A New Instrument for Preparative Gel Permeation Chromatography

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Synopsis

A preparative liquid-phase chromatograph was built for the purpose of obtaining sufficiently large quantities of very narrow fractions of different polymeric species, such as polystyrene, PVC, polybutadiene, and polyethylene. This apparatus allows the fractionation of approximately 20 g of polymer per day; the fractions so obtained have polydispersities of about 1.1 over a very wide range of molecular weights. Polydispersities of less than 1.01 were obtained after recycling of the sample.

INTRODUCTION

While gel permeation chromatography has evolved as an indispensable tool in the past few years for the characterization of high polymers, the possibilities of this method for the preparation of fractions have not yet been sufficiently explored. Analytical instruments (Waters Associates, Framingham, Mass.) presently available, employ a sample weight of about 5 mg, which is small to carry out a physicochemical study of the fractions.

We therefore undertook to construct an instrument suitable for fractionating samples of the order of 1 g per injection.

DESCRIPTION AND CHARACTERISTICS OF THE PREPARATIVE CHROMATOGRAPH

A preparative chromatograph can be divided into five components (Fig. 1) which are (1) the pumping system; (2) the sample injector; (3) the columns; (4) the detector; (5) the fraction collector. We shall not describe in this paper the pumping system and the detector, which are directly based on the Waters analytical instrument.

Introduction of Sample

The introduction system which we have constructed consists of a sampling loop, made of a tube 6.4 mm in diameter and having a volume of 100 ml, and of five two-way electrovalves, four of which are normally closed and one of which is normally open. Figure 2a shows the assembly of this system: in steady state, electrovalves 1, 2, 4, and 5 are closed,

electrovalve 3 is open, and the sample reservoir is under slight argon pressure.

Introduction of the sample requires two successive operations: filling of the loop and the introduction itself. The loop is filled by energizing electrovalve 1 and 5 which, upon opening, allow passage of the solute into the loop (Fig. 2b).

The loop being filled, its content is introduced into the columns by energizing electrovalve 3, which closes, and electrovalves 2 and 4, which open. Under these conditions, the loop is swept by the solvent and the solute is pushed into the columns (Fig. 2c).
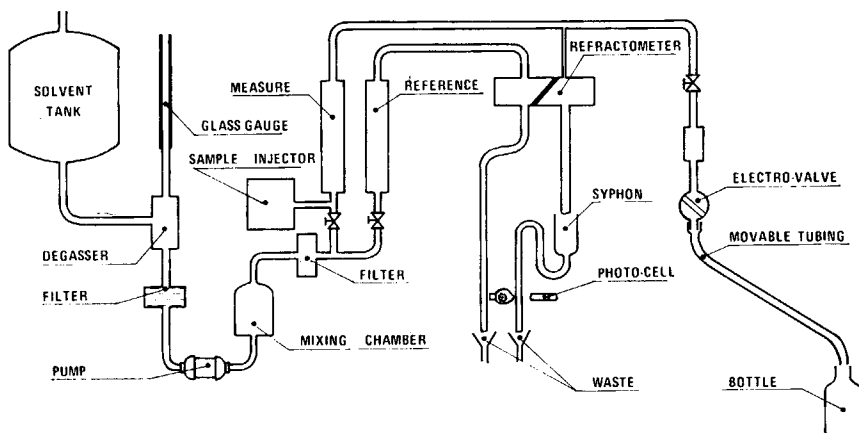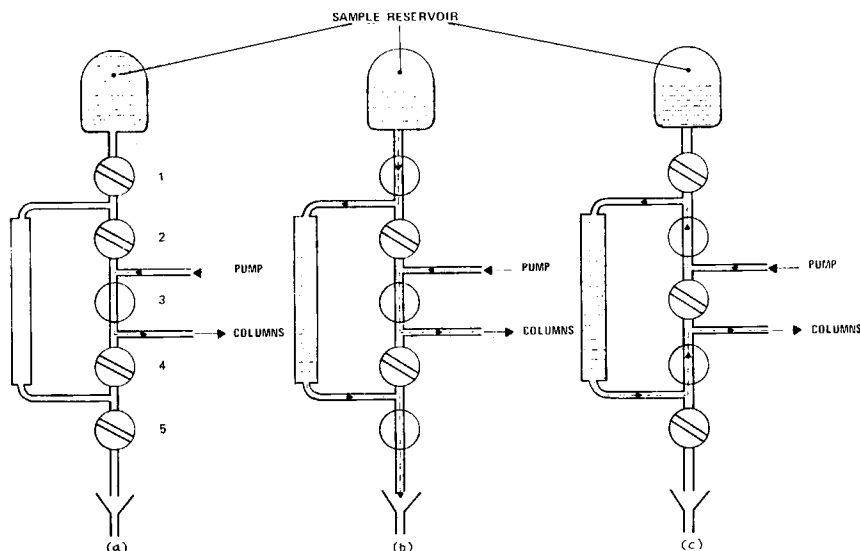

