

CHROM. 4540

FRACTIONATION OF POLYETHYLENE GLYCOL DERIVATIVES BY LINEAR ELUTION ADSORPTION CHROMATOGRAPHY*

C. CALZOLARI, L. FAVRETTO AND B. STANCHER

Istituto di Merceologia, Università di Trieste, Trieste (Italy)

(First received October 27th, 1969; revised manuscript received December 1st, 1969)

SUMMARY

Column chromatography with gradient elution permits the separation of mixtures of the polyethylene glycol derivatives $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$, as well as the determination of the molecular weight distribution up to a molecular weight of at least ≈ 1000 ($n = 17$).

INTRODUCTION

In the determination of the molecular weight distribution of polyethylene glycol derivatives, liquid-solid chromatography, called linear elution adsorption chromatography (LEAC) by SNYDER¹, is particularly suitable for the resolution of mixtures of oligomers with molecular weights exceeding 700–800. In this case the use of gas chromatography with temperature programming, though more accurate, is limited by the volatility of the derivatives^{2,3}. The application of gradient elution, furthermore, permits the separation of a large number of oligomers in a single sample, owing to the greater peak capacity in this chromatographic technique⁴.

The present aim is to illustrate the analytical possibilities of LEAC in the fractionation of mixtures of oligomers having the formula $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$ where n is the degree of polymerisation. Thanks to the presence of a chromophoric group, UV spectrophotometry can be used to determine accurately the concentration of the individual oligomers in the eluate.

Another aim was to study the effect of the deactivation of silica gel in the analysis of compounds of the type $\text{Ph} \cdot \text{O}(\text{CH}_2\text{CH}_2\text{O}) \cdot \text{Ph}$. In column chromatography, the degree of activation of silica gel has a marked effect on the separation of ethylene glycol oligomers. At a maximum activation, the wide distribution of the adsorption energy of the active silanol centres that form hydrogen bonds with the ether groups of the oligomers⁵ favours tailing⁶. The elimination of the more active centres by partial deactivation with water had already been used to reduce tailing and the retention volume of the peaks³, and we have now considered in particular the deactivation of the silanol groups by trimethylsilylation, which is a well-known technique used in the preparation of low-polarity silica supports for gas chromatography⁷.

* Translated by Express Translation Service, London S. W. 19.

EXPERIMENTAL

Compounds of the formulae $\text{Ph} \cdot \text{O}(\text{CH}_2\text{CH}_2\text{O})_n \cdot \text{Ph}$ (type I) and $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n \text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$ (type II) were prepared as before⁸ from commercial polyethylene glycols with a mean molecular weight of 400 and 600 (PEG 400 and PEG 600). Hexaethylene glycol derivatives, synthesised singly, were used as internal standards in the determination of n .

Data have already been reported^{9,10} for homogeneous compounds of types I and II with $n = 4-10$. Irrespective of the value of n , the UV spectra of compounds I recorded in 95% ethanol at 20° show the same behaviour, exhibiting two maxima (at 270.5 and 276 nm) the first of which was chosen for the detection of these compounds in the column chromatographic eluate. The UV spectra of compounds II recorded under the same conditions show a maximum at 253.5 nm, the coefficient of molar absorption ϵ_n (in $l \text{ cm}^{-1} \text{ moles}^{-1}$) being related to n by the following equation in the range $n = 4-10$:

$$\epsilon_n = \frac{154.2 (57.6 \pm 0.8)}{246.4 + 44.05 n}$$

In the interpolation of the experimental values by the least-square method, ϵ_n was assumed to be proportional to the molar fraction of the chromophoric group present in the oligomer. This equation was used for extrapolating the ϵ_n values up to $n = 17$.

The investigations were done with a column (I.D. 8.0 mm) kept at $20.0 \pm 0.1^\circ$ with the aid of circulating water and a thermostat. The column was connected to an Erba Fotocromacon AS-35 fraction collector so as to prevent eluate losses by evaporation upstream of the column. The adsorbent column itself weighed 7 g, was about 26 cm long, and had an interstitial volume of $V_0 = 6.5 \text{ ml}$.

