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DETERMINATION OF THE MOLECULAR WEIGHT OF CLINICAL DEXTRAN BY GEL PERMEATION CHROMATOGRAPHY ON TSK PW TYPE COLUMNS

R. M. ALSOP*
Fisons PLC, Pharmaceutical Division, Holmes Chapel, Cheshire (Great Britain)
and
G. J. VLACHOGIANNIS
Chemical Engineering Department, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (Great Britain)
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SUMMARY

The need for dextran fractions with a narrow molecular-weight distribution for clinical use is well known. The definition of such material has significantly improved by the use of gel permeation chromatography (GPC) techniques. However, an internationally accepted standard method of analysis giving accurate and reproducible results in different laboratories has yet to be defined. This paper reviews the GPC packings that can be or have been used for determining the molecular weight distribution of dextran polymers. The application of GPC to the determination of the molecular weight of dextrans on TSK PW type columns is described and its accuracy and reproducibility are discussed. This material appears to meet the criteria required for a standard method.

INTRODUCTION

Dextran is a glucose polymer and for many years it has been used in medicine as a blood plasma volume expander or blood flow improver. The current manufacturing process involves fermentation of sucrose to produce high-molecular-weight (*ca.* $5 \cdot 10^7$) native dextran, controlled hydrolysis of the native dextran to produce lower-molecular-weight products and carefully controlled fractionation procedures to isolate products of different weight-average molecular weights (*e.g.*, 40,000 and 70,000)[1,2]. For clinical purposes these heterogeneous dextran fractions should have narrow molecular-weight distributions (MWDs) because material with too small a molecular weight is rapidly lost from the circulation and is therefore therapeutically ineffective, and material with too high a molecular weight can interfere with normal coagulation processes of the blood.

Accurate and reliable methods for measuring MWDs are necessary and rapid methods are desirable so that on-line monitoring of the production process can be

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carried out. Clinical dextran is used widely throughout the world and methods for the characterization of molecular weight and MWD exist within various pharmacopoeias and official government specifications. With the exception of the Nordic Pharmacopoeia, these are largely based on light scattering and viscosity measurements. It has been proposed that gel permeation chromatography (GPC) be used internationally for such characterization[3], but clearly a method of proven reproducibility is required. Efforts in our laboratories and elsewhere have resulted in the development of improved analytical methods. The aim of this paper is to describe in more detail the GPC technique adopted by us for the determination of the MWD of clinical dextran and to check the accuracy and reproducibility of this technique in two separate laboratories.

THEORETICAL

Molecular weights and polydispersity

As no single number can adequately characterize the molecular weight of a polymer, such as dextran, various averages are used. Of the various molecular weight averages, the most commonly used to describe the dextran polymer are the weight-average molecular weight ($\bar{M}_w$) and the number-average molecular weight ($\bar{M}_n$):

$$\bar{M}_w = \frac{\Sigma n_i M_i^2}{\Sigma n_i M_i} \tag{1}$$

$$\bar{M}_n = \frac{\Sigma n_i M_i}{\Sigma n_i} \tag{2}$$

where n_i is the number of molecules of molecular weight M_i. $\bar{M}_w$ is particulary sensitive to changes in the high-molecular-weight content in the dextran polymer and gives an important indication of its *in vivo* behaviour. $\bar{M}_n$ is largely influenced by changes in the low-molecular-weight content of the sample.

The polydispersity term (D) is often used to describe the width of the MWD of the dextran polymers and is defined as

$$D = \frac{\bar{M}_w}{\bar{M}_n} \tag{3}$$

This ratio is always greater than unity, as $\bar{M}_w$ is always greater than $\bar{M}_n$ except for a monodisperse system, *e.g.*, glucose, where the ratio is unity.

Methods for determining molecular weight

Over the past 30 years a wide range of analytical methods have been used with varying degrees of success to provide data on the molecular weight of dextran. Absolute methods include light scattering, ultracentrifugation, osmometry and functional group analysis[4], but these methods gave only average molecular weights of the whole sample and therefore give only limited information regarding the polymer. The obvious requirement is for a method that gives information to enable a complete MWD of the polymer to be drawn. The first method used to obtain this MWD

involved fractional precipitation of 10–20 polymer fractions from the whole fraction by solvent precipitation. The weight and the average molecular weight of each fraction were then measured using one of the above techniques and on this basis an MWD curve was drawn.

Clearly this method is very time consuming, but this problem can be overcome by the use of GPC, which provides not only a rapid and sensitive method for the determination of weight-average and number-average molecular weights but also provides complete MWD data for the polymer under investigation.

