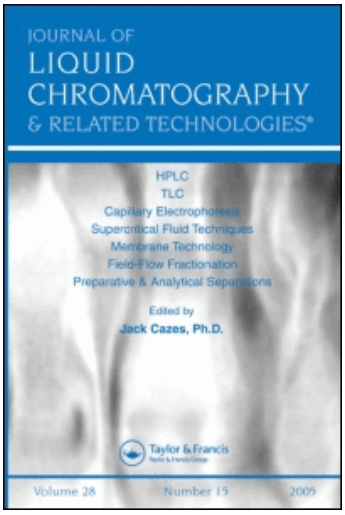

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Optimization of Peak Separation and Broadening in Aqueous Gel Permeation Chromatography (GPC). Dextrans

S. N. E. Omorodion[a]; A. E. Hamielec[a]; J. L. Brash[a]
[a] Department of Chemical Engineering, McMaster University Hamilton, Ontario, Canada

To cite this Article Omorodion, S. N. E. , Hamielec, A. E. and Brash, J. L.(1981) 'Optimization of Peak Separation and Broadening in Aqueous Gel Permeation Chromatography (GPC). Dextrans', Journal of Liquid Chromatography & Related Technologies, 4: 1, 41 — 50
To link to this Article: DOI: 10.1080/01483918108064795
URL: http://dx.doi.org/10.1080/01483918108064795

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(1), 41-50 (1981)

OPTIMIZATION OF PEAK SEPARATION AND BROADENING IN AQUEOUS GEL PERMEATION CHROMATOGRAPHY (GPC). DEXTRANS

S.N.E. Omorodion, A.E. Hamielec and J.L. Brash
Department of Chemical Engineering
McMaster University
Hamilton, Ontario, Canada

ABSTRACT

Herein is reported an experimental optimization of the aqueous size-exclusion chromatography of dextrans on untreated CPG-10 glass packings. The molecular weight calibration curve was independent of ionic strength and there was no evidence of polymer adsorption on the glass packings. However, in the absence of salt in the mobile phase, chromatograms did show a high molecular weight shoulder which is attributed to small negative charges on the dextran molecules resulting in ion exclusion from the pores of the negatively charged glass. These high molecular weight shoulders were completely eliminated with the addition of a small amount of salt (e.g. 0.05 M $NaSO_4$). A proper choice of pore sizes was essential to obtain good separation with minimal peak broadening giving a linear molecular weight calibration curve with a wide separation range and a small correction for imprefect resolution. Corrections to M_N and M_W were generally less than 5%. To establish an optimal column combination, it is recommended that single columns containing packing with one pore size be employed to establish the performance of a particular sized pore before a column combination is chosen.

INTRODUCTION

Many investigations of the aqueous size-exclusion chromatography of dextrans have been published recently (1-11). One of

the earliest studies was that of Bombaugh et al. (11), who used water at 65°C and 1 ml/min as mobile phase and deactivated Porasil as packing. Qualitatively the chromatograms indicated excellent peak separation for the molecular weight range of 11,000-150,000. The chromatograms for the higher molecular weight standards had shoulders near the void volume. This might have been due to size exclusion or possibly ion exclusion. Unfortunately salt was not added to the mobile phase to suppress ion exclusion. A careful study of adsorption showed that it was negligible. Cooper and Matzinger (4) found using CPG packing (a single 4 ft x 3/8 in column containing 75 A, 240 A and 2000 A pore diameters) and mobile phases containing 0.01 M, 0.1 M and 1.0 M phosphate at pH = 7.0 that the molecular weight calibration curve was independent of ionic strength. They showed that CPG packing materials can exhibit ion exclusion for polyelectrolytes in low ionic strength media. Spatorico and Beyer (5) chromatographed dextrans on CPG-10 packings (5 columns: 1250 A, 670 A, 500 A, 190 A and 75 A, each column 5 ft x 0.17 in) using 0.2 M and 0.8 M Na_2SO_4 as mobile phase. They observed that the molecular weight calibration curve was independent of salt concentration and flowrate. Peak separations were good and the corrections to $\bar{M}_N$ and $\bar{M}_W$ for imperfect resolution were apparently quite small although data were not presented. Buytenhuys and Vander Maeden (9) chromatographed dextrans on Lichrospher packings (untreated silica micropacking with particle diameter of about 10 microns and 100 A, 300 A, and 500 A pores) using water and also 0.5 M sodium acetate (pH = 5) as mobile phases. The use of the salt eliminated the high molecular weight shoulder caused by ion exclusion. These authors suggest that dextrans may have a few negative charges. A similar explanation would apply for the CPG packings when water is used as mobile phase. Soeteman, Roels, Van Dijk and Smit chromatographed dextrans on treated Porasil with water as the mobile phase. They apparently did not observe any high molecular weight shoulders attributable to ion exclusion. It may be that the negative charge on the treated Porasil is negligible. Their