The details of the analytical procedure were described before³. The samples weighed about 20 and 5 mg in the case of compounds I and II, respectively. 2.00-ml fractions of the eluate were evaporated on an air bath at 40° and 2 mm Hg. The residue was dissolved in 2.00 ml of ethanol, and the solution thus obtained was used to determine the spectral absorption in a Unicam SP-500 spectrophotometer with 1-cm cells. Eluate fractions containing the same compound were combined and diluted with ethanol to a known volume. This solution was then used again for the spectrophotometric analysis to determine the amount of the compound present.

The adsorbent was silica gel with a particle size of 0.044-0.088 mm, obtained by screening commercial silicic acid (Malinckrodt AR). Mixtures of acetone and methylene chloride were used as eluent.

The water-deactivated adsorbent was prepared as before³. The degree of activation was checked by determining the distribution coefficient $K = (V_R - V_0)/W$ (in ml/g) for naphthalene, eluted with n -pentane. The values obtained with the deactivated adsorbent containing up to 16 wt. % of water agreed with those reported for silica gel adsorbents¹¹.

For deactivation by silanisation, 20 g of silica gel were heated for 15 h at $200 \pm 2^\circ$ and then suspended in 50 ml of anhydrous toluene, containing trimethylchlorosilane (TMCS) in a certain wt. % on the silica gel. The mixture was agitated at room temperature for not less than 5 min and filtered at the pump. The adsorbent was washed with 100 ml of anhydrous toluene, dried at 200° for 15 h, and stored in a hermetically

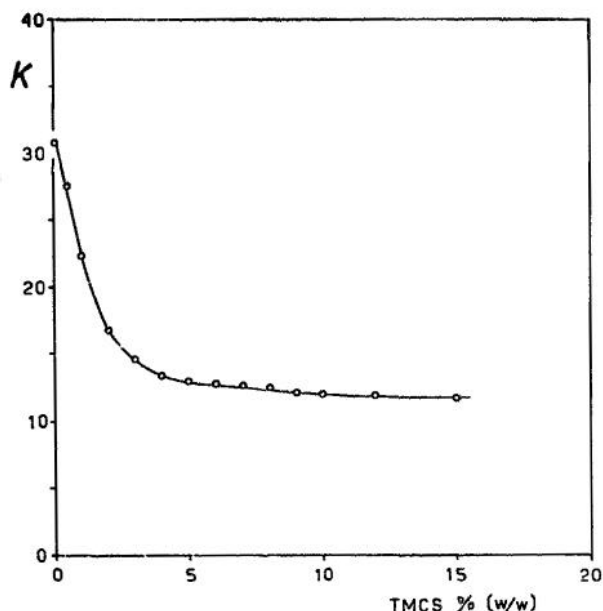


Fig. 1. Variation of the distribution coefficient K of naphthalene (eluted with *n*-pentane) with the amount of TMCS added to the adsorbent.

sealed container. Fig. 1 shows the relationship between the degree of activation (expressed by K) and the amount of TMCS. Since the latter reacts only with free silanol groups¹², K tends toward a limiting value of about 12 ml/g as the amount of this agent is raised.

The accuracy involved in determining K was independent of the magnitude of the latter, and was found to be on average ± 0.3 (expressed as the mean square deviation, calculated from the data on five samples for each degree of activation). The adsorbent, silanised by the method described above, showed no significant change in the degree of activation for at least a month.

Silanisation with dimethyldichlorosilane, used before to treat gas chromatographic supports⁷, was unsatisfactory, because washing the adsorbent with methanol and subsequent thermal treatment, prescribed by the method, irreversibly blocked some of the active silanol centres, probably by methylation¹³. The activity of the resulting adsorbent is very low even at a maximum activation ($K \approx 10$ ml/g), and depends closely on the thermal treatment. By contrast, the present method gave adsorbent samples whose K values varied continuously from 31 ml/g for the non-silanised form to about 12 ml/g for the silanised form.

A Wilkens Aerograph 1521-1 was used for the temperature-programmed gas chromatographic analysis under the same conditions as those used before⁸.

