Journal of Chromatography, 76 (1973) 149–157

CHROM. 6408

HIGH-SPEED GEL PERMEATION CHROMATOGRAPHY

APPLICATION OF LIQUID CHROMATOGRAPHIC TECHNIQUES

E. P. OTOCKA
Bell Laboratories, Murray Hill, N.J. 07974 (U.S.A.)
(First received August 4th, 1972; revised manuscript received October 2nd, 1972)

SUMMARY

The development of high-speed gel permeation chromatography has been undertaken by applying modern liquid chromatographic techniques. Reduction in column diameters and packing-particle sizes has yielded significant increases in efficiency.

Molecular-weight distributions have been determined in less than twenty minutes with good accuracy and precision.

INTRODUCTION

Gel permeation chromatography (GPC) has quickly become the most widely used analytical method for the determination of polymer molecular-weight distribution (MWD). Since its introduction, however, the execution of the technique has not changed appreciably: chromatographic hardware, columns, column packings, and detectors now in common use have been available for at least the past six years. Some recent work in the field indicates a number of new chromatographic techniques being explored for the determination of polymer MWD[1–7]. It appears that GPC is ready for the advent of "second generation" techniques. Significant improvements are sought in resolution, reproducibility, and elution time.

GPC is simply one method in the broad category of liquid chromatography (LC). The past five years have been marked by revolutionary advances in the other LC techniques of adsorption, partition, and ion-exchange. These methods have resulted in dramatic improvements in analyses[8,9]. A good understanding of the physical chemistry of these chromatographic processes has allowed a variety of approaches to optimum operating conditions[10–14].

In general, the major modifications so successful in LC, namely reductions in both packing-particle size and column diameter, have not been widely employed in GPC. This paper investigates the results obtained by this approach using rigid packings, and it compares the data with previous investigations employing other packing and column sizes.

EXPERIMENTAL

Several rigid, porous substrates were examined. Corning porous glasses CPG 10-240 and CPG 10-700 were chosen because of ready availability and extensive

characterization[15]. The CPG 10-240 was used in two particle sizes: 36–75 μ (available) and 36–44 μ (sieve cut from the above). The CPG 10-700 was also sieved and the 36–44-μ portion used. A newly available porous silica gel, MWE-1, was purchased from the Perkin-Elmer Liquid Chromatography Division (formerly the Nester/Faust Co.). This material is characterized as having a particle size of 36–44 μ and *ca.* 150-Å pores, and is surface-treated. (This surface treatment does not prevent adsorption.)

These materials were dry-packed into individual 0.5-m stainless-steel LC columns of 2.6 mm I.D. With the addition of each increment of packing material, compaction was achieved by tapping the column and brief use of a *ca.* 30-c.p.s. mechanical vibrator. In the CPG 10-700 column, tamping with a close-fitting metal rod was also used after each addition. After packing, the columns were flushed with solvent at 2 ml/min, and the end fitting was undone to examine the packing. Little ($\leqslant$ 2 mm) settling was found. The upper (injection) end was fitted with a 2.6-mm PTFE filter pad, and the columns were ready for use. The pressure drop across each column was $\leqslant$ 400 p.s.i. with tetrahydrofuran (THF) at 1 ml/min.

The instrument was a Perkin-Elmer Model 1210 liquid chromatograph (formerly the Nester/Faust Model 1210). The features of the instrument which pertain to the GPC investigation are: positive displacement syringe pumps of 500-ml capacity; on-column septum injection as well as valve/loop injection; tandem ultraviolet (UV) and refractive index detectors; cell volume of 12 μl; indicated total dead volume of 28 μl.

The instrument spreading was determined by attaching the septum injector directly to the UV detector using 30 cm $\times$ 0.23 mm I.D. connecting tubing with a 5-μl injection of 1 % xylene in THF. For the loop injection system the same connections were made and a 10-μl injection was employed.

