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Determination of the Molecular Weight Distribution of Cellulose on Calibrated Gel Columns

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Synopsis

CMC samples obtained by gel filtration on a preparative scale were used to calibrate an agarose column. The relation between molecular weight and the peak elution volume for monodisperse samples were calculated as well as the relation between band broadening and the peak elution volume. In the calculations of the molecular weight distribution curves of the studied CMC samples, consideration was given to the variable band broadening by proper transformations of the experimental data, thus making it possible to obtain numerical solutions free from oscillations. The method admits a rapid determination of the molecular weight distribution of cellulose and related materials.

INTRODUCTION

The purpose of this paper is to demonstrate the possibility of evaluating the molecular weight distribution of cellulose and cellulosic materials dissolved in Cadoxen by gel filtration chromatography. The development of this technique required determination of the relation between the molecular weight and the effluent volume and investigation of the band-broadening effects.

In the previous papers[1,2] it was shown that cellulose, sodium carboxymethylcellulose (CMC), and hemicellulose dissolved in diluted Cadoxen could be fractionated on different gels, swelled in the same solvent. It was found that agarose gels, swelled in diluted Cadoxen, were the best available gels for the fractionation of the different cellulosic materials. Applications of this method have been given in earlier articles.[3,4]

The determination of the molecular weight distribution of these materials at this stage was, however, subject to certain limitations. For monodisperse macromolecules, such as proteins, it has been shown that there is a direct relationship between peak effluent volume and the molecular weight of the polymer in question. Such a clear-cut relation does not exist for polydisperse materials like cellulose, CMC, and hemicellulose.

Before the molecular weight distribution can be calculated, the band-broadening effects also have to be corrected for. This difficulty can be overcome by using Tung's reverse flow method.[5] However, monodisperse or nearly monodisperse standard samples of the polymer in question are

necessary. For polymers like cellulose and its derivatives, such samples are not available.

In the present, paper, the band broadening was determined from experiments with the monomer glucose and high molecular weight cotton linters. The calibration curve was obtained from fractionated CMC samples. The computations, involving correction for the band broadening and calculation of the molecular weight distribution by the aid of the calibration curve, were performed according to Chang and Huang,[6] the experimental data being first transformed according to Vink.[7]

THEORY

Experimental and theoretical evidence has been presented by Tung and Runyon[8] that the normalized gel filtration chromatogram of a monodisperse polymer sample can be approximately represented by a Gaussian distribution curve:

$$C(V) = \sqrt{h/\pi} \cdot e^{-h(V-b)^2} \tag{1}$$

where b is the peak elution volume, V is the effluent volume, and h describes the spreading. If h is independent of b, it can be determined from the elution curve of the monomer which generally can be obtained in a pure state. However, h usually varies with b, and their relationship must therefore be known. According to the investigations recently performed by Tung and Runyon,[8] the leading halves of the chromatograms obtained from samples of very large molecular size can be represented by a Gaussian curve with an h value corresponding to a completely excluded sample. By performing experiments with the monomer and a very high molecular weight sample, it was thus possible to obtain two points on the curve representing the relation between h and b. In the present paper, this method was used, and it was further assumed that $h^{-1/2}$ bears a linear relation to b given by the equation

$$h^{-1/2} = A + B \cdot b \tag{2}$$

where A and B are constants. Theoretical evidence for such a type of relation can be found in Vink.[7]

With the relationship between h and b, it was theoretically possible to calculate the corrected chromatograms $f(b)$ defined by

$$C(V) = \int_{-\infty}^{\infty} \sqrt{h/\pi} \cdot e^{-h(V-b)^2} \cdot f(b)\, db \tag{3}$$

where $C(V)$ is the chromatogram of a polydisperse sample.

