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GEL PERMEATION CHROMATOGRAPHY IN COILED COLUMNS

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SUMMARY

Whereas in gas chromatography the use of coiled columns is the usual procedure, in liquid chromatography the use of straight columns is favoured.

Bent columns give an additional contribution to peak spreading which is of greater importance in liquid chromatography than in gas chromatography owing to smaller diffusion coefficients. The low linear velocities of the mobile phase used in gel permeation chromatography enable conditions to be found where the additional contribution of bending to peak spreading is small.

In the separation of oligomers, columns of 2-mm tube diameter and 20-cm coil diameter were used. With total lengths of 10 and 20 m, theoretical plate counts of *ca.* 70,000 and 150,000, respectively, could be obtained. The experimental conditions of column filling, etc., are discussed.

Examples are shown to demonstrate the potentialities of this technique. The main advantage is the low solvent requirement and the practicability. As predicted by theory, there is no advantage with respect to analysis time.

INTRODUCTION

In gel permeation chromatography (GPC), straight columns are used with a usual length of 1–2 m. If a greater column length is needed, several of these columns are combined by using sharply bent capillaries. The use of coiled columns should greatly reduce the separation efficiency in GPC, owing to the extremely small diffusion coefficients of macromolecules. This is probably the reason why coiled columns have not found frequent use in liquid chromatography (LC) nor especially in GPC. In this paper, we aim to show the potentialities and limitations of the use of coiled columns in GPC.

Advantages can be expected from ease of handling, owing to the small size of the column and the limited requirements of solvent and sample. The use of coiled columns should therefore be advantageous when expensive solvents are used, *e.g.*, fluorinated solvents. A disadvantage is that large sample sizes cannot be used as the column diameter in coiled GPC columns should be small.

In GPC, the volume in which the different peaks appear in the eluent is always smaller than the total volume of the column, and most efforts to increase the separation efficiency are therefore directed towards lowering the extent of peak spreading. Peak spreading is caused by different distortion effects, the variances of which and H values are additive.

According to Giddings[1], the additional contribution, H_c, due to coiling is

$$H_c = \frac{7 v_0 r_0^4}{48 R_0^2 D}$$

with v_0 = linear velocity; r_0 = column radius; R_0 = coiling radius; and D = diffusion coefficient.

Using a column of 1 mm radius and a coiling radius of 10 cm, the additional contribution due to coiling is calculated assuming a diffusion coefficient of 10^{-5} or

TABLE I

PEAK SPREADING IN COILED COLUMNS ACCORDING TO GIDDINGS[1]

Linear velocity, $v_0 = 0.007$ cm sec^{-1}; column radius, $r_0 = 0.1$ cm; coiling radius, $R_0 = 10$ cm; diffusion coefficient, D.

D (cm^2 sec^{-1})	H_c (cm)	H_t (cm)
10^{-5}	10^{-4}	10^{-2}
10^{-7}	10^{-2}	10^{-1}

10^{-7} in Table I. A diffusion coefficient of 10^{-5} corresponds to oligomers with molecular weights in the range of several hundreds, and 10^{-7} is obtained with molecular weights of about 10^6. The figures show that with oligomers, the additional contribution due to coiling is negligible if an elution time of about 5 h per metre of column length is used. The effect is in the region of 1%. With polymers, the effect is much larger, amounting to about 10% of the plate height of a straight column. As this contribution is very sensitive to the column radius, a further decrease in column radius seems to be of advantage for polymers.

EXPERIMENTAL

Experiments were carried out by using columns with the dimensions given in Table I and 10 m in length. The columns are made of PTFE tubing (wall thickness 1 mm) and easily withstand pressures of 50 atm. The gel must have a narrow particle size distribution, otherwise the flow resistance is too high, resulting in the total breakdown of the solvent flow. The mean diameter of the particles used was 40 μm and more than 95% of them had a deviation of less than 10% from the mean value. These narrow fractions of particle sizes were obtained by sedimentation[2].

