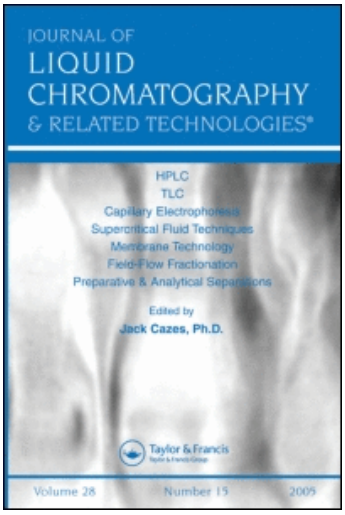

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Size Exclusion Chromatography of Poly(vinylpyrrolidone): I. The Chromatographic Method

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SIZE EXCLUSION CHROMATOGRAPHY OF POLY (VINYLPYRROLIDONE): I. THE CHROMATOGRAPHIC METHOD

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ABSTRACT

The optimum size exclusion chromatographic (SEC) method for poly-(vinylpyrrolidone) (PVP) was found to be based upon a stationary phase of diol derivatized silica of pore sizes 3000, 500, and 75Å and a mobile phase of 50:50 (v/v) MeOH/H_2O containing 0.1M-$LiNO_3$. Sample recovery under identical conditions varied for the commercial packings investigated and was found to be inversely related to molecular weight. The latter phenomenon was rationalized on the basis of a limited number of active substrate sites available for binding. Methanol was found to be a more effective mobile phase modifier than either dimethyl formamide or acetonitrile apparently due to its ability to function as a proton donor in hydrogen bonding with PVP. Chromatographic evidence for the existence of semipolyampholyte character in PVP is presented. A procedure for the construction of a column set log-linear in calibration and of extended dynamic range is described and is based upon hydrodynamic volume theory.

INTRODUCTION

Traditionally, the molecular weight of PVP has been characterized indirectly by means of the Fikentscher K-value, K,

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(1,3) which is defined in terms of the relative viscosity, η_{rel}, and the concentration c (in g/dl), of a given solution:

$$\frac{\log \eta_{rel}}{C} = \frac{75K_o^2}{1 + 1.5\ K_o C} + K_o$$

$$\text{and } K = 1000\ K_o$$

At present, PVP is produced in four basic K-value grades in the United States; namely, 15, 30, 60, and 90 which correspond to nominal viscosity-average molecular weights of 7,700, 38,000, 216,000, and 630,000 amu, respectively. These amounts are based upon a Mark-Houwink coefficient of 1.4×10^{-4} dl/g and an exponent of 0.7 for water at 25°C as described by Scholtan and coworkers (4,5).

Knowledge of the absolute molecular weight distribution of a polymeric material allows one to predict end-use properties which are not dependent upon and thus cannot be predicted by viscosity parameters such as K-value. The production of a molecular weight distribution by gel permeation chromatography (generally known as size exclusion chromatography) is inherently a two-part process: the development of a suitable chromatographic method and the establishment of an absolute means of calibration. The present paper will concern itself with the former aspect only for the case of PVP.

Chromatographic supports of crosslinked agarose (6) and dextran gels (7,9) have both been reported to successfully elute poly (vinylpyrrolidone). As a result of the poor mechanical strength of these gels, they cannot tolerate the normal flow rates (1-2 ml/min.) desirable for high performance liquid chromatography and, consequently, run times are long. They are also prone to irreversible swelling and deswelling phenomena which are tantamount to loss of resolution and efficiency. To overcome the difficulties associated with soft gels researches have resorted to the use of semi-rigid gels such as crosslinked poly(styrene/ divinyl benzene) in conjunction with a mobile phase

of N,N-dimethyl formamide (DMF) (10,11) or N-methyl pyrrolidone (NMP) (12) with or without the addition of 0.1M - LiBr.

This method has been found to be impractical by this laboratory from two standpoints. First, column efficiencies were observed to drop by more than 75% over several months of continuous use. This was attributed to irreversible swelling in either DMF or NMP as has been cited by one manufacturer of such gels, Waters Associates (Milford, MA). Second, as differential refractometry is the detection system most commonly employed and as PVP is particularly hygroscopic, a large negatively-oriented peak at the total permeation volume (V_t) is observed due to water. Because PVP is so similar in structure and thus in refractive index to either NMP or DMF, the residual water has a much higher response factor than the polymer in these solvents. These large water peaks have been noted to obliterate the low molecular weight tails of low K-value grades of PVP molecular weight distributions making quantitation extremely difficult.

