

CHROM. 4157

COMPARISON BETWEEN GAS-LIQUID CHROMATOGRAPHY AND COLUMN CHROMATOGRAPHY IN THE DETERMINATION OF THE MOLECULAR-WEIGHT DISTRIBUTION OF POLYETHYLENE GLYCOL DERIVATIVES

C. CALZOLARI, L. FAVRETTO AND B. STANCHER

Istituto di Merceologia, University of Trieste, Trieste (Italy)

(Received May 9th, 1969)

SUMMARY

In the determination of the molecular-weight distribution of polyethylene glycol derivatives, with the formula $\text{RO}(\text{CH}_2\text{CH}_2\text{O})_n\text{R}$, $\text{R} = p$ -(1,1,3,3-tetramethylbutyl)-phenyl, the fractionation by temperature-programmed gas-liquid chromatography gives accurate results up to $n = 7$ (mol. wt. 702.3). This could be confirmed by comparing the results with those obtained by linear elution absorption chromatography, which allows accurate fractionation at least up to $n = 12$ (mol. wt. 922.5).

INTRODUCTION

Temperature-programmed gas-liquid chromatography has been used before to determine the molecular-weight distribution of oligomeric ethylene glycol derivatives having the general formula $\text{RO}(\text{CH}_2\text{CH}_2\text{O})_n\text{R}'$, where R and R' denote *e.g.* hydrogen atoms, alkyl groups or alkylaryl groups and where n is the degree of polymerization. This work has been restricted to the more volatile derivatives, obtained by replacing the primary OH groups with methoxy¹, acetyl² or trimethylsilyl^{3,4} groups.

Under suitable conditions, GLC permits the resolution of these oligomers with molecular weights of up to about 1000, although above a molecular weight of 700, the results are progressively too low^{5,6}. The limited volatility severely restricts the use of GLC for the determination of the true molecular-weight distribution, and therefore we proposed before^{7,8} that it should be replaced for this purpose by a column-chromatographic technique, called linear-elution adsorption chromatography (LEAC) by SNYDER⁹.

The aim of the present work was to compare GLC and LEAC in their accuracy in the resolution of low-volatility oligomeric ethylene glycol derivatives with n of up to 12 or 13 and $\text{R} = \text{R}' = p$ -(1,1,3,3-tetramethylbutyl)phenyl (TMBP) group.

EXPERIMENTAL

The derivatives having the general formula $\text{RO}(\text{CH}_2\text{CH}_2\text{O})_n\text{R}$ where $\text{R} = \text{TMBP}$ were prepared and purified as before⁸, the starting material being commercial

polyethylene glycol (PEG) with a mean molecular weight of 400 ($n = 2-13$). In addition, the hexamer was synthesized, and its TMBP derivative was prepared for use as an internal standard in the determination of n of the individual oligomers by the two methods.

The tetramer, pentamer, heptamer and octamer were isolated from a polydispersion by preparative liquid-solid chromatography on a column. Thin-layer chromatography was used to determine the value of n and to check the degree of purity. Repeated elution gave homogeneous fractions, whose purity was finally confirmed by GLC. The characteristics of these compounds are listed in Table I. The purity was checked also by IR and UV absorption spectroscopy. The UV spectra had the same shape for these five compounds; they exhibited two maxima, one at 276 nm and the other at 283 nm. The first of these was used in monitoring the compounds in the LEAC eluate.

For these five compounds, n is connected with the molar absorption coefficient ϵ_n at 276 nm and 20° by the equation:

$$\log \epsilon_n = (-0.0122 \pm 0.0006)n + 3.5667$$

Extrapolation for $n = 9, 10, 11$ and 12 gave ϵ_n values of 2863, 2784, 2707 and 2631, respectively.

The eluate fractions containing the same compound were combined and diluted with ethanol to a known volume, and the spectral absorption was determined at 276 nm on these solutions.

The gas chromatographic work was done with a Wilkens Aerograph 1521-1, fitted with a flame-ionization detector and means for linear temperature programming. We used a stainless-steel column, having a length of 22 cm and an internal diameter of 1/8 in. The packing was silanized 60-80 mesh Gas-Chrom P (Applied Science Laboratories), impregnated with 5 wt.% of methylsilicone polymer UC W-98 (Hewlett Packard). The temperature of the injection port and of the detector was 400°. The heating from 150 to 350° was programmed at a rate of 8°/min. The flow rate of carrier nitrogen was 60 ml/min, the flow rate of hydrogen being 40 ml/min. Under these conditions, the column had an effective peak number¹⁰ of about 0.3 (relative to n -C₂₂/ n -C₂₃ paraffins).

