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MOLECULAR SIEVE EFFECTS IN THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A series of investigations was carried out to establish the experimental conditions under which fractionation by molecular size occurs due to molecular sieve effects in thin-layer chromatography with macroporous silica gels. Molecular sieve effects appear on chromatoplates pre-eluted with a developer before the sample development when adsorptive interactions between polymer and adsorbent are eliminated by choosing an appropriate solvent for the developer. It is pointed out that the phase ratio, defined as the weight of developer per unit weight of adsorbent, should be sufficiently high to induce molecular sieving. A quantitative discussion is given of the enhancement of the resolution with respect to molecular weight in the separation, compared with results obtained by gel permeation chromatography with the same silica gels.

INTRODUCTION

Hitherto reported applications of thin-layer chromatography (TLC) to polymer chemistry can be classified into two groups. One involves the possibility of separating copolymers and stereoregular polymers by differences in their compositions[1,2], monomer arrangement[3] and steric isomerism[4], which occur to a small extent depending on the molecular weight. The other group is related to the possibility of fractionating homopolymers by differences in molecular weights[5–8]. The mechanism for each type of separation has been discussed in detail[9–12].

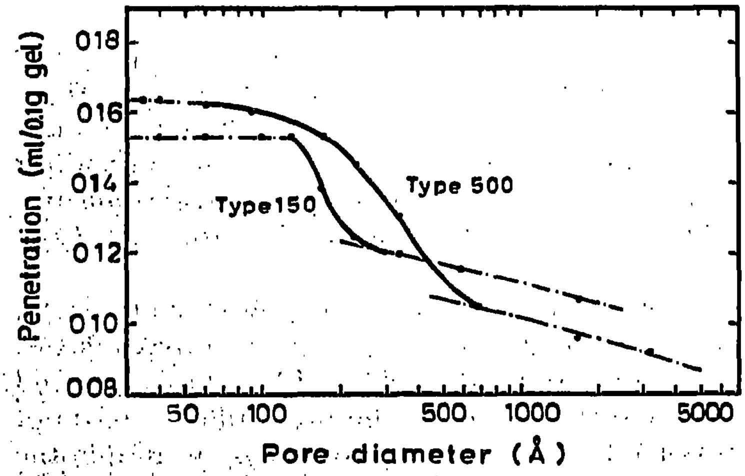
Recently, a third possibility has attracted the attention of polymer chemists, namely fractionation due to molecular sieve effects on thin-layer chromatoplates. JOHANSSON AND RYMO[13] were the first to apply Sephadex gels to TLC. This open gel bed system has since been utilized for the determination of the molecular weights of proteins[14]. HALPAAP AND KLATYK[15] reported that the fractionation of lyophobic polymers due to molecular sieve effects is possible by TLC with macroporous silica gels if the thin layer is pre-eluted with a developer before the sample development. BELENKII AND GANKINA[7] also described the appearance of these effects in conventional TLC with macroporous silica gels. More recently, OTOCKA *et al.*[12] investigated this technique and proposed a new name, thin-layer gel permeation chromatography (TLGPC), which is more specific to this technique than the term thin-layer gel chromatography used by DETERMANN[16].

In this paper we describe in detail investigations of the conditions under which molecular sieve effects appear most prominently and clearly in TLC. For this purpose, two approaches were studied, namely the chromatographic behaviour of polystyrenes in the absence and presence of adsorptive interactions between polymer and adsorbent. On the basis of the experimental results, two basic conditions that are necessary to induce molecular sieving effects were established: the chromatoplate coated with macroporous gel should be pre-eluted with developer to a certain level before the sample development, and the developer should be chosen so that adsorptive interactions between polymer and adsorbent, which will induce fractionation by molecular weight, are eliminated.