Tung[5,9,10] presented a numerical method for this purpose. As shown by Chang and Huang,[6] this method gave oscillations in the corrected chromatogram in certain cases. Presupposing a constant value of h, they presented a method reducing the oscillation problems. In the case of variable h, the method fails since the kernel of the integral in eq. (3) is unsymmet-

rical. Vink,[7] suggesting Fourier methods for the numerical solutions of eq. (3), pointed to the use of a convolution procedure in the case of variable h. However, the Fourier methods seem to suffer from the same weaknesses as the Tung method.

In the present paper, Vink's[7] method to introduce new variables defined by analogy was adopted to obtain an integral equation with a symmetrical kernel. This equation was then solved by the method of Chang and Huang.[6]

As explained by Vink,[7] the transformation (or definition)

$$x = 2 \int_0^b \sqrt{r(\xi)}\, d\xi \tag{4}$$

$$y = 2 \int_0^V \sqrt{r(\xi)}\, d\xi \tag{5}$$

where $r(b) = h$ gives rise to the convolution

$$C_1(y) = \frac{1}{2\sqrt{\pi}} \int_{-\infty}^{\infty} e^{-1/4(y-x)^2} \cdot \gamma(x) \cdot dx$$

where $C_1(y) = C(V)$ and

$$f(b) = \gamma(x) \cdot dx/db = \gamma(x) \cdot 2 \cdot \sqrt{r(b)}. \tag{6}$$

The kernel of eq. (6) is symmetrical and the equation can thus be solved according to Chang and Huang.[6]

In the specific case discussed in this paper, we have postulated

$$r(b) = (A + Bb)^{-2}. \tag{7}$$

The new variables x and y were calculated from eqs. (4) and (5). The corrected chromatogram was then obtained from eq. (6). A prerequisite for the use of Chang and Huang's computer program (the program was kindly put at our disposal by the authors) was the selection of values of equal intervals of the variable x. This was achieved by interpolation between the values calculated from the experimental chromatograms.

In order to determine the molecular weight distribution curve from the corrected chromatogram, the relation between b and the molecular weight must be known. When only polydisperse samples are available, the common way to let the peak volume of the chromatogram represent the b value is less satisfactory.

It was found that the relation between log M_w and the first moment of the chromatograms formed a practically straight lines. It was therefore assumed that there was a linear relationship between log M and b of the form

$$\log M = K_1 + K_2 b. \tag{8}$$

According to Almin,[11] K_1 and K_2 can be determined from the line giving the relationship between $^1/_2$ log $M_w M_n$ and the first moment I_1 of the

chromatogram. Benoit and co-workers[12,13] also found that the very similar expression, log $[\eta]$ M_w, formed a straight line when plotted against the peak elution volume. This fact supports the suggested method.

As the M_n values were unknown, M_w/M_n values were first assigned the value 1.5 for all fractions. The logarithms of the products of the weight-average molecular weight and the estimated number-average molecular weight were then plotted against the first moments. From the resulting straight line, preliminary values of K_1 and K_2 were calculated.

From the known $f(b)$ curves, the approximate molecular weight distribution curves $g(M)$ were calculated according to

$$g(M) = f(b) \cdot \left| \frac{db}{dM} \right| = \frac{1}{|K_2| \cdot M} \cdot f(b). \tag{9}$$

From these curves, improved M_w/M_n values were calculated for the different samples. These quotients were then used to determine a refined calibration curve. This procedure was repeated until agreement between assumed and calculated values was obtained.

MATERIALS AND METHODS

The CMC preparations investigated in this work were obtained from a CMC sample having a degree of substitution (*DS*) of 1.25 and a degree of polymerization (*DP*) of 480. The DS value was determined by combustion analysis of the CMC sample, dissolving the ash, and titrating with sulfuric acid according to Wilson.[14] The DP value was determined by the viscosity method in a Cadoxen solution as described below.

Preparation of Fractionated CMC Samples

In order to increase the accuracy of the calibration procedures, fractionated standard samples are advantageous. Even so, their polydispersity is too high to allow application of methods used for narrow-range polymers like polystyrene. To obtain standard CMC samples for the calibration procedure, the CMC sample described above was gel filtrated on a preparative gel column.

