

Determination of Molecular Weight Distribution of Polymeric Substances by Thin-Layer Chromatography

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ABSTRACT: Thin-layer chromatography (TLC) combined with concentration gradient development has been applied to the determination of the molecular weight distribution of polystyrene prepared by a radical polymerization. Developers obtained by mixing polar nonsolvents with moderately good solvents toward polystyrene were found to give complete separation of polymer according to molecular weight. It was proved that the molecular weight distribution deduced from the present TLC experiment was in good agreement with that from a gel-permeation chromatography experiment.

KEY WORDS Thin-Layer Chromatography / Gel-Permeation Chromatography / Molecular Weight Distribution / Polystyrene /

The potential of thin-layer chromatography in its application to polymer chemistry has been proved by our recent studies to be very promising on at least three counts. First, TLC allows us to separate given copolymer samples according to the chemical composition.¹ Second, differences in the sequential arrangement of copolymers as well as the steric isomerism of stereoregular polymers can be detected by TLC.²⁻⁵ Finally, such separations occur practically independent of the molecular weight of samples tested.

The last feature of TLC mentioned above appears to be irreconcilable with previous observations that adsorption of macromolecules onto a solid surface from a dilute solution depends on molecular weight.⁶ This, in turn, motivated us to make further application of TLC to the determination of molecular weight distributions of polymers. A number of attempts had, in fact, been made as well to fractionate polymers by molecular weight with chromatographic techniques,⁷⁻⁹ before gel-permeation chromatography (GPC) found continuous, heavy use.¹⁰ However, none of them has reached practical development, except the Baker—Williams procedure,^{8,9} whose principle is based on the solubility difference of polymer rather than adsorption.

Thus our efforts spent on the present work are to establish some experimental conditions that enable us to determine the molecular weight distribution of a polystyrene sample by using the TLC technique.

EXPERIMENTAL

Materials

Five monodisperse polystyrene samples with different molecular weights purchased from Pressure Chemical Co. Ltd., Pittsburgh, Pa. U.S.A., were used as reference samples for establishing the relation between molecular weight, M and rate of flow, R_f . A polystyrene sample was prepared in bulk at 60°C by using azobisisobutyronitrile (0.4 wt%) as an initiator, the conversion being 9%. Its molecular weight was established to be 18.8×10^4 from intrinsic viscosity, $[\eta]$ in butanone at 25°C by the following viscosity equation¹¹

$$[\eta] = 39 \times 10^{-5} M_w^{0.58}$$

The sample was fractionated into two parts by using chloroform and methanol as solvent and precipitant, respectively. Their molecular weights were again estimated by the above viscosity equation. Then, the lower molecular-weight fraction, coded as P-W, was used to test the applicability of the present method. The fraction P-W was expected to have a molecular weight

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Table I. Characterization of sample polymers

Sample code	M_w^a
P-002	2030
P-010	10300
P-050	51000
P-160	160000
P-500	498000
P-W	74300

^a Values of pressure chemical polystyrenes are their catalog (weight-average) molecular weights, and that for P-W is the viscosity-average value.

distribution just within the range of molecular weights covered by the reference samples. The results of the characterization of the samples are listed in Table I.

Reagent grade solvents, *n*-hexane, cyclohexane, benzene, butanone, acetone, and ethanol, were used as developers without further purification.

Thin-Layer Chromatographic Procedure

The details of the chromatographic procedure of TLC has been described before.¹ Silica gel, Kieselgel G (Merk), was applied as the stationary phase. The thickness of the gel layer was adjusted to 0.30 mm. It was dried at room temperature for one day, and was used without further activation. Stock solutions were prepared by dissolving each sample in cyclohexane, and the concentrations were adjusted to 2.0 and 4.0 g/dl for the reference samples and P-W, respectively. All chromatoplates were developed by the ordinary ascending technique in a jar at room temperature. The experiments combined with a concentration gradient development were also carried out; the details of technique will be given later. Each stock solution that involves *ca.* 20 and 50 μ g of polymer in the test for each reference sample and P-W, respectively, was spotted onto a thin layer. After development, chromatograms were visualized first by spraying with sat. thymol blue in water—ethanol mixture (1:1 by volume), followed by a 3N-sulfuric acid aqueous solution. Violet spots on a pale blue ground indicated the location of polymer. The chromatogram was photographed on high contrast film. A preliminary test proved that the blackness of film is proportional to the amount of polymer within the range of polymer concentrations studied here.

