Journal of Chromatography, 91 (1974) 237–245

CHROM. 7143

EFFECT OF SOLVENT ON THE SEPARATION OF OLIGOMER POLYETHERS ON SEPHADEX LH-20

D BEREK and D. BAKOŠ
Polymer Institute, Slovak Academy of Sciences, 809 34 Bratislava (Czechoslovakia)

SUMMARY

The selectivity of gel chromatographic separation of oligomers and low-molecular-weight compounds can be remarkably increased by the appropriate application of sorption effects. Effective fractionation of oligomers is achieved in systems where only the end groups of molecules are sorbed strongly on the gel. The extent of sorption can be influenced by the choice of a suitable separation system. The method is demonstrated by the fractionation of polyether oligomers on Sephadex LH-20 in various solvents.

INTRODUCTION

In the last decade the methods of gel and sorption liquid chromatography have undergone a rapid development. While in the case of gel chromatography differences in the sizes of molecules passing through a column filled with porous carrier are utilized, sorption liquid chromatography is based on the differences in interaction of mixture components with the column filling.

In the ideal case, in gel chromatography an inert carrier is assumed and the results should depend on experimental variables (temperature, type of solvent, etc.) mainly to the extent that these affect dimensions and mobility of separated molecules. In practice, particularly in the separation of oligomers and low-molecular-weight compounds, interactions of dissolved particles with gel[1], with solvent molecules[2,3] and also with one another[4] play, in many cases, quite an important role and cause some side-effects. However, the reproducibility of the results is very good. The separation process itself is associated with the porous structure of the carrier and its extent is determined by pore volume in a given system. The increase in pore volume because of column size increase or recycling causes zone broadening, prolongs the analysis, and makes inordinate demands on the apparatus used[5,6].

On the other hand, in sorption liquid chromatography, the surface of the carrier plays an important role, the capacity (the amount of separable compounds) of a column of given size being substantially higher than in gel chromatography[7]. Several experimental factors affect sorption and, consequently, often optimum conditions have to be ascertained by preliminary experiments. In partition liquid chromato-

graphy instability of the stationary phase and bleeding of the columns sometimes play a negative role.

It is attractive to try to combine the advantages of a separation on the basis of different gel permeability and sorption on the carrier. Already Heitz and Kern[8] have shown the role of interactions in gel permeation chromatography (GPC) of low-molecular-weight substances and Brown[9] has quantitatively studied the partitioning in various gel chromatographic systems.

Sorption properties of the carrier may be influenced in different ways. We chose change of solvent for this purpose. Sephadex LH-20 dextran gel was used in this study, as several authors have observed pronounced adsorption of separated compounds[10–23] and solvent dependence[24] on this carrier. The results were applied in the fractionation of polyether oligomers and in their separation from some low-molecular-weight admixtures. We supposed that in a system where oligomer end groups (but not the rests of the molecule) are strongly adsorbed, the GPC separation mode would be preserved but the selectivity of a given column would increase, at least in the region of lower molecular weights.

EXPERIMENTAL

The measurements were carried out on the apparatus described in ref. 23. As solvent, methanol, tetrahydrofuran (THF), acetone, N,N-dimethylformamide (DMFA) or water was used. The gel was Sephadex LH-20 from Pharmacia (Uppsala, Sweden). Glass columns (I.D. 15 and 28 mm) were sealed at each end by PTFE pistons. The lengths of gel bed varied between 65 and 125 cm. 2 ml of solution were injected via a six-port injection valve. In all cases the elution rate was about 40 ml/ h. Preliminary experiments showed that the results were not essentially influenced by changes in temperature of $\pm$ 2° and the work was carried out with non-thermostatted columns. Solute concentration in the eluate was measured by a Waters Model R4 differential refractometer (Waters Ass., Framingham, Mass., U.S.A.).

