Journal of Chromatography, 160 (1978) 59–71

CHROM. 11,046

COMPARISON OF SOME SOFT GELS FOR THE MOLECULAR-WEIGHT DISTRIBUTION ANALYSIS OF DEXTRAN AT ENHANCED FLOW-RATES

LARS HAGEL*
Pharmacia AB, Box 181, S-751 04 Uppsala (Sweden)
(Received March 28th, 1978)

SUMMARY

The performances of four packing materials, Sepharose 4B, CL-4B and CL-6B and Ultrogel AcA-34, for the molecular-weight distribution (MWD) analysis of dextran at enhanced flow-rates have been investigated. Whereas the elution volume, except for high-molecular-weight samples, decreases only slightly, the peak base width increases considerably on increasing the flow-rate. This leads to an impaired resolution of about 12% between 2.5 and 10 ml/cm^2·h. The plate height increases linearly with increasing flow-rate and the slope of this graph is related to column dispersion. When determining from the molecular weight correction factor, Sepharose 4B should be operated only at a low flow-rate (*e.g.*, 2.5 ml/cm^2·h), whereas the other gels can be operated at flow-rates of up to 10 ml/cm^2·h. For samples eluted near the void volume, however, the flow-rate must be kept low in order to reduce non-equilibrium effects irrespective of the gel in use. A limited capability study of a column packed with Sepharose CL-4B and operated at 10 ml/cm^2·h during a period of 4 months gave results in agreement with a current method for the MWD analysis of dextran.

INTRODUCTION

Gel permeation chromatography (GPC) is a powerful technique for the molecular-weight distribution (MWD) analysis of dextrans[1,2]. These analyses have been performed on soft permeation media at low flow-rates (*ca.* 4 ml/cm^2·h), requiring a long analysis time (15–20 h). Gels with improved mechanical strength, allowing higher flow-rates to be used with a consequent reduction in the time of analysis, have recently been introduced[3,4]. The aim of this investigation was to compare the performance of some of these media (Sepharose CL-4B and CL-6B and Ultrogel AcA-34) in the MWD analysis of dextran at enhanced flow-rates with that of the gel presently used (Sepharose 4B [2]).

* Present address: Pharmacia Fine Chemicals AB, Box 175, S-751 04 Uppsala, Sweden.

Several approaches have been described for the comparison of the separation properties of different packing materials. The concept of the number of theoretical plates used in liquid chromatography has been shown to be inappropriate for use in the GPC of high polymers[5,6]. The resolution[7], the specific resolution[8] or the resolution index[5] are readily determined but are only meaningful for samples with a very narrow MWD that are eluted in the linear region of the calibration graph[5,6]. The specific resolution factor, as defined by Hamielec[9], is claimed to be useful in evaluating gels and is also applicable to polydisperse samples. In Hamielec's method, the column is calibrated and a measure of the performance is determined from the agreement between the measured and absolute values of average molecular weights. This approach has been used extensively for calculations of column performance in terms of molecular weight correction factors[6,10–13]. In this paper, the performance of the packing materials is discussed in terms of resolution, molecular weight correction factor and variations in plate height as a function of flow-rate. The last relation can also give some valuable information on the permeation rates of high-molecular-weight compounds[14].

EXPERIMENTAL

Materials

The separation media used were (I) Sepharose 4B (agarose gel), (II) Sepharose CL-4B (batch No. 8152, cross-linked agarose gel), (III) Sepharose CL-6B (batch No. T 7067, as II) (all from Pharmacia Fine Chemicals, Uppsala, Sweden), and (IV) Ultrogel AcA-34 (batch No. 6905, polyacrylamide–agarose gel) (from LKB–Beckman Instruments, Stockholm, Sweden).

Seven fractions of dextran and the glucose monomer were used in the study of column performance (Table I). The eluent was 0.3% sodium chloride solution containing trichlorobutanol as a preservative.

TABLE I

DATA FOR THE SAMPLES USED IN THE PERFORMANCE STUDY[15]

Sample	$\bar{M}_w$*	$\bar{M}_n$**	$\bar{M}_w/\bar{M}_n$	*Concentration (%)*
1a	3500	3200	1.09	0.5
1b	175,000	129,000	1.36	0.5
2a	14,700	13,400	1.10	0.4
2b	409,000	303,000***	1.35	0.4
3a	39,800	33,600	1.18	0.4
3b	$1.08 \cdot 10^6$	873,000***	1.24	0.2
4	89,000	57,700	1.54	0.7
D-Glucose	180			

* Determined by light scattering.
** Determined by end-group analysis.
*** Estimated from GPC experiments.

