Large-Scale Polymer Fractionation by Gradient Elution.
Some Operating Principles

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The procedure for fractioning polymers to obtain fractions large enough for detailed measurements is inconvenient and often difficult. The well-established "batch" precipitation process can be very laborious, and there is therefore much contemporary interest in continuous methods based on fractional extraction or elution. One effective method, that of Baker and Williams,[1] has so far been described only on an analytical scale (~0.3 g. polymer). As shown below, this method has been found capable of development to a preparative scale (up to 8-g. samples) provided its operation is very carefully controlled. This paper offers an analysis of the factors governing the elution, which leads to practical rules for the operation. The elution gradient here considered is of the logarithmic form, but a similar analysis can be applied to any other definite form of gradient.

Basic Principles of the Method

The method utilizes both solution composition and temperature gradients, involving, in turn, (a) fractional extraction of the polymer sample by a mixture of solvent and nonsolvent liquids which is continuously enriched in solvent, and (b) continuous fractional precipitation of the solute down a column with descending temperature gradient.

The experimental arrangements devised by Baker and Williams and used on a larger scale in this work, are shown in Figure 1.

In principle, the extraction process alone could bring about fractionation, provided that at all times the whole of the polymer sample could be maintained in equilibrium with the solvent mixture. In practice, with finite rates of flow, such equilibration is impossible. Because of its slowness of diffusion through the gel, a proportion of the lower molecular weight material which should be soluble at a given solvent composition will always lag behind. The purpose of the thermal column is to
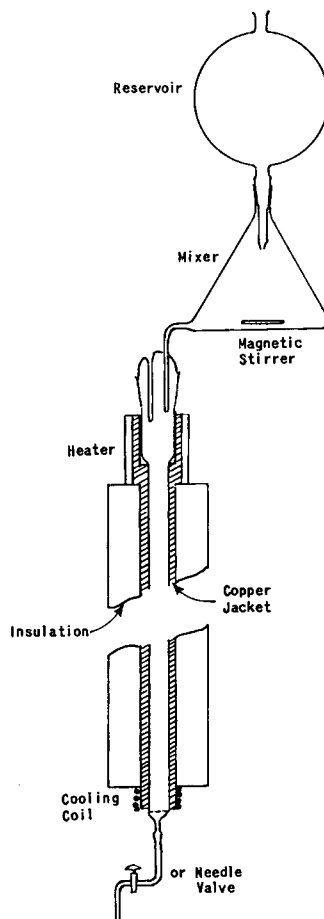

Fig. 1. Column arrangement.

correct this. By a continuous series of precipitations as the solution encounters cooler surfaces, and re-dissolutions as richer solvent follows, the less soluble fractions are retarded, and the more soluble ones accelerated in their progress down the column.

Problems of Design and Operation

The general criteria of efficiency of the fractionation are obvious enough. For the extraction

stages, the rates of eluant flow and change of composition must be as slow as possible. For efficiency in the precipitation stages, the polymer concentration must be very low. (To fractionate a large sample of polymer, the problem is to satisfy these conditions without excessively large apparatus, volumes, long experiments, etc.) Whatever the scale, the first condition presents no problems of control; the rates can always be made as slow as is experimentally tolerable. The second condition, however, will not be fulfilled without further adjustments, since at low rates of flow the solution will be approximately *saturated* at all stages. It is therefore necessary to control the elution *volume* (volume of eluant required for removing all the polymer sample) in order to keep it in reasonable proportion (which must be arbitrarily chosen) to the weight of polymer to be fractionated. This elution volume, for the logarithmic gradient used here, is a function not only of the gradient in eluant composition, but also of the volume of the eluant mixing vessel. For correct design of the apparatus, therefore, as well as for optimum operation, a detailed (if elementary) analysis of the gradient characteristics, etc., is necessary.

The Column

This is a most important element in the process, but the conditions of optimum performance (uniformity of column material and of temperature gradient, which should be maximum) are obvious and need no discussion. Once the dimensions and column material have been chosen, the only characteristic open to operational variation is the magnitude of the temperature gradient, and this is limited by the boiling points of the eluant mixture chosen.

For low molecular weight polymer, it may be necessary to use a very fine-grained column material, as the following considerations show. For any noncrystalline polymer, the precipitation equilibrium does not involve a true solubility but rather a partition between two essentially liquid phases, one dilute (the eluant) and one concentrated (the swollen polymer). When the molecular weight is low, the precipitated polymer phase may be a fairly mobile liquid. The success of the method will then depend on the ability of the column material to hold back this liquid precipitated phase with respect to the flowing eluant.

