

## POLYMER FRACTIONATION BY COLUMN METHODS

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Polystyrene and poly(methyl methacrylate) samples were fractionated by the following methods: column elution, precipitation chromatography and gel permeation chromatography. Fractionation efficiency of these three column methods was evaluated. Both chromatographic methods were found to be more efficient than the simple column elution method. In the case of polystyrene the fractionation efficiency of preparative precipitation chromatography was equivalent to that of analytical fractionation by gel permeation chromatography. For the fractionation of poly(methyl methacrylate) sample, which in comparison with polystyrene, had a considerably higher average molecular weight and more narrow molecular weight distribution, the precipitation chromatography, however, exhibited lower fractionation efficiency than the GPC method.

From the viewpoint of practical use the most important column fractionation methods for polymers are the column elution, precipitation chromatography and gel permeation chromatography.

The development of the column elution method<sup>1</sup> became a dividing line in the polymer fractionation techniques because it made possible to accelerate considerably the fractionation process and automate it. The endeavour to increase fractionation efficiency led to the development of precipitation chromatography<sup>2</sup>, which completed a simple elution by a multiple refractionation of polymer fractions moving through a column of a support. These two methods, both being based on a different solubility of macromolecules, were by the end of fifties supplemented by a gel permeation chromatography<sup>3</sup>, whose principle lays in a different ability of unevenly long molecules to permeate through a gel-filled column.

It is evident from the literature<sup>4,5</sup> that no unified view on the precipitation chromatography has been adopted. Some authors, including those who tried to treat the fractionation process of macromolecules by precipitation chromatography theoretically<sup>6,7</sup>, attribute to this method a higher fractionation efficiency than to a column elution one<sup>8-10</sup>, others experimentally prove either the opposite<sup>11</sup> or are critical to its possibilities<sup>12,13</sup>.

Also the conclusions of studies comparing the results of gel permeation chromatography with other column methods are different. Some authors prove that the separation efficiency of GPC is lower<sup>14</sup> and that the molecular weight distribution determined by it is broader than it is in reality<sup>15,16</sup> due to the effect of so-called axial dispersion<sup>17</sup>. In contrary, other authors assert that lower polydispersity values obtained from column elution or precipitation chromatography methods are due to their insufficient fractionation efficiency<sup>18</sup> and that the GPC method is more

efficient<sup>19</sup>. There are also papers which quote the agreement of results obtained by GPC and other column methods<sup>20-22</sup>, or only compare the fractionation efficiencies without more detailed analysis of the origin of some differences (*e.g.* ref.<sup>23</sup>).

The present work is also a contribution to the discussion about the fractionation efficiency of column methods. We have fractionated polystyrene and poly(methyl methacrylate) samples by the following methods: column elution, precipitation chromatography and gel permeation chromatography. The comparison of results is made and the differences observed are discussed.

## EXPERIMENTAL

### Polymers and Solvents

Polystyrene (PS) having molecular weight  $M_w = 2.4 \cdot 10^5$  and limiting viscosity number (toluene, 25°C) 75.0 ml/g was used. Poly(methyl methacrylate) (PMMA) had limiting viscosity number (benzene, 25°C) 134.0 ml/g. Analytical grade solvents were distilled on a metal packed distillation column having approximately 70 theoretical plates.

### Fractionation by Precipitation Chromatography and Column Elution Method

Fractionation by precipitation chromatography was performed according to a modified method<sup>24</sup>, which in contrast to the original variant by Baker and Williams<sup>2</sup> works with repeating temperature changes of the whole column (25–40°C, cycle duration being 1 h), or even with a programmed increase of the basic temperature of individual cycles at the final stages of fractionation, when there is no change of the elution mixture<sup>25</sup>. The same apparatus was used for column elutions but the column temperature was kept constant at 33°C.

