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PRECIPITATION CHROMATOGRAPHY FRACTIONATION: THE EFFECT OF THE RATIO SUPPORT WEIGHT/SAMPLE SIZE

J. M. Alvariño, María Luz Hernández, L. Lain,
M. de Renobales* and Alicia Torre
Departamento de Química Física, Facultad de Ciencias, Universidad de Bilbao,
Bilbao, Spain

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Abstract—The effect of the gravimetric ratio, packing beads/polymer (*r*), in the chromatographic fractionation of polystyrene has been studied. Anionic polystyrene of bimodal molecular weight distribution was fractionated in three experiments with *r* values of 25, 50 and 130; a value of about 50 provided the most efficient separation. A GPC self-consistency analysis of this fractionation has been done with very satisfactory results. On the other hand, GPC analysis could not show any significant broadening of the molecular weight distributions of the fractions in going from *r* values of 130 down to 25.

INTRODUCTION

Molecular weight distributions (MWD) of polymer samples are determined by the mechanism and kinetics of the polymerization reactions. A macromolecule is directly related to the elementary processes involved in its formation [1]. The best way to find the MWD is to perform a fractionation. Perhaps the most efficient fractionation method is precipitation chromatography, originally introduced by Baker and Williams [2]. The technique is unfortunately very laborious, although its great resolution makes it very suitable for detailed kinetic studies, particularly in anionic polymerization [3].

As an approach to the best experimental conditions we now report the results of three such fractionations performed by varying the amount of polymer deposited on a given weight of inert support, all other variables being held constant. References to earlier work on optimization of experimental conditions have been given [4]. We have fractionated a sample of anionic polystyrene of bimodal MWD in an attempt to investigate the resolving efficiency of the column under various conditions. With these experiments we try to decide which is the best ratio of inert support weight to polymer weight for obtaining the real MWD. To interpret the observed differences, we have also performed GPC analysis of some of the fractions.

EXPERIMENTAL

Products

A sample of anionic, bimodal MWD polystyrene was fractionated [5]. The viscosity average degree of polymerization was 719; the maxima of the two peaks corresponded, according to GPC analysis, to values of 423 and 960.

Conventionally purified benzene and methanol were used as solvent and non-solvent respectively. Toluene (Merck, reagent grade) was used as solvent for the viscosimetry measurements of the fractions without further purification.

The glass beads (100 μ nominal diameter) for packing of the column were repeatedly washed with conc. nitric acid and distilled water until no acid reaction was detected, and then with benzene and methanol. Finally they were dried at 110°.

Fractionation

The column (stainless-steel, 150 cm long and 2.5 cm inner diameter) has been described [6]. It was very carefully packed with the glass beads, a process performed over several days in order to achieve as compact a packing as possible. The top and bottom parts of the column were easily thermostatted, thus allowing a linear gradient of temperature to be established along the column. A 50°/20° (top/bottom) temperature gradient was used. The composition of the eluant, expressed as volume fraction of solvent, ranged from 0.50 at the beginning of every experiment to 0.72 at the end, i.e. we used a 50/72 eluting gradient. The final composition was asymptotically reached. These values were taken from the literature [6] as well as from our own experience. About half a liter of pure solvent was eluted at the end of each fractionation to extract the last fraction. The flow rate was about $25\,\text{cm}^3 \times \text{hr}^{-1}$. The duration of a fractionation ranged from 12 to 15 days.

Special attention was paid to the coating procedure. A given amount of glass beads was taken out of the column, dried and weighed. The weight of polymer to be fractionated was dissolved in benzene overnight and then the glass beads were poured into the polymer solution with careful, gentle stirring. The solvent was then evaporated. The entire operation was carried out slowly and with constant stirring to give the most uniform coat of polymer possible. Finally, a solid, hard crust was obtained. Methanol was added to separate the glass beads and apply them to the column. The collected fractions were redissolved in benzene, freeze-dried, weighed and characterized by viscometry and GPC. Weighing was carried out in an air thermostat with temperature constant to $\pm 0.2°$. Air humidity was also constant within 5%.

Viscosity measurements were performed in toluene at $20 \pm 0.1°$ in an Ostwald viscometer. The extrapolation and viscometric equations of Schulz–Blaschke [7] and Meyerhoff [8] respectively were used. The integral MWD's were constructed according to Schulz [9], but no last fraction

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* Present address: Division of Biochemistry, University of Nevada, Reno 89507, U.S.A.

corrections were applied. The GPC measurements were carried out on a liquid chromatograph (Waters Ass., Model ALC/CPC 202). Four μ-Styragel (Waters Ass.) filled, 1-ft long columns with nominal porosities of 10^6, 10^5, 10^4 and 10^3 Å were used. They were operated at $22 \pm 1°$. Conventionally dried tetrahydrofuran at a flow rate of $1.0\,\text{ml} \times \text{min}^{-1}$ was used as solvent. $80\,\mu\text{l}$ of a $0.25\%\,\text{g} \times \text{ml}^{-1}$ sample were injected every time. Polystyrene standards from Waters Ass. and Pressure Chemicals were used.