Fig. 1. Block diagram.
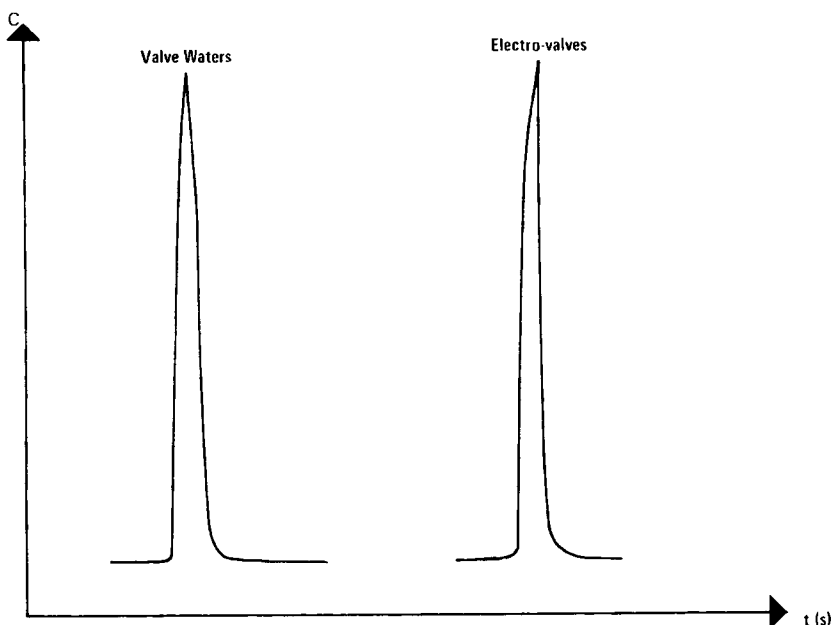

Fig. 2. Injection system.

Fig. 3. Comparison of dispersion introduced by the two injection systems.

The dispersion introduced by this injection system was compared with that provided by the manual valve in the Waters analytical instrument, by coupling the valve directly to the detector without going through the columns. Figure 3 shows the peaks obtained under these conditions. For manual injection, the flow rate was set at 1 ml/min, whereas it was set at 50 ml/min for the electrovalves. (It should be noted that in this case, not all the liquid passes into the refractometer due to a valve which provides for adjustment of its flow rate to a value identical with that used in analytical chromatography, Fig. 1). It can be seen that for an identical time of introduction (30 sec), the peak obtained from the preparative system is narrower. Therefore we can say that this system is quite satisfactory having, in addition, the advantage of being easily adaptable for automation.

The Columns—Packing material

Influence of Particle Size

Depending on the nature of the polymers to be fractionated, it may be necessary to change solvent and temperature conditions. Accordingly, porous silica beads manufactured by Pechiney-Saint-Gobain under the name Spherosil were chosen in preference to polystyrene gel. The rigidity of silica beads induces a very low pressure drop effect and especially a high insensitiveness to solvent and temperature changes.

The characteristics of various Spherosils we have used are summarized in Table I. Each type of silica is provided as beads having a diameter ranging

from 100 to 200 μ. De Vries et al.[1] have shown that the efficiency of a column is a function, for a given average particle diameter, of the spread in particle size distribution around this average value, the most satisfactory results corresponding to the narrowest distribution. We have reexamined this problem in order to determine whether a careful sieving was really indispensable. Using analytical columns 8 mm in diameter and 122 cm in length, the number of theoretical plates was measured for heptane (injection of 0.5 ml of a 0.25% toluene solution, flow rate 1 ml/min). The columns were dry packed while applying axial vibrations; this packing method gives reproducible results, the average values of which are summarized in Table II. The dispersion of the results is about 7%.