Using Pressure Chemical polystyrene (PS) in THF standards of 2 mg/ml, a standard 5-μl septum injection was adopted. Separate studies indicate injections of $>$ 100 μg can be used before obvious changes in the chromatogram result[16]. However, no systematic study of concentration effects has yet been made.

The exclusion behavior of the individual columns and the three-column series at THF flow-rates from 0.1 to 1.0 ml/min, and the efficiency for xylene as the solute were determined.

The validity of MWD determinations was tested in two ways. On the MWE-1 column the MWD of the 10,300 $\bar{M}$ PS standard was determined, and for the three-column series, the MWD of NBS-706 PS standard was determined. In the first case the flow-rate was 0.25 ml/min, and 0.5 ml/min for the second. The UV detector output was sampled at 10-sec intervals and computations were carried out using standard methods with the appropriate calibration curves. The appearance of a large quantity of monomeric material (presumably styrene) in the NBS-706 precluded accurately placing the baseline[17]. The baseline was artificially located at a cut-off corresponding to $M = 10^4$; it is thus anticipated that the determined $\bar{M}_n$ will be in error.

RESULTS AND DISCUSSION

The factor which limits chromatographic resolving power most severely is dispersion or peak spreading. All chromatographic processes are modeled as a series

of hypothetical steps, termed plates, within which the solute concentration is equilibrated between the mobile and stationary phases according to the distribution coefficient (K). The number of theoretical plates (N) is deduced from the chromatogram of a monodisperse solute by

$$N \equiv \left(\frac{V_r}{\sigma}\right)^2 \equiv \left(\frac{4V_r}{W_b}\right)^2 \tag{1}$$

where V_r is the retention volume, σ is the standard deviation, and W_b is the width of the peak at the base.

The observed width at the base is generated from a number of individual sources according to[10,18]

$$W_b = \sqrt{\Sigma w_i^2} \tag{2}$$

The individual w_i's arise from mobile phase dispersion, chromatographic dispersion, instrumental effects, and, naturally, polydispersity.

The capacity factor (k') in chromatography will be important in determining efficiency:

$$k' = \frac{V_r - V_0}{V_0} \tag{3}$$

In terms of k', GPC is much more limited than conventional LC. A simple examination of some factors explains this. For a regularly packed column ($d_{col}/d_p >$ 10, where d_{col} is the column diameter, and d_p is the packing-particle diameter), the void fraction (ε) is roughly 0.4. The void fraction of GPC packings, such as CPG 10, is roughly 0.6–0.7, so V_r (the elution volume of solvent sized solutes for which all the porosity is available) is effectively limited to $2V_0$ and k' to a value of *ca.* 1.0.

The linear flow-rate (u) in columns packed with porous supports is

$$u = \left(\frac{4F}{\pi d_{col}}\right)^2 \varepsilon_T \tag{4}$$

where F is the volume flow-rate, and ε_T is the total porosity with a value of *ca.* 0.8 (ref. 12).

Three final parameters which are important in LC are the reduced plate height (h) and reduced velocity (v) given by

$$h = \frac{H}{d_p} \tag{5}$$

$$v = \frac{ud_p}{D_m} \tag{6}$$

where D_m is the molar diffusivity of the solute.

Of great interest is how H or h depend upon u or v. This determines the loss of resolution through increased dispersion (for a given column) as the analytical

time is reduced by increasing the flow-rate. Numerous measurements of H *vs.* u have been made for LC columns and a variety of models[10,19,20] has been constructed to describe the data.

One of the models developed by KELLEY and co-workers[21–24] is given by the following expression

$$H = 2\left(\frac{\varphi D_m}{u} + \lambda d_p\right) + \frac{h\delta^2 d_p^2}{2\left(\frac{\varphi D_m}{u} + \lambda d_p\right)} + Cu \qquad (7)$$

where φ is a tortuosity factor, λ is an eddy diffusion proportionality constant, $h\delta^2$ is a dispersion parameter, and C is a permeation dispersion parameter. The first bracketed term in eqn. 7 represents molecular diffusion plus eddy diffusion dispersions while the second term describes dispersion arising from the velocity profile across the column. The final term represents the broadening arising from the permeation process.