Apparatus

The columns used had the dimensions 70 × 1.6 cm (K 16/70; Pharmacia Fine Chemicals) and were equipped with flow adaptors (A 16; Pharmacia Fine Chemicals). A motor valve (Labotron 002 540; Kontron, Zürich, Switzerland) governed by a home-made electronic device was used for automatic sample application, with a preload of four samples. The eluent reservoir was equipped with a heating coil to increase the temperature of the eluent slightly and thus reduce the risk of formation of air bubbles in the chromatographic bed. The effluent was continuously monitored with a refractive index detector (Multiref 901, 1-mm cell, sensitivity $50 \cdot 10^{-6}$; Optilab, Vällingby, Sweden). The capability of this instrument for the quantification of dextran in the column effluent has recently been investigated[16]. The flow-rate was controlled by a peristaltic pump (P-3; Pharmacia Fine Chemicals) or a syringe pump (Labotron LDP-13; Kontron). The column and detector were thermostatically controlled to within $\pm 0.1°$ using a Lauda thermostat. The continuous interferometer curves were digitized using a desk calculator (HP 9100B) with accessories (HP 9101A, HP 9107A)[16] and the data were processed by a computer system (HP 2120)[2].

Procedure

The gels were packed in the columns according to current practice[17] and were finally subjected to a sufficiently high operating pressure to give maximum flow-rates of 18 (I), 24 (II), 22 (III) and 25 (IV) ml/cm²·h (where I–IV refer to the type of gel), resulting in bed heights of 52.5 (I), 57.6 (II), 58.2 (III) and 58.0 (IV) cm. At this stage of packing the plunger was fixed and the flow-rate, obtained by weighing the effluent, was adjusted to 2.5 ml/cm²·h using the pump. After equilibration of the column, the void volume (V_0) and the total accessible liquid volume (V_t) were determined by eluting native dextran [0.08%, $\bar{M}_w > 10^7$ (ref. 18)] and sodium chloride. A 0.6-ml volume of the sample (Table I) was run once and a repeated determination of V_0 and V_t completed the test at each flow-rate used. After each increase in flow-rate the column was equilibrated with two total volumes before making further determinations.

The calibration of the columns was based on the chromatograms of samples 1–4 (a total of seven fractions) run at 2.5 ml/cm²·h. In the calibration procedure the MWD of each fraction was assumed, to a first approximation, to obey a Lansing–Kraemer distribution function[1]. The final calibration graphs were established by successive adjustments to give a deviation between the calculated and absolute values of $\bar{M}_w$ (as measured by light scattering) of less than 5%.

Evaluations

The plate heights were calculated from the chromatograms as[19]

$$H = \frac{L}{16}\left(\frac{W}{V_R}\right)^2 \tag{1}$$

where L is the bed height of the column, W is the peak base width as determined from the intersections of the tangents, drawn through the inflexion points, and the baseline, and V_R is the elution volume at the peak maximum. From the chromatograms of samples 1–3 the resolution was determined according to[6]

$$R_s = \frac{2(V_{R_1} - V_{R_2})}{W_1 + W_2} \tag{2}$$

where the subscripts denote solutes 1 and 2, respectively. The variations in the calibration graphs with changes in flow-rates were determined by calculations of the distribution coefficient from the equation[20]

$$K_D = \frac{V_e - V_0}{V_t - V_0} \tag{3}$$

where V_e, in this instance, is the elution volume corresponding to $\bar{M}_w$ as given by the Lansing–Kraemer distribution function.

The chromatograms were evaluated using the calibration graph established at 2.5 ml/cm^2·h and the molecular-weight correction factor[12] was calculated from

$$P = \left(\frac{\bar{M}_w/\hat{\mu}_w}{\bar{M}_n/\hat{\mu}_n}\right)^{\frac{1}{2}} \tag{4}$$

where $\hat{\mu}_w$ and $\hat{\mu}_n$ are the values of $\bar{M}_w$ and $\bar{M}_n$ determined by GPC[2].

RESULTS AND DISCUSSION

Flow-rate as a function of operating pressure

In the packing procedure for Sepharose, the maximum operating pressure temporarily exceeded that suggested by the manufacturer[3,21]. This was necessary in order to create a bed that would be stable throughout the range of flow-rates investigated and was considered not to be detrimental to the packing materials[22]. This statement is supported by Fig. 1, where the flow-rates of the gels at different operating pressures are illustrated. As has been indicated previously for a similar experiment using Ultrogel, a deformation of gel particles corresponds to a constant or decreasing flow-rate[23]. The flow-rate obtained with Ultrogel is about 40% less than that reported earlier[23], which might be due to the effect of using different experimental procedures (*e.g.*, the bedheight used in the previous study was only 15 cm) and variations in the

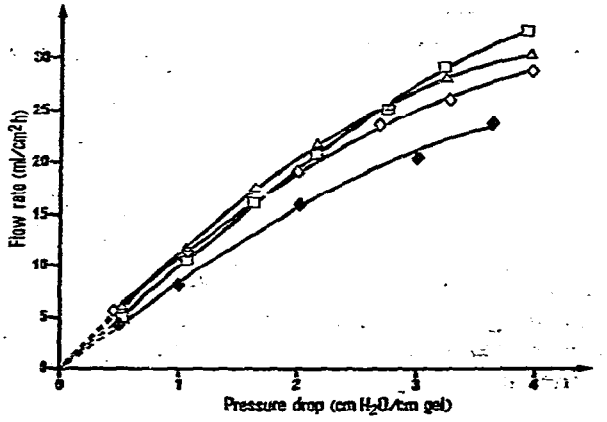

Fig. 1. Flow-rate as a function of operating pressure for Sepharose CL-4B(◆), Sepharose CL-6B (◇), Sepharose 4B (△) and Ultrogel AcA-34 (□).

flow properties of different gel batches (in this experiment the bed heights were 57–60 cm and the gels were allowed to equilibrate for 30 min after each increase in operating pressure). According to Fig. 1, no significant differences in the flow properties of the gels used were noted and no decrease in flow-rate was observed. The bed heights were found, for all gels, to decrease linearly with increasing pressure drop at a rate of 1.2 cm of gel/unit increase in pressure drop (cm H_2O/cm gel).