The procedure of column packing is illustrated in Fig. 1. The solvent is pumped into a metal cylinder, which contains a slurry of the gel. The outlet has a conical shape and is directly connected to the column end with a Hermeto fitting. The gel is transported by the solvent into the column. The solvent velocity during the packing procedure should not be much higher than the velocity during the use of the column. The

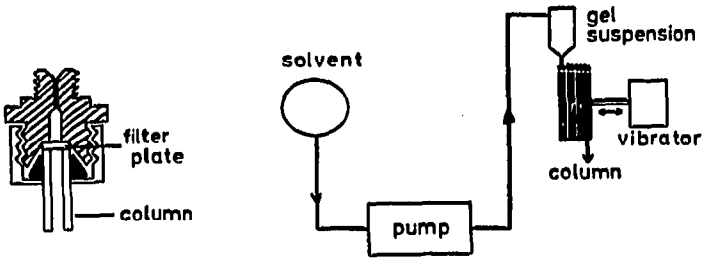


Fig. 1. Packing procedure and the end fitting of the column.

column is periodically vibrated in the direction of the coiling axis. The column end-fitting is made from Hermeto and Swagelok fittings in which a metal filter plate is inserted.

In the experimental arrangement for the separation, the solvent flows from the reservoir through the reference cell of the differential refractometer, to the pump, the probe inlet, the column and back again to the differential refractometer through the sample cell. The usual solvent velocity is 0.75 ml/h, corresponding to a linear velocity of 25 cm/h (0.007 cm/sec) and a total analysis time of 40 h. The pressure under these conditions is about 2 atm.

The pump used was of the double syringe type (Model LDP 13 A, Labotron Messtechnik, D-8191 Gelting, G.F.R.), giving a pulse-free solvent flow.

RESULTS AND DISCUSSION

Separation of oligomers

Fig. 2 shows examples of separations in coiled columns of oligostyrenes of different molecular weights prepared by the reaction of butyllithium with styrene. Each molecule contains one butyl group from the initiator. In Fig. 2a, the different degrees of polymerisation, represented by the numbers, are well resolved into peaks. The sample has an $\bar{M}_n$ value of 600. With increasing molecular weight, the peak distance between the different oligomers decreases as there is a logarithmic dependence of molecular weight on elution volume. Therefore, there is an unresolved background with higher molecular weights (Fig. 2b), and at a degree of polymerization of 14, the unresolved background amounts to about 50% of the total peak height. Up to a degree of polymerization of about 24, different oligomers can be recognized at least by a shoulder. On the high-molecular-weight side of the distribution, the sequence of peaks is so dense that a smooth curve results.

In order to obtain a good separation in the oligomeric range, a gel must be used with an exclusion molecular weight of about 4000. The gel which we used was a polyvinyl acetate gel (Merckogel 6000).

These separations can give information about the elementary steps of polymerization. Thus in radical polymerization, one can decide if the termination step is a combination or a disproportionation reaction. Combination results in a polymer homologous series with two initiator fragments as end-groups, whereas the disproportionation product has only one end-group originating from the initiator.