intrinsic viscosity measurements suggest that below a molecular weight of 50,000 the dextrans are essentially linear. At higher molecular weights the levelling off of intrinsic viscosity suggests that the molecules are highly branched. Treating the dextrans as linear up to a molecular weight of $\bar{M}_W$ = 532,000 has permitted accurate determinations of $\bar{M}_N$ and $\bar{M}_W$ by GPC by these workers. Perhaps the use of branched dextran standards to establish the molecular weight calibration curve has made the calculational procedure reasonable. It should be noted however that large errors in $\bar{M}_N$ and $\bar{M}_W$ would likely result if the calibration curve were used for an unknown dextran sample whose branching frequency were appreciably different.

The objective of the present investigation was to define a mobile phase and a combination of columns of CPG-10 packing for the efficient size separation of dextrans with corrections for imperfect resolution to $\bar{M}_N$ and $\bar{M}_W$ of less than about 5%.

EXPERIMENTAL

The polymers used in this study were a series of dextrans supplied by Pharmacia Fine Chemicals (Piscataway, N.J.). the molecular weight data supplied by Pharmacia are shown in Table 1.

The dextran standards are known to be highly branched, broadly distributed polymers. These standards are useful in an optimization study of peak separation and broadening. However, they should be used with caution for calibration purposes when dextran polymers with unknown branching characteristics are to be analyzed by aqueous GPC.

The liquid chromatograph employed in this study was a Waters Associates Model ALC/GPC 300 with a differential refractometer operated at room temperature. A 2 ml sample loop with polymer concentrations of 0.05-0.1 wt% and a 5 ml siphon were employed with mobile phase flowrates in the range, 1-8 ml/min. The columns were dry-packed with CPG-10 packing. Details of packing, column combination, mobile phase type and flowrate accompany the Figures showing the results of the investigation.

TABLE 1

Molecular Weights of Dextrans

Designation	$\bar{M}_N$	$\bar{M}_W$	$\bar{M}_W/\bar{M}_N$
T 2000	-	-	-
T 500	173.0	509	2.94
T 250	112.5	231	2.05
T 150	86.0	154	1.79
T 110	76.0	106	1.39
T 70	42.5	70	1.65
T 40	28.9	44.4	1.54
T 20	15.0	22.3	1.49
T 10	5.7	9.3	1.63

RESULTS AND DISCUSSION

The first mobile phase to be investigated was distilled water with no additives. Typical chromatograms obtained for the dextran standards in water are shown in Figure 1. A chromatogram for a high molecular weight nonionic polyacrylamide is also included to show the void volume. Most of the dextran chromatograms have a high molecular weight shoulder or are clearly bimodal. The shoulder or second peak is clearly not the result of solute exclusion from the largest pores on the basis of size as the retention volumes are considerably larger than the void volume. Also molecular aggregation is unlikely for branched dextrans. This phenomenon is observed with linear poly (vinyl chloride) synthesized at lower temperatures where syndiotactic sequences are of sufficient length to permit the formation of crystallites (12). These dissolve very slowly and are responsible for high molecular weight shoulders and bimodal chromatograms for PVC. The most likely explanation for the bimodal chromatograms for dextrans is ion exclusion. It is hypothesized that the dextran molecules have

