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COMPARISON OF POLY(ETHYLENE OXIDE), PULLULAN AND DEXTRAN AS POLYMER STANDARDS IN AQUEOUS GEL CHROMATOGRAPHY

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SUMMARY

Aqueous gel chromatography for poly(ethylene oxide), pullulan and dextran was carried out in 0.1 *M* aqueous sodium chloride solution at 25°C using a cross-linked hydrophilic vinyl polymer gel. From the results obtained, log M_w *vs.* $V_{r,w}$ calibration curves for both poly(ethylene oxide) and pullulan were linear over a wide molecular weight range, where M_w denotes the weight-average molecular weight of the sample and $V_{r,w}$ the retention volume at the centre of mass of the chromatogram peak. However, the corresponding calibration curve for dextran was non-linear.

Evidence in support of the universal calibration procedure was obtained for the three polymers since a single straight line could be drawn through all data points. It is concluded that poly(ethylene oxide) provides the best standards, while pullulan is also important because of the analogy of its chemical structure with those of other linear polysaccharides.

INTRODUCTION

Chromatographic separations according to the effective molecular size of polymers in solution are well described by the hydrodynamic volume concept, named the universal calibration procedure, proposed by Grubisic and co-workers[1,2]. In gel permeation chromatography (GPC), the effectiveness of the universal calibration procedure has been verified experimentally in various organic solvents, irrespective of polymer species and shapes, *e.g.*, linear and branched[3–9].

For water-soluble polymers, there are few articles[10–14] on the validity of the universal calibration procedure because of the lack of water-soluble polymer standards having a narrow molecular weight distribution, and for polymer standards molecular weights range from 10^3 to more than 10^6 g mol^{-1}. Although sodium poly(styrene sulphonate) (NaPSS) and dextran were used as the polymer standards in aqueous gel chromatography[11–14], their use has been criticized on the basis of their inherent features.

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The molecular size of NaPSS in solution depends on the added salt concentration[15], and thus its retention volume is dependent on the salt concentration of eluent used[10,16]. Moreover, the choice of eluent is restricted at high salt concentration in order to avoid the effects of high viscosity and to reduce the possibility of polymer adsorption on the column packing.

Dextran is a branched polymer and the molecular weight dependence of its molecular size becomes weak at higher molecular weights since the degree of branching increases with increasing molecular weight. Therefore, dextrans of high molecular weight have a broad distribution of both molecular weight and the degree of branching.

Recently, the standard poly(ethylene oxide) (PEO) with a narrow molecular weight distribution became commercially available. Pullulan, which is an extracellular polysaccharide of *Aureobasidium pullulans*, is a linear, stable and flexible polymer[17]. We recommend it as the standard sample for water-soluble polysaccharides.

Using the standard PEO, and carefully fractionated pullulan and dextran, we have carried out aqueous gel chromatographic measurements in 0.1 *M* aqueous sodium chloride solution at 25°C on a column system packed with porous polymer gel. On the basis of the experimental results thus obtained, we examine the practical usage of these polymers as the standard sample.

EXPERIMENTAL

Materials

The TSK-standard poly(ethylene oxide) (PEO) was prepared by the "living anion" polymerization technique and was purchased from Toyo Soda. The samples have very narrow molecular weight distributions and their molecular weights range from $24 \cdot 10^3$ to $1.3 \cdot 10^6$ g mol^{-1}. The molecular characterization was carried out by light scattering, GPC and viscometry by the manufacturer and also independently by the authors[18]. Two additional PEO samples having molecular weights of $2 \cdot 10^3$ and $8.3 \cdot 10^3$ g mol^{-1} were purchased from Wako. We characterized them by gel chromatography and viscosity measurement. Samples of dextran T40, T70, T500 and T2000 were purchased from Pharmacia and were well characterized by gel filtration. These samples were dissolved in water and were fractionated by addition of isopropanol as precipitant at 30°C. We selected eight appropriate fractions having narrow chromatograms and determined the molecular characteristics by light scattering and viscosity measurement. Dextran T10 (Pharmacia) was used as the sample of lowest molecular weight, without further treatment. The samples of pullulan were the products from partially hydrolysed starch (Hayashibara). They were carefully fractionated with water as solvent and isopropanol as precipitant at 30°C. The molecular characterization has been reported elsewhere[17].