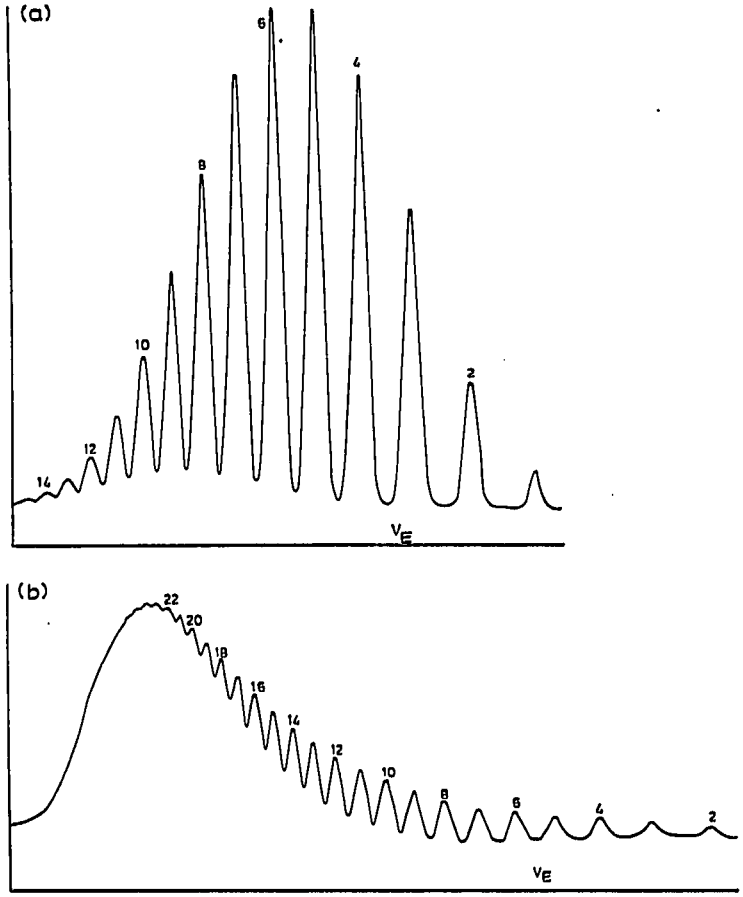


Fig. 2. Gel chromatogram of polystyrene, $C_4H_9-[CH_2-CH(C_6H_5)]_n-H$, in coiled columns. Numbers on the peaks correspond to the degree of polymerization. GPC conditions: column 0.2 × 1000 cm, Merckogel 6000, DMF; total analysis time 40 h. (a) $\bar{M}_n = 600$; (b) $\bar{M}_n = 2000$.

Oligomers prepared from styrene and bis-azoisobutyronitrile (AIBN) give a gel chromatogram as shown in Fig. 3. This product clearly consists of a single polymer homologous series. The analytical data for the single components confirm the given structure, with two nitrile end-groups.

A similar situation is found with oligomers prepared from methyl methacrylate (MMA) and AIBN. Again, there is a single polymer homologous series with two nitrile end-groups (Fig. 4). This result is in contrast to the findings in the usual polymerization of MMA, where disproportionation prevails. The reason is that oligomers are formed by the reaction of a macro-radical with a primary radical from the initiator, whereas in the usual polymerization reaction two macro-radicals react.

In contrast to this, oligomers prepared from butyl methacrylate and AIBN show two oligomeric series in GPC (Fig. 5.) It has been established that the series marked with asterisks has two nitrile end-groups. For oligomers with low molecular

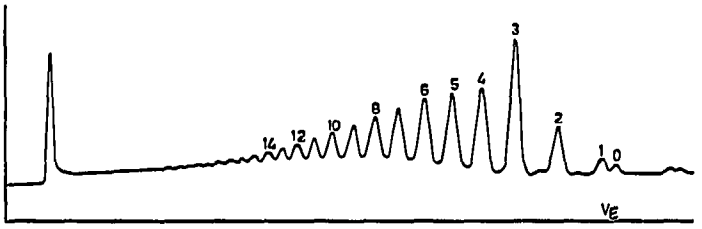
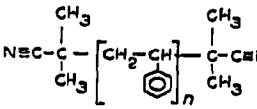


Fig. 3. Gel chromatogram of oligomers from styrene and AIBN,  , $\bar{M}_n$ = 600. GPC conditions as in Fig. 2.

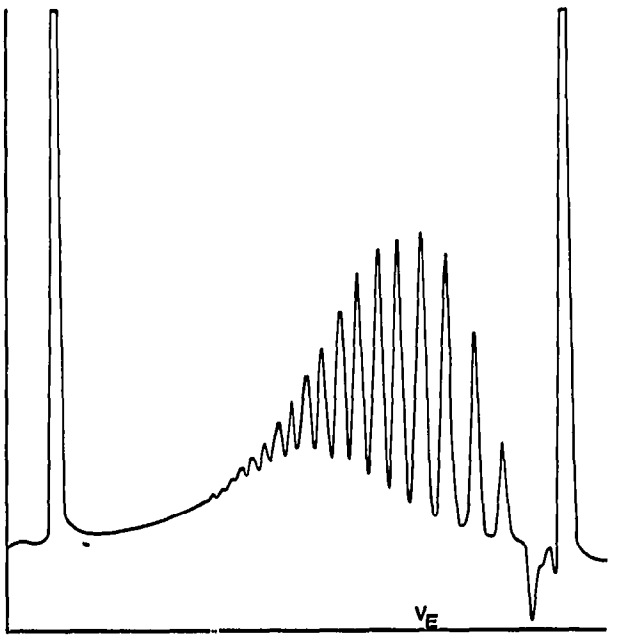


Fig. 4. Gel chromatogram of oligomers from methyl methacrylate and AIBN. GPC conditions as in Fig. 2.

weights, the main reaction occurs between a macro-radical and a primary radical, and approximately equal extents of combination and disproportionation occur (Fig. 5a). The main reaction between two macro-radicals from butyl methacrylate is disproportionation. If oligomers are prepared with higher molecular weights, this reaction is no longer negligible and so the extent of disproportionation increases, as shown in Fig. 5b. Again, the series with two nitrile end-groups is marked with asterisks, and it is the minor series. The main series has only one nitrile end-group.