The system benzene (solvent)–methanol (nonsolvent), having 50 vol.% of benzene in the initial and 75 vol.% in the final elution mixture, respectively, was used for fractionation of PS. Acetone as a solvent and methanol as a nonsolvent were used for PMMA fractionation, the initial and the final elution mixture contained 20 and 55 vol.% of acetone, respectively. Nonlinear elution gradient<sup>24</sup> was used in all cases. Weighed amount of polymer (5 g of PS or 6 g of PMMA) was dissolved in 250 ml of the solvent and deposited on a support in the upper part of the column<sup>24</sup> by evaporating the solvent. In the case of PS 100 ml of butanol was added to a polymer solution before deposition (diminishes the tackiness of the polymer film; partial selective deposition<sup>26</sup>).

The amount of polymers in individual siphons, containing eluates from the column (holding 160 ml), was determined by the evaporation residue method (aliquote volumes being evaporated at room temperature and than dried *in vacuo* for 3 h at 120°C). On the basis of the weight balance determined in this way the siphons in neighbourhood were joined to make fractions. Then the polymer was isolated by evaporating the elution mixture on a rotating vacuum evaporator and again dried in a vacuum oven at 120°C. The yield of polymer of individual fractionations was in the range of 98–103% with respect to the original amount of polymer; this is considered as an acceptable error<sup>27</sup>. The experimentally found value was therefore taken for 100% in further calculations.

### Molecular Weight Measurements

Molecular weights of PS and its fractions were measured by light scattering method on a Sofica apparatus. Toluene solutions were prior to measurements prefiltered through a sintered glass G4 and then filtered through a G5 sintered glass into dedusted cells. After measurements solution concentrations were again determined by the evaporation residue method as the original concentrations have changed after filtration. The solutions were measured in nonpolarised monochromatic light, the wavelength being 546.1 nm and the angles ranging from 30 to 150°. Experimental data were evaluated according to Zimm's method. For calculations published values of refraction index increments were used<sup>28,29</sup>.

Viscosities of benzene solutions of PMMA and its fractions were measured with the use of an Ubbelohde viscometer at  $25 \pm 0.03^\circ\text{C}$ . Limiting viscosity numbers were calculated from the single-point measurement values (concentration about 0.3 g/100 ml) according to Schultz and Blaschke's equation ( $1/k' = 5.34$  was employed<sup>30</sup>). Molecular weights were calculated from the equation  $|\eta| = 5.5 \cdot 10^{-3} \cdot M^{0.76}$  (where  $|\eta|$  is expressed in ml/g)<sup>31</sup>.

Schulz's method<sup>32</sup> was used for constructing integral distribution curves of molecular weights as the samples of PS and PMMA had sufficiently broad molecular weight distribution and the fractions obtained had low polydispersity.

### Determination of Molecular Weight Distribution by GPC Method

PS and PMMA samples and some of their fractions were analysed by GPC method at Centre de Recherches sur les Macromolécules, Strasbourg, using a Waters 200 apparatus. The system of four columns packed with styragel having the porosity of  $10^6$ ,  $10^4$ ,  $10^3$  and 25 nm, was employed. The flow rate was 1 ml/min; the concentration of solutions dosed into the apparatus through a special filter was approximately  $2 \cdot 10^{-3}$  g/ml. Chromatograms of PS samples were evaluated with the use of a calibration graph which was obtained by measuring PS standards supplied by Waters Comp. Chromatograms of poly(methyl methacrylate) were evaluated analogously; PMMA standards were obtained by precipitation chromatography. Then integral distribution curves of molecular weights, weight average and number average molecular weights were calculated from the chromatograms by the generally used methods.

## RESULTS AND DISCUSSION

To compare the efficiency of macromolecule separation at individual fractionation experiments we have to fulfill two conditions (within experimental errors)<sup>4,5</sup>: the total of fraction weights must be equal to the initial polymer weight and the weight mean of limiting viscosity number calculated from fractionation data must be equal to the limiting viscosity number of the original nonfractionated sample. Then it is possible to compare the results obtained by the same method or by the methods based on the same fractionation principle. If the principle is different it is necessary to consider to what extent the incidental phenomena, specific for the particular method, may influence the resulting shape of the distribution curve of molecular weights.