The Eluant Composition Gradient

Many different forms of gradient may be obtained, depending on the geometry of the reservoir–mixer assembly.[2] With the arrangements shown in Figure 1, the composition of eluant delivered by the mixer changes exponentially[3] with the volume passed (or with time, if the rate of flow is constant) between the limits set by the compositions initially in the mixer and reservoir.

This is easily seen in the special case where the mixer initially contains pure precipitant (subscript p) and the reservoir, pure solvent (subscript s). Then, in the ideal case where the liquids mix without volume change and for constant flow rate R and volume in the mixer V, the volume of precipitant in the mixer will fall with time at a rate proportional to its volume present at that time, that is:

$$-dv_p/dt = v_p R/V = kv_p$$

where $k = R/V$. Integrating,

$$v_p = v_p^0 e^{-kt}$$

Or in terms of the volume fraction $n_p = v_p = v_p/V$,

$$n_p = n_p^0 e^{-kt} = e^{-kt}$$

since $n_{p_0} = 1$. In terms of the total volume that has flowed through the mixer in time t ($v = R_1 t = kVt$)

$$n_p = e^{-v/V}$$

Correspondingly for the volume fraction of the solvent species,

$$n_s = 1 - n_p = 1 - e^{-v/V} = 1 - e^{kt}$$

In the general case, where the composition changes only from n^0 (that initially in the mixer) to n^∞ (that in the reservoir), the expressions for precipitant and solvent are identical in form.

$$n_p = n_p^0 + (n_p^\infty - n_p^0)(1 - e^{-kt})$$
$$n_s = n_s^0 + (n_s^\infty - n_s^0)(1 - e^{-kt})$$

the value of n_p falling (since $n_p^\infty < n_p^0$) and that of n_s rising correspondingly. $e^{-v/V}$ may be substituted for e^{-kt} in the last term.

Figure 2 shows these relationships (for volume fraction of solvent only) and illustrates how, at constant k ($= R/V$), the steepness of the gradient depends only on the separation of the composition limits ($n_s^\infty - n_s^0$). For a given separation, the gradient is directly proportional to the rate of flow (at constant V), and inversely proportional to the mixer volume (at constant rate). These relationships are of course intuitively obvious, but are worth stating explicitly, since the choice of practical
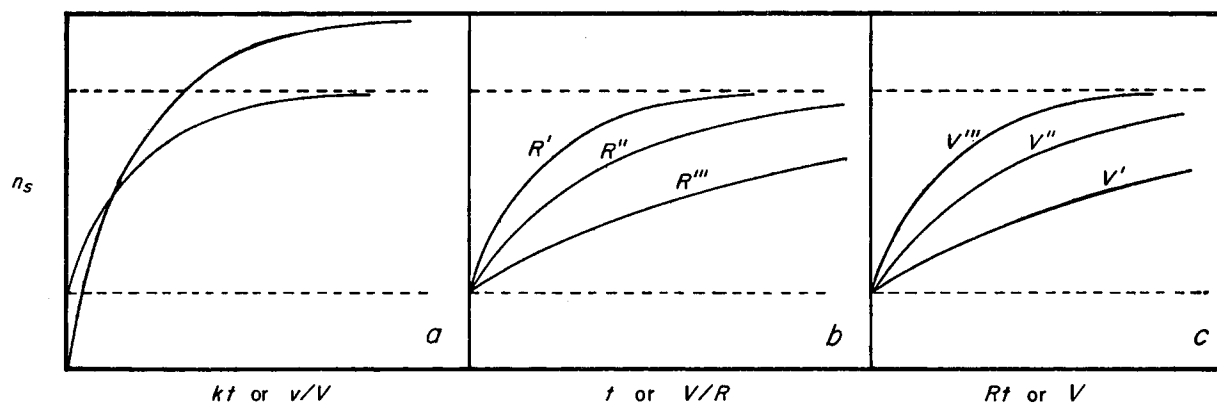
Fig. 2. (a) Effect of composition limits at constant V and R. (b) Effect of rate at constant V. $R' > R'' > R'''$. $v_{1/2}$ constant. $t_{1/2} \propto 1/R$. (c) Effect of mixer volume V at constant rate. $V' > V'' > V'''$. $t_{1/2}$ constant. $v_{1/2} \propto V$.

operating conditions involves decisions about all these parameters.

