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## COMPARISON BETWEEN COLUMN CHROMATOGRAPHY AND THIN-LAYER CHROMATOGRAPHY IN THE DETERMINATION OF THE MOLECULAR-WEIGHT DISTRIBUTION OF POLYETHYLENE GLYCOL DERIVATIVES

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## SUMMARY

The molecular-weight distribution of the polyethylene glycol derivatives  $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$  (where  $n$  is the degree of polymerisation) was determined by column chromatography combined with ultraviolet absorption spectroscopy, and by thin-layer chromatography combined with direct photometric evaluation after visualisation with iodine vapour. The second method was found to be less precise than the first but had a comparable accuracy and permitted the separation of oligomers between  $n = 6$  and  $n = 19$ .

## INTRODUCTION

Determination of the molecular-weight distribution (MWD) plays an important part in the quality control of polyethylene glycol (PEG). Thin-layer chromatography (TLC) combined with direct photometric evaluation of the spots after visualisation with iodine vapour has recently been proposed as a rapid method for this determination<sup>1,2</sup>.

Although the response of some compounds to iodine vapour varies with the degree of polymerisation  $n$ , the resulting systematic error can be corrected by means of a correction factor, found by examining the response of pure oligomers to iodine or by comparing the MWD with one obtained by an absolute method such as column chromatography (CC) combined with UV spectrophotometry<sup>3</sup>. This second method involves more work but is particularly promising in the fractionation of polyethylene glycols with chromophoric groups because—owing to the high peak capacity—gradient elution permits the separation of a great number of components<sup>4</sup>.

The present aim was to assess the accuracy and the precision of TLC in this field by comparing it with CC. The work was done on compounds with the general formula  $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$  ( $n = 1-19$ ), the molecular absorption coefficient of these compounds being sufficiently high to enable one to analyse 1-2 mg of the oligomer mixture by CC with gradient elution.

## EXPERIMENTAL

Commercial PEG with a number-average molecular weight of 600 was converted into the above-mentioned derivative, and the latter was purified<sup>5</sup>. In addition, pure compounds with  $n = 1-9$  were prepared from synthetic homogeneous ethylene glycol oligomers. The corresponding dichlorides were repeatedly distilled at reduced pressure, then reacted with sodium thiophenoxide, and the products were purified by CC (the monomer also by vacuum distillation). The purity was checked by temperature-programmed GLC<sup>5</sup>, IR and UV absorption spectroscopy, and NMR spectroscopy. The IR spectrophotometric analysis was done on a liquid sample with a Perkin-Elmer Infracord, and the NMR spectroscopic analysis with a Jeol C-60 instrument, the sample having been dissolved in deuterated chloroform (50%, v/v). The characteristics of these standards are shown in Table I.

TABLE I

DATA FOR THE PURE INDIVIDUAL MEMBERS OF THE SERIES  $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$  USED AS STANDARDS

The molar absorption coefficient in 95% ethanol at 253.5 nm and 20° is  $\epsilon = 15\,600 \text{ l} \cdot \text{cm}^{-1} \cdot \text{mole}^{-1}$ .

$n$	Degree of purity (% peak area)	Oligomers present as impurities (% peak area)	Elution temperature (°C)	$n_{D, 20}^{20}$ 880 nm
1	99.9 <sup>a</sup>	—	156	1.6169
2	99.9	—	173	1.5928
3	99.9	—	188	1.5782
4	99.5	0.5 ( $n = 3$ )	205	1.5671
5	99.9	—	221	1.5581
6	98.0	{ 0.8 ( $n = 5$ ) 1.2 ( $n = 7$ )	237	1.5508
7	99.0	1.0 ( $n = 6$ )	253	1.5438
8	97.0	{ 0.5 $n = 6$ 2.5 $n = 7$	269	1.5390
9	96.2	{ 0.2 ( $n = 7$ ) 3.6 ( $n = 8$ )	284	1.5359

<sup>a</sup> Boiling point: 171–173° at 0.03 mm Hg.