This model has been relatively successful in predicting the shape of H *vs.* u curves and the general magnitude of H (refs. 22, 23). The other models have met with similar success but in general they are too complicated for this limited discussion. For GPC theory the primary interest is in the last term of eqn. 7, but tests of permeation models must properly account for the overall mobile phase dispersions of macromolecular solutions flowing through packed columns in order to isolate the permeation dispersion accurately.

The efficiency data for the individual columns and the three-column series based on small-molecular solutes are presented in Table I. Also shown for comparison are the data of COOPER *et al.*[15] and GUDZINOWICZ AND ALDEN[7], who have also worked with CPG 10 series packings. The linear velocities and reduced velocities for all systems were calculated from eqns. 4 and 6 assuming $\varepsilon_T = 0.8$ and $D_m = 1 \cdot 10^{-5}$ cm²/sec. Since our septum injection/detector system gave a W_b of *ca.* 40 μl and a $V_r < 100$ μl, these corrections were not applied to our data. Corresponding results for the 10-μl loop injections are $V_r < 100$ μl and $W_b = 70$ μl. Accounting for instrument spreading would increase the plate counts shown in Table I by about 6%. Values for k' are approximate because of uncertainties in V_0.

The data in general show that the plate height decreases with decreasing particle size (columns 6, 3, and 4, for example). Even when reduced plate height is plotted *vs.* reduced velocity, differences in efficiency are seen (Fig. 1). Among particles of the same size, an increase in pore size appears to increase efficiency.

For speed of separation, several authors indicate that h/v is the crucial parameter. The data from columns 4, 5, and 6, which are similar in configuration, are compared in Fig. 2, which demonstrates the advantage of decreasing particle size. From a figure of this type we can place the optimum reduced velocity for GPC in the CPG 10 system in the range of 65–100. Above this reduced velocity, the pressure penalty far outweighs the decrease in experimental time.

Of particular concern is the behavior of polymeric solutes in these columns. Fig. 3 shows the molecular weight *vs.* retention volume curves for the individual 0.5-m columns with 36–44 μ packing. The familiar exclusion limits and resolving

TABLE I

GPC COLUMN BEHAVIOR FOR SMALL-MOLECULAR SOLUTES

Column packings: 1 = MWE-1, 36–44 μ; 2 = CPG 10-240, 36–44 μ; 3 = CPG 10-700, 36–44 μ; 4 = CPG 10-240, 36–75 μ; 5 = three-column (*i.e.* packings 1 + 2 + 3) series; 6 = five-column, CPG-10, series (ref. 15); 7 = seven-column, CPG-10, series (ref. 7).

Column	*Flow-rate (ml/min)*	*Column length (m)*	$d_p(\mu)$	$V_r(ml)$	$W_b(ml)$	$\eta_p = H/L$ *(Pl/ft.)*	H *(cm)*	k'
1	0.10	0.50	40	2.71	0.27	980		
	0.25	0.50	40	2.69	0.29	838	0.039	
	0.50	0.50	40	2.72	0.34	623	0.053	
	1.00	0.50	40	2.80	0.43	413		
2	0.10	0.50	40	2.80	0.27	1048		
	0.25	0.50	40	2.75	0.29	876	0.038	
	0.50	0.50	40	2.77	0.34	647	0.051	
	1.00	0.50	40	2.80	0.39	502	0.061	
3	0.10	0.50	40	2.80	0.23	1444		
	0.25	0.50	40	2.88	0.26	1196	0.028	
	0.50	0.50	40	2.84	0.29	935	0.034	
	1.00	0.50	40	2.89	0.36	628	0.048	
4	0.10	0.50	60	2.73	0.44	382		
	0.25	0.50	60	2.75	0.46	349	0.087	
	0.50	0.50	60	2.75	0.48	320	0.102	
	1.00	0.50	60	2.79	0.54	252	0.130	
5	0.25	1.50	40	8.29	0.49	894	0.037	*ca.* 1.1
	0.50	1.50	40	8.29	0.55	745	0.044	*ca.* 1.1
	1.00	1.50	40	8.30	0.66	614	0.053	*ca.* 1.1
6	1.00	6.10	100	235.2	19.48	117	0.124	*ca.* 0.8
7	0.728	6.40	47	22.4	1.06	337	0.09	*ca.* 0.9
	1.470	6.40	47	22.6	1.29	235	0.142	*ca.* 0.9

