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Method of Calculating Molecular Weight Distribution Function from Gel Permeation Chromatograms. II. Evaluation of the Method by Experiments

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Synopsis

The calculation scheme for correcting the broadening effect due to imperfect resolution on gel permeation chromatograms was compared with actual performances of a gel permeation chromatography (GPC) column. The experiment consisted of fractionating a high-density polyethylene on a GPC unit and then determining the chromatograms of the cuts collected. The chromatograms of the cuts were also computed from the starting chromatogram using experimentally determined resolution factors. The degree of agreement between the calculated and experimental chromatograms of the cuts shows convincingly that the previously proposed calculation scheme is satisfactory for the treatment of GPC data.

INTRODUCTION

A method of computing molecular weight distribution from gel permeation chromatogram has been proposed recently by one of us.[1] In the method the correction for the broadening of the chromatogram due to the imperfect resolution of columns of gel permeation chromatography (GPC) has been applied, and the method is expected to give the true molecular weight distribution of the sample.

In the present work the actual performance of a GPC unit was used to examine the proposed computation scheme. Briefly, the experiments consisted of collecting cuts of a polymer sample by a GPC unit and then comparing the chromatograms of these cuts with those obtained by calculation. The resolution of the GPC column was calibrated by a reverse-flow technique which did not rely on the distribution of any standard sample determined by other polymer fractionation methods. The analysis should therefore reflect the validity of the computation unequivocally. A molecular weight–eluent volume relationship which was calibrated by actual standard samples was used in the calculation to give the molecular weights of the cuts. This relationship did not enter in the main calculation for the chromatograms of the cuts, and a fictitious one would have served the same illustrative purpose.

EXPERIMENTAL

The GPC Unit

A commercial GPC unit made by Waters Associates was used. In the unit a special valve capable of instantly reversing the flow of eluting solvent was installed. The column consisted of three 4-ft. sections packed with resins of 10^6, 10^5, and 10^4 A. permeability (Waters designation) respectively. 1,2,4-Trichlorobenzene was the solvent. The overall plate count based on acetone in trichlorobenzene at room temperature was 680/ft. All runs were made at 130°C. and at an eluent flow rate of 1 ml./min.

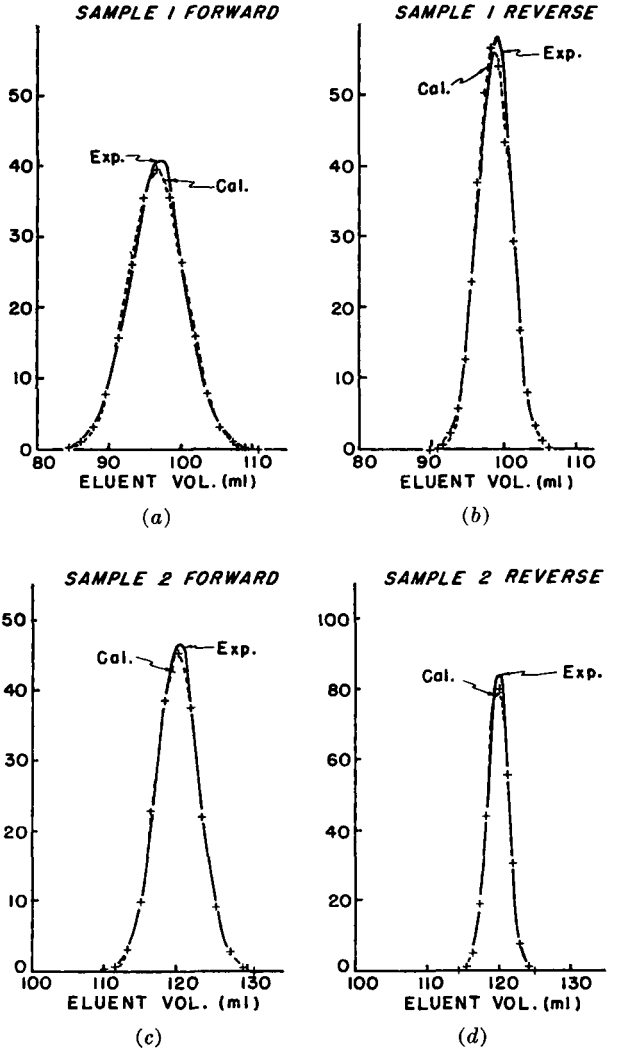


Figure 1. See caption, p. 1263.

