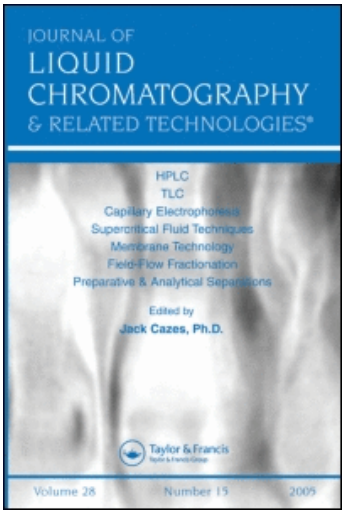

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HPSEC OF CATIONIC POLYMERS ON A POLYAMINE SUPPORT

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ABSTRACT

High performance steric exclusion chromatography of polyvinylpyridines (PVP) on silica particles coated with polymerized amine (SynChropak CATSEC) is described. PVP standards were analyzed first on a conventional neutral support (SynChropak GPC), and the results were compared with the amine coated silica. Conditions for analysis were then examined for optimization. Linear recovery was observed for a PVP standard of M.W. 3,000.

INTRODUCTION

Over the past few years, high performance steric exclusion chromatography (HPSEC) on silica based supports has become an accepted technique for polymer characterization. Methodologies for analyzing neutral and anionic polymers have been established by numerous research groups; however, the analysis of cationic polymers has been a problem. Positively charged molecules ionically bind to negative silanols on silica surfaces. When silica has a chemically-bound neutral layer covering the surface, the negative character of the silica is reduced but not eliminated (1-4). When Pfannkoch et al. examined this phenomena for a glycerylpropylsilyl support (SynChropak GPC), they found that

adjusting the pH or the ionic strength of the mobile phase would allow certain cationic proteins such as lysozyme and cytochrome c to elute by size (1). Alternatively, organic solvents have been used by Rand et al. to aid elution (2). Barth found that he could analyze cationic polymers on SynChropak GPC columns by preconditioning them with a cationic homopolymer (3). Highly charged polymers such as polyvinylpyridines (PVP) exhibited adsorption or, at best, tailing, under most conditions on neutrally bonded columns (4).

One way to eliminate the negative silanol groups and the adsorptive effects of cationic polymers is to bond an amine to the silica surface to neutralize the silanols. The resulting support would have a net positive charge and would exhibit anion exchange characteristics for appropriate molecules. Talley and Bowman used such an approach when they reacted controlled porosity glass with 3-aminopropyltriethoxysilane which was subsequently quaternized (5). PVP standards eluted from this support without tailing when an acid eluent was used.

This paper describes the use of SynChropak CATSEC columns which are composed of high performance silica coated with polymerized amine. PVP standards elute from these columns according to size, and they exhibit Gaussian peak shapes when appropriate operating conditions are used.

EXPERIMENTAL

Apparatus: SynChropak CATSEC 100 and CATSEC 1000 (250 x 4.6mm ID) and SynChropak GPC 100 (250 x 10mm ID) were obtained from SynChrom,

Inc. (Linden, IN). A ConstaMetric IIG liquid chromatograph (Laboratory Data Control, Riviera Beach, FL) with a Model 7125 injection valve (Rheodyne, Berkeley, CA) and a Chem Research Model 2020 multiple wavelength detector (Instrumentation Specialties Company, Lincoln, NE) were used for the analyses.

Reagents: Trifluoroacetic acid (TFA) was purchased from Pierce Chemical Company (Rockford, IL). Sodium chloride and sodium phosphate monobasic were from Mallinkrodt (Paris, KY). The glycyl-l-tyrosine and cytidine were from Sigma Chemical Company (St. Louis, MO). The poly (2-vinylpyridine) (PVP) standards were obtained from Larry Rosen at Pressure Chemical Company (Pittsburgh, PA).

Procedure: The 0.1% trifluoroacetic acid/0.2M sodium chloride solution, the 1.0% trifluoroacetic acid/0.2M sodium chloride solution, and 0.1% trifluoroacetic acid solution used for buffers were prepared in water. The 0.02M sodium phosphate/0.2M sodium chloride solution was adjusted to pH 2.0 with hydrochloric acid. The glycyltyrosine and cytidine standards and poly (2-vinyl-pyridine) standards were prepared in buffer and sonicated where necessary to effect solution. Duplicate samples of 2.5-10µl were injected into the liquid chromatograph with the isocratic buffer at a flow rate of 0.5ml/min. Full scale absorbance of the detector was set at 0.16 and at a wavelength of 254 nm.

