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CALCULATION OF AVERAGE MOLECULAR WEIGHTS OF OLIGOMERS IN HIGH-PERFORMANCE GEL PERMEATION CHROMATOGRAPHY

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SUMMARY

Most chromatograms of synthetic oligomers in high-performance gel permeation chromatography consist of two parts: one represents a low-molecular-weight fraction where oligomers are separated into individual peaks and the other a high-molecular-weight fraction with one broad curve. An example is the chromatogram of polystyrene 600 (nominal molecular weight 600), which was separated into 13 peaks from the dimer to the tetradecamer when recycle operation was applied or longer column lengths were employed. Average molecular weights in this instance were $\bar{M}_w = 688$ and $\bar{M}_n = 598$. Average molecular weights calculated from the norma chromatogram of polystyrene 600 by using several methods were $\bar{M}_w = 691$–742 and $\bar{M}_n = 605$–648. The preferred and most practical procedure involved the calculation of average molecular weights for the low-molecular-weight fraction of the chromatogram by measuring each peak area and by knowing its molecular weight, calculation of those for the high-molecular-weight fraction in a similar manner as in high polymers, and then calculation of the overall average molecular weights.

INTRODUCTION

Recent developments in liquid chromatography have accelerated the reduction of the separation time in gel permeation chromatography (GPC) and high-performance (high-speed) GPC columns are now commercially available[1–3]. High-performance GPC columns packed with polystyrene gels of narrow pore sizes can be used for the separation of oligomers and low-molecular-weight compounds[4–6], and have been applied to a wide variety of samples as a first choice instead of using adsorption or partition chromatography. It is also useful for the initial exploratory separation of unknown samples by the successive application of more than one liquid chromatographic technique. The main applications of GPC to low-molecular-weight compounds are to separate individual components and to establish approximately their proportions. In the application of GPC to synthetic oligomers, the main aim is usually the determination of average molecular weights.

In high-performance GPC, the complete separation of oligomers with molec-

ular weights of less than 500 is easily attainable. However, oligomers with molecular weights above about 1000 require many columns in order to obtain very high theoretical plate numbers for the complete separation, and it is usually impossible to separate a mixture of such oligomers into individual components. It is not practical to use a large number of GPC columns for the complete separation, because they are expensive. The principles of high-resolution recycle GPC[6–8] are well known and the effect of recycle operation is equivalent to an increase in the number of columns. However, recycle operation is time consuming and complete separation is limited to a few samples or special cases, in addition to the small range of molecular weights.

Synthetic oligomers are mixtures of species with the same monomer unit and ranging in molecular weights from 100 to several thousands. Chromatograms of these oligomers obtained by the usual high-performance GPC with columns packed with narrow pore-size gels generally consist of two parts: one is a low-molecular-weight fraction where oligomers are separated into individual components and the other a high-molecular-weight fraction where the recorder trace shows one broad curve. When separation is complete, that is, the number of peaks is equal to the number of components, average molecular weights can be calculated in the usual manner by measuring each peak area and by knowing the molecular weights of the corresponding oligomers. If a chromatogram of an oligomer mixture shows only one broad peak, the same method of calculation applied to synthetic polymers can be used. If a chromatogram is a combination of one broad peak and several finely separated peaks, as indicated above, the calculation procedure should be different from that for polymers, but no-one has discussed this problem so far.

In this work, GPC was applied to an oligostyrene mixture (nominal molecular weight 600) and average molecular weights were calculated by using several procedures and compared. The comparison of procedures for the calculation of average molecular weights of oligomers from partially separated chromatograms is discussed and practical aspects of the calculation procedure without using recycle operation or a large number of GPC columns are emphasized.

EXPERIMENTAL

The apparatus used in GPC was a Model LC-08 high-performance preparative liquid chromatograph (Japan Analytical Industry Co., Mizuho-cho, Tokyo, Japan) equipped with a differential refractometer, a sample loop valve and a recycle valve. Two JAI GEL 2H columns packed with polystyrene gel were used. These columns are equivalent to Shodex H202 (Showa Denko Co., Minato-ku, Tokyo, Japan) and the exclusion limit of molecular weight of the gel is 8000 for polystyrene. The column dimensions were 60 cm × 20 mm I.D. GPC was performed at room temperature. The eluent was chloroform at a flow-rate of 2.88 ml/min and the chart speed was 10 mm/min. The sample used for comparing several calculation procedures was commercial standard polystyrene 600 (nominal molecular weight 600), purchased from Pressure Chem. (Pittsburgh, Pa., U.S.A.). The sample concentration was 3% (w/v) and the injection volume was 3 ml.

