Journal of Chromatography, 389 (1987) 103–113
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

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HYDROLYTIC STABILITY OF AMINOPROPYL STATIONARY PHASES USED IN THE SIZE-EXCLUSION CHROMATOGRAPHY OF CATIONIC POLYMERS

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(First received July 16th, 1986; revised manuscript received October 22nd, 1986)

SUMMARY

The size-exclusion chromatography of cationic polymers on silica-based supports is difficult when residual active sites are present. The hydrolysis of hydrophilic bonded phases, with the concomitant exposure of free silanols, is also detrimental to the chromatography. Attempts to minimize these harmful solute–support interactions has led to the use of weak cationic bonded phase which provide limited charge repulsion, but not total ion exclusion. An approach was found to greatly improve the hydrolytic stability of cationic stationary phases to the extent that reproducible chromatography was achieved in day-to-day operations on the same column over a period of several months. This was achieved by reacting aminoalkylsilane groups with diepoxides.

INTRODUCTION

Hydrophilic stationary phases bonded to silica gel have been useful in a number of chromatographic separation modes including normal phase, ion-exchange, hydrophobic interaction, and aqueous size-exclusion chromatography (SEC). In each of these separation modes, the bonded phase contributes to the interfacial interaction required for the operation. However, the function of the stationary phase in the size-exclusion mode is unique, since its purpose is to eliminate interfacial interactions at the silica surface, thus allowing the steric interactions of the solute with the porous support material to dominate the mode of separation. Unfortunately, bonded stationary phases used in SEC do not completely eliminate adsorptive interactions between silaceous matrices and some solutes, resulting in unwanted adsorption and mixed-mode separations[1]. Also, the bonded phase itself may act as a site for hydrophobic interactions with polymers which possess hydrophobic regions. These non-size-exclusion effects are often very troublesome, particularly when multisite solute support interactions occur, such as with cationic polymers.

0021-9673/87/$03.50

Semirigid polymeric packings have been used to overcome some of the problems associated with silica in the SEC of cationic polymers[2,3]. The weak anionic nature of residual carboxyl groups in these polymeric packings has been minimized with high ionic strength and/or ion suppression with an acidic mobile phase. Potential problems encountered with polymeric supports included increased hydrophobic interactions, particularly at high ionic strength, and decreased efficiency compared to rigid microparticulate silicas.

A stationary phase that eliminates, or at least minimizes, the interaction of cationic polymers with silica surfaces would be useful in SEC. Some desirable qualities for such a stationary phase include: (1) extensive surface coverage, (2) low hydrophobicity to minimize interaction with cationic polymers containing hydrophobic moieties, and (3) high hydrolytic stability. The high degree of surface coverage required to overcome active sites on the silica surface has been approached by using polymeric-bonded phases[4], rather than monolayer coverages which are reportedly patchy[5,6]. The polymeric approach is presumably used with the popular TSK-SW column[7]. A potential problem with the polymeric coverage is increased hydrophobicity. Also, experience in this laboratory indicates that residual active sites are present, even with polymeric coverages. Indeed, all of the commercially available bonded silicas which have been evaluated in this laboratory have been unsuitable for SEC of certain cationic polymers, especially those with both hydrophobic and cationic groups along the polymer chain.

Cationic stationary phases bonded to silica have been successfully used in the SEC of cationic polymers[8–11]. These bonded phases provide electrostatic repulsion to polymers of the same charge, thus minimizing adsorption. The degree of ion exclusion experienced by charged polymers in the presence of a cationic silica surface is controlled by the ionic strength and pH of the mobile phase[12–14]. Thus, size-exclusion separations can occur when mobile phase conditions minimize charge exclusion sufficiently for polymers to permeate into the porous matrix of the silaceous support. Unfortunately, the advantage of decreased ion exclusion with increasing ionic strength may be offset by increased hydrophobic interactions that occur at the higher ionic strengths[1].