RESULTS AND DISCUSSION

Analysis of PVP standards on a neutral support: In order to evaluate the chromatography of the cationic polymers on the

polyamine support, it was first necessary to run them on silica with a neutral bonded layer. A SynChropak GPC 100 column which has a glycerylpropylsilyl bonded layer was chosen for the study. A mobile phase of 0.1% trifluoroacetic acid was used to suppress ionic effects. The PVP standards did elute under these conditions; however, some adsorption was occurring. Initial injections of the samples seemed to coat the reactive sites but these adsorbed polymers appeared to leach off over time. Subsequent samples

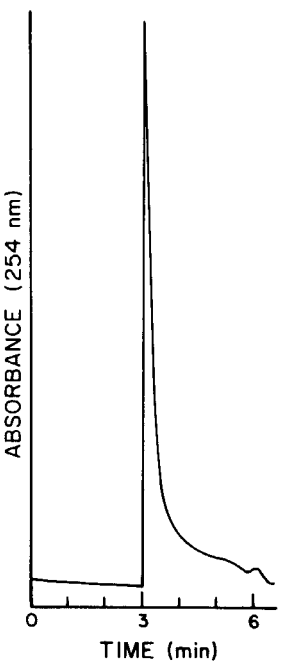

FIGURE 1

Analysis of a PVP standard (MW 20,000) on a SynChropak GPC 100 column (25 cm x 1 cm I.D.). Mobile phase: 0.1% trifluoroacetic acid; flowrate: 3.0 ml/min.

did elute according to size; however, some tailing of the peaks was present as seen in Fig. 1. Although these neutral supports could be used for the analysis of cationic polymers, the adsorption, sample leaching, and tailing made them less than ideal for this application.

Analysis on a polyamine support: A new column packing material, SynChropak CATSEC, was developed for the SEC of cationic polymers. This is a porous silica which has a thin layer of polyamine polymerized with a neutral hydrophilic crosslinker to increase stability. When PVP standards were run on these columns, linear recovery of the PVP was observed as seen in Fig. 2. With lower

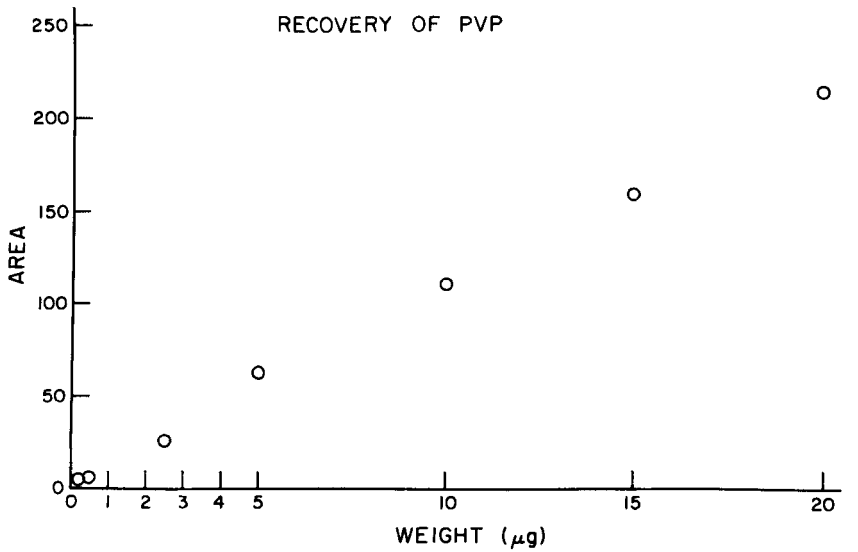

FIGURE 2

Recovery of a PVP standard (MW 3,000) from a SynChropak CATSEC 1000 column (250 mm x 4.6 mm I.D.). Mobile phase: 0.1% trifluoroacetic acid, 0.2M sodium chloride; flowrate: 0.5 ml/min.

concentrations, slight adsorption was observed. Once the feasibility of using these columns was determined, conditions for analysis were examined for optimization.

