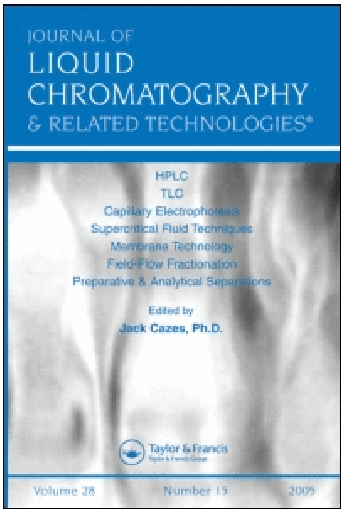

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Determination of Functionality and Molecular Weight Distribution by Orthogonal Chromatography

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DETERMINATION OF FUNCTIONALITY AND MOLECULAR WEIGHT DISTRIBUTION BY ORTHOGONAL CHROMATOGRAPHY

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ABSTRACT

During polymerization oligomers, telechelics and macromers with different endgroups are formed as a result of various mechanisms (starting, termination, cyclization, transfer reactions).
The characterization of the resulting products is possible by using orthogonal chromatography. In order to characterize 1,3,6-trioxocane polymers, synthesized in presence of benzyl alcohol, Liquid Adsorption Chromatography (LAC) under "critical conditions" is combined with Size-Exclusion Chromatography (SEC). This method includes a preparative chromatographic separation of the product which is followed by SEC and LAC under "critical conditions" and vice versa. Results advantages and problems of this type of orthogonal chromatography are discussed.

INTRODUCTION

Main problems of polymer characterization are often related to the overlapping of different distribution functions, for example molar mass and branching or chemical composition, sequence length and molar mass in the case of copolymers. One major approach to solve these problems is the combination of different chromatographic methods. A nearly complete characterization of copolymers according to molar mass and chemical composition is possible by coupling SEC and LAC with gradient elution, the so-called "cross fractionation". Other orthogonal chromatographic techniques have been developed, including two SEC modes with different eluents, in order to characterize polymers regarding molar mass and sequence length distribution or branching and molar mass distributions (1-5). The characterization of oligomers, telechelics, macromers and polymers containing different functional groups is possible by LAC under critical conditions (6). This method allows the chromatographic separation of molecules on different functionality and the determination of the functionality type distribution (FTD) accordingly. The molar mass distribution can be distinguished from the functionality distribution by coupling SEC and LAC under critical conditions. The results of this coupling procedure are presented in this paper. First a preparative fractionation is carried out followed by either SEC-LAC or LAC-SEC. The results are compared and the optimization of the method is discussed.

MATERIALS

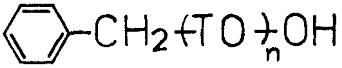
To pursue the polymerization reaction of 1.3.6-trioxocane (TO) in the presence of benzyl alcohol, i.e. the formation of TO-oligomers with different endgroups, the SEC has been used.

The chromatograms of such a reaction product obtained with two detectors (UV- and RI-detector) , see FIGURE 1, show overlapping of different molar mass distributions.

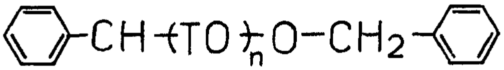
Details of the mechanism of this reaction will be published in another paper (7). As a result, oligomer mixtures are expected besides monofunctional endgroups, oligomer series II, and even oligomers with two hydroxy-endgroups, series I, or two benzyloxy-endgroups, series III.

I: $\mathrm{HO{-}Et{-}O\ Et(TO)_{n}OH}$

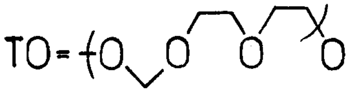
II:



III:

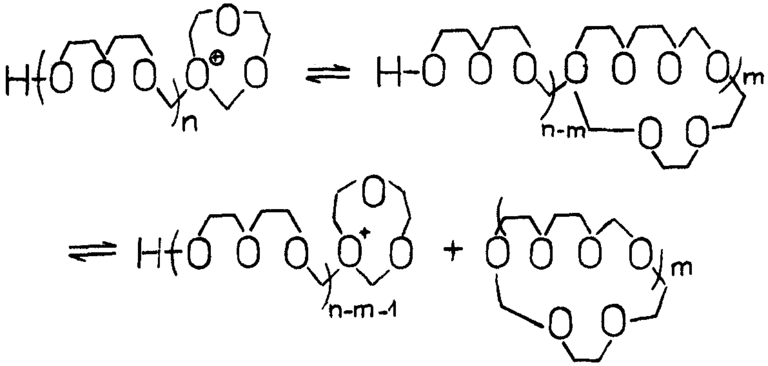


n = 0,1,2···



Additional cyclic oligomers are possible by intramolecular back- biting reaction, series IV.