Elution characteristics of several low-molecular-weight model compounds and oligomeric polyethers were measured. Polyethylene oxides (PEO) and polypropylene oxides (PPO) were from Union Carbide (New York, N.Y., U.S.A.), Hüls, Fluka (Buchs, Switzerland), and Chemical Works-CHZWP (Nováky, Czechoslovakia). PEO adducts of *p*-nonylphenol (let us denote them $NF(EO)_n$, where n is the number of ethylene oxide moles per mole of *p*-nonylphenol) as well as the lower members of the analogous series PEO, PPO, $NF(EO)_n$ were prepared at the Research Department of CHZWP Nováky[23,25]. Evaluating the results, we used mean values of molecular weights of oligomers indicated by manufacturers and checked by some authors[25–27] by means of vapour pressure osmometry and chemical methods. The equality of mean number molecular weight and GPC peak molecular weight was assumed for higher, polydisperse samples to a first approximation. Analytical-grade low-molecular-weight model compounds were used. The solvents were dried with sodium or $CaCl_2$, or on molecular sieves Nalsit 13 (Chemical Works-CHZID, Bratislava, Czechoslovakia), and distilled. Peroxides in THF were removed by shaking with a solution of iron(II) sulphate. As a bacteriostat 0.02 w/w% sodium azide was added to water.

RESULTS AND DISCUSSION

Basic variables considered in GPC are dimensions and elution volumes (V_e) of molecules. They are associated by the relation called calibration dependence, which is usually determined for the given system by means of model compounds (standards).

Since the mechanism of the process has so far not been elucidated, it is difficult to define the molecular dimensions controlling the separation. For macromolecules, the variables proportional to their hydrodynamic volume[28] and for low-molecular-weight compounds their molar volumes[29] are used as "universal" dimensional parameters. The hydrodynamic volume should be applied from the region of oligomer molecular weight, where the molecules in solution begin to possess the shape of statistical coils. However, the limits of such regions change from one system to another and, moreover, it is disadvantageous to work with two parameters. Therefore, for oligomers, it appears to be most simple to work with molecular weight instead of dimensional characteristics, even just to avoid complications in the comparison of results for various types of separated molecules. Comparison of elution volumes obtained in various systems is also complicated as V_e depends on the volume of solvent in which the chromatographic process takes place. This refers primarily to the volume of gel pores (V_i) and to the interstitial volume (V_o). Both these volumes are a function of column geometry as well as of external and internal gel structures. In the literature[22] are found several suggestions on how to solve this problem. In this work we used the partition coefficient, K_{av}[30], defined by the expression $K_{av} = (V_e - V_o)/(V_t - V_o)$, where V_t is the total volume of gel bed. For molecules excluded from gel pores, the coefficient K_{av} has the value 0, whereas its values > about 0.6–0.9, according to gel type, suggest the retardation of eluted molecules caused by some kind of interaction.

We will deal successively with the results obtained in individual solvents. The data obtained on low-molecular-weight model compounds will be treated in detail elsewhere[31]; here, we will refer to them only to the extent to which they are related to the elution behaviour of studied oligomers. The calibration dependences of log M_w on V_e have been constructed using chromatograms of polydisperse samples (*cf.* Fig. 1 or 8). The peaks of low-molecular-weight oligomers have been identified by means of corresponding pure model substances[23].

Methanol

In methanol many compounds are retarded. In particular, polynuclear aromatic hydrocarbons exhibit high elution volumes, which even increase with increasing molecular weight ("inversion"). In this case, there is a simultaneous effect of interaction of π-electrons of aromatics, particularly with ether groups of the gel[13], and of low solubility of aromatics in methanol, which causes their partition in favour of the gel phase. There is a considerable difference between the behaviours of aliphatic and aromatic hydroxyl groups. Phenols are eluted much later than alcohols and it is obvious that besides π-electrons the hydroxyl groups of phenols are also sorbed. Similarly, if at least one of the substituents in an ether possesses aromatic character, the retardation increases to a considerable extent. These results are similar to data using methanol[16] and isopropanol[12,18] and suggest high elution volumes for phenolic antioxidants and stabilizers based on benzophenone and the possibility of their separ-