Choice of Composition Limits

It is obvious (see Fig. 2a) that for given rates, etc., the most gradual change of composition is obtained by setting the composition limits as close together as possible, i.e., no wider apart than is necessary for initial complete insolubility and final complete solubility of the polymer. It is therefore worthwhile to determine these limits by a separate experiment. On the conventional precipitation curves shown in Figure 3, only those eluant com-

positions between Y (onset of solubility at the upper temperature) and X (complete solubility at the lower temperature) will be effective in fractionation.

In practice it will of course be necessary to set the gradient limits slightly outside the X and Y compositions to allow for inaccuracies in determinations of X and especially of Y and to permit complete elution in a finite time and volume.

The above considerations are relevant to the choice of the solvent and the nonsolvent. On general grounds, one would choose the pair that gave the most gradual precipitation–composition
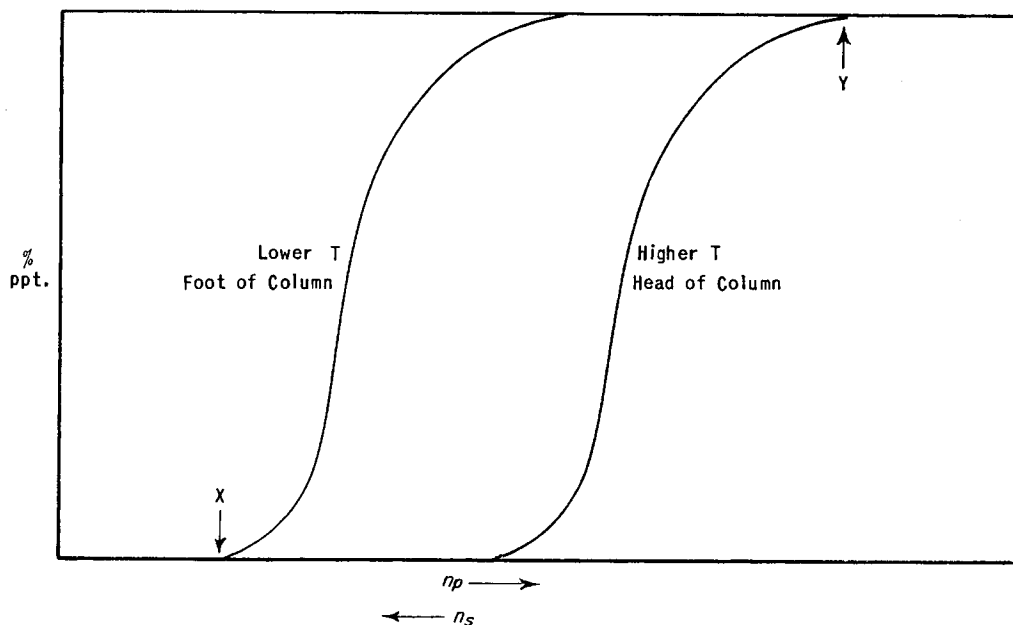

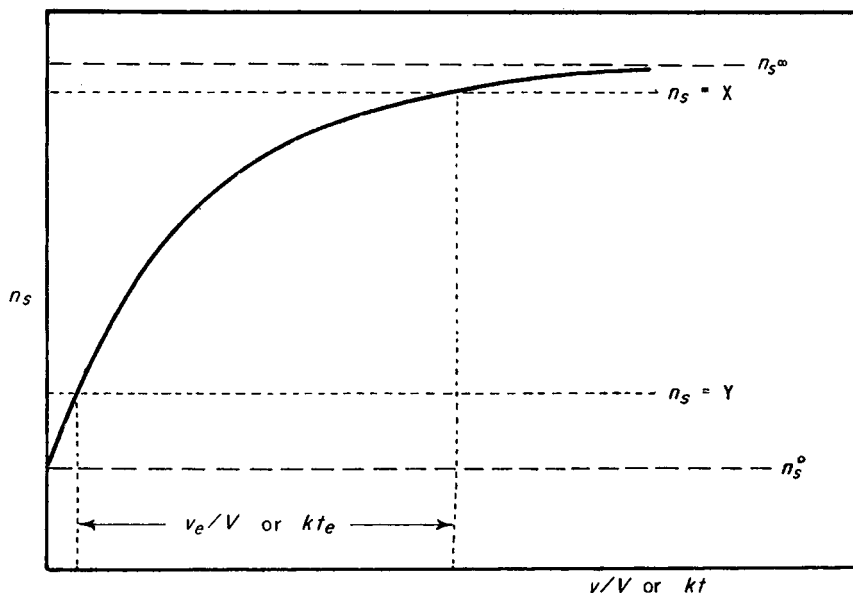Fig. 3. Percentage precipitation–composition curves.