IV:



As model substances for the investigation of separation phenomena poly-(1.3.6-trioxocanes) with different molar

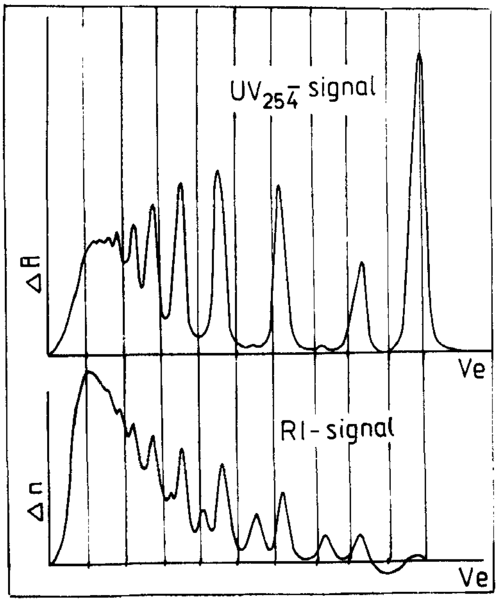


FIGURE 1
Comparison of size exclusion chromatograms of sample A (TABLE 1) with UV - and RI - detection, two columns Ultrastyragel (WATERS, 10 nm, 300 x 8 mm ID) in THF

TABLE 1

Molar masses of poly-(1.3.6-trioxocanes) used for the determination of critical conditions in HPLC (in g/mol)

TO-polymer	M_n	M_w
1	770	940
2	1640	2100
3	3740	4450
4	21900	26500
5	71000	82600

TABLE 2

Synthesis parameters and molar masses of the samples studied (BA/initiator ratio: 84.9 mol BA/mol initiator)

sample	TO g	BA g	OH-number mg KOH/1g S	M_n g/mol
A	47.2	21.6	164	385
B	47.2	13	137	520

masses with OH-endgroups (TABLE 1) and samples with different relations of trioxocane to benzyl alcohol (TABLE 2) were synthesized.

EXPERIMENTAL

HPLC

The measurements were performed on an HPLC equipment including two high-pressure pumps LC- 6A, a gradient controller SCL-6A and a UV-VIS detector SPD-SAV(=254nm) by Shimadzu, an RI detector RIDK-101 by Laboratorni Pristroje Praha (Czechoslovakia) and the integrator Shimadzu CR-6A.

Stainless steel columns (250 X 4.6 mm ID) were used, with an RP-18 separation phase (average particle size 10 µm), selfpacked according to the slurry-method. Pore distribution was determined by inverse chromatography with polystyrene in THF. The solutions (3 wt%) were injected by a loop doser Rheodyne 7125 (20 µl).

The solvent acetonitrile, especially pure for HPLC, and quartz-distilled water were mixed and degassed before measurement.

SEC

The SEC investigations were carried out on a microcomputer-coupled SEC measurement device (8). The system consists of a KNAUER-pump, KNAUER UV- and RI-detector and KNAUER injection system. Both detectors were coupled on-line via a self-made interface using the microcomputer MC 80 from VEB Messelektronik Gera. With regard to instrumental broadening, the chromatograms were corrected by a program developed by Opfermann (9). As mobile phase THF was used after chemical purification and fresh distillation. The molar masses were calculated from the chromatograms using calibration curves of each homologous series (see FIGURE 5) and an analysis program (9).