RESULTS AND DISCUSSION

Three fractionations F-1, F-2 and F-3 were performed. The experimental conditions common to all of them have been previously indicated, viz. a 50/20 temperature gradient and a 50/72 eluting gradient. The intrinsic viscosity of the unfractionated polymer was $0.396\,\text{dl} \times \text{g}^{-1}$. Other initial data and some results are collected in Table 1.

The agreement between values of both $\bar{P}_w$ and $\bar{P}_n$ from the various experiments are satisfactory. On the other hand both r_m and r_η are very close to 1, proof of the internal selfconsistency of every fractionation. However, a point deserving attention is the fact that r_m is greater than 1 in every case; usually in fractionations $r_m < 1$ because of loss of polymer. We explain our finding as due to a small quantity of glass beads going through the filter in the valve at the bottom of the column. Indeed, the glass beads had a distribution of sizes; about 8% of their weight corresponds to diameters $< 75\,\mu$. Unfortunately, there was no sieving before the column was loaded with the support, so this point is open to discussion. It does not, however, affect our major conclusions.

A better insight may be gained by MWD's plots. Figure 1 shows the integral distribution curves corresponding to the three fractionations performed.

The bimodal MWD of this anionic polystyrene allows investigation of the fractionation efficiency or resolving power of the column and comparison of the results of F-1, F-2 and F-3. This peculiar MWD is due to the one-side killing of the bifunctional, electron-transfer initiator at the initiation step [5,10]. The elution curve (solute concentration vs elution volume) provides us with a better means of inspecting the efficiency of the fractionation than the integral MWD curve. Figure 2 shows the reduced, or normalized, elution curves of F-1, F-2 and F-3.

The normalization is done as follows: first, the actual F-1 curve is drawn. Then, the ordinates of the actual F-2 and F-3 curves are divided by the quotient between the values of the F-2 and F-3 solute concentrations at the principal maximum and the same quantity for F-1. Likewise, a reduction of the abscissae of F-2 and F-3 was performed. The elution volume for which the solute concentration attained a vanishingly low value (we adopted $0.01\,\text{g} \times \text{l}^{-1}$ as such concentration) played in this case the role of the solute concentration at the principal maximum. The results for the graphical calculation of $\int_0^\infty c\,dV_E$ for the three curves as derived from Fig. 2 agree within 5%. This is taken as proof of our normalization procedure.

Table 1. Internal and mutual self-consistency of the fractionations

	r	N	r_m	r_η	$\bar{P}_w$	$\bar{P}_n$
F-1	133	18	1.07	0.99	721	613
F-2	57.7	46	1.08	1.00	756	581
F-3	26.6	65	1.03	1.02	741	617

$r \equiv$ weight of beads/weight of polymer; N, number of fractions; $r_m \equiv \Sigma m_i/m$, m weight of whole polymer, m_i weight of ith fraction; $r_\eta \equiv \Sigma\omega_i[\eta]_i/[\eta]$, ω_i weight fraction of ith fraction, $[\eta]_i$ intrinsic viscosity of ith fraction, $[\eta]$ intrinsic viscosity of the whole polymer; $\bar{P}_w$, $\bar{P}_n$, weight and number average polymerization degrees as derived from fractionation data.

Now, if resolution is *defined* as the product of the separation between the maxima and the depth of the valley relative to the maxima, the conclusion from Fig. 2 is that the order of efficiency of separation is F-2 > F-3 $\simeq$ F-1. The selfconsistency of the F-2 fractionation was checked by a more rigorous method than the mere r_m and r_η values as defined in Table 1. This is a very cumbersome method and is not frequently used. In particular we feel this is the first time the method has been used for a fractionation with so many fractions. The method involves a comparison of the GPC chromatogram of the unfractionated polymer with the weighted sum of the chromatograms of the 46 fractions. The product of the weight fraction of every fraction, ω_i, by the ratio of areas of the chromatograms of the whole polymer, S_0, to that of the ith fraction, S_i, was used to correct the experimental measured height of the chromatogram for this fraction at a given elution volume, $h_i(V_E)$. The sum of these 46 corrected heights, i.e.

