

INVESTIGATION OF THE FRACTIONATION EFFICIENCY OF THE MODIFIED METHOD OF PRECIPITATION CHROMATOGRAPHY. INFLUENCE OF THE ELUTION GRADIENT

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Within the framework of the optimization of the fractionation process by the modified method of precipitation chromatography, the influence of the elution gradient on the fractionation efficiency was studied experimentally using a poly(methyl metacrylate) sample of a known molecular weight distribution. The decisive role of the limiting compositions of the elution mixture, in particular of its final composition, was found. The choice of the course of change of the elution mixture composition between two correctly chosen limiting values was found to be less critical.

One of the column methods used in the fractionation of polymers is the modified method of precipitation chromatography¹. It differs from the original method of Baker and Williams² in the first place by using temperature as a factor enabling the re-precipitation of polymer fractions during their passage through the support column, wherefrom the method acquires its chromatographic characteristics. Instead of using a longitudinal temperature gradient it employs periodical temperature changes of the entire column. It has been shown that their effect is analogous to the temperature gradient effect³.

The modified method of precipitation chromatography was used in the past years in the fractionation of a series of polymers⁴⁻⁹. Also, a comparison of its fractionation efficiency with that one of the original Baker-Williams method¹⁰ as well as of the column elution methods and the gel permeation chromatography¹¹ (GPC) was made. Despite the fact that the modified method of precipitation chromatography exhibited in most cases a good fractionation efficiency, a systematic investigation of the effect of various experimental conditions on the fractionation process was needed. Theoretical studies were published concerning the mechanism of the separation of polymer fractions of various molecular weights resulting from a combined influence of the elution and thermal gradient¹²⁻¹⁴, however, the results of these studies have found only a limited application in selecting concrete fractionation conditions^{15,16}. Recently, Schulz and coworkers¹⁷ have shown that experimental studies of the fractionation efficiency of precipitation chromatography can be still fully justified.

The aim of this study has been to investigate the effect of those experimental factors which may play a significant role in the fractionation process of precipita-

tion chromatography. A series of fractionations was carried out of a poly(methyl metacrylate) sample of a known molecular weight distribution¹¹. With the exception of the factor under study, all other fractionation conditions were kept constant and "optimized", *i.e.* such which were known to lead to good results either from earlier experience or from the experiments described here.

This communication summarizes results of the investigation of the effect of elution mixture composition changes on the fractionation efficiency, including both the choice of the limiting compositions of the elution mixture and the course of its composition change between these limiting values.

EXPERIMENTAL

Chemicals and Apparatus

The poly(methyl metacrylate) (PMMA) sample was used whose benzene solution had at 25°C the limiting viscosity number 134.0 ml/g; the distribution of its molecular weights was described earlier¹¹. All solvents used were of analytical purity and all were in addition distilled on a distillation column filled with a metal packing of about 70 theoretical plates.

The apparatus used was described in our earlier communications^{4,18}.

Fractionation

The total of eight preparative fractionations was carried out using acetone as the solvent and methanol as the precipitant. 6 g of the sample was dissolved in 250 ml of acetone and deposited on the support in the upper part of the column by evaporating the solvent. The method of filling the column with the support carrying the polymer as well as the entire manipulation with the apparatus was described in detail earlier⁴.

Table I summarizes the fractionation conditions used in the particular fractionations. The letter in the line denoted "Mixing Program" corresponds to the program of mixing the liquids from both reservoirs of the mixing device shown in Fig. 1 (*i.e.* of two mixtures acetone-methanol of the initial and final composition). Fig. 2 shows the course of changes of the elution mixture

TABLE I
Experimental Conditions^a Used in PMMA Fractionations

Fractionation	1	2	3	4	5	6	7	8
X_a (initial) ^b	10	20	20	20	35	20	20	20
X_a (final) ^b	70	70	55	50	60	55	55	55
Mixing program ^c	A	A	A	A	A	B	C	D

^a Sample weight 6 g, temperature cycles 25–40°C, cycle period 1 h, cycle shape saw-tooth, symmetrical, flow rate 160 ml/h; ^b volume % of acetone in initial (final) elution mixture; ^c see Fig. 1A–D.

compositions in dependence on the volume passed through the column (so called elution programs), as calculated from the respective mixing program and chosen limiting compositions of the elution mixture. Its real course was checked throughout the fractionations by measuring the refraction index of the elution mixture sampled directly from the mixing flask and by determining its composition from the calibration graph. A good agreement with the calculated values was found.