TABLE I
Characteristics of Spherosil Silical Beads

Type of Spherosil	Pore volume, ml/g	Pore diameter, Å
A	0.7–1.0	<100
B	0.7–1.0	100–200
C	0.5–0.7	200–400
D	0.5–0.7	400–800
E	0.5–0.7	800–1,500
F	0.3–0.5	~3,500
G	0.8	~16,500

TABLE II
Influence of Particle Size on the Number of Theoretical Plates N

Particle size, μ	N
100–125	1,400
125–160	1,000
160–200	600
100–200	1,300

It can be seen that the number of theoretical plates decreases very rapidly as the average particle diameter increases, which is in agreement with the results of Le Page, Beau, and De Vries.[2] However, the most interesting fact is that very few theoretical plates are lost by using particles with a size distribution ranging from 100 to 200 μ. This observation makes it possible to use the Spherosils as supplied by the manufacturer.

Resolution of Spherosil Columns

The simplest method for determining the range of selectivity of a set of columns in gel permeation chromatography consists in drawing the curve shown in Figure 4 in which the logarithm of the molecular weight of different monodisperse samples is plotted against elution volumes. It is obvious that the smaller the slope, the greater the resolution of the system.

Five analytical columns were filled with gels B, C, D, E, and F, and, using polystyrene standards marketed by Waters, curve (a) shown in

Figure 4 was plotted.   Comparing this curve with the calibration curve (b) for five Styragel columns designated by the manufacturer as 10^6, 10^6, 3.10^5, 3.10^4, and 700 Å, it can be seen that the curve obtained with Spherosil is linear over the entire range of molecular weights from 5,000 to 2.5 millions and especially that its slope is definitely smaller than that of curve (b). It seems, therefore, at first sight that Spherosil should have a definitely higher separating power than Styragel.
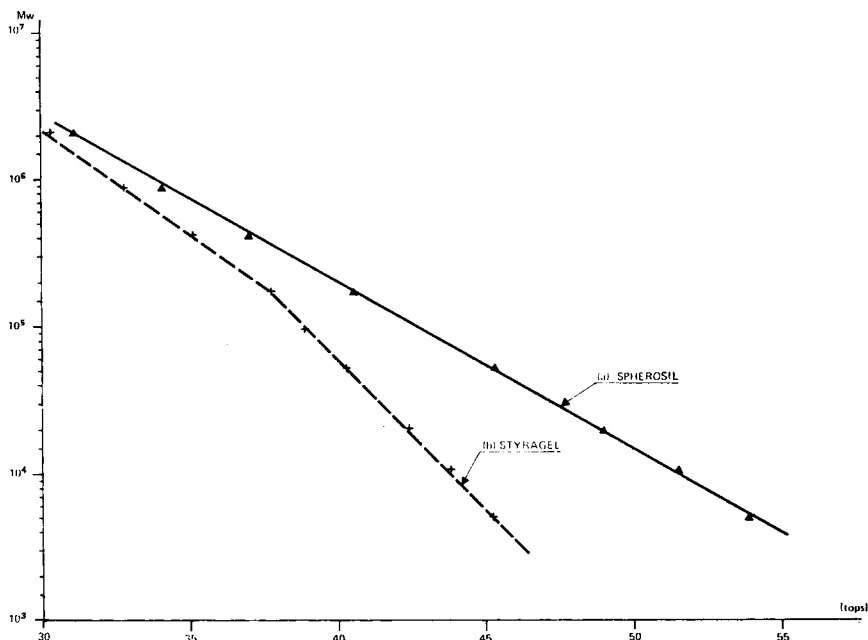

Fig. 4. Calibration curves obtained from two packing materials.