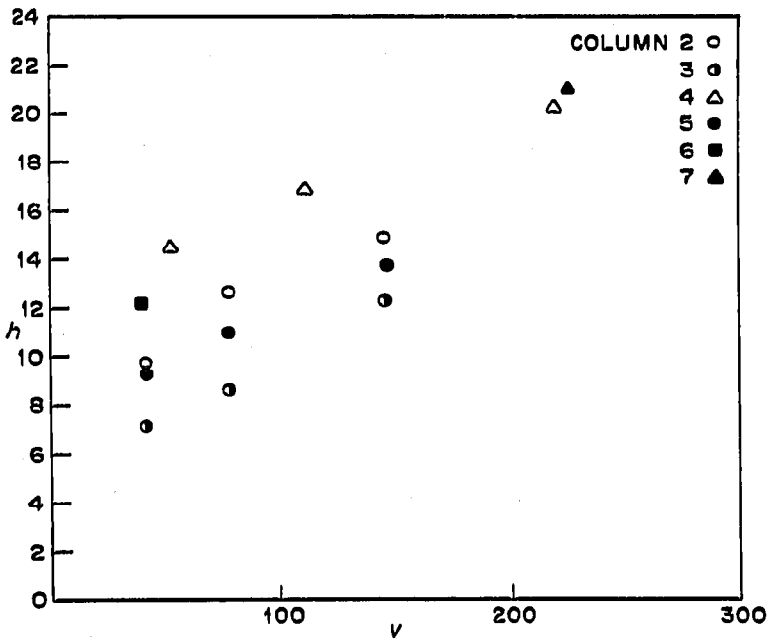

Fig. 1. Reduced plate height (h) *vs.* reduced velocity (v) for various CPG 10 column systems. See Table I for key.

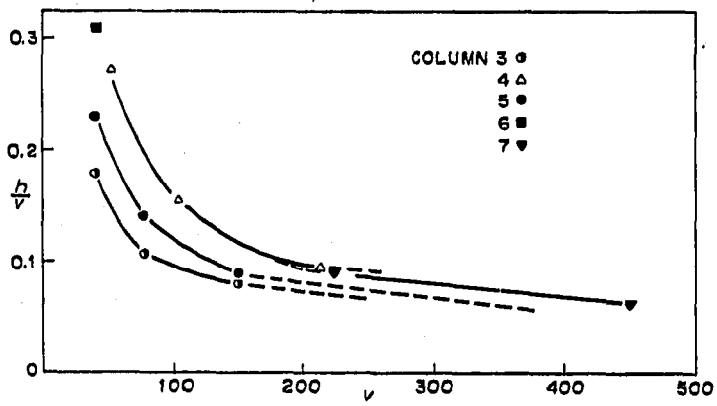

Fig. 2. Packing efficiencies (h/v) as a function of reduced velocity (v) for various CPG 10 column systems. See Table I for key.