EXPERIMENTAL

Stationary phase

Two types of macroporous silica gels, designated Type 150 and 500, obtained from E. Merck AG, Darmstadt, G.F.R., were used to prepare the TLC stationary phase. The pore size distribution was determined with a Mercury Intrusion Porosimeter (American Instrument Co., Silver Spring, Md., U.S.A.). The integral distribution curves thus obtained for each type of gel are shown in Fig. 1. The maximum



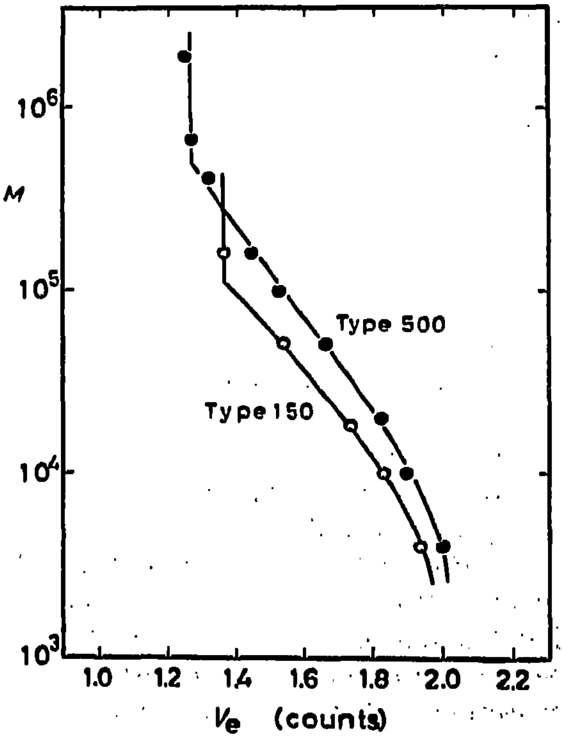


Fig. 1. Integral pore size distribution curves obtained by means of mercury intrusion for Type 150 and 500 silica gels.

Fig. 2. GPC plots of molecular weight against elution volume (in counts) established for narrow-distribution polystyrenes with Type 150 and 500 gels.

pore diameters were estimated to be *ca.* 300 and 700 Å for Type 150 and 500 gels, respectively, and the pore sizes were found to be distributed around a diameter of 170 Å for the former and 300 Å for the latter.

The permeability limits (PL) of the gels were determined by using a Shimadzu gel permeation chromatograph, Model 1-A, equipped with a column 1 ft. long and 8 mm I.D. Narrow distribution polystyrenes were used as reference samples. Tetrahydrofuran (THF) was used as the eluent, and the flow-rate was adjusted to 0.5 ml/min. The results of gel permeation chromatography (GPC) are given in Fig. 2, from which it can be seen that the PL values on the molecular weight scale are $1.0 \cdot 10^5$ and $5.0 \cdot 10^5$ for type 150 and 500 gels, respectively.

To prepare thin-layer chromatoplates, each gel was slurried with distilled water and applied to thick glass plates of dimensions 20 × 10 and 20 × 20 cm^2. The thickness of the thin layer was adjusted to 0.25, 0.5 and 1.0 mm for different experiments. The chromatoplates were dried in an open vessel for at least two days and used without activation by heating.

Development procedure

From various reports concerning molecular sieve effects in TLC[14], we found that such effects appear only when the chromatoplate was wetted with eluent before the sample development. The aim of such pre-treatments of the chromatoplates with Sephadex gels was to produce swelling equilibrium of the gel particles. For silica gel, HALPAAP AND KLATYK[16] proposed that pre-elution with a developer should be carried out up to a distance of at least 1 or 2 cm from the starting point of the sample spots. In accordance with this proposal, we investigated the extent to which the pre-elution should be carried out so as to induce molecular sieving.

To minimize adsorptive interactions between polymer and adsorbent (silica gel), THF and polystyrene were used as the developer and sample polymer, respectively; interactions were no longer significant owing to the highly polar nature of THF[1,10]. However, we shall later give some results in which molecular sieve effects and adsorption effects act simultaneously when using cyclohexane and benzene as the developer system[8,15].

Either ascending or descending development was carried out at 25° in a closed chamber. In the former method the chromatoplate was dipped into THF (developer) to a depth of 1 cm (dip level) from its lower edge, and pre-elution was carried out with THF up to the desired level. As soon as the solvent front had reached this level, the polymer sample dissolved in THF was introduced with the aid of a micropipette having a J-form at its tip. The sample size was adjusted to give an amount of polymer ranging from 10 to 20 µg and the starting point was taken as 1 cm above the dip level. The development was continued until the solvent front had reached a distance of 18 cm from the starting point, instead of 10 cm as usually used.