However, in order to correctly estimate the separating power, it is indispensable to take into consideration the width of peak corresponding to a monodisperse system, i.e., the number of theoretical plates.   It is therefore necessary to characterize a set of columns by a resolution index R_s which takes into account both the slope of the calibration curve and the number of theoretical plates.   In accordance with Altgelt's notation,[3] the following equation applies:

$$R_s = \frac{\Delta V_e}{(w_1 + w_2)/2}$$

where ΔV_e is the difference in the elution volume of two standards and $(w_1 + w_2)/2$ is the average width of the two peaks on the baseline.   As soon as R_s becomes less than unity, there is an overlap of the two chromatograms and the separation is no longer perfect.

The resolution index was calculated for two molecular weight standards having a molecular weight of 411,000 and 98,200:

For Styragel: $R_s = 1$

For Spherosil: $R_s = 0.95$

The two values are practically identical. The Spherosil columns have fewer theoretical plates (for styragel $N = 1200/ft$) but show a greater variation of the elution volume as a function of molecular weight, thus making them equivalent to the Styragel columns as far as their resolving power is concerned.

Efficiency of Preparative Columns

The development of preparative chromatography, at present, is still limited by a fundamental difficulty, which is the loss in efficiency of the columns when attempts are made to increase their diameter, particularly above a value of 15 mm.[4]

This loss in efficiency is commonly attributed to packing heterogeneities and, in particular, to a segregation of particles upon filling, the larger ones having a tendency to accumulate near the wall thus leading to a radial permeability gradient.

The good results obtained with the packing method described by Bayer et al.[5] seem to confirm this hypothesis. Those authors indeed obtain a substantial gain in efficiency by applying axial vibrations to the column which tend to provide a segregation such that the heavy particles move toward the extremity of the column, the resulting longitudinal variations in velocity being much less harmful.

Taking this result into account, our columns were packed while applying axial vibrations only. The columns consisted of stainless steel tubes 122 cm long and 60 mm in diameter and comprised at each extremity a connecting flange. In order to correct for the curvature of the elution front, the connecting flange was made conical and a sintered metallic component with a 20-μ porosity was mounted on its base, making it possible to retain the gel in the column and providing a good distribution of the liquid flow.

The efficiency of these columns was measured by injecting 25 ml of a 0.25% heptane solution in toluene, the flow rate being set at 50 ml/min. Under these conditions, the number of theoretical plates N ranged very reproducibly from 3,000 to 3,500 per column. It seems surprising that the value of N was more than twice that obtained for analytical columns, which is contrary to the result currently observed, i.e., a loss of efficiency with increasing diameter. This phenomenon deserves to be studied more systematically, and it would also be valuable to determine the influence of the junction angle of the cone connecting the columns to the tubing as well as that of the packing technique.

Fraction Collector

The fractions were collected in 20 10-liters bottles arranged in a circle. The liquid coming out of the columns arrives at the center of this circle, and a tube, mounted on a movable arm, directs it into the various bottles. The electrovalve shown in Figure 1 cuts the flow during passage of the arm from one bottle to the next, and a dead volume of 30 ml, at atmospheric pressure, prevents the pressure from rising in the upstream circuit when the electrovalve is closed.

Automation

The various operations involving sampling, introduction, and advance of the collector are carried out automatically using a 24-channel Coreci programmer: a motor causes the unwinding, at constant velocity, of a suitably punched card and the appearance of a hole on the channel controls an operation. Both extremities of the card are connected to each other, and the time necessary for it to carry out one revolution corresponds to the output time of the useful part of the chromatogram.

EXPERIMENTAL RESULTS

Fractionation of Polystyrene PSL 134

The first results were obtained on a polystyrene PSL 134 sample which served to check the instrument and evaluate its possibilities. This sample, which was already studied previously in this laboratory, had the following characteristics:

$$\bar{M}_w = 255,000$$

$$\bar{M}_n = 127,000$$

$$\frac{\bar{M}_w}{\bar{M}_n} = P = 2.$$

For this first fractionation, a set of four columns was used, each containing gel B, C, D, or E, which corresponds to a porosity range of 100 to 1,500 Å. Using a 50 ml/min flow rate, 100 ml of solution at a polymer concentration of 1%, or 1 g of polymer, was introduced. The fractions collected in the various bottles were concentrated using a rotating evaporator, and were subsequently analyzed by analytical GPC, the instrument being provided with five Styragel columns designated as 10^6, 10^6, 3.10^5, 3.10^4, and 700 Å.