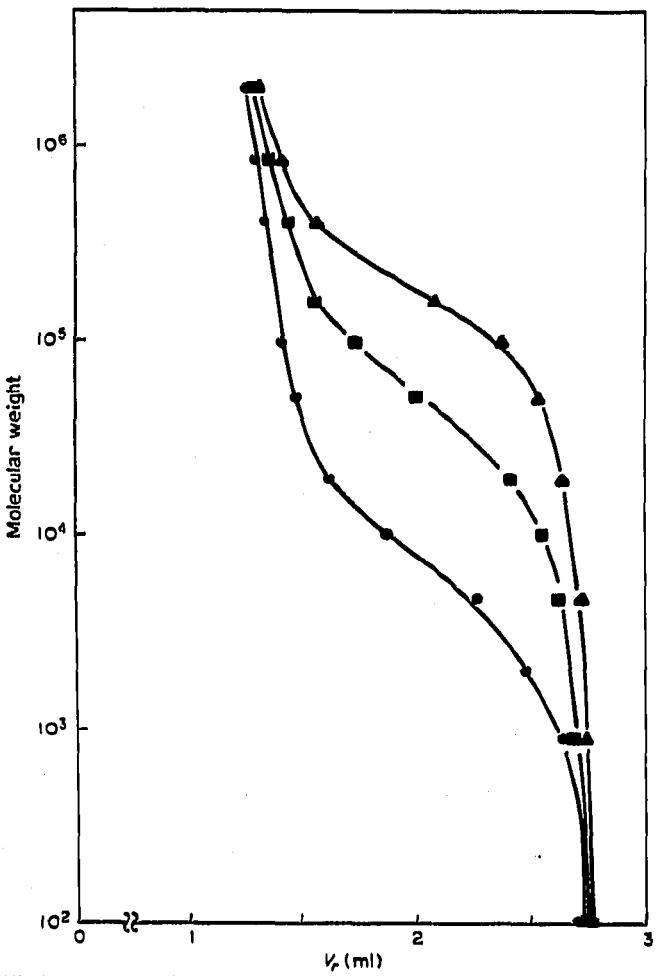

Fig. 3. Molecular weight *vs.* retention volume (V_r) curves for individual columns. ●, column 1; ■, column 2; ▲, column 3.

region are demonstrated in each case. CPG 10 and other porous glass substrates have traditionally exhibited low plate counts for narrow polymer standards[15,24].

The present column/column packing configuration overcomes this deficiency. Table II compares the plate counts for narrow polystyrene standards, which fall roughly in the middle of the resolving range of column(s) in question. The system used in this work is about four times as efficient as the systems representative of current practice in terms of macromolecules dispersion. This is important because it drastically reduces the need for laborious mathematical correction of the chromatogram for narrow MWD samples[25,26].

TABLE II

COLUMN EFFICIENCIES FOR MACROMOLECULAR SOLUTES

Column	*Flow-rate (ml/min)*	*Polystyrene ($\bar{M}$)*	V_r *(ml)*	W_b *(ml)*	η_p *(Pl/ft.)*
1	0.50	10 300	1.87	0.51	131.2
2	0.50	51 000	2.00	0.50	156.2
3	0.50	160 000	2.10	0.49	179.2
5	0.50	51 000	6.1	0.96	131.1
6	1.00	160 000	180.37	29.04	30.8
7	0.728	160 000	15.5	1.78	57.8
	1.470	160 000	15.6	2.73	24.9

The determination of MWD was demonstrated for column 1 with the 10,300 $\bar{M}$ PS standard at 0.25 ml/min. Using calibration data from 900 to 97,200 (Fig. 3) the following results were achieved in 12 min without applying any corrections:

$\bar{M}_w = 10{,}580$; $\bar{M}_n = 9{,}800$; $\bar{M}_w/\bar{M}_n = 1.08$.

The three-column series gave elution curves represented by Fig. 4. Using a baseline anchored prior to the monomeric component the elution of NBS 706 on the

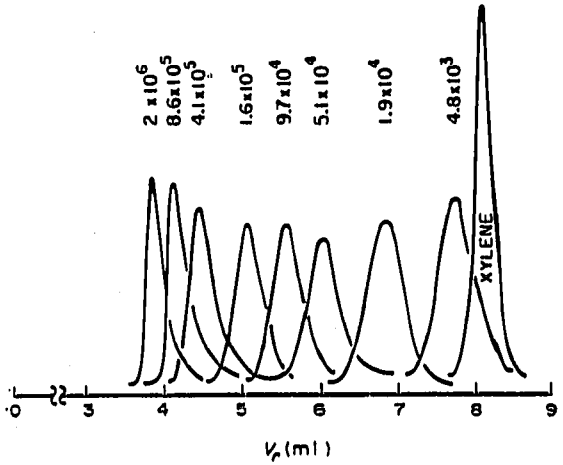

Fig. 4. Polystyrene standards eluted from the three-column series and UV-detected.