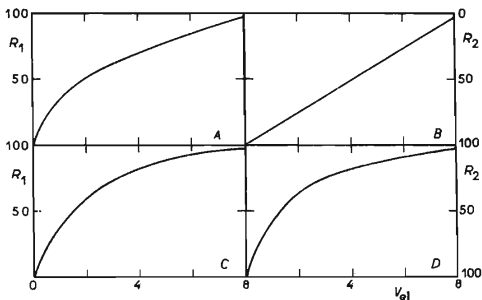


FIG. 1

Mixing Programs

R_1 is the volume % of the elution mixture of final composition; R_2 volume % of the elution mixture of initial composition; V_{el} (l) is the elution volume; A, B, C, D explication see text.

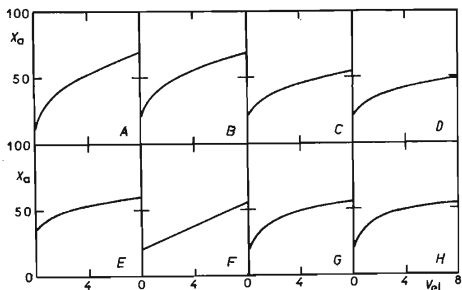


FIG. 2

Elution Programs

X_a is the volume % of acetone in the elution mixture, V_{el} (l) is the elution volume; A - H explication see text.

The amount of the polymer in the particular siphons containing the eluate from the column was determined by the evaporation residue method. The procedure used in determining the weight yield of the fractionations and in isolating the fractions was described earlier¹¹.

Determination of Molecular Weights of Fractions and Construction of Integral Molecular Weight Distribution Curves

Viscosities of benzene solutions of the fractions were measured at $25 \pm 0.03^\circ\text{C}$ by means of the Ubbelohde viscometer. Limiting viscosity numbers of the fractions were calculated from the values measured at one polymer concentration (about 0.3 g/100 ml) using the Schulz-Blaschke equation ($1/k' = 5.34$ was used¹). Molecular weights were calculated with the use of equation $[\eta] = 5.5 \cdot 10^{-3} M^{0.76}$ ($[\eta]$ in units ml/g)¹⁹. Integral distribution curves of the molecular weights were constructed using the Schulz method. This method is sufficiently accurate for a mutual comparison of the results concerning the samples of a not too narrow molecular weight distribution, separated into fractions of a sufficiently low polydispersity²⁰.

RESULTS AND DISCUSSION

Limiting Composition of the Elution Mixture

In the column fractionations based on different solubilities of polymer fractions of different molecular weight the correct choice of the elution gradient is of prime importance^{21,22}. The question of the determination of limiting compositions of an elution mixture has been discussed in many papers^{3,16,21,22}. However, experimental information on how this choice of the limiting compositions influences the course of fractionation are very scarce¹⁷, though it is very probable — in view of the complexity of the system solvent-precipitant-*n*-component polymer sample — that usual methods used in their determination may give more or less erroneous results which would need further corrections. Therefore, we found it useful to show, how an incorrect choice of the limiting compositions of an elution mixture may influence the resulting shape of the molecular weight distribution curve.

Fig. 3A–H show the integral distribution curves of molecular weights of a PMMA sample constructed from the data of fractionations 1–5, where this effect was studied (Table I). For comparison, with each distribution curve the distribution curve determined by the GPC method¹¹ is shown. The latter serves as an orientation measure of the fractionation efficiency of the particular fractionation experiments. Another criterion for their mutual comparison were the values of the polydispersity indices¹¹ $(\bar{M}_w/\bar{M}_n)^*$ calculated in the standard manner; they make it possible to judge more objectively the fractionation efficiency than the (\bar{M}_w/\bar{M}_n) values calculated directly from the fractionation data. These indices are given in Table II together with the value of \bar{M}_w^* and \bar{M}_n^* ; their correlation with the values of the same quantities obtained with the use of the GPC method is shown, too.