In descending development, a method proposed by MORRIS[17] was adopted: the chromatoplate was inclined at an angle of 30° to the horizontal and the developer was supplied to the upper edge of the chromatoplate from a solvent tank with the aid of strips of filter-paper. The sample was introduced in a manner similar to that for ascending development. The chromatograms were stained with thymol blue saturated in a mixture of water and ethanol (1:1, v/v), and concentrated sulphuric acid[8].

TABLE I

DESIGNATION AND MOLECULAR WEIGHTS OF SAMPLES USED

Code	M_w[a]
PS-1	4 000
PS-2	10 000
PS-3	19 800
PS-4	51 000
PS-5	160 000
PS-6	670 000
PS-I[b]	97 200
PS-II[b]	411 000
PS-III[b]	1800 000

[a] Nominal values indicated by the Pressure Chemical Co.
[b] Used only for GPC calibration experiments.

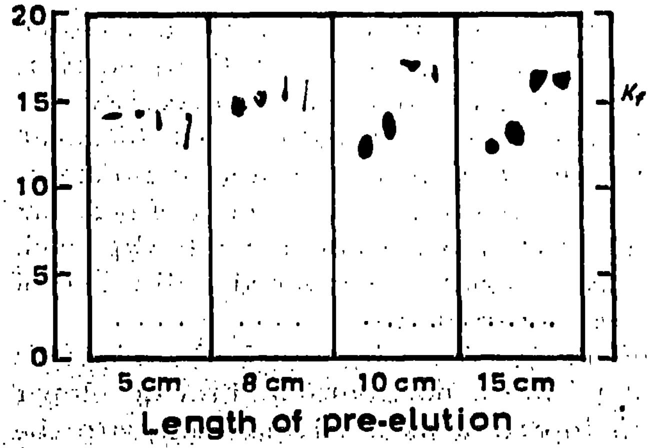
Materials

Reagent grade solvents were used without further purification as the developer. Narrow-distribution polystyrene samples were obtained from the Pressure Chemical Co., Pittsburgh, U.S.A., and designations and nominal molecular weights are listed in Table I.

RESULTS AND DISCUSSION

Pre-elution and molecular sieve effects

The relation between pre-elution and the appearance of molecular sieve effects was investigated by using the ascending development technique and THF as the developer. Fig. 3 shows a chromatogram that was obtained for samples PS-1 PS-2, PS-5 and PS-6 on a thin layer of Type 150 gel with a thickness of 0.5 mm. From Fig. 3 it can be seen that when the level to which pre-elution was carried out



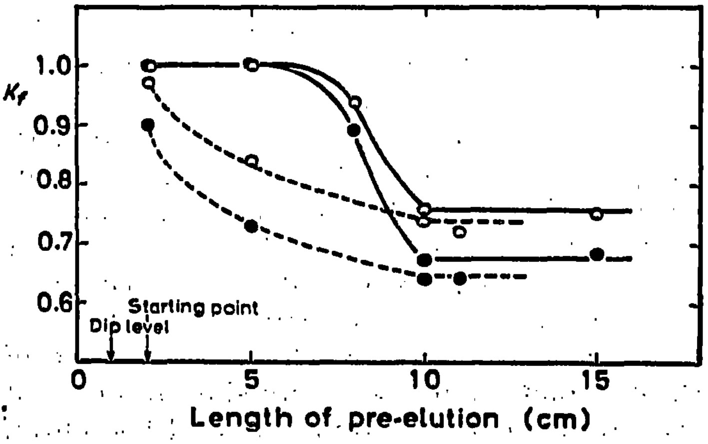


Fig. 3. Chromatograms obtained for polystyrenes with a thin layer of Type 150 gel and THF under various pre-elution conditions.

Fig. 4. Plots of K_f found for PS-1 (●) and PS-2 (○) against length of pre-elution. Unbroken and broken lines show the results found by ascending and descending development, respectively.