Rigid packings based upon porous silica are not subject to the irreversible swelling/deswelling phenomena exhibited by both the soft and semi-rigid gels. A method for PVP involving a hydrophobically modified silica was investigated by this laboratory. This material (produced by E. I. DuPont de Nemours & Co., Inc., and consisting of porous silica deactivated by chlorotrimethylsilane) was utilized with a mobile phase of DMF containing 0.1M - LiBr. While this method was found to provide reproducible chromatograms, high resolution, and high sample recovery, it also suffered from the interference of residual water peaks. In addition, it did not offer resolution of material greater than 1.5 million amu due to the inadequacy of the largest pore size used, 1000 Å.

Recently, several groups of workers have reported on a variety of SEC methods applicable to PVP based upon an aqueous mobile phase. A novel hydrophilic semi-rigid polymeric gel containing the group $\{CH_2CHOHCH_2O\}$ was introduced under the name TSK-GEL type-PW (Toya Soda Co., Japan). The fractionation of PVP

using an aqueous mobile phase in conjunction with this packing material has been reported (13). While the water peak was eliminated here, column efficiency appeared to decline markedly over several months of use (14).

Engelhardt and Mathes described a bonded (protic) amide stationary phase and a mobile phase of 0.1M - TrisHCl buffer (pH = 8.0) with 10% $^{v}/_{v}$) ethylene glycol adjusted to an ionic strength of 0.5 with Li_2 SO_4(15). The purpose of the ethylene glycol was to eliminate partition due to a hydrophobic interaction between the stationary phase and PVP. This packing material is not commercially available. An interesting material which was found to be incapable of eluting PVP was PVP-coated silica (16). While the mobile phase was not specified, the adsorption of PVP was said to be a result of the dipolar interaction between the two PVP phases. As PVP is an aprotic amide its failure as a stationary phase is at odds with the work of Engelhardt and Mathes.

The primary purpose of this work was to describe a successful method for the aqueous size exclusion chromatography of PVP based upon a diol-bonded silica packing (also referred to as possessing glyceryl, glycol or carbohydrate functionality). This work was produced independently with respect to that of Herman, Field and Abbott who report a similar separation (17). The two methods are contrasted in the body of this report.

EXPERIMENTAL

The chromatograph employed in this study was a Waters Model 150C GPC operated at ambient temperature. Data collection was achieved with a Perkin-Elmer Sigma 10 data station. SEC stationary phases investigated included commercial diol columns (250 x 4.6 mm ID) obtained from E. Merck (LiChrospher-DIOL) and Synchrom, Inc. (Syn Chropak GPC) as well as bulk packing from Electro-Nucleonics, Inc., (Glyceryl-CPG). The former materials are based upon nominal 10μm diameter spherical silica while

the latter consists of crushed irregular glass particles in the 37-74μm range. The Glyceryl-CPG material was dry-packed into Waters 300 x 7.8 mm ID S.S. columns by the "tap-fill" method of Snyder and Kirkland (18).

The poly(vinylpyrrolidone) samples studied represent typical batches of the four major grades produced by GAF Corporation. The narrow polystyrene calibration standards were obtained from Pressure Chemical Co. The methanol, water, tetrahydrofuran, and acetonitrile used were distilled-in-glass solvents obtained from Burdick & Jackson, Inc. LiBr, $LiNO_3$, and KH_2PO_4 were reagent grade materials obtained from J.T. Baker Co. The Glyceryl-CPG column bank was operated at a flow rate of 2.0 ml/min. which corresponds to a nominal analysis time of 30 minutes. Unless indicated otherwise in the figure captions, the injections consisted of 160μl volumes of 0.25% ($^{w}/v$) solutions for all molecular weight grades. (K-90 grade PVP was studied at a 0.1% $^{w}/v$ concentration as well. No peak shift ascribable to viscous fingering was detected.) The development of the mobile phase composition is presented in the Results/Discussion section.

RESULTS AND DISCUSSION

A. Glyceryl-CPG Column Bank Design

Apart from increasing resolution and minimizing adsorptive effects, there are two main considerations in constructing a well-designed SEC column set. These are the maximization of dynamic range (selective permeation) to include all molecular weight components of interest and the optimization of the shape of the calibration curve to be log-linear in character over a broad range with respect to the dependence of molecular weight upon elution volume (V_e). The latter allows the abscissa of a molecular weight distribution plot to be divided into equal increments corresponding to decades of molecular weight and facilitates the use of polydisperse (broad) standard calibration. A non-log-linear column bank can cause a symmetric, Gaussian peak shape to

appear skewed and resolution to be a function of elution volume in the selective permeation range.

In this study the column bank was required to possess a selective permeation range extending from several hundred to ten million amu (or five decades). It has been demonstrated that a column set constructed from only two pore sizes can span a linear dynamic range of four but not five decades and that the pore volumes of each size should be equal for highest linearity (19). The latter stems from the dependence of calibration slope upon pore volume in the range covered by a particular pore size. Therefore to span five decades, at least three pore sizes are required.