In the fractionation 1 the widest range of the elution mixture composition was employed (Table I, Fig. 2A). The molecular weight distribution curve (Fig. 3A) is considerably distorted over the entire range and in the high-molecular-weight

region exhibits a second, false inflex. The ratio $(\bar{M}_w/\bar{M}_n)^*$ has the lowest value of all. The unnecessarily wide range of change of the elution mixture composition shows itself in the fractionations with a constant elution volume (here, 50 siphons of 160 ml, *i.e.* 8 l) as an effective lowering of the real volume. In this fractionation it was only about 50% of the real value. As a result, the concentration of the polymer in the

TABLE II

Values \bar{M}_w^* , \bar{M}_n^* and $(\bar{M}_w/\bar{M}_n)^*$ Calculated from the Data of the Particular Fractionations by Means of Precipitation Chromatography and Gel Permeation Chromatography

Fractionation	$\bar{M}_w^* \cdot 10^{-5}$	$\bar{M}_n^* \cdot 10^{-5}$	$(\bar{M}_w/\bar{M}_n)^*$
1	6.44	2.88	2.24
2	6.46	2.80	2.31
3	6.41	2.40	2.67
5	6.42	2.78	2.31
6	6.45	2.68	2.41
7	6.42	2.64	2.43
8	6.43	2.73	2.36
GPC, ref. ¹¹	6.59	2.32	2.84

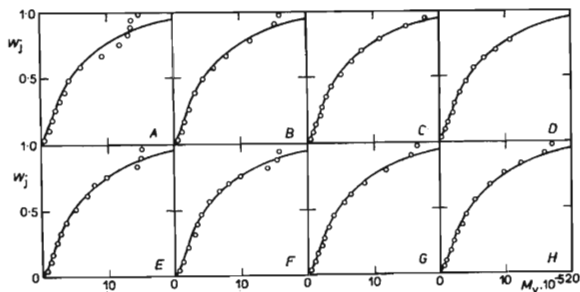


FIG. 3

Molecular Weight Distribution Curves of the PMMA Sample Obtained in the Particular Fractionations by Precipitation Chromatography (○) Compared with the Distribution Curve Obtained by the GPC Method (solid curve); A — H for explication see text.

eluate increased and the conditions for an effective fractionation were in general worse²²⁻²⁴. Also, the slope of the elution program was too large in the critical high-molecular-weight region and this contributed to the non-selective fractionation of the high-molecular-weight fractions²⁵.

In fractionation 2 the solvent content in the initial elution mixture was increased (Table I). The resulting elution program is shown in Fig. 2B. However, the effective elution volume increased only negligibly and also the slope of the elution program in the high-molecular-weight region decreased very little. It can be seen from Fig. 3B that the fractionation efficiency remains low. This is confirmed by the low value of the polydispersion index, too (Table II).

Only a substantial lowering of the solvent content in the final elution mixture in fractionation 3 (Table I, Fig. 2C) led to an almost 90% exploitation of the real elution volume, to a prominent lowering of the elution program slope over its entire region and, consequently, to a considerable improvement of the fractionation efficiency. The shape of the resulting molecular weight distribution curve (Fig. 3C) and the high value of the polydispersity index approaching that one determined by the GPC method, corroborate this conclusion. Referring to the detailed discussion from ref.¹¹, the results of this fractionation can be regarded as satisfactory.

Further lowering of the solvent content in the final elution mixture (fractionation 4; Table I, Fig. 2D) resulted in a failure to eluate the high-molecular-weight fractions at the given column temperature regime. However, the available section of the distribution curve (Fig. 3D) was practically identical with the corresponding section of the distribution curve from the preceding fractionation.

In the last fractionation (fractionation 5) the content of the solvent in the initial elution mixture was substantially increased. The final mixture was enriched by the solvent, too (Table I, Fig. E). The effective elution volume was lower again, and the fractionation efficiency decreased conspicuously (Fig. 3E). Also, the polydispersity index had a very low value in this fractionation (Table II), practically the same as in fractionation 2 and only somewhat higher than in fractionation 1. This finding indicates that again the influence of a higher solvent content in the final elution mixture played the dominant role. If the high acetone content in the initial elution mixture considerably worsened the fractionation efficiency in the region of low and medium molecular weights, the value of \bar{M}_n^* should have been higher and, consequently, $(\bar{M}_w/\bar{M}_n)^*$ lower than that one found in fractionations 1 and 2.

In fractionations 1 and 5 small reversals in the molecular weight course of the final fractions were observed.