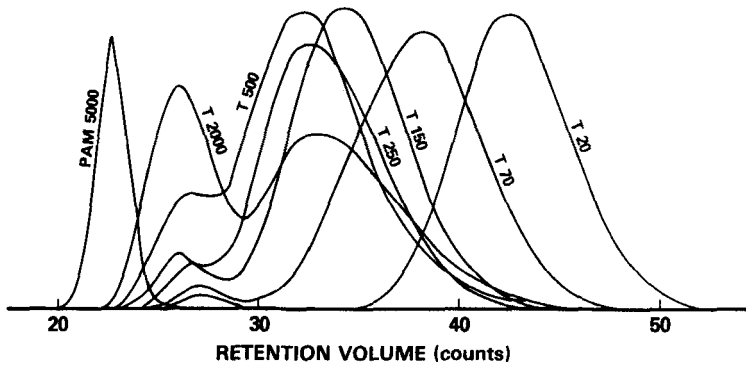

FIGURE 1: Chromatograms of dextrans on CPG 10 packing (one column, 4' x 3/8", of each of pore sizes: 125 A, 240 A, 370 A, 2000 A) in distilled water showing partial ion exclusion. PAM 5000 (a high MW polyacrylamide) shows void volume of column system.

a small negative charge and that the larger of these polymer molecules have substantially reduced available pore volume due to charge repulsion near the glass surface. Buytenhuys and Vander Maeden (9) have made a similar suggestion. The addition of salt to the mobile phase eliminates the bimodalities and gives unimodal peaks as shown in Figure 2a. Also shown is a salt peak due to ion inclusion. The electrolyte added to the mobile phase presumably screens the charge on the polymer molecules and compresses the electrical double layer associated with the glass surface. The available pore volume for the larger dextran molecules is thus increased. The molecular weight calibration curves obtained for water and two salt solutions as mobile phase are shown in Figure 2b. The column combination employed was the same for the data presented in Figures 1, 2a and 2b. It was observed that the peak retention volumes are independent of electrolyte concentration. Moreover, the addition of other additives including salts, acid, and, nonionic surfactants (e.g. Tergitol, a low MW polyether) also had no effect on elution volumes. This suggests that adsorption of dextran on the CPG surface is negligible. It was concluded

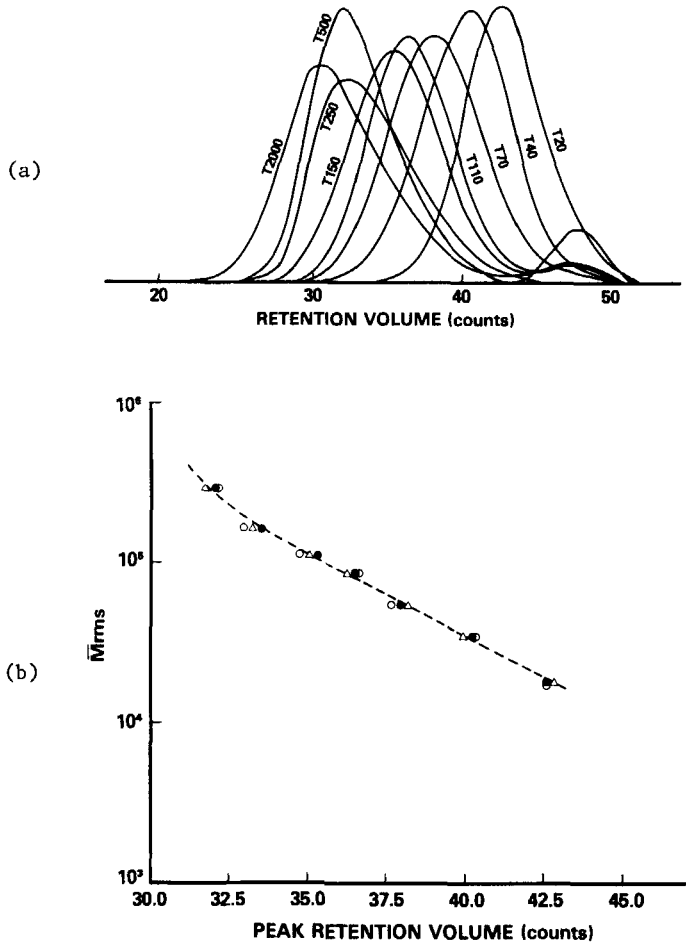

FIGURE 2: (a) Chromatograms of dextrans on CPG 10 packing (same columns as Figure 1) with addition of 0.05 M Na_2SO_4. A salt peak is now apparent. (b) MW calibration curves for dextrans in water (O), 0.1 M KBr (●) and 0.05 M Na_2SO_4 (Δ), showing lack of dependence on electrolyte type and concentration.

that an aqueous solution 0.05 M in Na_2SO_4 is as effective as any other mobile phase, and should be used for further development of a GPC system for these polymers.

