

CHROM. 19 193

CHARACTERIZATION OF POLYMERIC AMINOPROPYL DERIVATIZED SILICA SUPPORT FOR THE SIZE-EXCLUSION CHROMATOGRAPHY OF CATIONIC POLYMERS

ELIZABETH V. PATTON

Analytical Technology Division, Eastman Kodak Company, Rochester, NY 14650 (U.S.A.)
and

DAVID M. WONNACOTT*

Research Laboratories, Life Sciences Division, Eastman Kodak Company, Rochester, NY 14650 (U.S.A.)
(First received July 16th, 1986; revised manuscript received October 22nd, 1986)

SUMMARY

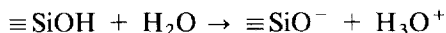
An experimental analysis of a size-exclusion chromatography support material was designed to define the chemical characteristics of the pore surface. This support material consisted of silica which had been derivatized with aminopropyltriethoxysilane and subsequently cross-linked with butadiene diepoxide. The characteristics of this material include a complex combination of acidic sites arising from the presence of underivatized silanols, basic amine sites on the polymeric derivatized phase, and hydrophobic characteristics associated with the polymer carbon chains. An understanding of the interactive properties of the stationary phase allowed the design of mobile phases to minimize these interactions for specific polymers.

INTRODUCTION

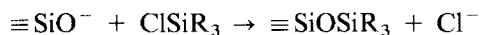
High-performance size-exclusion chromatography (SEC) of organic polymers, also referred to as gel-permeation chromatography, is a well understood, fairly routine procedure in which rigid styrene-divinylbenzene gels, or microparticulate silica that has been derived with a neutral organic phase, provides a porous, non-interactive packing material. The elution of a polymer is controlled by its size, and non-size separation effects are small. In contrast, the exclusion chromatography of water-soluble polymers is still somewhat of an art in which non-size separation effects are often not fully eliminated^{1,2}. Water-soluble polymers encompass a wide range of characteristics; neutral, polyanions, polycations, copolymers containing hydrophobic and hydrophilic moieties, and more complex polymers such as proteins which are composed of cationic, anionic, and hydrophobic groups.

This paper describes a support material which has been prepared for the SEC of cationic polymers³. The chromatography of such polymers is an especially difficult problem. There are several mechanisms for non-steric interaction between cationic polyelectrolytes and SEC porous packing materials.

(1) *Electrostatic adsorption.* Bare, underivatized silica in aqueous solution carries a negative charge due to the dissociation of surface silanol groups.



Colloidal silica has been found to have a measurable anionic charge density down to a pH of 4 (ref. 4). Polycations and polybases will adsorb to these sites electrostatically. Since there are multiple cationic sites on the polymer, the adsorption is cooperative and may be irreversible. The silica surface may be derivatized, *e.g.*,



to eliminate these sites. Because of steric crowding, however, derivatization is usually incomplete. The effect of this anionic charge may be lessened by the addition of electrolytes to the eluent; these compete for the anionic sites and reduce the Debye interaction distance. It has been suggested that quaternized ammonium salts may act as effective mobile phase modifiers, reversibly derivatizing the surface silanols, and allow the chromatography of cationic polyelectrolytes on underivatized silica⁵; this has not been demonstrated experimentally.

(2) *Electrostatic exclusion.* If the surface of the porous packing material carries a charge, ions of similar charge will be partially or wholly excluded from the pore volume. For example, the negative charges present on bare silica in aqueous solution, derivatized silica with residual underivatized silanol groups, and some hydrophilic polymeric gels will cause early elution of salts and anionic polyelectrolytes⁶⁻¹¹. These effects are eliminated by the use of ionic strengths greater than 0.01 *M*⁸.