three-column series gave $\bar{M}_z$ = 353,000; $\bar{M}_w$ = 263,000; $\bar{M}_n$ = 162,000, which is in very good agreement (except $\bar{M}_n$) with the NBS data. On three successive injections the departure from baseline all occurred on the same counts, and values of $\bar{M}_z$, $\bar{M}_w$, and $\bar{M}_n$ agreed within ± 5, ± 3, and ± 2 %, respectively.

From the efficiency data available for the small-molecule solutes, an approximation of optimum GPC conditions may be generated, using a reduced particle size/column diameter concept. The porous glasses appear to have relatively uniform porosity and in principle, at least, should not suffer any loss in properties down to particle sizes of 10 μ. Assuming a d_p of 20 μ with $h \leqslant 10$ and $0.3 \leqslant u \leqslant 0.6$ cm/sec, plate counts approach 2000 Pl/ft. for small molecules. The same line of reasoning gives nearly 500 Pl/ft. for polymeric solutes. A single 6-ft. column, 0.2 cm in diameter (ΔP = *ca.* 4000 p.s.i. at F = 1 ml/min) would then be capable of resolution satisfactory for many separations. The V_0 = *ca.* 3.0 ml and V_t = *ca.* 6.0 ml putting the experimental time at 6 min.

Practical considerations of current hardware limitations make such operating conditions somewhat unattractive. The near future is likely to see reliable pumps, detectors and injectors which will be capable of extended operation at such elevated pressures. Another consideration arises, however. It has been mentioned that porous styrene/divinyl benzene packings do not perform well in small bore columns[27]. It has also been reported that high flow-rates in conventional columns reduce elution volume[28]. An understanding of these observations has not been reached. However, semi-rigid packings are susceptible to compression at high pressures.

New models for the fractionation of polymeric solutes by GPC have been advanced, modified, discussed, and reviewed[20–35]. As indicated above, a great portion of the difficulty in testing any model with experimental data arises in separating the polymeric solute dispersion arising from mobile phase effects and dispersion arising from the permeation process. With the data of this work (column 5) and of GUDZINOWICZ AND ALDEN (column 7) on small molecule solutes, a value of C in the last term of eqn. 7 was calculated, using appropriate values for linear velocity. Neither case gave a truly constant value for C over the flow-rate range studied, in conflict with the previous success of this calculation[23]. Further investigations along this line are planned.

One of the noteworthy results found here is the increase in packing efficiency with increase in pore size. It would be inviting to suggest that this is an indication of eluent flow through the pores. However, it may be pointed out that these packings do not meet the criteria set forth in ref. 32 which defines the onset of flow through the pores, namely

$$\log (2D_a/l < V_p >_s) \leqslant 0 \qquad (9)$$

where $D_a = D_m$, l = length of the pore in packing particle and $\langle V_p \rangle_s$ = average velocity of solute particles.

CONCLUSIONS

Reduction of particle sizes and column diameters yields increased GPC efficiencies with porous glass packings. Extrapolation of the data indicate experiment

times on the order of five minutes for molecular-weight distribution determinations. The necessity for mathematical correction of chromatograms appears greatly reduced.

ACKNOWLEDGMENTS

Mrs. M. Y. HELLMAN and P. M. MUGLIA performed much of the experimental work. The Perkin-Elmer Liquid Chromatography Division (formerly Nester/Faust) kindly provided the sieve fractions of CPG 10-240 and CPG 10-700.