Gel-Permeation Chromatography (GPC)

GPC measurements were carried out by using a Shimadzu gel permeation chromatograph model 1-A with a series arrangement of four columns of upper porosity rating 10^3 , 10^4 , 10^5 , and 10^6 Å. Tetrahydrofuran was used as solvent at room temperature, its flow rate of *ca.* 1 cc/min. being employed throughout. Samples dissolved in tetrahydrofuran were injected for 1 min, yielding an approximately 5-mg sample (concentration of 6 g/l). Calculation of the molecular weight distribution was made by referring to a calibration curve established for this series of columns without any further correction. In this connection it is mentioned that the linearity of the calibration curve was ranging from *ca.* 3×10^3 to 2×10^5 in molecular weight.

RESULTS AND DISCUSSION

TLC Development with Single Solvents and Their Mixtures

In order to search for an appropriate developing condition, the monodisperse reference samples, P-002, P-010, P-050, P-160, and P-500, were chromatographed by using single solvents with different dielectric constants. The results are summarized in Table II, which may suggest what types of solvents are suitable for the present purpose. From Table II it is seen that for the development of polystyrene a good dissolving power of developer toward polystyrene appears to be a necessary condition. Further to discuss this problem we classify the developers tested above into four categories according to their thermodynamic property toward polystyrene. They are: (1) nonsolvents with low dielectric constants, *e.g.*, *n*-hexane; (2) poor solvents, *e.g.*, cyclohexane; (3) good solvents, *e.g.*, benzene, and butanone, and (4) nonsolvents with high dielectric constants, *e.g.*, acetone, and ethanol. Under the above classification our experimental experience indicates that only the developers belonging to (3) can promote the migration of sample. However, none of these developers allowed us to make a perfect fractionation of polystyrene samples according to molecular weight.

On the basis of the above result, development with mixtures of the developers was attempted.

Table II. Results of chromatographic development with single solvents having different dielectric constants

Developer	Dielectric constant		R_f				
			P-002	P-010	P-050	P-160	P-500
<i>n</i> -Hexane	1.89	N ^a	0	0	0	0	0
Cyclohexane	2.02	S ^a	0(u) ^b	0(u)	0	0	0
Benzene	2.28	S	>0.90	>0.90	>0.90	>0.90	>0.90
Butanone	18.5	S	>0.90	>0.90	>0.90	>0.90	>0.90
Acetone	20.7	N ^c	>0.90	>0.90	0	0	0
Ethanol	24.3	N	0	0	0	0	0

^a Notation N and S mean nonsolvent and solvent toward polystyrene, respectively.

^b Tailing-up of spots on chromatogram.

^c Solubility of polystyrene in acetone depends markedly on molecular weight (for the details, see text).

Table III. Results of chromatographic development with mixtures of benzene and acetone

Volume ratio of benzene and acetone	R_f				
	P-002	P-010	P-050	P-160	P-500
0 : 20	>0.90	>0.90	0	0	0
2 : 20	>0.90	>0.90	0.29—0.55	0—0.13	0
3 : 20	>0.90	>0.90	>0.90	0.47—0.61	0—0.29
4 : 20	>0.90	>0.90	>0.90	>0.90	0.78—1.00

Table IV. Results of chromatographic development with solvent mixtures of benzene (a), butanone (b), acetone (c), and ethanol (d)

Volume ratio of mixture (a : b : c : d)	R_f				
	P-002	P-010	P-050	P-160	P-500
3 : 1 : 6 : 4	0.80—1.00	0.42—0.52	0.03—0.09	0	0
4 : 2 : 6 : 4	0.74—1.00	0.46—0.61	0.24—0.50	0.06—0.16	0
5 : 3 : 6 : 4	0.70—1.00	0.40—0.70	0.30—0.49	0.14—0.28	0—0.28

Six possible combinations of two developers which belong respectively to different categories were tested. The developments with following systems proved to be able to separate the samples according to molecular weight; these were the systems (1) + (3), (2) + (3), and (3) + (4), which were practically *n*-hexane and butanone, cyclohexane and butanone, and benzene and ethanol or acetone, respectively. It should be noted here that every system useful for the present purpose contains developers belonging to the category (3). With the other systems no migration of the sample occurred, except for the system (2) + (4), in this case cyclohexane and ethanol, which showed a slight trend of separa-

tion by molecular weight at a certain composition of the mixture. Thus it may be concluded that for good separation the developer should have polar characteristics as well as poorer dissolving powers toward polystyrene. In this connection we mention that in acetone, samples P-002 and P-010 were soluble, whereas P-050, P-160, and P-500 were insoluble.