The Glyceryl-CPG material pore sizes chosen were nominally 75, 500, and 3000 Å. The 75Å material is the smallest commercially available pore size and is capable of resolving components in the several hundred amu range. The remaining sizes were chosen so that their individual selection permeation ranges barely overlapped. The spacing between the three pore sizes to insure maximum linearity was dictated by the following consideration.

The diameter of an individual pore can be thought of as equivalent to twice the Stoke's radius of the largest random coiled polymer molecule (Gaussian chain) which can penetrate it. (The SEC mechanism operates upon the molecular hydrodynamic volume.) According to the Flory-Fox equation the hydrodynamic volume, $[\eta] M$, of such a molecule can be expressed by

$$[\eta] M = \Phi \langle r^2 \rangle^{3/2}$$

where, $[\eta]$ = the intrinsic viscosity

Φ = a universal constant

$\langle r^2 \rangle$ = the mean square end-to-end distance (equal to six times the mean square radius of gyration)

Utilizing the Mark-Houwink equation which empirically relates $[\eta]$ to M by

$$[\eta] = K'M^{a}$$

where K' and a are the Mark-Houwink coefficient and exponent respectively, one may substitute for $[\eta]$ in the Flory-Fox equation to yield

$$K'M^{1+a} = \Phi\langle r^2\rangle^{3/2}$$

Assuming that K', a, and Φ are essentially independent of molecular weight and taking the logarithm of both sides of this equation, the following corollary is obtained, viz..

$$\log M \propto \log r$$

or any other linear dimension of such a Gaussian chain.

Thus, the difference in the logarithms of the three pore sizes must be equal in order for the log M values corresponding to their respective exclusion limits to be equally spaced yielding a log-linear calibration curve. In this case,

$$\log 3000 - \log 500 = 0.778$$
$$\log 500 - \log 75 = 0.824$$

i.e., the differences are nearly equivalent, indicating proper selection.

Because the pore volume of the 75Å material was reported to be approximately half that of the 500 and 3000 Å materials by their manufacturer, the bed volume of the former was doubled. The Glyceryl-CPG column set consisted of two columns of the 75Å and one each of the 500Å and 3000Å materials. The calibration curve corresponding to this column set as defined by a narrow

polystyrene standards in a THF mobile phase is depicted in Figure 1. A correlation coefficient of 0.9981 was achieved, indicative of high linearity (n=13).

B. Mobile Phase Development

The first mobile phase attempted in conjunction with the Glyceryl-CPG column set consisted of 5% DMF ($^{V}/v$), 95% H_2O ($^{V}/v$) and 0.1 M -LiBr. The purpose of the salt was to counteract any residual ionic electrolyte character of the PVP as well as to screen any remaining anionic $-SiO^-$ sites on the support. DMF was included to interact with the remaining silanol sites via hydrogen bonding in order to eliminate PVP absorption. While commercial K-90, -60, -30, and -15 samples were found to elute under these conditions in correct SEC order, the K-90 and K-60 peak shapes were highly skewed to low molecular weight. This apparent adsorptive effect could not be overcome by either increasing the DMF concentration or by substitution of the DMF by NMP.

In order to overcome this effect, a different approach was taken. Methanol was substituted for the DMF. The former was expected to interact via hydrogen-bonding with the carbonyl groups of PVP leaving the unreactive methanol methyl group exposed to the support surface. What was not known a priori was what concentration of methanol was required for optimum efficiency in shifting the equilibrium between methanol and water to the left:

$$\backslash C = O \cdots HOCH_3 + HOH \quad \rightleftharpoons \quad \backslash C = O \cdots HOH + HOCH_3$$
/ /

A series of aqueous 0.1M-LiBr mobile phases were prepared containing 10, 25, 40, and 50% ($^{V}/v$) of methanol (MeOH) in water. (The pH of these mixtures was normally in the range of 6 and was not adjusted.) The 10% composition resulted in total retention of all PVP samples injected. The chromatography observed for the compositions 25, 40 and 50% ($^{V}/v$) MeOH is depicted in Figure 2 and the peak crest times are reported in