The deleterious effects of hydrophobic interaction chromatography to size-exclusion separations can be minimized by decreasing the hydrophobicity of the stationary phases, and/or increasing the organic content of the mobile phase. However, decreasing the hydrophobicity of the stationary phase often gives bonded phases which lack hydrolytic stability due to their increased wettability and solubility in aqueous mobile phases. Interestingly, recent reports suggest that hydrophilic stationary phases bonded to zirconium-treated silica exhibit increased hydrolytic stability over non-zirconium-treated silica, presumably due to modification of surface silanols[15]. Stable stationary phases have also been prepared by adsorbing polymers to the silica surface followed by cross-linking of the polymers[16].

The use of 3-aminopropylsilanes as stationary phases for SEC of cationic polymers is presented here. One of the major problems encountered with this phase is its hydrolytic instability. It is surprising that this stability problem is not more widely discussed considering the popularity of amino columns for weak anion-exchange chromatography, sugar separations, etc. A number of attempts are described here to increase the hydrolytic stability of aminoalkyl functionalities bonded to silica, with the most successful being the use of diepoxide treatments.

EXPERIMENTAL

Chromatographic apparatus

The equipment consisted of a Waters Model 6000A liquid chromatography pump, a Waters Model 440 UV detector (Waters Assoc., Milford, MA, U.S.A.), and a Rheodyne Model 7125 injector (Berkely, CA, U.S.A.). The columns were precision-bore stainless steel, 250 × 4.6 mm.

Chemical and packing materials

Aminopropyltriethoxysilane, aminopropyldimethylsilane, and aminobutyldimethylsilane were obtained from Petrarch Systems (Levittown, PA, U.S.A.). Allyl glycidyl ether and 1,3-butadiene diepoxide were obtained from Aldrich (Milwaukee, WI, U.S.A.). 1,4-Butanediol diglycidyl ether, adenosine diphosphate, inorganic buffer salts, spectro-grade toluene, and carbon tetrachloride were obtained from Kodak Laboratory Chemicals (Eastman-Kodak Company, Rochester, NY, U.S.A.). Li-Chrosphere silica (particle diameter 10 μm) of various pore diameters was obtained from E. Merk (Darmstadt, F.R.G.) and Zorbax silica (particle diameter 5 μm) was obtained from DuPont (Wilmington, DE, U.S.A.). Poly(2-vinylpyridine) (PVP) standards were purchased from Pressure Chemicals (Pittsburgh, PA, U.S.A.).

Silica derivatization

The silica and silylating reagents were used as received from the manufacturer without further purification. Silica was silylated with 10% solutions of alkoxysilanes in refluzing toluene or carbon tetrachloride. The refluxing was typically carried out for 15 h under anhydrous conditions. Following silylation, the silica was washed thoroughly with toluene and isopropanol. A series of slurrings, settlings, and decantations from isopropanol were used to remove the silica fines. Epoxide treatment of amino silylated silica was performed by gently warming the silica in a 5–10% solution of epoxide in tetrahydrofuran (THF) for 24 h. The completeness of the epoxide treatment was qualitatively determined with ninhydrin. Amino-silane treated silica reacts in a warmed ninhydrin solution to give the characteristic dark blue color, whereas the silica which had thoroughly reacted with epoxides did not give color. When the aminopropyl silica was extensively reacted with the diepoxides and thoroughly washed with trifluoroacetic acid (TFA), the residual epoxide groups were treated with 0.1 *M* nitric acid for 24 h in order to form the diol.

Aminopropyl silica was also prepared from 5% solutions of 3-aminopropyltriethoxysilane in water. With this procedure, the silica was slurried in the aqueous silane solution, degassed under vacuum, and warmed at 40°C for 3 h. The silica was filtered, washed with isopropanol, and air dried.