The chromatographic column had an internal diameter of 5.0 mm, was thermostatically controlled at  $20.0 \pm 0.1^\circ$ , and was connected to an Erba Fotocromacon AS-35 fraction collector, preventing losses by evaporation. The adsorbent column had a height of about 27 cm and an interstitial volume  $V_0$  of 6.5 ml; it was prepared by sedimenting a suspension of 3 g of the adsorbent in 10 ml of the starting eluent. A nitrogen pressure of about 500 mm Hg upstream of the column ensured an eluent flow rate of 0.7–0.8 ml/min. About 2 mg of the sample was placed on a stationary-phase disk not thicker than 1 mm at the top of the column with the aid of a teflon capillary connected to a 10- $\mu$  Hamilton microsyringe.

The 2.00-ml fractions were evaporated in a drying cabinet at 40° and 2 mm Hg; the residue was redissolved in 2.00 ml of 95% ethanol, and the solutions were subjected to spectrophotometric determination at 253.5 nm in 1-cm cells, the results being used to obtain the elution pattern in the form of a histogram. Eluate fractions

containing the same compound were combined, diluted with ethanol to a known volume, and quantitatively analysed by spectrophotometry. The same absorption coefficient ( $\epsilon = 15\,600 \text{ l} \cdot \text{cm}^{-1} \cdot \text{mole}^{-1}$ ) was taken for all the compounds so that the molar fraction was proportional to the spectrophotometric absorption for all the members of the polymer mixture.

The degree of polymerization of the above fractions was found by comparing the latter with pure standards with the aid of TLC and GLC, carried out on the residues left behind after the evaporation of the ethanolic solutions. Compounds with  $n = 2-5$  could not be separated by CC and were separated and estimated by GLC.

The silica gel used as the adsorbent in CC was obtained by screening commercial silicic acid (Mallinckrodt AR); it had a particle size of 0.035–0.050 mm, permitting better resolution than the granulometric fractions used before<sup>4</sup>. The eluents were mixtures of methylene chloride (Carlo Erba, freshly distilled over  $\text{P}_2\text{O}_5$ ) and acetone (Erba chromatographic reagent).

To reduce the retention volumes  $V_R$  (in ml), the adsorbent, maximally activated by heating for 15 h at  $180 \pm 5^\circ$ , was partially deactivated with 3% (w/w) water or by silanisation (addition of 3% trimethylchlorosilane) as described previously<sup>4</sup>. The degree of activation was found by the following two methods:

(a) Determination of the distribution coefficient  $K$  (in ml/g) at  $20^\circ$  for the compounds with  $n = 4-7$ , fractionated with a 95:5 (v/v) mixture of methylene chloride and acetone;  $K = (V_R - V_0)/W$ , where  $W$  is the weight of the adsorbent in g);

(b) Determination of the adsorption isotherm of the compounds with  $n = 4-6$ , in the presence of the same eluent at  $20^\circ$  by spectrophotometric measurement of the oligomer concentration in the liquid phase before and after the establishment of equilibrium with the adsorbent<sup>6</sup>.

For TLC, optical-grade glass plates ( $20 \times 2.5$  cm) were coated with 20–40  $\mu$  Silica Gel G (Merck) by means of an applicator with a fixed 200- $\mu$  slit. 40  $\mu\text{g}$  of the oligomeric mixture, or not more than 5  $\mu\text{g}$  of each pure oligomer in a chloroform solution, were applied to the plate with a 10- $\mu\text{l}$  Hamilton microsyringe mounted on a micrometer support, the sample spots having a diameter of not more than 3 mm. The plates were developed vertically to a run of 17.0 cm in a cylindrical Desaga tank ( $20 \times 5$  cm), equilibrated at  $20 \pm 1^\circ$ , the developer being a 90:10 (v/v) mixture of butanone and water. Other details of the process have been described before<sup>2</sup>.

The eluted spots were visualised with iodine vapour in the course of 1 h, then protected with a glass plate and subjected to photometric evaluation with a Joyce Loebel Chromoscan set for measuring the transmission. This was done by scanning in the elution direction at  $\lambda = 430$  nm (glass filter), with  $5 \times 1$ ,  $6 \times 1$ , and  $7 \times 1$  mm slits. The recorder response, linearised in absorbance in the range of 0–1 A, permitted the determination of the integrated absorbance of peaks, whose area was found planimetrically.