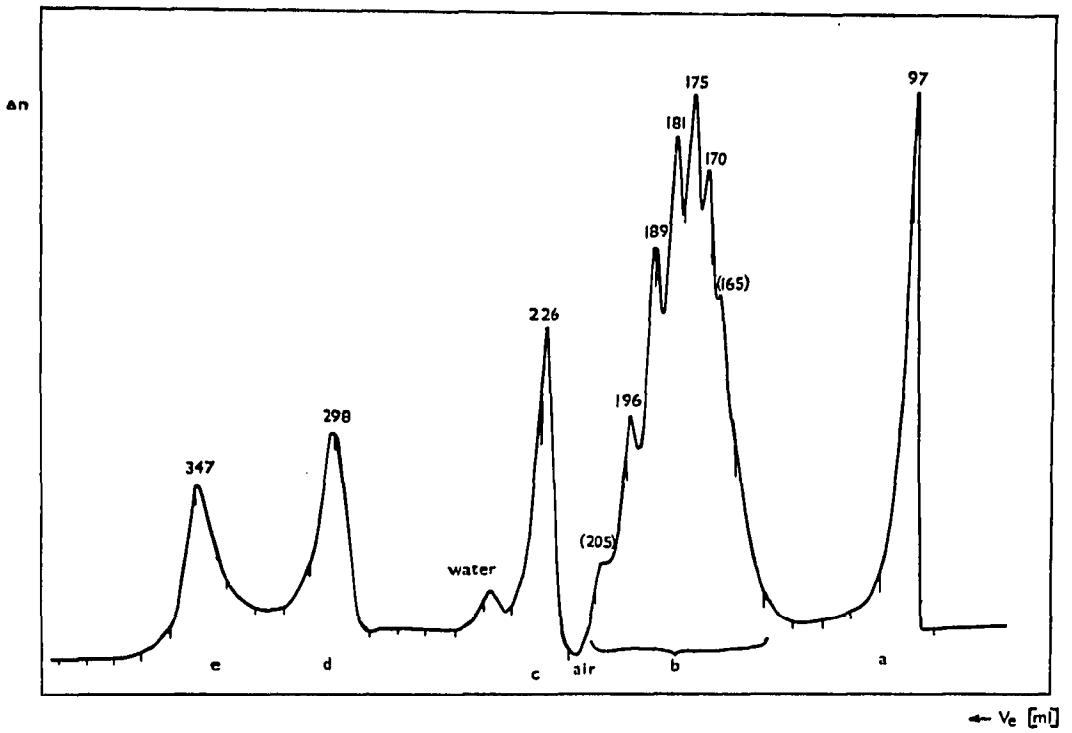

Fig. 1. Chromatogram of PEO ($\bar{M}$ = 4000, curve a; and $\bar{M}$ = 300, curve b), antioxidant: 2,6-di-*tert.*-butyl-4-methylphenol (curve c), 2-hydroxybenzophenone (curve d), and 2,4-dihydroxybenzophenone (curve e) on Sephadex LH-20 in methanol. Gel bed, 65 × 2.8 cm.

ation from mixtures with molecules of similar dimensions but exhibiting lower adsorptions[23] (Fig. 1).

Calibration dependences for the polyethers investigated, lower paraffins and primary alcohols are shown in Fig. 2. The differences in densities and consequently also in molar volumes of the lowest members of compared homologous series are relatively small. Thus the differences in corresponding elution volumes may primarily be attributed to interactions in the system. Low-molecular-weight alcohols are chemically similar to both the gel and the solvent, which accounts for their low elution volumes. On the other hand, the possible association of alcohol molecules either mutually or with eluent would also reduce the V_e values. Owing to their low solubility in methanol, paraffins may be expected to partition in favour of the gel and thus be retarded. In the case of polyethers, their retardation increases in the sequence PPO < PEO < NF(EO)$_n$, obviously due to their adsorption on the gel. The selectivity of Sephadex LH-20 in methanol for NF(EO)$_n$ derivatives is relatively high and enables good fractionation of polyadducts of this type[23].