(length of pre-elution) is increased to 8 cm from dip level, molecular sieve effects begin to appear; the sample with the lowest molecular weight, PS-1, migrates the least, the sample with the second lowest molecular weight, PS-2, migrates beyond PS-1, while PS-5 and PS-6 migrate to the same height beyond PS-1 and PS-2. It should be noted that the behaviour of samples PS-5 and PS-6 can be interpreted in terms of the PL determined for the gel (see Fig. 2). The result is in agreement with those reported by other workers[12,15]. The necessity for pre-elution implies that the phase ratio, defined as the weight of solvent retained per unit weight of adsorbent, should be sufficiently high to induce molecular sieving.

A series of investigations similar to the above were carried out with the descending development technique. Considering that the appearance of molecular sieve effects is closely related to the phase ratio, the experiment was conducted by changing the number of strips of filter-paper that are used to transfer solvent to the upper edge of the chromatoplate. With chromatoplates of Type 150 gel, it was demonstrated that molecular sieving began to appear even with one strip of filter-paper, and occurred most readily when this number was increased to four.

To represent the above results quantitatively, a quantity K_f can be used, defined by the ratio of two over-all migration rates:

$$K_f = (d/T)/(d_m/T) = d/d_m$$

where T is the development time and d and d_m are the distances of migration found simultaneously for a sample being investigated and for a sample whose molecular weight exceeds PL, respectively. Fig. 4 shows the relationships between the K_f values found for PS-1 and PS-2 and the length of pre-elution established by ascending and descending development. In descending development the number of strips of filter-paper was fixed at four and the length of pre-elution was varied. From Fig. 4 it can be seen that molecular sieve effects appear in a different manner for the different development techniques.

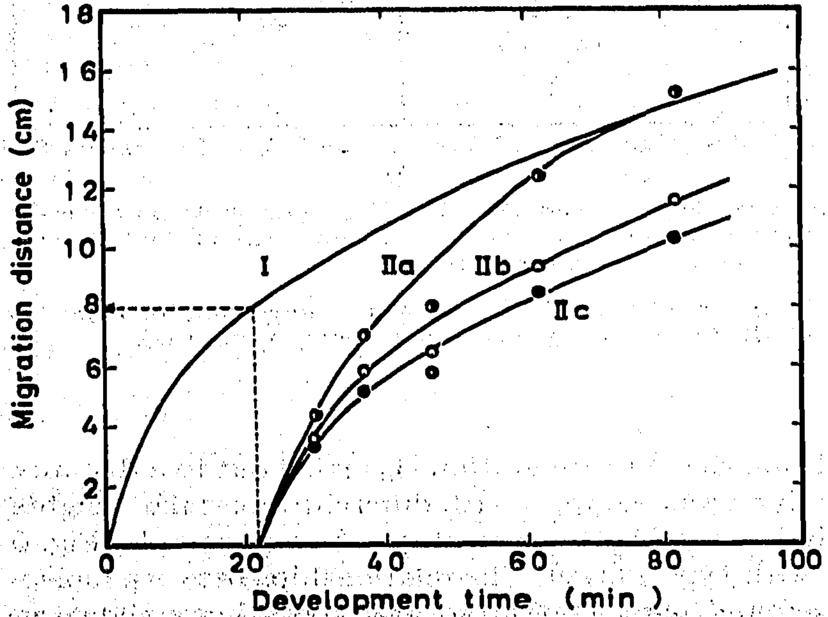
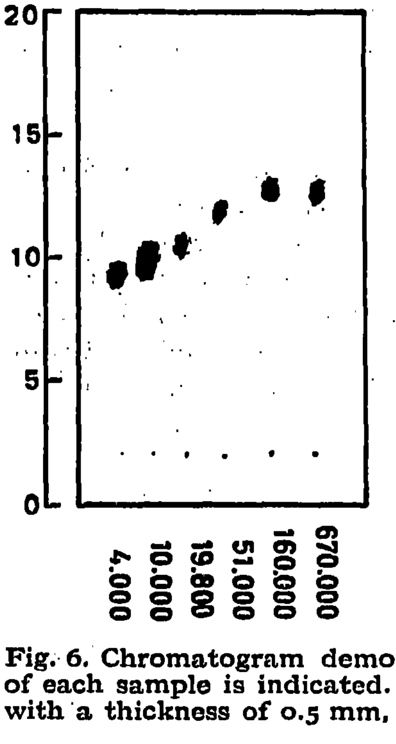