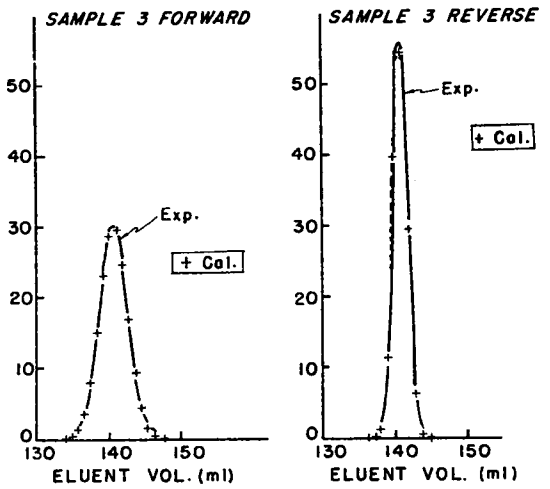


Fig. 1. Calibration chromatograms.

Calibration of Column Resolution

When a polydispersed sample is being eluted through a GPC column, its chromatogram is broadened by two processes, a desirable process due to the difference in molecular size of the species and an undesirable process due to mixing in the longitudinal direction. The second broadening process impedes the resolution of the column. If the elution of a sample is allowed to proceed to some part of the column and then the direction of flow is reversed the chromatogram of the eluent reflects only the effect of the second process. The resolution of a column can thus be calibrated.

In our computation scheme the chromatogram of the undesirable broadening is assumed to be Gaussian, i.e., the chromatogram of a monodispersed sample is assumed to be represented by the equation

$$F(v) = Ae^{-h(v-v_0)^2} \tag{1}$$

where $F(v)$ is the function representing the chromatogram, v is the eluent volume, v_0 is the eluent volume at the peak of the chromatogram, A is a constant related to the concentration of the sample, and h is the resolution factor related to the width of the Gaussian curve. The chromatogram obtained from the reverse-flow technique should therefore fit eq. (1) regardless of the degree of polydispersity of the sample.

Three samples of different molecular weights were used in the present calibration. The peak positions of the samples were predetermined. Then each sample was injected twice in the actual calibration. The first injection was made when the flow of the eluting solvent was in a normal direction. As the eluent volume reached one-half of that for the peak position of the sample, the flow was immediately reversed. The resulting chromatogram was used to compute the resolution for the front half of

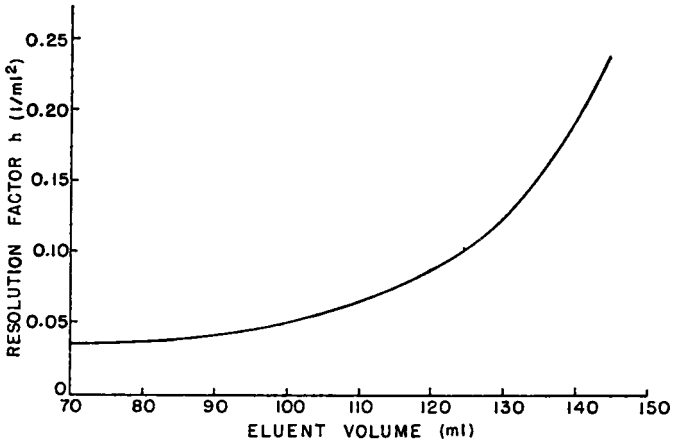


Fig. 2. Variation of the resolution factor h with respect to the eluent volume.

the column. The process was repeated with a second injection of the sample when the flow was in a reverse direction. The chromatogram produced was used to determine the resolution for the second half of the column. The constants in eq. (1) were calculated from the chromatograms by the method of moments. Thus

$$v_0 = \mu_1/\mu_0 \tag{2}$$

$$h = \mu_0^2/2(\mu_2\mu_0 - \mu_1^2) \tag{3}$$

$$A = \mu_0\sqrt{h/\pi} \tag{4}$$

and

$$\mu_i = \int_{-\infty}^{+\infty} F(v)v^i dv \tag{5}$$

Figures 1*a*–1*f* show the fit between the chromatograms and the calculated curves for the constants determined from eqs. (2)–(5). The overall resolution factor for a sample was calculated from the formula

$$h = 2/[(1/h_f) + (1/h_r)] \tag{6}$$

where h_f is the resolution factor for the front half of the column and h_r is that for the rear half of the column. Table I shows the results of the calibration for the present column.