N,N-Dimethylformamide

The sorption of aromatics in this system is low, but not negligible. Unlike Streuli[24], we also found inversion, *i.e.* an increase in V_e with increasing molecular weight. Phenolic hydroxyl groups cause only a slight retardation. Calibration curves

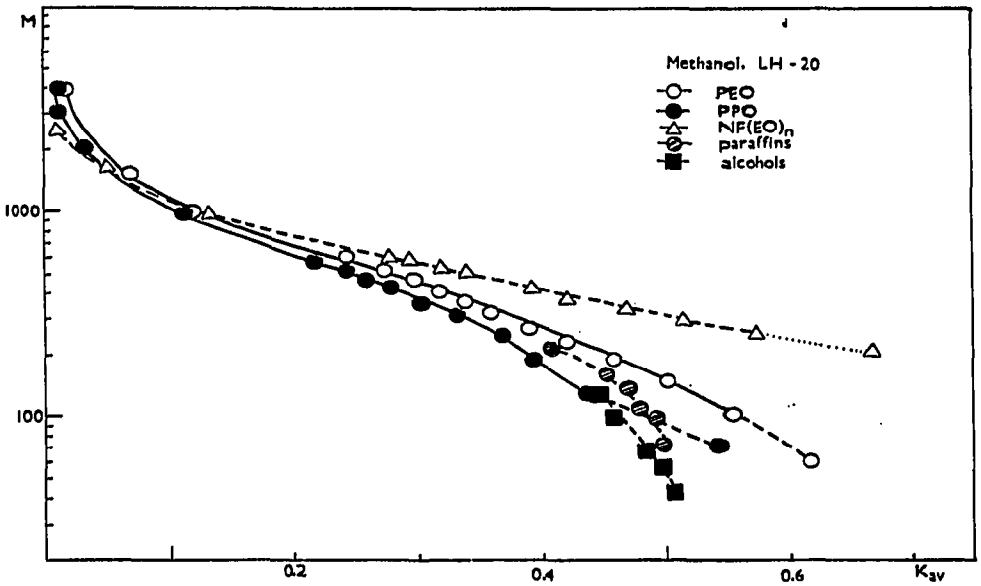

Fig. 2. Calibration curves (log M *vs.* K_{av}) for the system Sephadex LH-20/methanol.

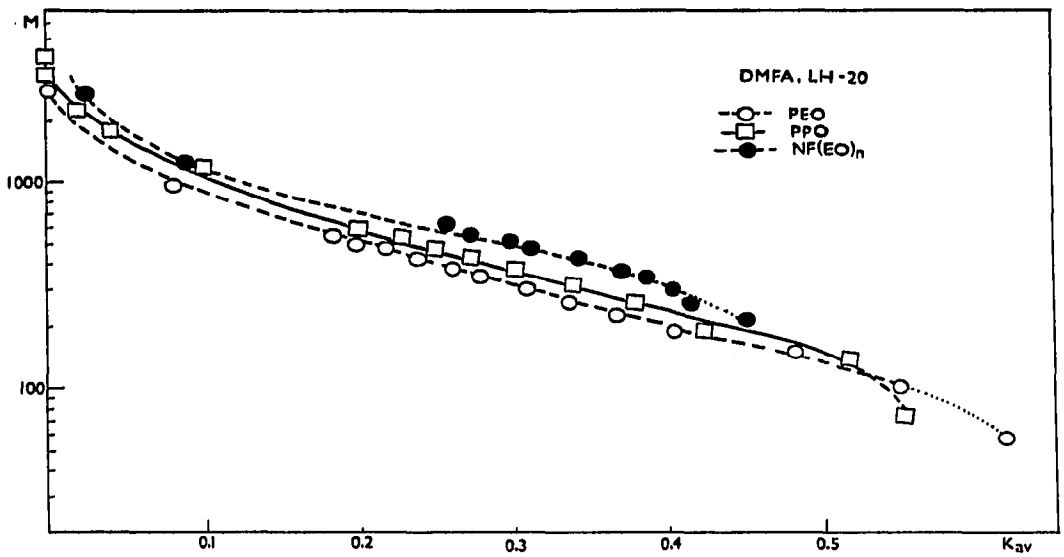

Fig. 3. Calibration curves for the system Sephadex LH-20/DMFA.

for polyethers are shown in Fig. 3. Obviously, the separation proceeds primarily according to dimensions of molecules and its selectivity is not very high.