GEL-PERMEATION CHROMATOGRAPHY

Principles

GPC is a liquid chromatographic technique for the separation of molecules according to their size. Because the gel (packing material) has a porous structure, small molecules penetrate it to a greater extent than large molecules and therefore elute more slowly. The chromatography can be defined mathematically by the following equation:

$$K_d = \frac{V_e - V_0}{V_t - V_0} \tag{4}$$

where

K_d = fraction of the pore volume penetrated by the molecule;
V_e = elution volume of the molecule;
V_0 = void volume of the column (*i.e.*, the elution volume of a molecule too large to penetrate the pores);
V_t = total bed volume (*i.e.*, the elution volume of a molecule small enough to penetrate all the pores).

These parameters are shown diagrammatically in Fig. 1. As $V_e - V_0$ represents the volume within the pores available to the molecules eluting at V_e and $V_t - V_0$ represents the total pore volume, K_d is equivalent to a distribution coefficient normally quoted in other forms of chromatography.

Packing materials

The many improvements in liquid chromatography over the last decade have been due not only to innovation in instrumentation but, of greater importance, to the development of new packing materials, especially those capable of use at high pressures. Molecular weight determinations of water-soluble polymers were first carried out using gels such as Sephadex (a cross-linked dextran) and Sepharose (an agarose gel)[5]. These materials are still used widely in biochemical studies, including dextran characterization, but because they are soft they can withstand only low pressures and low mobile phase flow-rates must be used, which result in long analysis times.

However, improved packings for GPC with aqueous solvents, have been developed. The most widely used are based on silica (*e.g.*, Spherosil, Porasil, Zorbax PSM). These packings have been used by us for dextran analysis[6,7] but their suitability for long-term reproducible usage has yet to be demonstrated. The main problems are their solubility in aqueous mobile phases (weight losses of about 1 % per day

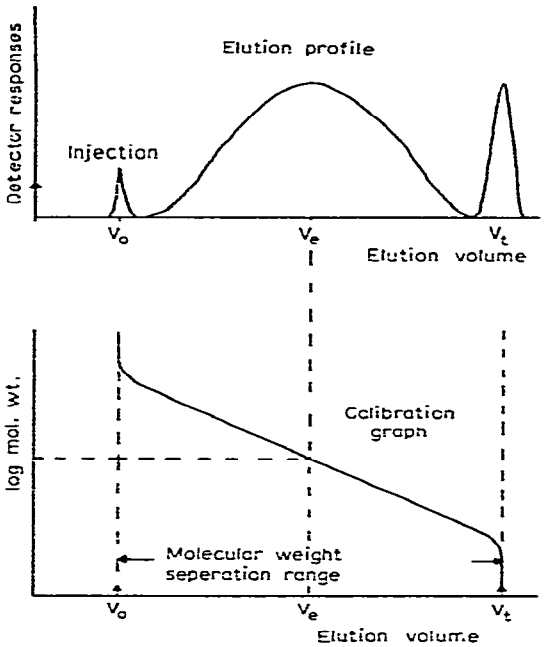

Fig. 1. Diagrams showing the relationship between the elution profile and the calibration graph in gel permeation chromatography.

at pH 7 have been reported[8] and non-specific adsorption, which may occur on silica surfaces[9].

Because of these doubts about long-term stability and adsorption effects of the silica packings we have chosen to use the rigid organic packings that have been developed for aqueous GPC. These are essentially water-compatible cross-linked organic polymers. In our laboratories Hydrogel (Waters Assoc.), a highly cross-linked polymer of ethylene glycol dimethacrylate, was used for dextran analysis[6]. The advantages of this packing were its long stability and its rigidity, which allows operation at pressures of up to 3000 p.s.i. This enables high flow-rates to be maintained and therefore a complete MWD to be obtained in less than 1 h. Unfortunately, the Hydrogel range of GPC packings was withdrawn from the market about 2 years ago owing to poor batch reproducibility, although stable packed columns remain in use in our laboratories that still give excellent results, albeit in a limited molecular weight range.

Alternative materials, *e.g.* Spheron, falling within this same class of organic polymers have also been used successfully[10] for the determination of dextran MWD but similar variations in supply have been noted.

