

A FRACTIONATION EFFICIENCY STUDY OF THE MODIFIED METHOD OF PRECIPITATION CHROMATOGRAPHY. INFLUENCE OF FRACTIONATED POLYMER AMOUNT, FLOW RATE, AND TEMPERATURE CYCLES

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In a study of optimizing the fractionation process of the modified method of precipitation chromatography, a poly(methylmethacrylate) sample of a known distribution of molecular weights was used to investigate experimentally the influence of the fractionated sample amount, of the flow rate, and of temperature cycles on the fractionation efficiency. It has been shown that all these factors had a pronounced effect on the fractionation process and that they decreased the efficiency, if their values surpassed a certain limit. However, the use of large flow rates indicated the possibility of the separation of high-molecular-weight fractions based on different dissolution rates.

Similarly as in the case of other column methods, the fractionation process by the modified method of precipitation chromatography¹ is to a large extent influenced by the choice of fractionation conditions^{2,3}. Two types of papers have appeared which are related to this problem; studies of the first group aim at using simplified models in describing the separation mechanism of macromolecules according to their molecular weight during the passage through the column and at evaluating by calculations the influence of various fractionation factors³⁻⁶; papers of the second group investigate experimentally the course of the column fractionation process and try to find the optimum regime of operation⁷⁻¹⁰.

Our previous communication¹⁰ belongs to the latter group. We studied experimentally the influence of the elution gradient on the fractionation efficiency and we showed that the decisive role had the limiting compositions of the elution mixture, in particular its final composition. The aim of this communication is to demonstrate experimentally the influence of other factors which may play a more significant role in the fractionation process of precipitation chromatography: the amount of fractionated polymer, flow rate, and temperature range within which the column temperature varies.

EXPERIMENTAL

Chemicals and Apparatus

The poly(methylmethacrylate) (PMMA) sample was used whose benzene solution had the limiting viscosity number of 134.0 ml/g at 25°C; its molecular weight distribution was described earlier¹¹.

All solvents used were of analytical purity; they were distilled over a distillation column filled with metal packing of about 70 theoretical plates.

The apparatus used was described in detail in our earlier communications^{12,13}.

Fractionation

Seven preparative fractionations of the PMMA sample were carried out using acetone as the solvent and methanol as the precipitant. If not stated otherwise, the same elution gradient was used in the fractionations as that one in fractionation C of the preceding communication¹⁰ (20 vol.% of acetone in the initial elution mixture, 55 vol.% of acetone in the final elution mixture, a non-linear mixing program). The samples were dissolved in 250 ml of acetone and deposited on the support in the upper part of the column¹² by evaporating the solvent. The method of filling the column with the support carrying the polymer and all the manipulation with the apparatus was described in detail earlier^{12,13}.

Table I summarizes the fractionation conditions used in the particular fractionations. 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 $25 \pm 0.03^\circ\text{C}$ in the Ubbelohde-type viscometer. The procedure used in determining the values of limiting viscosity numbers,

TABLE I

Experimental Conditions^a Used in Fractionations of Poly(methylmethacrylate)

Fractionation	A ^b	B	C	D	E	F	G	H
Sample weight, g	6	9	12	6	6	6	6	6
Flow rate, ml/h	160	160	160	320	480	160	160	160
Temperature cycles ^c , °C	25—40	25—40	25—40	25—40	25—40	25—30	25—50	25—50

^a Elution program used in fractionations A—G was the same as that one of fractionation C from the preceding communication¹⁰, in fraction H the acetone content in the initial elution mixture reduced to 10 vol.%; ^b experiment A is identical with experiment C from the preceding communication¹⁰; ^c temperature cycles of sawtooth shape, symmetrical, cycle period 1 h, only in experiment F 0.5 h.

in calculating molecular weights of the fractions, and in constructing the integral molecular weight distribution curves was the same as in the preceding communication¹⁰.

RESULTS AND DISCUSSION

Amount of the Fractionated Polymer

The aim of most preparative fractionations is to prepare narrow fractions of a required molecular weight in an amount as large as possible. However, the sample weight of the fractionated polymer is limited first of all by technical parameters of the particular fractionation apparatus and also by requirements based on theory¹⁴, determined experimentally^{7,15-18}, and verified by calculations^{19,20}, which are related to this fraction factor. At a given elution volume and at a chosen elution gradient the amount of polymer used in the fractionation determines the amount of polymer in the eluate which in turn co-determines a successful outcome of the fractionation^{19,20}. Therefore, we wanted to demonstrate, how the sample weight of a polymer influences the fractionation efficiency of the modified method of precipitation chromatography.