(3) *Hydrophobic partitioning.* If the packing material used for aqueous SEC is partially organic in nature, either because the column is made up of an organic gel or of silica which has been derivatized, polymers may be retained through hydrophobic partitioning. This process may be quite weak, in which case the polymers may still elute from the column in a size separated mode. Such delayed elution has been described by Dawkins and Hemming¹² with the relationship

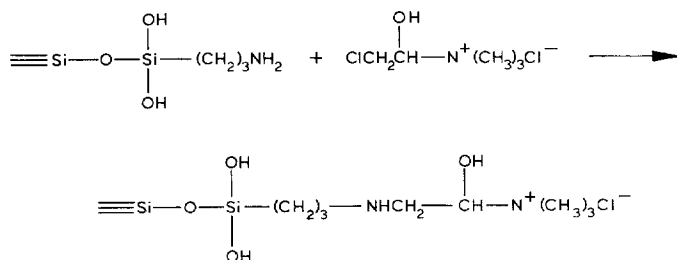
$$V_e = V_0 + K_p K_{\text{SEC}} V_i \quad (1)$$

in which V_0 is the column void volume, V_i the pore volume, K_{SEC} the coefficient describing the size separation, and K_p is a coefficient describing the distribution of polymer between the stationary and mobile phases¹². Pfannkoch *et al.*² has demonstrated that derivatized phases which have been designed to be hydrophilic may have measurable hydrophobic character. The high ionic strengths of mobile phases that are designed to minimize the electrostatic effects discussed above will increase the hydrophobic retention of polymers. The amount of interaction depends on the polarity of the polymer and that of the derivatized phases.

Currently available SEC support materials

Recently, several commercial materials have been developed for the SEC of aqueous polymers. Only a few have been designed specifically for use with cationic polymers. Talley and Bowman¹³ derivatized controlled porosity glass, a low-pressure

porous support that is similar to silica in surface characteristics, with 3-aminopropyltriethoxysilane which they subsequently quaternized.



The quaternary ammonium group provides a positively charged pore surface which prevents cationic polymers from approaching residual silanols. A set of characterized poly(2-vinylpyridine) (PVP) narrow molecular-weight-distribution standards were size separated on this column.

Synchro has developed a high-performance, silica-based support derivatized with a polymeric amine¹⁴. In low pH eluents, this also provides a cationic surface for the elution of cationic polymers. In buffers containing 0.1% trifluoroacetic acid and 0.2 *M* sodium chloride, PVP standards eluted according to size¹⁴. The separation was very dependent on ionic strength; the nature of this dependency was not investigated.

A high-performance, water-compatible gel, TSK-PW, manufactured by Toyo-Soda, has been designed for use primarily with water-soluble polyelectrolytes. This porous packing material has a proprietary structure believed to be a cross-linked, hydroxylated polyether gel¹⁵. The particle sizes range from 10 μm for the small pore-sized materials, to 25 μm for the largest. Because of the large particle size and dispersity present in a set of columns, the efficiency is lower than for porous silica microsphere-based columns. There are some electrostatic charges present, apparently because of residual groups on the gel matrix. It is suggested that ionic strengths greater than 0.3 *M* be used for the analysis of very polar polymers¹⁶. Size separation has been demonstrated on these materials for characterized polybases, polyethyleneimine and polyvinylamine, when these are chromatographed as the free bases¹⁷. Broad molecular-weight-distribution cationic polyelectrolytes also eluted from the columns with no apparent tailing.

The diversity of the types of non-ionic interactions which may potentially occur in aqueous chromatography suggests that the conditions for SEC of any polymer must be optimized for the characteristics of the specific polymer on the specific column. This paper describes the characterization of a high-pressure silica support derivatized to be used with polymers containing cationic moieties. The silica is reacted with aminopropyltriethoxysilane, which subsequently cross-linked with butadiene diepoxide. The derivatization procedure is described in detail in an accompanying article³. The results of this characterization were used to optimize the eluent conditions for the chromatography of a polymer which is cationic under the conditions used for SEC, poly[N(1,1-dimethyl-3-imidazolylpropyl)acrylamide] (PDIA).

EXPERIMENTAL

Materials

PVP standards were purchased from Pressure Chemicals (Pittsburgh, PA, U.S.A.). These are anionically polymerized, narrow molecular-weight-distribution polymers. Quaternary ammonium salts were from Kodak Laboratory Chemicals (Eastman-Kodak, Rochester, NY, U.S.A.), and were used without further purification. The polymer (PDIA) was synthesized by I. Ponticello, and was solvent fractionated by T. Handel, both from Research, Eastman Kodak^{18,19}. Weight-average molecular weights of the polymer were determined by light scattering in methanol containing 0.01 *M* tetrabutylammonium bromide. The solution properties of these fractions are described elsewhere^{18,19}.