Water

Some of the results are shown in Fig. 4. With the decreasing solubility of higher polypropylene glycols and alcohols in water, a partition in favour of gel phase takes place.

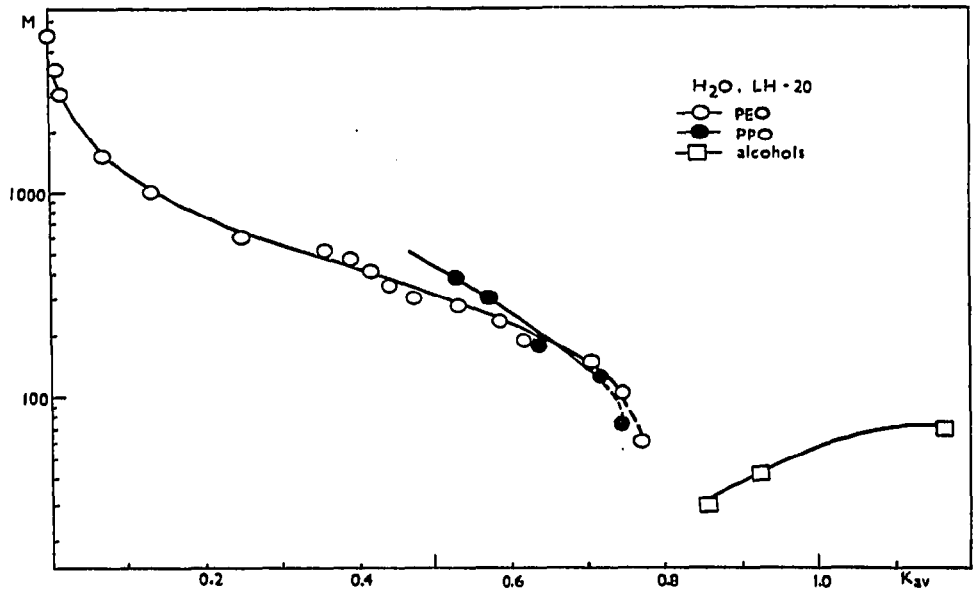

Fig. 4. Calibration curves for the system Sephadex LH-20/water.

Acetone

Adsorption of aromatic compounds in this system is relatively low but inversion is still present (Fig. 5). Retardation of alcohols and phenols is extremely high. Owing to the adsorption of hydroxyl end groups in PPO and PEO, there is a strong

