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DETERMINATION OF RESOLUTION IN GEL PERMEATION CHROMATOGRAPHY

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SUMMARY

The determination of the chromatographic resolution is based on the shape of and the distance between two neighbouring peaks produced by two homogeneous components of the sample. The application of this test to the gel permeation chromatography (GPC) of polymers using the peaks of calibration standards is influenced by the choice of sample. A new measure of the resolving power, which is corrected for sample heterogeneity and distance, is discussed and compared with already known tests. The numerical value of the proposed measure of resolving power indicates that ratio of molar masses which would be separated with a 4 σ resolution. The comparison is based on data measured with dextran standards in water and with polystyrene standards in methyl ethyl ketone or tetrahydrofuran. The aim of this work was the evaluation of the efficiency of a given GPC apparatus before and after re-swelling the gels, and the comparison of columns packed with particles either 40–60 μm or 10 μm in size.

INTRODUCTION

Tests of chromatographic resolution are necessary in order to compare different equipment or methods, to ascertain whether there is a change in the separation power of a given device with time, and to determine the possible influence of any alteration of the apparatus or the method.

The height equivalent to a theoretical plate, or the plate number, $N = 16(V_e/W)^2$, are often used as a measure of resolution estimated from the peak width, W, and elution volume, V_e, of one component. In addition to the influence of sample viscosity and hence of molar mass, an influence which is of special importance in polymer research, it has already been pointed out that different chromatographic columns with identical plate numbers do not always show identical separation efficiencies. A reliable test of separation power should refer to data obtained by means of *two* components[1]. This holds for the resolution, R_s, calculated by means of eqn. 1 from the peak widths, W, and the elution volumns, V_e, of two components, I

and II. As $V_{e,II} > V_{e,I}$, in GPC, the molar mass of the component I is higher than that of II, $M_I > M_{II}$.

$$R_s = \frac{2(V_{e,II} - V_{e,I})}{W_I + W_{II}} \tag{1}$$

In order to adapt eqn. 1 to the investigation of polymer samples, Bly[2] introduced the heterogeneity of the specimens, $H = \overline{M}_w/\overline{M}_n$, and formulated the corrected resolution:

$$R_{corr} = \frac{2(V_{e,II} - V_{e,I})}{W_I/H_I + W_{II}/H_{II}} \tag{2}$$

where $\overline{M}_w$ is the weight-average and $\overline{M}_n$ the number-average of molecular mass distribution. Bly[2] also suggested the specific resolution:

$$R_{sp} = \frac{2(V_{e,II} - V_{e,I})}{(W_I/H_I + W_{II}/H_{II})\log(M_I/M_{II})} \tag{3}$$

as a standard for GPC efficiency. This equation involves the selectivity term of exclusion chromatography:

$$S = \frac{V_{e,II} - V_{e,I}}{\log(M_I/M_{II})} \tag{4}$$

which also represents the slope of the GPC calibration graph:

$$V_e = A - S \log M \tag{5}$$

The limiting value

$$\lim R_{sp} = S/W \tag{6}$$

$$H_I = H_{II} \to 1$$
$$W_I = W_{II}$$

has been named the "resolution index" by Bly[2], whereas Cooper and Kiss[3] suggested the quantity

$$RI = (M_{II}/M_I)^{1/R_s} \tag{7}$$

as the resolution index.

The aim of our study was the evaluation of the separation power of a commercial GPC apparatus with Spheron columns and water as the eluent, the comparison with the separation power after re-swelling the gels in methyl ethyl ketone (MEK) and re-packing the columns, and the evaluation of the separation power of a set of LiChrospher® columns in combination with the same extra-column equipment as used previously.

EXPERIMENTAL

Apparatus

A gel and liquid chromatograph (LC/GPC 5050) with a differential refractometer (2025/50) and a UV photometer (4040) (Knauer, Oberursel, G.F.R.) was used.