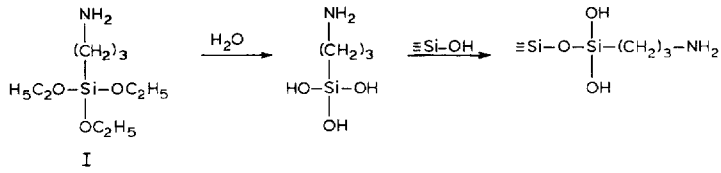
Stability of bonded phases

The hydrolytic stability of aminoalkyl silylated stationary phases was determined by running the columns as weak anion exchangers. As the cationic phase was lost from the column, there was a concurrent drop in the retention of adenosine diphosphate, the anionic solute. Indications of bonded phase deterioration were also apparent from increased adsorption of cationic polymers, although these observations were more qualitative than quantitative. The mobile phase used during loss of

the bonded phase is indicated in the respective figures. Adjustments in ionic strength were made with potassium nitrate.

RESULTS AND DISCUSSION

Silica bonded with the aminopropylsilane functionality (APS silica), is one of the more common commercially available weak anion exchangers used in high-performance liquid chromatography (HPLC). The bonding chemistry of aminopropyltriethoxysilane likely involves intramolecular base-catalyzed hydrolysis of the ethoxy groups to give free silanols which subsequently condense with surface silanols[17]. The water that participates in the hydrolysis may be available in the bulk phase, or may be adsorbed on the silica surface[18,19].



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Wide pore LiChrosphere and Zorbax silicas were derivatized with a solution of aminopropyltriethoxysilane and the resulting packings were used in the separation of PVP standards. The weak cationic stationary phase may be used for size-exclusion separations shortly after derivatization, as illustrated in Fig. 1A. After only a few days, however, separations deteriorated significantly (Fig. 1b–c). Several observations led to the conclusion that the column degradation illustrated in Fig. 1 was caused by an unstable stationary phase; for example: (1) a positive ninhydrin and/or *o*-phthalaldehyde (OPA) reaction was continually observed with the column eluent,

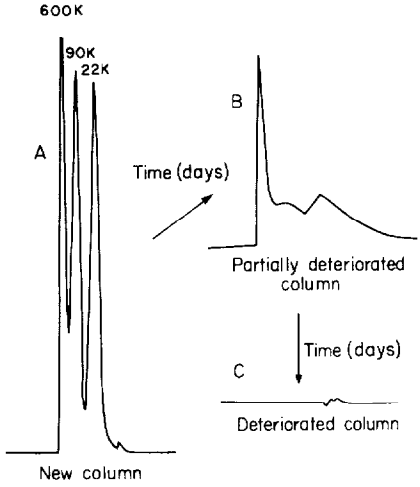


Fig. 1. Size-exclusion chromatography of 600, 90, and 22 K (K = kilodalton) PVP standards on an APS silica column as a function of column usage. Mobile phase: 0.1 *M* nitric acid and 0.2 *M* potassium nitrate.

indicating the presence of free amino groups. The colored or fluorescent products formed by the reaction of amines with ninhydrin or OPA, respectively, were observed with mobile phases in contact with the APS silica in the pH range of 2–8, and they were particularly noticeable when using the column after standing for a period of time. (2) When the APS columns were used in an ion-exchange mode, the exchange capacity dropped after usage, again indicating depletion of the cationic functionality. (3) Analysis of the packing material after column usage showed a drop in carbon content. (4) Retreating the silica with an aminopropyl silylating reagent restored the chromatography to its initial state.

Cationic polymers were irreversibly adsorbed following initial column deterioration. However, the adsorptive nature of partially deteriorated APS columns could be compensated for by lowering the ionic strength or pH of the mobile phase. The effect of pH and ionic strength on the performance of a one-week-old column that initially could be operated at a pH of 2.3 and an ionic strength of 0.3 is illustrated in Fig. 2. It was observed that decreased polymer adsorption occurred when the pH and/or ionic strength of the mobile phase was lowered. The charge density of the cationic polymer and the cationic bonded phase is likely to be increased with decreasing pH, resulting in a greater repulsion at the interface and less solute adsorption. Reducing the ionic strength of the mobile phase also increases the Debye interaction length at the interface and increases the solute's hydrodynamic volume so that there is less access to pores where irreversible adsorption occurs. Unfortunately, the ability to improve chromatographic performance of partially deteriorated APS columns by changing the mobile phase was not only impractical but also short lived, for, within a matter of days, the APS columns were totally deteriorated and useless.