Fig. 1 shows the integral molecular weight distribution curve of the PMMA sample (sample weight 6 g); the elution gradient and other fractionation factors were suitably chosen¹⁰. This distribution curve, identical with the distribution curve

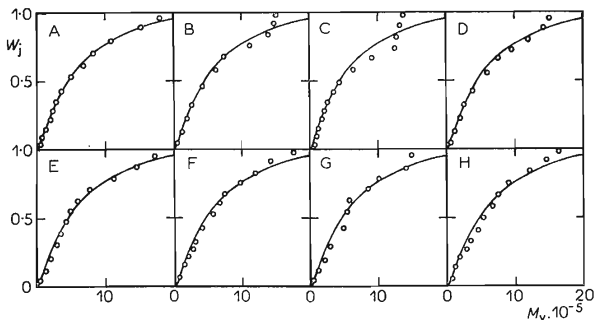


FIG. 1

Molecular Weight Distribution Curves of the Poly(methylmethacrylate) Sample Obtained in the Particular Fractionations by the Modified Method of Precipitation Chromatography (○) Compared with the Distribution Curve Obtained by the GPC Method¹¹ (full line)

in Fig. 3C of the previous communication¹⁰, is almost identical with the molecular weight distribution curve obtained by the analytical version of gel permeation chromatography¹¹. It is evident that lowering of the polymer sample weight would lead to a further – though certainly insignificant – improvement of the fractionation efficiency¹¹, with all other fractionation parameters preserved. However, from the preparative point of view this gain is not a sufficient compensation for the lowering of the sample weight of the fractionated sample. Therefore, in further experiments only the influence of larger sample weight was investigated.

Fig. 1B and 1C show the integral molecular weight distribution curves obtained in the fractionation of a 9 g and a 12 g sample of PMMA. In both cases the curves are deformed in the high-molecular-weight region. While with the 9 g sample this deformation starts at $W_j = 0.70$, the sample weight increase to 12 g results in spreading the deformation towards the lower-molecular-weight region.

Also, polydispersity indices $(\overline{M}_w/\overline{M}_n)^*$ calculated by the standard procedure¹¹ decrease in the experiment series A–C (Table II).

The observed decrease of the fractionation efficiency with the increasing sample weight confirms the calculations of Kamide and coworkers¹⁹. These authors found, when simulating mathematically the successive precipitation (*i.e.* the process which decides the quality of macromolecule separation in precipitation chromatography), that the fractionation effect decreases sharply and eventually disappears entirely, if a certain limiting concentration of polymer fractions in the solution is surpassed whose value is indirectly proportional to the molecular weight. In our case, *e.g.*, the final four fractions of experiment C show a very similar value of the molecular weight; this confirms that the fractionation efficiency in this stage of the experiment was very low indeed. However, the efficiency in the region of lower molecular weights was influenced much less even with the 12 g sample. This finding indicates that from the preparative point of view the maximum sample weight allowing still an efficient fractionation represents a variable factor depending on the molecular weight of the polymer and on its molecular weight distribution.²¹

Flow Rate

In the studies of the flow rate effect on the fractionation efficiency we were interested in the possibility of shortening the duration of the fractionation process by increasing the flow rate. Figs 1A, D, and E show integral molecular weight distribution curves obtained in PMMA sample fractionations with a different flow rate. With the flow rate of 160 ml/h (Fig. 1A) the fractionation efficiency was good over the entire molecular weight range^{10,11}. Doubling the flow rate results in a slight decrease of the fractionation efficiency over the entire molecular weight range which is visually most obvious in the region of high molecular weights (Fig. 1D). However, the deformation of the distribution curve is not as striking as with the doubled sample

weight of the polymer. Only tripling the flow rate (Fig. 1E) leads to a distinct decrease of the fractionation efficiency in the region of low and medium molecular weights. The polydispersity index $(\bar{M}_w/\bar{M}_n)^*$ (Table II) sharply decreases and the distribution curve becomes straightened. However, the fractionation effect in the high-molecular-weight region improves again in comparison with the doubled flow rate, the shape of the resulting molecular weight distribution curve is in this region similar to that one from experiment A.

Most authors who dealt with studies of this fractionation parameter concluded that for each particular fractionation apparatus there was an optimum region of flow rates²¹. Out of this region a decrease of the fractionation efficiency takes place, either because of the backward diffusion of low-molecular-weight fractions (small flow rates) or because of entirely non-equilibrium conditions in the column (large flow rates).

