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Fractionation of polyethylene glycol by thin-layer chromatography* Studies on the choice of derivatives and eluent systems

In the determination of the molecular-weight distribution of polyethylene glycols (PEG), $\text{HO}\cdot(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$, by adsorption chromatography on silica gel, the conversion of the terminal hydroxyl groups into hydrophobic groups reduces the adsorption energy of the polymers and thus allows the separation of members with a higher degree of polymerization (n). The conversion of PEG into the dichloro derivatives has already been used for the subsequent fractionation of mixtures with values of n up to 20 by thin-layer chromatography (TLC) combined with multiple elution¹.

This paper describes recent work on selecting the best derivative and the best eluent for the fractionation of PEG by TLC, and the analytical advantages of the best choice are discussed.

The derivatives tested were $\text{RS}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{SR}$, where R = phenyl, n -hexyl, n -octyl and n -decyl; and $\text{Cl}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{Cl}$ and $\text{RO}(\text{CH}_2\text{CH}_2\text{O})_n\text{R}$, where R = phenyl and triphenylmethyl (trityl).

The derivatives were compared by estimating the free energy of adsorption of the $\text{CH}_2\text{CH}_2\text{O}$ group and the other hydrophobic groups from the R_F values by Martin's equation². Such calculations have recently been applied in the chromatographic study of non-ionic polyoxyethylene surfactants^{3,4}.

The eluents were the C_3 - C_7 2- n -alkanones and their aqueous mixtures, and in particular 2-butanone-rich mixtures of 2-butanone, acetone and water.

Experimental

Methods reported in the literature⁵ were used to prepare the derivatives of a commercial polydisperse PEG (Chemische Werke Hüls), having a number-average degree of polymerization $\bar{n} = 13.2$. To prepare the ditrityl derivative⁶, 4 mmoles of this glycol were dried at 80° and 0.2 mm Hg, dissolved in 20 ml of anhydrous pyridine and mixed with 8.8 mmoles (10% excess) of certified Fisher triphenylchloromethane under a stream of nitrogen. The mixture was kept for 50 h at 50° and then heated for 1 h on a steam-bath. Water (100 ml) was added, the mixture was extracted with three 20-ml volumes of diethyl ether, and the combined ether phase was washed with two 10-ml volumes of water. The solvent was evaporated and the residue was heated at 60° and 0.2 mm Hg to eliminate traces of pyridine. The resulting crude product was used in the form of its 2% (w/v) solution in chloroform.

Homogeneous oligomers, prepared from hexaethylene glycol, were used as standards; the purities of these were determined by gas-liquid chromatography (GLC) and TLC (cf. Table I).

The R_F values, which varied between 0.12 and 0.70, were determined by eluting at 23° not more than 15 μg of the sample on 200- μ layers of 10-40- μ Merck Silica Gel G by the ascending technique to a height of 17.0 cm after equilibrating the plates with the vapour-saturated atmosphere of the developing tank. The spots

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TABLE I
 DATA FOR THE PEG DERIVATIVES
 The R_M values were obtained at 23°.

Compounds	Standard compound		$R_M = an - b$		F_{EO} (cal mole ⁻¹)	F (cal mole ⁻¹)	
	n	Purity (% peak area)	a	b			
RS(CH ₂ CH ₂ O) _n CH ₂ CH ₂ SR, R =	n -hexyl	5	96.2 (GLC)	0.098	1.04	133	-1060
	n -octyl	5	95.0 (GLC)	0.101	1.16	137	-1220
	n -decyl	5	92.0 (GLC)	0.102	1.22	138	-1300
	phenyl	5	99.9 (GLC)	0.98	0.94	132	- 920
Cl(CH ₂ CH ₂ O) _n CH ₂ CH ₂ Cl	5	99.0 (GLC)	0.105	0.95	142	- 940	
RO(CH ₂ CH ₂ O) _n R, R =	phenyl	6	98.8 (GLC)	0.106	1.10	144	-1140
	triphenylmethyl	6	88 (TLC)	0.096	1.27	130	-1380

were visualized with iodine vapour, and then evaluated with a photometer (Joyce-Loebl Chromoscan), the transmission being measured^{1,7}.

The pure homogeneous polymers were used as internal and external standards in determining the degree of polymerization of the samples.

Choice of derivative

Fig. 1 shows a linear relationship between n and $R_M = \log[(I/R_F) - 1]$ for the case of elution at 23° with 2-butanone saturated with water. Table I gives the values of the constants a and b for the straight line $R_M = an - b$, which were calculated by the method of least squares.