In order to separate a sample of oligomer mixture into individual components, recycle GPC was performed by setting the recycle valve to the recycle position. The components of the back parts of the chromatogram in cycle n were eluted from the

system by turning the recycle valve to the collect position so as to prevent re-mixing of the back parts of the chromatogram in cycle n and the front parts of the chromatogram in cycle $n + 1$.

A calibration graph for normal GPC was constructed by using oligostyrenes from the dimer ($i = 2$) to the octamer ($i = 8$) in a sample of polystyrene 600 and several low-molecular-weight standard polystyrenes.

Calculations of average molecular weights

Method A. Complete separation by recycle. The recycle operation was continued until separation of the bands of the sample was completed. Average molecular weights were calculated from the contents of individual components and their molecular weights by the use of the equations

$$\bar{M}_w = \frac{\Sigma w_i M_i}{\Sigma w_i} \tag{1}$$

and

$$\bar{M}_n = \frac{\Sigma w_i}{\Sigma (w_i/M_i)} \tag{2}$$

where $\bar{M}_w$ and $\bar{M}_n$ are weight- and number-average molecular weights, respectively, w_i is the weight percentage of i-mer, M_i the molecular weight of i-mer (*e.g.*, 266 for $i = 2$, 370 for $i = 3$ and 474 for $i = 4$, as the end group for the oligomers is the butyl group). $\Sigma w_i = 100$. The weight percentage of i-mer can be replaced by a peak area percentage of i-mer. The peak area calibration procedure involved duplication of the peak contour on the chart paper on paper of constant weight, cutting the peak from the trace and weighing it. The areas of all of the peaks were added and the percentage area (the percentage weight) of each peak in relation to the total was calculated.

Method B. Measurement of height at peak maximum of each oligomer. The peak height from the baseline to the peak maximum was measured for peaks that were completely or partially separated. The distance from the baseline to the recorder trace at the elution position of each i-mer was measured for the part of chromatogram that was not separated into peaks at all. The latter elution positions were determined by use of a calibration graph. Eqns. 1 and 2 were used for calculation, the weight percentage being replaced by the peak height of the i-mer.

Method C. Calculation of peak area of each oligomer. The portion of chromatogram that was surrounded by the recorder trace, baseline and two perpendiculars drawn from valleys on both sides of the peak of the i-mer to the baseline was regarded as the peak area of the i-mer, if the peaks were completely or partially separated. For the portion of the chromatogram that was not separated but showed one broad trace, two perpendiculars were drawn, one from the mid-point of the elution positions of the i-mer and $(i + 1)$-mer and the other from the mid-point of the elution positions of the i-mer and $(i - 1)$-mer. These elution positions were determined in the same manner as in method B. Peak areas were measured by the peak cutting and weighing technique as in method A.

Method D. Division of the chromatogram on the chart into equal parts every 0.25 min and measurement of the height at each point. The purpose of this method was to divide the chromatogram into equal parts as finely as possible. The chromatogram in this study was divided into 89 points, a division every 0.25 min corresponding to dividing the chromatogram every 0.72 ml. Average molecular weights were calculated in the usual manner for GPC by measuring the heights and by obtaining molecular weights from a calibration graph.

Method E. Division of the chromatogram on the chart into equal parts every 1.0 min and measurement of the height at each point. The division was coarser than that in method D. The chromatogram in this study was divided into 21 points, corresponding to a division every 2.88 ml.

Method F. Division of the chromatogram on the chart into equal parts every 1.5 min. The division was coarser than that in method E. The chromatogram in this study was divided into 15 points, corresponding to a division every 4.32 ml.

Method G. Division of the chromatogram on the chart into equal parts every 2.0 min. The chromatogram in this study was divided into 11 points.

Method H. Division as in method G, but with a shift of the divided points.