If the chromatograms of the fractions are Gaussian and if the logarithm of the molecular weight is a linear function of the elution volume, the distribution of molecular weights within a given fraction can be represented by the normal generalized logarithmic function

$$W(M) = \frac{1}{\sigma\sqrt{2}} \frac{1}{M} \exp - \frac{1}{2} \frac{\log M/M_0}{\sigma_M}.$$

In this expression, σ_M represents the standard deviation and M_0 is the molecular weight at the apex of the peak, and it can be shown that[6]

$$\bar{M}_w = M_0 e^{\sigma_M{}^2/2\pi}, \quad \bar{M}_n = M_0 e^{-\sigma_M{}^2/2} \text{ and } P = e^{\sigma_M{}^2}.$$

However, the quantity σ_M is different from the standard deviation which is calculated from the chromatogram: the latter is widened as a result of axial dispersion. Hendrickson[7] has shown that if the causes of the widening as assumed to be independent, the variances contributing to the total variance can be added. If we let $\sigma_t{}^2$ be the total variance (experimental), and $\sigma_M{}^2$ and $\sigma_D{}^2$ the variances due to the molecular weight distribution and axial dispersion, the following applies:

$$\sigma_M{}^2 = \sigma_t{}^2 - \sigma_D{}^2. \tag{1}$$

(In order to go from the half-widths measured at the level of the inflection point of the chromatogram at σ_t, the relation $\sigma = SB$ is used, where B is the slope of the calibration line, $\log M = A - BV_e$.)

The variation of $\sigma_D{}^2$ as a function of the molecular weight was determined using Waters polystyrene standards whose polydispersity P, and therefore their corresponding standard deviation σ_M, are known. Applying relation (1), it is possible to determine the quantity σ_D for each standard.

A simple method of calculation is thus available which allows us to compare polydispersities. When the chromatograms are not symmetrical, the presence of a dissymmetry is to be specified for it makes this method of calculation less reliable.

Twenty fractions of polystyrene PSL 134 were collected, and the results obtained with six of them only are summarized in Table III, for they are characteristic of the variation of the polydispersity P with the molecular weight of the fraction.

It can be noted that the polydispersity of the central fractions is lower than that of the end fractions. Examination of the chromatograms indeed shows that the central fractions are symmetrical, whereas the first show a tail of low molecular weights and the latter, a tail of high molecular weights. These tails are probably due, in the first fraction, to insufficient separating

TABLE III
First Fractionation of PSL 134[a]

Fractions	$M_0 \times 10^{-3}$	P
2	504	1.28
8	366	1.20
10	200	1.21
11	185	1.21
14	160	1.21
16	55	1.53

[a] Columns: E-D-C-B; flow rate: 50 ml/min; concentration: 1%; quantity injected: 100 ml; solvent: toluene; room temperature; volume of the fractions 250 ml.

power in the high molecular weight range and in the latter fractions, to adsorption of the highest molecular weights on Spherosil, this adsorption increasing their elution volume.

In order to increase resolution, the same sample was refractionated, using this time the set of columns B/C, D, E, E, F which is more efficient in the high molecular weight range.

Tetrahydrofuran (THF) was also used as the solvent; its polarity being greater than that of toluene, adsorption of polystyrene is lower. The polydispersity of the fractions collected was determined, on the one hand, according to the analytical GPC method already used, and on the other hand, using the absolute values of $\bar{M}_w$ and $\bar{M}_n$ obtained by light scattering and osmometry. These various results are collected in Table IV, in which an asterisk shows those fractions in which a tail was present.

It is noted, first of all, that except for fractions 9, 12, 13, and 14, the number-average molecular weights $\bar{M}_n$ are consistently higher than the corresponding weight-average molecular weights $\bar{M}_w$. However, the deviation between $\bar{M}_w$ and $\bar{M}_n$ is not higher than 11%, which is compatible with the 5% accuracy attributed to these absolute measurements. The lack of accuracy of these measurements is confirmed by an IUPAC report[8] and shows that it is futile to try to determine, using this type of method, the polydispersity of narrow fractions.