METHODS

1. Liquid adsorption chromatography under "critical conditions"

Liquid adsorption chromatography (LAC) under "critical conditions" has become an established method for the characterization of oligomer mixtures including a distribution by functionality in addition to the molar mass distribution. The method of LAC under "critical conditions" differs from size exclusion or adsorption mode, since the monomeric units in the chains do not contribute to retention by entropy and free adsorption enthalpy. This means that the retention is independent of the molar mass and determined exclusively by the hydrophilic or hydrophobic character of the endgroups. For the system studied, first of all the critical conditions of TO were determined at which the separation into functionality, nature and number of endgroups takes place. Poly-(1.3.6-trioxocanes) with two OH-endgroups and different molar masses (TABLE 1) were used to determine the critical conditions for poly-(1.3.6- trioxocanes). In the case of benzyloxy endgroups the synthesized polymers are more hydrophobic than TO-monomer units. Therefore , as stationary phase a reversed phase should be used. To optimize the detection a typical reversed-phase binary eluent should be used, e.g. acetonitrile/water. Acetonitrile is such a typical eluent for SEC of this polymer type, and water is a typical wrong eluent for the adsorption and partition chromatography. For the RP-18 column (250 X 4.6 mm ID, 10 μm particle size) used the dependence of the retention volume on the molar mass of the samples in acetonitrile was estimated by SEC mode. The water content of the eluent was increased in steps. By this mode , the differences in the elution volume between samples with different molar masses became smaller. At critical conditions , all samples were eluted at the same volume. As a result, the peak is small and symmetric. If there are different functional distributions in the sample, separated peaks are obtained. If the water content is higher than 50%, the system is working in the adsorption mode and the elution order is inverted. The retention volume increases with the growing molar mass. That is dangerous for the column, because an irreversible sorption becomes possible. It should be remarked that for the same reason a thermostating of the column is necessary. The critical conditions for 1.3.6-trioxocane were determined using an eluent mixture of 49.5 vol% acetonitrile and 50.5 vol% water (FIGURE 2). Two products were separated under these conditions (FIGURE 3). The peak at 6.17 min (FIGURE 3) was assigned to the TO-oligomers with two OH-endgroups. For identification poly-(1.3.6-trioxocanes) with different molar masses were used, which have the same elution volumes. The little peak at 7.03 min corresponds to

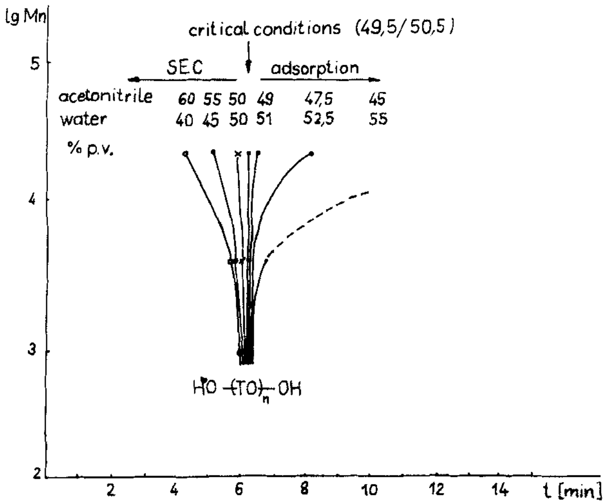


FIGURE 2
Estimation of critical conditions of LAC from the dependence of retention time on lg M of the samples (TABLE 1) in acetonitrile/water mixtures on an RP-18 column (10 um, 250 x 4.6 mm ID)

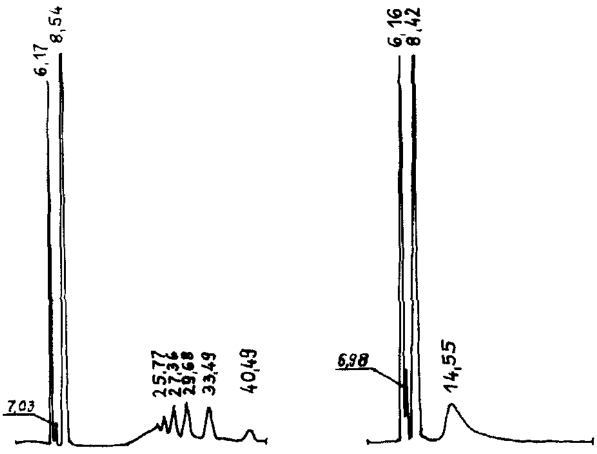


FIGURE 3
LAC under critical conditions of sample A and B (TABLE 1), experimental conditions see FIGURE 2

cyclic TO-oligomers without any end functionality (IV). The proof was furnished by reference substances (monomeric TO, different crown ethers), which were eluated at the same time. The peak at 8.54 min was assigned to the oligomers with one benzyl- and one OH-endgroup (II). Benzyl alcohol, the most low-molecular representative of this oligomer series (n=0), was used as reference substance. The oligomers with two benzyl-endgroups (III) do not appear as a single peak, but show a separation on molar mass even under critical conditions.