The instability of aminopropyl phases in aqueous systems was not unique to those prepared in this laboratory. An evaluation of commercially available amino

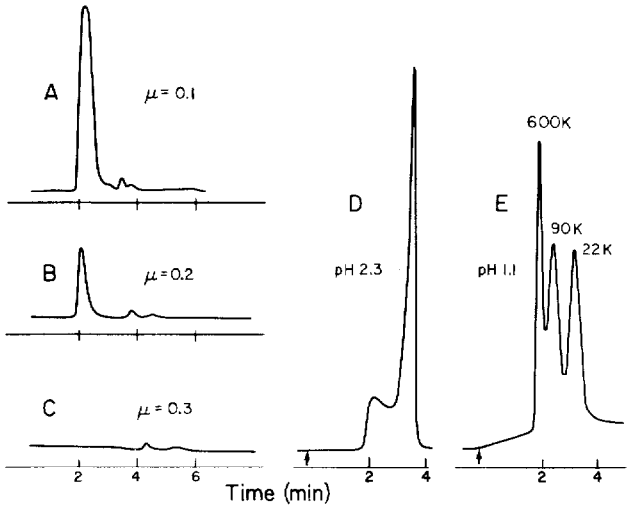


Fig. 2. Effect of pH and ionic strength on the elution of PVP standards from a one-week-old APS silica column. Chromatograms A, B, and C illustrate the elution of 600 K PVP at pH 1.1 and ionic strengths of 0.1, 0.2, and 0.3, respectively. Chromatograms D and E illustrate the elution of 600, 90, and 22 K PVP at pH 2.3 and 1.1, respectively (ionic strength = 0.2).

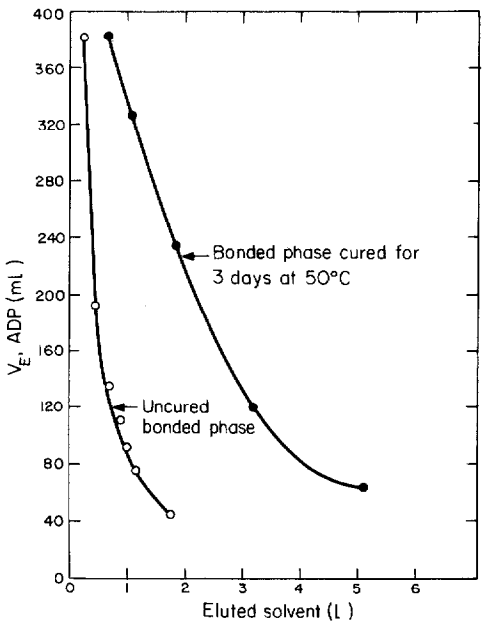
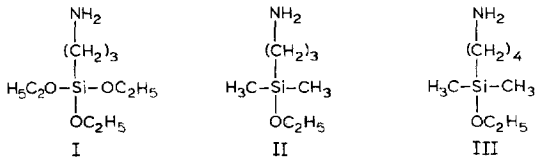


Fig. 3. The influence of curring on the stability of APS silica. The elution volume of adenosine diphosphate (ADP) decreases as a function of column usage (eluted solvent). Mobile phase: 0.01 *M* phosphate buffer, pH 7.8.

columns revealed the same problem with all of them; namely, hydrolytic instability. This is not a reflection on the ability of various vendors to bond the aminopropyl phase, but rather an observation of the nature of the chemistry of APS silica.