Similar separations can be obtained with other oligomers, *e.g.*, polyethylene oxide, polyepoxides and surfactants. These examples show that coiled columns give excellent separations of oligomers.

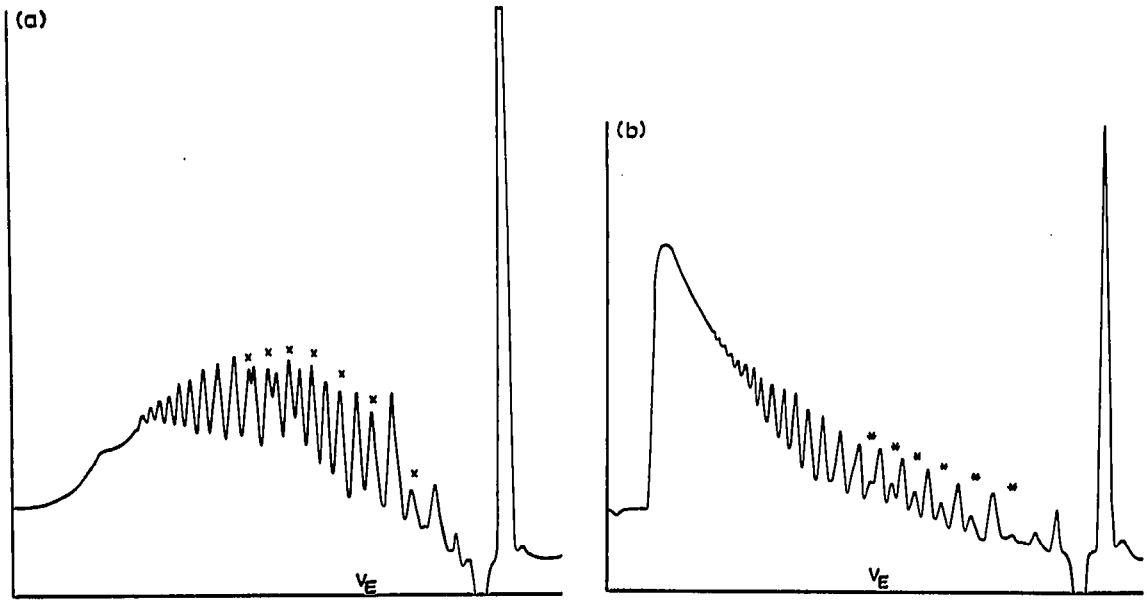
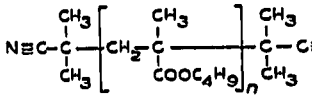


Fig. 5. Gel chromatogram of oligomers from butyl methacrylate and AIBN. GPC conditions as in Fig. 2. Peaks marked with asterisks represent the polymer homologous series



, (a) $\bar{M}_n = 600$; (b) $\bar{M}_n = 4000$.

Separation of polymers

The proof of the efficiency of separation of polymers is difficult. We have no truly monodisperse samples, so we must use indirect methods for the determination of peak spreading. The reverse flow technique proposed by Tung *et al.*[3] has the limiting assumption that peak spreading is independent of the direction of flow. Peak spreading must be calculated by using polymers with a known molecular-weight distribution. Narrow distributions can be approximated by a log-normal distribution, so we can apply the additivity of variances:

$$\sigma^2_{exp} = \sigma^2_{inst} + \frac{1}{B^2} \cdot \log\left(\frac{\bar{M}_w}{\bar{M}_n}\right) \tag{1}$$

where B is the slope of the log M/V_E curve* and $\bar{M}_w$ and $\bar{M}_n$ are the weight- and number-average molecular weights, respectively.

In Fig. 6, superimposed traces of polystyrene curves are shown; these are the commonly used polystyrene standards. As they are anionically prepared, they should have a Poisson distribution with $\bar{M}_w/\bar{M}_n = 1+1/p$ as the lowest limiting value. In practice, the distribution is broader. The unsymmetrical peak form of polystyrene with $\bar{M}_n = 19{,}850$ is also found with other gel and column types. Using the data provided by Pressure Chem. Co. (Pittsburgh, Pa., U.S.A.) for $\bar{M}_n = 10{,}000$ ($\bar{M}_w/\bar{M}_n = 1.06$),

* M = Molecular weight; V_E = elution volume.