Fig. 4. Ideal elution volume (time).

curve. The above analysis indicates that this is unnecessary; provided the limits X and Y can be determined, the eluant composition may be controlled to traverse this effective region as slowly as desired. The choice of liquids can therefore be made on other grounds (suitability of boiling points, etc.). Of course, the nature of the liquid chosen may be important for other reasons, especially in so far as it affects the degree of swelling of the polymer deposited from it on cooling.

The Elution Volume and Time

As illustrated in Figure 4, the volume v_e and time t_e necessary to change the composition through the effective range can now be simply expressed. This gives the "ideal" elution volume and time for complete extraction of the polymer in an ideal experiment, i.e., one in which equilibration is achieved at all stages.

$$n_s = n_s^0 + (n_s^\infty - n_s^0)(1 - e^{-v/V})$$

$$[(n_s^\infty - Y)/(n_s^\infty - X)] =$$
$$-(v_Y - v_X)/V = v_e/V$$

Hence,

$$v_e = V \ln [(n_s^\infty - Y)/(n_s^\infty - X)]$$

and

$$t_e = \frac{v_e}{R} = \frac{V}{R} \ln \frac{(n_s^\infty - Y)}{(n_s^\infty - X)} = \frac{1}{k} \ln \frac{(n_s^\infty - Y)}{(n_s^\infty - X)}$$

The Mixing Volume V

In the above expression for the elution volume, the logarithm term is fixed for any given polymer-eluant system by the solubility characteristics (values of X and Y), and the choice of n_s^∞ close to X. The elution volume can then be varied only by variation of V. It is evident that a minimum value is imposed on V by the condition that the elution volume must be large enough to keep the polymer concentration low. One cannot, of course, control the polymer concentration at all stages of the elution since the solubility varies continuously, but one can fix an arbitrary average concentration. In fractionation by batch precipitation, it is considered that the concentration of polymer in solution should not exceed 1/2% and should preferably be lower for polymer of very high molecular weight. If a similar limit is used here one can conclude that the elution volume should be at least 200 times the weight w of polymer to be fractionated*

$$v_e \geqslant 200w$$

$$V \geqslant \frac{200w}{\ln (n_s^\infty - Y)/(n_s^\infty - X)}$$

$$t_e \geqslant \frac{200w}{R}$$

fixing a minimum value for the mixing volume.

* Strictly speaking, it is the *volume* concentration of the polymer which is important. But since the equations are by nature approximate, they have for convenience been written in terms of weight concentration.

The magnitude of the logarithm term will of course be different for different polymer–eluant systems, but it does not in fact vary very greatly. For most systems and practical "settings" of the composition n_s^∞ this logarithm term has values between 1.7 and 2.5. It is therefore possible to make a practical rule for the design of apparatus taking as a compromise an apparatus mean value of 2, and concluding that the mixing volume should always be at least 100 times the weight of the polymer sample, and preferably larger.

Permissible Loading and Rates of Flow

Certain of the operating parameters for any given column and polymer, e.g., the maximum loading with polymer that the column can handle without blocking and the maximum eluant flow rate which still permits equilibration, cannot be predicted by simple theoretical analysis and must be found by trial. The equation for the ideal elution volume does, however, provide a valuable check on the latter point. If the observed volume necessary to elute all the polymer is appreciably different from the ideal, then obviously the rate of flow is too great for equilibration. If we define an equilibration coefficient $\beta = v_e(\text{obs.})/v_e(\text{theor.})$, then $\beta > 1$ implies a condition of undersaturation, i.e., the extraction stages are not in equilibrium, and $\beta < 1$ implies a condition of supersaturation, i.e., the precipitation stages are not in equilibrium.

EXPERIMENTAL

The foregoing theory of the column operation has not been given rigorous testing by experiments explicitly designed for the purpose. However, the following results, obtained during the semi-empirical development of the method, provide some confirmation of the theory's validity. The polymers examined were all polystyrenes and of low to medium molecular weight (9–290,000).