The molecular characteristics of all samples used in this study are listed in Table I. The third column of the table shows the *z*-average square radii of gyration, $\langle S^2 \rangle_z$, for PEO and dextran samples measured by an Union Giken LS 601A automatic light scattering analyser in 0.1 *M* aqueous sodium chloride solution at 25°C. The $\langle S^2 \rangle_z$ values of pullulan samples measured in water at 25°C[17] were assumed to be the same, within experimental error, as those in 0.1 *M* sodium chloride since no salt-concentration dependence of intrinsic viscosity was observed for the samples.

Sodium chloride was reagent grade and water was doubly distilled.

Aqueous gel chromatography

The chromatographic system used was specially designed for aqueous solutions by Toyo Soda. The instrument was comprised a solvent vessel, an Altex Model 100A pump, a Rheodyne SV-7125 syringe-loading sample injector valve, a TSK RI-8 differential refractometer, and four TSK-GEL PW-type columns (600 × 7.5 mm I.D.) covered with an urethane foam cylinder as thermal insulator. These devices were assembled in a large incubator and were thermostatted at 25.0 ± 0.5°C. Data processing was carried out by a TSK HLC-CP8 Model III microcomputer. TSK-GEL PW-type columns with different pore sizes were commercially available and were packed with semi-hard spherical particles (*ca.* 15 ± 2 μm in diameter) of cross-linked hydrophilic vinyl polymer[19]. These columns had more than 4000 theoretical plates per ft. The column combination was one G2000PW, one G3000PW and two G5500PW

TABLE I

MOLECULAR CHARACTERISTICS OF POLY(ETHYLENE OXIDE), PULLULUAN, AND DEXTRAN SAMPLES

Sample	$M_w(10^3\ g\ mol^{-1})$	$\langle S^2\rangle_z$ $(10^{-12}\ cm^2)$	$[\eta](dl\ g^{-1})$	M_w/M_n	$V_{r,w}(ml)$	$V_{r,max}(ml)$	$W(ml)$
Poly(ethylene oxide)							
PEO-130	1300	37.9	6.03	1.12	44.0	44.1	4.4
PEO-70	730	23.4	4.05	1.10	46.3	46.4	3.8
PEO-30	320	8.4	2.28	1.06	49.6	49.7	3.7
PEO-16	160	4.1	1.49	1.04	52.4	52.4	3.4
PEO-8	80	–	0.93	1.03	55.2	55.2	3.2
PEO-4	40	–	0.65	1.03	58.2	58.2	3.3
PEO-2	21	–	0.41	1.15	59.9	70.0	4.3
PEO-8200	8.2	–	0.21	1.01	64.0	64.0	3.0
PEO-2000	2.0	–	0.09	1.01	69.4	69.5	3.0
Pullulan							
PF-802	1500	30.0	2.67	1.35	45.4	45.2	6.7
PF-302	959	17.7	1.96	1.28	47.3	47.3	5.9
PF-303	611	10.0	1.44	1.18	48.6	48.8	6.1
PF-304	432	6.5	1.14	1.18	50.3	50.4	5.9
PF-305	291	4.4	0.82	1.16	52.3	52.1	5.8
PF-306	180	–	0.62	1.13	53.9	54.0	5.1
PF-307	134	–	0.48	1.30	55.2	55.2	6.7
PF-308	86	–	0.35	1.32	57.1	57.0	7.0
PF-105	48	–	0.29	1.25	59.4	59.5	6.6
Dextran							
T2F1	5193	28.1	0.81	2.67	45.2	45.3	10.3
T2F2	1127	10.6	0.62	2.31	49.3	49.7	8.8
T5F2	578	5.9	0.55	1.76	50.7	51.3	7.8
T5F4	320	3.5	0.44	1.37	53.1	53.2	6.1
T5F7	154	–	0.34	1.28	55.4	55.5	5.7
T2F5	81	–	0.26	1.32	58.1	58.1	6.4
T7F7	43	–	0.20	1.20	60.2	60.5	5.3
T4F4	38	–	0.18	1.19	60.8	61.0	5.4
T10	9.4	–	0.10	1.72	65.9	66.7	9.4