TABLE I
Resolution Factors of the GPC Column

Calibration sample	h_f	h_r	h overall	Eluent volume v_0, ml.
1	0.0324	0.0899	0.0476	97.7
2	0.0547	0.2204	0.0876	120.2
3	0.1248	0.4800	0.1981	140.7

Figure 2 shows the plot of h versus the eluent volume of the column based on the results in Table I.

Calibration of Molecular Weight

The method described in Waters manual was used in calibrating the molecular weight–eluent volume relationship. A total of ten high-density polyethylene samples of known molecular weight were used. The result can be represented by the equation

$$v = 211 - 21(\log M) \tag{7}$$

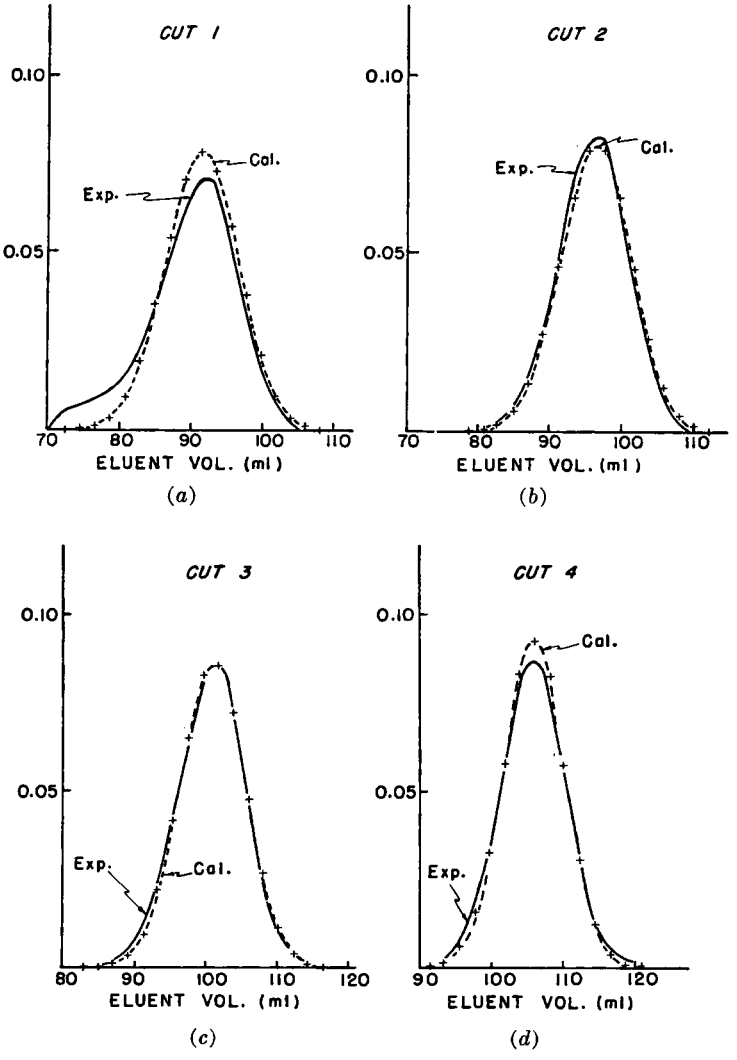


Figure 3. See caption, p. 1267.