In practice, a number of parameters is used to compare the fractionation efficiency<sup>5</sup>. All of them have, however, only a relative and limited validity and therefore

for the sake of objective evaluation it is wise to compare fractionation experiments by using several parameters simultaneously.

One of the most used parameters of fractionation efficiency is the shape of distribution curve of molecular weights. It is approximately valid that the more efficient separation according to molecular weights proceeds if the resulting integral distribution curve of molecular weights covers a wider range of molecular weights and if the slope at the inflexion point is smaller<sup>33</sup>. Even if this criterion is only qualitative, it is good enough, in case of a larger difference in the quality of separation, for judging which experiment gives the results resembling most closely the real molecular weight distribution of a nonfractionated polymer. It has also the advantage that the course of integral distribution curve of molecular weights constructed according to Schulz's method does not depend on the number of fractions<sup>34</sup>, providing that their number does not become so small as to make uncertain the graphical construction of the curve itself.

The other, seemingly quantitative parameter of fractionation efficiency, is the index of polydispersity, *i.e.* the ratio of the weight and number average molecular weight calculated from the fractionation data. Again, it approximately holds, that the more efficient fractionation experiment is that which gives higher value of this ratio. However, a very often overlooked fact is that the value of this ratio is to a large extent influenced by the number of fractions and also by the weight distribution of polymer parts in them. Therefore, it is not possible to compare polydispersity indexes directly from the fractionation data because both the number of fractions and the weight distribution of a polymer in them vary from experiment to experiment. Table I

TABLE I

Values of  $\overline{M}_w$ ,  $\overline{M}_n$  and  $\overline{M}_w/\overline{M}_n$  Calculated from Distribution Curve of Molecular Weights of PMMA Sample Determined by GPC Method for Different Fictitious Distributions of the Polymer in Fractions

A 20 fractions by 5%; B 10 fractions by 10%; C 5 fractions by 20%; D 10 fractions: fract. 1-4 by 5%, fract. 5-8 by 10%, fract. 9 + 10 by 20%; E 10 fractions: fract. 1 + 2 by 20%, fract. 3-6 by 10%, fract. 7-10 by 5%.

Distribution	$\overline{M}_w \cdot 10^{-5}$	$\overline{M}_n \cdot 10^{-5}$	$\overline{M}_w/\overline{M}_n$
GPC original	6.64	1.90	3.49
A	6.70	2.18	3.07
B	6.59	2.32	2.84
C	6.39	2.60	2.46
D	6.40	2.18	2.94
E	6.70	2.60	2.58

gives an example. There are presented values of weight and number average molecular weights and their ratios obtained directly from GPC-chromatogram of PMMA sample and also the values calculated on the assumption that the resulting integral distribution curve of molecular weights identical with the experimental one would be constructed from a different number of fractions and different percentual proportions in them. The value of polydispersity index calculated from the chromatogram (reading: 0.5 count) corresponds to 29 fractions in which the polymer was distributed similarly as in the case D (Table I).

Further useful parameter of fractionation efficiency which can be employed in the case of preparative fractionation methods is the width of molecular weight distribution of fractions determined by different methods.

Fractionation experiments included in this work fulfilled all the fundamental requirements necessary for their mutual comparison. It was, however, necessary to evaluate to what extent this comparison is objective if one of the methods is the GPC method. It is generally known that at this method the neglect of the axial dispersion<sup>17</sup> may lead to erroneous conclusions regarding the width of molecular weight distribution. It has been shown<sup>35</sup>, however, that the axial dispersion markedly influences the width of polydispersity of samples being not so much polydispersed. Samples having  $\overline{M}_w/\overline{M}_n > 3$  are practically unaffected. Therefore, in our case the neglect of axial dispersion could manifest itself in smaller extension of molecular weight distribution of PMMA sample.

The integral distribution curves of molecular weights of PS sample (Fig. 1a) constructed from the data obtained by gel permeation chromatography and by precipitation chromatography have very similar course. This indicates an approximately equal fractionation efficiency. A slightly worse efficiency in the whole range of molecular weights has been observed when column elution method is applied. In the case of the last mentioned method the polymer was intentionally deposited only on the support from the upper part of the column and not from the column entire. Thus.

