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DETERMINATION OF THE MOLECULAR-WEIGHT DISTRIBUTION OF POLYETHYLENE GLYCOLS BY THIN-LAYER CHROMATOGRAPHY COUPLED WITH DENSITOMETRY

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SUMMARY

Thin-layer chromatography with multiple one-dimensional elution was used to determine the molecular-weight distribution of polyethylene glycols of the general formula $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$ after conversion into their dichloro derivatives. The adsorbent was silica gel, the developer was based on a mixture of 2-butanone and water, and the spots were visualised with iodine vapour, and then evaluated with the aid of an optical densitometer. This method permitted the quantitative fractionation of polyethylene glycols with degrees of polymerisation (n) of up to 21.

INTRODUCTION

Chromatographic techniques have proved particularly useful for the determination of the molecular-weight distribution of polyethylene glycol (PEG) with the general formula $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$, where n is the degree of polymerisation. The molecular-weight distribution is an essential part of the characterisation of polyethylene glycol products, which are of growing industrial importance.

Temperature-programmed gas chromatography (GLC) of the trimethylsilyl derivatives permits the fractionation of the lower members extending up to $n = 18$ (refs. 1-3), but the results are accurate only for $n < 14$ (ref. 4).

The limitations of gas chromatography, which are imposed by the low volatility of the compounds, do not apply to liquid-solid chromatography, and, indeed, thin-layer chromatography (TLC) has given very good results in the fractionation of surface-active polyethylene glycols⁵⁻⁹. The present authors¹⁰ have recently used TLC in conjunction with the direct densitometric evaluation of the spots. The latter were visualised with iodine vapour, which has been recommended^{11,12} before in the qualitative detection. As n increases, the compound absorbs more iodine, and thus the photometric determination is progressively improved¹⁰.

In the thin-layer chromatographic analysis of polyethylene glycol derivatives, the adsorbent is activated silica gel and the eluent is a mixture based on 2-butanone and water. This system is highly selective for n , but cannot be used for the direct fractionation of polyethylene glycols. This is because the energy of adsorption of the

primary OH groups on the silica gel is high, and so the sample does not migrate ($R_F < 0.1$).

In the present work, the distribution of the molecular weights or — what amounts to the same thing — the distribution of the degrees of polymerisation was determined after conversion of the polyethylene glycols into the dichloro derivative $\text{Cl}(\text{CH}_2\text{CH}_2\text{O})_{n-1}\text{CH}_2\text{CH}_2\text{Cl}$ (n is invariably the degree of polymerisation of the parent polyethylene glycol). This was carried out with the aid of thin-layer chromatography and by the direct photometric evaluation of the spots, which were visualised by means of iodine vapour. Use was also made of multiple one-dimensional elution, whereby one can separate high- n and low- n members on the same plate, at least within the peak capacity of the chosen migration distance. The advantages of this technique have been discussed before in detail, together with its theoretical and practical aspects^{13, 14}.

EXPERIMENTAL

The four commercial polyethylene glycol samples used had the following stated mean degrees of polymerisation: $\bar{n} = 8.2, 13.2, 17.8$ and 22.3 .

We synthesised homogenous ethylene glycol oligomers with $n = 6, 7$, and 8 , and then prepared their dichloro derivatives. After repeated distillation of the latter under reduced pressure, the degrees of purity of the resulting three standards, determined gas chromatographically², were 99.0, 95.5, and 94.1 %, respectively.

Polyethylene glycol was chlorinated with thionyl chloride in pyridine¹⁵. To determine the amount of thionyl chloride (excess) needed to ensure a complete reaction, the chlorination of polyethylene glycol with $\bar{n} = 22.3$ was checked by IR spectroscopy, NMR spectroscopy, and TLC.