## RESULTS AND DISCUSSION

### *Column chromatography*

Fig. 1 shows  $\log K$  vs.  $n$  in the case of deactivated and maximally activated

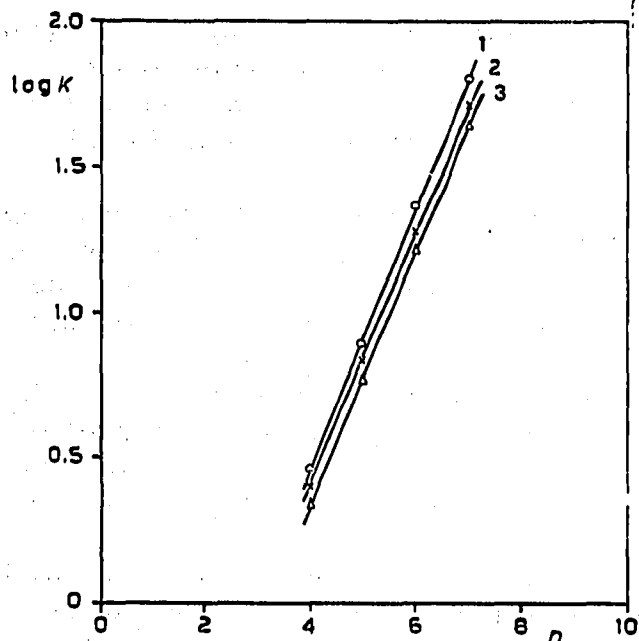


Fig. 1. Variation of  $\log K$  with the degree of polymerisation ( $n$ ) of compounds  $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n \text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$  in the case of silica gel adsorbents with various degrees of activation, and a 95:5 (v/v) mixture of methylene chloride and acetone as eluent. 1, maximally activated adsorbent; 2, adsorbent deactivated with 3% (w/w) water; 3, adsorbent deactivated with 3% (w/w) trimethylchlorosilane (TMCS).

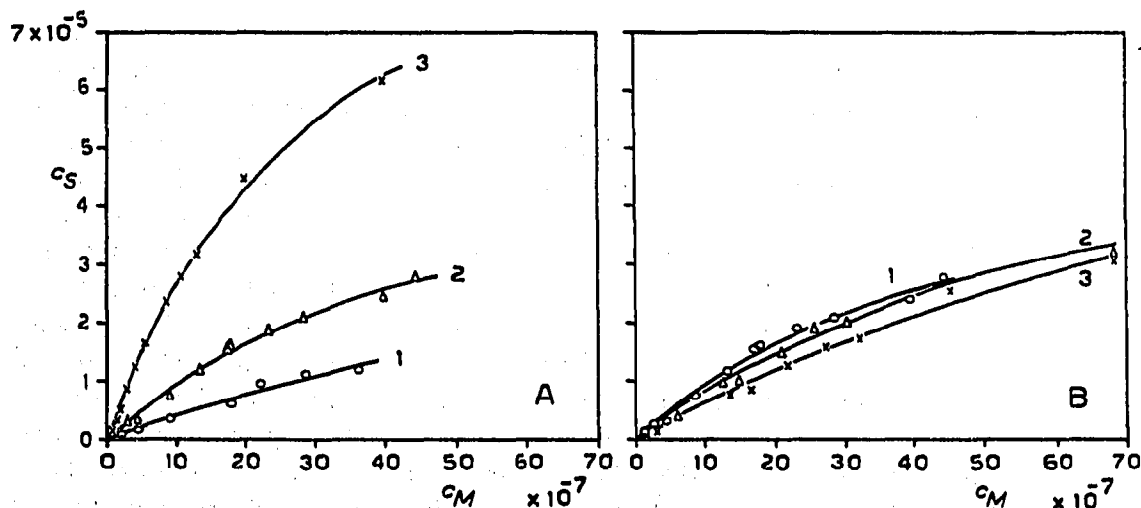


Fig. 2. Adsorption isotherms of pure members of the series  $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n \text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$  at  $20^\circ$ , the liquid phase being a 95:5 (v/v) mixture of methylene chloride and acetone. Diagram A:  $n = 4$  (curve 1),  $n = 5$  (curve 2), and  $n = 6$  (curve 3); adsorbent: maximally activated silica gel. Diagram B:  $n = 5$ ; adsorbent: maximally activated silica gel (curve 1), silica gel deactivated with 3% water (curve 2) and silica gel deactivated with 3% trimethylchlorosilane (curve 3).  $c_M$  is the equilibrium concentration of the adsorbate in the liquid phase (mole/ml), and  $c_S$  is the specific adsorption (mole/g). The continuous curves connecting the experimental points were obtained by least squares on the assumption that the Langmuir isotherm is obeyed.