Elution volume as a function of flow-rate

The elution volumes decreased with increasing flow-rate. The decrease was very small except for the high-molecular-weight fractions able only partially to penetrate the gel (sample 3b for Sepharose CL-4B and Sepharose 4B and sample 2b for Sepharose CL-6B and Ultrogel AcA-34; Figs. 2 and 3), for which the decrease in elution volume was 6–9%. This molecular weight dependence of the change in elution volume with a change in flow-rate has been attributed to a non-equilibrium distribution of the polymer molecules between the mobile and stagnant phases at high flow-rates[13,24].

Plate height as a function of flow-rate and sample molecular weight

Due to an increase in base widths, the plate heights of the elution curves increased linearly with increasing flow-rate, except for the high-molecular-weight fractions. This is illustrated for some of the samples in Fig. 4, and the shapes of the curves are in close agreement with the adoption of Hermans' theory of permeation dispersion[25] for a run of polystyrene on a Styragel column at high flow-rates[14]. Thus,

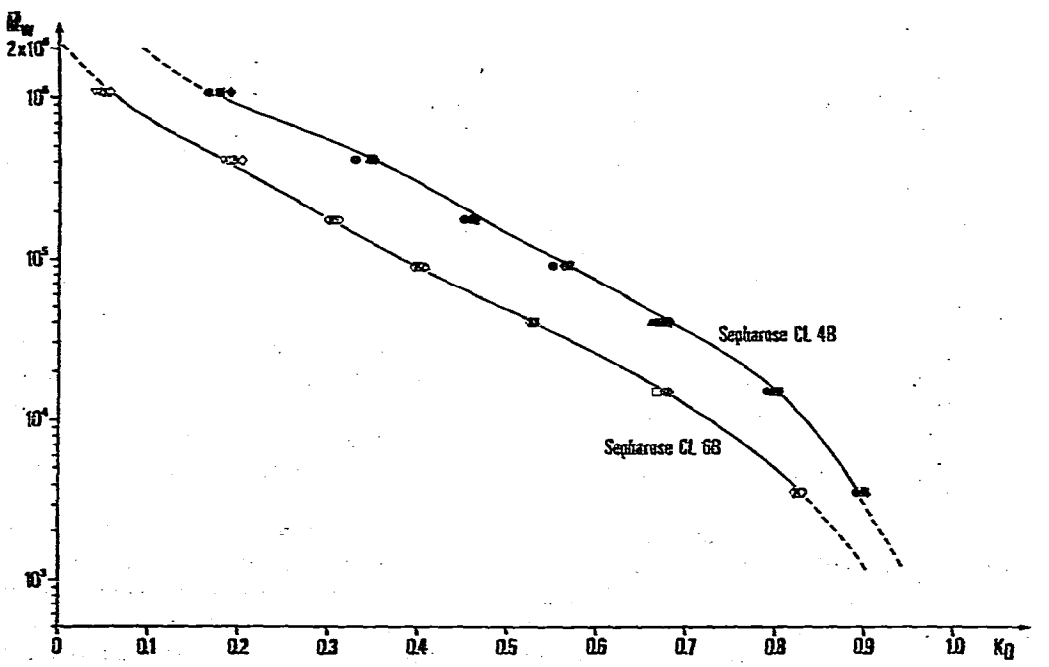

Fig. 2. Calibration graphs for dextran on Sepharose CL-4B and Sepharose CL-6B at 2.5(◆, ◇), 5 (■, □), 10 (●, ○), 13.5 (▲), 15 (△) and 20 (▽) ml/cm²·h.