The molecular weight was determined with a Hewlett-Packard 302 vapour-pressure osmometer at 20° after dissolving the samples in toluene to a concentration of 4×10^{-3} M.

RESULTS

Comparison between water and trimethylchlorosilane as deactivator

Fig. 2 shows the variation of $\log K$ with n in the case of compounds I and II

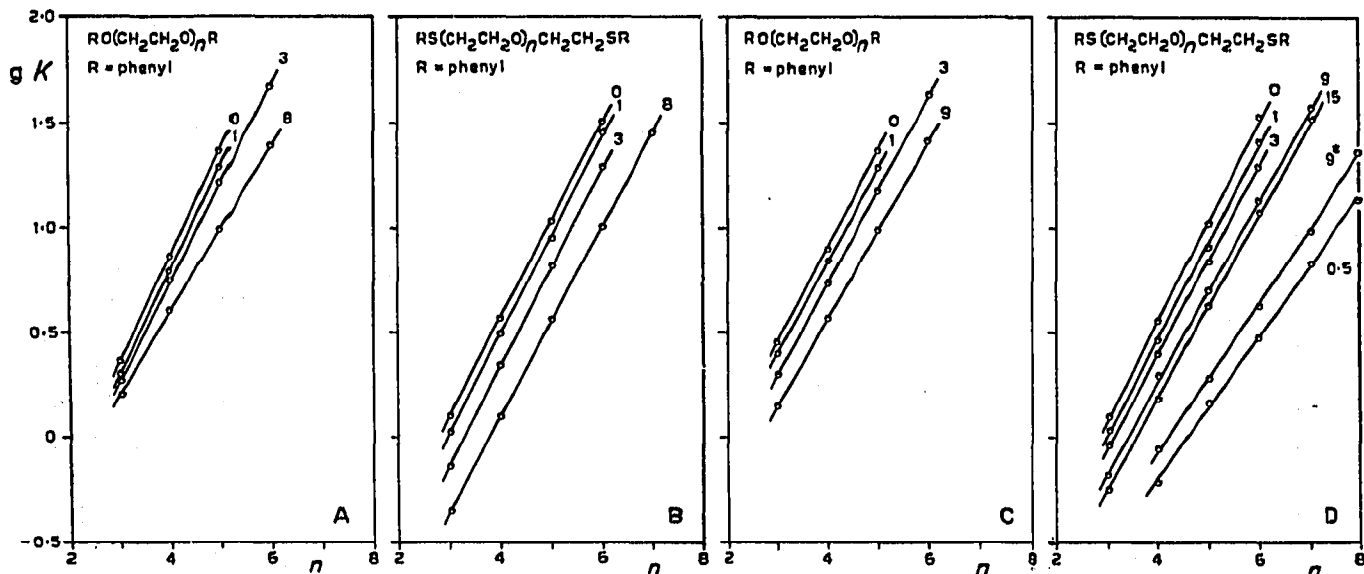


Fig. 2. Variation of $\log K$ with the degree of polymerisation n . The adsorbent was deactivated with water (sections A and B) TMCS (sections C and D), used in quantities specified in wt. % by each line. The volumetric ratio between methylene chloride and acetone in the eluent was 95:5, except for lines marked by 9* and 0.5, when it was 92:8 and 88:12, respectively. Compounds with $n = 3$ could not be separated from compounds with $n = 1$ and 2.

fractionated with the same eluent on silica gel, progressively deactivated with water (sections A and B) or by silanisation (sections C and D). In both cases, a decrease in the activity is seen to cause a reduction in the K value of the oligomer with a certain degree of polymerisation.