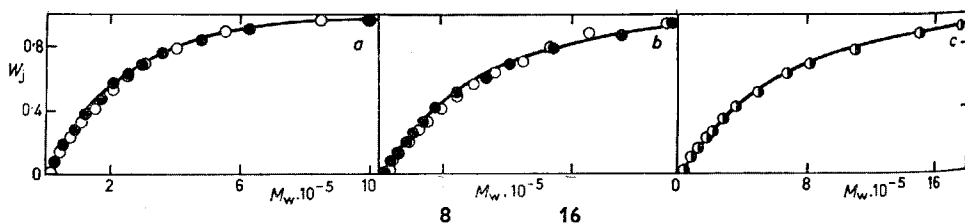


FIG. 1

Integral Distribution Curves of Molecular Weights of Polystyrene (a) and of Poly(methyl methacrylate) (b, c)

Method: — GPC, ○ column elution, ●, ● precipitation chromatography (● corrected for superposition of molecular weight distributions of fractions).

we were able to show that by changing the separation process from a simple column elution to a chromatography, the selectivity of separation of macromolecules under other conditions preserved increases. But, in this comparison the column elution method was slightly disadvantaged.

Table II summarizes the values of weight and number average molecular weights and their ratios calculated directly from the fractionation data and by the standard method; the latter makes the comparison more objective. Ten values of molecular weights for  $W_j$  0.05, 0.15 ... 0.95 were taken from the integral distribution curves of molecular weights and used for calculating the  $\bar{M}_w^*$ ,  $\bar{M}_n^*$  and  $(\bar{M}_w/\bar{M}_n)^*$  values. For PS the values of polydispersity indexes obtained by the described procedure confirm the evaluation of fractionation methods stated above. Due to the reasons given earlier the polydispersity index calculated directly from GPC-chromatogram has, however, a considerably higher value and proves how unreliable criterion of the fractionation efficiency it is.

PS fractions, prepared by the column elution method were also analysed by the GPC method. The values of polydispersity indexes calculated from chromatograms were about 1.25. They differ from the real values because narrow fractions were used and they are therefore greatly influenced by the axial dispersion<sup>35</sup>. Cantow and coworkers<sup>21</sup> have shown that the correct value of the polydispersity index is in such case lower by about 0.15–0.20 than the value given by the chromatogram. Benoit and coworkers<sup>36</sup> came to a similar conclusion. Therefore, it can be assumed that the real values of these indexes for fractions prepared by column elution method are in the 1.05–1.1 range and for the fractions prepared by precipitation chromato-

TABLE II

Values of Average Molecular Weights and of Indexes of Polydispersity Calculated Directly from Fractionation Data and by Standard Procedure (denoted by \*) for Individual Fractionation Experiments

Polymer	Fractionation method	$\bar{M}_w \cdot 10^{-5}$	$\bar{M}_n \cdot 10^{-5}$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w^*$	$\bar{M}_n^*$	$(\bar{M}_w/\bar{M}_n)^*$
PS	column elution	2.48	0.78	3.18	2.47	0.84	2.94
	precipitation chromatography	2.60	0.76	3.42	2.55	0.79	3.23
	GPC	2.59	0.60	4.32	2.46	0.75	3.28
PMMA	column elution	6.38	2.62	2.44	6.37	2.74	2.32
	precipitation chromatography	6.45	2.25	2.87	6.41	2.40	2.67
	precipitation chromatography + correction	6.60	1.87	3.53	6.66	2.34	2.85
	GPC	6.64	1.90	3.49	6.59	2.32	2.84

graphy the values are close to unity. In the case of PS samples this finding confirms a high fractionation efficiency of column methods based on a different solubility.