$$h_i(V_E) \equiv I(V_E) = \sum_{i=1}^{46} \omega_i \frac{S_0}{S_i} h_i$$

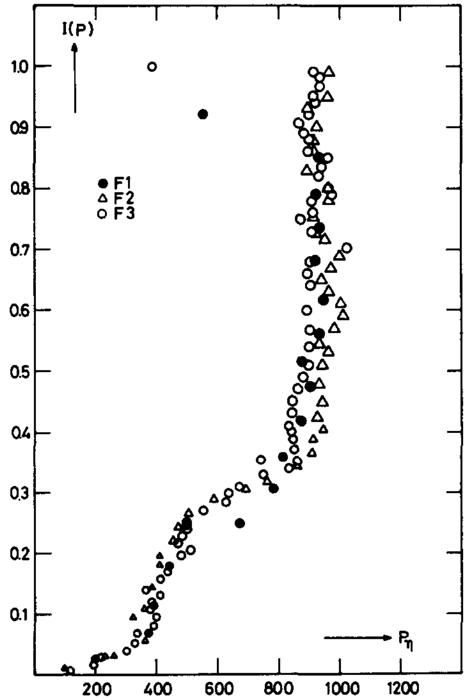

Fig. 1. Integral MWD (fractionation order) of the F-1, F-2 and F-3 fractionations.

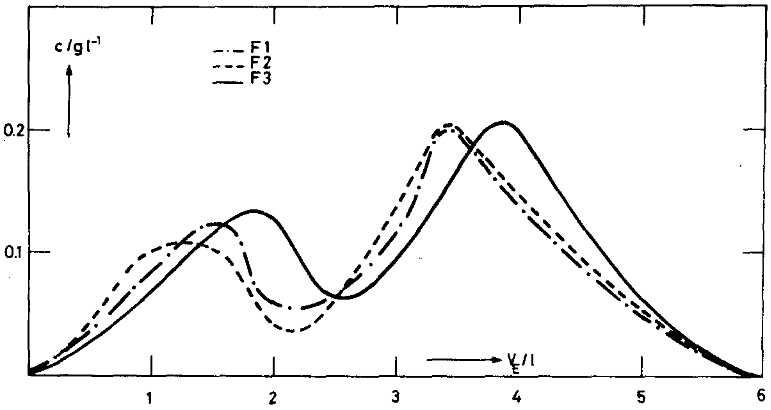

Fig. 2. Normalized elution curves of the F-1, F-2 and F-3 fractionations.

was determined for 11 arbitrarily selected values of V_E plus the values of V_E for the two maxima. The values of V_E were determined precisely by weighing the total solution collected up to the peak and then transforming distances on the graph paper on both sides of the maximum into volumes with the help of V_E (peak) and the flow rate. One thus gets the $I(V_E)$ vs V_E sum chromatogram depicted in Fig. 3 together with the directly measured curve of the unfractionated polymer.

The agreement is good, indicating the selfconsistency of the fractionation and also the good working of the GPC techniques.

Incidentally, calculated $\bar{P}_w/\bar{P}_n$ values of the fractions ranged from 1.60 for the 1st fraction and 1.15 for the 2nd, 3rd and 4th, down to 1.04 to 1.06 for fraction nos. 11 to 46. These low values also confirm the efficiency of the fractionation.

The conclusions derived from Fig. 2 with respect to the relative resolutions of F-1, F-2 and F-3 can be independently checked. For this purpose, we recall the rigorous mathematical concept of a distribution function. It must be a continuous, monotonically increasing curve [11], in our case meaning that, if $P_{i+1} > P_i$, then $I(P_{i+1}) > I(P_i)$. This condition is not fulfilled by some of the points in Fig. 1, i.e. when one puts the fraction in the output order off the column (fractionation order). It is then justifiable to transform the data accordingly, and the corresponding representation is given in Fig. 4, where $I'(P)$, which is obviously different from $I(P)$ from Fig. 1, stands for this corrected integral distribution function.

Clearly a better separation is obtained under the F-2 conditions in accordance with data from the elution curves (Fig. 2). It is perhaps obvious to note that the change from Fig. 1 to Fig. 4, i.e. from $I(P)$ to $I'(P)$ does not affect the results in Table 1: only the numbering, that is, the labelling of the fractions, is changed.