The gel filtration technique has been described earlier.[1,2] The preparative gel column was filled with a 2% agarose gel (Sepharose 2B, Pharmacia Fine Chemicals AB, Uppsala, Sweden). The column dimensions were 5 × 49 cm². The solvent was 0.2*M* aqueous NaCl solution. A 0.50% CMC solution, 12 ml, was loaded on the column for each run. The flow rate was held at about 3 ml/hr per cm² by a siphoning head of 10 cm. The effluent volume was plotted as shown in Figure 1. To obtain enough material, the procedure had to be repreated seven times, i.e., 420 mg CMC was needed. Along the effluent volume axis, the eluted volume was divided into seven pools numbered 1 to 7, as indicated in Figure 1 by dotted lines. The tails were rejcted because of too low concentration. Every pool or CMC fraction had a volume of about 400 ml. The fractions were concentrated by

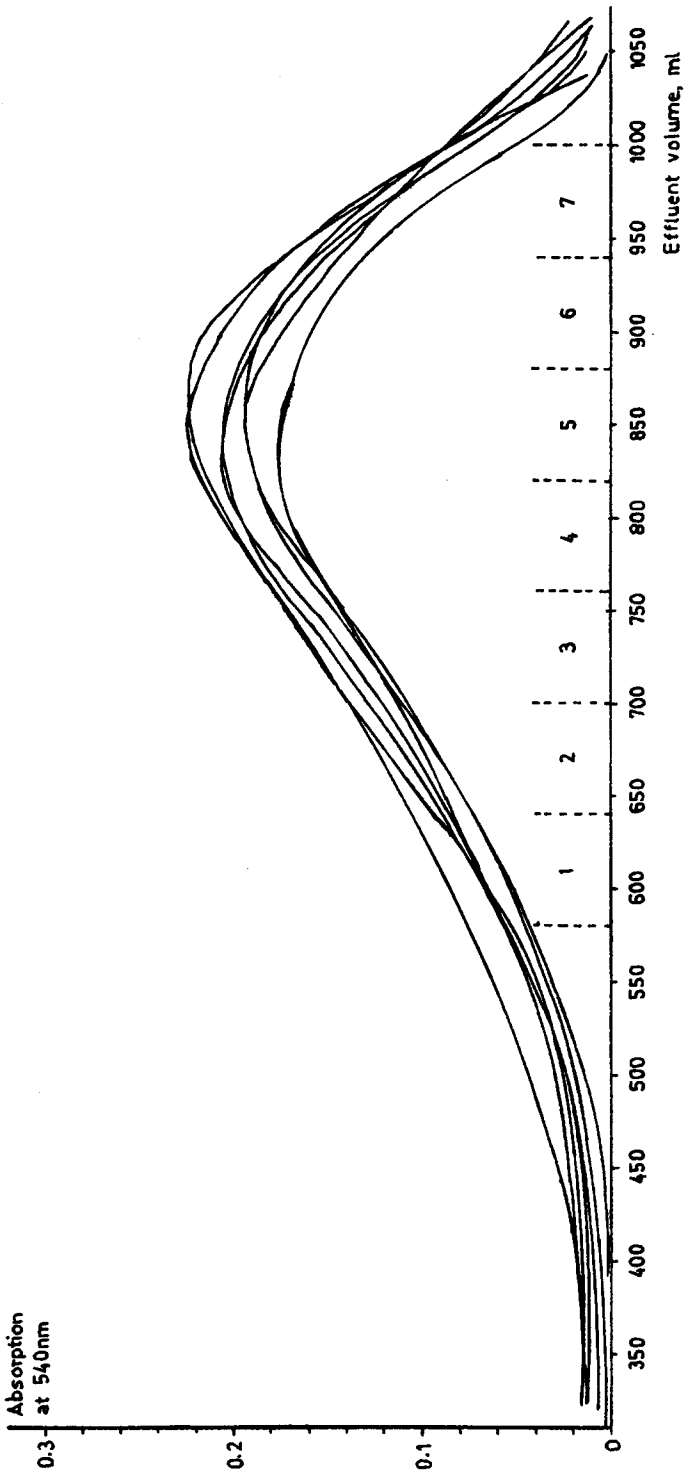
Absorption at 540nm
0.3
0.2
0.1
0
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
1050
Effluent volume, ml
1
2
3
4
5
6
7