A greater difference in fractionation efficiency of the individual methods was found for PMMA sample. This is evident not only from the course of integral distribution curves of molecular weights (Fig. 1b) but also from the values of  $(\overline{M}_w/\overline{M}_n)^*$  given in Table II. Both criteria show that the fractionation efficiency of the methods decreases in this order: GPC, precipitation chromatography, column elution. This fact can be taken as a valid one only for a mutual comparison of the column elution and the precipitation chromatography taking into account the exception discussed earlier in the case of PS. It was, however, necessary to decide whether the difference of fractionation efficiency between the GPC and the precipitation chromatography is a real one or if it is an artifact caused by neglecting the axial dispersion when evaluating the GPC-chromatogram. If the fractionation efficiency of both methods were the same also in the case of PMMA and if the integral distribution curve of molecular weights determined by the GPC method were extended only due to an uncorrected axial dispersion then the polydispersity index values of PMMA fractions obtained by the precipitation chromatography and analysed by the gel chromatography should again be lower than 1.25, *i.e.* below the value obtained for PS fractions (about the same number of fractions, similar shape of distribution curve, small difference in distribution width). Chromatograms of three PMMA fractions having low, medium and high molecular weights gave, however, higher values ranging from 1.3 (low molecular weight fraction) to 1.4 (high molecular weight fraction). The width of molecular weight distribution of these fractions was after subtraction of an approximative contribution of the uncorrected axial dispersion<sup>21</sup> characterised by polydispersity index having the value of 1.1–1.2. Therefore, it is quite evident, that the fractionation of PMMA by the precipitation chromatography was less efficient than that by the gel chromatography.

To confirm this conclusion we have tried to correct the course of the integral distribution curve of molecular weights determined by the precipitation chromatography for the superposition of molecular weight distributions of fractions by a procedure analogous to that suggested by Haseley<sup>37</sup>. The integral distribution curves of molecular weights of PMMA fractions obtained by the GPC method were in accord with Tung's distribution function<sup>38</sup>

$$W_j = 1 - e^{-yM^z},$$

where  $W_j$  is the cumulative weight function,  $M$  represents molecular weight and  $y, z$  are two adaptable parameters from which only  $z$  is a function of distribution width<sup>35</sup>. Therefore, we have selected several suitable values of  $z$  and for each of them, with the use of values of molecular weights of fractions  $M_j$  (attributed to  $W_j = 0.5$ ), calculated the values of the second parameter  $y$  for all fractions obtained by the

precipitation chromatography. Then we could for each value of  $z$  construct integral distribution curves of molecular weights of fractions; the same width of distribution being their common feature. For each series of these distribution curves, we have, according to the procedure proposed by Haseley<sup>37</sup>, determined the values of  $W_j$  corrected for a corresponding superposition of distributions of molecular weights of fractions, then plotted the integral distribution curve of molecular weights and compared it with the curve obtained by the GPC method.

Fig. 1c shows an example when one of the corrected curves of the integral distribution of molecular weights obtained by the precipitation chromatography closely resembles the distribution curve obtained by the GPC method. Also the value of  $\overline{M}_w/\overline{M}_n$ , calculated for this corrected distribution from the  $\overline{M}_w$  and  $\overline{M}_n$  values of fractions and the value of  $(\overline{M}_w/\overline{M}_n)^*$  are in good agreement with corresponding values determined for the GPC method (Table II). The width of superimposed distributions was, however, for this particular case characterised by the value of  $z$  being 2.5, which corresponds to the  $(\overline{M}_w/\overline{M}_n)^*$  value of 1.24, therefore, to a higher value than we have estimated for the real fractions from the GPC chromatogram. This gives the evidence that the observed difference in fractionation efficiency of the GPC and the precipitation chromatography is in the discussed case only partly real; to some extent, the uncorrected axial dispersion at the gel chromatography participates in it.

It is known<sup>39</sup>, that the separation efficiency at one-step phase separation depends upon the average molecular weight of the polymer, the width of molecular weights distribution, the total polymer concentration and the coefficient of interaction between the polymer and the solvent. We think, that in this case the lower fractionation efficiency of the precipitation chromatography in the case of PMMA sample is caused by the first two factors mentioned. The PMMA sample in comparison with the PS one, had a higher average molecular weight and a narrower distribution of molecular weights, *i.e.* the properties predicting less efficient separation of macromolecules according to molecular weights<sup>34</sup>.

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