The chlorination was carried out as follows: the polyethylene glycol sample was dried for 1 h at 60° and 10 mm Hg, and 0.5 g of it were dissolved in 0.5 ml of warm anhydrous pyridine in a 10-ml test-tube. The solution was cooled to 30° , and 0.2 ml of thionyl chloride were added dropwise over a period of 1/2 h, with continuous agitation. A further 0.4-ml portion of thionyl chloride was then added quickly, and the reaction mixture was heated for 3 h at $80 \pm 2^\circ$. The mixture was first extracted with at least eight 10-ml portions of diethyl ether, then taken up in 5–10 ml of water, and the resulting aqueous solution was extracted with two 5-ml portions of ether. The ether extracts were combined and distilled first at atmospheric pressure and then at 10 mm Hg for 1 h at 60° . This gave the crude dichloro derivative, which was used without further purification.

The plates were prepared by applying Silica Gel G 20–40 μ (Merck) to optical glass supports (20×2.5 cm) by means of a spreader with a slit fixed at 200 μ . The silica gel was first made into an aqueous slurry as recommended by the manufacturers. At the wavelength at which the optical density was determined, the absorbance of the resulting plates was about 2.0. To eliminate any bubbles from the silica gel layer, the plates were activated by raising the temperature to $180 \pm 5^\circ$ over the course of 1 h, and by keeping them at this temperature for 1/2 h.

The sample was made into a 2 % w/v solution in chloroform, and this solution was applied to the plate with a 10- μ l Hamilton microsyringe, so as to form a spot not bigger than 3 mm in diameter. The eluents were chromatographic solvents RS (Carlo Erba, Milan). The plates were developed to a distance of 17.2 cm by the ascending

technique in cylindrical Desaga tanks (20 × 5 cm) having a vapour-saturated atmosphere. The spots were visualised in similar tanks, which were placed in a horizontal position, and whose atmosphere was saturated with iodine vapour by placing a few iodine crystals at the bottom. The best contrast of the spots was obtained by first drying the plates in air for 1/2 h, and then exposing them to the iodine vapour at 20° for at least 1/2 h. When the spots had been visualised the plate was immediately covered by another glass plate of the same size, and the edges were sealed with adhesive tape to minimise the losses of iodine by evaporation.

The plates were then subjected to photometric evaluation with the aid of a Chromoscan Joyce Loebel apparatus, set for measuring the transmission. The plates were scanned along the direction of the migration. The measurements were made at