Chromatography

Chromatographic equipment consisted of a Waters Assoc. Model 6000A chromatography pump, a Rheodyne Model 7125 injector and a Waters Assoc. Model R401 refractive index detector. Fabrication of the columns used for separation is described in the accompanying article³. All chromatograms were run at ambient temperature.

Viscometry

Intrinsic viscosities were determined with a Ubbelohde viscometer at 25°C.

Column analysis

The experimental analysis of the polymeric derivatized support was designed to define the chemical characteristics of the pore surface of the derivatized silica. These include a complex combination of acidic sites arising from the presence of underivatized silanols (which are always present because of the steric inability to achieve complete derivatization), the basic amine sites on the polymeric derivatized phase, and the hydrophobic characteristics associated with the polymer carbon chains. An understanding of the interactive properties of the stationary phase may allow the design of mobile phases to minimize these interactions for specific polymers.

RESULTS

Analysis for electrostatic adsorption

Anionically polymerized, narrow molecular-weight-distribution PVP standards were used to probe the interactive characteristics of a Zorbax PSM 1000 column which had been derivatized according to the procedure described in the accompanying article. These polymers are bases, which are partially charged under the eluent conditions employed in this work. The degree of protonation, α , was determined spectrophotometrically in one of the systems used, 0.10 *M* sodium formate adjusted to pH 3.50 with formic acid, by monitoring the absorbance of the polymer at 262 nm, at which there is an absorbance characteristic of the pyridinium ion²⁰. The absorptivity of the polymer in ethanol, A_{EtOH} , was assumed to be that of the neutral polymer; that in 2 *M* hydrochloric acid, A_{HCl} , was assumed to be that of the totally protonated form. The degree of protonation was calculated using the relationship

$$\alpha = \frac{A - A_{\text{HCl}}}{A_{\text{EtOH}} - A_{\text{HCl}}}$$

in which A is the absorptivity of the polymer in the eluent. Under the conditions described, α was found to be 0.45.

In order to investigate the possibility of electrostatic retention by small surface concentrations of underivatized silanols, several concentrations of polymer were injected at different ionic strengths and the eluted peak shapes were compared. The elution behavior of PVP with molecular weight 92 000 is illustrated in Fig. 1 for 100- μl injection volumes at concentrations of 0.1%, 0.01%, and 0.001% polymer. The peak sizes have been normalized for comparison. The peak shapes of the eluted polymers, when injected at 0.1% in the eluents containing 0.50 and 0.10 M buffer, reveal little difference in peak shape and neither of these peaks indicate that a significant amount of polymer adsorption is occurring. However, the elution curves of lower injected concentrations of polymer shows that there is some adsorption occurring at a small number of sites. This adsorption is more severe for the polymer in 0.50 M buffer; it results in less tailing in the 0.10 M buffer.

The permeation of the pores of this derivatized material must be considered to be that of a cationic polymer into a porous medium that carries a cationic charge covering an anionic pore wall. The distance of the approach of the polymer to the pore wall is controlled by the Debye length; the distance over which the charges interact. In 0.50 M buffer, the Debye length is short and the polycations are able to approach the pore surface closely enough to interact with any underivatized silanol sites. In 0.10 M buffer, the distance over which repulsion occurs between the polymer and the derivatized phase is greater and there is minimal adsorptive interaction occurring; less than 1% of the injected polymer is significantly retained. When the ionic strength of the buffer was further decreased to 0.01 M , all concentrations of PVP eluted from the column as sharp peaks. Also, the calibration curve representing the elution volumes of PVP's of different molecular weights moved to smaller volume and became very steep; the size-differentiating ability of the porous material became less. At this low ionic strength, the polymers were partially excluded from the porous volume of the column so that they were not as well size separated.