grade columns. The chromatographic solvents used were 0.1 *M* aqueous sodium chloride solution, water with 0.02% (w/w) sodium azide and 0.08 *M* Tris–HCl buffer (pH = 7.9)[17,18]. No sample degradation was observed in all three solvents within a week judging from the viscosity measurements. Therefore, in this study, all the results were obtained in 0.1 *M* aqueous sodium chloride solution. All sample solutions injected were freshly made each time.

The eluent flow-rate was 1.0 ml/min and total pressure drop was less than 40 kg/cm^2. The injection volume was 0.5 ml and the sample concentrations were 0.2 mg/ml for PEO and 0.5 mg/ml for pullulan and dextran, respectively. The solvent flow through the reference side of the refractometer cell was not pumped and was achieved with a 20-cm hydrostatic head difference between the inlet and outlet. Measurements for NaPSS standard samples in the some solvent were unsuccessful because of adsorption of NaPSS on the packed gel.

Viscosity

Intrinsic viscosity values, $[\eta]$, of the polymer samples at 25°C in 0.1 *M* aqueous sodium chloride solution were determined on an Ubbelohde type capillary viscometer. The results are listed in Table I. The kinetic energy correction was always negligible. Intrinsic viscosities were evaluated from plots of both η_{sp}/c against c and ln η_r/c against c, where η_{sp} and η_r are the specific viscosity and the relative viscosity, respectively.

RESULTS AND DISCUSSION

Figs. 1–3 show the chromatograms for all samples used in this study. All the chromatograms had narrow and near-symmetrical distributions except for some chromatograms of dextran. The last column in Table I lists the baseline width of each chromatogram, W, expressed in terms of retention volume (ml), as illustrated in Fig. 1.

First we established the relationship between the retention volume and the weight-average molecular weight, M_w, rather than the molecular size in solution, in

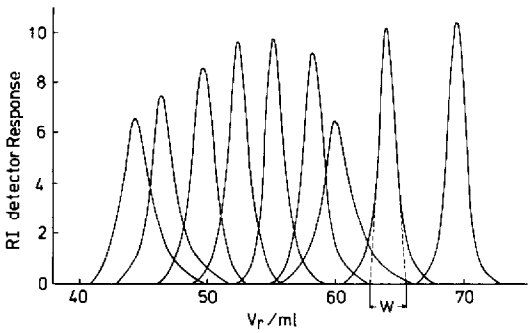

Fig. 1. Chromatograms for poly(ethylene oxide) in 0.1 *M* aqueous sodium chloride solution at 25°C. Samples are listed in Table I. Molecular weights of samples decrease from left to right. As an example, the baseline width of the chromatogram, W, is defined for PEO-8200. The vertical axis denotes refractive index (RI) detector response (normalized).

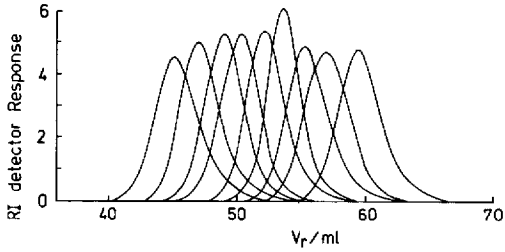

Fig. 2. Chromatograms for pullulan fractions in 0.1 *M* aqueous sodium chloride solution at 25°C. Samples are listed in Table I.

order to calculate the molecular weight distributions from the chromatograms. We assumed that the representative retention volume of the sample is the retention volume at the centre of mass of the chromatogram peak, $V_{r,w}$, corresponding to the retention volume where M_w occurs; $V_{r,w}$ is related to the retention volume representing the peak maximum, $V_{r,max}$, if the molecular weight distribution of the sample is narrow[20].