Method J. Combination of methods B and D. Method B was applied to the portion of the chromatogram representing low-molecular-weight components, where the chromatogram was separated into several peaks, and method D was applied to the other part of the chromatogram representing high-molecular-weight components, where the chromatogram showed one broad peak. Average molecular weights of the whole species were calculated by use of eqns. 3 and 4.

$$\bar{M}_w = \frac{(\bar{M}_w)_L A_L + (\bar{M}_w)_H A_H}{A_L + A_H} \tag{3}$$

$$\bar{M}_n = \frac{A_L + A_H}{A_L/(\bar{M}_n)_L + A_H/(\bar{M}_n)_H} \tag{4}$$

where A_L and A_H are the peak areas of the parts of the chromatogram representing low- and high-molecular-weight components, respectively, $(\bar{M}_w)_L$ and $(\bar{M}_w)_H$ are weight-average molecular weights of low- and high-molecular-weight components, respectively, and $(\bar{M}_n)_L$ and $(\bar{M}_n)_H$ are the corresponding number-average molecular weights. Peak areas were measured by the use of the peak cutting and weighing method.

Method K. Combination of methods C and D. Method C was used instead of method B in method J.

Method L. Separation of oligomers with longer column lengths. Polystyrene 600 could be completely separated up to the tetradecamer ($i = 14$) with longer column lengths. The pentadecamer ($i = 15$) and/or above might be present in negligibly small amounts. Average molecular weights were calculated from a chromatogram in the literature[5]. Peak height was employed in the calculation instead of peak area, assuming that the band widths remained constant throughout the chromatogram[7].

RESULTS AND DISCUSSION

Calculated values of the average molecular weights of polystyrene 600 are given in Table I. Preferred procedures for the calculation of the average molecular weights

TABLE I

RESULTS OF CALCULATION OF AVERAGE MOLECULAR WEIGHTS OF POLYSTYRENE 600

The average molecular weight stated by the manufacturer is $\bar{M}_n = 585 \pm 7\%$ by vapour pressure osmometry (chloroform solvent). Data sheet No. 112 (Pressure Chem.).

Method	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
A	688	598	1.15
B	726	632	1.15
C	707	614	1.15
D	695	606	1.15
E	697	607	1.15
F	704	617	1.14
G	706	625	1.13
H	742	648	1.15
J	723	635	1.14
K	691	605	1.14
L	688	596	1.15

of oligomers are methods A and L, because the components of a sample are separated into individual peaks and the contents or peak areas of the *i*-mers can be easily determined. However, recycle operation limits effective separations to a few samples, and it is not practical to increase the number of columns because GPC columns are expensive. Oligomers with molecular weights higher than about 2000 in general cannot be separated from each other, even if longer column lengths are employed. Thus, for practical use, it is necessary to select the most appropriate procedure for calculation from methods B–K.

Recycle chromatograms of polystyrene 600 are shown in Fig. 1. In recycle operation, it is sometimes necessary to withdraw a fraction of low-molecular-weight components from the system in order to prevent overlapping of the end of the chromatogram in cycle n with the front in cycle $n + 1$. Consequently, it was impossible to record the trace of the completely separated peaks of all components as a single chromatogram after several recycles. The procedure for recycle and calculation of the contents of oligomers in method A is described below.

In cycle 2 (the first recycle), the eluate from cycle 1 was returned to the columns. Peak areas of the dimer ($i = 2$) and trimer ($i = 3$) (cross-hatched area in Fig. 1b) were measured and the areas of these peaks as percentages of the total were calculated. When the peak of the trimer appeared in the detector (the arrow in Fig. 1b), the recycle valve was switched to the normal position to allow a fraction of dimer to be drawn off. In cycle 3, the peak area of the tetramer ($i = 4$) was measured and the percentage area was calculated. As the total area in cycle 3 corresponds to 86% of that in cycle 2, the percentage area for the tetramer in cycle 3 multiplied by 0.86 is the percentage of the tetramer in the sample. The peak of the trimer in Fig. 1c represents a small portion of trimer in the sample, and it was necessary to draw off the trimer so as to leave some in the system in order to prevent the loss of any portion of tetramer before going to the next cycle. The same precaution must be taken in every recycle. In cycle 4, the peak area for the pentamer ($i = 5$) as a percentage of the total was determined. The percentage area of the pentamer in cycle