Fig. 3 shows for compounds I and II with $n = 4-6$ the variation of K with the

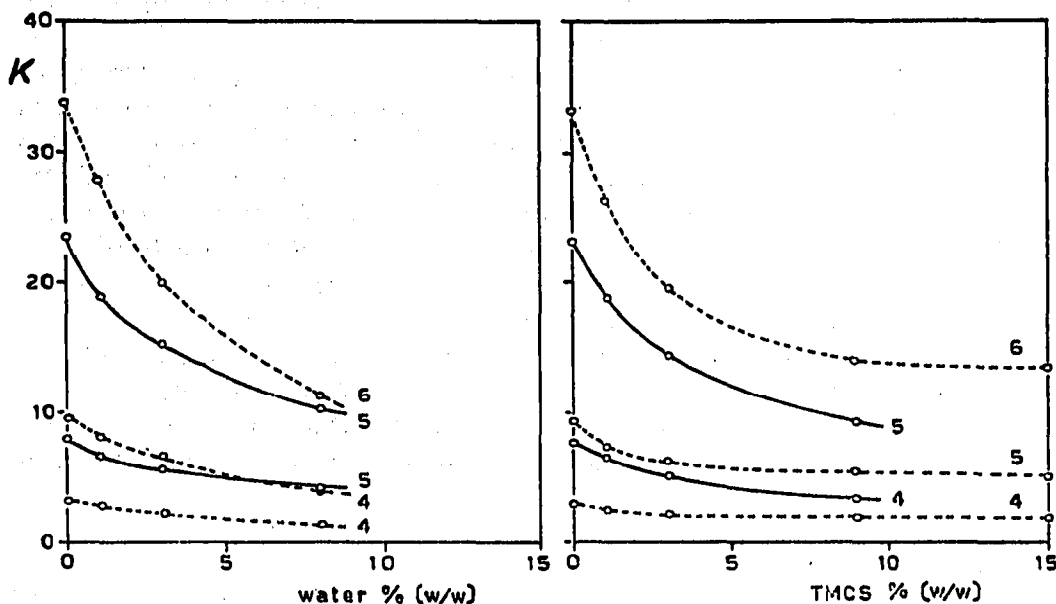


Fig. 3. Variation of K with the amount of deactivator for compounds of type $\text{Ph} \cdot \text{O}(\text{CH}_2\text{CH}_2\text{O})_n \cdot \text{Ph}$ with $n = 4-5$ (full line) and for compounds of type $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n \text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$ with $n = 4-6$ (broken line). The n values are given by the curves.

