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High Resolution Gel Permeation Chromatography—Using Recycle*

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Summary

High resolution in gel permeation chromatography has been accomplished by use of long columns, since commercial rigid gels afford relatively fixed capacity ratios and since plate numbers of equivalent columns are additive. To overcome the high cost and high pressure requirement of long columns often required to resolve discrete species, high resolution gel permeation chromatography may be attained in commercial GPC equipment by recycle operation through the reciprocating pump, GPC column, and detector. However, peak width increases with the number of cycles (ν). Since the contained volume of the closed recycle system is constant, as ν increases the peak width (W) of the distribution will eventually exceed the volume of the system and peak overlap will occur. This presentation considers the increase in W and provides a method of "flush and draw off" to prevent sample overlap. Analytical and preparative scale separations were investigated, using both small and macromolecules. The effects of sample load and flow rate on resolution with recycle operation were investigated.

INTRODUCTION

Gel permeation chromatography, developed by Moore as a method of fractionating macromolecules, has also been extended to small molecules by a number of workers (*2–8*). Most of this work was done either at low resolution, or with species affording large alpha prime

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values where alpha prime is equal to $V_2/V_1 \geq 1.1$, where V_2 and V_1 equal the elution volume of the two solutes.

To resolve species with alpha prime (relative retention) values less than 1.1, an increase is required, either in plate number of the system or in the capacity ratio (K') of the gel

$$K' = \frac{V_e - V_0}{V_0}$$

where V_e is the elution volume of the solute and V_0 is the interstitial (dead) volume of the column.

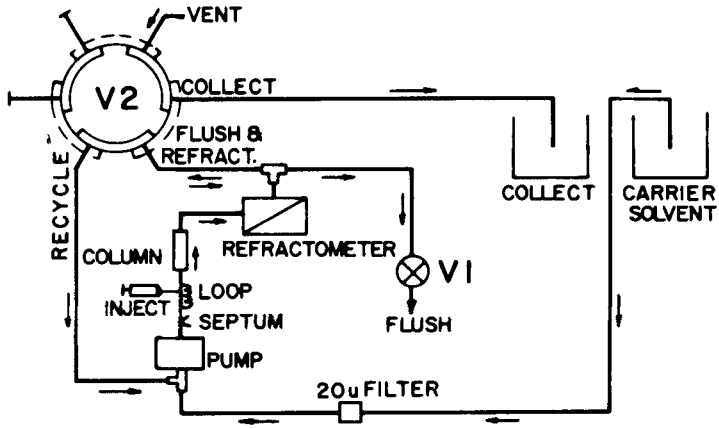
Commercial rigid gels offer a fixed K' of 0.8 to 1.2. Therefore, high resolution is accomplished in GPC by increasing the plate number (N). In an earlier work it was shown that high resolution was attainable in GPC by "brute force," using multiple gel columns in series at high pressure (*3*). This was possible since plate numbers of equivalent columns were additive (*1*). It was further shown that similar results could be attained by multiple use of columns in commercial GPC equipment by means of recycle operation through the reciprocating pumps, GPC columns, and detectors (*9*). The initial work was done with a narrow distribution pair of single species to demonstrate feasibility and to optimize the system for minimal band spreading. However, with broad distributions, a method of "draw off" was needed to prevent overlap, since band width increases with effective column length. Because the inlet from the solvent tank was left open during the recycle operation to prevent cavitation in the pumps, it was also necessary to devise a procedure to flush the inlet tee as well as the interconnecting tubing to prevent reinjection of minute quantities of sample which diffuse into the tees and remain in the interconnecting lines.

Recycle is an effective method of obtaining high resolution at heavy load for preparative separations of both small and macromolecules. Therefore, consideration of the effect of sample load on resolution was studied at both constant concentration and constant sample volume. The effect of flow rate on cycle requirement for resolution was also considered.

EXPERIMENTAL

Apparatus and Procedure

A Waters Associates ALC 100, adapted for recycle operation, was used to fractionate Triton X100, Triton X45, and C_{22-28} alpha olefins.