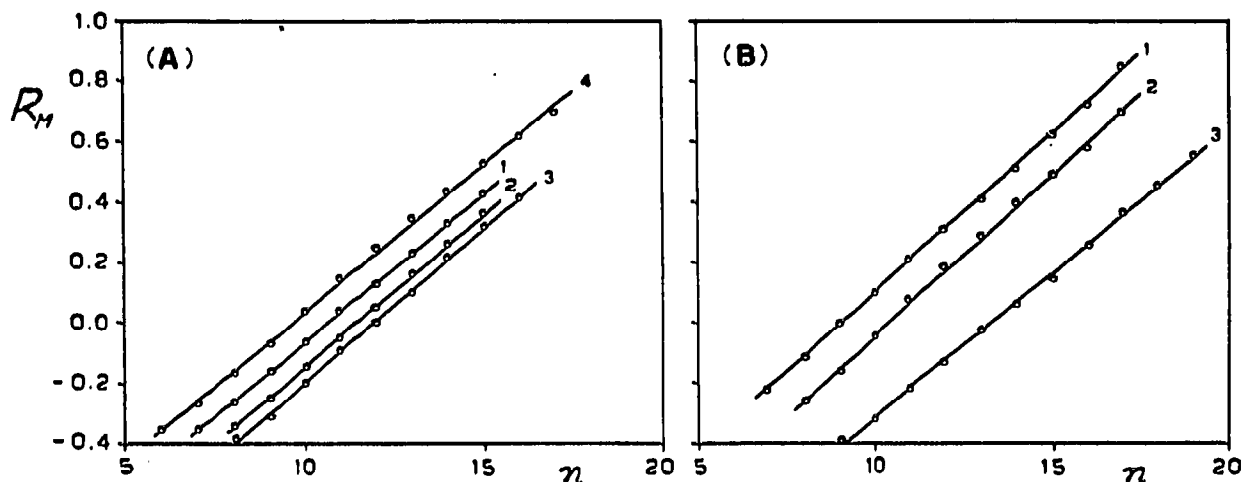


Fig. 1. Relationship between R_M and the degree of polymerization (n) for some PEG derivatives: (A), $RS(CH_2CH_2O)_nSR$, where $R = n$ -hexyl (curve 1), n -octyl (curve 2), n -decyl (curve 3) and phenyl (curve 4); (B), $Cl(CH_2CH_2O)_nCH_2CH_2Cl$ (curve 1) and $RO(CH_2CH_2O)_nR$, where $R =$ phenyl (curve 2) and trityl (curve 3). The interpolated lines were obtained by the method of least squares.

For every elution, the experimental points deviate little from the interpolated straight line, for the mean-square deviation of the slope a is about 2×10^{-4} and that of the intercept b is about 2×10^{-2} . For a given type of compound, the intercept varies from elution to elution with a mean-square deviation of about 3×10^{-2} .

The following relationship holds for a reversible process²:

$$R_M = (\Delta F_0 + n\Delta F_{EO}) / (2.302 RT) + \log(I/\rho)$$

where ΔF_{EO} and ΔF_0 are the changes in the free molar energy of adsorption for a CH_2CH_2O group and for the rest of the molecule, respectively, R is the universal gas constant ($1.9872 \text{ cal mole}^{-1} \text{ deg}^{-1}$), T is the absolute temperature (296°K), and ρ is the layer constant (1.80), determined experimentally. In these approximate calculations, ρ was assumed to remain constant over the range of R_F values considered.

The calculated ΔF values are listed in Table I. The free energy of adsorption for a CH_2CH_2O group is about 130 – $140 \text{ cal mole}^{-1}$, *i.e.* close to the values found by HAYANO *et al.*^{3,4} for the polyoxyethylene derivatives of nonylphenol and fatty alcohols with the same chromatographic system at 20° (144 – $159 \text{ cal mole}^{-1}$).

As the hydrophobic character of the terminal groups is increased, ΔF_0 decreases, and thus more members can be eluted from the starting line. The ditrityl derivative

is the best from this point of view, permitting the separation of the compound with $n = 22$ ($R_F \approx 0.12$) in a single elution.

Choice of the eluent system

The eluents were evaluated on the basis of the slope $a = \Delta R_M / \Delta n$ of the graph of R_M vs. n and on the basis of the value of $n_0 = b/a$, corresponding to $R_M = 0$ (the slope was found by regression). The value of n_0 is a measure of the resolving power of the eluent; in the fractionation of polyoxyethylenes by TLC, the members that are present in larger amounts should have a value of R_M of almost zero for maximum resolution. Derivatives with $R =$ trityl were used in these experiments.