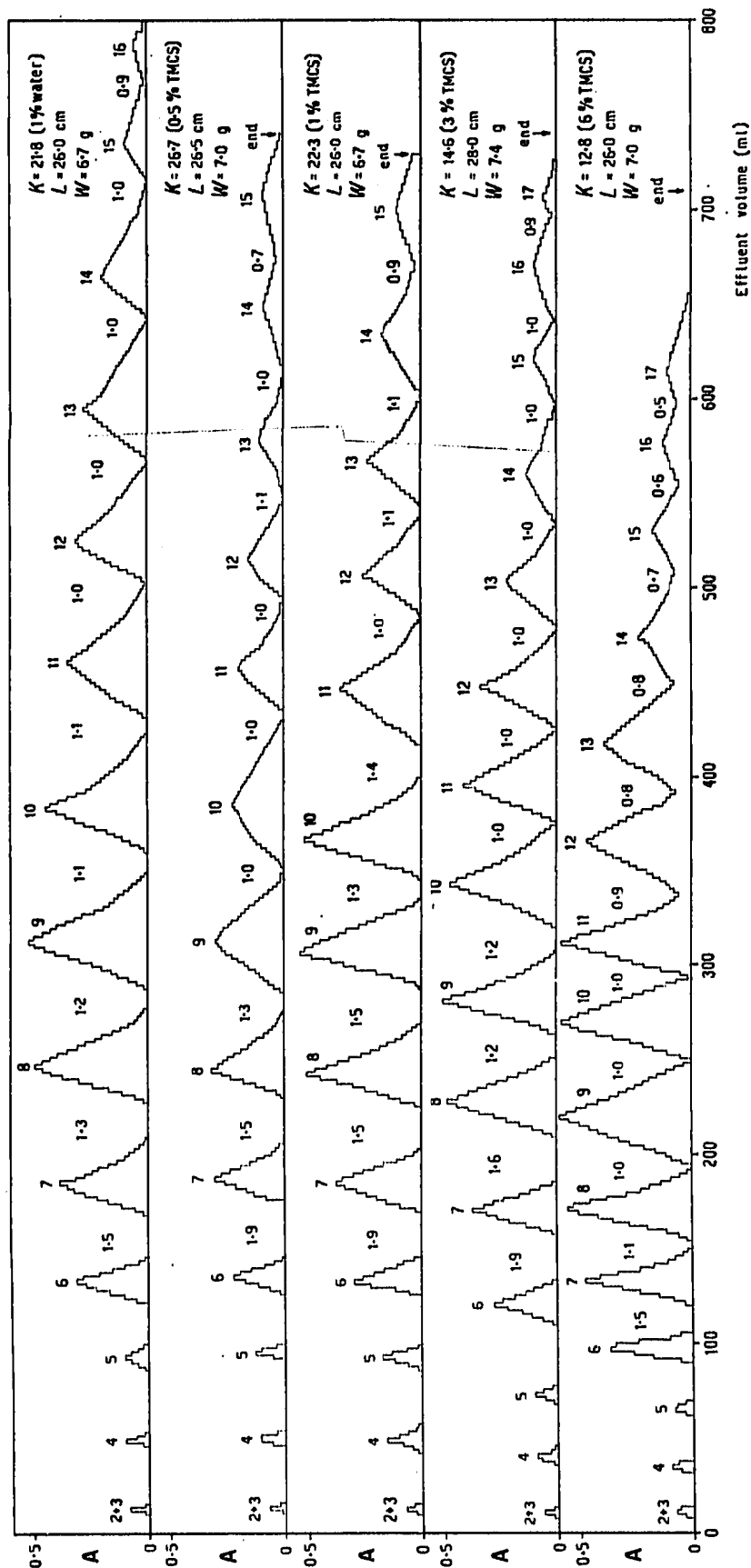


Fig. 4. Column chromatogram obtained by gradient elution and spectrophotometric detection of a mixture of compounds of type $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$ on silica gel deactivated with water or TMCS (for elution gradient, see text). A, L and W denote the spectral absorption at 253.5 nm, the height of the adsorbent column (cm), and the weight of the adsorbent column (g), respectively. The degree of activation is indicated by the distribution coefficient K for naphthalene, eluted with *n*-pentane. The values in brackets refer to the amount of water and the silanising agent in %. The figures at and between the peaks give the value of n and the separation coefficient, respectively.

degree of activation of the adsorbent, expressed by the amount of water or TMCS (wt.%) on the silica gel. The attainment of limiting values when the silanisation is enhanced indicates that there remain some active centres which are not blocked even by excess TMCS. By contrast, deactivation with water tends to be more complete.

Log K varies linearly with n in a wide range, irrespective of the degree of activation, the nature of the deactivator, and the nature of the eluent (at least up to an 88:12 mixture of methylene chloride and acetone). With silanisation and an eluent composed of a 95:5 mixture of methylene chloride and acetone, the slope of the log K vs. n lines is practically constant at 0.45 for both series of compounds. With water as deactivator, this applies to compounds II (slope: 0.47), but not to compounds I, where the slope decreases from 0.5 to 0.4 as the activity decreases.

On the basis of the peaks with a corrected retention volume $V_R' = V_R - V_0$ of 40–70 ml, the number of theoretical plates of the column was about 70.

Determination of the molecular weight distribution

To ensure a better spectrophotometric sensitivity, we analysed derivatives of type II, obtained from PEG 600 (*cf.* Fig. 4). The eluent gradient obeyed the equation

$$\log P = 0.66 \times \log V - 0.48$$

where P is the amount of acetone in methylene chloride (in vol. %), and V is the volume of the effluent (in ml). With this elution gradient, the value of V_R' varies linearly with n between $n = 8$ and at least $n = 17$.