Our findings concerning the influence of the limiting compositions of the elution mixture on the fractionation efficiency are in agreement with recently published results of Böhm, Casper, and Schulz¹⁷. They show that attention should be paid to their preliminary estimation in precipitation chromatography and column elu-

tion. Any substantial deviation of the chosen value from the optimum one, namely in the final elution mixture, is unfavourably reflected in the high-molecular-weight part of the distribution curve. In view of the non-linear relation between the polymer solubility and its molecular weight²¹, a small change in the composition of the elution mixture may substantially change the range of molecular weights passing into solution²⁶ and thus it may negatively influence the fractionation efficiency. For example, Glöckner and Kuhnhardt¹⁰ – in comparing the fractionation efficiency of the modified method of precipitation chromatography¹ with the original procedure of Baker and Williams² on a polystyrene sample – used temperature cycles between 40°C and 25°C and a linear temperature gradient 50–15°C; *i.e.* not entirely commensurable temperature regimes. The limiting compositions of the elution mixtures were identical in both cases. However, the final elution mixture dissolving high-molecular-weight fractions at the lowest column temperature, 15°C, contains too much of the solvent, when using temperature cycles with the lower operating temperature 25°C, and this was – according to our opinion – the reason of the observed lower fractionation efficiency of the modified method in the high-molecular-weight region.

Mixing Program

Among the correctly chosen limiting values of the elution mixture composition there exist one linear and an unlimited quantity of non-linear courses of the composition change. In view of the above mentioned dependence of the polymer solubility on their molecular weight²¹, only those of them have been used characterized by a continuous decrease of the slope with the increasing value of the elution volume. However, published literature offers only scarce data concerning the influence of this factor on the fractionation efficiency^{26–29}. Therefore, we studied this factor more in detail.

While in fractionations 1–5 always the same mixing program A was used (Fig. 1A) at various limiting compositions, in fractionations 3 and 6–8 four various mixing programs were employed (Fig. 1A–D) keeping the limiting compositions of the elution mixtures constant (Table I).

The non-linear mixing program used in fractionation 3 (Fig. 1A) gave the molecular weight distribution curve discussed earlier (Fig. 3C) which shows a satisfactory fractionation efficiency¹¹. Therefore, it may be assumed that this mixing program, in combination with the remaining fractionation conditions, was a suitable one.

In fractionation 6 a linear mixing program was used (Fig. 1B) and also the resulting elution program was linear (Fig. 2F). The molecular weight distribution curve (Fig. 3F) is again distorted in the high-molecular-weight region. In order to eluate the first ten percent of the polymer almost 50% of the entire elution volume was consumed, while the last 30% of the elution volume released from the column almost

70% of the polymer. Therefore, with the exception of the beginning of the distribution curve its entire course is a result of a fractionation under unfavourable concentration conditions, with too large an increment of the solvent in the elution mixture in the final stage of the fractionation. The lower value of $(\bar{M}_w/\bar{M}_n)^*$ calculated for this fractionation confirms this conclusion.

In fractionation 7 we used again a non-linear mixing program C (Fig. 1C) which differed from program A in a steeper starting and a gentler final part. The resulting elution program is shown in Fig. 2G. The molecular weight distribution curve obtained from these fractionation data (Fig. 3G) is similar to that one from the preceding fractionation, despite the great differences in the elution programs, and the same holds for the polydispersity indices. However, the value of the number average molecular weight \bar{M}_n^* is obviously higher in comparison with the corresponding value from fractionation 3 and indicates that the steep start of the mixing program C impaired the separation in the region of lower molecular weights.

In the last fractionation another non-linear mixing program D was used (Fig. 1D) with a very steep beginning and a sharp transition to the limiting region. The corresponding elution program is shown in Fig. 2H. The molecular weight distribution curve of this fractionation (Fig. 3H) again does not differ too much from that one from fractionation 7, however, the low value of the polydispersity index shows a further decrease of the fractionation efficiency.

To use a non-linear change of the elution mixture composition has been most often recommended in the literature^{21,22,27}. The reason is a non-linear dependence of the solubility on the molecular weight²¹, and also because the generally used constant volume mixer offers such a change of the elution mixture composition²². However, several authors have shown that for certain polymers this change was not suitable²⁶ and that the linear elution gradient was more convenient²⁸.

In our case one of many possible non-linear gradients proved itself to be the best one. It was thus confirmed that the choice of a suitable course of change of the elution mixture composition depends mainly on the system polymer-solvent-precipitant and on the distribution of molecular weights of the polymer fractionated²⁹. It follows from our results, too, that — if we exclude extreme elution gradient courses — in complex estimating the influence of the elution gradient on the fractionation efficiency in precipitation chromatography the leading factor is the correct choice of the limiting compositions of the elution mixtures, and only then the choice of the course of composition change between these values.

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