Fig. 4 shows the log M_w *vs.* $V_{r,w}$ calibration curves thus obtained. The peak position calibration method is available since the $V_{r,w}$ values coincide with the $V_{r,max}$ values within experimental error, as listed in Table I. The equations of the calibration curves for PEO and pullulan were obtained by linear regression as:

$$\log M_w = 10.87 - 0.1081\ V_{r,w} \text{ (for PEO)} \quad (1)$$

$$\log M_w = 10.97 - 0.1058\ V_{r,w} \text{ (for pullulan)} \quad (2)$$

The form of the calibration curve for dextran appears to be non-linear. This difference may be attributable to the broad distribution of the chromatograms shown in Fig. 3 and also to the branching of dextran. The effect of the broad distribution may be compensated by assuming that plots of log $(M_w/M_n)^{1/2}$ against the first moment of the chromatogram are approximately adequate as the calibration curves for samples having broad and asymmetrical distributions, as reported by Linden[21]. However, we found that the log $(M_w/M_n)^{1/2}$ *vs.* $V_{r,max}$ calibration for dextran was also not a straight line. Taking into account the coincidences of $V_{r,w}$ with $V_{r,max}$ in Table I even for dextran, the broad distribution is not the only origin of the curved calibration line.

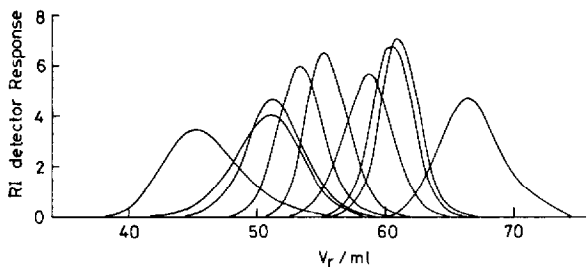

Fig. 3. Chromatograms for dextran fractions in 0.1 *M* aqueous sodium chloride solution at 25°C. Samples are listed in Table I.

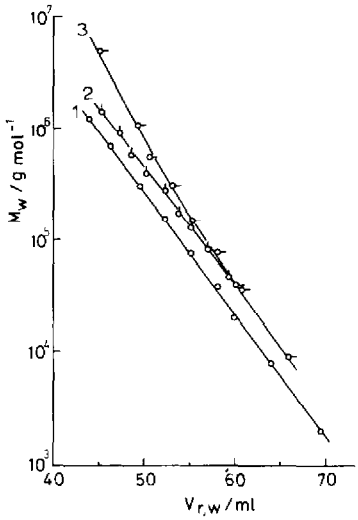

Fig. 4. The log M_w *vs.* retention volume calibration curves for poly(ethylene oxide) (1), pullulan (2) and dextran (3). Solid lines show the plots of log M_w against $V_{r,w}$ for all samples.

Recently, the use of the log M_w *vs.* $V_{r,w}$ calibration curve was recommended in practice since direct molecular weight monitors for GPC, such as a low angle laser light scattering photometer (LALIS), became available[22]. The reliability of this calibration had been confirmed experimentally[19,23]. Thus, we have estimated M_w/M_n ratios for all samples using the calibration curves in Fig. 4; the values are listed in the fifth column in Table I. Evidently, PEO standards have very narrow molecular weight distributions. The average molecular weights of dextran T40, T70 and T500 were estimated from the chromatograms using the curved calibration line in Fig. 4 and are listed in Table II in comparison with the data obtained by the distributor of the samples. The two sets of data are in good agreement. Therefore, the log M_w *vs.* $V_{r,w}$ calibration curve for dextran can be used to estimate molecular weights of samples.