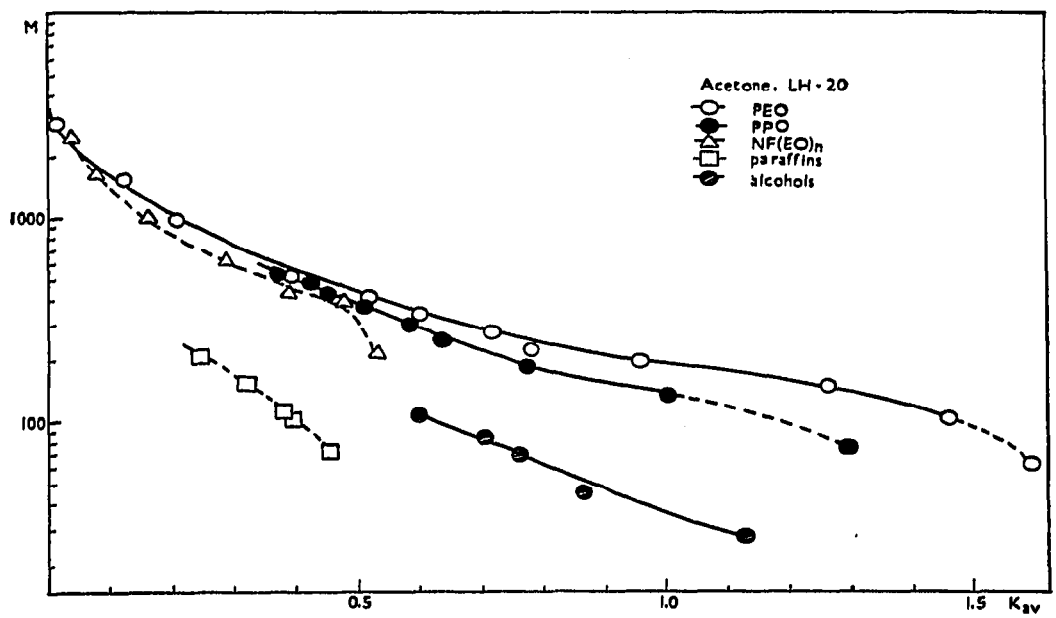

Fig. 5. Calibration curves for the system Sephadex LH-20/acetone.

increase in their fractionation selectivity. The partition of paraffins in favour of the gel phase is obvious from Fig. 5.

Tetrahydrofuran

The results obtained in THF are similar to those in acetone. However, the retardation of aromatics was less and no inversion was observed. The sorption of aliphatic and aromatic hydroxyl groups is very large, which leads to high separation selectivities for the corresponding substances (Fig. 6).

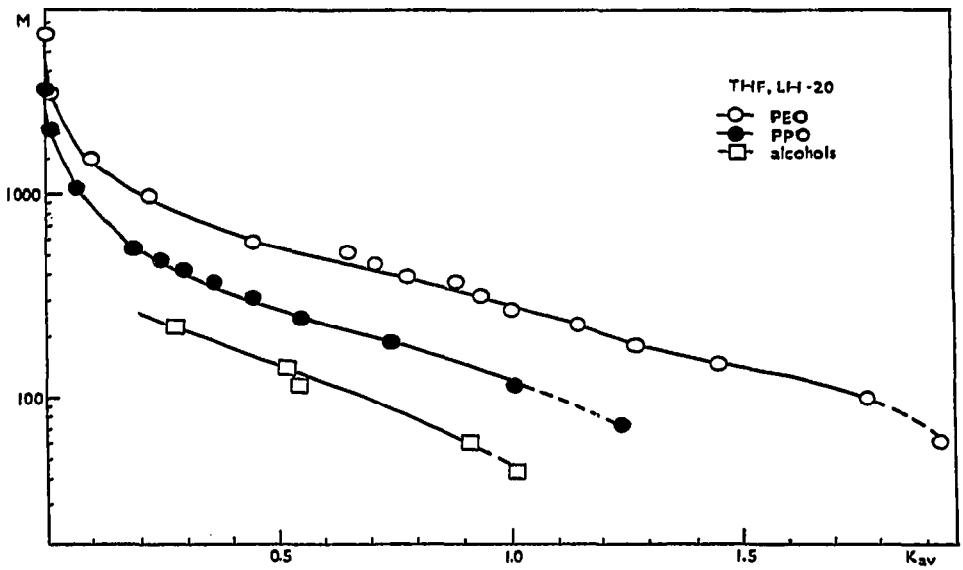

Fig. 6. Calibration curves for the system Sephadex LH-20/THF.