Another organic rigid material suitable for the analysis of dextran is the TSK PW type gel[11] (Toyo Soda, Japan) (Fig. 2). This gel is available in many pore sizes and is sold in packed columns. It has an efficiency of 17,500 plates/m and it allows operating pressures of up to 1000 p.s.i., which enable high flow-rates to be maintained and therefore a complete MWD to be obtained in less than 1 h. Long-term stability of

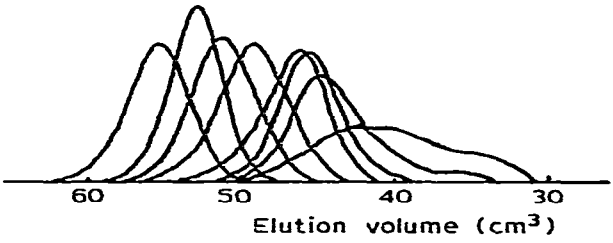

Fig. 2. Elution curves for dextran standard measured with a TSK PW type column set, G3000 PW and two G5000 PW. Samples are, from right to left, T2000, T500, T250, T150, T70, T40, T20 and T10.

the material has been demonstrated in our laboratories, where the columns have been in continuous operation for over 2 years.

Data handling

The complex part of GPC is the production of the final MWD curve and the weight-average and number-average molecular weights from the chromatographic data.

The raw data consist of an elution profile of detector response against elution volume. The detector response is proportional to weight of polymer present, and the molecular weight of the polymer is a logarithmic function of K_d, a function of the elution volume (eqn. 4). In order to relate the elution volume (through K_d) to the molecular weight, calibration of the GPC column is necessary. This is carried out by chromatographing standard samples of known molecular weight. Ideally these should be as near as monodisperse as possible and should also consist of the same polymer as that being characterized. The former simplifies the procedure and the latter ensures that no doubt can be expressed concerning the validity of the calibration.

In GPC with organic eluents, polystyrene standards of this type are available and are widely used as calibrants for a variety of polymers in addition to polystyrene. This requires the use of the universal calibration, a concept derived from studies of the properties of polymers in dilute solution, which have also thrown light on the mechanism of GPC. The separation achieved by GPC is dependent on the effective volume of the polymer molecule in solution. For a monodisperse polymer this hydrodynamic volume is proportional to the product of the intrinsic viscosity (η) and its molecular weight (M). Thus a plot of log (ηM) against elution volume has provided a universal calibration graph found to be valid for many polymers.

However, polystyrene standards are not suitable for aqueous GPC and in our case dextran is the only satisfactory molecule for calibration. Dextran fractions of low polydispersity are not readily available and have to be produced by fractional precipitation or preparative GPC, normally in the laboratory requiring them. Lansing and Kraemer[12] developed a method in which standards with a polydispersity of less than 1.1 are first characterized by measuring their $\bar{M}_w$ values by light scattering and their $\bar{M}_n$ values by end-group analysis. The fractions are then assumed to have a Gaussian distribution (weight *versus* log molecular weight) and by using their derived equation the theoretical MWD is calculated. The samples are then chromatographed and the characteristics thus obtained are equated with those from the calcu-

lated MWD. By this means a series of suitable standards may be used to produce the calibration graph (Fig. 1).

Alternatively, broader fractions of polymer can be used. This necessitates a different approach, as was used by Nilsson and Nilsson[13]. In this instance, "broad" fractions are analysed by GPC, their $\bar{M}_w$ values having been obtained by light scattering measurements. Each elution curve is divided into at least twenty vertical sections, the areas and elution volumes are measured, and elution volume (V_e) converted to a K_d value. Nilsson and Nilsson then assumed that the molecular weight (M_i) and the K_d value were related by the following equation:

$$M_i = b_5 + \exp[b_0 + b_1 (K_d) + b_2 (K_d)^2 + b_3 (K_d)^3] \quad (5)$$

Values of the constants b_1–b_5, which give the optimum agreement between the actual values of $\bar{M}_w$ measured by light scattering for each standard fraction and the calculated values obtained from the elution profile using eqns. 1 and 5, and where i refers to the ith vertical section, are then determined by computation. This minimization of the difference is carried out using Hartley's modification of the Grauss–Newton method[14]. The form of eqn. 5 thus derived is used to calculate the molecular weight corresponding to each value of K_d and a calibration graph is drawn (Fig. 1).

This is the preferred calibration procedure as any one set of (K_d,M) coordinates is estimated from the several overlapping profiles of the dextran standards. Also, this calibration can be easily incorporated into an automated calculation. We have found that the inclusion of glucose and an oligosaccharide in the calibrating standards is advantageous in obtaining accurate calibration at low molecular-weight values.

ANALYTICAL METHOD FOR DEXTRAN CHARACTERIZATION

Equipment

The two analytical systems that were used are shown diagrammatically in Fig. 3.