When time was given for the trifunctional silane (Structure I) to cure following the initial bonding, additional stability was imparted to the bonded phase. This was demonstrated by reacting LiChrosorb Si 60 silica with an aqueous solution of aminopropyltriethoxysilane. Following bonding, the silica was allowed to air dry for a day, and was then divided into two portions, one of which was cured an additional 3 days at 50°C. Columns were packed with these APS silicas and were evaluated in a relatively hostile ion-exchange mode (pH 7.8) using adenosine diphosphate as a probe molecule (retention of the probe molecule decreases as cationic bonded phase is lost from the column). Fig. 3 illustrates that the more fully cured bonded phase exhibited greater stability, possibly due to the increased silanol condensation giving a more stable network than the bonded phase which did not receive the extra curing. However, it was only a matter of time before the bonded phase in the more fully cured column also experienced the same serious deterioration.

The instability of APS silica prompted an investigation into ways of creating more stable bonded amino phases. One approach involved bonding aminoalkylsilanes with increased hydrophobicity and increased steric hindrance at the siloxane bond. Thus, aminopropyldimethylethoxysilane (Structure II) and aminobutyldimethylethoxysilane (Structure III) were used to derivatize LiChrosphere Si 500 silica and the resulting packing material was compared to a column derivatized with aminopyropyltriethoxysilane (I).



The dimethylbutylsilane molecule (Structure III) has twice the hydrocarbon content after hydrolysis as the propylsilane molecule (Structure I) and both silane II and III provide the added steric hindrance of two methyl groups adjacent to the siloxane bond at the silica surface. Although the trifunctional silane I, does not have the steric hindrance or hydrophobic resistance to hydrolysis, it is potentially capable of forming two bonds with the surface and/or reacting with adjacent silanes to give a more highly cross-linked coverage than with the monofunctional silanes II and III, which could only form a monolayer coverage having one siloxane bond per aminoalkyl group[18]. Silica derivatized with the trifunctional silane in this experiment, however, gave only a 1–2% increase in carbon content over the monofunctional silanes due to the anhydrous reaction conditions.

A mixture of PVP standards was used to compare the deterioration of the stationary phases I–III. As the bonded phase is hydrolyzed and washed from the column, a decrease in charge repulsion and an increase in free silanols occurs and the cationic polymers experience increased adsorption, as previously described for APS silica (Fig. 1). Table I shows the number of days each of the columns performed in a manner similar to that illustratyed in Fig. 1. It was initially thought that increased steric hindrance and hydrophobicity of the monofunctional silanes would increase the longevity of the stationary phase, but this was not the case; the silica treated with the trifunctional silane lasted more than twice as long as those treated with monofunctional silanes. Apparently, the multiple siloxane attachments which occurred with the trifunctional derivatizing reagent I, contribute more to stationary phase stability than steric hindrance and the modest increase in hydrophobicity provided by silanes II and III.

The foregoing data suggest that the bonding of aminoalkylsilanes to microparticulate silica is unstable even when polymerization of the stationary phase sil-

TABLE I

ELUTION OF POLY(2-VINYLPYRIDINE) ON MONO- AND TRIFUNCTIONALLY BONDED AMINOALKYL STATIONARY PHASES

*Performance level**	*Performance period (days)***		
	Aminobutyl dimethyl siloxane (III)	*Aminopropyl dimethyl siloxane (II)*	*Aminopropyl siloxane (I)*
New column	1	1	1–7
Partially deteriorated column	2	2	7–10
Deteriorated column	>3	>3	>10

* See Fig. 1 for examples of performance levels.