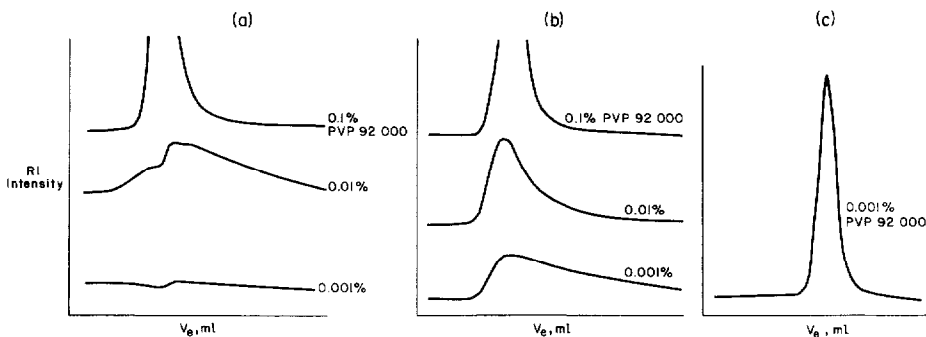


Fig. 1. Elution curves of PVP (mol. wt. = 92 000) injected at different concentrations in pH 3.5 sodium formate buffers of ionic strengths: (a) 0.50 M ; (b) 0.10 M ; (c) 0.010 M .

TABLE I
MARK-HOUWINK CONSTANTS FOR PVP IN CHROMATOGRAPHY SOLVENT SYSTEMS

	a	K
0.10 M sodium formate pH 3.50	0.93	$2.5 \cdot 10^{-5}$
0.50 M sodium formate pH 3.50	0.83	$5.0 \cdot 10^{-5}$
0.10 M sodium formate, 20% methanol, pH 3.55	0.95	$1.7 \cdot 10^{-5}$

SEC of PVP standards

The experiments needed to analyze the effect of solvent composition on non-steric interactions between the polymer and the packing material are complicated by the fact that intramolecular electrostatic effects cause the hydrodynamic volume of a polyelectrolyte to be heavily dependent on the eluent ionic strength. The elution volume of the polymer is, therefore, affected by this as well as adsorptive effects. In this work the effects of polymer expansion are accounted for by the use of universal calibration plots in which the hydrodynamic volume, V_h of the polymer is used as the variable, rather than the molecular weight. Grubisic *et al.*²¹ and others have demonstrated the validity of the universal calibration concept for polymers in organic solvents. Beyer and co-workers have extended this concept to aqueous systems²² and to mixed aqueous-methanol systems²³. Based on these findings, we have adopted the universal calibration concept for our column analysis. Shifts in plots of $\log V_h$ vs. elution volume for different polymers, or the same polymers in different eluents, are assumed to reflect changes in non-steric interaction parameters, rather than polymer hydrodynamic volume.

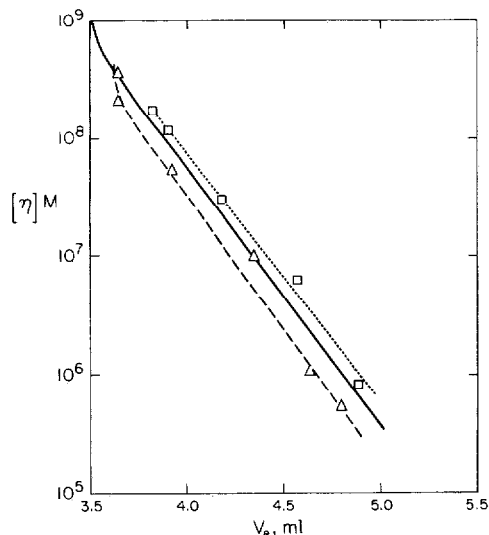


Fig. 2. Universal calibration plots of PVP in pH 3.50 sodium formate buffers as an eluent of two different ionic strengths, (---) 0.10 M , (····) 0.50 M , and of (—) polystyrene in THF on a 1000 Å pore size Zorbax column which has been derivatized with a polymeric amine phase.

Intrinsic viscosities of polymer standards

The intrinsic viscosities of each of the PVP standards were determined in each of the eluents in which they were used; the Mark-Houwink constant, a , for each system is listed in Table I. The high value of a indicates that the polymer is charged and highly solvated in each of the solvent systems.