METHOD OF OPERATION

RECYCLE TO COLLECT SEQUENCE
(V2 IN RECYCLE POS.)

1. TURN PUMP AND RECORDER OFF.
2. FLUSH WITH APPROXIMATELY 15 ml OF SOLVENT BY OPENING & CLOSING V1.
3. TURN V2 TO "COLLECT" POSITION.
4. TURN PUMP AND RECORDER ON.

COLLECT TO RECYCLE SEQUENCE
(V2 IN COLLECT POS.)

1. TURN PUMP AND RECORDER OFF.
2. TURN V2 TO "RECYCLE" POSITION.
3. FLUSH AS "RECYCLE TO COLLECT" SEQUENCE.
4. TURN PUMP AND RECORDER ON.

FIG. 1. Schematic diagram of recycle operation.

(A mixture of paraffins was used to evaluate the flush and draw off procedure described below.) Triton X100 was fractionated through six cycles at 3.1 and 0.5 ml/min. Five columns, 4 ft $\times$ 3/8 in., containing 60 and 100 Å Poragel were used to fractionate the smaller molecules. A schematic diagram of the recycle system and a mode of operation is shown in Fig. 1. The effect of sample load on resolution with recycle operation was investigated, using the new Waters Associates Chromatoprep. This is a new preparative scale liquid chromatograph, designed for recycle operation. The unit can accommodate four columns 2½ in. in diameter by 4 ft in length. Pump capacity is 10 to 120 ml/min. Sample injection is made through the solvent pump. The fraction collector employs an optically indexed 40-port valve controlled by an automatic time based programmer. A schematic diagram of the unit is shown in Fig. 2.

To determine the effect of sample load on resolution, equal parts polystyrene 51K and 10.3K were fractionated at constant volume (100

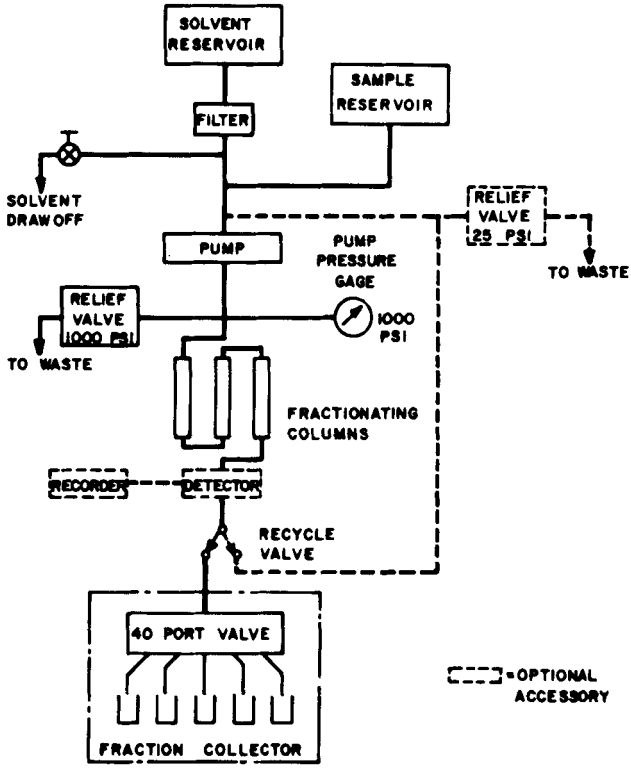


FIG. 2. Schematic diagram of Chromatoprep.

ml), varying concentration as shown in Table 1, and at constant concentration (10 mg/ml), as shown in Table 2. Resolution values were calculated for each cycle, using the equation

$$R = 2\left(\frac{V_2 - V_1}{W_1 + W_2}\right)$$

Resolution at various flow rates was determined at a 1-g load using a 100-ml sample at a concentration of 10 mg/ml.