TABLE II

VALUES OF THE CONSTANTS  $A$  AND  $B$  IN THE LANGMUIR ISOTHERM OF  $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$  COMPOUNDS AT  $20^\circ$

The liquid phase is a 95:5 (v/v) mixture of methylene chloride and acetone.

$n$	Degree of activation of the silica gel adsorbent	$A$ ( $\text{ml} \cdot \text{g}^{-1}$ )	$B$ ( $\text{ml} \cdot \text{mole}^{-1}$ )
4	Maximum	4.24	$5.46 \times 10^4$
5	Maximum	11.2	$1.82 \times 10^5$
	Deactivated with 3% water	9.30	$1.32 \times 10^5$
6	Deactivated with 3% TMCS	6.71	$6.27 \times 10^4$
	Maximum	33.7	$2.87 \times 10^5$

adsorbents. In accordance with MARTIN's equation<sup>7</sup>, the adsorption energy is seen to vary linearly with the degree of polymerisation of the adsorbate in all three cases.

Fig. 2 shows the adsorption isotherms for the various systems at  $20^\circ$ . The experimental values were connected into continuous curves on the assumption that the Langmuir isotherm is obeyed, *i.e.* that  $c_S = Ac_M/(1 + Bc_M)$ , where  $c_S$  is the

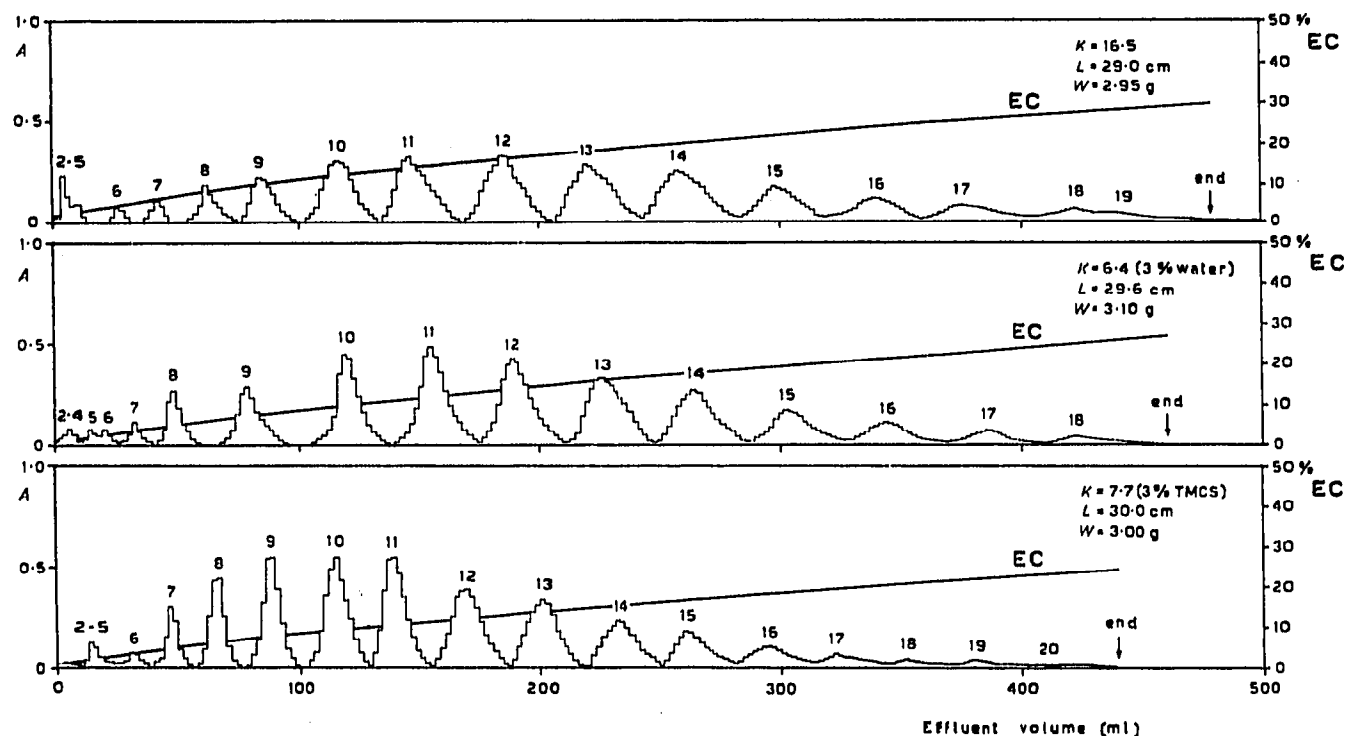


Fig. 3. Column chromatograms obtained for  $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$  by gradient elution and spectrophotometric evaluation. Adsorbents: maximally activated silica gel (top), silica gel deactivated with 3% water (middle), and silica gel deactivated with 3% trimethylchlorosilane (bottom). Eluent: mixtures of methylene chloride and acetone. The eluent composition at the top of the adsorbent column is given by a straight line in conjunction with the ordinate on the right, which shows the amount of acetone in the mixture (% v/v).  $A$  = spectrophotometric absorbance at 253.5 nm;  $L$  = height of the adsorbent column (cm), and  $W$  = weight of the adsorbent column (g). The degree of activation of the silica gel is expressed by the distribution coefficient  $K$  of naphthalene, eluted with  $n$ -pentane. The number over each peak is the corresponding degree of polymerisation  $n$ .