In conclusion, it may be stated that sorption of individual compounds on Sephadex LH-20 gel may be affected to a considerable extent by choice of solvent. Beside adsorption, it is necessary to consider in some cases also the partition of separated compounds in the system. The use of solvents enhancing the selective adsorption of end groups of an oligomer can remarkably increase the selectivity of its fractionation into individual homologous series members. This is shown in Fig. 7, where calibration curves for PEO in the solvents investigated are summarized. Of course, the selectivity of individual systems is to some extent given also by the value of molecular weight excluded from the gel, *i.e.* by the swelling ratio of Sephadex LH-20 in the given solvent. However, as is obvious from Fig. 7, the sorption effects have a decisive influence on the separation selectivity in many solvents. An example of fractionation in selective system is given in Fig. 8, where a chromatogram is shown of PPO with average molecular weight 315 separated on a gel bed of Sephadex LH-20, 65 × 2.8 cm, in THF. In this column, with about 3,000 theoretical plates (on benzene), a fractionation was achieved comparable with that obtained[5,6] on a set of forty 122-cm-long columns filled with polystyrene gel (separating over about the same range of molecular weights as Sephadex LH-20 in THF) with about 180,000 theoretical plates.

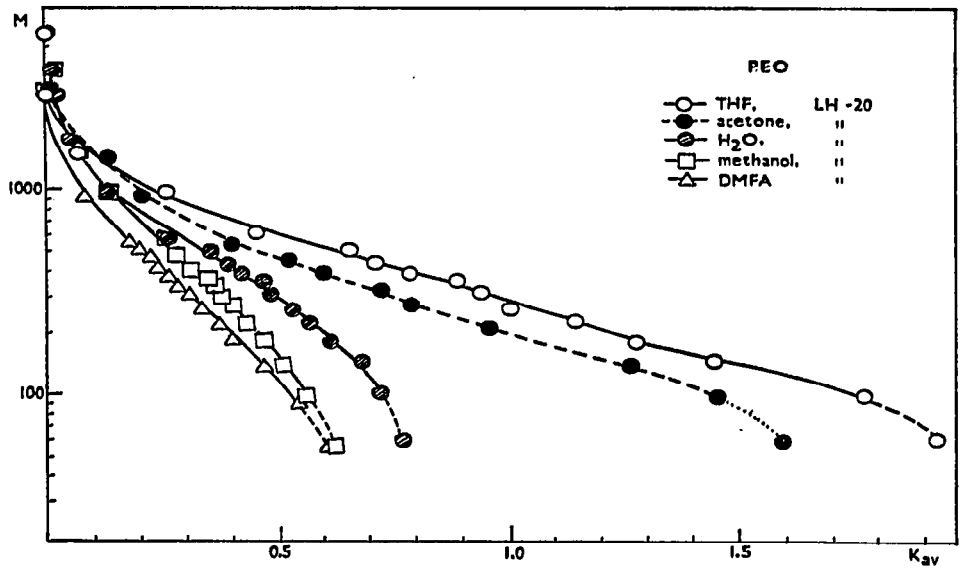

Fig. 7. Calibration curves for PEO on Sephadex LH-20 in various solvents.

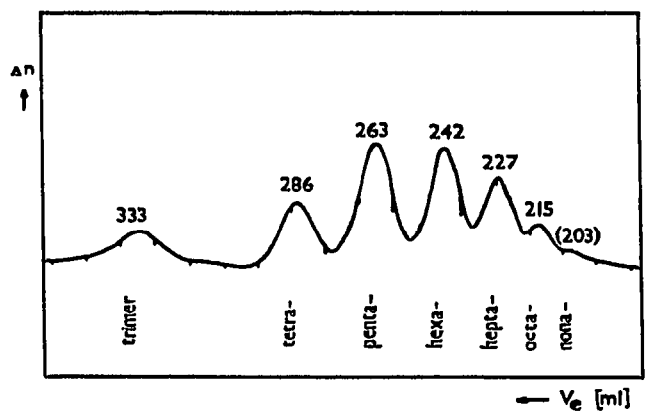

Fig. 8. Chromatogram of PPO, $\bar{M} = 315$, on Sephadex LH-20 in THF. Gel bed, 65 × 2.8 cm.

ACKNOWLEDGEMENTS

The authors thank Dr. J. C. Galin and Dr. Z. Gallot of the Centre de Recherches sur les Macromolécules, Strasbourg, for the samples of purified PEO and PPO and Dr. L. Novák of the Research Institute of CHZWP, Nováky, for samples of PEO, PPO and NF(EO)$_n$.

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