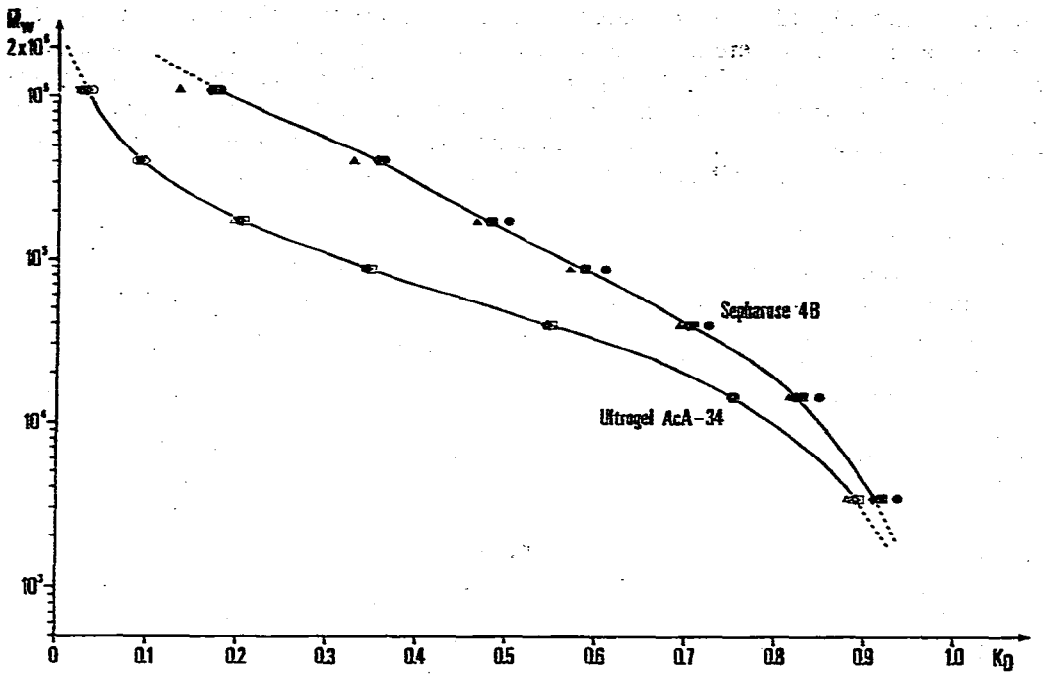

Fig. 3. Calibration graphs for dextran on Sepharose 4B and Ultrogel AcA-34 at 2.5(◆, ◇), 5 (■, ▽), 10 (●, ×), 13.5 (▲), 15 (△), 20 (□) and 24 (○) ml/cm²·h.

the increase in plate height with increasing sample molecular weight is attributed to the decrease in the diffusion coefficient. For large sample molecules, however, the equilibrium between the mobile and the stagnant phases becomes more dependent on the flow-rate and for high flow-rates the elution peak is skewed and the plate height curve declines. This effect is related to the K_D value of the sample, as illustrated by the rapid decline of the plate height curve of $\bar{M}_w$ $1.08 \cdot 10^6$ on Sepharose CL-6B and the decline at high flow-rate of the plate height curve of $\bar{M}_w$ 409,000 on Ultrogel AcA-34, while the plate height of $\bar{M}_w$ $1.08 \cdot 10^6$ on Sepharose CL-4B still gives a straight line. The decline of the curves was also accompanied by a pronounced skewing of the elution peaks. When the sample is totally excluded from the stationary phase, the sample dispersion is independent of the flow-rate, as shown by the sample of $\bar{M}_w$ $1.08 \cdot 10^6$ on Ultrogel AcA-34. This is attributed to the coupling of molecular diffusion and eddy diffusion with the velocity profile effects to yield a dispersion independent of flow-rate in the mobile phase for samples with low diffusion coefficients[26].

Even though the absolute values of plate height do not correlate well with separation properties in GPC, the slope of the plate height curves gives information about the flow sensitivity of peak dispersion over the working range of each type of gel (Fig. 5). The shapes of the curves are very similar for Sepharose but they are displaced with respect to K_D. The unexpected difference between the curves for Sepharose 4B and CL-4B might reflect the difference in the mechanical strengths of these two types of gels. This results in a slight deformation of Sepharose 4B caused

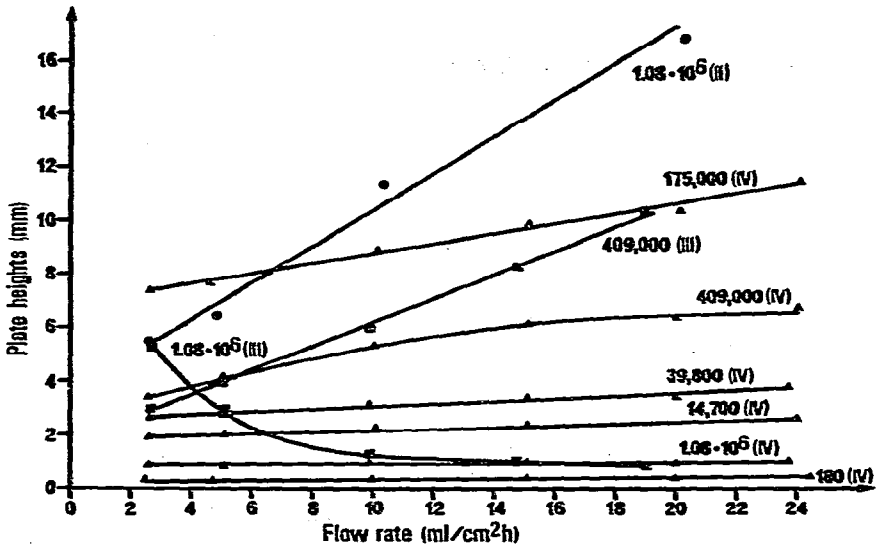

Fig. 4. Plate heights of dextran as a function of flow-rate on Sepharose CL-4B (II, ●), Sepharose CL-6B (III, ■) and Ultrogel AcA-34 (IV, ▲). Figures denotes $\bar{M}_w$ of samples used.

by the high operating pressure during the packing procedure, which would lead to irregularities in the flow pattern and an increase in the velocity profile constant. The plate heights for D-glucose were smaller for all gels than that reported for porous glass (CPG-10 [27], Table II). The low slopes found on Ultrogel AcA-34 might be partially attributed to the smaller particle diameter of this gel[28].