The final point of this distribution can be assigned to the most low-molecular species (n=0), to dibenzyl formal. Dibenzyl formal, synthesized as a model substance, confirms the elution time of the peak in the chromatogram.

The separation of the oligomer series III under critical conditions of TO as individual peaks for small oligomers can be explained by the pore size of the stationary phase (30 nm).

For that behaviour, the relation between the pore size of the stationary phase and the dimension of solvated molecules of this type (III) is responsible. If the medium pore diameter is larger than the solvated α,ω-dibenzyl-oligo-(1.3.6-trioxocane), only one benzyl-endgroup can interact with the RP-18 phase. The second benzyl-endgroup is specifically solvated compared with the TO-chain. This solvation depends on the molar mass of the oligomer so that a separation can be observed for this oligomer series III.The second sample in FIGURE 3 contains a higher molar mass portion of the oligomer series III, while the lower molar mass oligomers were extracted by diethyl ether.

By calibrating the chromatographic peak areas of the different oligomer series with reference samples and osmotically determined molar mass the mass portions

TABLE 3

Mass portions of the functionality fractions (wt%)

sample	I 	cycl. 	II 	III 
A	18.26	0.66	51.89	29.19
B	33.43	1.13	57.75	7.69

could be quantified for the four functionality fractions of both samples using the method of external standard (TABLE 3). Both samples show that beside the oligomer series I-III also cyclic TO-oligomers could be found, however only in little amounts. The major part in both samples is fraction II. The amounts of fraction I and III resulted from transacetalization reactions. In TABLE 3 the mass portions of functionality fractions are listed.

The chromatographic results of the separation according to LAC under critical conditions are in accordance with the gravimetric analysis in the preparative mode. The procedure developed for the quantitative determination of functionality distribution was applied to describe the kinetics and to control the course of polymerization (7). For the preparative separation a semipreparative RP 18 column (250x16 mm ID), filled with the identical 10 µm RP-material as in the analytical mode, was used. 200 µl of the sample solution (200 mg/ml) were injected. The rechromatography of the preparatively separated fractions on the analytical column gave a nearly complete separation of the oligomer series I, II and III. The fraction of cyclic TO-oligomers (series IV, FIGURE 4) still contains 30 wt% of I and 20 wt% of II. After a second preparative separation, this fraction was also pure (FIGURE 4). The fractions were collected, evaporated and then separated in THF by SEC.

2. Size exclusion chromatography

For SEC two columns (300 x 8 mm ID) filled with Ultrastyragel, 10 nm (WATERS), separating in the molar mass range up to 10 000 g/mol were used. As mobile phase THF was used. The resulting calibration curves (FIGURE 5) were obtained from the oligomer peaks of the preparatively separated fractions in LAC mode. As models for the four smallest species (n=0) diethylene glycol (I), benzyl alcohol (II), dibenzyl formal (III) and 1.3.6.-trioxocane (IV) were used. Different calibration curves were obtained for the investigated series, as shown in FIGURE 5.Comparing the elution volumes of different series at the same molar mass it could be observed, that the series I with two OH-endgroups was eluted at lower counts than the series II,IV and III.The reason for this phenomenon is the solvation of the OH-endgroups by THF (10).

For the analytical separation 20 µl of the sample solution (0.1 wt%) were injected. For the preparative fractionation 20 mg (injection volume 200 µl) were separated in one run. Fractions of 10 runs were collected, evaporated and separated according to functionality with the corresponding mobile phase for LAC under critical conditions.

3. Coupling of methods - orthogonal chromatography

The results obtained by the individual methods, SEC and LAC show, that the polymers investigated are distributed in molar mass and functionality. Only by combining of