Columns. (a) Four stainless-steel tubes, 600 mm × 7.8 mm I.D., were packed with Spheron gels in water by the manufacturer (Knauer KG). The gels were Spheron P 40, P 100, P 300 and P 1000, particle size 20–40 μm. The total void volume was 50.0 ml and the accessible volume (sodium chloride peak) 95.4 ml. (b) The same tubes were re-packed by Laboratorní Přístroje, Prague, Czechoslovakia (by courtesy of Dr. J. Čoupek), with the gels re-swollen in MEK. The void volume was 53.6 ml and the accessible volume (benzene peak) 100.0 ml. (c) Five stainless-steel tubes, 250 mm × 4 mm I.D., were packed with LiChrospher gels by the manufacturer. The gels were LiChrospher Si 100, Si 500 (two), Si 1000 and Si 4000. The void volume was 6.6 ml.

Samples.

(a) Dextran standards were obtained from Pharmacia, Uppsala, Sweden. The molecular mass (M) and heterogeneity (H) as given by the supplier are shown in Table Ia. (b), (c) Polystyrene standards were obtained from Knauer KG. The molecular mass (M) and sample heterogeneity (H) are given in Table I (b, c).

Solvents

(a) Distilled water; (b) methyl ethyl ketone (MEK), reagent grade, distilled; and (c) tetrahydrofuran (THF), reagent grade, purified and distilled, were used.

Working conditions

(a), (b) A flow-rate of 1.5 ml/min, injection volume 1.5 ml and sample concentration 0.01% were used; (c) a flow-rate of 1.5 ml/min, injection volume 10 μl and sample concentration 0.25% were used.

RESULTS

The elution volumn (V_e) refers to the position of the peak, and the peak width (W) is the distance between the points at which the inflection-tangents of a peak cross the baseline. These data are given in Table I.

DISCUSSION

The peak width of polydisperse polymer samples is influenced by chromatographic dispersion, extra-column effects and sample heterogeneity. If all of these contributions are Gaussian, the additivity rule is

$$\sigma^2_{\text{total}} = \sigma^2_{\text{apparatus}} + \sigma^2_{\text{polymer}} \tag{8}$$

The quantity $\sigma_{\text{apparatus}}$, which refers to the standard deviation of column and extra-column effects, corresponds to the peak width $W = 4\sigma$ needed for the evaluation of resolution by means of the equations cited.

TABLE I

EXPERIMENTAL RESULTS

System	*Sample*			*Peak*		*Resolution tests*				
	No.	$M \cdot 10^{-3}$	*H*	V_e *(ml)*	*W (ml)*	R_S	R_{corr}	R_{sp}	*RI*	*T*
(a) Dextrane-water-Spheron[4]	1	234	2.13	58.5	41.4	0.18	0.31	0.90	0.012	13.0
	2	106	1.39	65.3	34.2	0.13	0.19	1.15	0.055	7.4
	3	72.5	1.49	70.2	40.5	0.16	0.25	1.06	0.035	8.7
	4	42	1.62	76.4	35.6	0.17	0.27	0.98	0.026	10.5
	5	22.3	1.49	82.0	29.3	0.20	0.33	1.11	0.030	8.0
	6	11.2	1.96	87.6	27.9					
(b) Polystyrene-methyl ethyl ketone-Spheron	1	2000	1.04	54.00	10.35	0.17	0.17	(0.36)	(0.001)	(580)
	2	670	1.03	56.02	13.95	0.16	0.18	0.75	0.033	22
	3	390	1.19	58.27	14.40	0.54	0.70	1.28	0.095	6.1
	4	110	1.4	67.72	20.70	0.56	0.72	0.99	0.048	10.3
	5	20.4	1.2	78.30	17.33	0.46	0.54	0.77	0.030	20
	6	4	1.14	86.40	17.55	0.35	0.41	(0.50)	(0.005)	(97)
	7	0.6	1.20	92.93	19.35					
(c) Polystyrene-tetrahydrofuran-LiChrospher	1	2000	1.04	6.60	1.28	0.29	0.30	(0.64)	(0.024)	(37)
	2	670	1.03	6.94	1.05	0.27	0.29	(0.63)	(0.019)	(39)
	3	233	1.11	7.35	2.03	0.54	0.59	1.81	0.252	3.6
	4	110	1.04	8.25	1.28	1.26	1.31	2.76	0.420	2.3
	5	37	1.04	9.90	1.35	0.59	0.64	1.89	0.265	3.4
	6	17	1.12	10.91	2.10	0.72	0.79	3.41	0.478	2.0
	7	10	1.05	12.15	1.35	0.83	0.85	2.15	0.330	3.0
	8	4	1.01	13.05	0.83					

The heterogeneity correction used in eqn. 2, $W_{corr} = W/H$, is only an approximation. The thick line in Fig. 1 shows the degree of improvement corresponding to this approximation, whereas the set of thin lines demonstrate corrections obtained with eqn. 8. If the heterogeneity is not too high, the approximation will work satisfactorily, especially for equipment with a calibration graph that is not too steep.