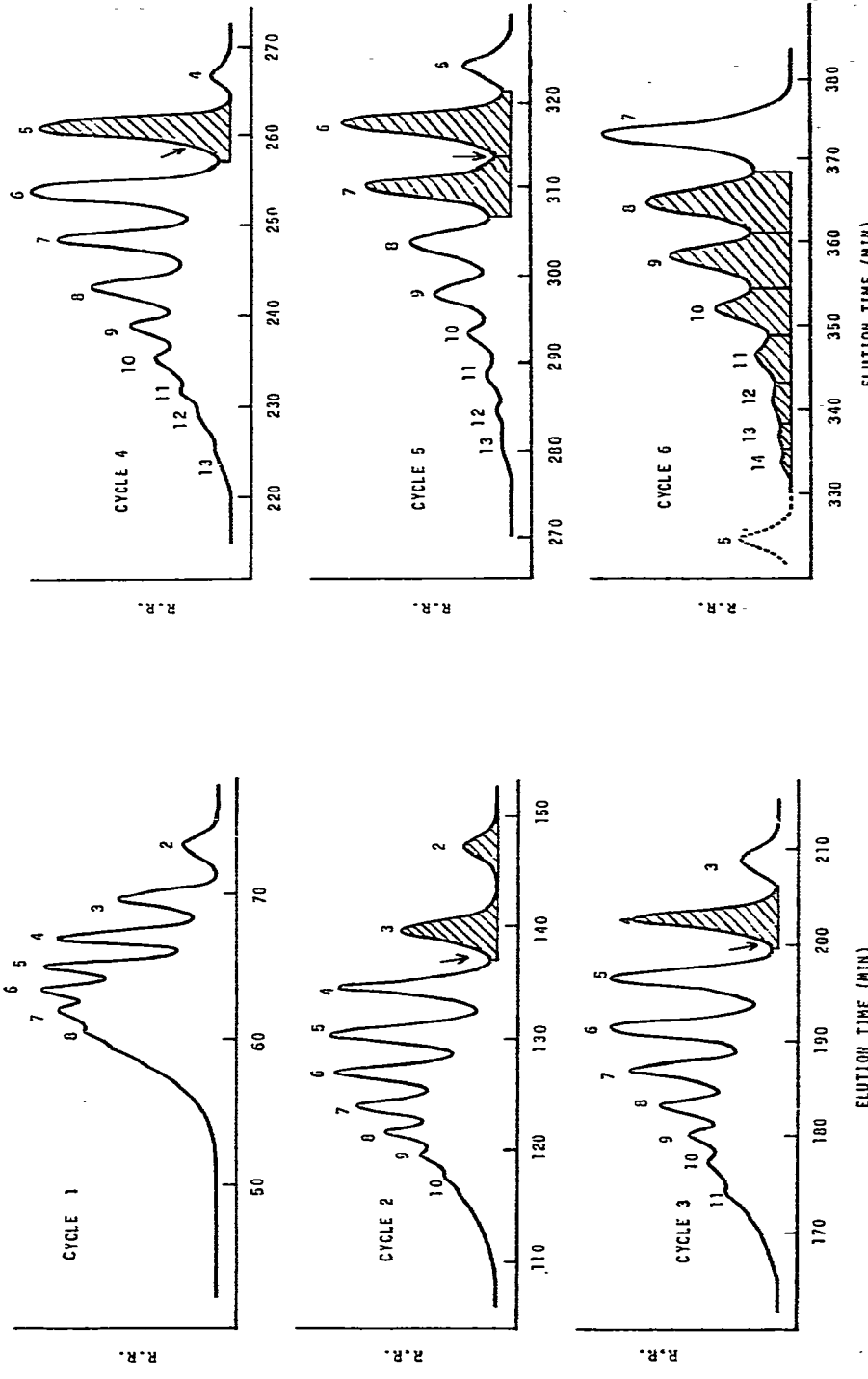

Fig. 1. Recycle gel permeation chromatograms of polystyrene 600.

4 multiplied by 0.72 is the percentage of the pentamer in the sample. The pentamer was drawn off in cycle 4. In cycle 6, the peak areas of the octamer ($i = 8$) up to the tetradecamer ($i = 14$) as percentage of the total were determined and multiplied by 0.25. The percentages of each oligomer obtained in this way are listed in Table II.

TABLE II

CONTENTS OF *i*-MERS IN POLYSTYRENE 600

i	*Method A*	*Method B*	*Method C*	*Method L*
2	3.9	3.0	3.4	4.0
3	10.1	8.6	9.0	10.4
4	14.5	12.7	13.9	14.6
5	16.2	15.1	16.2	16.4
6	16.3	15.3	16.5	15.8
7	13.5	13.6	12.5	13.1
8	10.3	11.1	9.2	9.6
9	6.2	8.3	8.1	6.8
10	3.4	5.8	5.9	4.2
11	2.7	3.5	3.0	2.6
12	1.7	2.0	1.6	1.4
13	0.7	0.6	0.4	0.7
14	0.5	0.4	0.3	0.4