Fig. 5 shows the double logarithmic plots of the intrinsic viscosity, $[\eta]$, against

TABLE II

AVERAGE MOLECULAR WEIGHTS OF DEXTRAN SAMPLES

A, data obtained by Pharmacia; B, data estimated from the calibration curve in Fig. 4.

Sample		$M_w \cdot 10^{-3}$	$M_n \cdot 10^{-3}$	$M_z \cdot 10^{-3}$	M_w/M_n	M_z/M_w
T40	A	41.0	28.0	–	1.46	–
	B	40.7	29.1	55.8	1.40	1.37
T70	A	67.6	37.2	–	1.82	
	B	70.5	41.2	109	1.71	1.55
T500	A	478	193	–	2.48	
	B	449	210	1090	2.13	2.43

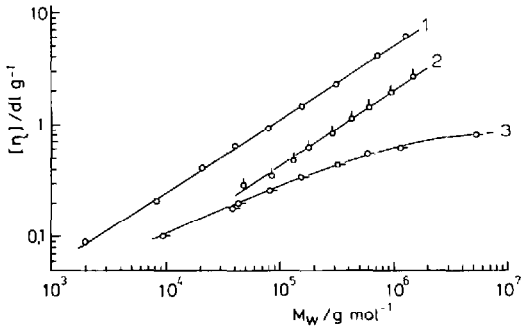

Fig. 5. The double logarithmic plots of the intrinsic viscosity, $[\eta]$, against M_w for all samples in 0.1 M aqueous sodium chloride solution at 25°C. Numbers as in Fig. 4.

M_w for all samples in 0.1 M aqueous sodium chloride solution at 25°C. The Mark–Houwink–Sakurada equations obtained were:

$$[\eta] = 5.88 \cdot 10^{-4} M_w^{0.65} \quad \text{(for PEO)} \tag{3}$$

$$[\eta] = 1.79 \cdot 10^{-4} M_w^{0.67} \quad \text{(for pullulan)} \tag{4}$$

$$[\eta] = 1.36 \cdot 10^{-3} M_w^{0.47} \quad \text{(for dextran)} \tag{5}$$

Eqn. 5 was estimated for molecular weights lower than 10^5 g mol^{-1} and the plots for dextran appear to level off at higher molecular weights. From the results shown in Figs. 4 and 5, the molecular weight dependence of the molecular size in solution for dextran appeared to be different from that for linear polymers such as PEO and pullulan. It may be concluded that this difference results from the inherent branching of dextran. Therefore, we point out that the optimal column combination for aqueous gel chromatography was erroneously selected by Wu *et al.*[14] in terms of the calibration curve for dextran standards. The polymer standards with linear calibration curves may be used in order to check the column combination.

Next we discuss the relationship between the molecular size in solution and the retention volume, $V_{r,w}$. The molecular dimension may be represented by the z-average square radius of gyration, $\langle S^2 \rangle_z$, measured by light scattering and the hydrodynamic volume estimated by the product of $[\eta]$ and M_w[1,2]. Plots of log $\langle S^2 \rangle_z^{3/2}$ against $V_{r,w}$ for PEO, pullulan and dextran shown in Fig. 6. All points are reduced on a straight line. The $\langle S^2 \rangle_z^{3/2}$ values may represent the effective molecular sizes in solution governing the separation.

Fig. 7 shows plots of the log $[\eta]M_w$ against $V_{r,w}$. The universal calibration procedure is also supported experimentally since a single straight line may be drawn by linear regression through all points for PEO, pullulan and dextran. The equation of the curve was:

$$\log [\eta]M_w = 14.69 - 0.1781 V_{r,w} \tag{6}$$

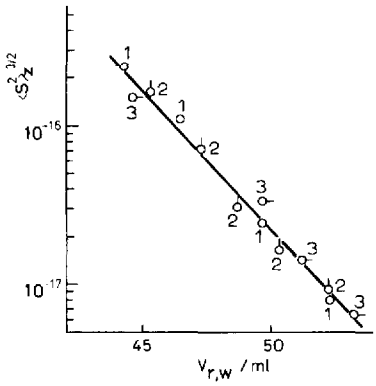

Fig. 6. The plots of log $\langle S^2 \rangle_z^{3/2}$ against $V_{r,w}$ for poly(ethylene oxide), pullulan and dextran. Numbers as in Fig. 4.