Fig. 1.

freeze drying. The resulting white powder, consisting mainly of NaCl, was suspended in a small amount of water and dialyzed in collodion tubes against distilled water. Usually another concentration step by rolling evaporation at 40°C was necessary. New CMC solutions with a Cadoxen:water ratio of 1:1 as solvent were prepared for the viscosity measurements and for calibration of the gel column.

Gel Filtration Chromatography

The column was filled with the same type of gel as used in earlier studies,[2-4] a 2% agarose gel, Biogel A-50m, 50–100 mesh (Kemila-Preparat, Stockholm, Sweden). The siphoning head was 15 cm, giving a flow rate of 4–5 ml/hr per cm^2. CMC solution, 3 ml (concentration 0.1–0.5%), was loaded on to the column.

Viscosity Measurements

The viscosities of the different CMC preparations in Cadoxen solutions (1:1) were determined at 25°C in an Ostwald-type viscometer with the following constants: effluent volume, 0.5 ml; mean hydrostatic head, 136 mm; capillary length, 98 mm; capillary diameter, 0.4 mm. The efflux time of Cadoxen:water 1:1 was 124.5 sec. No viscometer corrections were needed. Instrinsic viscosity $[\eta]$ was determined by plotting η_{sp}/c against c and extrapolating to $c = 0$. The weight-average degree of polymerization was calculated according to the formula given by Brown et al.[15]:

$$[\eta] = 2.0 \times 10^{-2} \cdot \overline{DP}_w^{0.73}.$$

Assays

The CMC concentration in the fractions was determined with the modified orcinol method,[1] which requires 0.5 ml of the sample solution.

EXPERIMENTAL RESULTS

The seven CMC standard samples were gel filtrated in the Cadoxen solvent as described above. The results from the viscosity measurements are shown in Table I, column 2. The corresponding weight averages as calculated according to Brown et al.[15] are seen in Table I, column 3.

The chromatograms are shown in Figure 2. It is seen that fractions no. 2 to 6 appear similar to the Gaussian distribution curve but that the fractions no. 1 and 7 are skewed. For fraction no. 1, this is due to a partial exclusion of the large molecules. This effect is also evident in the chromatogram of the original CMC preparation. In order to determine parameter h in eq. (1), a Cadoxen solution containing very high molecular weight cotton linters ($DP = 7000$) together with glucose, the monomer, was fractionated. The elution curve is shown in Figure 3. The main part of the cotton linters was obviously excluded from the gel matrix and ap-

TABLE I
Viscometric and Chromatographic Characterization of the CMC Samples[a]

Designation of sample	Intrinsic viscosity, dl/g	DP_w (ref. 15)	I_1, ml	DP_w calcd.	DP_n calcd.	DP_w/DP_n calcd.
Fract. no. 1	2.4	710	183	798	545	1.46
no. 2	1.95	520	190	643	472	1.36
no. 3	2.15	600	192	624	449	1.39
no. 4	1.7	440	205	520	350	1.49
no. 5	1.4	350	222	350	219	1.60
no. 6	(0.60)	(110)	230	307	199	1.54
no. 7	0.45	71	279	86	62	1.39
CMC 5	1.80	480	214	535	210	2.55

[a] I_1 = The first moment of the chromatographic elution curve; DP_w calcd. = weight average molecular weight calculated from the molecular weight distribution curves; DP_n calcd. = number average molecular weight calculated from the molecular weight distribution curves.

pears with the void volume of the column. The glucose peak assumed the expected symmetrical shape.