Universal calibration plots

PVP standards were eluted from a column containing DuPont Zorbax PSM 1000 Å silica which had been derivatized with aminopropylsilane and reacted with butadienediepoxyde (APS-BD) as described in the accompanying article³. Aqueous buffers at two different ionic strengths were used. The universal calibration plots for these two elutions are included in Fig. 2 along with a similar plot for the elution of polystyrene in tetrahydrofuran (THF) on this column. It is assumed that the elution of polystyrene-THF is representative of a simple size separation. A buffer concentration of 0.50 M causes the elution of PVP to occur at somewhat greater volume than that of polystyrene-THF, indicating that some non-steric retention mechanism, is operative. A buffer concentration of 0.10 M causes the PVP elution to be at lower volume than that of the polystyrene; this is characteristic of an exclusion mechanism. Both of these effects are weak and cause only a shift of the size-separation curve, an effect similar to that described by eqn. 1.

The mechanism for retention of PVP at high ionic strengths (Fig. 1a) may be ascribed to a solvophobic interaction between the polymer and the derivatized phase. Although both materials are cationic, they contain some organic segments which could form transient hydrophobic domains. This type of interaction between eluting polymer and derivatized sites results in a simple shift of the calibration plot of the polymer to increased elution volumes. The slope of the plot is approximately the same, indicating that the cause of the shift is a weak partitioning of the polymer. The five-fold increase in ionic strength will increase the driving force toward the formation of hydrophobic domains, and is a consistent mechanism to explain this.

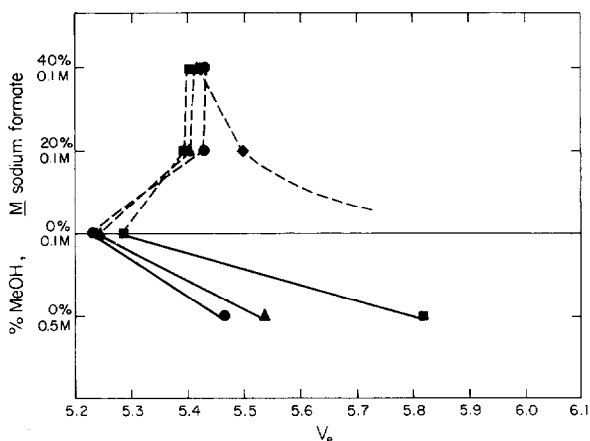


Fig. 3. Elution volumes of tetraalkylammonium salts in eluents of varying composition: 0.10 M sodium formate buffer, pH 3.50 containing 0, 20, and 40% methanol, and 0.50 M sodium formate buffer, pH 3.50. (●) Tetraethylammonium; (▲) tetrabutylammonium; (■) tetrahexylammonium; and (◆) hexadecyltrimethylammonium.

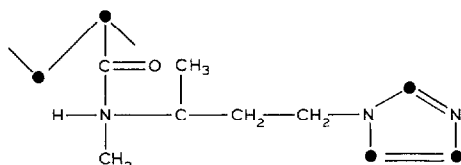
Elution of quaternary ammonium salts

The possibility of solvophobic partitioning between the derivatized sites and a molecule with both hydrophobic and cationic characteristics was further investigated by the elution of a series of quaternary ammonium salts in eluents whose makeup differed in ionic strength and solvent composition. These salts are cationic and hydrophobic in nature, as are the PVP standards. Fig. 3 is a plot of the elution volumes of each of these salts; tetraethyl, tetrabutyl, tetrahexyl, and hexadecyltrimethyl in buffers made up of 0.50 *M* and 0.10 *M* buffer containing 0, 20, and 40% methanol by volume. The possibility of micellization in the case of the hexadecyltrimethyl salt was avoided by using concentrations well below its critical micelle concentration.

It is obvious from this plot that several factors are operative in the retention of these salts. The elution behavior of the hexadecyltrimethylammonium cation, with a long hydrocarbon chain, is clearly dominated by hydrophobic effects. It does not elute from the column when no methanol is present; addition of 20% methanol solvates the ion well enough that it elutes as a symmetric peak in the approximate range of the total column volume, and addition of 40% methanol to the eluent causes it to elute at the same elution volume as the less hydrophobic cations. The elution of the symmetric cations is more complicated. In the high ionic strength eluent, the order of the elution volumes is dominated by hydrophobic interactions, with the ions eluting in order of chain length. This order persists at 0.10 *M*, but the elution volumes are more similar, and very short. The ions all have essentially the same elution volumes in 20 and 40% methanol. The retardation of the ions at 20 and 40% methanol compared to 0% is difficult to explain. It may be related to interactions between these small cations and the bare silanol sites to which they have more access. The addition of 20% methanol to 0.10 *M* buffer has no effect on the position of the universal calibration curve of PVP. This suggests that a combination of ionic strength and organic solvent can be optimized for the separation of organic polymers containing cationic groups, and for calibration with PVP.