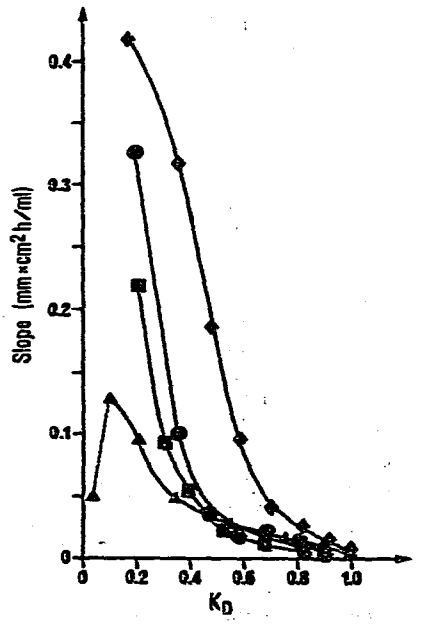

Fig. 5. Slope of the plate height curves as a function of K_D. Gel type used: ◆, Sepharose 4B; ●, Sepharose CL-4B; ■, Sepharose CL-6B; ▲, Ultrogel AcA-34.

TABLE II

PLATE HEIGHT FOR 1% D-GLUCOSE AT 5 ml/cm²·h

Separation medium	*Plate height (mm)*
I	0.27
II	0.35
III	0.24
IV	0.32
CPG 10*	0.54

* Flow-rate 4 ml/cm²·h (ref. 27).

Resolution as a function of flow-rate

The resolution decreased with increasing flow-rate (Tables III–VI). The decrease is, however, largest between 2.5 and 10 ml/cm²·h and is about 12% for all gels except Sepharose 4B, which shows an average decrease of 23%. As can be expected, the resolution increases with a decrease in the slope of the relationship $\log M = f(K_D)$ (*i.e.*, in the order Sepharose 4B ≈ Sepharose CL-4B < Sepharose CL-6B < Ultrogel AcA-34). The resolution of sample 3 on Sepharose CL-6B was not calculated owing to the pronounced skewness of the elution peak of sample 3b, as discussed above.

Molecular weight correction factor as a function of flow-rate

As it is not obvious how a decrease in resolution affects the MWD analysis of

TABLE III

PERFORMANCE OF SEPHAROSE 4B AT VARIOUS FLOW-RATES

Quantity measured	*Sample*	*Flow-rate (ml/cm²·h)*				
		2.5	*5*	*10*	*10**	*13.5*
R_s	1	1.99	1.78	1.43		1.35
	2	1.96	1.71	1.43		1.39
	3	2.04	1.84	1.80		1.78
$\hat{\mu}_w/\hat{\mu}_n$	1a	2.13	2.60	3.97	2.30	4.24
	2a	1.94	1.82	3.54	2.21	2.00
	3a	1.14	1.23	1.41	1.32	1.39
	4	1.24	1.28	1.52	1.46	1.56
	1b	1.22	1.28	1.47	1.42	1.55
	2b	1.21	1.27	1.46	1.41	1.55
	3b	1.28	1.39	1.64	1.65	1.47
P	1a	0.72	0.65	0.52	0.69	0.51
	2a	0.75	0.78	0.56	0.70	0.74
	3a	1.02	0.98	0.92	0.95	0.92
	4	1.12	1.10	1.01	1.03	0.99
	1b	1.06	1.03	0.96	0.98	0.94
	2b	1.06	1.03	0.96	0.98	0.93
	3b	0.98	0.94	0.87	0.86	0.92

* Values calculated from the calibration graph at 10 ml/cm²·h.

TABLE IV

PERFORMANCE OF SEPHAROSE CL-4B AT VARIOUS FLOW-RATES

Quantity measured	*Sample*	*Flow-rate (ml/cm²·h)*			
		2.5	*5*	*10*	*14*
R_s	1	1.97	1.92	1.87	1.69
	2	1.98	1.83	1.77	1.46
	3	2.01	2.02	1.74	1.61
$\hat{\mu}_w/\hat{\mu}_n$	1a	1.64	1.74	1.66	2.40
	2a	1.61	1.63	1.59	1.84
	3a	1.23	1.14	1.17	1.21
	4	1.26	1.26	1.27	—
	1b	1.24	1.22	1.25	1.32
	2b	1.16	1.18	1.24	1.33
	3b	1.36	1.30	1.54	1.52
P	1a	0.82	0.79	0.81	0.67
	2a	0.82	0.82	0.83	0.77
	3a	0.98	1.02	1.00	0.99
	4	1.11	1.11	1.10	—
	1b	1.04	1.05	1.04	1.01
	2b	1.08	1.07	1.04	1.01
	3b	0.95	0.98	0.90	0.90

a polydisperse dextran sample, all chromatograms were evaluated according to the calibration graph established at 2.5 ml/cm²·h. For this to be a valid approach, the calibration graph must not vary significantly with the flow-rates used[24]. As can be seen from Figs. 2 and 3, this is approximately true for all gels except Sepharose 4B.