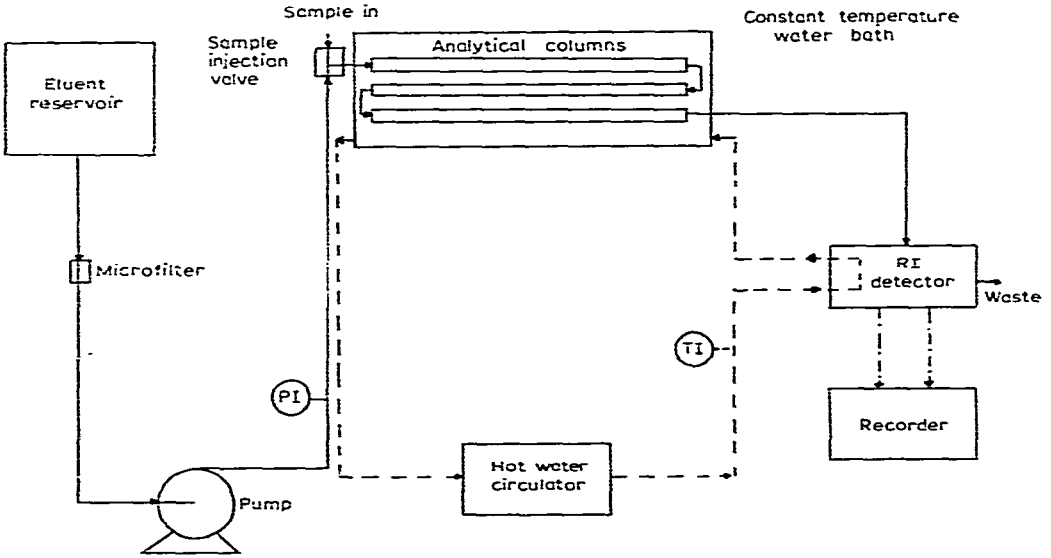

Fig. 3. Schematic diagram of the analytical system.

Both systems consisted of an eluent reservoir, microfilter, pump, a sample injection valve, a set of TSK PW type columns, hot water circulator, water-bath, detector, recorder, pressure indicator and temperature indicator.

The eluent used was a 0.02% (w/v) solution of sodium azide in distilled water. The sodium azide solution was used as a bacteriostat and to suppress any ionic exclusion peaks[15]. The samples (2% w/v) to be injected were made up to a concentration of 0.02% (w/v) sodium azide to prevent the presence of any negative (absence of azide) peaks on the chromatograph.

The eluent was pumped with a positive displacement pump (Series II, Metering Pumps Ltd.) at Aston University and a Constametric pump at Fisons.

Samples were injected using a sample injection valve (Type 30-501, Spectroscopic Accessory Co.) fitted with a constant-volume (100 μl) sample loop. All the samples were filtered before being injected using a Millipore syringe filter that was fitted with a 0.45-μm disposable filter.

The set of columns used for dextran analysis consisted of two TSK G5000 PW columns and a TSK G3000 PW column. The columns were connected in series in sequence of descending pore size and were enclosed in a larger glass column (70 × 7.5 cm I.D.) filled with water and heated by passing it through a hot water circulator (C-400; Tecam, Cambridge, Great Britain), so that operation of the analytical system at higher temperatures was allowed, in order to create better resolution and lower the pressure across the system. The operating temperature of the system was 35°C and the flow-rate of the mobile phase was about 1 $cm^3\ min^{-1}$.

The eluate from the columns passed into a differential refractometer [Model 1107LJ (Laboratory Data Control) at Aston University, and an R401 (Waters Assoc.) at Fisons], and the resulting change in the eluate concentration was registered on a flat-bed recorder [Venture Servoscribe (Smiths Ltd.) at Aston University and a Jobling CR 100 at Fisons]. The resulting chromatograms were measured manually, at Aston, in order to convert them to MWD and average molecular weights later.

At Fisons, the signal from the differential refractometer was converted to the digital mode and recorded by a cassette data logger (Digitronix) so that data could be entered into the computer immediately for the MWD and average-molecular-weights calculations.

Data conversion

To convert a chromatogram into an MWD and average molecular weights, it was necessary to calibrate the column packing. The Nilsson and Nilsson[13] calibration approach was used to calibrate the TSK gel PW type columns.

Dextran T fractions (Pharmacia, Sweden) were used as calibrants, as their $\bar{M}_w$ values obtained by light scattering measurements are known and they are widely available. Glucose and very-high-molecular-weight dextran ($>2 \cdot 10^6$) were used to provide the total liquid ($V_0 + V_i$) and void (V_0) volumes, respectively. Using these dextran T fractions and the Nilsson and Nilsson iterative calibration approach[13], the values of the constants b_1–b_5, which give the optimum agreement between the $\bar{M}_w$ values measured by light scattering and the values obtained by GPC, were calculated.