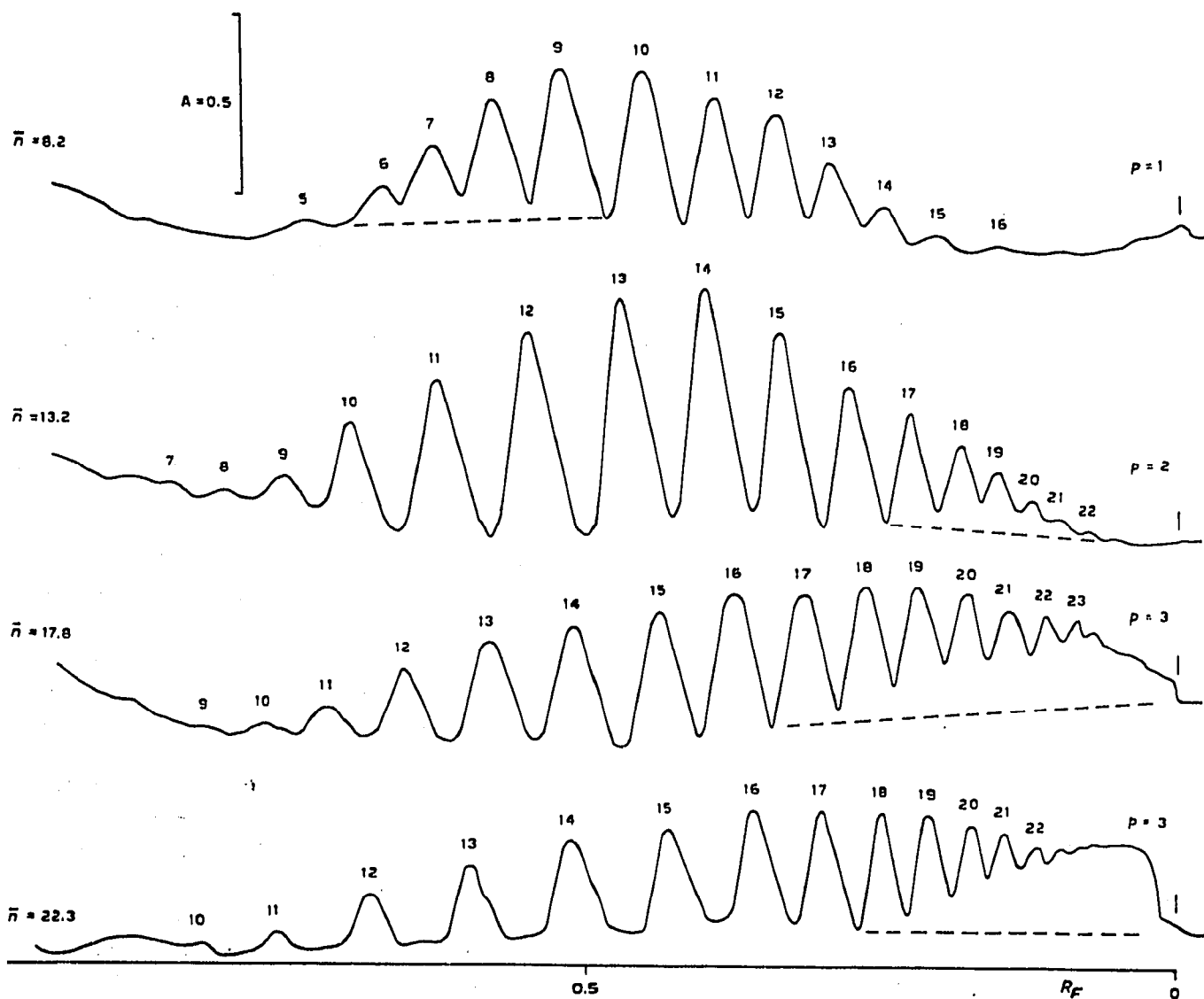


Fig. 1. Thin-layer chromatograms of the dichloro derivatives of polyethylene glycols, eluted at 20° with a 90:10 mixture of 2-butanone and water (upper curve), and with an 87.0:9.7:1.4:1.9 v/v mixture of 2-butanone, water, chloroform, and methanol (ratio before mixing) (other curves). Amount of sample applied to the plates: 15 μ g (upper curve), and 20 μ g (other curves). \bar{n} is the degree of polymerisation of the parent polyethylene glycol.

a wavelength of 490 nm (green filter) and at slits of 5, 6, 7 \times 1 mm. The response of the recorder was linearised for absorbances in the range of 0-1, and hence it was possible to determine the total absorbance of a peak from the area, which was found planimetrically.

The spectrophotometric determinations were carried out with a Perkin-Elmer Infracord apparatus, and the NMR spectra were obtained by means of a Jeol C-60 HL apparatus.

RESULTS AND DISCUSSION

Experiments with the three standards have shown that the total absorbance corresponding to the peaks varies linearly with the amount of solute placed on the sample (at least in the range 1-10 nmole). Furthermore, the mean square deviation of the gradient of the calibration curve is of the same order of magnitude as the error involved in the use of the microsyringe¹⁰. The linearity also holds for spots eluted over different migration distances, but the gradient varies from plate to plate, because the visualising conditions cannot be fixed in a fully reproducible manner. It has also been found that, when the three standards are eluted on the same plate, the recovery varies linearly with the value of n (the recovery is the ratio between the integrated absorbance, expressed in area units, and the amount of the sample in nmoles).

The chromatograms in Fig. 1 were obtained by eluting the sample up to three times with the same eluent and to the same distance. Although further repetition of the elution — we tried up to five elutions — slightly improves the resolution, the photometric homogeneity of the plates tends to deteriorate progressively after the third elution.

The polyethylene glycol samples with $\bar{n} = 8.2$ and 13.2 are seen to have been almost fully resolved. Even for some incompletely separated head and tail members, the separation factor is such as to permit their resolution by graphical means.