TABLE V

PERFORMANCE OF SEPHAROSE CL-6B AT VARIOUS FLOW-RATES

Quantity measured	*Sample*	*Flow-rate (ml/cm²·h)*				
		2.5	*5*	*10*	*15*	*19*
R_s	1	2.66	2.54	2.33	2.13	2.03
	2	2.11	2.00	1.82	1.65	1.59
	3	2.63	—	—	—	—
$\hat{\mu}_w/\hat{\mu}_n$	1a	1.22	1.25	1.29	1.31	1.33
	2a	1.20	1.22	1.24	1.29	1.28
	3a	1.09	1.09	1.11	1.14	1.15
	4	1.20	1.20	1.24	1.26	1.28
	1b	1.20	1.23	1.28	1.32	1.37
	2b	1.18	1.25	1.32	1.45	1.56
	3b	1.29	1.37	1.43	1.48	1.35
P	1a	0.95	0.93	0.92	0.91	0.91
	2a	0.95	0.95	0.94	0.92	0.92
	3a	1.04	1.04	1.03	1.02	1.01
	4	1.13	1.13	1.12	1.11	1.10
	1b	1.06	1.05	1.03	1.01	0.99
	2b	1.07	1.04	1.01	0.97	0.93
	3b	0.98	0.95	0.93	0.92	0.96

TABLE VI

PERFORMANCE OF ULTROGEL AcA-34 AT VARIOUS FLOW-RATES

Quantity measured	*Sample*	*Flow-rate (ml/cm²·h)*					
		2.5	*5*	*10*	*15*	*20*	*24*
R_s	1	2.88	2.80	2.56	2.45	2.33	2.28
	2	2.90	2.78	2.56	2.50	2.51	2.49
	3	3.07	3.05	2.81	2.72	2.73	2.58
$\hat{\mu}_w/\hat{\mu}_n$	1a	1.61	1.84	1.91	2.22	2.27	2.27
	2a	1.38	1.37	1.45	1.49	1.50	1.51
	3a	1.10	1.10	1.11	1.11	1.12	1.13
	4	1.20	1.22	1.20	1.22	1.23	1.22
	1b	1.19	1.21	1.24	1.25	1.28	1.29
	2b	1.23	1.27	1.34	1.35	1.39	1.40
	3b	1.25	1.36	1.33	1.34	1.26	1.26
P	1a	0.82	0.76	0.76	0.70	0.69	0.69
	2a	0.89	0.90	0.87	0.86	0.85	0.85
	3a	1.04	1.04	1.03	1.03	1.03	1.02
	4	1.13	1.12	1.13	1.12	1.12	1.12
	1b	1.07	1.06	1.04	1.04	1.03	1.03
	2b	1.04	1.03	1.00	1.00	0.99	0.98
	3b	0.99	0.95	0.96	0.96	0.99	0.99

Therefore, the run at 10 ml/cm²·h on this gel was also evaluated according to a calibration graph established at 10 ml/cm²·h. The results of the evaluations are given in Tables III–VI. As the calibration graphs are constructed by minimizing the error in $\hat{\mu}_w$, the calculations of $\hat{\mu}_w/\hat{\mu}_n$ and P at 2.5 ml/cm²·h thus represent deviations of $\hat{\mu}_n$ from $\bar{M}_n$ (ref. 12). The calculated polydispersity increased with increasing flow-rate, which was mainly due to a decrease in $\hat{\mu}_n$. In order to evaluate the data presented in Tables III–VI, the random error must be known. The repeatability was calculated from seven runs with sample 1 on Sepharose CL-4B at 10 ml/cm²·h and the relative standard deviation was less than 2% for $\hat{\mu}_w/\hat{\mu}_n$ and less than 1% for P. Thus, allowing a ΔP of 0.02, from the P values in Tables III–VI it can be concluded that Sepharose 4B should only be operated at low flow-rates (2.5 ml/cm²·h), whereas the other gels can be operated at flow-rates of up to 10 ml/cm²·h. This limit is dependent on the desired working range of the gel; sample 3a can thus be run at over 15 ml/cm²·h. It should be noted, however, that when running high-molecular-weight samples the flow-rate must be kept low for all of the gels owing to the non-equilibrium effects outlined above. It must also be emphasized that possible differences between the gel batches were not included in this study.