TABLE IV
Second Fractionation of PSL 134[a]

Fractions	Weight, mg	Cumulative percentage by weight	$\bar{M}_{wDDL} \times 10^{-3}$	$\bar{M}_{nOsmo} \times 10^{-3}$	$M_0 \times 10^{-3}$	P_{GPC}
1*	380.1	100	702	—	620	1.17
2	292.4	97.02	540	—	525	1.10
3	431.5	94.73	466	—	430	1.13
4	624.5	91.35	432	—	380	1.13
5	823.0	86.45	369	—	325	1.13
6	1029.7	80.00	328	366	275	1.13
7	1217.0	71.93	245	258	235	1.15
8	1341.1	62.40	200	213	200	1.14
9	1410.0	51.89	167	164	170	1.16
10	1338.9	40.84	135	144	140	1.16
11	1168.9	30.35	96.7	115	108	1.17
12	939.8	21.19	80.7	80.4	84	1.18
13	669.1	13.82	71.1	60	64	1.17
14	465.3	8.58	48.2	43	45	1.17
15	296.1	4.93	—	31.7	32	1.19
16	180.6	2.61	—	20.2	30.5	1.18
17*	95.3	1.20	—	15	13	1.25
18*	58.0	0.45	—	—	10	—

[a] Columns: B/C, D, E, E, F; flow rate: 25 ml/min; concentration: 1%; quantity injected: 100 ml; solvent: THF; room temperature; volume of the fractions: 250 ml.

Although a very low polydispersity was not reached, since it ranges from 1.1 to 1.2, the fractions show a symmetrical pattern in a very wide range of molecular weights, and slight tails are noted only for fraction 1 with high molecular weights and fractions 17 and 18 with low molecular weights. All adsorption phenomena have therefore not been entirely eliminated.

Recycling

With the instrument provided with the set of columns B, C, D, E and in the presence of toluene (conditions which, let us recall, were not the best adapted for fractionation of PSL 134), the variation in polydispersity of the fractions with concentration of the solution injected was studied, again for this sample (Table V).

TABLE V
Influence of Concentration on Polydispersity

$M_0 \times 10^{-3}$	Polydispersity P					
	1%	2%	3%	4%	5%	6%
366	1.20	1.21	1.20	1.20	1.21	1.24
175	1.21	1.22	1.27	1.29	1.30	1.37
140	1.19	1.21	1.24	1.29	1.30	1.34

Polydispersity increases with concentration; however, even at a concentration of the order of 6%, which corresponds to the fractionation of 6 g of polymer, it is still acceptable, at least for the central fractions, since its value is approximately 1.3. This is very advantageous since it makes possible to appreciably increase the quantity of polymer fractionated in a given experiment without unduly decreasing the efficiency of the fractionation.

On the basis of these results, 20 g of polystyrene PSL 134 at a concentration of 5% were fractionated, which made it possible to obtain central fractions in rather large quantities to allow two refractionations. Thus, fraction F 10 with a polydispersity of 1.32 was refractionated, and the polydispersity of subfraction F'10 was equal to 1.12. Fraction F'10 was refractionated again and the fraction obtained, F"10, had a polydispersity of 1.07. All of these subfractionations are summarized in Table VI.

TABLE VI
Recycling

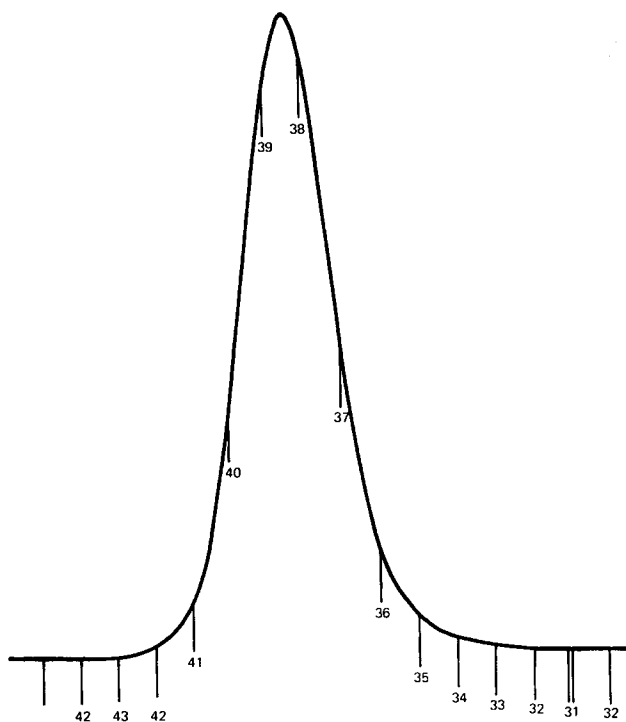| Fraction	P
PSL 134	2
F10	1.32
F'10	1.12
F"10	1.07