TABLE I

MOLAR FRACTIONS (X_n IN %) OF COMPOUNDS OF TYPE $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$, AS OBTAINED IN THE ANALYSIS OF A MIXTURE BY COLUMN CHROMATOGRAPHY AND GAS CHROMATOGRAPHY
The \bar{X}_n values are means of three determinations, and the s_n values are the mean square deviations.

n	LEAC		GLC (temperature programmed)
	Deactivation with 1% water X_n	Deactivation with 3% TMCS $\bar{X}_n \pm s_n$	X_n
1	—	—	0.0034
2	{ 0.0028	{ 0.0029 ± 0.0002	0.0055
3			0.0069
4			0.0076
5	0.0154	0.0097 ± 0.0003	0.0121
6	0.0493	0.0363 ± 0.0004	0.0443
7	0.0882	0.0698 ± 0.0011	0.0887
8	0.1224	0.1044 ± 0.0004	0.1350
9	0.1460	0.1337 ± 0.0029	0.1563
10	0.1444	0.1573 ± 0.0032	0.1723
11	0.1412	0.1562 ± 0.0025	0.1544
12	0.1145	0.1334 ± 0.0019	0.1074
13	0.0837	0.1007 ± 0.0019	0.0631
14	0.0490	0.0527 ± 0.0004	0.0290
15	0.0258	0.0207 ± 0.0005	0.0099
16	0.0130	0.0122 ± 0.0005	0.0041
17	—	0.0055 ± 0.0004	—

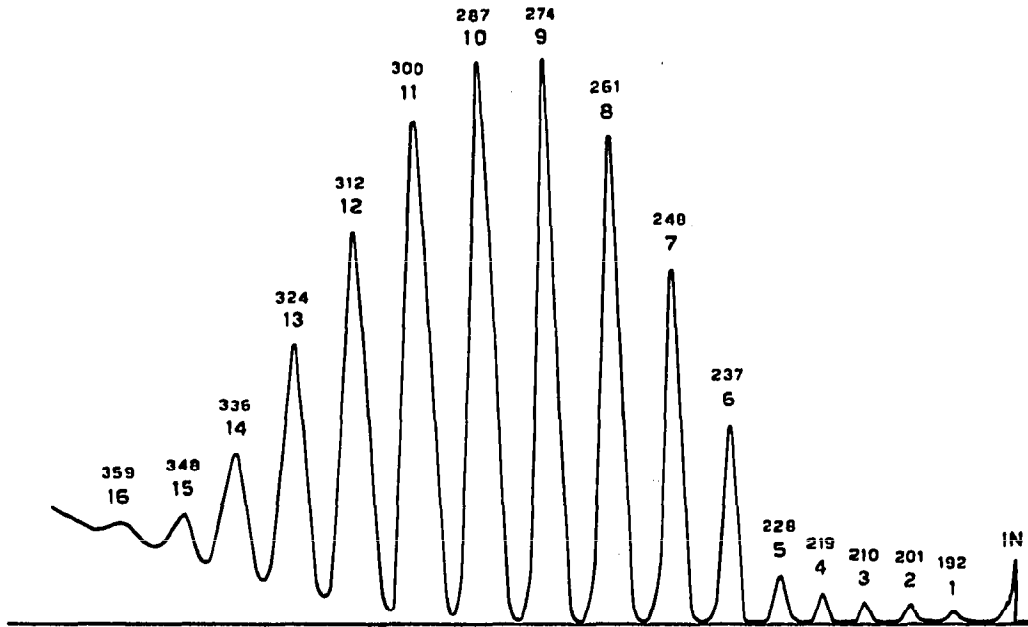


Fig. 5. Gas chromatogram obtained in the analysis of a mixture of compounds of type $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$. The upper and the lower numbers over the peaks denote the temperature at which the peaks emerged (in °C) and the degree of polymerisation n , respectively.

We have specified in Fig. 4 the separation coefficients for adjacent peaks with $n \geq 6-17$ in the case of variously silanised silica gel samples. The separation is best on silica gel treated with 1% of TMCS. For practical purposes, however, silica gel treated with 3% of TMCS is preferred: this still permits a complete resolution of nearly all the compounds and gives smaller retention volumes. As a result, the compound with $n = 17$ can be fractionated with less than about 700 ml of eluent, and the broadening of the peaks is reduced.