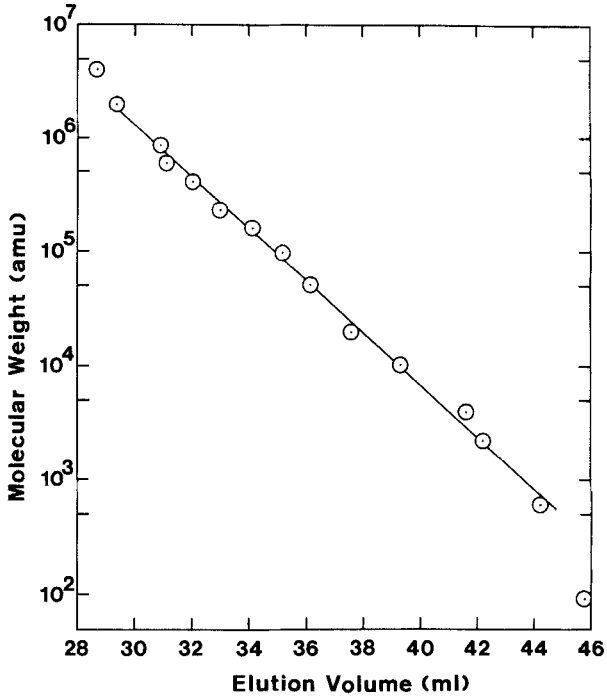

FIGURE 1. Calibration curve for the Glyceryl-CPG column set consisting of 2x75Å, 500Å, and 3000Å, (300 x 7.8mm) columns using narrow polystyrene standards in THF. Conditions: flow rate of 2.0 ml/min., injection volume of 80 µl, concentration of 0.1% W/v.

Table 1. The peak symmetry of the K-90 and -60 materials was found to improve as the methanol concentration was increased while the peak crest retention times monotonically decreased (although minimally from 40 to 50%). As adsorption appears to have been minimized at the 50% methanol level, this composition was subsequently maintained. At this point in the development of the method, $LiNO_3$ was substituted for LiBr because of the potential of corrosion of stainless steel tubing in the prolonged presence of bromide ions. Equimolar replacement was found to have no effect upon the observed chromatography as shown in Figure 3. The 2000 Å column utilized initially to perform the chromatography

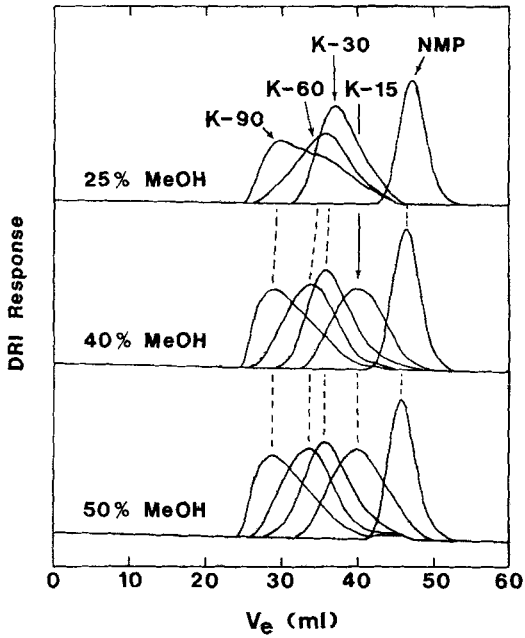

FIGURE 2. The effect of methanol concentration in the mobile phase of PVP peak shape and position. Conditions: MeOH/H_2O mobile phases containing 0.1 M-LiBr, column bank of Glyceryl-CPG 2x75Å, 500Å, and 2000Å (300 x 7.8mm), flow rate of 2.0 ml/min., injection volume of 80 µl, concentration of 0.25% $^{W}/_{V}$.

TABLE 1

Peak Crest Times (in min.) of Various Grades of PVP as Observed on a Glyceryl-CPG Column Bank

Sample	% Methanol ($^{V}/_{v}$) in Mobile Phase		
	25%	40%	50%
K-90	14.6	14.2	14.1
K-60	17.5	16.7	16.4
K-30	18.4	17.7	17.4
K-15	-	19.7	19.7
NMP	23.4	22.7	22.5

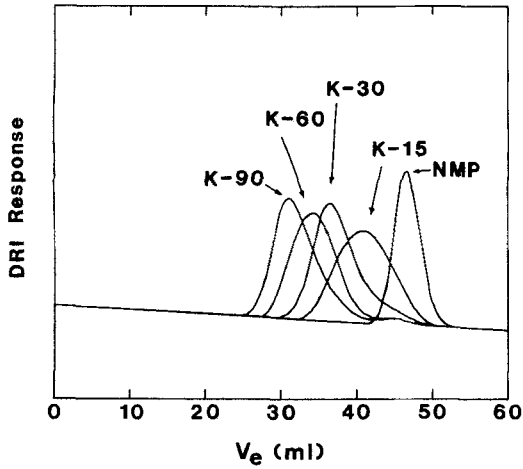

FIGURE 3. Size exclusion chromatography of PVP under the final chromatographic conditions: a mobile phase of 50:50 v/v MeOH/H_2O containing 0.1M-$LiNO_3$, a column bank of Glyceryl-CPG 2x75Å, 500Å, and 3000Å (300 x 7.8mm), flow rate of 2.0 ml/min., injection volume of 160 µl, concentration of 0.25% w/v.

depicted in Figure 2 was replaced by a 3000Å column in Figure 3 as per part A of this Discussion.