The analysis by LEAC was carried out with the aid of a glass column having an I.D. of 8 mm. Its temperature was kept thermostatically at 20.0 ± 0.1° with the aid of circulating water. It was connected to an Erba Fotocromacon AS-35 fraction collector in such a way as to prevent eluate losses by evaporation upstream of the detector.

The column packing was prepared by making 8.0 g of silica gel into a slurry with 30 ml of the eluent. The slurry was allowed to settle for 2-3 h, and the column was stabilized at the operating pressure, which was so chosen as to give an outflow rate of 0.4 ml/min. The resulting column in the glass tube had a height of about 27 cm and an interstitial volume of 7.0 ml.

An approximately 15-mg sample was placed on the column and was distributed with a few drops of the eluent on a disc of the adsorbent ($h \leq 2$ mm). The elution was then started, and 2.00-ml fractions were collected. The eluent was evaporated first on an air bath at 40° and then under a vacuum of 2-5 mm Hg at room temperature. The residue was taken up in 2.00 ml ethanol, and this solution was used to measure the

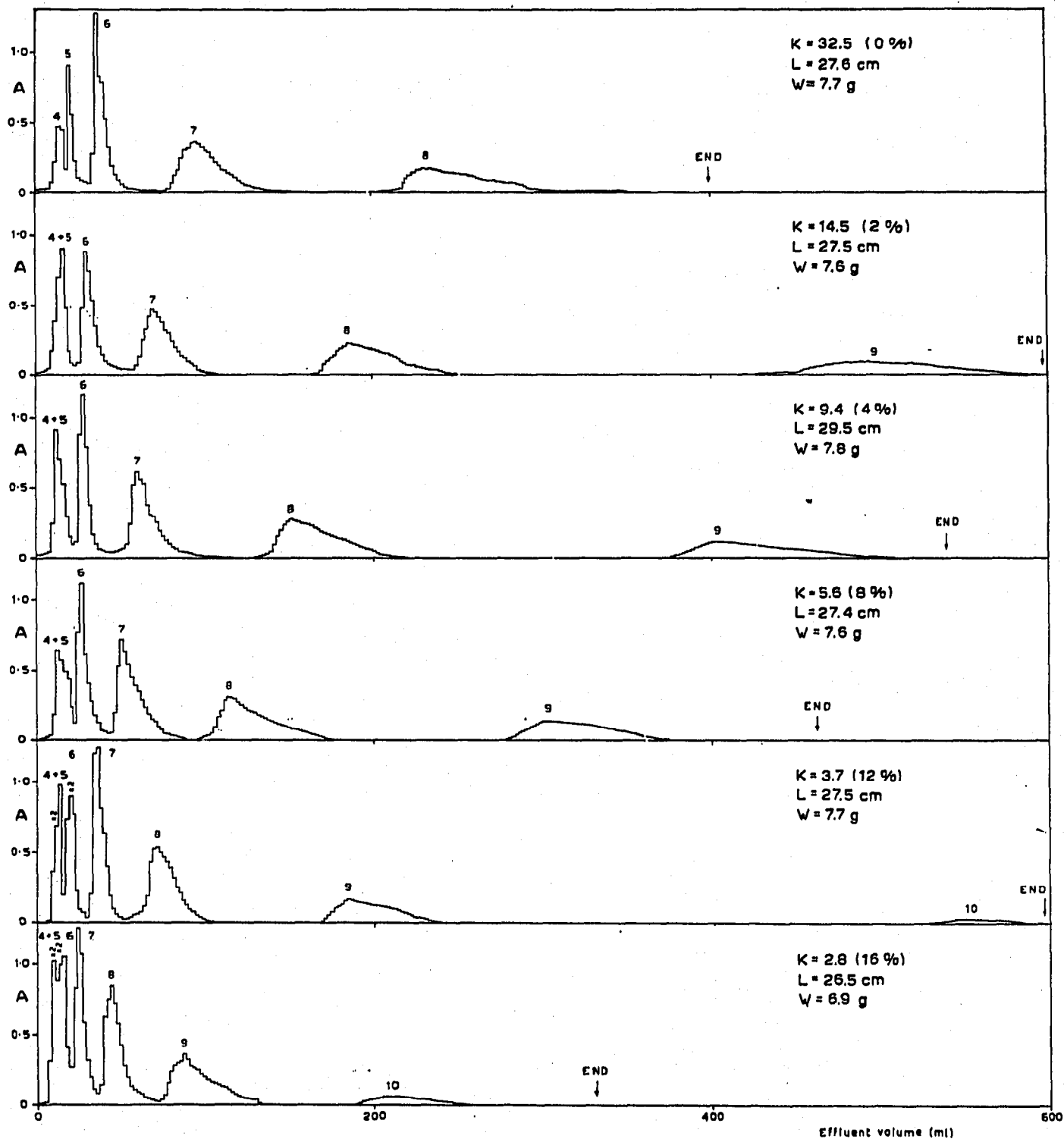


Fig. 1. Spectrophotometrically monitored elution patterns for the column chromatographic resolution of a mixture of TMBP derivatives of polyethylene glycols with 95:5 (v/v) methylene chloride-acetone on a column of silica first activated and then deactivated with water to various extents. *A*, spectral absorption at 276 nm; *K*, a measure of the degree of activation of silica gel (water content in brackets); *L*, height of the silica gel column (cm); *W*, weight of the silica gel column, (g); *n*, degree of polymerization, given above the peaks (the peak with $n = 4$ comprises also small amounts of the previous two compounds).

TABLE I
 DATA FOR THE TMBP DERIVATIVES OF POLYETHYLENE GLYCOLS, PREPARED AS SEPARATE COMPOUNDS
 The molar absorption coefficient ϵ_n was recorded at 276 nm in ethanol at 20°; the R_F values were obtained by TLC.

<i>n</i>	Degree of Oligomers purity (%)	Elution tempera- ture (°C)	n_{20}° 589 nm	Chemical analysis				ϵ_n (l cm ⁻¹ mole ⁻¹)	R_F