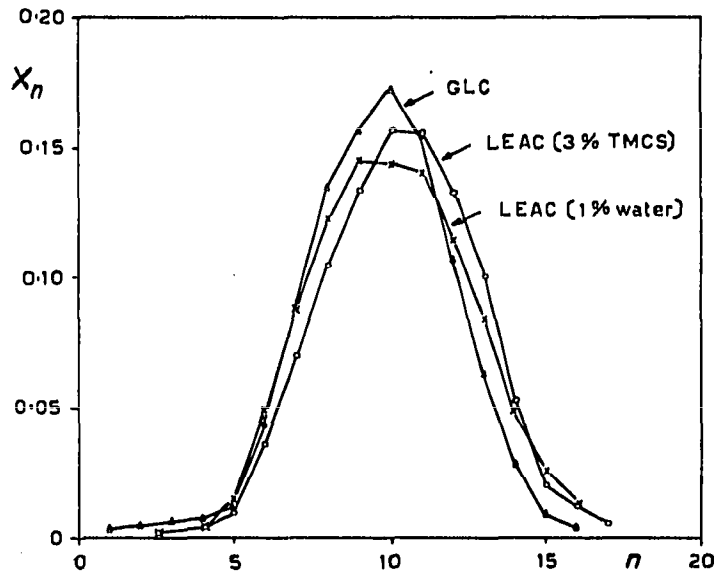


Fig. 6. Molecular weight distribution curves based on column chromatographic and gas chromatographic results. X_n is the molar fraction of the compound with a degree of polymerisation n .

The data listed in Table I illustrate the reproducibility. Since the volatility decreases with increasing degree of polymerisation, gas chromatography tends to give too low values for compounds with $n > 11-12$ (cf. the gas chromatogram in Fig. 5). This emerges from a comparison with the distribution curves in Fig. 6, and from a comparison of the number-average degree of polymerisation (\bar{n}) as measured osmotically and as calculated from the column chromatographic and the gas chromatographic values ($\bar{n} = \sum nX_n$). Thus, osmometric measurement, column chromatography on silica gel with 3% of TMCS, the same on silica gel with 1% of water, and gas chromatography gave \bar{n} values of 11.1, 10.4, 10.0, and 9.7, respectively. The best agreement with the osmometric value was obtained by column chromatography on silanised silica gel, in which case the discrepancy did not exceed 7%.

ACKNOWLEDGEMENT

The authors wish to thank Chemische Werke Hüls (Marl, Kreis Recklinghausen, G.F.R.) for supplying the polyethylene glycol samples.

REFERENCES

- 1 L. R. SNYDER, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968, pp. 75-97.
- 2 C. CALZOLARI, L. FAVRETTO AND B. STANCHER, *J. Chromatog.*, 39 (1969) 318.
- 3 C. CALZOLARI, L. FAVRETTO AND B. STANCHER, *J. Chromatog.*, 43 (1969) 207.
- 4 C. G. HORVATH AND S. R. LIPSKY, *Anal. Chem.*, 39 (1967) 1893.
- 5 K. BÜRGER, *Z. Anal. Chem.*, 196 (1963) 259.
- 6 L. R. SNYDER, *J. Chromatog.*, 5 (1961) 430.
- 7 E. C. HORNING, K. C. MADDOCK, K. J. ANTHONY AND W. J. A. VANDENHEUVEL, *Anal. Chem.*, 35 (1963) 526.
- 8 C. CALZOLARI, B. STANCHER AND L. FAVRETTO, *J. Chromatog.*, 38 (1968) 7.
- 9 L. FAVRETTO, A. LOKAR AND F. CARLILE, *Tec. Ital.*, 32 (1967) 1.
- 10 L. FAVRETTO AND B. STANCHER, *Rass. Chim.*, 20 (1968) 59.
- 11 L. R. SNYDER, in F. D. SNELL AND C. L. HILTON (Editors), *Encyclopedia of Industrial Chemical Analysis*, Vol. I, Interscience, New York, 1966, pp. 78-98.
- 12 V. YA. DAVYDOV, A. V. KISELEV AND L. T. ZHURAVLEV, *Trans. Faraday Soc.*, 60 (1964) 2254.
- 13 M. L. HAIR, *Infrared Spectroscopy in Surface Chemistry*, Marcel Dekker, New York, 1967, p. 122.

J. Chromatog., 47 (1970) 209-216