Mobile phase selection: When a mobile phase of 0.1% trifluoroacetic acid was used for analysis, the standards appeared to have

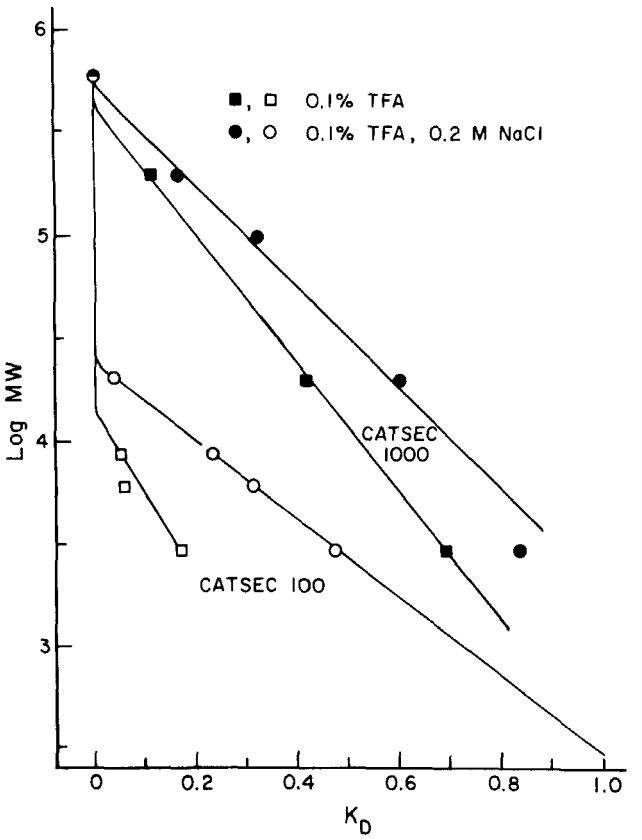

FIGURE 3

Ion-exclusion effect shown by the calibration curves for PVP standards on SynChropak CATSEC 100 and 1000 columns.

lower retention times than would be expected on columns of these pore diameters (100 Å and 1000Å, respectively). This phenomenon would be symptomatic of ion-exclusion, which would be expected with pores containing a charged surface and an eluent with inadequate ionic strength. The density of positive charges within the pore repel the positively charged polymers and prevent them from penetrating properly. The effect was more severe for the 100 Å than the 1000 Å column due to the much higher surface area of the 100 Å support. Figure 3 illustrates the ion-exclusion effect on the calibration curves of each support. A mobile phase of 0.1% trifluoroacetic acid containing 0.2M sodium chloride was used for comparison.

Eluents containing 0.2M salt appeared to obviate ion exclusion on columns of all pore diameters. Mobile phases of several different pH values were used and all gave similar results when 0.2M sodium chloride was added: 1) 1.0% trifluoroacetic acid, 0.2M sodium chloride (pH 1.4), 2) 0.1% trifluoroacetic acid, 0.2M sodium chloride (pH 1.6), and 3) 0.02M sodium phosphate, 0.2M sodium chloride (pH 2.0). Figure 4 shows the analysis of three PVP standards on a 100Å SynChropak CATSEC 100 column using 0.1% trifluoroacetic acid and 0.2M sodium chloride. Figure 5 exhibits the analysis of four PVP standards using the 0.02M sodium phosphate (pH 2.0) buffer with 0.2M sodium chloride. No differences are seen between the eluents as regards peak shape. When 0.5M sodium chloride was used, some added retention was seen. This was probably due to a hydrophobic interaction or a salting-out

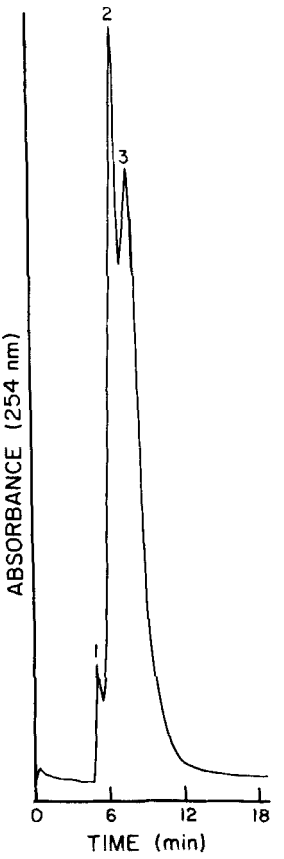

FIGURE 4

Analysis of PVP standards on a SynChropak CATSEC 100 column (250 mm x 4.6 mm I.D.). Mobile phase: 0.02M sodium phosphate, 0.2M sodium chloride, pH 2.0; flowrate: 0.3 ml/min; PVP standards: 1. MW 600,000; 2. MW 11,000; 3. MW 3,000.