Analysis of PDIA

A column set consisting of one column containing derivatized 1000 Å PSM and a column of the same size packed with derivatized 100 Å PSM was assembled. This procedure resulted in a bimodal set of columns which have two pore sizes that differ by a factor of 10 and have similar total pore volumes. Such a combination of columns has been recommended by Yau *et al.*²⁴ to establish a linear fit over a wide range of molecular weight. Calibration of this set with polystyrene in THF and with PVP in 0.10 *M* buffer both give calibration plots with a correlation coefficient to a linear fit of > 0.99. This set was used for the analysis of PDIA.



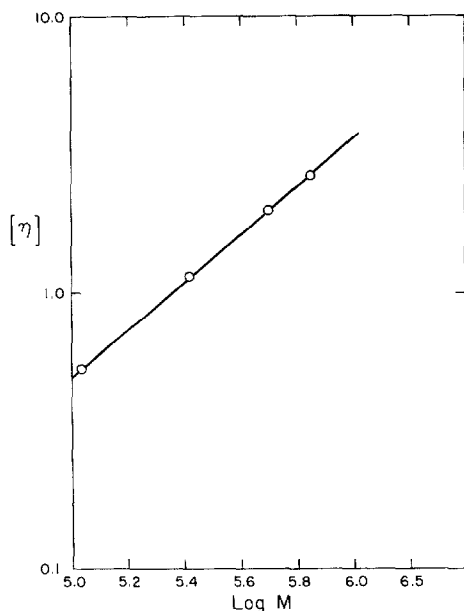


Fig. 4. Mark-Houwink plot of PDIA in 0.10 *M* sodium formate buffer, pH 3.55, containing 20% methanol as an eluent. $K = 2.98 \cdot 10^{-5}$, $a = 0.85$.

The preparation and fractionation of this polymer is discussed elsewhere^{18,19}. Seven fractions were separated; of these, fractions 1, 2, and 3 had too high a molecular weight to be completely size separated in the pore volume of this column set. The remaining four fractions were characterized by SEC using PVP as a calibrant, and the resulting weight-average molecular weights compared with those determined using light scattering in methanol and 0.01 *M* tetrabutylammonium bromide.

The eluent for SEC was 0.10 *M* sodium formate containing 20% methanol. The elution of PVP is not affected by the addition of methanol, universal calibration data points for each eluent composition lie on the same curve. PDIA must be assumed to have a considerably more hydrophobic character than PVP because of the greater number of carbon which make up the side chain. The results of the quaternized salt experiment indicate that this amount of methanol concentration minimizes hydrophobic partitioning on this derivatized phase.

TABLE II

LIGHT SCATTERING AND SEC MOLECULAR WEIGHTS OF PDIA

<i>Fraction</i>	<i>Mol. wt., light scattering</i>	<i>Mol. wt., SEC</i>
F4	703 000	723 000
F5	504 000	590 000
F6	265 000	292 000
F7	109 000	127 000

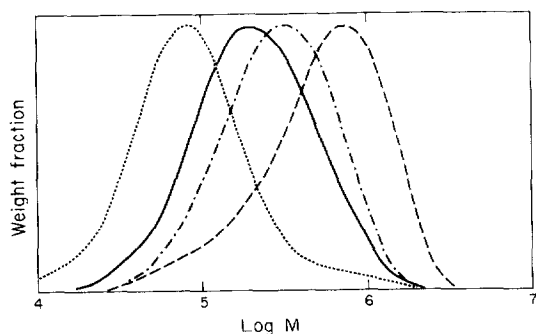


Fig. 5. Molecular weight distribution of PDIA based on universal calibration with PVP. PDIA fractions: (---) F4, (-·-·-) F5, (—) F6, (····) F7.

The Mark-Houwink coefficients for PDIA in this eluent were determined by measuring intrinsic viscosities in the eluent and plotting these against the molecular weights determined for the fractions with light scattering. The resulting plot and constants are contained in Fig. 4.