Column efficiency was measured using an analogous totally permeating species, NMP, and performing the test at normal operating conditions (i.e., 2.0 ml/min flow rate, all four columns, 50% MeOH/50% H_2O containing 0.1M-$LiNO_3$, ambient temperature). Using the 5σ method (peak width measured at 4.4% of peak height from baseline) the column bank efficiency was found to be 720 plates. This result is not surprising considering the particle size and particle irregularity of the packing material.

C. Comparison to Other Systems

Herman, Field and Abbott have demonstrated the use of a mobile phase consisting of 40% CH_3CN / 60% H_2O in 0.01 M-KH_2PO_4 adjusted to pH = 2.5 in conjunction with a 100Å diol column to successfully perform SEC on a K-15 grade PVP(17). Sample recovery was reported to be 100%. The function of the

acidity (low pH) was to suppress the ionization of PVP which was said to be an acidic polyelectrolyte. Our laboratory has to date found no viscometric evidence to support the existence of polyelectrolyte character in unmodified PVP as would be revealed by the nonlinear dependence of inherent or reduced viscosity upon concentration in water (3). A minimal electrophoretic mobility has been observed for PVP and ascribed to carboxyl end groups (20).

The effect of removing salt from a 50:50 $^{v}/_{v}$ MeOH/H_2O mobile phase was investigated for K-90, K-30, K-60, K-15, and NMP on Glyceryl-CPG columns as depicted in Figure 4. These chromatograms should be compared to the 50% MeOH chromatograms of Figure 2. The distribution of the K-90 material exhibits a distinct shoulder whereas the K-60, K-30, and K-15 grade samples possess bimodal distributions: one peak at the total exclusion volume and one corresponding to the expected SEC peak. This phenomenon is consistent with a polyelectrolyte effect despite the lack of supporting viscometric evidence. However, PVP has been reported to be a semi-polyampholyte on the basis of conductometric titration (21,22) and ^{13}C-NMR data (23). A hydrolysis/ring-opening reaction results in a Zwitterionic (amino acid) form whose positive charges on the nitrogens are screened by concomitant negatively charged carboxyl groups which are furthest from the backbone. Mutual repulsion between such negative charges and with respect to residual $-SiO^-$ groups on the silica substrate would result in increased hydrodynamic volume in the absence of free cationic counterions and is consistent with a portion of the PVP eluting at the total exclusion volume. The bimodal nature of these chromatograms is not completely understood, however, and is under further investigation. The frequency of the Zwitterionic form has been reported to increase dramatically at both high and low pH values (21).

Thus, the adjustment of the mobile phase to low pH is unwarranted and can be as detrimental to the stability of the

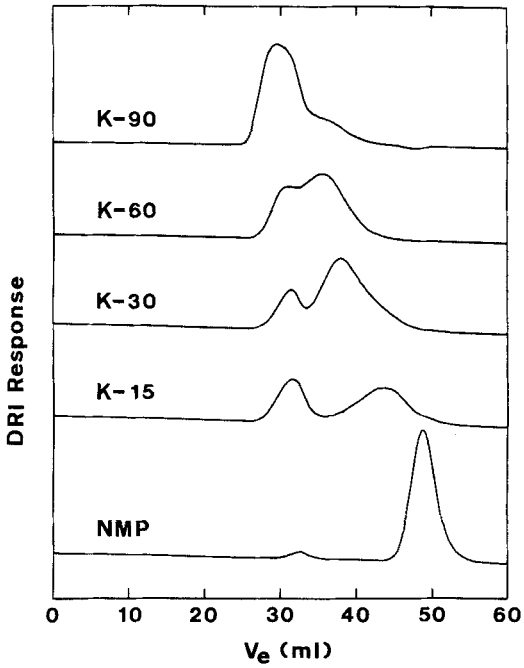

FIGURE 4. The effect of the removal of salt from the mobile phase upon PVP peak shape and position. Conditions: a mobile phase of 50:50 $^{v}/_{v}$ MeOH/H_2O, column bank of Glyceryl-CPG 2x75Å, 500Å, and 3000Å (300 x 7.8 mm), flow rate of 2.0 ml/min., injection volume of 160 µl, concentration of 0.25% $^{w}/_{v}$.

bonded phase as high pH is to the silica substrate (24). Residual ionic effects exhibited by either the polymer or chromatographic support are better counteracted by the addition of an adequate quantity of a neutral salt. The acetonitrile incorporated into the mobile phase of Herman et. al. can participate in hydrogen-bonding although, unlike methanol, strictly as a proton acceptor.