				obs. (%)		calc. (%)			
				C	H	C	H		
4	95.0	233	1.5165	—	—	75.81	10.26	3297 ± 12	0.80
5	96.7	245	1.5132	—	—	74.29	10.17	3191 ± 10	0.76
6	98.1	258	1.5098	72.85	10.18	72.98	10.12	3125 ± 9	0.68
7	96.2	271	1.5075	—	—	71.82	10.05	3040 ± 9	0.58
8	97.3	284	1.5058	70.90	10.01	70.81	9.99	2935 ± 18	0.50

spectral absorption at 276 nm in a Unicam SP-500 spectrophotometer, fitted with 1-cm cells. The use of blanks showed that the thorough elimination of the eluent reduced the background absorption to negligible values ($A < 0.005$).

Preliminary tests were carried out with various eluent mixtures and adsorbents having various particle sizes. On the basis of the separation of the first members, we chose 0.088–0.044-mm silica (obtained by screening silicic acid (Mallinkrodt, AR)), and a mixture of methylene chloride and acetone as the eluent. The advantage of this eluent is that it can be rapidly removed by evaporation.

The methylene chloride was obtained by distilling the laboratory reagent (Erba, RP) over P_2O_5 . Acetone was a commercial product (Erba, RS) for chromatographic use. The adsorbent was first activated for 15 h at $200 \pm 2^\circ$, and then deactivated by adding to it calculated amounts of water and allowing the system to reach equilibrium in a closed flask for some days. The degree of activation, remaining after the addition of various amounts of water, was found by determining the partition coefficient $K = (V_R - V_0)/W$ (ml/g) for naphthalene, eluted with *n*-pentane. The values obtained with water contents of up to 16% (w/w) agreed with those reported for siliceous adsorbents¹¹.

RESULTS AND DISCUSSION

Fig. 1 shows the spectrophotometrically monitored elution pattern of a mixture of the TMBP derivatives of polyethylene glycols, obtained by column chromatography on silica gel packings having different degrees of activation. A decrease in the activity is seen to reduce the retention volume V_R' (corrected for the interstitial volume V_0) of the oligomer with a given *n* value. Irrespective of the degree of activation and the