By way of example, Table III is given to show the result obtained with the system benzene and acetone. As is seen from the table, an increase in the content of benzene accompanied an increase in R_f -value for every sample with a different molecular weight, and a set of the samples, whose molecular weights lie within a

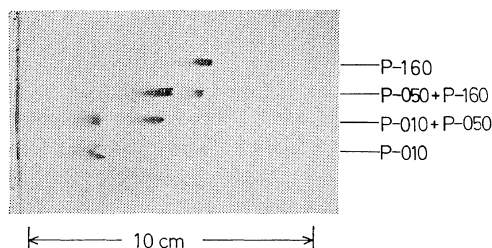


Figure 1. Chromatogram obtained for testing the resolving power in molecular weight (for the details, see text).

limited range, can be separated with a mixture of benzene and acetone at a certain composition. However, it is obvious that these types of mixture with any composition cannot give complete separation of all the reference samples without some samples remaining on the start line and the others reaching the solvent front.

In order to accentuate the effect of molecular weight on chromatographic migration, mixtures of benzene, butanone, acetone, and ethanol have been adopted. The results obtained with these types of mixture are given in Table IV. Inspection of this table indicates that the mixture with a certain composition given in the last row of the table permits us to achieve complete separation according to molecular weight so far as the reference samples are concerned. For practical purposes, however, these types of mixture were still unsatisfactory because of the blurring-over nature in the spot shape (tailing phenomena), as seen in Figures 1 and 2, which will lower the accuracy in reading off the R_f -value on the chromatogram.

Concentration Gradient Development

As was stated above, the tailing phenomena will surely disturb the determination of the molecular weight distribution, for samples with wide polymolecularities will, in principle, exhibit blurring-over of the spot shape. Thus for suppressing such trends, a concentration gradient development has been applied. At the beginning of the experiment, a mixture of benzene (5 ml), acetone (15 ml), and ethanol (10 ml) was set in a container. After the solvent front had reached the height of the starting line, an equivolume mixture of benzene and butanone (15 ml) was added dropwise in such a way that the rate of

addition decreased with the development time. The final composition of the mixture reached the same as that given in the last row of Table IV, the solvent loss during the development being ignored. The chromatogram thus obtained was found to result in more distinct shapes of spots. Further experiments were, therefore, carried out by making use of this procedure. Prior to the application of the concentration gradient method to radically polymerized polystyrene (P-W), the resolution of TLC with respect to molecular weight was investigated. A blended species of P-010 and P-050 and that of P-050 and P-160 were subjected to concentration gradient development. The chromatogram showed complete separation into components, as shown in Figure 1. It should be noted, however, that the introduction of the concentration gradient method to the present case was not the necessary condition as required in the TLC study of copolymers^{1,2} but only a condition for achieving effective separation.

Determination of Molecular Weight Distribution Curve

Molecular weight distribution of radically polymerized polystyrene, P-W, was then determined by developing it with the concentration gradient method. Samples P-002, P-010, P-050, P-160, and P-500 were developed simultaneously on the same chromatoplate. A photograph of the chromatogram shows that the sample P-W is developed not as a spot but as an extending band, showing a definite distribution of blackness (see Figure 2). For each spot of the reference samples, values of R_f at upper and lower sides were read off and plotted against $\log M$ of the samples (M , molecular weight). Figure 3 shows this plot, which is in agreement with that reproduced from the data given in the last row of Table IV, though not represented in the figure.

The first step for the determination of molecular weight distribution is to know the relation between the R_f -value and the apparent blackness on the film (B_i), which may directly be converted to a relative amount of polymer (X_i), because B_i is proportional to X_i as described previously. Then with the aid of the relation between R_f and M , the relative weight fraction

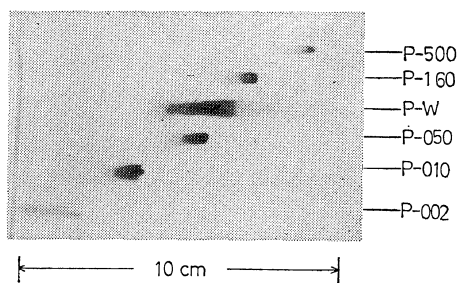


Figure 2. Chromatogram obtained for the determination of the molecular weight distribution of polystyrene sample coded P-W. Monodisperse polystyrene samples, P-002, P-010, P-050, P-160, and P-500 are developed as references for establishing the relation between R_f and M .