Fig. 5. Time dependences of sample migration observed for PS-6 and PS-5 (Curve IIa), PS-2 (IIb) and PS-1 (IIc) with THF on a thin layer of Type 150 gel pre-eluted to a distance of 8 cm from the dip level. Curve I represents the ascending solvent front.

Improvement of resolution

To investigate whether or not the resolution in the separation with respect to molecular weight could be improved by prolonging the development time, the distance migrated in ascending development was measured as a function of time with a thin layer of Type 150 gel and THF. When the solvent front had reached a distance of 10 cm from the dip level, the samples were loaded and the migration distance was determined at several time intervals. The results are plotted in Fig. 5.

In Fig. 5 curve I indicates the time-dependence of the ascending solvent front. Curve IIa is for PS-6 and PS-5, and curves IIb and IIc are for PS-2 and PS-1, respectively, and show that after a certain time the rate of migration for PS-6 and PS-5, whose molecular weights exceed PL, is similar to that of the solvent ascending front, while the rates for PS-2 and PS-1 remain almost unchanged, independent of the time. It can therefore be concluded that no distinct improvement in the resolution can be expected by prolonging the development time.



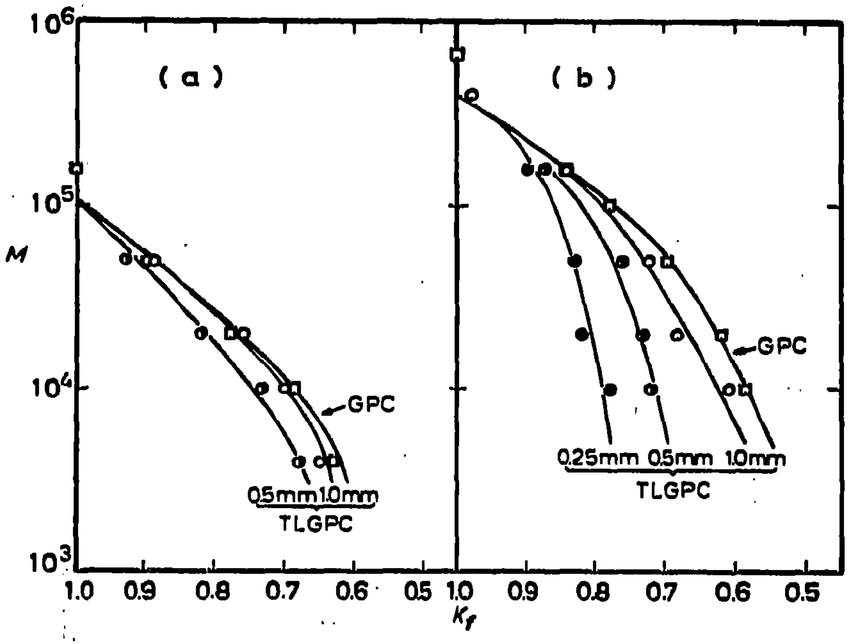


Fig. 6. Chromatogram demonstrating molecular sieve effects (TLGPC). The molecular weight of each sample is indicated. Development conditions: non-activated thin layer of Type 150 gel with a thickness of 0.5 mm, and THF; length of pre-elution, 10 cm.

Fig. 7. Comparison of TLGPC results with those from GPC for (a) Type 150 gel and (b) Type 500 gel. In TLGPC, thin layers of different thicknesses (0.25, 0.5 and 1.0 mm) were used.

Comparison with GPC results

Under the established condition that the pre-elution is carried out to a distance of 10 cm from the dip level, the polystyrene samples with different molecular weights were developed with THF on thin layers of both types of gel. As an example, Fig. 6 shows a chromatogram obtained with type 150 gel. The relationships between molecular weight (M) and K_f values found with Types 150 and 500 gel are shown in Figs. 7a and b, respectively. In these figures it can be seen that the gradient of the plots decreases as the thickness of the thin layer increases. Enhancement of the resolution with respect to molecular weight in TLGPC can therefore be expected when the thickness of the thin layer is increased.