RESULTS AND DISCUSSION

Peak Spread—System Capacity

Peak width (W) in GPC is proportional to the effective length (L) of the column by the relationship

$$L = 16H\left(\frac{V_r}{W}\right)^2$$

where V_r = retention volume and W is measured at the peak base. With equivalent columns in recycle operation, height equivalent to a theoretical plate, H may be considered constant since, with recycle operation, L and subsequently V_r, is a function of the number of cycles. W increases with the number of cycles (ν) by the relationship

$$W^2 = a\frac{V_r^2}{\nu} = a\frac{\nu_n^2}{\nu} = a\nu$$

or

$$W = W_0\sqrt{\nu}$$

where W_0 is the peak width in milliliters of the first cycle.

Since the contained volume of the closed recycle system is constant as ν is increased, the peak width of the distribution will eventually exceed the volume of the system and peak overlap will occur. To prevent overlap a "draw off" procedure is needed. The procedure is illustrated in Fig. 3, where Triton X45 is run through six cycles. The

TABLE 1

Resolution at Various Sample Loads Using Constant Volume (100 ml)

g	Concentration (mg/ml)	Resolution		
		Cycle 1	Cycle 2	Cycle 3
1.0	10	1.06	1.31	1.47
2.0	20	0.59	1.13	1.29
3.5	35	0.34	0.77	1.14
5.0	50	0.25	0.54	0.92

TABLE 2

Resolution at Various Sample Loads Using Constant Concentration (10 mg/ml)

g	Volume (ml)	Resolution		
		Cycle 1	Cycle 2	Cycle 3
1.0	100	1.06	1.31	1.47
2.2	220	0.70	1.20	1.34
3.5	350	0.39	0.91	1.14

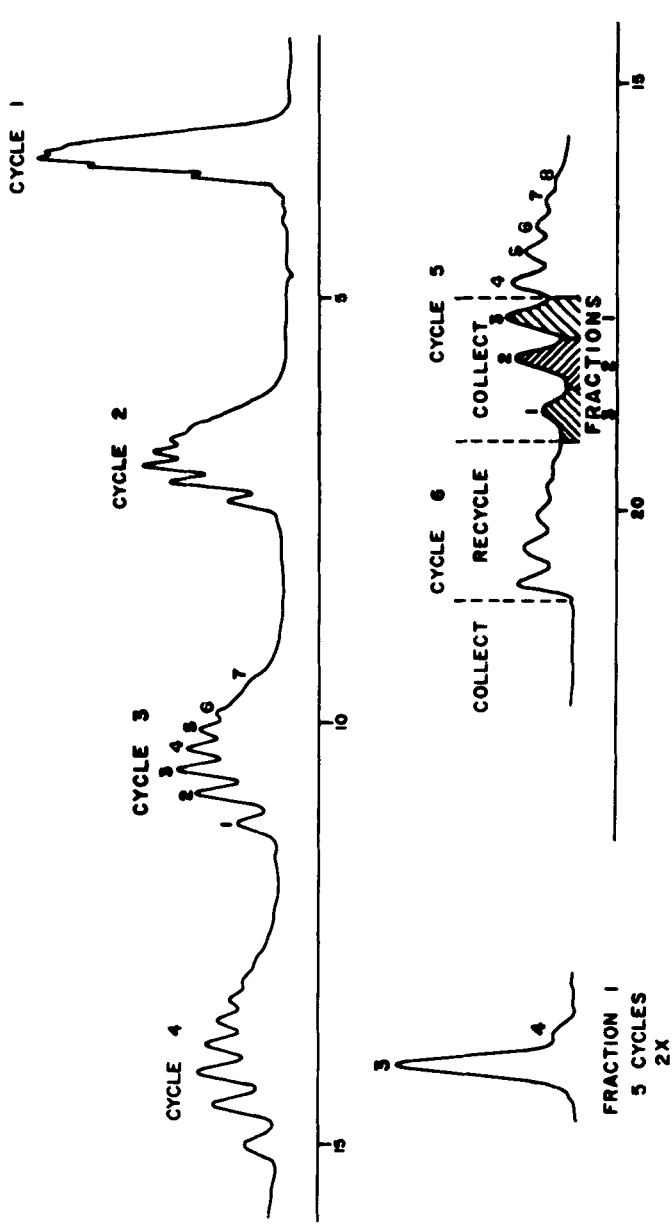


FIG. 3. Effect of cycle number on resolution. Sample: Triton X-45. Concentration: 50%. Injection: 30 μl. Solvent: THF. Flow rate: 0.48 ml/min. Columns: Stragel 60 Å (15 ft).