Samples 1–3 were analysed according to the procedure described by Nilsson and Nilsson[2] and the results are presented in Table VII. The P values thus obtained are in close agreement ($\pm$ 0.03) with those presented in Tables III–VI, except for samples 1a and 2a. This discrepancy is presumably due to an uncertain extrapolation of the calibration graph from the few calibration points in the low-molecular-weight range. The high P value for sample 4 indicates that the value of $\bar{M}_w/\bar{M}_n$ is too high. The agreement of the data in Tables VII and III (at 2.5 ml/cm²·h) also supports the

TABLE VII

ANALYSES OF SAMPLES 1–3 ON SEPHAROSE 4B AT 3.5 ml/cm²·h ACCORDING TO REF. 2[29]

Quantity measured	*Sample*					
	1a	*1b*	*2a*	*2b*	*3a*	*3b*
R_s	2.16		1.99		2.04	
$\hat{\mu}_w/\hat{\mu}_n$	1.41	1.20	1.30	1.19	1.17	1.30
P	0.88	1.06	0.92	1.07	1.01	0.98

opinion that the high operating pressure used in the packing of the columns was not detrimental to the performance of the gels.

As flow-rate variations result in incorrect measurements of elution volumes and consequently misleading values of $\hat{\mu}_w$ and $\hat{\mu}_n$, especially at high flow-rates[30], the constancy of the pumps at the flow-rate of 10 ml/cm²·h was examined. For the peristaltic pump used, the flow-rate was checked by weighing the effluent every half hour, 16 times in all, giving a relative standard deviation (R.S.D.) of 0.1%. The long-term stability, determined by measuring the flow-rate 19 times during 10 days, was 0.16% (R.S.D.). For the syringe pump used, the flow-rate was determined on nine occasions during 14 days, giving an R.S.D. of 0.08%. The flow-rate variations are thus less than the limit given by Bly *et al.*[30] (0.3%).

For reasons of baseline stability, the detector used was operated at a lower sensitivity range than in the chemical anthrone analysis utilized earlier[2,16], which is why the sample concentration was increased 4–5-fold. To ascertain that the increased sample load would not cause any viscosity effects, the method for studying overloading effects suggested by Moore[31] was used. This was performed by running 0.6 ml of sample 1b in the concentration range 0.16–5% on Sepharose CL-4B at 10 ml/cm²·h. The operating range over which overloading effects can be neglected is obtained from a plot of $[\eta]vc$ against W/W_0, there $[\eta]$ = intrinsic viscosity, v = sample volume, c = sample concentration and W_0 is the base width for the lowest concentration applied.

$[\eta]vc$ was calculated using $[\eta]$ = 41 ml/g (ref. 32). As can be seen from Fig. 6, the safe operating range seems to be $[\eta]vc < 0.25$ ml, which for sample 1b corresponds to a load of 6 mg (1%) and for the high-molecular-weight sample (3b), with $[\eta]$ = 76.3 ml/g (ref. 32), to a load of 3 mg (0.5%). It can therefore be concluded that overloading effects are not to be expected with the concentrations used in this study (Table I).

After the completion of the gel test (which lasted approximately 1 month for each type of gel), a reduction of 5–7.5 ml/cm²·h in the maximum flow-rate of the gels at the operating pressure used was noted. To examine the long-term stability of the gel, a column was packed with Sepharose CL-4B at 12.5 ml/cm²·h and then run at 10 ml/cm²·h. During a period of 4 months two samples (Dextran 40 and 70) were run four times each and the calculations gave variations of estimates ($\hat{\mu}_w$, $\hat{\mu}_n$, etc.) in agreement with earlier reported values[2] and, further, no variations of the estimates due to ageing were observed[29].

During the period of this study, some new rigid materials for aqueous permeation chromatography have been introduced and have also been used for the MWD

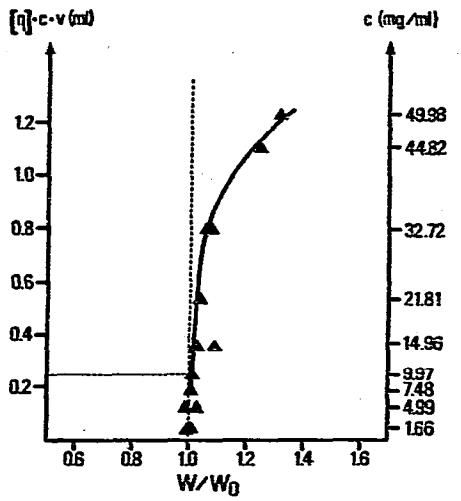

Fig. 6. Relative base width, W/W_0, as a function of solvated molecular volume of sample load, $[\eta]\,c\,v$.

analysis of dextran[33–38]. It was shown, however, that the flow-rate should also be kept low with some of these materials[39]. It has been pointed out that an improved column efficiency is obtained by using a small particle size[40], and rapid GPC analysis (15 min) on columns packed with very small particles (diameter 7–10 μm), which also eliminates the correction for column dispersion, has been reported[28]. Such small particle size media have also recently been introduced for aqueous GPC[33,37,41–43] and one of these is under investigation in this laboratory according to the scheme outlined in this paper.

ACKNOWLEDGEMENTS

The author is grateful to Professor Bengt Nygård and Dr. Kirsti Granath for valuable discussions of this manuscript. The fractions of dextran were kindly supplied by Dr. Granath, and thanks are due to her and her "boys" at the Department of Polymer Physical Chemistry, Pharmacia, for helpful advice during the course of this work. The author also thanks Dr. John Brewer for valuable comments on the manuscript.