The percentage areas for each oligomer in every cycle corrected to the percentage area of the whole sample are given in Table III. Peak areas of the fraction of oligomers whose peaks were not separated were measured in a similar manner to method C. The results in Table III suggest that in the incompletely separated

TABLE III

WEIGHT PERCENTAGES (PERCENTAGES OF PEAK AREAS) OF OLIGOMERS IN EACH CYCLE IN RECYCLE OPERATION

i	*Cycle**					
	1	*2*	*3*	*4*	*5*	*6*
2	3.4	*3.9*	(3.9)	(3.9)	(3.9)	(3.9)
3	9.0	*10.1*	(10.1)	(10.1)	(10.1)	(10.1)
4	13.9	14.2	*14.5*	(14.5)	(14.5)	(14.5)
5	16.2	16.7	16.3	*16.2*	(16.2)	(16.2)
6	16.5	16.5	16.4	16.4	*16.3*	(16.3)
7	12.5	14.7	14.6	15.3	*13.5*	(13.5)
8	9.2	11.2	11.2	11.5	9.6	*10.3*
9	8.1	5.5	5.8	6.1	6.2	*6.2*
10	5.9	3.4	2.1	2.1	3.2	*3.4*
11	3.0	2.5	2.5	1.9	2.4	*2.7*
12	1.6	1.0	1.6	1.0	2.3	*1.7*
13	0.7	0.3	1.0	1.0	1.1	*0.7*
14					0.7	*0.5*

* Numbers in italics represent percentages of the shaded peak areas in Fig. 1.

chromatogram, the contents of oligomers in the inside portion of the chromatogram were over-estimated and those in the outside portion of the chromatogram were under-estimated.

The peak heights of oligomers represent their contents in the sample in method B. The solid lines drawn vertically from the chromatogram to the baseline in Fig. 2 indicate the peak heights of the oligomers at the corresponding elution volumes. The peaks of oligomers from the dimer to the octamer were separated and the confirmation of their peak positions was not difficult. The heights of the peaks at their maxima were measured as peak heights. For oligomers larger than the nonamer, the peak positions were estimated by a calibration graph that was obtained by plotting elution volume against the logarithm of molecular weight for oligomers from the dimer to the octamer. As the calibration graph was linear for this range of oligomers, it was extrapolated to determine the peak positions of oligomers larger than the nonamer. Peak widths in GPC generally remain approximately constant throughout the chromatogram[9]. If the peaks are separated, the contents of the components can be measured from the peak heights (method L). However, as in the method B, when separation is incomplete, the overlapping of some portion of adjacent peaks influences the peak heights, and the correlation between peak height and content is reduced.

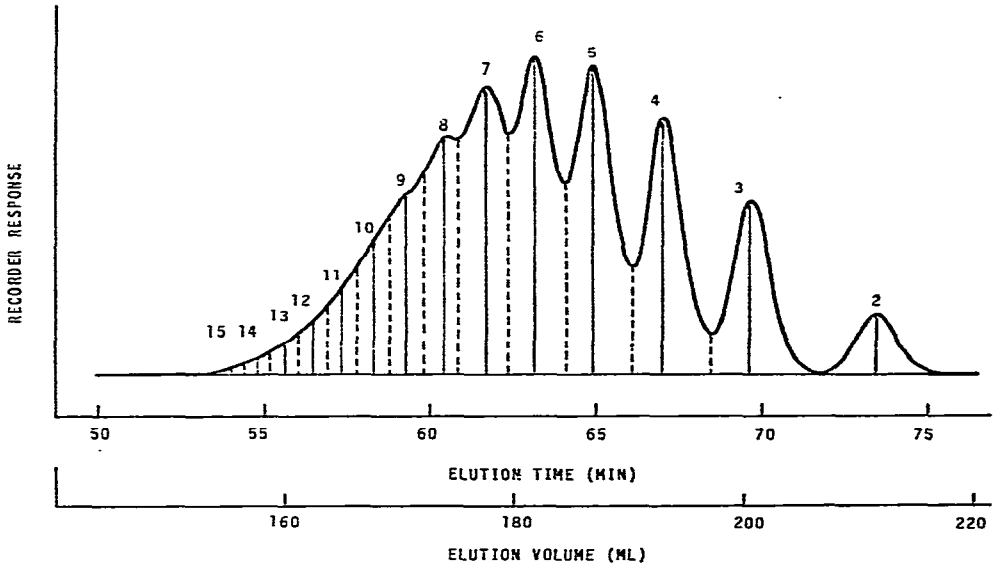

Fig. 2. Gel permeation chromatogram of polystyrene 600 using methods B and C. The numbers on the chromatogram refer to the numbers of monomer units in the oligostyrenes.