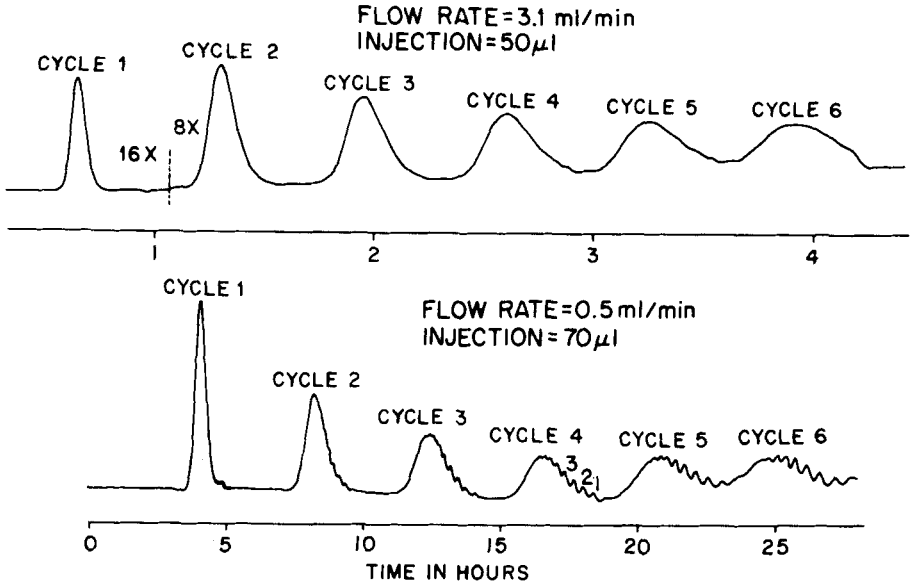


FIG. 4. Effect of flow rate on capacity requirement. Sample: Triton X-100. Concentration: 50%. Solvent: THF. Columns: Styragel 100 Å (8 ft); 60 Å (12 ft).

low molecular weight end is resolved first. Peak 1 could be removed after the third cycle, but in this work was removed along with Peaks 2 and 3 during the fifth cycle, just before overlap occurs from the high molecular weight end of cycle six. (After removal of the resolved low molecular weight species, the high molecular weight portion is recycled additional cycles until resolution is virtually complete.) Also shown is a chromatogram of Peak 3 (Fraction 1) as obtained by recycling five times on the same system. Relative areas indicate that Fraction 1 contained 92% of Peak 3 and 8% of Peak 4.

System Capacity—Flow Rate

Since W increases with ν, it is essential to provide enough system capacity to get the desired resolution at the low molecular weight end of the distribution before peak overlap occurs. This is illustrated in Fig. 4. The top chromatogram of Triton X100 was obtained at 3.1 ml/min; the bottom chromatogram at 0.5 ml/min. At the high flow rate, six cycles were completed in slightly over 4 hr. However, peak overlap resulted before evidence of discrete peaks was observed. At the