To assess the relative efficacy of the two mobile phases, a direct comparison of sample recovery was performed using the same diol column bank, that of the Glyceryl-CPG. Peak areas of 160µl injections made on these columns were compared to 10µl injections made on a flow restrictor coil (in the absence of the columns)

with compensation for differences in injection volume, chart speed, flow rate, and detector sensitivity. The detector response for the case of the flow restrictor alone is taken to represent 100% recovery. The results of this study are summarized in Table 2. In both cases a generally monotonic increase is observed for % recovery as molecular weight is decreased. At every molecular weight studied, the methanol based mobile phase resulted in a substantially higher recovery than that based upon acetonitrile. This is particularly evident for the K-90 grade material. The greater efficacy of the former mobile phase modifier is evidence for the role of proton donation in overcoming adsorption in this system.

The inverse dependence of percent recovery upon molecular weight is not surprising. An individual mer segment on a low molecular weight PVP molecule has an equal probability of interacting with an active substrate site as does a mer segment on a high molecular weight molecule. However, once such an interaction occurs, the mass of PVP retained is greater for the high molecular weight case. (This argument presumes a limited number of active substrate sites. In the event that the number of sites is very high, as in unmodified silica, all PVP molecules are retained eliminating the molecular weight dependence.) This molecular weight dependence is contrary to what would be expected from end-group effects alone. The high recovery of K-15 material in the study of Herman et. al. is consistent with the selection of a low molecular weight test sample and a lesser amount of packing material than in the present study.

An effort was made to investigate the characteristics of commercially available 10μm particle size diol columns such as SynChropak-GPC and LiChrospher-DIOL in order to improve efficiency while retaining the good features of the Glyceryl-CPG columns: log-linear calibration and high sample recovery. A 250 x 4.6 mm ID column containing 500 Å pore size SynChrom material was compared chromatographically to a similar column of the LiChrospher material. These comparative chromatograms appear in

TABLE 2

% PVP Sample Recovery as Affected by Mobile Phase Composition

Sample	Mobile Phase of Present Study[1]	Mobile Phase of Herman et al[2]
K-90	87.1	37.3
K-60	93.3	60.7
K-30	92.8	59.7
K-15	98.1	75.1
NMP	100	62.2

(1) 50% MeOH/50% H_2O (v/v) containing 0.1M-$LiNO_3$ (pH 6).

(2) 40% CH_3CN/60% H_2O (v/v) containing 0.01M-KH_2PO_4, pH = 2.1.

Figure 5. While recovery of K-30 and K-15 materials appeared to be adequate and comparable for the two columns, K-90 eluted well from the LiChrospher column but was almost completely retained by the Synchrom column. This result indicates that the diol derivatization of the Glyceryl-CPG and LiChrospher materials is more complete than that of the SynChrom material. Sample recovery of K-90, -60, -30, -15 and NMP was determined to be 100% for a LiChrospher-DIOL column bank consisting of one 100 Å, one 500 Å, and one 4000 Å column. The efficiency of this column bank was determined to be 2060 theoretical plates by the 5σ method at a 0.8 ml/min flow rate.

The 100 Å packing represents the smallest, commercially available pore size LiChrospher-DIOL. According to the pore size selection scheme developed in part A of this section, the correct

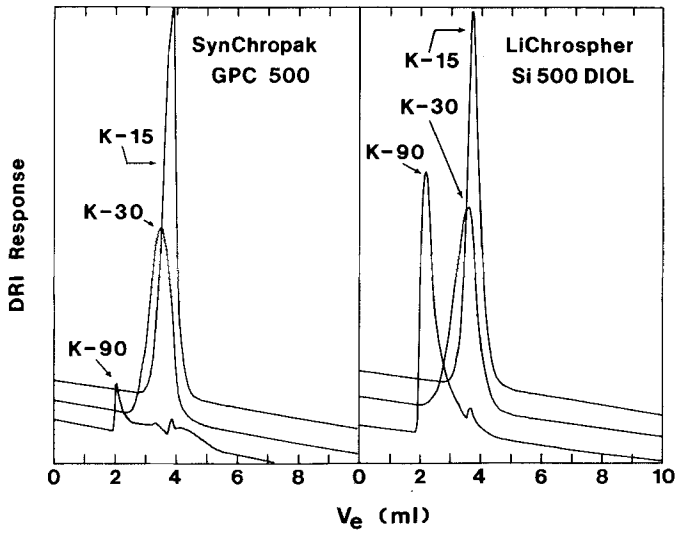

FIGURE 5. Comparison of two commercially-available 10 µm particle size 500Å (250 x 4.6mm) diol columns with regard to PVP recovery. Conditions: a mobile phase of 40:60 $^{v}/_{v}$ MeOH/H_2O containing 0.1M-$LiNO_3$, flow rate of 1.0 ml/min., injection volume of 20 µl, concentration of 0.25% $^{w}/_{v}$.

pore size to mate with 4000 Å and 500 Å pore sizes would be 60 Å (assuming equal pore volumes.) A calibration curve for the LiChrospher column set used was constructed from narrow polystyrene standards in a THF mobile phase and is depicted in Figure 6. The non-linearity of this calibration curve (it appears to be comprised of three distinct linear segments of different slope) is indicative of both poorly matched pore volumes and the absence of a sufficiently small pore size. That a 60 Å size would be a clear improvement is seen by the lack of selective permeation below 500 amu.