As regards polyethylene glycol samples with higher \bar{n} values, three elutions give

TABLE I

R_F VALUES OBTAINED AFTER SINGLE, DOUBLE, AND TRIPLE ELUTION WITH THE SAME ELUENT AND TO THE SAME DISTANCE AT 20°.

p is the number of elutions. The sample was the dichloro derivative of polyethylene glycol with $\bar{n} = 22.3$. (n is the degree of polymerisation of the parent polyethylene glycol.) The eluent was an 87.0:9.7:1.9:1.4 v/v mixture of 2-butanone, water, methanol, and chloroform (ratio before mixing).

n	$(R_F)_p$					
	$p = 1$		$p = 2$		$p = 3$	
	Obs.		Obs.	Calc.	Obs.	Calc.
10	0.46		0.70	0.69	0.82	0.84
11	0.39		0.64	0.63	0.76	0.77
12	0.33		0.56	0.55	0.70	0.70
13	0.27		0.47	0.47	0.62	0.61
14	0.23		0.40	0.41	0.53	0.54
15	0.19		0.32	0.34	0.46	0.47
16	0.15		0.26	0.28	0.37	0.38
17	0.13		0.22	0.24	0.31	0.34
18	0.11		0.18	0.21	0.25	0.29

a practically complete separation of the pair with $n = 17$ and $n = 18$, and qualitative identification is possible up to $n = 22-23$.

The $(R_F)_p$ values observed after p successive elutions are in good agreement with the values calculated as follows from $(R_F)_1$, obtained after the first elution:

$$(R_F)_p = 1 - [1 - (R_F)_1]^p$$

Table I shows the observed and the calculated $(R_F)_p$ values for successive elutions of the dichloro derivative of the polyethylene glycol sample with $\bar{n} = 22.3$.

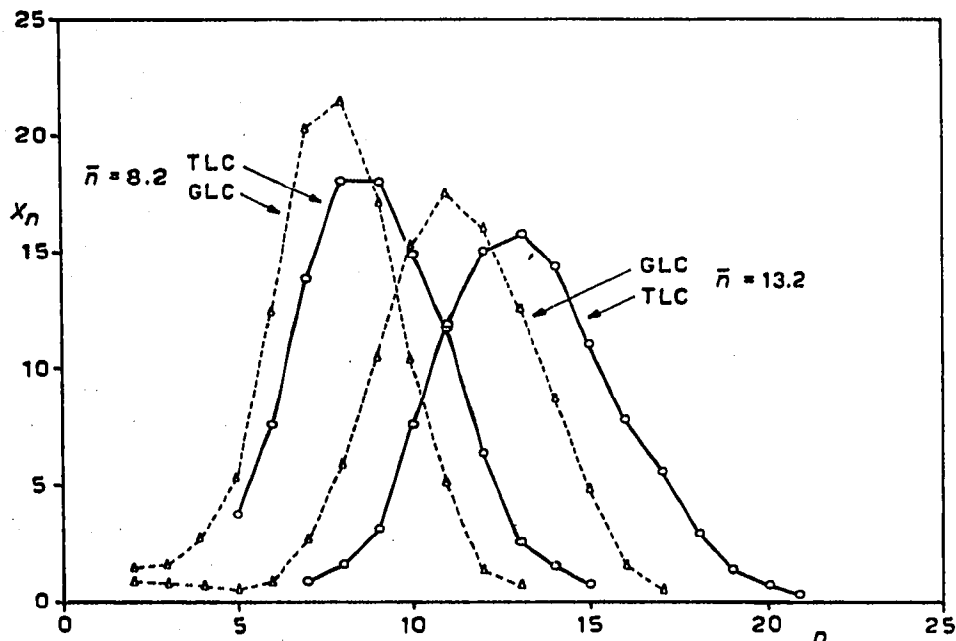


Fig. 2. Degree of polymerisation distribution as determined by TLC (full-lines) and by GLC (broken-lines) for the dichloro derivatives of polyethylene glycols $[\text{Cl}(\text{CH}_2\text{CH}_2\text{O})_{n-1}\text{CH}_2\text{CH}_2\text{Cl}]$. n is the degree of polymerisation of the parent polyethylene glycol; \bar{n} is its mean degree of polymerisation; and x_n is the average molar fraction in % (cf. Table II).