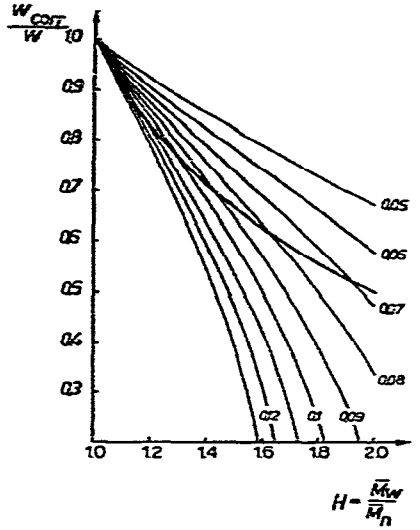

Fig. 1. Ratio of peak width corrected for sample heterogeneity (W_{corr}) to peak width (W) as taken from a chromatogram *versus* sample heterogeneity (H). Thick line: $W_{corr} = W/H$. Thin lines: W_{corr}/W as calculated by means of the additivity rule, eqn. 8, assuming samples with logarithmic normal distribution of molar mass, $\sigma^2_{polymer} = (S/\ln 10)^2 \ln H$. The parameter used in plotting the set of thin lines has the meaning $K = (S/\ln 10)^2$ and refers to the selectivity, S, of the chromatographic system under investigation.

The original equation for chromatographic resolution (eqn. 1) refers to adjacent peaks of components with crucial separability. The heterogeneity correction which is introduced in eqn. 2 qualifies this equation for calculations based on the peak width of slightly polydisperse specimens, *e.g.*, standard samples as used in GPC calibration. However, the peaks of such samples cannot be regarded as neighbouring peaks in the strict sense. As a consequence, the values of R_{corr} depend on the choice of sample. For demonstration purposes, we calculated R_{corr} using some different combinations of the data compiled in Table I. Consecutive samples yield lower values than are obtained from alternate specimens. Combination of every third sample produced even higher results in all instances investigated (Table II).

In order to eliminate the undesired influence of sample distance, this quantity was introduced into eqn. 3 as $\log(M_I/M_{II})$. The specific resolution is insensitive to sample distance, as demonstrated by the values under $\bar{R}_{sp}$ in Table II. This holds for that range of molecular masses governed by the fairly straight part of the calibration graph. However, as the change in V_e caused by a certain change in $\log M$ decreases towards the limits of the separation range, the specific resolution also reaches too low a value here, whereas the corresponding exponential quantities are too high,

TABLE II

AVERAGE VALUES OF SOME RESOLUTION TESTS CALCULATED FROM THE DATA IN TABLE I

Systems: (a) dextran–water–Spheron; (b) polystyrene–MEK–Spheron; (c) polystyrene–THF–LiChrospher. Calculations: (A) calculated by means of combination of consecutive samples, [(a) 1 + 2, 2 + 3, 3 + 4, 4 + 5, 5 + 6; (b) 2 + 3, 3 + 4, 4 + 5, 5 + 6; (c) 3 + 4, 4 + 5, 5 + 6, 6 + 7, 7 + 8]; (B) calculated by means of combination of alternate samples, [(a) 1 + 3, 2 + 4, 3 + 5, 4 + 6; (b) 2 + 4, 3 + 5, 4 + 6, 5 + 7; (c) 2 + 4, 3 + 5, 4 + 6, 5 + 7, 6 + 8]; (C) calculated by means of combination of every third sample, [(a) 1 + 4, 2 + 5, 3 + 6; (b) 1 + 4, 2 + 5, 3 + 6, 4 + 7; (c) 2 + 5, 3 + 6, 4 + 7, 5 + 8].