specific adsorption (in moles of adsorbate per g of adsorbent),  $c_M$  is the equilibrium concentration (in moles per ml of liquid phase), and  $A$  and  $B$  are constants whose values are found by the method of least squares (*cf.* Table II). As  $c_M$  approaches zero,  $A$  comes to represent the slope of the linear isotherm. Deactivation of the adsorbent reduces both  $A$  and  $B$ , and thus increases the  $c_M$  range in which the isotherm is approximately linear.  $\log A$  increases linearly with  $n$ , the line being parallel to that obtained by plotting the chromatographic  $\log K$  values against the  $n$  values. The silanised adsorbent seems to be best for the compounds under consideration.

Fig. 3 shows some elution patterns obtained in the gradient elution for a PEG preparation with  $\bar{n} = 12.2$ . The partially deactivated adsorbents are seen to have permitted a better separation of the high- $n$  compounds.

#### Thin-layer chromatography

As may be seen from Fig. 4, there is a linear relationship for  $n = 1-9$  between the sample size (in  $\mu\text{g}$ ) and the integrated absorbance of the peak, measured planimetrically on the same plate after visualisation with iodine vapour. Such straight lines are obtained for various visualisation times. In the case of one and the same plate, the ratio between the slopes of the straight-line calibration curves for two compounds is constant and approaches unity for  $n > 7$ .

In Fig. 5  $r$  is the ratio between the slope of the calibration curve for a compound with a polymerisation degree  $n$  and the slope of the calibration curve for the nonamer, this second slope being taken as 1. The correction factors to be applied to the integrated absorbance of compounds with  $n < 9$  are as follows (the value of  $n$  is indicated in parentheses): 0.35 (1), 0.46 (2), 0.57 (3), 0.63 (4), 0.70 (5), 0.76 (6), 0.87 (7), and 0.95 (8). These correction factors are calculated as  $1/r$  in each case. It has