To convert a chromatogram of a sample to average molecular weights and MWD it was necessary for the heights measured at regular intervals along the chromatogram, the elution volumes at these heights, the constants b_1–b_5 found by the

calibration programme and the void and total liquid volumes for the fractionating columns to be entered into a computer program that combined eqns. 1–5 to obtain the average molecular weights, polydispersity and MWD.

DISCUSSION

The same selection of T fractions (Table I) that gave calibration GVT6 on the GPC analytical system at Aston University was used to produce the calibration 315Z at Fisons.

TABLE I

COMPARISON OF MOLECULAR WEIGHTS FOUND BY LIGHT SCATTERING AND OBTAINED BY GPC FOR CALIBRATION 315Z

Fraction	*Batch No.*	*Molecular weight*		*Difference (%)*
		Light scattering	*GPC*	
T500	3202	490,000	491,232	0.25
T250	1343	231,000	228,168	1.20
T150	921	154,000	155,706	1.10
T70	5403	72,000	71,473	0.70
T40	2540	44,400	44,646	0.60
T40	2514	41,800	42,125	0.80
T20	7968	22,300	22,076	1.00
T10	3205	9300	9318	0.20
Stachyose		667	667	0.00
Glucose		180	180	0.00

Although the calibration graphs were not identical (Fig. 4), owing to the different properties of the two sets of TSK columns, when for comparison purposes the dextran T40 and T70 calibration standards were analysed using the respective elution profile from each calibration, good agreement was obtained for both the GPC systems (Table II). Hence reproducible results can be obtained in determining the average molecular weights and MWD of dextrans by two different GPC systems that contain an identical set of TSK gel PW type columns when they are operated under similar conditions.

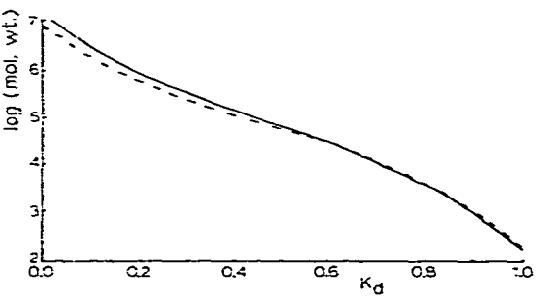

Fig. 4. Calibration graphs for 315Z (broken line) and GVT6 (solid line).

TABLE II

MOLECULAR WEIGHT PROFILES OF DEXTRAN T40 AND T70 ON BOTH ASTON AND FISONS SETS OF PW TYPE COLUMNS

Integral distribution (%)	*MW profile of dextran T40 at Aston (A)*	*MW profile of dextran T40 at Fisons (B)*	*MW profile of dextran T70 at Aston (C)*	*MW profile of dextran T70 at Fisons (D)*	$\frac{A}{B}\cdot 100$	$\frac{C}{D}\cdot 100$
5.0	11,425	11,502	16,034	17,009	99	95
10.0	14,689	14,897	21,252	22,120	99	96
15.0	17,250	17,583	25,403	26,374	98	97
20.0	19,577	19,972	29,191	30,253	98	97
25.0	21,740	22,221	32,820	34,016	98	97
30.0	23,923	24,425	36,475	37,757	98	97
35.0	26,126	26,600	40,217	41,547	98	97
40.0	28,441	28,864	44,131	45,452	98	97
45.0	30,839	31,360	48,375	49,588	98	98
50.0	33,469	33,977	52,814	54,054	99	98
55.0	36,299	36,831	58,012	58,992	99	99
60.0	39,505	39,951	63,587	64,470	99	99
65.0	43,019	43,410	70,108	70,712	99	99
70.0	47,273	47,362	77,957	78,111	100	100
75.0	51,966	52,073	87,287	87,110	100	100
80.0	58,195	58,063	99,068	98,936	100	100
85.0	66,325	66,076	115,182	114,745	100	100
90.0	78,007	77,342	139,498	138,945	101	100
95.0	99,841	99,110	182,075	186,104	101	98

Dextran	$\bar{M}_w$		
	Light scattering data	*GPC (Fisons)*	*GPC (Aston)*
T40	41,800	42,125	42,012
T70	72,000	71,473	70,088

The Nordic Pharmacopoeia requires that all of the calibration standards shall have individual molecular weights obtained by GPC within 90–110% of those obtained by light scattering before one proceeds with the analysis of samples by GPC. Our calibrations provided a much better agreement (less than 2.5% deviation) between $\bar{M}_w$ values found by GPC and those measured by light scattering (Table I).