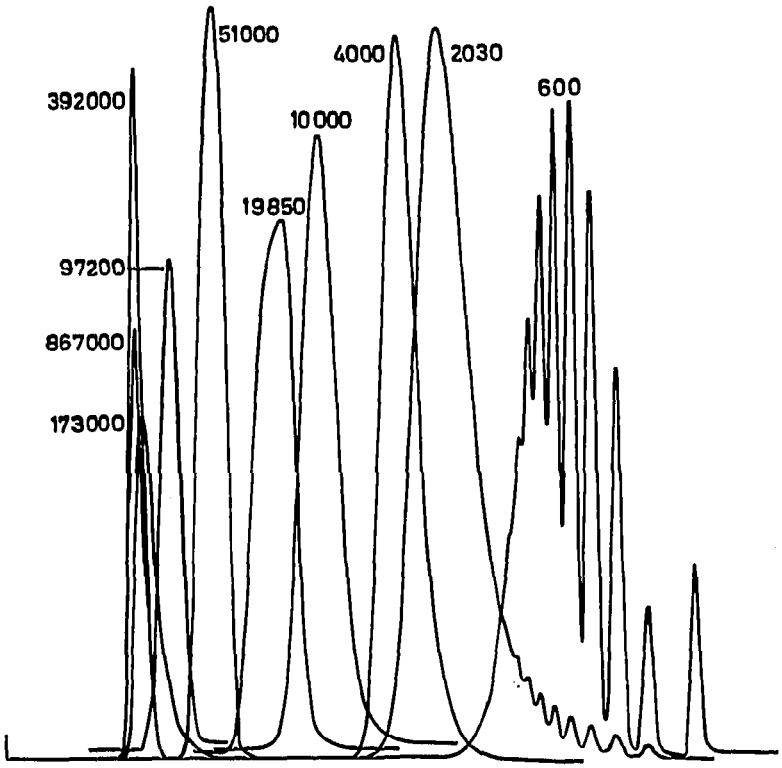


Fig. 6. Superimposed gel chromatogram of polystyrene standards. Numbers on the peaks represent $\bar{M}_n$. GPC conditions: column 0.2 × 1000 cm; Merckogel 80,000, DMF; total analysis time 40 h.

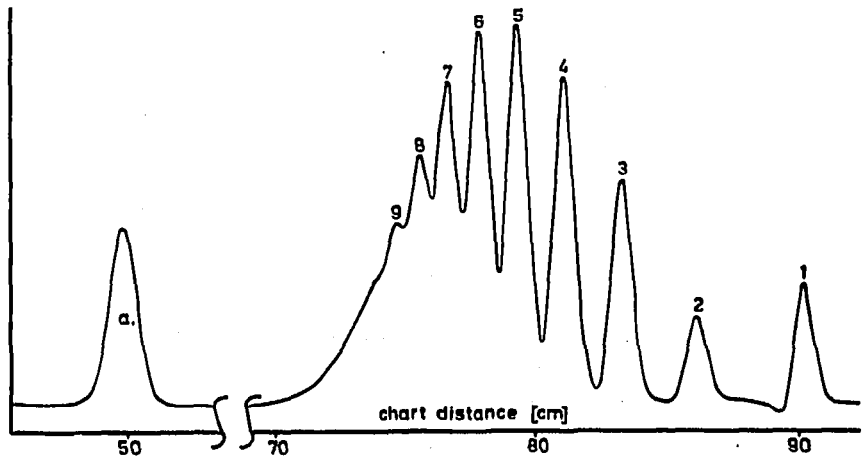


Fig. 7. Peak width of a Baker–Williams fraction of polystyrene ($\bar{M}_n$ = 36,400; $\bar{M}_w/\bar{M}_n \sim 1.001$) and of the oligomers in polystyrene ($\bar{M}_n$ = 600). GPC conditions as in Fig. 6.