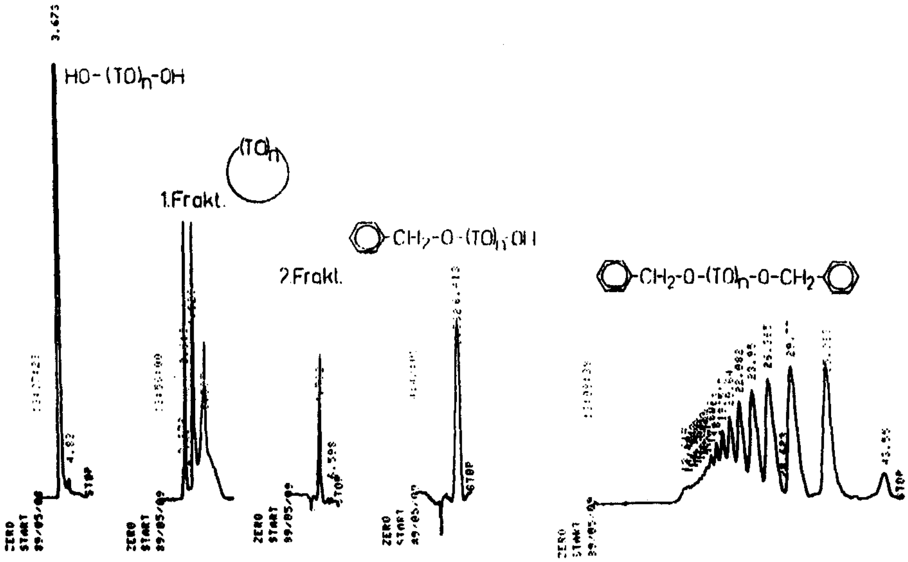


FIGURE 4
Rechromatography of preparatively separated fractions by LAC under critical conditions

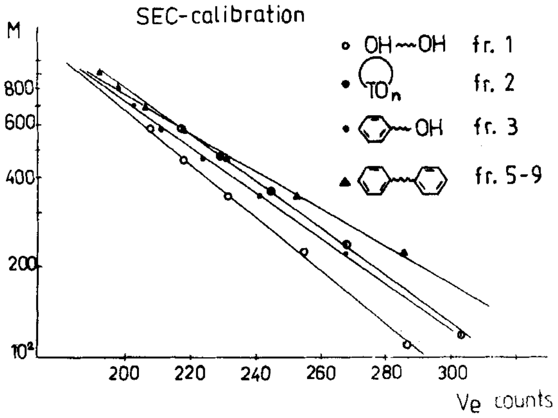


FIGURE 5
SEC calibration curves of investigated series I to IV in the oligomer range, experimental conditions see FIGURE 1

both modes of liquid chromatography the nonuniformity can be completely described.

Experimental investigations were necessary to establish the optimal mode for the first dimension of separation and the following second separation (SEC or LAC under critical conditions or vice versa). In particular, aspects of compatibility of the mobile phases, detectibility of species and the injected amount of sample solution must be taken into consideration. In FIGURE 6, the results of the orthogonal chromatography, first dimension LAC under critical conditions and second one analytical SEC of the fractions, are presented. The SEC

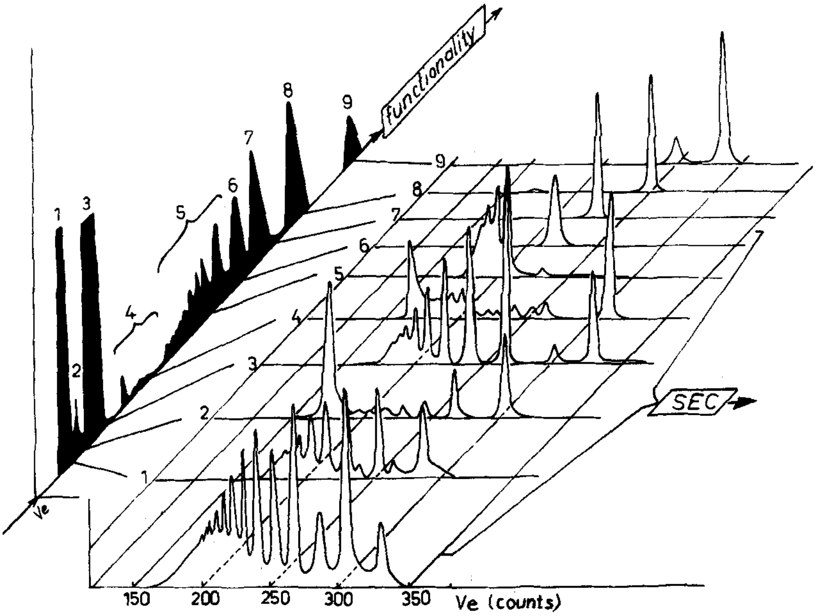


FIGURE 6
Orthogonal chromatographic separation , first dimension: preparative LAC under critical conditions, second dimension: analytical SEC