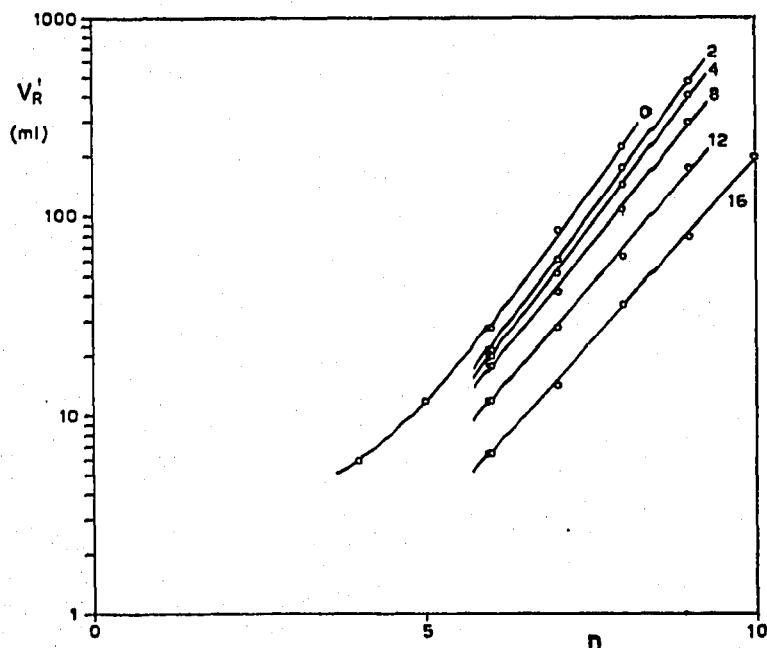


Fig. 2. Variation of the retention volume V_R' with the degree of polymerization *n* of TMBP derivatives of polyethylene glycols. The numbers at the curves denote the amount of water in the adsorbent (in %).

composition of the eluent (up to 12% v/v of acetone), $\log V_R'$ varies linearly with n over a wide range, which indicates that the adsorption isotherm is linear at a good approximation.

The graphs in Fig. 2 were obtained by plotting V_R' against n on a semi-logarithmic scale for the case when the eluent was a 95:5 (v/v) mixture of methylene chloride and acetone. The number of theoretical plates of the column, calculated from the peaks with $V_R' = 20-150$ ml was about fifty.

Fig. 3 illustrates the decrease in the partition coefficient $K_n (= V_R'/W, \text{ml/g})$ with the increasing deactivation of the adsorbent at various n values. Fig. 4 shows that, except for $n = 2-4$, all the components up to $n = 12$ can be resolved on a column by suitable gradient elution with a mixture of methylene chloride and acetone. Fig. 5 shows a typical gas chromatogram for the sample, while Table II gives the composition of the sample, as determined by LEAC (with gradient elution) and GLC in repeated analyses.

An earlier check on the accuracy of GLC showed⁶ that, apart from fragmentation occurring in the preparation of the derivatives, the results are reliable up to a molecular weight of about 700. This is confirmed by the present results. Comparison between the molecular-weight distribution curves obtained by GLC and LEAC (Fig. 6) shows that the former gives lower values than the latter for the compounds with $n > 7$ (the molar fraction of the heptamer with a molecular weight of 702.3 is taken as unity). In the determination of the molecular-weight distribution under the present conditions, therefore, LEAC with gradient elution is appreciably more accurate than GLC in

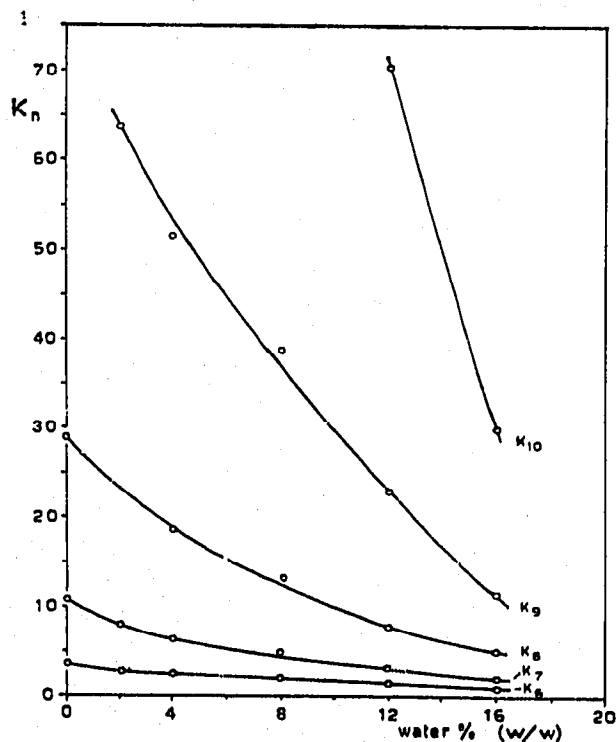


Fig. 3 Variation of the partition coefficient K_n with the water content of the adsorbent (inversely related to the degree of activation) in the case of the TMBP derivatives of polyethylene glycols with $n = 6-10$.