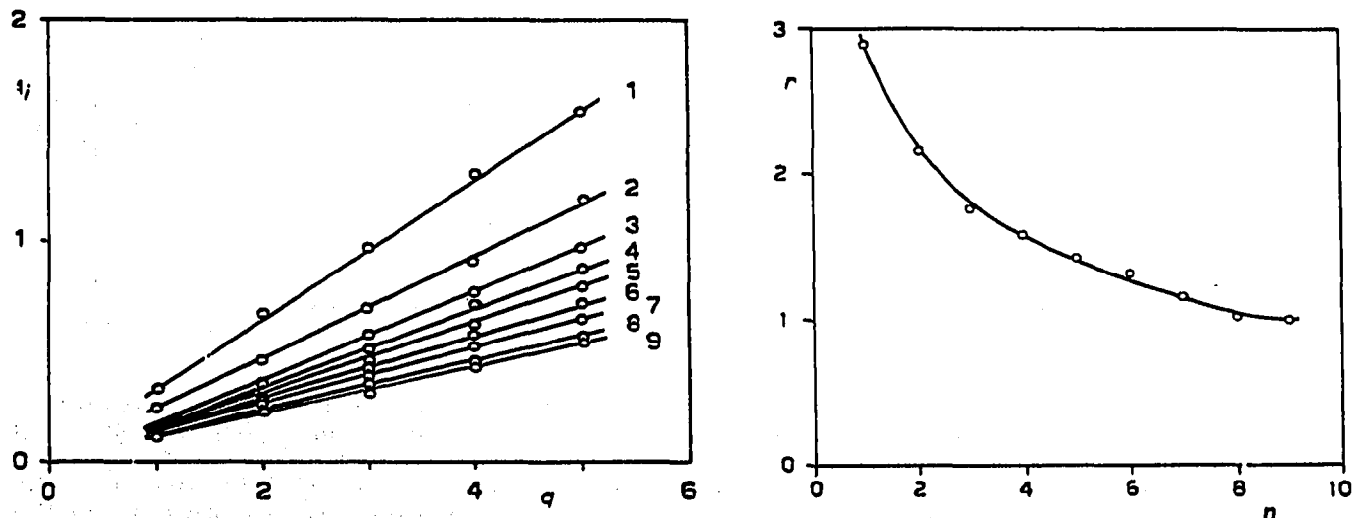


Fig. 4. Relationship between the amount of sample  $q$  ( $\mu\text{g}$ ) placed on the plate and the integrated absorbance  $A_i$  (in arbitrary units), measured planimetrically.

Fig. 5. Ratio  $r$  between the slope of the calibration curve for a compound with a degree of polymerization  $n$  and the slope of the calibration curve for the nonamer ( $r$  is always the mean of three measurements).

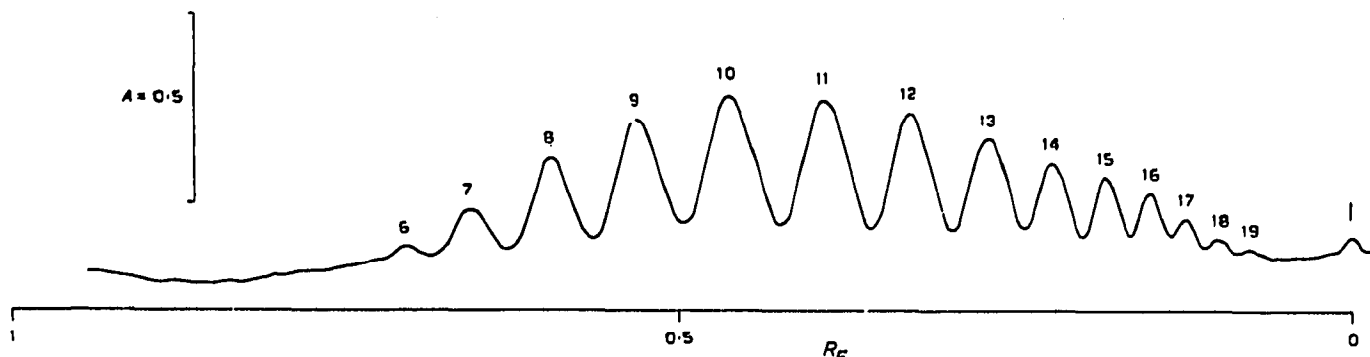


Fig. 6. Thin-layer chromatogram of PEG derivatives with  $\bar{n} = 12.2$ . Single elution with a 90:10 (v/v) mixture of butanone and water at 20° ( $A$  = absorbance).

been assumed that the compounds with  $n > 9$  respond to the iodine vapour similarly to the nonamer.

Fig. 6 shows a chromatogram obtained by the single elution of a mixed PEG with  $\bar{n} = 12.2$ . The resolution of the individual compounds is seen to be practically complete up to  $n = 19$ .