Fig. 5. Chromatogram of polystyrene fraction, $M_0 = 41{,}000$.

The value of recycling a sample immediately upon its exit from the last column is evident. The operation can be carried out automatically on our preparative chromatograph, provided several minor modifications, which we propose to put into effect, are introduced.

Preparation of Monodispersed Fractions

When narrow fractions are to be prepared in a given molecular weight range, it is essential to start with a polymer showing, in its distribution curve, a relatively substantial abundance of molecular weights in that range.

For low molecular weight fractions, it is possible to obtain, in certain cases, a polydispersity of the order of 1.01. Thus starting with a product having a molecular weight $\bar{M}_w = 274{,}800$ and a polydispersity of 6, and using the set of columns B, B/C, C, D, and E, which is efficient in the low molecular weight range, a fraction of molecular weight $M_0 = 41{,}000$ and polydispersity 1.01 (Fig. 5) was obtained.

The problem is more difficult for higher molecular weights. In trying to obtain fractions which might be used as standards, 20 g of the already studied PSL 134 was used as a starting point, 2 g of which was selected by fractional precipitation in a first fraction having a molecular weight of $\bar{M}_w = 476{,}000$ and a polydispersity of 1.58. This fraction was fractionated
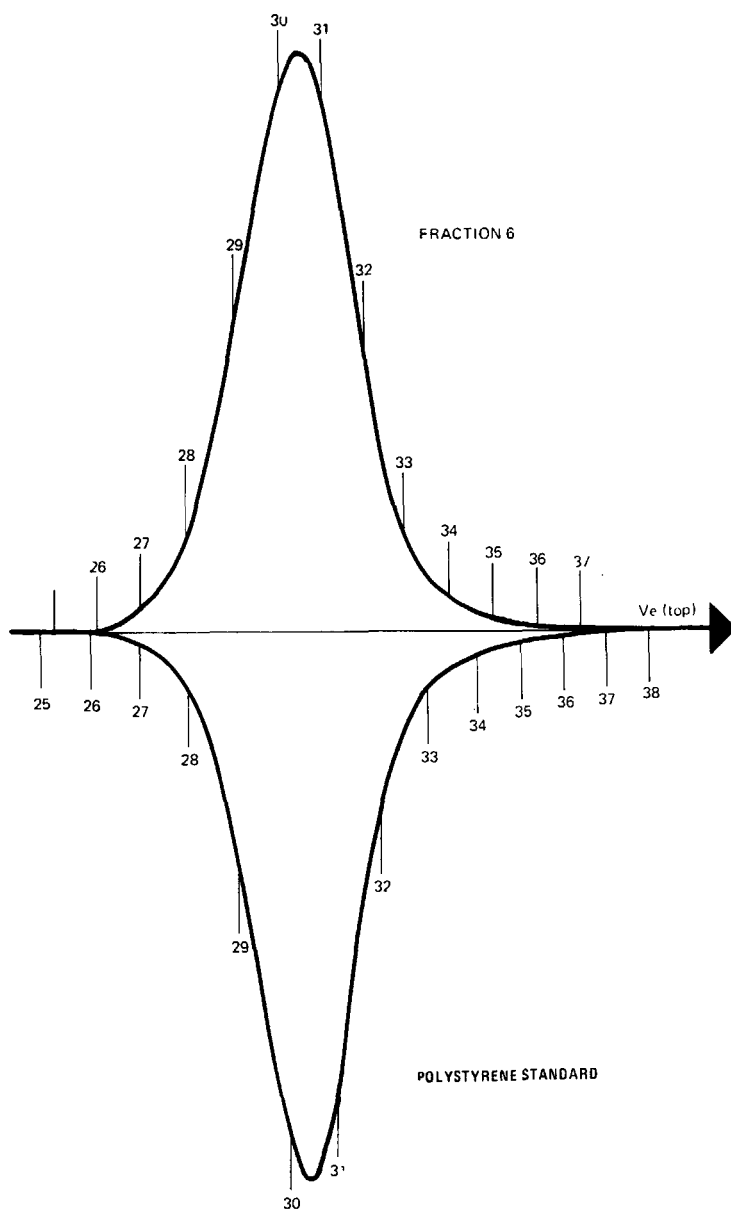
Fig. 6. Comparison of chromatograms of fraction 6 and Waters standard, $M_0 = 867,000$.

