SEC calibration by MALDI-TOF-MS. The last detector in this mode should be an ELSD.

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References

1. S. G. Entelis, V. V. Evreinov, and A. V. Gorshkov, *Adv. Polym. Sci.*, **76**, 129 (1986).
2. G. Schulz, H. Much, H. Krüger, and C. Wehrstedt, *J. Liq. Chrom.*, **13**, 1745 (1990).
3. A. V. Gorshkov, H. Much, H. Becker, H. Pasch, V. V. Evreinov, and S. G. Entelis, *J. Chromatogr.*, **91**, 523 (1990).
4. H. Krüger, H. Much, G. Schulz, and C. Wehrstedt, *Makromol. Chem.*, **191**, 907 (1990).
5. H. Pasch, H. Much, G. Schulz, and A. V. Gorshkov, *LC GC Int.*, **5**, 38 (1992).
6. R. -P. Krüger, H. Much, and G. Schulz, *J. Liq. Chrom.*, **17**, 3069 (1994).