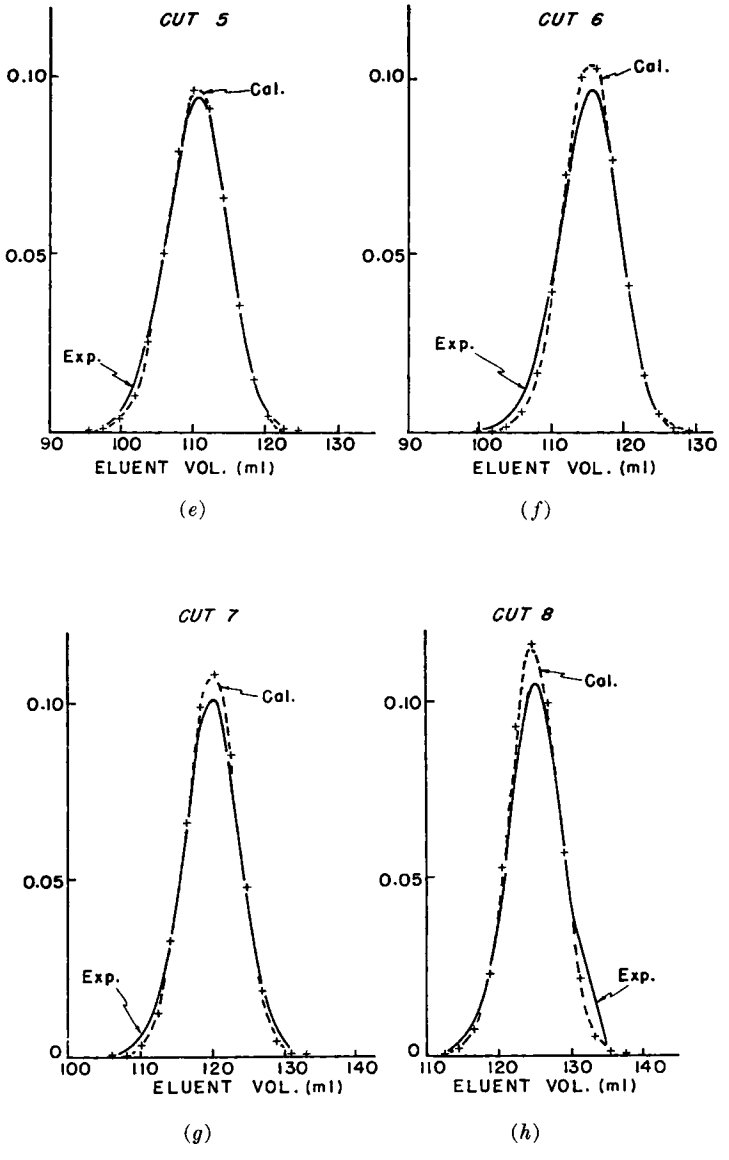
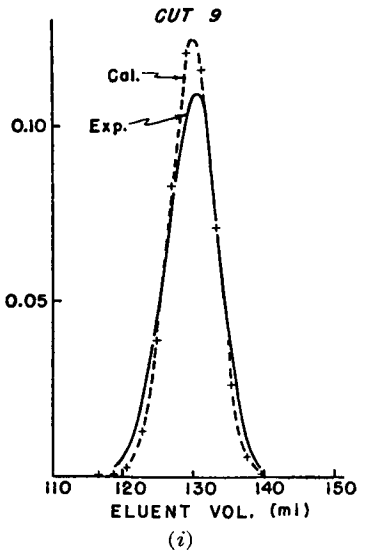


Figure 3. (*continued*)

Collection of Cuts

The starting sample used for collecting the cuts was a commercial high-density polyethylene of weight-average molecular weight 190,000. Table II shows the eluent intervals at which cuts were made.

The elution was repeated 20 times to collect enough sample for the cuts. The chromatograms of the starting polymer and the cuts are summarized in Table III.



(i)

Fig. 3. Chromatograms of the cuts.

TABLE II
Eluent Intervals of the Cuts

Cut	Eluent interval, ml.
1	90–95
2	95–100
3	100–105
4	105–110
5	110–115
6	115–120
7	120–125
8	125–130
9	130–135

CALCULATION SCHEME

The relation between an experimental chromatogram $F(v)$ and the chromatogram $W(y)$ at infinitely large resolution is given by the integral equation

$$F(v) = \int_{v_a}^{v_b} W(y)\sqrt{h/\pi}e^{-h(v-y)^2}\,dy \tag{8}$$

where y is the variable representing eluent volume under the integral sign, v_a is the initial eluent volume, and v_b is the final eluent volume. The variable y rather than v has a one-to-one correspondence with the molecular weight of the species in the sample, and $W(y)$ may also be considered as the distribution function in terms of the eluent volume. Let $W_i(y)$ denote the corresponding $W(y)$ function for the ith cut. Then

$$W_i(y) = \int_{v_{ia}}^{v_{ib}} W(y)\sqrt{h/\pi}e^{-h(v-y)^2}\,dv \tag{9}$$

TABLE III
Chromatogram Readings of Whole Polymer and Cuts[a]