of the fractions 1-5 shows the molar mass distribution of the oligomer series. The chromatogram of fraction 1 corresponds to oligomer series I, fraction 2 to series IV, fraction 3 to series II and fractions 5-9 to series III. In addition to the earlier chromatogram (FIGURE 3), fraction 4 was obtained. This fraction indicates that, in the time between the chromatographic measurements, the benzyl alcohol is partly oxidized to benzaldehyde. According to NMR-results, the benzaldehyde reacts in competition with formaldehyde, which is a component of 1.3-dioxacyclanes. As a result, an additional molar mass distribution has been observed.

The average number molar masses of the total samples were calculated from those of the fractions using their mass portions (TABLE 4). The portions of fractions 2 and 4, which are below 1 wt%, were neglected.

The calculations were carried out on the basis of the SEC calibration curves (FIGURE 5). Only SECs with RI-detection were taken into consideration.

The superposition of the molar mass distributions of the individual fractions explains the multimodal distribution curve of the oligomer mixture in good agreement. Thus, the complete description of the oligomer

TABLE 4

List of average number molar masses (g/mol)

	Total sample		Fractions		
	SEC	Osmosis	I	II	III
A	391	385	290	385	520
B	548	520	410	645	815

system is successful if SEC and functionality chromatography are combined. In FIGURE 6 the results of the second possibility of orthogonal chromatography, first dimension SEC and second one LAC under critical conditions, are presented. The molar mass (exactly the hydrodynamic volume) of fractions decreases with increasing fraction number. The distribution of every SEC fraction is presented. The advantage of this combination consists in the possibility to recognize the superposition of different functionalities in every SEC fraction. For the verification the sequence of the first species of oligomers this figure is helpful. The calculation of molar mass averages of functionally different series is complicated because of the higher error of estimation for the amount of each peak. Experimental errors, i.e. loss in weight during the off-line technique (evaporation, dilution), are unavoidable.

DISCUSSION

In FIGURE 6 and 7 the graphical relationship of the distribution of MMD and FTD is presented. Based on the perfect separation (FIGURE 4) regarding functionality, the algorithms of calculating of M_n and M_w can be applied for every functionality fraction . The same is more complicated in the second case (FIGURE 7) owing to different calibration functions. After preparative SEC fractionation, nearly every fraction consists of a superposition of molar mass and functionality distribution. Obviously, it is not possible to solve this problem using the universal calibration. Another possibility of presentation, as suggested in other two-dimensional separation mechanisms (1,3), is the graphic reduction of the three-dimensional presentation on a two-dimensional area by contour maps. The results of the subject inves-

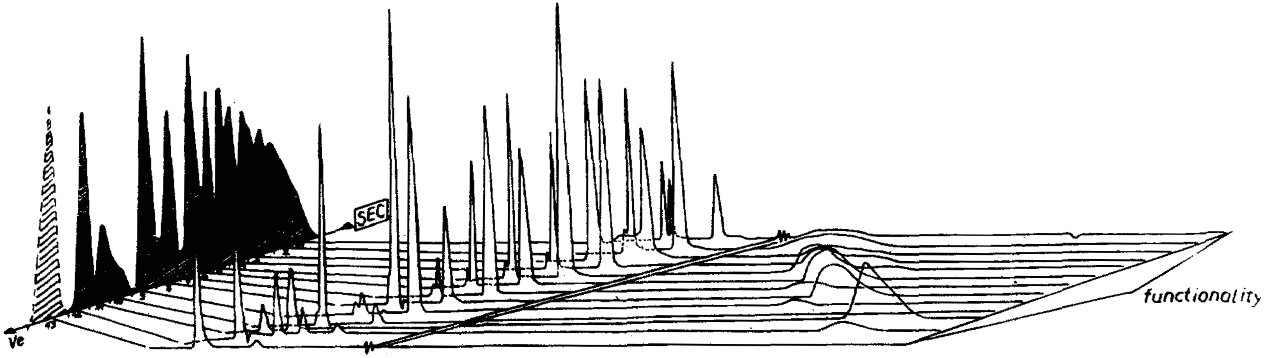


FIGURE 7
Orthogonal chromatographic separation, first dimension: preparative SEC, second dimension: analytical LAC under critical conditions