To assess further the accuracy of the calibrations, the standard deviation of the ratio of the molecular weights obtained by GPC divided by those obtained by light scattering for the calibration standards, multiplied by 100, was calculated and plotted against the residual sum of squares obtained from the iterative calibration procedure. These results from several calibrations are plotted in Fig. 5. The smaller the standard deviation and the residual sum of squares are, the better is the agreement between the molecular weights for the calibration standards obtained by GPC and light scattering and the more reliable is the calibration.

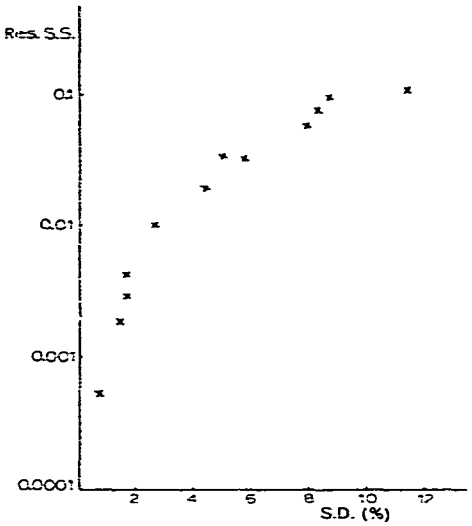

Fig. 5. Graph of residual sum of squares (Res. S.S.) *versus* the standard deviation of the ratio of light scattering molecular weight divided by the GPC molecular weight × 100.

Similar accuracies of calibration have been achieved by other workers in Sweden using Sepharose gels. Their data[16] give a standard deviation of 1.14, corresponding to a residual sum of squares of approximately $0.1 \cdot 10^{-2}$ (very similar to calibration GVT6).

One of the most significant sources of error in the above procedure can result from the use of inaccurate V_0 and V_t determinations in the calibration and sample analysis procedures. Wherever possible, it is our practice to add V_0 and V_t markers to the calibration standards and samples under test, provided baseline separation between the three components can be obtained. The elution profiles in Fig. 6 show the

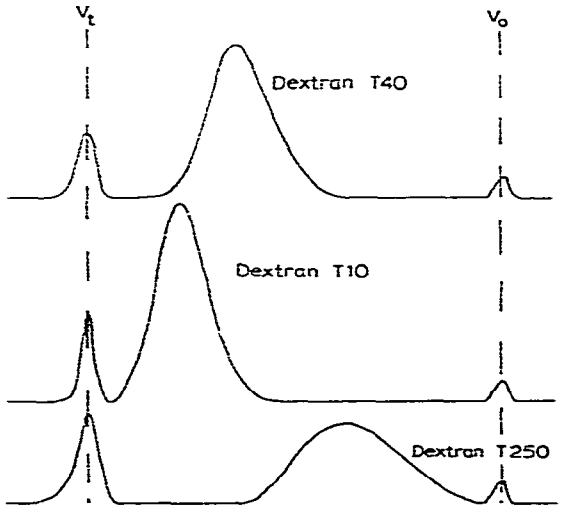

Fig. 6. Separation of calibration standards from V_0 — V_t markers.

capability of the proposed TSK PW column system to resolve a T fraction of molecular weight 10,000 completely from glucose and a T fraction of molecular weight 250,000 from a native dextran V_0 marker. A T fraction of molecular weight 40,000 is also shown for comparison.

The effects of variation in V_0 and V_t are shown in Figs. 7 and 8 for the calibration system previously described. In these instances a standard calibration (K_d *versus* molecular weight) has been produced where the V_0 and V_t values were 409 and 838 units, respectively. In Fig. 7 the calibrations have been re-run using the standard