Eluent vol., ml.	Whole polymer	Cut 1	2	3	4	5	6	7	8	9
70.0	0.0	0.0								
72.5	2.5	2.5								
75.0	4.5	3.5								
77.5	5.8	5.0								
80.0	9.5	6.5	0.0							
82.5	12.8	11.2	1.2							
85.0	16.5	17.5	2.8	0.0						
87.5	20.2	25.5	7.5	1.2						
90.0	25.5	33.0	14.5	4.2						
92.5	31.2	35.0	25.5	10.0	0.0					
95.0	36.0	27.5	32.2	21.0	5.0					
97.5	41.8	17.6	33.5	33.0	13.5	0.0				
100.0	48.0	8.5	24.5	43.5	27.0	5.0	0.0			
102.5	54.2	3.0	13.5	44.5	47.0	14.5	1.8			
105.0	61.0	0.0	5.8	30.8	61.2	34.0	5.8	0.0		
107.5	66.5		1.7	15.8	59.0	59.0	16.0	1.0		
110.0	72.0		0.0	5.8	40.0	80.2	35.5	4.0		
112.5	74.2			1.8	20.5	75.0	61.5	11.5	0.0	
115.0	75.5			0.0	7.0	46.5	81.0	27.5	2.5	
117.5	73.6				3.0	22.7	75.5	49.5	7.5	0.0
120.0	67.5				1.0	6.0	43.5	64.5	22.5	2.0
122.5	59.0				0.0	1.5	17.5	55.0	42.5	7.3
125.0	49.5					0.0	3.0	29.5	55.5	19.0
127.5	39.5						0.0	11.0	46.5	33.5
130.0	29.5							2.5	22.0	43.5
132.5	23.0							0.0	11.5	35.3
135.0	13.6								0.0	16.0
137.5	7.5									4.5
140.0	2.5									0.0
142.5	0.0									

[a] The above are recorder readings. They do not reflect the relative amount of one cut to another as the concentrations and the attenuation adjustment of the refractometer were not kept constant for all cuts.

Now v is the variable under the integral sign; v_{ia} to v_{ib} is the ith cut interval. The corresponding chromatogram $F_i(v)$ for the ith cut is then

$$F_i(v) = \int_{v_a}^{v_b} W_i(y)\sqrt{h/\pi}e^{-h(v-y)^2}\,dy \qquad (10)$$

The functions used in eqs. (8)–(10) are not normalized.

In carrying out these calculations the numerical method described earlier[1] was used to solve for $W(y)$ from eq. (8). Equation (9) was then integrated numerically by using a 32-term Gaussian quadrature formula. Finally eq. (10) was integrated by a polynomial method similar to that described in the earlier work[1] using an h value corresponding to the peak of $W_i(y)$. There was no particular advantage of using the lengthy poly-

nomial method for the integration except that a computer program readily adaptable to such use was at hand.

RESULTS AND DISCUSSION

The result of an initial calculation showed that the positions of the peaks of the calculated chromatograms $F_i(v)$ were higher in eluent volume than those of the experimental chromatograms by about 2 ml. This difference in peak position was constant for all cuts. In the calculation scheme the assumed Gaussian function is symmetrical and the correction was applied symmetrically except for the variation of h in eqs. (8), (9), and (10). This difference, which was asymmetrical with respect to the chromatograms, therefore must be caused by systematic errors in the experiments and not by any inconsistency in the calculation scheme. Part of the difference, about $^1/_2$ ml., can definitely be traced to the hold-up of liquid in the line between the refractometer and the syphon tube. As the test of our calculation scheme is the main concern, the final calculations were made by assuming that the entire 2 ml. was due to the hold-up in the instrument and the cut intervals were adjusted accordingly. Thus cut 1 was taken to be the sample collected in the interval between 88 and 93 ml. of the eluent volume, cut 2 between 93 and 98 ml., etc. The calculated chromatograms with these adjustments and the experimental chromatograms are shown in Figures 3*a*–3*i*. The curves shown are normalized chromatograms, not direct recorder readings. Table IV shows the molecular weights and the weight-average to number-average molecular weight ratios computed for the experimental and calculated chromatograms of the cuts using the relationship of eq. (7). These quantities do not reflect the true distribution of the cuts as they were calculated directly from $F_i(v)$ and not from $W_i(y)$.