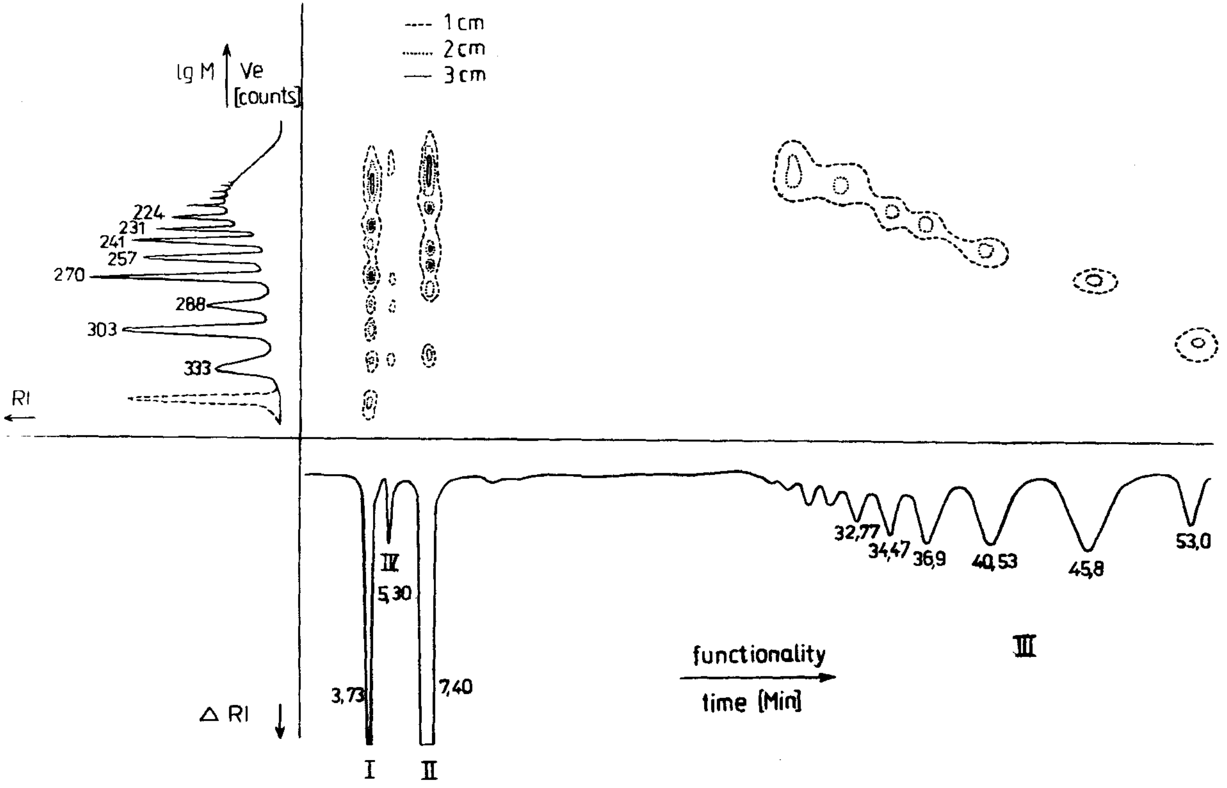


FIGURE 8
Contour map of the two-dimensional distribution of sample A by combination of LAC under critical conditions and SEC

tigated in such a way are shown in FIGURE 7. The three-dimensional presentation (x=functionality, y=molar mass and z=frequency) is reduced to a two-dimensional description by means of projecting the lines of equal frequency to the x-y area. The lines of equal frequency are indicated by different dashed curves. Hence, for different series deformed ellipses exist, which are closed in the case of complete separation of the series I,II and IV. For series III, the contour lines of lower frequency run together. The differences in the contour map comparing series I,II and IV with series III are caused by the complicated propagation mechanism.

CONCLUSIONS

A useful combination of different chromatographic methods for the complete characterization of 1.3.6.-trioxocane polymers has been developed and evaluated. By orthogonal chromatography of SEC and LAC under critical conditions a separation of molar mass distribution and functionality distribution is realized. The preparative separation in the LAC under critical conditions in the first dimension, followed by analytical SEC in the second dimension, should be preferred. This method is helpful and important for the investigation of polymerization mechanisms and kinetics. The further development of this orthogonal chromatography can be expected by online coupling of these two chromatographic modes.

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