Fig. 2 shows the variations of the slope a and of n_0 with the number of carbon atoms in the 2- n -alkanone used as the eluent. The slope is seen to increase with increasing carbon number, while n_0 decreases, reaching a limiting value of 6-7. Among the ketones tested, 2-butanone proved to be the best, as it permits satisfactory separation of polymers with $n_0 \approx 9-10$.

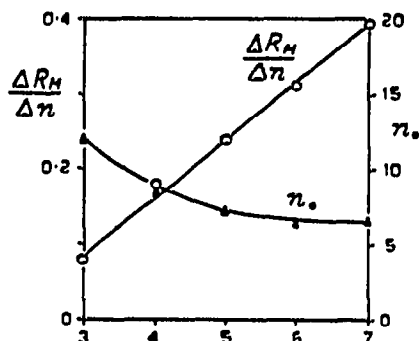


Fig. 2 Variation of the slope $a = \Delta R_M / \Delta n$ and of n_0 for the trityl derivatives of PEG with the number of carbon atoms (3-7) in the 2- n -alkanones used as eluents at 23°.

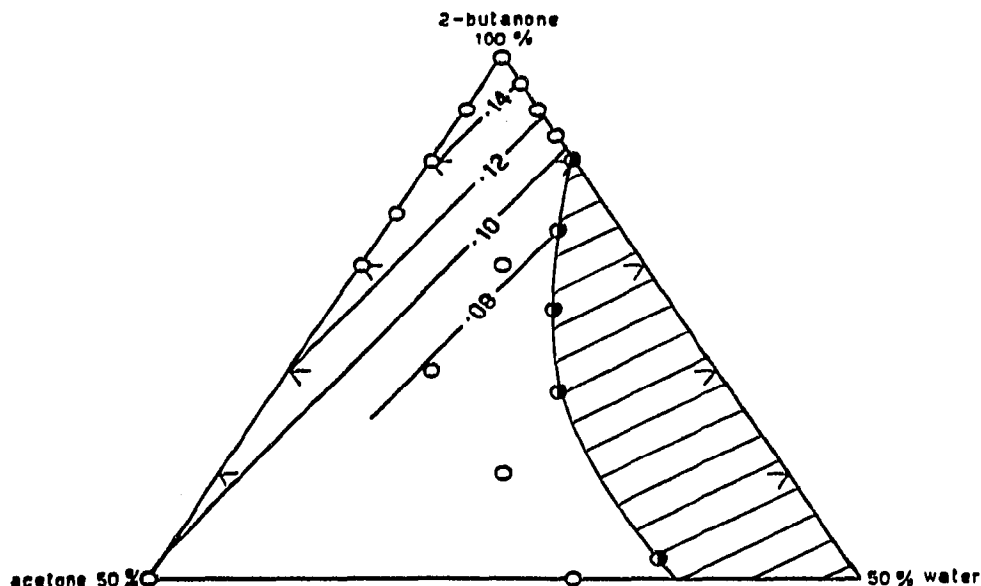


Fig. 3. Variation of the slope $a = \Delta R_M / \Delta n$ with the eluent composition for the trityl derivatives of PEG at 23° for ternary mixtures of 2-butanone, acetone and water. The figure shows only ternary systems containing $\geq 50\%$ of 2-butanone. The composition of the eluents at 23° is expressed in vol. % before mixing. The composition of the mixtures at the limit of the miscibility gap was found by the addition of water until a turbidity appeared; these mixtures are represented by the symbol \bullet .

Fig. 3 illustrates the variation of a with the eluent composition at 23° in the case of ternary mixtures of 2-butanone, acetone and water rich in 2-butanone. The addition of water to 2-butanone markedly improves this eluent, for it reduces the slope to a minimum of about 0.100 (in the case of 2-butanone saturated with water), while increasing n_0 to 13. A further increase in the water content by the addition of acetone does not offer any appreciable advantage. This is because under the conditions used, a minimum slope of about 0.100 is required for the separation of polymers to be complete or almost complete (at $R_M = 0$), and thus for the photometric estimation of the quantities to be possible. Slopes of up to about 0.080 can still be used for qualitatively identifying the members in the polydispersion, but below this value compounds with higher degrees of polymerization tend to accumulate, and the polydispersion migrates as a single unresolved spot. This undesirable effect of a further addition of water is accompanied by the appearance of two solvent fronts.

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

L. FAVRETTO
L. FAVRETTO GABRIELLI
G. PERTOLDI MARLETTA

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