The experimental chromatograms are all somewhat broader in distribution than the calculated chromatograms. As the cuts were collected from many repeated runs, a slight shift of the position of the cuts would broaden the distributions. This difference is therefore expected. Nevertheless, the agreement between the experimental and calculated chromatograms of the middle cuts is well within the reproducibility of the experi-

TABLE IV
Molecular Weights and $\bar{M}_w/\bar{M}_n$ Ratios of the Chromatograms $F_i(v)$

	Experimental chromatogram			Calculated chromatogram		
Cut	$\bar{M}_w \times 10^{-3}$	$\bar{M}_n \times 10^{-3}$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w \times 10^{-3}$	$\bar{M}_n \times 10^{-3}$	$\bar{M}_w/\bar{M}_n$
1	746	466	1.60	578	424	1.36
2	349	263	1.32	330	246	1.34
3	204	157	1.30	197	153	1.29
4	116	90.4	1.29	114	91.0	1.25
5	68.0	54.2	1.25	66.7	54.5	1.22
6	41.1	33.4	1.23	38.8	32.5	1.19
7	23.9	19.6	1.22	23.1	19.7	1.17
8	13.5	11.2	1.20	13.5	11.7	1.16
9	8.10	6.88	1.18	7.87	6.95	1.13

ments. The end cuts contained less amounts of sample and the broadening effect was hence more noticeable. If the resolution factor was not considered the calculated cuts would be represented by slices of the chromatogram of the starting sample and would be vastly different from the experimental chromatograms. The agreement found in the present work therefore shows convincingly that our proposed method for calculating molecular weight distribution function from gel permeation chromatograms is satisfactory.

Recently Hess and Kratz[2] have proposed an unsymmetrical function for representing the mixing in longitudinal direction in GPC. Their function was based on the treatment by Danckwerts[3] and by Carberry and Bretton[4] for axial dispersion of fluids in fixed beds. The curves shown in Figures 1*a*–1*f* are symmetrical because of the reverse-flow technique used in the calibration. Hence the present results do not exclude the possibility that such function is unsymmetrical but they do show that the symmetrical Gaussian function given by eq. (1) is a satisfactory approximation for treating GPC data.

References

1. L. H. Tung, *J. Appl. Polymer Sci.*, **10**, 375 (1966).
2. M. Hess and R. F. Kratz, *J. Polymer Sci. A-2*, in press.
3. P. V. Danckwerts, *Chem. Eng. Sci.*, **2**, 1 (1953).
4. J. J. Carberry and R. H. Bretton, *A.I.Ch.E. J.*, **4**, 367 (1958).

Résumé

Le schéma des calculs en vue de corriger l'effect d'elargissement dû à une résolution imparfaite des chromatogrammes par perméation sur gel a été comparé avec les performances actuelles d'une colonne de chromatographie par perméation sur gel. L'expérience consistait dans le fractionnement d'un polyéthylène de haute densité sur une unité GPC et ensuite la détermination des chromatogrammes des fractions collectées. Les chromatogrammes des fractions ont également été évalués au départ du chromatogramme initial utilisant les facteurs de résolution déterminés expérimentalement. Le degré d'accord entre les chromatogrammes calculés expérimentaux montre de façon convaincante que le schéma des calculs proposés précédemment est satisfaisant pour le traitement des donées de GPC.

Zusammenfassung

Das Berechnungsschema zur Korrektur des durch die unvollkommene Auflösung in einem Gelpermeationschromatogramm bedingten Verbreiterungseffekts wurde mit dem tatsächlichen Veralten einer gelpermeationschromatographischen (GPC) Säule verglichen. Der Versuch bestand in einer Fraktionierung eines Polyäthylens hoher Dichte an einer GPC-Einheit und der darauffolgenden Bestimmung der Chromatogramme der gesammelten Schnitte. Die Chromatogramme der Schmitte wurden auch aus dem Ausgangschromatogramm mit experimetell bestimmten Auflösungsfaktoren berechnet. Der Grad der Übereinstimmung zwischen den berechneten und den experimentellen Chromatogrammen der Schmitte zeigt in überzeugender Weise, dass das früher vorgeschlagene Berechnungsschema für die Behandlung von GPC-Daten brauchbar ist.

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