on the preparative chromatograph provided with three columns, E, E, and F, which were selective in the high molecular weight range. The fractions obtained had a polydispersity of the order of 1.09 for molecular weights of about 1 million.

Figure 6 shows the chromatograms of fraction 6 and of the Waters polystyrene standards with a molecular weight of 867,000. These two fractions

are directly comparable, for they have the same elution volume; it is noted that they also have the same polydispersity.

It can therefore be seen that it is very easy to obtain narrow fractions in a large molecular weight range and in large quantities; and the instrument, provided with only three columns, can fractionate approximately 20 g of polymer daily under these conditions. This result is also given by Waters.[9,10]

Fractionation of Polybutadiene and Poly(vinyl Chloride) (PVC) Samples

In order to check that the method is applicable without any further problems to polymers other than polystyrene, two samples of polybutadiene and PVC were fractionated whose characteristics are given, with the results, in Table VII.

TABLE VII
Fractionation of Polybutadiene and PVC[a]

PVC[b]			Polybutadiene[c]		
Fraction	$M_0 \times 10^{-3}$	P	Fraction	$M_0 \times 10^{-3}$	P
6	145	1.09	2	350	1.19
7	118	1.13	3	270	1.18
11	66	1.15	6	160	1.13
12	55	1.14	8	130	1.15
15	22	1.13	12	80	1.24
16	16	1.15	13	67	1.30

[a] Columns: B/C, D, E, E, F; flow rate: 50 ml/min; concentration: 1%; quantity injected: 100 ml; solvent: THF; room temperature; volume of fractions: 250 ml.
[b] $\bar{M}_w = 89,700$; $\bar{M}_n = 37,000$; $P = 2.4$.
[c] $\bar{M}_w = 364,100$; $\bar{M}_n = 137,800$; $P = 2.64$.

The results are entirely comparable with those obtained for polystyrene, and this is not one of the least contributions of this technique, for the preparation of fractions of such products has always raised problems which have never been completely satisfactorily solved.

CONCLUSIONS

This study has shown that gel permeation chromatography extrapolated to a preparative scale is a relatively simple technique to use and does not involve any insurmountable difficulties. Indeed, it provides for the fractionation of large quantities of product of up to 20 g daily if a polydispersity for the fractions of the order of 1.1 is acceptable. The quantities thus obtained are sufficient already now for rheological experiments on a laboratory scale.

On the other hand, if fractions with a polydispersity about 1.01 are desired, it is necessary to recycle the sample, which amounts to increasing the

length of the column corresponding to each type of gel.   For a polydispersity of 1.01, three passages are sufficient, and it is possible to fractionate further up to 10 g of product daily, which gives quantities of the order of 0.5 g of such fractions.

However, if the efficiency of our packing method is confirmed for columns with a diameter greater than 60 mm, these quantities treated on a daily basis might be largely increased.   Indeed, it is known that they vary as a function of the square of the column diameter.

Finally, this method is applicable to most polymers, and since the instrument we have set up can function up to a temperature of 150°C, it should be possible soon to prepare very narrow polyethylene fractions; for it is known that for this product difficulties due to adsorption are never encountered.   This is of considerable value, for it is the only method available to characterize in a precise fashion the extent of branching of high-pressure polyethylenes.   It is therefore believed that this technique will undergo substantial developments during the next few years.

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