Column Characteristics

Two columns were investigated; these had the characteristics shown in Table I.

Both columns were operated with the same upper and lower temperatures (65 and 12°C.), but in the short column the gradient could be maintained only over the upper half (since the jacket characteristics were inadequate, the wall and insulation being too thin). The large column was shown to have a linear temperature gradient down its whole length.

Exploratory Trials

A series of trials was carried out on low molecular weight (11,200) samples of a single polymer preparation to explore the main variables, e.g., column material, eluant system, etc. These trials were made before the theory was developed, and the operating conditions, summarized in Table II, were often ill-controlled and far from ideal. They provide however, an opportunity to correlate approach to "ideality" and success in fractionation.

A fractionation was deemed a success if the molecular weight of the successive fractions increased smoothly and continuously, with no irregularities. The departures from ideal operating conditions are expressed by the coefficients $\alpha = V(\text{expt.})/V(\text{theor.})$, and $\beta = v_e(\text{obs.})/v_e(\text{theor.})$. It can be seen that some correlation does exist, at least in a negative sense. Fractionation has been effected under a wide variety of conditions, but the one characteristic common to the experiments that failed is that the mixing volume was very low (e.g., $\alpha \sim 0.2$) giving a correspondingly high concentration of polymer in the eluant. It seems that with this very low molecular weight polymer it is not necessary for α to be greater than 0.5 (i.e., the average concentration in the solution can be allowed to reach 1%, instead of the arbitrary limit of $\frac{1}{2}\%$).

The ratio β is in every case except Experiment 7 greater than unity, implying that equilibration was never fully achieved. This can be attributed to the high rates of flow in all cases except Experiment 2, where it seems possible that 'channelling' in the rather nonuniform sand packing of the column may have been responsible.

Despite these indications of inadequate control,

TABLE I

| No. | Diameter, cm. | Length, cm. | T gradient, °C./cm. | Copper jacket | | Maximum polymer loading, g. |
				Wall thickness, in.	Insulation, and thickness, in.	
1	2	50	2	$\frac{1}{16}$	asbestos, 1/4	2
2	2.5	90	0.6	$\frac{1}{8}$ later $\frac{1}{4}$	vermiculite, $1\frac{1}{2}$	8

TABLE II
Trial Fractionation
(Ethanol as nonsolvent)

Exp. No.	Column No.	Column Material	Solvent	n_s^0	n_s^∞	Polymer, g.	R, ml./hr.	V, ml.	k, hr.$^{-1}$	α	β	c_{max}[a] % (w/v)	Result
1	1	Quartz sand (0.3 mm.)	benzene	1.0	1.0	2	17–40	100	0.17–0.40	0.2	—	1.8	failure
2	1	Quartz sand (0.3 mm.)	benzene	0.2	1.0	1	3–10	250	0.012–0.032	1.06	1.45	0.9	partial success
3	1	Quartz sand (0.3 mm.)	MEK	0.43	1.0	1.0	10	175	0.017–0.07	0.78	1.38	0.5	success
4	1	Ballotini (0.1-mm. glass spheres)	MEK	0.43	1.0	1.22	25	175	0.14	0.64	1.33	0.7	success
5	1	Copper powder	MEK	0.43	1.0	1.23	30–50	175	0.17–0.28	0.63	1.43	0.8	success
6	2	Ballotini	benzene	0.25	1.0	3.95	35–60	200	0.175–0.30	0.21	1.72	1.5	failure
7	2	Ballotini	benzene	0.22	0.75	3.90	4–10	180	0.022–0.056	1.51	1.01	0.9	success
8	2	Ballotini	benzene	0.22 0.5	0.5[b] 0.75	7.95	5–7	180	0.02 0.04	0.2 0.1	— —	2 5	partial success in early stages only

[a] The concentration of polymer in the eluant changes continuously, reaching a maximum c_{max} in the middle of the fractionation.