** Time period at which specified level of performance was observed.

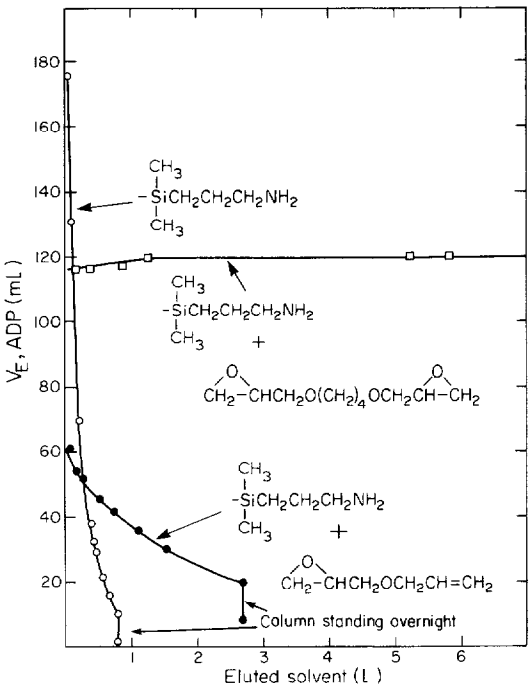
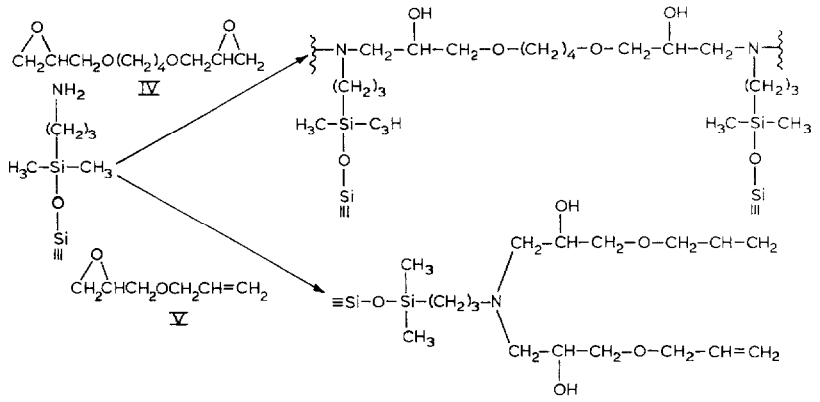


Fig. 4. Stability of untreated and epoxide-treated silica bonded with aminopropyldimethylsilane. Mobile phase: 0.01 *M* phosphate buffer, pH 7.8. The retention of adenosine diphosphate (ADP) decreases as the cationic-bonded phase is lost from the column.

oxanes provides multiple surface attachments. One approach used in our laboratory for overcoming the inherent instability of APS silica has involved cross-linking the bonded phase through the amine groups. Exceptionally stable stationary phases are formed by reacting APS silica with diepoxides. The stability of silica (LiChrosorb Si 60) derivatized with aminopropyldimethylsilane, II (a stationary phase previously demonstrated to be very unstable, Table I) with two stationary phases which were prepared by reacting bonded phase II with epoxides are compared in Fig. 4. In this experiment, silica was treated with aminopropyldimethylethoxysilane (Structure II) and the resulting product was divided into three lots; one of which was treated with 1,4-butanediol diglycidyl ether (Structure IV) another with allyl glycidyl ether (Structure V) and the third was untreated. Since allyl glycidyl ether does not give cross-linking under the reaction conditions employed, it provided a model for comparison to the reaction chemistry of the diepoxide.

Several reaction products are potentially formed when the surface bound aminopropyl groups are treated with epoxides. Since each amino group is capable of reacting with more than one epoxide, it is likely that treatment with diepoxides results in cross-linking of the aminopropyl groups at the silica surface. This is strongly suggested from the stability of the diepoxide *versus* the monoepoxide treated stationary phase illustrated in Fig. 4.