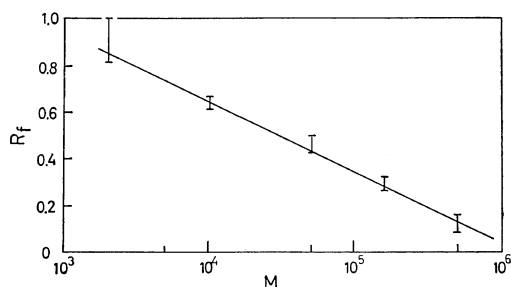


Figure 3. Semilogarithmic plot of R_f against M constructed with samples, P-002, P-010, P-050, P-160, and P-500.

of a polymer species with known molecular weight can be estimated. To illustrate the process and final results of analysis Table V is given. In Figure 4, the molecular weight distribution curve thus obtained is drawn with a solid line. For comparison, the dotted line in the same figure describes a molecular weight distribution curve for the same sample, which was deduced from the GPC measurement. No clear-cut distinction may be seen between these results from two different methods. Such a visual coincidence is further confirmed by computing the weight, and the number average molecular weight (M_w and M_n , respectively) of sample P-W by making use of the distribution curves. Values of M_w and M_n deduced from the TLC experiment were 76600 and 45400, respectively; while 78100 and 39300 were obtained from the GPC experiment. Thus the numerical agreement was also found to be excellent.

Table V. Calculation process of molecular weight distribution curve

R_f	$M \times 10^{-4}$	$B_i (\propto X_i)$	$(X_i / \sum X_i) \times 100$
0.135	49	0.5	0.33
0.163	39	1.0	0.67
0.190	32	1.7	1.15
0.217	26.2	2.1	1.42
0.244	21.3	2.8	1.89
0.271	17.1	4.5	3.02
0.298	14.0	4.7	3.17
0.325	11.6	9.4	6.36
0.352	9.8	16.4	11.09
0.379	7.6	20.0	13.52
0.406	6.1	19.1	12.91
0.436	4.8	16.7	11.29
0.461	4.0	14.1	9.58
0.488	3.3	10.4	7.03
0.515	2.66	8.4	5.68
0.542	2.17	6.1	4.12
0.569	1.78	3.9	2.64
0.596	1.42	2.6	1.76
0.623	1.17	2.1	1.42
0.651	0.96	1.1	0.74
0.677	0.77	0.3	0.20

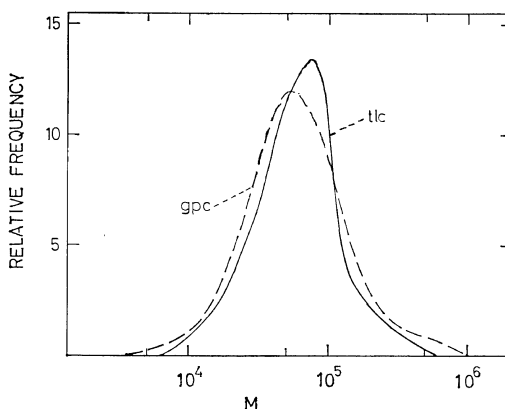


Figure 4. Comparison of the molecular weight distribution curve for sample P-W deduced from the TLC experiment with that from the GPC experiment.

In summary, TLC combined with concentration gradient development has been shown to be applied successfully to the determination of the molecular weight distribution of polystyrene. In so far as the present experiment is concerned, the separation mechanism appears to relate to thermodynamic equilibria, *viz.*, the

phase equilibrium between liquid and polymer, and the equilibrium in adsorption of polymer on the interface between the liquid and stationary phases. The reason for the superior fractionation ability proved by this method is rather complex; factors affecting R_f -values are now being investigated in our laboratory. Preliminary experiments imply that among characteristic factors, an important one would be a change in the ratio of liquid to solid phase of the chromatoplate. In this connection, it should be mentioned that nearly monodisperse polystyrene samples, when developed, exhibited peculiar spot shapes which may be expressed by the phrase "tailing-up" with the tail clearly cut off (see Figures 1 and 2). Results of further investigations of the spot shape in relation to the separation mechanism will be reported in a subsequent publication.

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