The leading halves of these two peaks between 25% and 75% of the peak heights were assumed to follow a Gaussian distribution curve, and thus allowed the determination of the h values in accordance with Tung and Runyon.[8] Because of the unsymmetrical shape of the chromatogram, the

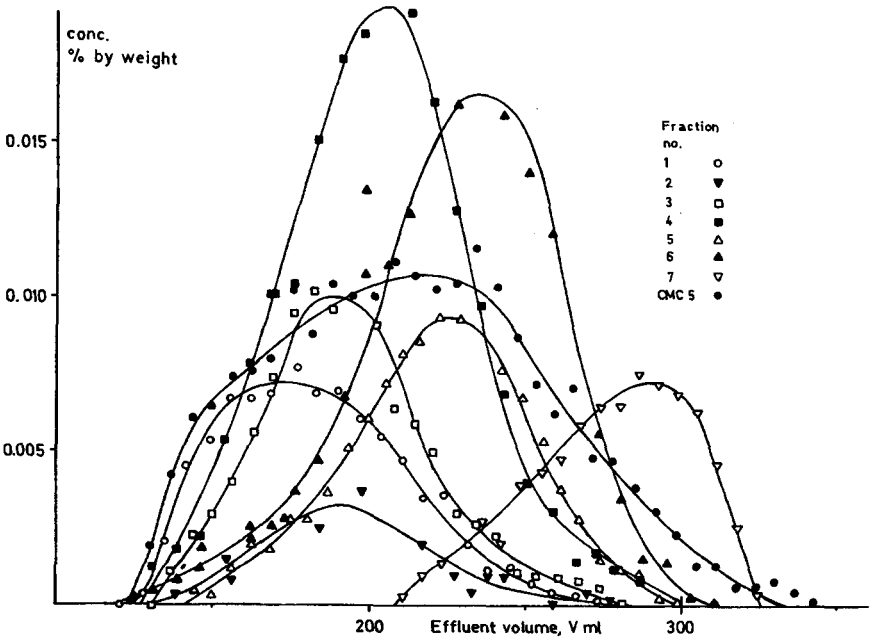

Fig. 2.

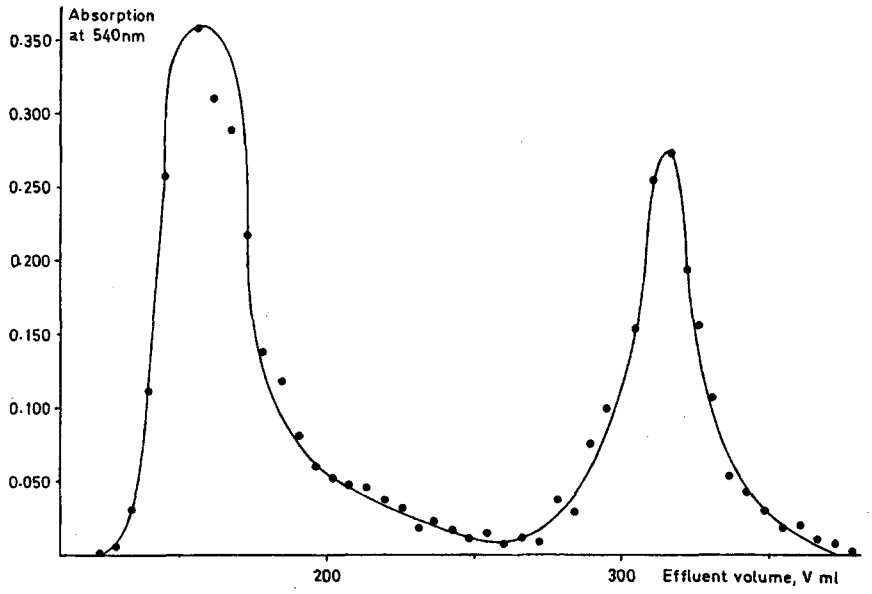

Fig. 3.

corresponding b value was calculated from this leading part of the peak. The b value of the glucose peak was estimated from Figure 3.