Results from conventional column GPC are usually represented by plotting log M against the elution volume, V_e. Hence, in order to directly compare TLGPC results with those from GPC, it is necessary to convert V_e into K_f values. According to the definition given for K_f, this conversion can be made by using the equation

$$K_f = (1/V_e)/(1/V_e^i) = V_e^i/V_e$$

where V_e^i is the interstitial volume, that is, the elution volume for samples whose molecular weights exceed the PL of gel used. The results shown in Fig. 2 are re-plotted in this manner in Figs. 7a and b. It can be seen from these figures that the gradient is less than that found with thin layers of 1.0 mm thickness. The difference in the gradients, and therefore in the resolutions, observed in TLGPC and GPC, can probably be attributed to the difference in the phase ratios. In addition, better linearity of the plot for TLGPC can be achieved by using a thin layer prepared with a mixture of gels that have different pore sizes.

Molecular sieve effects in adsorption TLC

HALPAAP AND KLATYK[15] investigated the chromatographic behaviour of polystyrene samples with macroporous silica gels and cyclohexane and benzene developers and reported that molecular sieve effects in the separation were observed only when the chromatoplate had been pre-eluted. On the other hand, BELENKII AND GANKINA[7] pointed out that molecular sieve effects could be observed in adsorption TLC without the pre-elution if the development conditions were chosen appropriately.

Following the work of HALPAAP AND KLATYK[15], we also investigated the conditions under which the molecular sieve effects appear in adsorption TLC. For this purpose, mixtures of cyclohexane and benzene of three different compositions, namely 50:50, 55:45 and 60:40, v/v, were used as the developer with Type 150 gel. These developer systems are good solvents for polystyrene, and no phase separation of the polymer takes place during the sample development[9,10]. For the developer of each composition, two chromatoplates were prepared, only one of which had been pre-eluted up to a distance of 10 cm from dip level. Then the samples were developed on these chromatoplates simultaneously under the same conditions.

The chromatograms are illustrated in Fig. 8, in which it can be seen that the

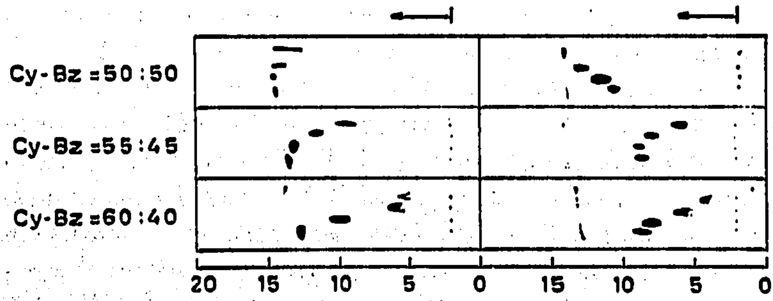


Fig. 8. Chromatograms obtained with Type 150 gel and cyclohexane–benzene developer systems with different compositions. On each chromatogram, the polymer samples developed are PS-6, PS-5, PS-2 and PS-1 from top to bottom. The three chromatograms on the left-hand side were obtained without pre-elution, and those on the right-hand side were obtained with pre-elution.

chromatograms on the left-hand side (no pre-elution) are characteristic of adsorption chromatography, so that the migration distance increases with decreasing molecular weight[6,9,10] except for the 50:50 mixture. The chromatogram obtained with pre-elution at this composition shows the typical features of molecular sieving. This proves that only if adsorption effects between polymer and adsorbent are eliminated is the sample migration determined uniquely by molecular sieve effects. The chromatograms on the right-hand side (with pre-elution) are different for different compositions of the developer system, indicating that both adsorption and molecular sieve effects influence the sample migration simultaneously. These observations are in agreement with those reported by other workers[12,15]. The results obtained by Belenkii and Gankina[7] could not be reproduced, however, in the present work.

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