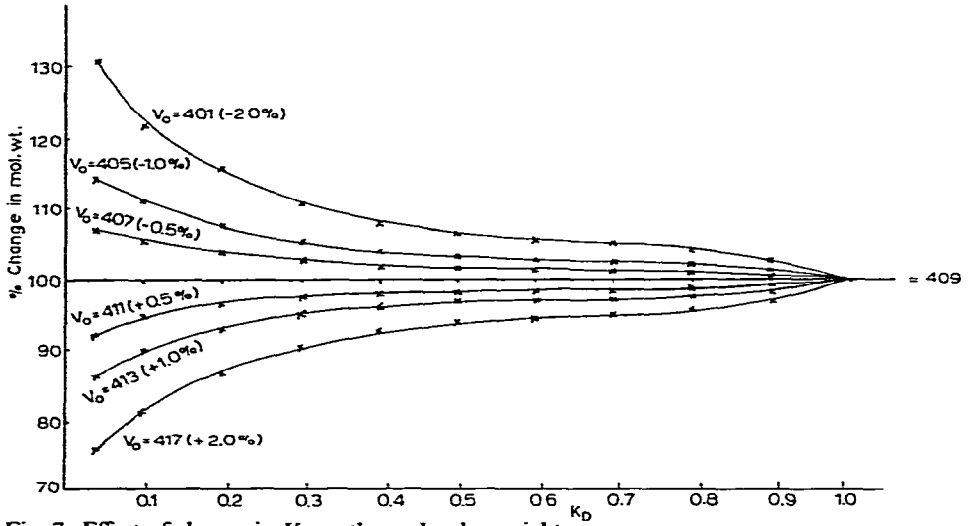

Fig. 7. Effect of change in V_0 on the molecular weight.

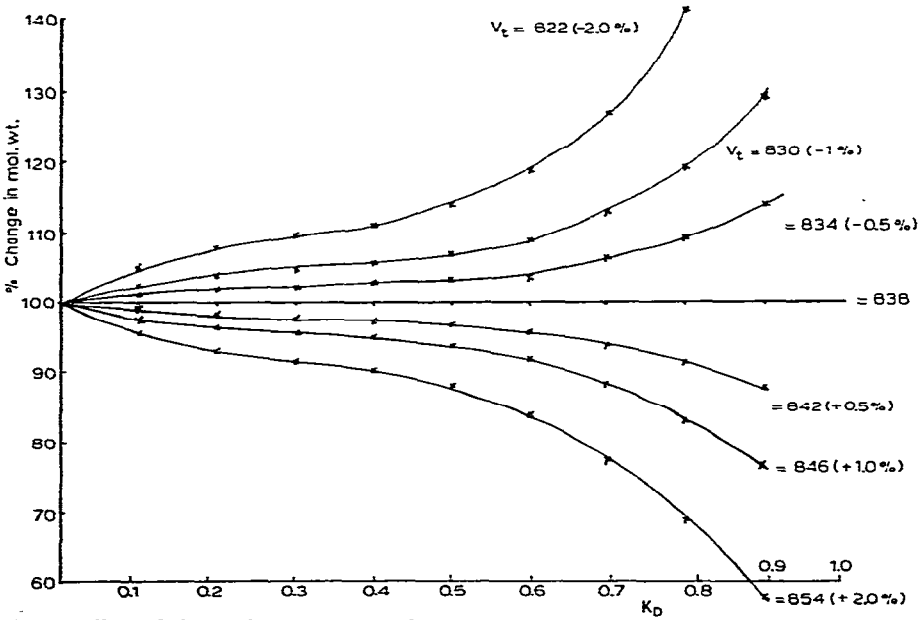

Fig. 8. Effect of change in V_t on the molecular weight.

V_t value but introducing erroneous values for V_0. It can be seen that a small deviation of 0.5% in V_0 can produce variations of >5% in the molecular weight read from the calibration graph at K_d values <0.1. Deviation of 2% in V_0 produced highly distorted values for molecular weight from the calibration at $K_d < 0.3$.

Similarly in Fig. 8 the calibration has been re-run using the standard V_0 value but introducing erroneous values for V_t. Highly distorted values for molecular weights at $K_d > 0.7$, are obtained with a V_t deviation of 2%.

In our opinion, a reproducibility of V_0 and V_t expressed as coefficient of variation, (standard deviation · 100)/mean value, of <0.5% is required in the determination of these values over a several-month period in order to obtain a satisfactory system for clinical dextran analysis. Data for V_0 and V_t values over a typical 2-month period for the Fisons TSK PW gel system give a coefficient of variation for V_t of 0.216 and for V_0 of 0.278 based on 35 determinations.

Another source of error in GPC measurements of this type is produced by the degree of diffusion of individual molecular species that occurs on the column. Previous workers[4,17] have applied a diffusion correction procedure to the calculation of MWDs from elution profiles based on the diffusion obtained with the V_t marker glucose. Similarly, the glucose profile can be used to calculate the number of theoretical plates in the column using the expression $16 (V_m)^2/b$, where V_m is the elution volume at the peak maximum and b is the peak width at the baseline.