The numerical values of h and b for the peaks of cotton linters and glucose were determined to be: $h = 0.84 \times 10^{-2}$ ml^{-2}, $b = 151$ ml; and $h = 0.48 \times 10^{-2}$ml^{-2}, $b = 315$ ml, respectively. These values allowed the calculation of A (7.68) and B (2.14×10^{-2}) in eq. (2).

In Figure 4, the logarithm of the weight-average degree of polymerization versus the first moment of the chromatograms is given for the seven fractions. The following straight line was obtained:

$$\log DP_w = 4.716 - 0.01016 \cdot I_1. \tag{10}$$

As outlined by Almin,[11] eq. (10) was based on the assumption that $^1/_2 \cdot \log DP_w \cdot DP_n$ versus the first moment I_1 of the chromatogram forms a straight line. The first term of the right side in eq. (10), 4.716, is therefore equal to $K_1 + {}^1/_2 \log DP_w/DP_n$. The ratio DP_w/DP_n was first suggested to be 1.5 for all the fractions. This gave $K_1 = 4.628$ and $K_2 = 0.01016$. Refined values of the ratio DP_w/DP_n were calculated for each sample, and DP_n was thereafter determined from the original DP_w values. A new plot of $^1/_2 \log DP_w \cdot DP_n$ versus I_1 was made from which the values of constants K_1 and K_2 were determined to be 4.629 and 0.01013, respectively. Further refinement was found unnecessary.

In Table I, the calculated molecular weight averages DP_w and DP_n and their ratio DP_w/DP_n are reported for the seven fractions. Calculated values of the original CMC preparation are given as well. In Figure 5,

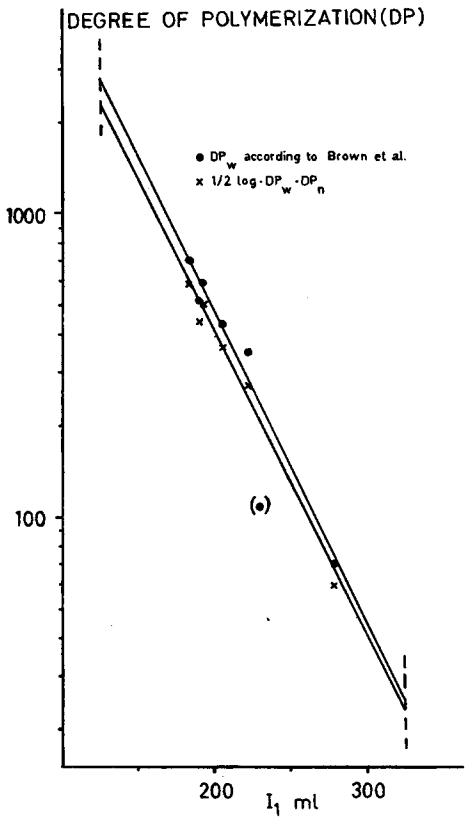

Fig. 4.

the corrected chromatogram, the $f(b)$ curve, of fraction no. 3 is compared to the experimental chromatogram. The final logarithmic molecular weight distribution curves are drawn in Figure 6. It is believed that the deviations from a unimodal shape of the curves are mainly due to weakness in the experimental technique.

DISCUSSION

The method developed in this paper makes it possible to correct the gel filtration curves of cellulose and cellulosic materials for band broadening. One of the basic assumptions is that under proper experimental conditions, the elution curve of a hypothetical monodisperse sample forms a Gaussian curve. Skewing of the elution curves is known from investigations of other polymers. According to Tung and Runyon,[8] the assumption of Gaussian spreading function does not cause serious errors. They also claim that until an experimental method is developed for determining the extent of skewing, such errors are not possible to avoid.