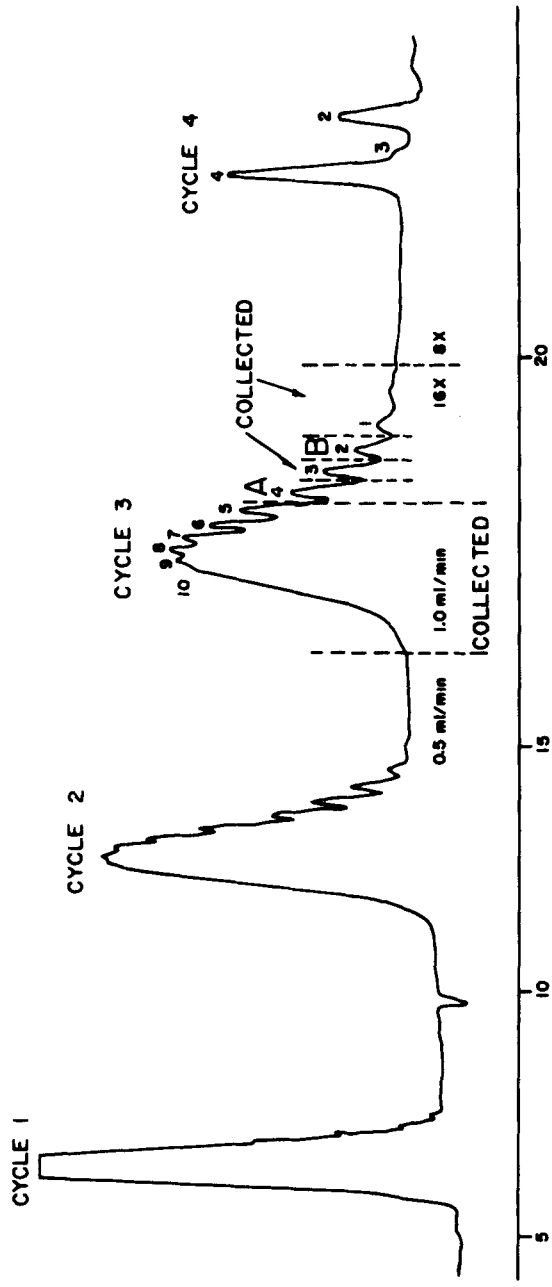


FIG. 5. Effect of system capacity on cycle requirement. Sample: Triton X-100. Concentration: 50%. Injection: 70 μl. Solvent: THF. Columns: Styragel 100 Å (8 ft) and 60 Å (24 ft).

low flow rate a small, low molecular weight peak is observed in the first cycle, which required 4½ hr. Clear evidence of individual species is seen in the second and fourth cycles (19 hr) are completed before overlap occurs. Peaks 1 through 3 could be removed during the fourth cycle, permitting additional cycles without overlap. In this manner additional components from the low molecular weight end of the distribution could be drawn off and recycling continued until resolution is complete and all species are isolated.

By increasing the columns from five (20 ft) to eight (32 ft), fewer cycles are necessary (and somewhat higher flow rates may be used) as is illustrated in Fig. 5. At this condition resolution is observed in the first cycle. Note that virtual baseline resolution of Peaks 2, 3, and 4 was attained on the third cycle. During Cycle 3, Peaks 1, 3, and 5 through 10 were drawn off. Peaks 2 and 4 remained in the system and were run through Cycle 4. High purity material remained, as only a trace of Peak 3 is seen between Peaks 2 and 4.

When the distribution is narrow, fewer columns are required, or more cycles can be run without draw off. The separation of C_{22-28} alpha olefins, using five columns as shown in Fig. 6, was run through five cycles without concern for overlap. Components are removed from both sides of the distribution until only Peak 2 remains.

Effect of Sample Load on Resolution, Using Large Diameter Columns (2½ in.)

The effect of sample load on resolution, as determined at a constant injection volume of 100 ml, is shown in Table 1, and the effect of sample load on resolution at constant concentration (10 mg/ml) is shown in Table 2. A plot of R vs. ν for these data shows that poor resolution is obtained at >2 g with one cycle. However, at three cycles a 5-g load is resolved. At either constant volume or constant concentration, a 3.5-g load shows slightly higher resolution at three cycles than a 1-g load with a single cycle. In terms of through-put, therefore, a 3.5-g load at three cycles would yield more product than three separate 1 g injections run for one cycle. A comparison of constant concentration versus constant volume shows that better resolution is obtained at lower concentration (10 mg/ml) at two cycles. Lower resolution is obtained for the 3.5-g injection at a concentration of 35 mg/ml, probably due to viscosity effects. However, the points merge at three cycles.