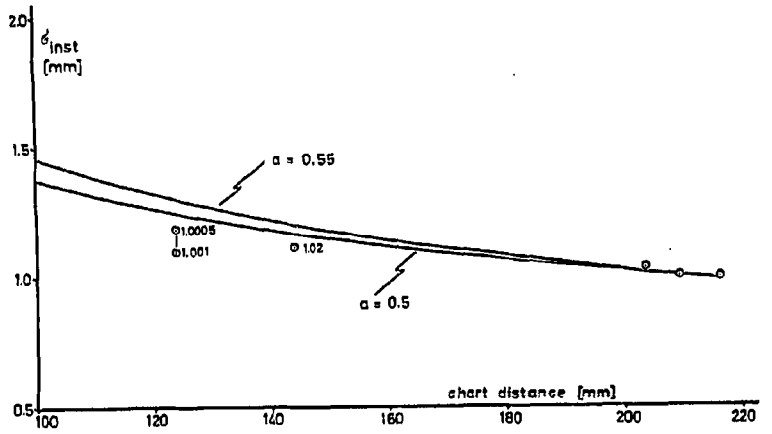


Fig. 8. Peak spreading in GPC. Dependence of the instrumental mean deviation (σ) on the elution volume according to eqn. 3. Figures at the exp. points are $\bar{M}_w/M_n$.

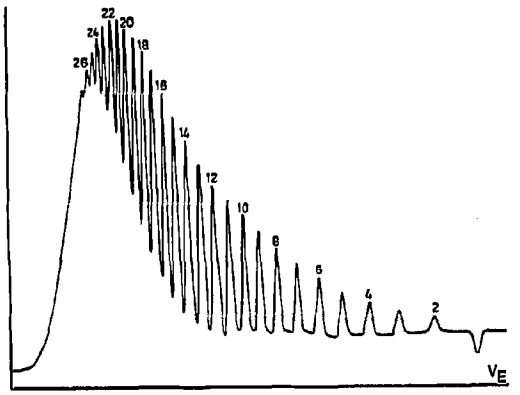


Fig. 9. Gel chromatogram of polystyrene, $\bar{M}_n = 2000$ (the same product as in Fig. 2b). GPC conditions: column 0.2 × 2000 cm; Merckogel 6000, DMF; total analysis time 20 days.

the peak spreading due to the molecular-weight distribution should be larger than the total spreading observed.

Peak spreading has been checked with a narrow fraction obtained by Baker–Williams fractionation* with $\bar{M}_w/\bar{M}_n \sim 1.001$. In Fig. 7, the peak spreading of this fraction with $\bar{M}_n = 36{,}400$ is compared with that of low-molecular-weight oligomers. The peak of the fraction is not much broader than those of low-molecular-weight substances. It can therefore be concluded that additional peak spreading in coiled columns is small in this molecular-weight range.

* Kindly supplied by L. Böhm, Physikalisch-chemisches Institut der Universität Mainz, Mainz, G.F.R.

Polymers have a small diffusion coefficient, so a linear dependence between the reduced plate height and the reduced velocity can be assumed if identical experimental conditions are applied:

$$H = K \cdot \frac{1}{D} \qquad (K = \text{constant}) \tag{2}$$

Inserting the diffusion coefficient/molecular weight relationship $D = KM^{-a_D}$ and from GPC $\log M = AWBV_E$, we obtain

$$\frac{\mathrm{d}\log H}{\mathrm{d}V_E} = -a_D \cdot B \tag{3}$$

In Fig. 8, two curves are plotted assuming $a_D = 0.5$ and $a_D = 0.55$, respectively, and a degree of polymerization of 2 as a fixed point. Peak spreading of the Baker–Williams fraction agrees approximately with this curve.

Limitations

The separation efficiency can be increased by using longer columns and analysis times. Fig. 9 shows a separation using a 20-m column and an analysis time of 20 days. The oligomers can be seen up to a degree of a polymerization of 26. At a degree of polymerization of 19, the unresolved background is half of the total peak height.

A further increase in analysis time is limited by the insufficient baseline stability at these slow flow-rates. Higher separation efficiencies can also be obtained by using smaller particle sizes of the stationary phase. Using cross-linked organic polymers as the stationary phases, it has been found experimentally that a large decrease in column efficiency occurs if a pressure greater than a certain critical value is applied during packing. This critical value is strongly dependent on the gel type. For Merckogel 6000, pressures higher than 10 atm gave lower separation efficiencies. Under the conditions applied (10-m column length, 40-h total analysis time), particle diameters down to 15 μm should be applicable.

ACKNOWLEDGEMENTS

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REFERENCES

1 J. C. Giddings, *J. Chromatogr.*, 3 (1960) 520.
2 W. Heitz and J. Čoupek, *J. Chromatogr.*, 36 (1968) 290.
3 L. H. Tung, J. C. Moore and G. W. Knight, *J. Appl. Polym. Sci.*, 10 (1966) 1261.