REFERENCES

1 F. KAMIYAMA, H. MATSUDA AND H. INAGAKI, *Polym. J.*, 1 (1970) 518.
2 H. INAGAKI, F. KAMIYAMA AND T. YAGI, *Polym. J.*, 4 (1971) 133.
3 E. P. OTOCKA, *Macromolecules*, 3 (1970) 691.
4 E. P. OTOCKA, M. Y. HELLMAN AND P. M. MUGLIA, *Macromolecules*, in press.
5 R. S. PORTER, *Winter DHPP Meeting of APS, Cambridge, 1971.*
6 N. CATSIMPOOLAS AND J. KENNEY, *J. Chromatogr.*, 64 (1972) 77.
7 B. J. GUDZINOWICZ AND K. ALDEN, *J. Chromatogr. Sci.*, 9 (1971) 65.
8 J. J. KIRKLAND (Editor), *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971.
9 F. BOWMAN (Editor), *Basic Liquid Chromatography*, Varian Associates, Palo Alto, Calif., 1971.
10 J. F. K. HUBER AND J. A. R. HULSMAN, *Anal. Chim. Acta*, 38 (1967) 305.
11 J. F. K. HUBER, *J. Chromatogr. Sci.*, 7 (1969) 85.
12 I. HALÁSZ AND M. NAEFE, *Anal. Chem.*, 44 (1972) 76.
13 T. W. SMUTS AND V. PRETORIUS, *Anal. Chem.*, 44 (1972) 121.
14 B. L. KARGER, in J. J. KIRKLAND (Editor), *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971, Ch. 1.
15 A. R. COOPER, A. R. BRAZZONE, J. H. CAIN AND E. M. BARRALL, II, *J. Appl. Polym. Sci.*, 15 (1971) 571.
16 M. TELEPCHAK AND R. YOST (Perkin-Elmer), private communication.
17 H. L. WAGNER, private communication.
18 J. G. HENDRICKSON, *J. Polym. Sci.*, A-2, 6 (1968) 1903.
19 J. C. GIDDINGS, *Dynamics in Chromatography*, Part I, Marcel Dekker, New York, 1965.
20 J. C. GIDDINGS AND K. L. MALLIK, *Anal. Chem.*, 38 (1966) 997.
21 F. W. BILLMEYER, JR., G. W. JOHNSON AND R. N. KELLEY, *J. Chromatogr.*, 34 (1968) 316.
22 R. N. KELLEY AND F. W. BILLMEYER, JR., *Anal. Chem.*, 41 (1969) 894.
23 R. N. KELLEY AND F. W. BILLMEYER, JR., *Anal. Chem.*, 42 (1970) 399.
24 R. N. KELLEY AND F. W. BILLMEYER, JR., *Separ. Sci.*, 5 (1970) 291.
25 L. H. TUNG AND J. R. RUNYON, *J. Appl. Polym. Sci.*, 13 (1969) 2397.
26 A. E. HAMIELEC, *J. Appl. Polym. Sci.*, 14 (1970) 1519.
27 K. BOMBAUGH, in J. J. KIRKLAND (Editor), *Modern Practice of Liquid Chromatography*, Wiley-Interscience, New York, 1971, Ch. 7.
28 W. W. YAU, *J. Polym. Sci.*, A-2, 7 (1969) 483.
29 E. F. CASASSA AND Y. TAGAMI, *Macromolecules*, 2 (1969) 14.
30 E. A. DIMARZIO AND C. M. GUTTMAN, *J. Polym. Sci., Part B*, 7 (1969) 267
31 E. A. DIMARZIO AND C. M. GUTTMAN, *Macromolecules*, 3 (1970) 131.
32 E. A. DIMARZIO AND C. M. GUTTMAN, *Macromolecules*, 3 (1970) 681.
33 F. H. VERHOFF AND N. D. SYLVESTER, *J. Macromol. Sci. Chem.*, 4 (1970) 979.
34 J. J. HERMANS, *J. Polym. Sci.*, A-2, 6 (1968) 1217.
35 E. F. CASASSA, *J. Phys. Chem.*, 75 (1971) 3929.