[b] This experiment was conducted in two stages: in the first the reservoir composition, n_s^∞, was 0.5; and in the second, 0.75.
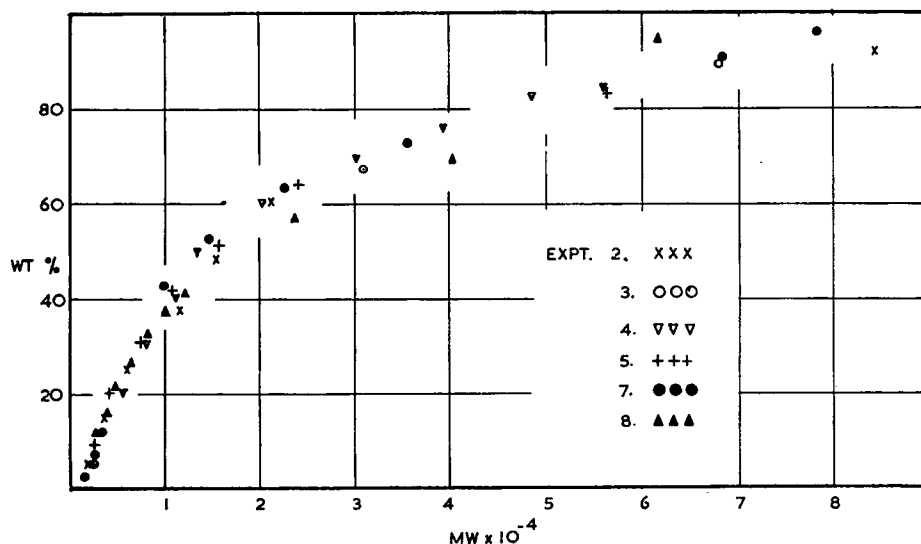

Fig. 5. Cumulative weight distribution.

the "successful" experiments all give similar degrees of fractionation, i.e., the cumulative distribution curves derived all lie very close to one another (see Fig. 5). This indicates that the fractionation method is feasible with any of the column materials and eluant mixtures tested.

Of the column packings, it seems that the quartz sand is inferior to both the ballotini and the copper powder. The last material was chosen for its high thermal conductivity, and it is likely that its relatively successful performance (permitting fractionation at very high rates of flow) was due to its improvement of the thermal gradient. For further development of the method, however, the ballotini packing was chosen because of its inertness and uniformity of particle size.

Of the solvents used, methyl ethyl ketone appears to be superior to benzene, under the conditions of our experiment. It has a theoretical superiority also in that its density (0.81 at 15°) is closer to that of the nonsolvent ethanol (0.79) than is benzene (0.88); this should minimize the tendency for "channelling" to develop in the column. However, a more than counterbalancing disadvantage is that its viscosity (~0.42 centipoise) is much further from that of ethanol (1.2 cp.) than is that of benzene 0.7 cp.); this makes it much more difficult to control the rate of flow as the composition changes.

Experiments with Different Polystyrene Samples

The large column was used to fractionate a series of polystyrenes in the molecular weight range 8,000–290,000, prepared by using cationic, anionic, and free radical initiators. In all experiments the column material was ballotini, the eluant mixture ethanol–benzene, the loading 4 g. of polymer, and the temperature gradient 0.6°/cm. Table III shows the operating conditions and success of the various experiments.

Most of these experiments were controlled in the sense that the values of α were chosen by preliminary calculation of the 'theoretical' mixing volume. There were fewer failures, but again the previous pattern was observed, i.e., fractionation fails when the mixing volume is too small. It seems confirmed that α need not have values greater than 0.5 for very low molecular weight polymer in the sample being fractionated but must be appreciably greater than unity for molecular weights > 100,000: for the lowest molecular weights an average concentration of 1% in the eluant is tolerable, but less than a third of this concentration is permissible at the highest molecular weights.

The values of β reveal that the process of extraction becomes much slower for high molecular weight polymer, as expected. For example, in Experiment 7, a polymer of M.W. 11,200 is equilibrated ($\beta = 1.01$) at a k value of 0.04 ($R = 8$ ml./hr.), whereas in Experiments 16 and 17 in which the M.W. was 288,000, extraction equilibrium was not established ($\beta = 1.35$) with a lower value of k and a slower rate. Attainment of equilibrium in the sense that $\beta = 1$ does not seem to be critical. Indeed, a value of $\beta > 1$, implying that the solution is less than saturated, may be an advantage for the solution stages. A value of $\beta < 1$, implying supersaturation of the eluant, would be expected to impair the fractionation seriously, and may be the explanation for the

TABLE III

Fractionation of Polystyrenes

(Medium, ballotini; eluant, ethanol–benzene; loading, 4 g.; rate of eluant flow, 10 ml./hr.)