REFERENCES

1 K. A. Granath and B. E. Kvist, *J. Chromatogr.*, 28 (1967) 69.
2 G. Nilsson and K. Nilsson, *J. Chromatogr.*, 101 (1974) 137.
3 *Sepharose CL, for Gel Filtration and Affinity Chromatography*, Pharmacia Fine Chemicals, Uppsala, 1975.
4 *Ultrogel, Pre-swollen Polyacrylamide/Agarose Gel Beads for High-Speed Gel Filtration*, Publication No. 5210(A), LKB-Beckman Instruments, Stockholm, 1974.
5 W. V. Smith and G. A. Feldman, *J. Polym. Sci., Part A-2*, 7 (1969) 163.
6 W. W. Yau, J. J. Kirkland, D. D. Bly and H. J. Stoklosa, *J. Chromatogr.*, 125 (1976) 219.
7 J. C. Giddings, *Dynamics of Chromatography*, Marcel Dekker, New York, 1965, p. 34.
8 D. D. Bly, *J. Polym. Sci., Part C*, No. 21 (1968) 13.
9 A. E. Hamielec, *J. Appl. Polym. Sci.*, 14 (1970) 1519.

10 W. V. Smith, *J. Appl. Polym. Sci.*, 18 (1974) 925.
11 P. C. Christopher, *J. Appl. Polym. Sci.*, 20 (1976) 2989.
12 F. L. McCrackin, *J. Appl. Polym. Sci.*, 21 (1977) 191.
13 M. R. Ambler, L. J. Fetters and Y. Kesten, *J. Appl. Polym. Sci.*, 21 (1977) 2439.
14 W. W. Yau, C. P. Malone and H. L. Suchan, in K. H. Altgelt and L. Segal (Editors), *Gel Permeation Chromatography*, Marcel Dekker, New York, 1971, p. 105.
15 K. Granath, Department of Research, Pharmacia, Uppsala, personal communication.
16 L. Hagel, *Anal. Chem.*, 50 (1978) 569.
17 *Sephadex, Gel Filtration in Theory and Practice*, Pharmacia Fine Chemicals, Uppsala, 1973, p. 35.
18 G. Arturson and K. Granath, *Clin. Chim. Acta*, 37 (1972) 309.
19 D. J. Harmon, in K. H. Altgelt and L. Segal (Editors), *Gel Permeation Chromatography*, Marcel Dekker, New York, 1971, p. 40.
20 J. M. Goodson, V. Distefano and J. C. Smith, *J. Chromatogr.*, 54 (1971) 43.
21 *Beaded Sepharose 2B-4B-6B*, Pharmacia Fine Chemicals, Uppsala, 1971.
22 J. Brewer, Pharmacia Fine Chemicals, Uppsala, personal communication.
23 E. Boschetti, R. Tixier and R. Garelle, *Sci. Tools*, 21 (1974) 35.
24 W. W. Yau, H. L. Suchan and C. P. Malone, *J. Polym. Sci., Part A-2*, 6 (1968) 1349.
25 J. J. Hermans, *J. Polym. Sci., Part A-2*, 6 (1968) 1217.
26 R. N. Kelley and F. W. Billmeyer, Jr., *Anal. Chem.*, 41 (1969) 874.
27 A. M. Basedow, *Ph.D. Thesis*, Heidelberg, 1973.
28 J. J. Kirkland, *J. Chromatogr.*, 125 (1976) 231.
29 S. Hultström, Department of Quality Control, Pharmacia, Uppsala, personal communication.
30 D. D. Bly, H. J. Stoklosa, J. J. Kirkland and W. W. Yau, *Anal. Chem.*, 47 (1975) 1810.
31 J. C. Moore, in K. H. Altgelt and L. Segal (Editors), *Gel Permeation Chromatography*, Marcel Dekker, New York, 1971, p. 157.
32 T. Andersson, Department of Research, Pharmacia, Uppsala, personal communication.
33 C. D. Chow, *J. Chromatogr.*, 114 (1975) 486.
34 H. Pitz, *Anaesthesist*, 24 (1975) 500.
35 A. M. Basedow and K. H. Ebert, *Infusionstherapie*, 2 (1975) 261.
36 A. L. Spatorico and G. L. Beyer, *J. Appl. Polym. Sci.*, 19 (1975) 2933.
37 M. Vondruška, M. Šudřich and M. Mládek, *J. Chromatogr.*, 116 (1976) 457.
38 J. J. Kirkland and P. E. Antle, *J. Chromatogr. Sci.*, 15 (1977) 137.
39 A. M. Basedow, *Königsteiner Tagung, Königstein, October 1977.*
40 P. Flodin, *J. Chromatogr.*, 5 (1961) 103.
41 *Corning, Controlled Pore Glass*, Pierce Eurochemie, Rotterdam, The Netherlands, 1977.
42 R. Vytášek, J. Čoupek, K. Macek, M. Adam and Z. Deyl, *J. Chromatogr.*, 119 (1976) 549.
43 R. V. Vivilecchia, B. G. Lightbody, N. Z. Thimot and H. M. Quinn, *J. Chromatogr. Sci.*, 15 (1977) 424.