In conclusion, the experimental conditions followed in the F-2 fractionation seem to be the most appropriate for analytical purposes. Smaller quantities (down to about 400 mg as in the case of F-1) can of course be fractionated with the same efficiency by retaining the value 50 for r, by using a quantity of beads correspondingly smaller. Anyway, it must be

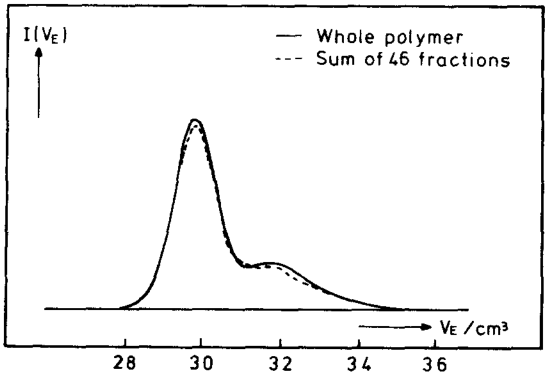

Fig. 3. GPC chromatograms of the whole polymer and of the weighted sum of the fractions of F-2.

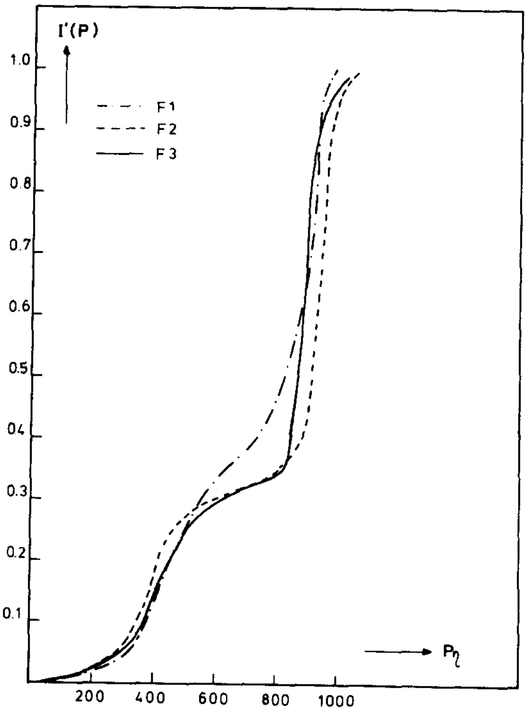

Fig. 4. Integral MWD (mathematical definition) of the F-1, F-2 and F-3 fractionations. The curves are drawn without experimental points for the sake of clarity.

noted that the number of fractions one can collect in a given fractionation is limited by the uncertainties in their experimental characterization. About 10 mg seems to be the limit for a reliable weighing and one-point viscometry for not too low molecular weights.

The GPC-measured MWD of the fractions of F-3 shows that an r value of 25 can still lead to rather monodisperse fractions. If the temperature gradient is important [6], one should deposit the polymer in the thermostatted top part of the column. In our case the weight of glass beads in that part amounts to about 100 g, i.e. about $\frac{1}{25} \times 100 = 4$ g could at least be fractionated for preparative purposes.

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REFERENCES

1. M. Morton, *J. chem. Educ.* **50,** 704 (1973).
2. C. A. Baker and R. J. P. Williams, *J. Chem. Soc.* 2352 (1956).
3. L. L. Böhm, M. Chmelir, G. Löhr, B. J. Schmitt and G. V. Schulz, *Adv. Polym. Sci.* **9,** 1 (1972).
4. J. M. Hulme and L. A. McLeod, *Polymer* 3 (153) 1962; R. S. Porter and J. F. Johnson, In *Polymer Fractionation*" (Edited by M. J. R. Cantow), Academic Press, New York (1967); A. L. S. Spatorico and B. Coulter, *J. Chromatogr.* **79,** 121 (1973); J. F. Johnson, M. G. Macphail, A. R. Cooper and A. R. Bruzzone, *Separ. Sci.* **8,** 577 (1973).
5. J. M. Alvariño, M. Chmelir, B. J. Schmitt and G. V. Schulz, *J. Polymer Sci. Symposium No.* **42,** 155 (1973).
6. G. Meyerhoff and J. Romatowski, *Makromolek. Chem.* **74,** 222 (1964); L. L. Böhm, R. H. Casper and G. V. Schulz, *J. Polym. Sci.* (*Physics*) **12,** 239 (1974).
7. G. V. Schulz and F. Blaschke, *J. Prakt. Chem.* **158,** 130 (1941).
8. G. Meyerhoff, *Z. Physik. Chem. NF* **4,** 344 (1955).
9. G. V. Schulz, *Z. Physik. Chem.* **B47,** 155 (1940).
10. J. M. Alvariño, A. Bello and G. M. Guzmán, *An. R. Soc. Esp. Fís. Quim.* **66,** 105 (1970).
11. H. Cramer, *Mathematical Methods of Statistics.* Ch. 6. Almqvist & Wiksells, Uppsala (1950).