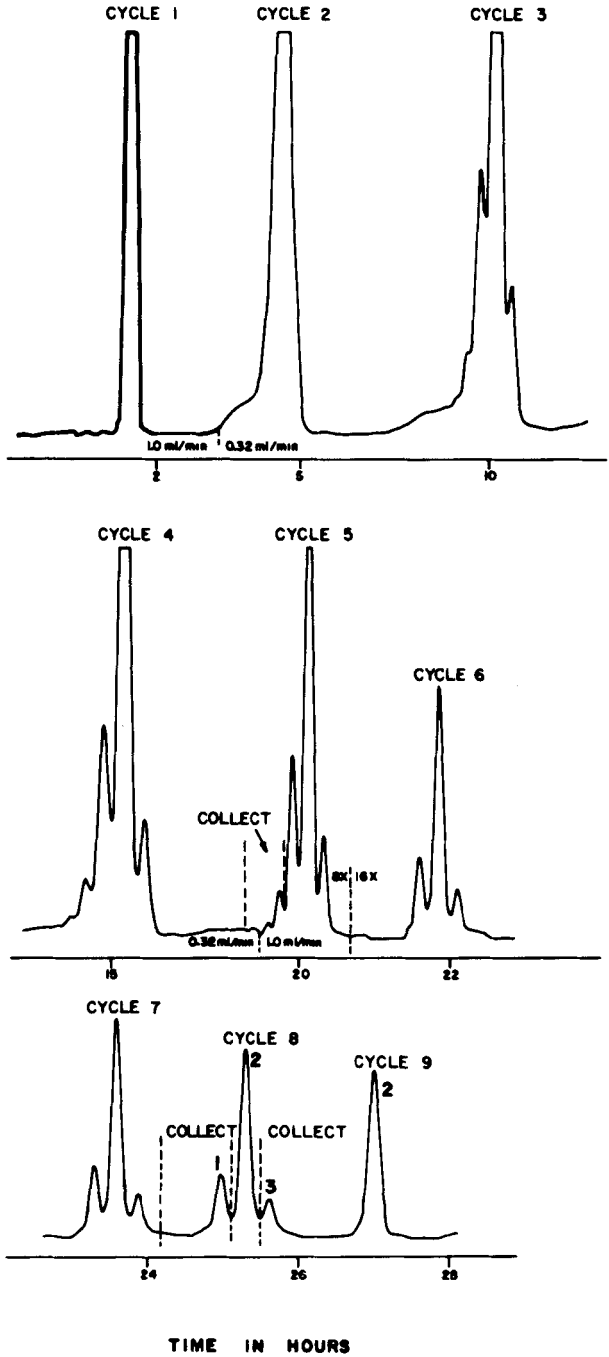


FIG. 6. Effect of sample distribution on capacity requirement. Sample: α-olefin mixture. Concentration: 60 mg/ml. Load: 90 mg. Solvent: toluene. Columns: Styragel 60 Å (15 ft).

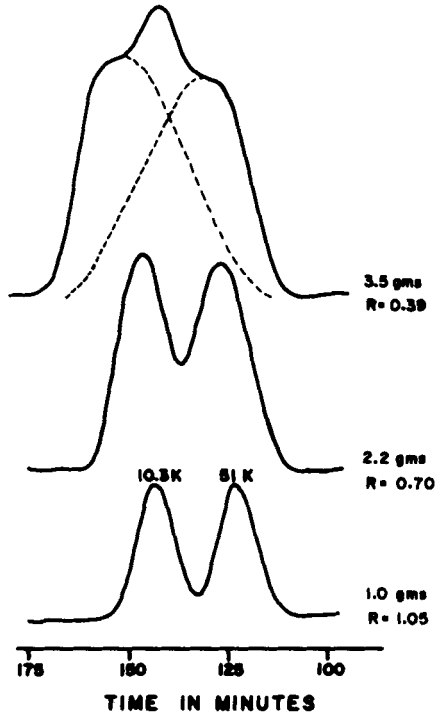


FIG. 7. Effect of load on resolution. Sample: Polystyrene mixture 51K + 10.3K (1 — 1). Concentration: 10 mg/ml. Injection: varied. Solvent: toluene. Flow: 14.4 ml/min. Column: Styragel 2.5×10^4 Å (4 ft).