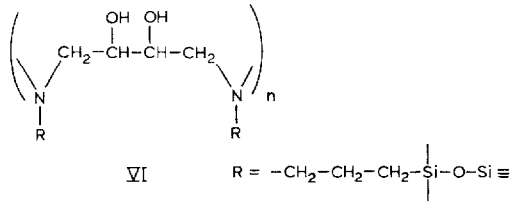
The retention of adenosine diphosphate as a function of column usage (volume



of mobile phase eluted through column) is shown in Fig. 4. As the bonded amino phase is hydrolyzed and washed from the column, the retention of the adenosine diphosphate decreases. As expected, the unmodified monofunctional silane and the monoepoxide-treated silane were very unstable under the evaluation conditions. However, the bonded phase which was formed by reacting the diepoxide with the monofunctional amino silane was exceptionally stable, as illustrated in Fig. 4.

The remarkable stability of the diepoxide-treated bonded phase may be explained, in part, because of multipoint attachment to the silica surface. If the diepoxide reaction couples several aminopropyl groups which are bonded to the silica surface, it would be necessary to simultaneously hydrolyze all of their surface siloxane bonds before the polymerized phase could experience dissolution and wash away. Since dissolution/deposition is an equilibrium process[20], the cross-linking potentially prevents the stationary phase from washing away before siloxane recondensation occurs. Even at pH 7.8, there was no noticeable loss of stationary phase over prolonged periods (Fig. 4).

Following the development of a method for imparting hydrolytic stability into an APS silica, it was possible to prepare weak cationic stationary phases with various diepoxides. Columns prepared by reacting APS silica with butadiene diepoxide (Structure VI) *in situ*, exhibited excellent hydrolytic stability and were particularly suitable for the size exclusion of cationic polymers which have hydrophobic regions in their polymer chain. These columns gave limited hydrophobic interactions, presumably due to a high hydroxyl–alkyl ratio.



The resolution of PVP standards on APS LiChrosphere silica treated with butadiene diepoxide is illustrated in Fig. 5 and is discussed further in the accom-

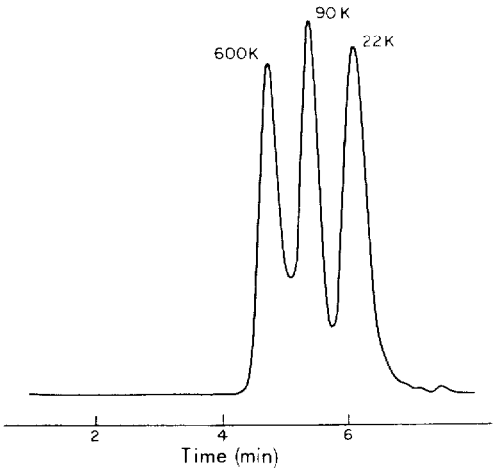


Fig. 5. Elution of PVP standards on LiChrosphere Si 400 and Si 500 silica treated with APS and butadiene diepoxide. Mobile phase: 0.1 *M* nitric acid, Flow-rate: 0.5 ml/min.

panying paper[21]. The performance of these columns did not deteriorate after months of usage and the carbon content did not drop, again illustrating that loss of bonded phase was largely responsible for cationic polymer adsorption.

CONCLUSIONS

Cationic polymers which adsorb to silica bonded with neutral stationary phases, can be separated with size-exclusion chromatography on silica bonded with cationic stationary phases. Unfortunately, aminoalkyl silanes which have been used extensively in the synthesis of silica-based, weak ion exchangers do not lend themselves to this type of chromatography due to their hydrolytic instability.

The hydrolytic stability of aminoalkyl columns was found to be better with trifunctional silanes, which potentially cross-link and form multipoint attachments to the silica surface, than with columns derivatized with monofunctional silanes. However, columns prepared with trifunctional aminopropylsilanes are still relatively unstable and show noticeable change within a matter of days. Hydrophilic, cationic, stationary phases which were extremely resistant to dissolution from the silica surface were prepared by reacting the bonded amino groups of APS silica with diepoxides. These bonded phases provide excellent surface coverage, as determined by the elution of cationic polymers which are difficult to elute on other chromatographic media. Columns prepared in this manner have been used in day-to-day operations over many months without any indication of loss of surface coverage.

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