Chromatograms of all four grades of PVP as well as NMP obtained for the LiChrospher column bank recovery study are presented in Figure 7. The highly skewed appearance and sharp high molecular weight cutoff of the K-60 and K-90 peaks appeared unusual in light of the peak shapes observed for these materials

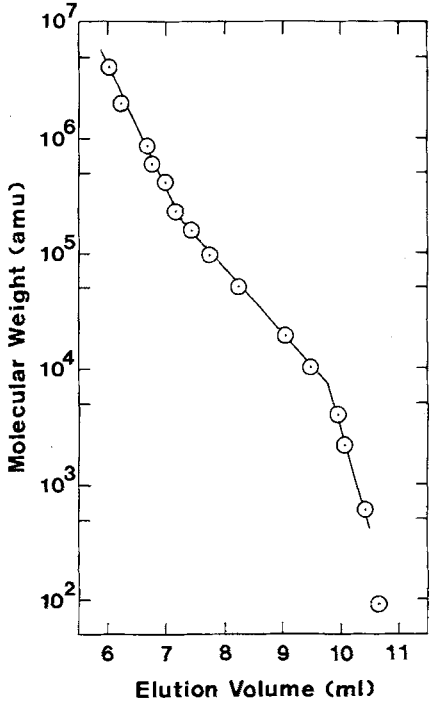

FIGURE 6. Calibration curve for the LiChrospher DIOL column set consisting of 100Å, 500Å, and 4000Å, (250 x 4.6mm) columns using narrow polystyrene standards in THF. Conditions: flow rate of 1.0 ml/min., injection volume of 20 µl, concentration of 0.1% w/v.

in conjunction with the Glyceryl-CPG column. In addition, a 4000 Å column would be expected to show good selectivity for the high molecular weight tail of PVP K-90 whose weight average molecular weight has been reported to be in the range of 1.5 million amu (25). According to the polystyrene calibration curve given in Figure 6, total exclusion appears to occur prior to 4.1 million amu (6.03 ml). The peak crest retention volume of 7.1 ml (and leading edge retention volume of 6.4 ml) for the K-90 peak representing 20µl of a 0.25% solution was significantly later than the estimated lower limit of the total exclusion volume. For a 200µl injection volume of a 0.15% solution of the same material

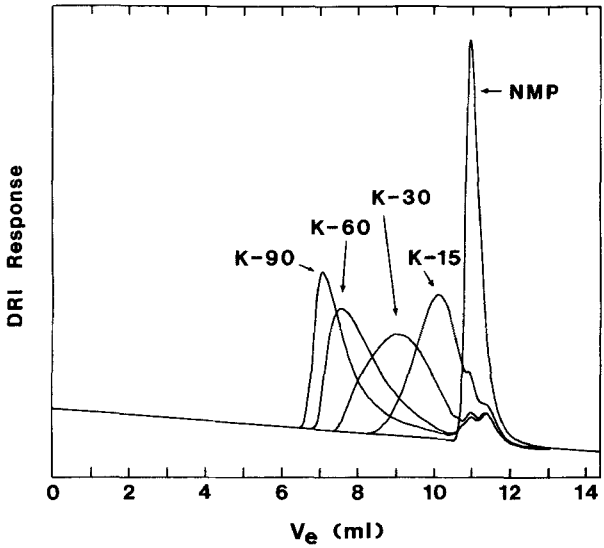

FIGURE 7. Size exclusion chromatography of PVP using the LiChrospher column bank of 100Å, and 500Å, and 4000Å (250 x 4.6mm). Conditions: a mobile phase of 50:50 $^{V}/_{V}$ MeOH/H_2O containing 0.1M-$LiNO_3$, flow rate of 0.8 ml/min., injection volume of 20 µl, concentration of 0.25% $^{W}/_{V}$.

the peak crest retention volume of 7.6 ml (and leading edge retention volume of 7.0 ml) reflects even further retardation and a dependence upon concentration. These facts imply an undesirable mixed mode separation of SEC and partition or adsorption of PVP for the LiChrospher material.