System	R_s			R_{corr}			R_{sp}			RT			T		
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
a	0.17	0.32	0.50	0.27	0.53	0.82	1.04	1.06	1.10	0.032	0.032	0.034	9.6	9.0	8.1
b	0.37	0.81	1.33	0.46	0.98	1.60	0.95	0.93	0.92	0.052	0.050	0.054	14.6	17.6	14.1
c	0.64	1.30	2.27	0.68	1.39	2.38	2.40	2.25	2.44	0.35	0.33	0.36	2.8	3.1	2.8

e.g., the resolution index, eqn. 7. This is the reason why some results obtained using samples within the boundary regions were omitted when averaging (see footnote to Table II).

For a baseline separation, the resolution is 1.5, but even a value of 1.0 indicates a good separation, with only 3% overlapping of peak areas. One of the aims in chromatography is to increase the resolution to 1.0 or at the most to 1.5, but not above 1.5 as adjacent peaks are then too far apart.

The substitution of $\log(M_I/M_{II})$ in eqn. 3 relates the result to the condition $M_I/M_{II} = 10$. In consequence, the numerical values of the specific resolution can be unusually high, *e.g.*, 2 or more, as is the case with our system (c), Table II.

The resolution index, eqn. 7, yields values in the range 0–1. The lower limit corresponds to an extremely poor resolution. Our experimental work with Spheron columns led to small *RI* values, but the values for system (b) (polystyrene–MEK) are markedly higher than those for system (a) (dextrane–water). Unfortunately, this is not evidence of better resolution, as the heterogeneity of the polystyrene samples is much less than that of the dextran specimens and, in eqn. 7, the non-corrected resolution, R_S, is used as an exponent. In order to overcome this drawback of eqn. 7 we used the expression

$$T = (M_I/M_{II})^{1/R_{corr}} \tag{9}$$

for calculation of the separation power. The quantity T is insensitive to the distance between the samples chosen for evaluation, as demonstrated in Table II. Further, it has a straightforward graphical meaning, as it indicates the ratio of molecular masses which would be separated with a 4σ resolution. For example, $T = 3$, which holds for our system (c), means that two species with a 1:3 ratio of molecular masses can be separated almost completely, whereas our system (a) provides the same level of separation only with a 1:9 ratio. As eqn. 9 defines a measure of the greatest separation and as the term "resolution index" has already been used, the quantity T might be called "separation power" (Trennvermögen). From the figures in the last column in Table I it can be seen that T does not vary with the molecular mass of the samples used for determination. Apart from the figures produced by samples too near the limits of the separation range, the efficiency can be regarded as constant. This is demonstrated most clearly by the T data for system (c) in the range $233{,}000 \geqslant \bar{M}_w \geqslant 4000$, and to some extent also by all the further values of T and R_{sp} in Table I.

It is worth mentioning this constancy, as the height equivalent to a theoretical plate which is a one-sample test exhibits a marked dependence on molecular mass in accordance with the viscosity change[5]. The constancy of T and R_{sp} therefore indicates an improved separation of the high-molecular-mass components of the sample, a feature which has already been observed in preparative GPC.

Finally we come back to the starting questions: was the separation of the GPC equipment altered by re-packing the columns with Spheron gels re-swollen in MEK, and what is the separation given by the apparatus in combination with columns packed with 10-μm LiChrospher particles?

The answer to the second question is clear, because all of the tests taken into account indicate that the equipment with the LiChrospher columns exhibits a higher resolution.

The influence of the re-swelling of the Spheron gels is harder to detect. The values of R_{corr} are higher for the re-packed columns, but it must be kept in mind that these data might have been influenced by the choice of sample. This influence is unavoidable, as two different sets of samples are needed when working with either water or MEK. The specific resolution indicates that the re-filling caused a slight degeneration. The *RI* test yields the opposite result, but this is misleading as two different sets of specimens, dextran and polystyrene, with different values of heterogeneity, were used in the two investigations. The figures in column A under *RI* in Table II demonstrate clearly the drawback of eqn. 7. Finally, the quantity T leads to a clear answer, which is in accord with the indication of the R_{sp} test: the Spheron columns exhibited a greater efficiency with the water-swollen gels, but the re-packing had only a small influence.

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