The next step in system development was to optimize pore size selection to produce an effective column combination. The procedure used was to calibrate single columns containing one pore size or a relatively narrow pore size distribution. The results of these calibrations are shown in Figure 3. It is clear that pores of 1000 A or greater are too large to give appreciable peak

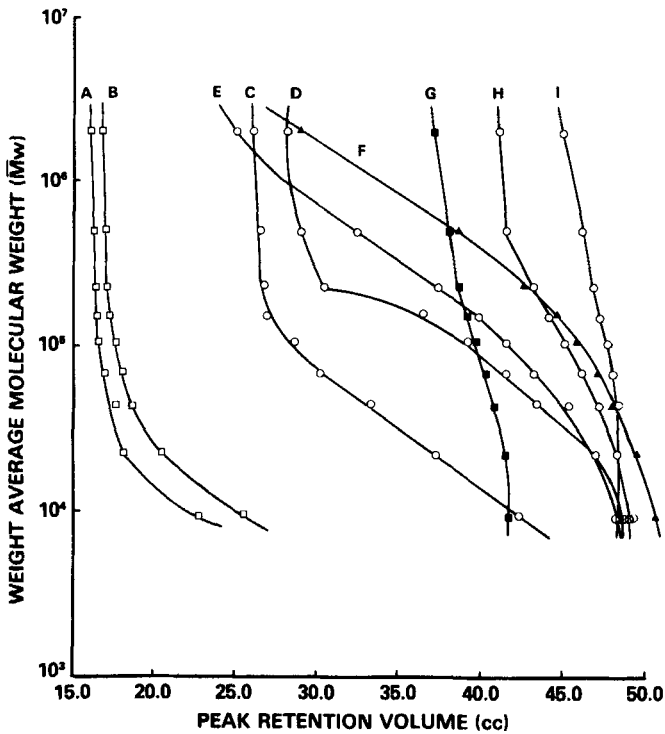

FIGURE 3: MW calibration curves for dextrans on CPG 10 using single columns (each 4' x 3/8") with 0.05 M Na_2SO_4 as mobile phase. A - 88 A, B - 120/88 A, C - 240/120 A, D - 370/327 A, E - 700/500/370 A, F - 727/700 A, G - 1000 A, H - 2000 A, I - 3000 A.

separation for the relatively small dextran molecules. It may be seen that the slopes of the molecular weight calibration curves for the 727/700 A, 700/500/370 A, 370/327 A, 240/120 A and 88 A columns are approximately the same in their linear regions and that there are no molecular weight gaps. This suggests that a column combination with one column of each of these pore sizes should give a linear molecular weight calibration curve having the same slope and a very wide molecular weight separation range. This is borne out by the molecular weight calibration curves shown in Figure 4. The true molecular weight calibration curve was

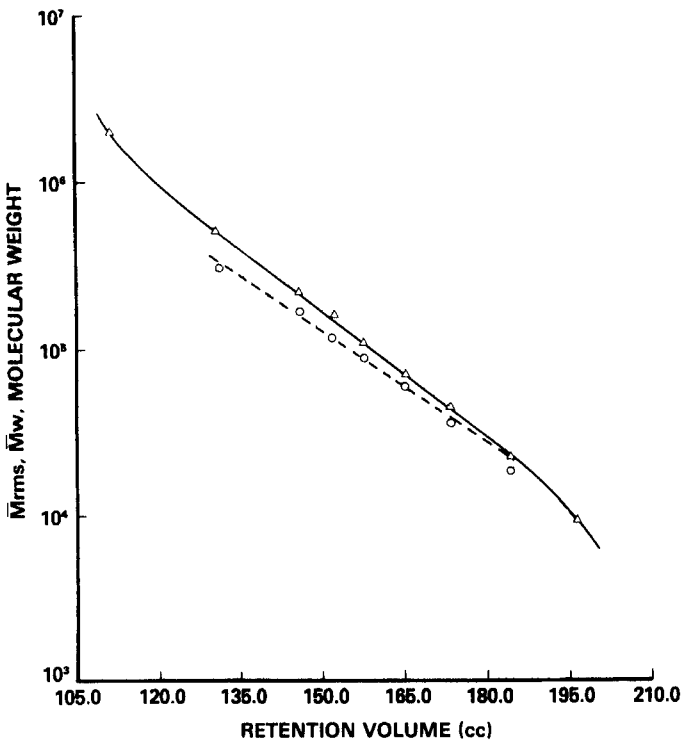

FIGURE 4: MW calibration curve for "optimum" column combination (88 A, 240/120 A, 370/327 A, 700/500/370 A, and 727/700 A) and mobile phase (0.05 M Na_2SO_4). O = M_{rms}; Δ = M_w; ---- theoretical calibration curve using two broad standard method.