TABLE III

DISTRIBUTION OF THE DEGREE OF POLYMERISATION FOR A  $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$  PREPARATION WITH  $\bar{n} = 12.2$ , AS OBTAINED BY CC (NUMBER OF DETERMINATIONS  $N = 2$ ) AND TLC ( $N = 3$ ).

$\bar{x}_n$  is the molar fraction (mean of  $N$  determinations), and  $s$  is the mean-square deviation. The variation coefficients (in %) are given in brackets. The last line shows the calculated number-average degree of polymerisation  $\bar{n}_{\text{calc.}} = \sum \bar{x}_n n$ .

$n$	$\bar{x}_n \pm s$			Thin-layer chromatography ( $N = 3$ )
	Column chromatography ( $N = 2$ )			
	0% water	3% water	3% TMCS	
2 <sup>a</sup>	0.0026 ± 0.0001	0.0037 ± 0.0001	0.0042 ± 0.0002	—
3 <sup>a</sup>	0.0037 ± 0.0001	0.0049 ± 0.0001	0.0057 ± 0.0002	—
4 <sup>a</sup>	0.0044 ± 0.0003	0.0064 ± 0.0002	0.0074 ± 0.0001	—
5 <sup>b</sup>	0.0164 ± 0.0006	0.0190 ± 0.0004	0.0111 ± 0.0003	—
6	0.0499 ± 0.0006	0.0437 ± 0.0005	0.0404 ± 0.0001	0.0073 ± 0.0015
7	0.0848 ± 0.0006	0.0716 ± 0.0030	0.0715 ± 0.0009	0.0406 ± 0.0069
8	0.1163 ± 0.0005 (0.5)	0.1079 ± 0.0007 (0.6)	0.1039 ± 0.0002 (0.2)	0.1022 ± 0.0082 (8.0)
9	0.1525 ± 0.0007 (0.5)	0.1436 ± 0.0006 (0.4)	0.1269 ± 0.0009 (0.7)	0.1525 ± 0.0136 (8.9)
10	0.1558 ± 0.0010 (0.6)	0.1458 ± 0.0022 (1.5)	0.1362 ± 0.0003 (0.2)	0.1737 ± 0.0100 (5.7)
11	0.1416 ± 0.0009 (0.6)	0.1333 ± 0.0007 (0.5)	0.1322 ± 0.0002 (0.1)	0.1691 ± 0.0105 (6.2)
12	0.1155 ± 0.0003 (0.3)	0.1125 ± 0.0004 (0.4)	0.1159 ± 0.0007 (0.6)	0.1299 ± 0.0052 (4.0)
13	0.0812 ± 0.0009 (1.1)	0.0707 ± 0.0100 (14.1)	0.0859 ± 0.0009 (1.0)	0.0994 ± 0.0136 (13.6)
14	0.0425 ± 0.0003	0.0533 ± 0.0003	0.0600 ± 0.0003	0.0592 ± 0.0130
15	0.0174 ± 0.0008	0.0374 ± 0.0003	0.0455 ± 0.0005	0.0314 ± 0.0067
16	0.0087 ± 0.0001	0.0234 ± 0.0157	0.0283 ± 0.0003	0.0215 ± 0.0032
17	0.0044 ± 0.0002	0.0089 ± 0.0006	0.0148 ± 0.0005	0.0091 ± 0.0014
18	0.0023 ± 0.0002	0.0039 ± 0.0003	0.0076 ± 0.0002	0.0042 ± 0.0012
19	trace	—	0.0025 ± 0.0001	0.0019 ± 0.0006
20	—	—	trace	—
$\bar{n}_{\text{calc.}}$	9.98	10.31	10.56	10.88

<sup>a</sup> Values obtained by GLC.