The effect of the diffusion correction, expressed as σ, the standard deviation of the glucose distribution on the molecular weight results, can be calculated for various degrees of diffusion corresponding to known numbers of theoretical plates in the column[4]. Thus a particular elution profile obtained with 1360 theoretical plates (analysis 2, Table III), processed using different values of σ, indicates the variation of molecular weight at these different σ values and at the corresponding number of theoretical plates. Each analysis should only be directly compared with the uncorrected result ($\sigma = 1$) in Table III. It must be remembered that, for example, the results of analysis 5 in Table III are not derived from the actual profile obtained with a column of 2987 theoretical plates but are the results of a standard profile corrected for

TABLE III

RESULTS OF THE ANALYSES OF PROFILE 027.88

Analysis No.	*Number of theoretical plates*	*σ (glucose)*	*Molecular weight* × 10^{-3}					
			$\bar{M}_w$	$\bar{M}_n$	M_5	M_{10}	M_{90}	M_{95}
1	8 × 10^5	1	43.3	26.2	10.7	14.4	81.7	106.1
2	1360	243	41.4	28.7	13.1	16.7	73.1	91.8
3	1505	231	41.6	28.4	12.8	16.5	74.0	93.2
4	2008	200	42.0	27.9	12.2	15.9	76.0	96.6
5	2987	164	42.5	27.3	11.7	15.4	77.9	99.8
6	3984	142	42.7	27.0	11.4	15.2	78.9	101.4
7	5970	116	42.9	26.8	11.1	14.9	79.9	103.0
8	9918	90	43.1	26.6	10.9	14.7	80.6	104.2
9	15075	73	43.1	26.4	10.8	14.6	81.0	104.8
10	20240	63	43.2	26.4	10.8	14.6	81.1	105.1

the diffusion that would occur on a system of 2987 plates and compared with the same uncorrected profile.

From examination of Table III it can be seen that the differences in the various molecular weight values for diffusion-corrected and uncorrected results are not significant with columns of more than 10,000 theoretical plates. With column systems containing only 1000–1500 theoretical plates large variations in the M_5 and M_{95} values occur.

The reproducibility of the system can be illustrated by the analysis of batch BT62D run on fifteen occasions during March 1981 on the Fisons columns and Batch BT1J run during August 1981. The data are shown in Tables IV and V.

TABLE IV

REPRODUCIBILITY FOR BT62D

Date	*Injection No.*	V_0	V_t	$\bar{M}_n$	$\bar{M}_w$
March 3, 1981	310-91	424	841	22,149	31,544
March 5, 1981	310-95	420	834	22,427	31,543
March 6, 1981	310-97	420	835	22,633	31,474
March 10, 1981	311-19	422	840	23,182	31,556
March 11, 1981	311-20	424	840	23,241	31,540
March 11, 1981	311-27	424	842	21,659	31,139
March 12, 1981	311-30	424	840	22,703	31,348
March 13, 1981	311-38	424	840	23,150	31,479
March 13, 1981	311-42	424	841	22,677	31,287
March 16, 1981	311-43	423	838	23,170	31,618
March 17, 1981	311-48	422	838	22,758	31,473
March 17, 1981	311-55	424	839	22,771	31,480
March 18, 1981	311-60	423	838	22,424	31,137
March 18, 1981	311-63	424	836	22,446	31,362
March 19, 1981	311-64	423	837	22,749	31,746
$\bar{x}$		423	838.6	22,676	31,448
σ		1.414	2.324	413	162

TABLE V

REPRODUCIBILITY FOR BT1J

Date	*Injection No.*	V_0	V_t	M_{10}	M_{90}	$\bar{M}_n$	$\bar{M}_w$
August 17, 1981	315-38	410	837	14,582	67,874	25,918	38,104
August 19, 1981	315-42	410	838	14,282	69,770	25,687	38,523
August 21, 1981	315-43	410	839	14,073	68,499	25,307	38,428
August 26, 1981	315-44	410	842	14,249	69,674	25,519	38,752
September 1, 1981	315-46	409	838	14,423	69,131	25,911	38,343
$\bar{x}$		410	839	14,322	68,989	25,668	38,430
σ		0.447	1.924	171	719	234	213

CONCLUSIONS

Two similar simple GPC analytical systems that each contain a set of TSK gel PW type columns were used for the determination of the MWD of clinical dextran. The accuracy and reproducibility of these GPC systems in the determination of the MWD of dextrans together with the speed of the GPC method make this method eminently suitable as a basis for the international control of the MWD of clinical dextran products.

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NOMENCLATURE

b	peak width at baseline
b_{1-5}	calibration constants
D	polydispersity
K_d	distribution coefficient
M_i	molecular weight of species i
$\bar{M}_n$	number-average molecular weight
$\bar{M}_w$	weight-average molecular weight
MWD	molecular weight distribution
n_i	number of molecules of molecular weight M_i
V_i	pore volume
V_m	peak maximum volume
V_0	Void volume
V_e	elution volume
V_t	total liquid volume ($V_i + V_0$)

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