Expt. No.	Polymer			α	k, hr.$^{-1}$	β	$c_{max.}$, w/v	Result
	No.	M.W.	Initiator					
11	P6	8,750	BzO$_2$	1.11	0.04	1.00	1.1	success
9	P1	11,000	H$_2$SO$_4$	0.55	0.06	0.96	2.5	partial failure
7	P13	11,200	H$_2$SO$_4$	0.51	0.04	1.01	0.9	success
12	P75	21,000	Ph$_3$CNa	1.12	0.03	1.01	0.9	success
13	P72	42,000	Ph$_3$CNa	0.92	0.026	1.32	0.4	failure
14	P72	42,000	Ph$_3$CNa	1.8	0.022	1.20	0.6	success
10	P4	79,000	Na	0.65	0.067	1.56	0.7	success
15^a	SP10	125,000	C$_{10}$H$_7$Na	1.08	—	1.37	0.9	partial failure
16^a	SP10	125,000	C$_{10}$H$_7$Na	2.94	0.05	1.75	0.3	success
17^a	SP5	288,000	C$_{10}$H$_7$Na	2.98	0.022	1.35	0.41	success

^{a} Fractionations were performed by Dr. A. Albert-Martinez of this department.

failure in Experiment 9, where conditions were otherwise just barely favorable ($\alpha = 0.55$).

The concentration of polymer in successive eluted fractions varied continuously in each run from initially very small values, through a peak concentration (c_{max} quoted in the tables), to small values at the end of the run. This variation obviously causes the efficiency of fractionation to vary, but it cannot be entirely eliminated since it is determined by the properties of the polymer sample (the peak in the weight distribution) as well as by the eluant composition gradient. Its undesirable effect can be minimized if the mean concentration (w/v_e) is kept so low that even the peak concentration of polymer does not reach an undesirable value.

CONCLUSIONS

The above results indicate that the most important single factor influencing the performance of the column is the concentration of polymer in the eluant; furthermore, providing that this is controlled by use of at least the "theoretical" mixing volume, then relatively large samples of polymer can be satisfactorily fractionated.

The formal theory appears to be adequate to describe the performance of the column and to provide useful control parameters. The fact that the theory takes into account only the characteristics of the composition gradient emphasizes that this is the most significant element in the overall process of fractionation, important though the thermal gradient in the column may be. Further refinements in the method may be expected when a composition gradient of a form more perfectly adapted to that of the polymer molecular weight–solubility relationship is found.

References

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Synopsis

A detailed analysis of the factors operating in polymer fractionation by gradient elution column methods is offered. A comparison between experimental fractionations of a range of polystyrenes indicates that the most important single factor is the concentration of polymer permitted in the eluant. For the logarithmic composition gradient considered here, the average concentration in the eluant may be controlled through control of the volume of the eluant in the mixing vessel. This volume should be not less than 200 times the volume of the polymer sample, and considerably larger if the polymer is of high molecular weight.

Résumé

Une analyse détaillée des différents facteurs influençant les méthodes de fractionnement de polymère par colonnes à gradient d'élution a été effectuée. Une comparaison avec des fractionnement expérimentaux d'une série de polystyrène indique que le facteur simple le plus important est le concentration en polymère possible dans l'éluant. Avec le gradient logarithmique considéré ici, la concentration moyenne dans l'éluant peut être controlée par mesurant le volume du récipient contenant le mélange éluant. Celui-ci ne sera pas moindre à 200 fois le valeur de l'échantillon de polymère, et encore beaucoup plus grand si le polymère est de haut poids moléculaire.

Zusammenfassung

Eine ins einzelne gehende Analyse der wirksamen Faktoren bei der Methode der Polymerfraktionierung durch eine Gradientelutionssäule wird gegeben. Ein Vergleich mit Fraktionierungsversuchen an einer Reihe von Polystyrolen zeigt, dass der wichtigste Einzelfaktor die im Eluierungsmittel zugelassene Polymerkonzentration ist. Mit dem hier betrachteten logarithmischen Gradienten, kann die mittlere Konzentration im Eluierungsmittel durch Überwachung des Volums des Mischgefässes für das Eluierungsmittel unter Kontrolle gehalten werden. Dieses Volumen sollte nicht kleiner als das 200-fache des Volumens der Polymerprobe sein, beträchtlich grösser aber, wenn das Polymere ein hohes Molekulargewicht besitzt.