The effect of load on resolution is shown by Fig. 7, where chromatograms from three different sample loads are shown. The apparent trimodal distribution is the sum of the unresolved bimodal. The effect of improved resolution by recycle for the same sample load is shown in Fig. 8, where in the second cycle the apparent trimodal resolves into a bimodal distribution.

Effect of Flow Rate

The effect of flow rate on resolution for various cycle numbers is shown in Table 3. A plot of these data shows that higher resolution is obtained with three cycles at 121 ml/min than for one cycle at 14.4 ml/min. Therefore, for optimum resolution/time (R/t), the preferred modus operandi should employ maximum load and maximum flow rate, provided adequate system capacity is provided to prevent overlap.

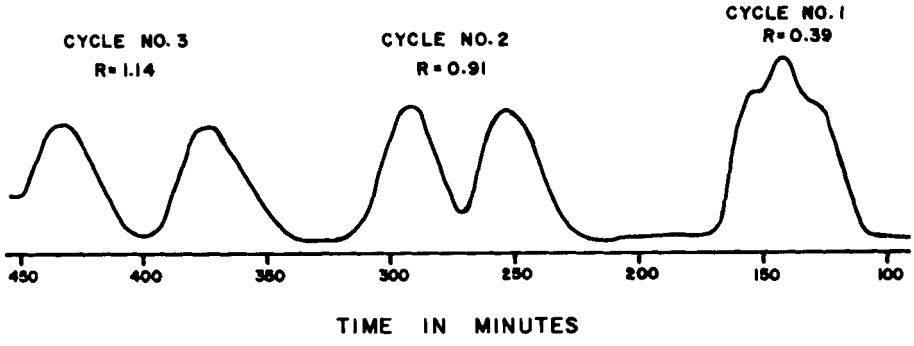


FIG. 8. Effect of recycle on resolution at heavy load. Sample: polystyrene mixture 51K + 10.3K (1 — 1). Concentration: 10 mg/ml. Injection 350 ml. Load: 3.5 g. Solvent: toluene. Flow: 14.4 ml/min. Column: Styragel 2.5×10^4 Å (4 ft).

Adequate resolution of the species at the low end of the distribution should be obtained before overlap occurs, if resolution is to be accomplished on a single injection. Where this is not practicable, it may be necessary to use multiple fractionations, whereby the sample is pre-cut sequentially into progressively more narrow fractions. By this procedure, high resolution can be obtained, with broad distribution over virtually any molecular weight range.

TABLE 3

Resolution at Various Flow Rates

Flow rate	Resolution		
	Cycle 1	Cycle 2	Cycle 3
14.4	1.06	1.31	1.47
29.6	0.95	1.30	1.40
59.1	0.81	1.03	1.11
92.0	0.69	1.04	1.13
121.0	0.57	0.95	1.09

REFERENCES

1. J. C. Moore, *J. Polym. Sci., Part A*, **2**, 835 (1964).
2. K. J. Bombaugh, W. A. Dark, and R. F. Levangie, *Z. Anal. Chem.*, **236**, 443 (1968).

3. K. J. Bombaugh, W. A. Dark, and R. F. Levangie, *Separ. Sci.*, **3**, 375 (1968).
4. J. Cazes and D. R. Gaskill, *Separ. Sci.*, **2**, 421 (1967).
5. J. G. Hendrickson and J. C. Moore, *J. Polym. Sci., Part A-1*, **4**, 167 (1966).
6. J. G. Hendrickson, *Anal. Chem.*, **40**, 49 (1968).
7. J. L. Spell, *Fourth International GPC Seminar*, Waters Associates, Framingham, Massachusetts, 1967.
8. W. Heitz, B. Bömer, and H. Ullner, *Makromol. Chem.*, **121**, 102 (1969).
9. K. J. Bombaugh, W. A. Dark, and R. F. Levangie, *J. Chromatogr. Sci.*, **7**, 42 (1969).

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