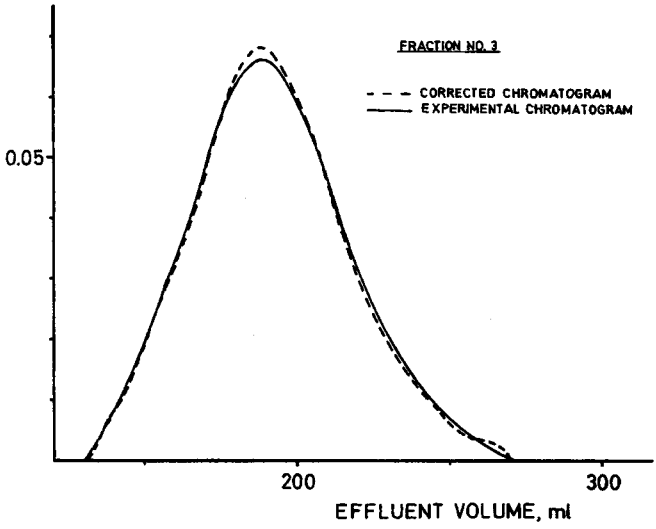

Fig. 5.

The fact that the spreading parameter h was assumed to have a definite shape, as shown in eq. (2), is of course a serious limitation. Experimental results by Tung and Runyon[8] showed that in their experiments with standard styrene polymers, the parameter h formed a unimodal curve when plotted against the peak elution volume. The lack of experimental methods to determine h values in more than two extreme points, however, made it impossible to decide whether a maximum or a minimum might appear in the curve. A monotonous relationship as used in this paper was therefore

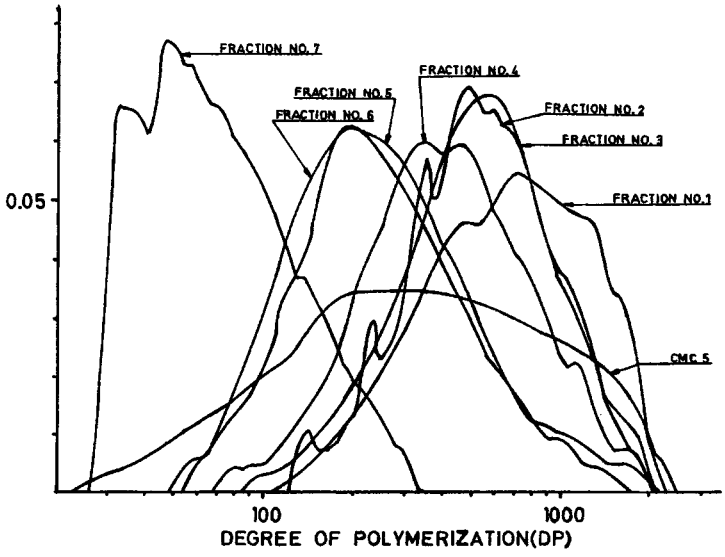

Fig. 6.

assumed. Vink's[7] theoretically derived expression supports this assumption.

The possibility of determining further points on the curve representing the relation between h and b must involve a new experimental technique. In a forthcoming paper, such a method will be proposed. With this technique, the effluent volume is collected in a series of tubes, and a progressively greater fraction of the fluid from each successive tube is withdrawn. These fractions are then pooled. The fractionated polymer in the pooled liquid will again be gel filtrated on the same column. From the results of such an experiment, it would be theoretically possible to establish an improved relationship between h and b.

In this paper a linear log M-versus-b plot has been used. The experimental results supported this procedure.

The computer method for the determination of the corrected chromatogram functioned satisfactorily. However, the extent to which the approximation involved in the transformations in eqs. (4) and (5) influences the result is at present unknown. A method free from oscillations in the corrected chromatogram was, however, preferred. Studies of these questions are planned.

The gel used in this work had negligible adsorption effects. With cellulosic materials such gels are rare. On the other hand, it would be desirable to obtain higher resolving power, especially for high molecular weight species, e.g., paper pulp. New gel types would therefore be of benefit.

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