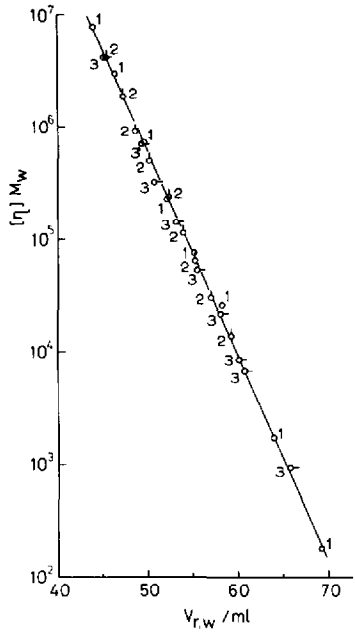

Fig. 7. The plots of log $[\eta]M_w$ against $V_{r,w}$ for all samples in 0.1 *M* aqueous sodium chloride solution at 25°C. Numbers as in Fig. 4.

Therefore, it is practically possible to estimate the absolute molecular weights for pullulan by a combination of $[\eta]$ data for pullulan and the universal calibration curve drawn by using PEO samples as polymer standards. Moreover, substituting eqn. 4 for

$$\log M_w = 11.01 - 0.1064\, V_{r,w} \tag{7}$$

This calibration curve is good agreement with eqn. 2.

On the basis of the universal calibration procedure, the viscosity equation constants, K and α in $[\eta] = KM^{\alpha}$, for an unknown polymer may be evaluated by using the several methods reported in literature[3,24–26]. The method proposed by Cael *et al.*[26] may be convenient because it does not require measurement of the viscosity for the polymer in question. We examine this method using the data for pullulan in Table I and also eqns. 1 and 3 for PEO. At the same retention volume, the following equations may be derived

$$K_s M_s^{1+\alpha} = K_p M_p^{1+\beta} \tag{8}$$

$$\log M_p = \frac{1}{1+\beta} \cdot [\log K_s + (1+\alpha)\log M_s] - \frac{1}{1+\beta} \cdot \log K_p \tag{9}$$

where the subscript s denotes the value for PEO standards as $[\eta] = K_s M_s^{\alpha}$ and p

denotes that for pullulan as $[\eta] = K_p M_p^{\beta}$. Plotting log M_p against [log K_s + (1 + α) log M_s], we can estimate $K_p = 1.62 \cdot 10^{-4}$ and $\beta = 0.68$ from the values of the slope and intercept of eqn. 9. These values are in accord with eqn. 4. As discussed above, it is clear that the molecular characteristics measured in this study are accurate and reliable. Therefore, the experimental data for PEO are easily convertible into those for pullulan based on the validity of the universal calibration procedure, and *vice versa*.

In conclusion, either PEO or pullulan is suitable as a polymer standard in aqueous gel chromatography. The added-salt concentration dependences of the molecular size in aqueous solution for both polymers were negligibly small and there was no tendency for polymer adsorption on the packed gel. PEO in aqueous solution was less stable than pullulan, while the addition of sodium azide (0.02%, w/w) to aqueous solvent was recommended in order to prevent the bacterial degradation of pullulan[17]. Taking into account the wide retention volume range in Fig. 4, the wide M_w range of linearity of the plots in Fig. 5 and the very narrow molecular weight distribution obtained by the "living anion" technique, we may conclude that PEO standards are the best. Nevertheless, pullulan is an important polymer standard based on the analogy of its chemical structure and properties with other linear polysaccharides. The dextran standards may be used as standards for branched polymers.

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