Our experiments in the range of large flow rates in principle confirm this conclusion, too. An attempt to shorten the fractionation time by increasing the flow rate affects negatively the quality of separation. However, using a very large flow rate (experiment E) indicates a specific possibility of fractionating polymers of a very high molecular weight by the column method. (We mentioned this possibility earlier in another connection¹³.) Due to strongly non-equilibrium conditions in the column the kinetics of dissolution may play a decisive role. It is known²² that the differences in dissolution rates of macromolecules (with the increasing molecular weight the dissolution rate decreases) exist even in the region of very high molecular weight,

TABLE II

Values of \bar{M}_w^* , \bar{M}_n^* and $(\bar{M}_w/\bar{M}_n)^*$ calculated from the Data of the Particular Fractionations by the Modified Method of Precipitation Chromatography and by Gel Permeation Chromatography¹¹

Fractionation	$\bar{M}_w^* \cdot 10^{-5}$	$\bar{M}_n^* \cdot 10^{-5}$	$(\bar{M}_w/\bar{M}_n)^*$
A	6.41	2.40	2.67
B	6.42	2.59	2.48
C	6.45	2.70	2.39
D	6.40	2.60	2.46
E	6.43	2.85	2.26
F	6.36	2.57	2.47
G	6.33	2.85	2.22
H	6.36	2.70	2.36
GPC	6.59	2.32	2.84

where the differences in solubilities disappear entirely^{2,3}. However, more detailed investigations of these phenomena are beyond the scope of this study.

Temperature Cycles

In fractionations by the modified method of precipitation chromatography temperature cycles play an analogous role as the longitudinal temperature gradient in the original variant of Baker and Williams^{2,4}: they guarantee the multiple re-precipitation of polymer fractions passing through the column. It can be expected, therefore, that they will have a considerable influence on the fractionation process, too.

We have shown in one of our earlier communications that the fractionation efficiency of the modified method of precipitation chromatography was higher than that of the simple column elution^{1,1}. Also, analogous findings concerning the original variant of Baker and Williams were published⁹. Therefore, in this paper we concentrated only on investigating the effect of the temperature limits between which the column temperature changed. The results are given in Figs 1A and 1F–H.

The molecular weight distribution curve in Fig. 1A was obtained by using temperature cycles between 25°C and 40°C (Table I). This temperature regime was normally used in routine fractionations and led to good results^{1,1}. Fig. 1F shows the molecular weight distribution curve of the PMMA sample when the 25–30°C temperature cycles were used and the cycle period was reduced to one half (Table I). The shape of the curve is without any visible deformations, however, it is somewhat steeper than in Fig. 1A; also, the polydispersity index value is lower (Table II). The increased number of refractionations obtained by shortening the cycle period thus does not compensate for the efficiency loss due to the temperature range lowering. Otherwise, however, even in this fractionation the efficiency was higher than in the simple elution at a constant temperature^{1,1}.

Fig. 1G shows the integral molecular weight distribution curve obtained when temperature cycles 25–50°C were used. It can be seen both from the shape of the curve and from the polydispersity index value (Table II) that a sharp decrease of the fractionation efficiency occurred, namely in the middle part of the curve. The temperature increase by 25°C during one cycle has approximately the same effect on the PMMA solubility as the increase of acetone content in the elution mixture by 13 vol.% (ref.³). This effect interferes with the elution program; it causes passing into the solution of even higher-molecular-weight fractions at the maximum column temperature shortly after the beginning of the fractionation, while at the minimum column temperature only low-molecular-weight fractions remain in the solution. Thus, in a relatively small elution volume large amount of polymer of a wide molecular weight distribution passes through the column and is re-precipitated; this all occurs under very complicated conditions, when – besides others – a prominent role plays actual concentration of fractions of a different molecular weight. Therefore,

too large range of the limiting cycle temperatures can negatively influence the fractionation process.

As pointed out in our previous communication¹⁰, the limiting compositions of the elution mixture must be adjusted to the two limiting temperatures. In both preceding experiments this requirement was not fulfilled. In experiment F this fact could not substantially influence the fractionation process, but in experiment G even the initial elution mixture was too solvent-rich. Therefore, in the following fractionation process the solvent content in the initial elution mixture was lowered, while the cycles 25 to 50°C were preserved (Table I). The resulting molecular weight distribution curve is shown in Fig. 1 H. However, it is evident from its course as well as from the polydispersity index value (Table II) that only a partial improvement of the fractionation efficiency occurred. Our experiments thus show that — with respect to the fractionation system used and to the chosen elution gradient — there is a certain optimum range of limiting temperatures of cycles; outside of this range a decrease of the fractionation efficiency was observed.

Despite the fact that only an analogy exists between the temperature cycle effect and the longitudinal temperature gradient effect, and in detail the mechanism of its influence differs³, the conclusions made by us concerning the influence of temperature cycles on the fractionation efficiency are in agreement with the data published on the effect of the longitudinal temperature gradient. It was found even in the case of the original variant of precipitation chromatography that with a mild temperature gradient better fractionation results could be achieved than when using too large temperature differences between both column ends^{2,5}.

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