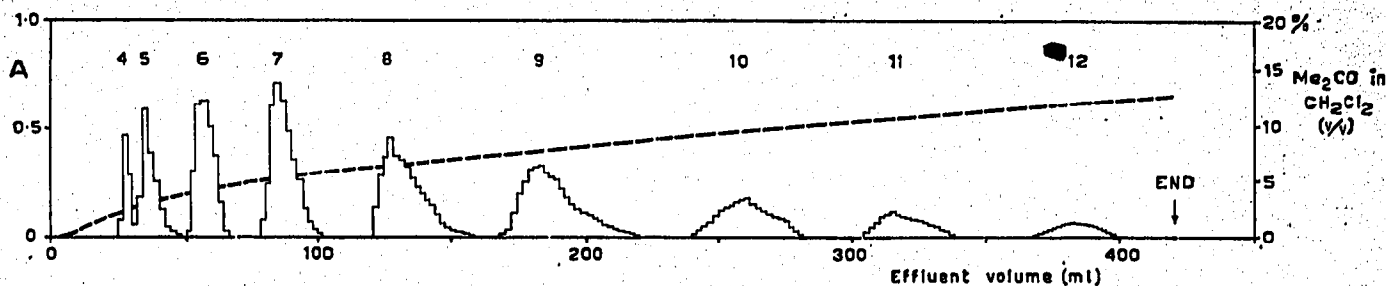


Fig. 4. Elution pattern of the TMBP derivatives of polyethylene glycols, obtained on a silica gel column with a water content of 0.5% and gradient elution with a mixture of methylene chloride and acetone. The broken-line curve gives the composition of the eluent over the column. *A*, spectral absorption at 276 nm; $L = 30.0$ cm; $W = 8.1$ g; the numbers above the peaks refer to the corresponding degree of polymerization n .

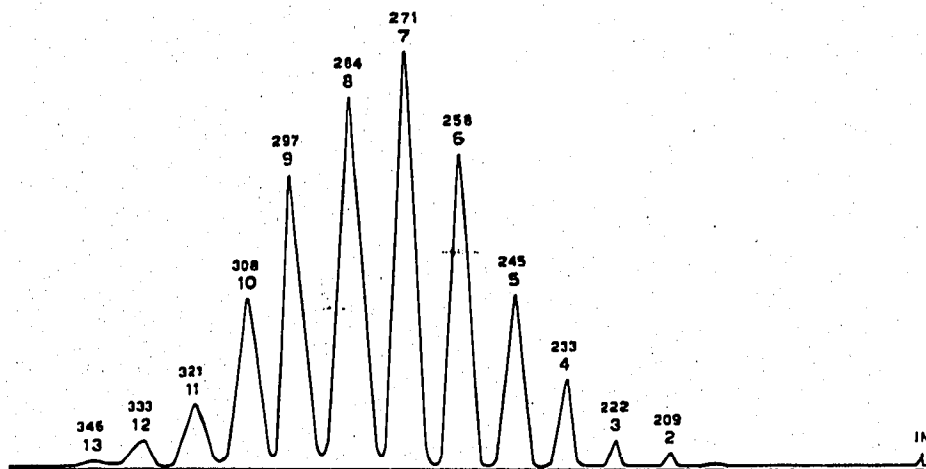


Fig. 5. Gas chromatogram for a mixture of the TMBP derivatives of polyethylene glycols. The upper values refer to the temperature of the appearance of the peak ($^{\circ}\text{C}$), while the lower values refer to the degree of polymerization n .