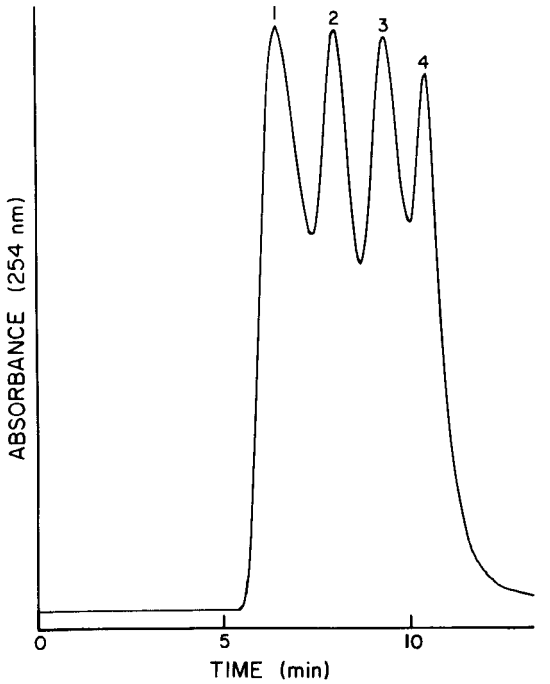

FIGURE 5

Analysis of PVP standards on a SynChropak CATSEC 1000 column (250 mm x 4.6 mm I.D.). Mobile phase: 0.1% trifluoroacetic acid, 0.2M sodium chloride; flowrate: 0.3 ml/min; PVP standards: 1. MW 600,000; 2. MW 100,000; 3. MW 20,000; 4. MW 3,000.

phenomenon and has been observed elsewhere (3). Some folding and extending of charged polymers occurs with changes in salt concentrations so it is important to maintain the same operating conditions for all analyses in order to get valid molecular weight comparisons and calibration curves.

Choice of totally included solute: One problem which occurred during these studies concerned the choice of a probe used to deter-

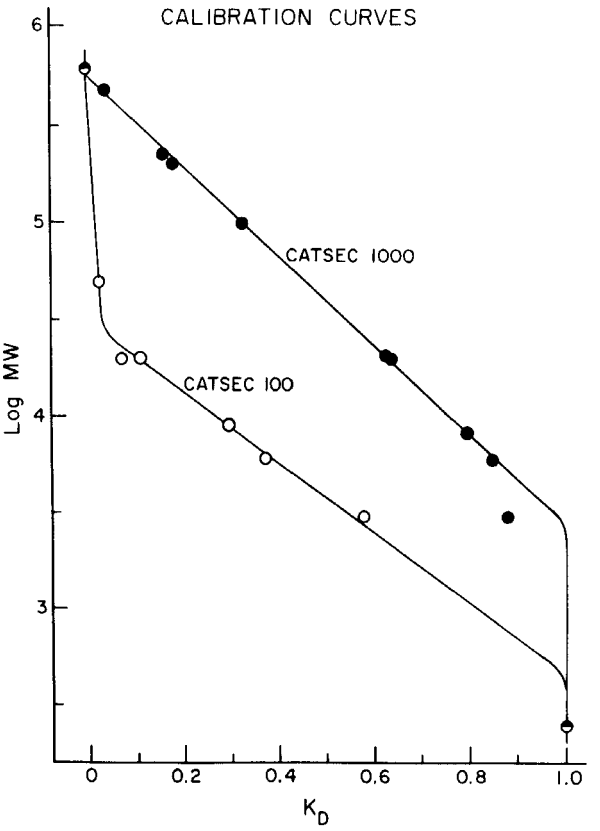

FIGURE 6

Calibration curves for SynChropak CATSEC 100 and 1000 columns using cytidine as the total volume indicator. Mobile phase: 0.1% trifluoroacetic acid, 0.2M sodium chloride; flowrate: 0.5 ml/min.

mine the total column volume. Pfannkoch et al. stressed the importance of finding a molecule which exhibits no interaction whatsoever with the column (1). Glycyltyrosine, which had been successfully used with glycerylpropyl columns, was initially used for these columns. The amphoteric character of this dipeptide caused it to be retained slightly on this polyamine column in all cases except when the mobile phase with the lowest pH (pH 1.4) was used. This phenomenon was not immediately obvious since the calibration curves were linear. Changes in the structure of the PVP polymers or the polyamine coating were blamed for the observed changes in K_D or internal volume of the support. When a more suitable small molecule, cytidine, was chosen, parameters such as internal volume and K_D were reproducible for mobile phases with different pH. Figure 6 shows the calibration curve for the PVP standards using cytidine instead of glycyltyrosine, which was used in Fig. 1.

CONCLUSIONS

Cationic polymers such as polyvinylpyridines can be analyzed by steric exclusion chromatography on SynChropak CATSEC columns. The polyamine coating on these columns neutralizes negative silanol groups and prevents adsorption of the polymers. Acidic eluents containing 0.2M salt eliminate ion-exclusion and interaction between the polymers and the supports. Cytidine was shown to be a good indicator of the total volume of the column.

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