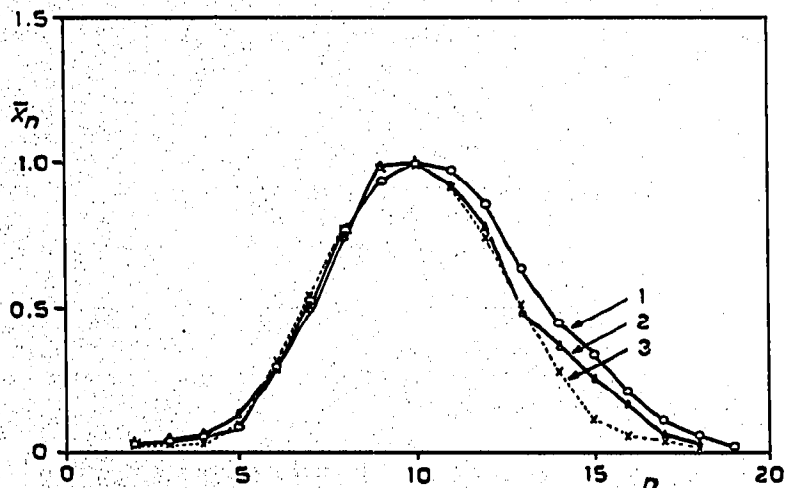


Fig. 7. Distribution of the degree of polymerisation in a  $\text{Ph} \cdot \text{S}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{S} \cdot \text{Ph}$  preparation, as obtained by CC with maximally activated silica gel (curve 3), silica gel deactivated with 3% TMCS (curve 1), and silica gel deactivated with 3% water (curve 2).  $\bar{x}_n$  is the molar fraction (mean of two determinations);  $\bar{x}_{10}$  was taken as 1.

#### Comparison between CC and TLC

The data in Table III show that CC is more precise than TLC. In particular, CC with an adsorbent deactivated by silanisation permits the determination of the individual compounds in the mixture with a variation coefficient of about 0.5%, the corresponding value in the case of TLC being about 8%. Furthermore, the results obtained by CC with such an adsorbent are also more accurate than those obtained by CC with the other adsorbents examined; the progressive underestimation

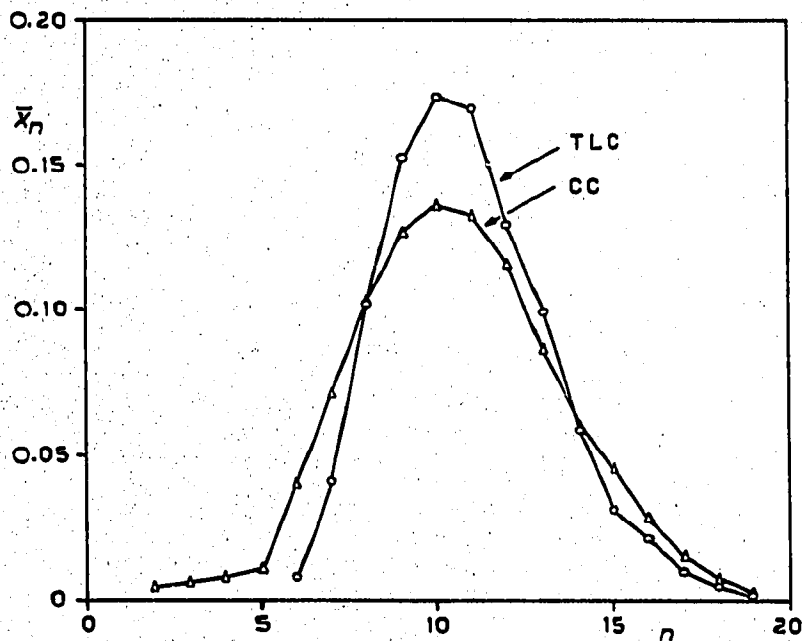


Fig. 8. Distribution of the degree of polymerisation in PEG derivatives with  $\bar{n} = 12.2$ , as obtained by CC and by TLC.  $\bar{x}_n$  is the molar fraction (cf. Table III).



of high- $n$  compounds, seen in the three distribution curves calculated with  $\bar{x}_{10} = 1$  (cf. Fig. 7), is least pronounced with the silanised adsorbent and most pronounced with the maximally activated one. Though less precise, TLC has an accuracy comparable to that of CC, and it offers the means of rapid analysis of samples even smaller than 0.1 mg. Fig. 8 shows that the two distribution curves are of the same type, and the calculated  $\bar{n}$  values agree in the two cases, even though TLC does not respond sensitively enough to compounds with  $n < 6$ .

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