In method C, peak areas represent the contents of oligomers. Each area bounded by two adjacent dotted lines in Fig. 2 represents an oligomer. Table II shows that method C gives better results than method B.

In methods D–H, the chromatogram was divided in equal parts, and average molecular weights were calculated from the peak height and molecular weight at each

point. This procedure resembles that for polymers. In method D the chromatogram was divided into the smallest parts (Fig. 3), *e.g.*, the peak for the dimer was divided into 14 parts and that for the trimer into 13 parts. As a result of the gradually changing molecular weights, the influence of overlapping of adjacent peaks could be cancelled and the values calculated were similar to those obtained by method A. However, the molecular weights used for calculation were nominal values obtained from a calibration graph and not identical with those for the corresponding oligomers, which make it difficult to attach any significance to the average molecular weights calculated.

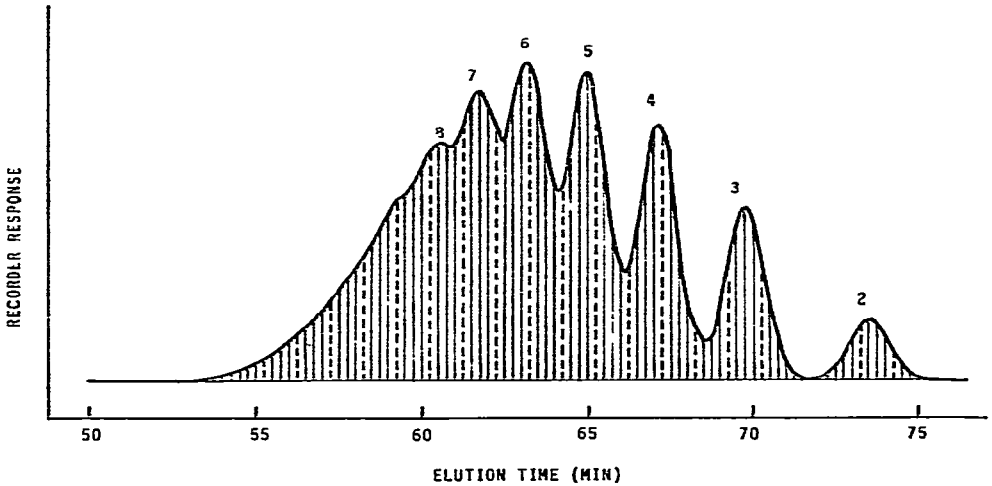

Fig. 3. Gel permeation chromatogram of polystyrene 600 using methods D and E.

The greater the number of parts into which the chromatogram is divided, the more accurate is the value calculated. Method E is acceptable, but method F is not. When a chromatogram is divided more coarsely, as in methods G and H, the dividing points at which the height is measured is far from the peak maximum (see every other broken line in Fig. 3). In method G the dividing points were near the peak maxima, whereas in method H the dividing points were far from the peak maxima and relatively small values of the peak height were used for calculation. Unreasonably high values for the average molecular weights were obtained when the chromatogram was divided as in method H. In this respect, it is not wise to divide a chromatogram coarsely if the chromatogram has partially separated peaks.

Using methods B and C, a significance can be attached to the calculated average molecular weights, but the value are not precise. It is not easy to determine the elution volumes of *i*-mers in a high-molecular-weight fraction. Using methods D and E one can hardly assign any significance to the average molecular weights obtained, but the values are more precise. Methods J and K are compromises between methods B (or C) and method D (or E) and can be used to assign a significance to the values and to obtain more precise values.

In the comparison of the various calculation methods in Table I, the uncertainty in the molecular weight averages reported by the manufacturer ($\pm 7\%$) is

greater than most of the values calculated. However, it might be possible to establish that the values calculated by methods A and L are the most reliable values, if the differences in the refractive indices of the oligomers were corrected. The effect of the molecular weight–refractive index dependence must be considered when calculating average molecular weights[10]. The refractive indices of oligomers increase with increasing molecular weight and the response of the refractometer for each oligomer must be corrected in order to give a more precise molecular weight determination. However, for comparison of the various calculation methods, this effect could be neglected and from a practical point of view, one can say that the most accurate method is the one that gives the value nearest to that obtained by methods A or L. In conclusion, the preferred procedure is method K, followed by method D.

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