obtained using the two broad standards method (13). It is of interest to note that for dextrans the use of the root mean square molecular weight ($M_{rms} = \sqrt{M_N \cdot M_W}$) gives a molecular weight calibration curve which is in good agreement with the true calibration curve. This would not be true in general for any molecular weight distribution but apparently works reasonably well for the dextran standards. The use of $\bar{M}_W$ obviously gives the incorrect calibration curve. The true molecular weight calibration curve was then used to calculate $\bar{M}_N$ and $\bar{M}_W$ using the chromatograms of the standards. These calculated molecular weight averages along with molecular weight averages provided by the supplier are shown in Table 2. The agreement is excellent for the intermediate molecular weight standards with poorer agreement at the high and low molecular weight ends where the calibration curve is non-linear.

TABLE 2

MW Data for Dextrans: GPC Values Compared to Values of Manufacturer

	Values Supplied by Manufacturer (Pharmacia)			GPC Values Uncorrected for Imperfect Resolution		
Sample	$\bar{M}_N \times 10^{-3}$	$\bar{M}_W \times 10^{-3}$	$\bar{M}_W/\bar{M}_N$	$\bar{M}_N \times 10^{-3}$	$\bar{M}_W \times 10^{-3}$	$\bar{M}_W/\bar{M}_N$
T 10	5.70	9.3	1.63	8.73	12.27	1.41
T 20	15.00	22.3	1.49	15.56	22.66	1.46
T 40	28.90	44.4	1.54	27.22	44.69	1.64
T 70	42.50	70.0	1.65	43.02	70.85	1.69
T 110	76.00	106.0	1.39	70.95	104.65	1.48
T 150	86.00	154.0	1.79	89.38	151.99	1.70
T 250	112.50	231.0	2.05	124.89	230.82	1.85
T 500	173.00	509.0	2.94	206.78	421.27	2.04

In summary it has been shown that addition of small amounts of electrolyte (e.g. 0.05 M $Na_2 SO_4$) to water eliminates high molecular weight shoulders from the chromatograms of dextrans on CPG-10 glass. Using this mobile phase a column combination having pore sizes in the range 88-727 A gives excellent resolution in the molecular weight range $22,000 < \bar{M}_w < 230,000$.

ACKNOWLEDGMENT

The financial support of the National Sciences and Engineering Research Council of Canada is gratefully acknowledged.

REFERENCES

1) A.R. Cooper, and D.S. Van Derveer, J. Liquid Chromatography, 1, 693, 1978.
2) A.R. Cooper and D.P. Matzinger, J. Liquid Chromatography, 1, 745, 1978.
3) A.R. Cooper and D.P. Matzinger, Am. Lab., 9, 12, 1977.
4) A.R. Cooper and D.P. Matzinger, J. Applied Polymer Sci., 23, 419, 1979.
5) A.L. Spatorico and G.L. Beyer, J. Applied Polymer Sci., 19, 2933 (1975).
6) A.A. Soeteman, J.P.M. Roels, J.A.P.P. Van Dijk and J.A.M. Smit, J. Polymer Sci., 16, 2147, 1978.
7) R.R. Vrijbergen, A.A. Soeteman and J.A.M. Smit, J. Applied Polymer Sci., 22, 1267, 1978.
8) J.A.P.P. Van Dijk, W.C.M. Henkens and J.A.M. Smit, J. Polymer Sci., 14, 1485, 1976.
9) F.A. Buytenhuys and F.P.B. Van der Maeden, J. Chromatography, 149, 489, 1978.
10) T. Hashimoto, H. Sasaki, M. Aiura and Y. Kato, J. Polymer Sci., 16, 1789, 1978.
11) K.J. Bombaugh, W.A. Dark and J.N. Little, Analytical Chem. 41, 1337, 1969.
12) A.H. Abdel-Alim and A.E. Hamielec, J. Applied Polymer Sci., 17, 3033, 1973.
13) A.E. Hamielec, and S.N.E. Omorodion, ACS Symposium Series, in press, Washington, 1979.