TABLE II

MEAN POLAR FRACTIONS (\bar{X}_n IN %) OF THE COMPONENTS OF A MIXTURE OF POLYETHYLENE GLYCOLS RESOLVED AS THE TMBP DERIVATIVES BY LEAC AND GLC

α_n gives the confidence limits at $P = 0.95$; N denotes the number of analyses.

n	LEAC (gradient elution) ($N = 3$)		GLC ($N = 4$)	
	\bar{X}_n	$\pm \alpha_n$	\bar{X}_n	$\pm \alpha_n$
2	5.15	± 0.31	0.53	± 0.02
3			0.89	± 0.02
4			3.58	± 0.02
5	6.68	± 0.25	9.47	± 0.04
6	14.59	± 0.10	18.76	± 0.01
7	18.72	± 1.66	23.25	± 0.07
8	16.81	± 1.97	18.48	± 0.07
9	13.49	± 0.04	13.90	± 0.08
10	10.76	± 0.21	7.17	± 0.13
11	7.41	± 0.55	2.62	± 0.01
12	6.27	± 0.75	1.32	± 0.01

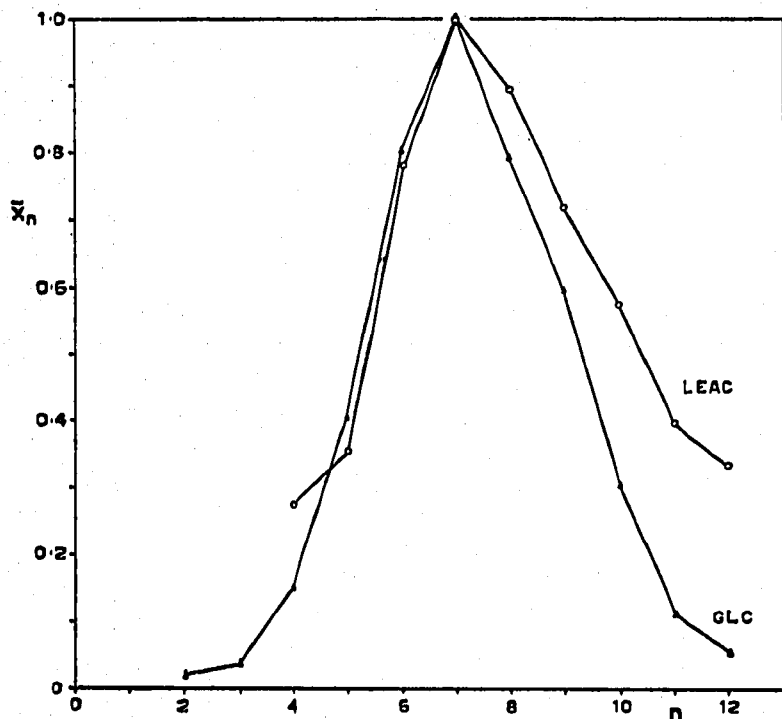


Fig. 6. Comparison between the molecular-weight distribution curves for the TMBP derivatives of polyethylene glycols, as obtained by GLC and LEAC (the molar fraction \bar{X}_7 of the heptamer was taken as unity).

the case of the higher members (at least up to $n = 12$), although it is obviously less precise than GLC.

ACKNOWLEDGEMENT

The authors wish to thank Chemische Werke Hüls (Marl, Kreis Recklinghausen, Germany) for supplying the polyethylene glycol sample.

REFERENCES

- 1 T. NAKAGAWA, H. INOUE AND K. KURIYAMA, *Anal. Chem.*, 33 (1961) 1524.
- 2 L. GILDENBERG AND J. R. TROWBRIDGE, *J. Am. Oil Chemists' Soc.*, 42 (1965) 69.
- 3 J. TORNQUIST, *Acta Chem. Scand.*, 21 (1967) 2095.
- 4 M. K. WITHERS, *J. Gas Chromatog.*, 6 (1968) 242.
- 5 C. CALZOLARI, B. STANCHER AND L. FAVRETTO, *J. Chromatog.*, 38 (1968) 7.
- 6 C. CALZOLARI, L. FAVRETTO AND B. STANCHER, *J. Chromatog.*, 39 (1969) 318.
- 7 L. FAVRETTO, A. LOKAR AND F. CARLILE, *Tec. Ital.*, 12 (1967) 1.
- 8 L. FAVRETTO AND B. STANCHER, *Rass. Chim.*, 2 (1968) 59.
- 9 L. R. SNYDER, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, pp. 75-97.
- 10 R. A. MURREL AND S. G. PERRY, *Nature*, 196 (1962) 571.
- 11 L. R. SNYDER, IN F. D. SNELL AND C. L. HILTON (Editors), *Encyclopedia of Industrial Chemical Analysis*, Vol. 1, Interscience, New York, 1966, pp. 78-98.