By comparison the polystyrene calibration curve shown in Figure 1 for the Glyceryl-CPG column bank indicates that total exclusion is not expected before 28.7 ml (corresponding to the 4.1 million amu standard). The K-90 peak crest retention volume was 31.2 ml but the leading edge retention volume was 25.1 ml or less than the estimated total exclusion volume. This result confirms the presence of a very high molecular weight component in K-90 grade PVP (and indicates axial dispersion in this column set) which is consistent with a predominantly SEC mechanism.

CONCLUSIONS

The optimum size exclusion chromatographic method for poly(vinylpyrrolidone) was found to be one based upon a stationary phase of diol derivatized silica and a mobile phase of 50:50 (v/v) MeOH/H_2O with 0.1M-$LiNO_3$. Sample recovery under identical conditions varied for the commercial packings investigated but was found to be acceptable for two. This variation underscores the requirement of complete surface deactivation of the silica substrate. The percent recovery was found to be inversely related to molecular weight. This phenomenon was rationalized on the basis of a limited number of active substrate sites available for binding. Methanol was found to be a more effective mobile phase modifier than either DMF or acetonitrile which appears due to its ability to act as a proton donor in hydrogen bonding with PVP. Chromatographic evidence for the existence of semipolyampholyte character in PVP has been reported. A procedure for the construction of a log-linear (in calibration) column set of extended dynamic range has been described and is based upon hydrodynamic volume theory.

The primary drawback to the method described lies in the relatively low column efficiency exhibited by 37-74 m packing material. Unfortunately, the commercially available, prepacked 10μm (high efficiency) column materials were found to be inferior to the above with regard to PVP recovery, mixed-mode separation character, mismatched pore volumes and lack of availability of a crucial pore size (60 Å).

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REFERENCES

1. H. Fikentscher, Cellulose - Chem., 13, 58 (1932).

2. G. M. Kline, Mod. Plast., 22, 157 (1945).

3. E. G. Malawer, J. K. DeVasto, and S. P. Frankoski, Pharmacopeial Forum, 9 (1), 2651 (1983).

4. W. Scholtan, Makromol. Chem., 7, 209 (1951).

5. J. Hengstenberg and E. Schuch, Makromol. Chem., 7, 236 (1951).

6. G. E. Fleig and F. Rodriguez, Chem. Eng. Commun., 13, 219 (1982).

7. H. Nogami, T. Nagai, and A. Kondo, Chem. Pharm. Bull., 18, (11), 2290 (1982).

8. B. G. Belenkii, L. Z. Vilenchik, V. V. Nesterov, V. J. Kolegov, and S. Ya. Frenkel, J. Chromatogr., 109, 233 (1975).

9. Yu, E. Kirsh, T. M. Karaputadze, V. I. Svergun, T. A. Sus', O. I. Sutkevich, I. I. Iverdokhlebova, and V. P. Panov, Khim. - Farm. Zh., 14 (7), 107 (1980).

10. F. Comelli, R. Francesconi, and E. Lanzi, Polym. Eng. & Sci., 20 (5), 370 (1980).

11. M. Ezrin, private communication (Springborn Laboratories, Inc., Enfield, CT).

12. H. N. Cheng, T. E. Smith, D. M. Chiou, and E. Malawer, unpublished data. (A GAF method involved the use of NMP at 80°C in conjunction with a bank of Waters μ-Styragel columns.)

13. T. Hashimoto, H. Sasaki, M. Aiura, and Y. Kato, J. Polym. Sci., Polym. Phys. Ed., 16, 1789 (1978).

14. P. L. Dubin, Polym. Prepr., 22, (1), 132 (1981).

15. H. Engelhardt and D. Mathes, J. Chromatogr., 185, 305 (1979).

16. L. Letot, J. Lesec, and C. Quivoron, J. Liq. Chromatogr., 4, (8), 1311 (1981).

17. D. P. Herman, L. R. Field, and S. Abbott, J. Chromatogr. Sci., 19, 470 (1981).

18. L. R. Snyder and J. J. Kirkland, "Introduction to Modern Liquid Chromatography," 2nd ed., (1979), Wiley-Interscience, p. 206-207.

19. W. W. Yau, J. J. Kirkland, and D. D. Bly, "Modern Size-Exclusion Liquid Chromatography," (1979), Wiley-Interscience, p. 265 ff.

20. L. May, M. Hines, L. Weintraub, J. Scudder, and S. Graff, Surg., 35, 191 (1954).

21. H. P. Frank, J. Polym. Sci., 12, 565 (1954).

22. A. Conix and G. Smets, J. Polym. Sci., 15, 221 (1955).

23. H. N. Cheng, D. M. Vitus, and T. E. Smith, unpublished data (GAF Corporation).

24. N. H. C. Cooke and K. Olsen, Am. Lab., 11, (8), 45 (1979).

25. BASF product bulletin B382e, "Kollidon Grades: Polyvinylpyrrolidone for the Pharmaceutical Industry," (May, 1982).