The SEC data were reduced with a computer program which has been adapted to do universal calibrations. Mark-Houwink coefficients, a , for both the calibrant and sample are entered, and the molecular weight for each chromatogram elution volume, M , is calculated using the relationship

$$\log M_2 = \frac{1}{1 + a_2} \log \frac{K_1}{K_2} + \frac{1 + a_1}{1 + a_2} \log M_1$$

The subscript 1 refers to the calibrant PVP and 2 refers to the polymer PDIA. Weight-average molecular weights were calculated from the value for each increment in the normal way. The values for these four samples are listed in Table II along with the results from light scattering. The results are within 20% of each other in every case. Fig. 5 contains a plot of the molecular weight distribution of the samples.

CONCLUSIONS

A method for the chromatographic molecular weight characterization of a cationic polymer which is also very hydrophobic in nature has been developed. A silica support material was derivatized with aminopropylsilane which was subsequently cross-linked with butadiene diepoxide. A solvent system which reduced the effects of hydrophobic and electrostatic interactions was found by monitoring the elution of model monomers.

REFERENCES

- 1 H. G. Barth, *J. Chromatogr. Sci.*, 18 (1980) 409.
- 2 E. Pfannkoch, K. C. Lu, F. E. Regnier and H. G. Barth, *J. Chromatogr. Sci.*, 18 (1980) 430.
- 3 D. Wonnacott and E. Patton, *J. Chromatogr.*, 389 (1987) 103.
- 4 G. H. Bolt, *J. Phys. Chem.*, 61 (1957) 1166.
- 5 F. A. Butenhuys and F. P. B. Van der Maeden, *J. Chromatogr. Sci.*, 149 (1978) 489.

- 6 P. A. Neddermeyer and L. B. Rogers, *Anal. Chem.*, 40 (1968) 755.
- 7 P. A. Neddermeyer and L. B. Rogers, *Anal. Chem.*, 41 (1968) 91.
- 8 A. Domard, M. Rinaudo and C. Rochas, *J. Polym. Sci.: Part A-2*, 17 (1979) 673.
- 9 C. Rochas, A. Domard and M. Rinaudo, *Eur. Polym. J.*, 16 (1980) 135.
- 10 M. Rinaudo, J. Desbrieres, *Eur. Polym. J.*, 16 (1980) 849.
- 11 M. Rinaudo, J. Desbrieres and C. Rochas, *J. Liq. Chromatogr.*, 4 (1981) 1297.
- 12 J. V. Dawkins and M. Hemming, *Makromol Chem.*, 176 (1975) 1795.
- 13 C. P. Talley and L. M. Bowman, *Anal. Chem.*, 51 (1979) 2239.
- 14 D. L. Gooding, M. N. Schmuck and K. M. Gooding, *J. Liq. Chromatogr.*, 5 (1982) 2259.
- 15 Y. Kato, K. Komiya, H. Sasaki and T. Hashimoto, *J. Chromatogr.*, 193 (1980) 311.
- 16 T. V. Alfredson, C. T. Wehr, L. Tallman and F. Klink, *J. Liq. Chromatogr.*, 5 (1982) 489.
- 17 P. L. Dubin and I. J. Levy, *J. Chromatogr.*, 235 (1982) 377.
- 18 T. M. Handel, I. S. Ponticello, J. S. Tan and T. M. Bruemmer, *International Symposium on Macromolecules, 1985*, p. 209.
- 19 T. M. Handel, I. S. Ponticello and J. S. Tan, *Macromolecules*, (1987) in press.
- 20 T. Kitajima-Yamishata, *Polym. J.*, 4 (1973) 262.
- 21 Z. Grubisic, R. Temppe and H. Benoit, *J. Polym. Sci., Part B*, (1967) 753.
- 22 A. Spatorico and G. Beyer, *J. Appl. Polym. Sci.*, 19 (1975) 2933.
- 23 G. Beyer and B. Contestable, *Polym. Prepr. Am. Chem. Soc. Div. Polym. Chem.*, 20 (2) (1979) 20.
- 24 W. W. Yau, C. R. Ginnard and J. J. Kirkland, *J. Chromatogr.*, 149 (1978) 465.