In the analysis of polyethylene glycol samples with $\bar{n} = 8.2$ and 13.2 , there is a systematic discrepancy between the molecular-weight distribution found by TLC and that found² by GLC (cf. Table II and Fig. 2). This discrepancy increases with the mean molecular weight of the sample, as can also be seen by comparing the numerical average molecular weights. One reason for this lies in the fact that, for compounds with $n > 14$, the gas chromatographic analysis of the trimethylsilyl derivatives gives values that drop below the correct figures more and more as n increases. The other reason is that, in thin-layer chromatography, the affinity to iodine and the stability of the colour are lower for components with low n values. The influence of these two factors is clearly seen from the data in Table III, which shows the molar fractions as obtained by TLC and GLC for adjacent oligomer pairs (chosen for similar distributions so as to minimise the accidental error). The best agreement is found for the pair with $n = 12$ and 13 . The reproducibility of the thin-layer chromatographic data is about 5-8%, expressed as the mean square deviation from the average molar fractions.

Although the preparation of the derivatives is laborious, the present method scores over GLC by giving more accurate results for the molecular-weight distribution of polyethylene glycols with $n > 14$ –15. The resolution of the dichloro derivatives is

TABLE II

DEGREE OF POLYMERISATION DISTRIBUTION OF POLYETHYLENE GLYCOL, AS FOUND BY TLC AND GLC
 \bar{n} (calc.) is the numerical-average degree of polymerisation, calculated from the distribution;
 \bar{x}_n is the average molar fraction (%); s_n is the attendant mean square deviation found in five determinations; and n is the degree of polymerisation of the parent polyethylene glycol.

n	$\bar{n} = 8.2$		$\bar{n} = 13.2$	
	TLC (dichloro deriv.) $\bar{x}_n \pm s_n$	GLC (TMS deriv.) x_n	TLC (dichloro deriv.) $\bar{x}_n \pm s_n$	GLC (TMS deriv.) x_n
2	—	1.4	—	0.9
3	—	1.5	—	0.7
4	—	2.8	—	0.6
5	3.8 ± 1.3	5.3	—	0.5
6	7.5 ± 3.1	12.5	—	0.9
7	14.0 ± 2.3	20.2	0.9 ± 0.3	2.7
8	18.1 ± 2.9	21.6	1.5 ± 0.2	6.0
9	18.1 ± 1.9	17.1	3.1 ± 0.7	10.5
10	15.0 ± 1.9	10.4	7.8 ± 1.2	15.3
11	11.8 ± 2.0	5.2	11.9 ± 1.1	17.6
12	6.7 ± 1.7	1.3	15.1 ± 1.4	16.0
13	2.6 ± 0.4	0.7	15.8 ± 0.6	12.5
14	1.7 ± 0.8	—	14.4 ± 0.8	8.8
15	0.7 ± 0.2	—	11.0 ± 1.2	4.9
16	traces	—	7.8 ± 1.3	1.6
17	—	—	5.5 ± 0.6	0.5
18	—	—	2.8 ± 0.4	—
19	—	—	1.4 ± 0.3	—
20	—	—	0.7 ± 0.2	—
21	—	—	0.3 ± 0.2	—
22	—	—	traces	—
\bar{n} (calc.)	8.98	7.74	13.24	11.08

TABLE III

DISTRIBUTION OF ADJACENT OLIGOMER PAIRS, AS FOUND BY TLC AND GLC

\bar{x}_n is the average molar fraction (%); s_n is the attendant mean square deviation found in three determinations; and n is the degree of polymerisation of the parent polyethylene glycol.

Starting PEG	n	TLC (dichloro deriv.) $\bar{x}_n \pm s_n$	GLC (TMS deriv.) x_n
$\bar{n} = 8.2$	7	43.0 ± 6.8	48.3
	8	57.1 ± 7.2	51.7
$\bar{n} = 13.2$	12	50.5 ± 4.8	52.4
	13	49.5 ± 5.2	47.6
	16	58.8 ± 7.6	74.5
	17	41.8 ± 8.1	25.5

no longer quantitative beyond about $n = 20$, but this limit may be raised with other derivatives, prepared by substituting the OH groups with non-polar groups, and particularly with hydrophobic groups. Work in this direction is being carried out in our Department.

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