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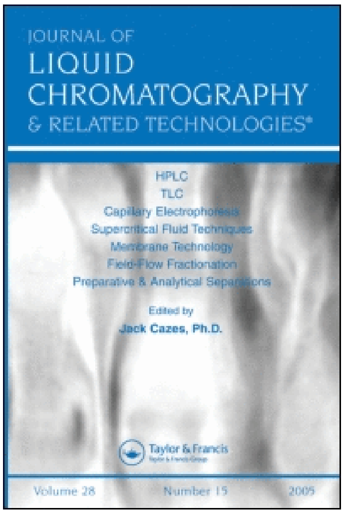

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A NEW PACKING FOR AQUEOUS SIZE EXCLUSION CHROMATOGRAPHY POLYVINYLPYRROLIDONE-COATED SILICA.

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ABSTRACT

Adsorption of polyvinylpyrrolidone on silica in aqueous medium is studied. Adsorption isotherms, thermogravimetry and elementary analysis show that a complete surface coverage is reached with large pore microbeads. The coating stability and its ability to prevent non-steric effects when used as packing in exclusion chromatography are demonstrated. Four different pore size packings were prepared for chromatographic evaluations. It is shown that water soluble polymers like polyethylene oxide, polyvinylalcohol, polyacrylamide and hydroxyethylcellulose can be chromatographied in pure water according to the steric exclusion mechanism.

INTRODUCTION

For rapid size exclusion chromatography of water soluble polymers, inorganic gels (microparticles of porous silica or controlled pore glass) seem to be the best way for high mechanical strength packings. In order to prevent the strong adsorption of samples on the surface of these materials, silica particles bonded with various molecules, have been recently prepared (1-3). Two micropackings are now commercially available (4-5) but the

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nature and the covalent structure of these bonded phases were not published. Another technique consists in pretreating silica beads by polyethylene oxide (6), but the coverage is not very stable.

By contrast, it is well known that polyvinylpyrrolidone (PVP) is strongly adsorbed onto silica (7-8). The presence of this polymer on a silica surface must reduce the adsorption of macromolecular solutes in GPC experiments ; this led us to check the chromatographic properties of PVP-coated silica in exclusion chromatrography of water soluble polymers.

EXPERIMENTAL

Apparatus

A Waters Associates model ALC/GPC 201, equipped with a M 6000A solvent delivery system, a U6K injector and a R 401 differential refractometer, was used throughout this work. Stainless steel columns (30cm length, 48mm I.D.), capped with Parker end fittings (2 micron frits) were packed by the slurry technique.

Materials

The silica used in our experiments was Lichrospher , purchased from Merck (Darmstadt, RFA). This spherical porous material has an average particle diameter of 10 microns and a controlled, narrow pore size distribution. To cover the whole range of molecular weight solutes, four different porosities were studied : 100 Å, 500 Å, 1,000 Å and 4,000 Å. The coating material was polyvinylpyrrolidone (PVP K 15), supplied by Fluka (Buchs, Switzerland) with a molecular weight near 10,000. The chromatographic properties of our PVP-coated silicas were mainly investigated with two sets of standards : dextrans from Sigma (St Louis, USA) and polyethylene oxides from Toyo Soda (Tokyo, Japan). In all experiments, desionized water was produced by a Milli-Q system supplied by Millipore (Bedford, Mass, USA).

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PVP Adsorption

Adsorption of PVP onto silica was studied by stirring a silica microbead suspension in aqueous solutions of PVP. Some kinetic experiments have shown that adsorption equilibrium was reached within 6 hours. After this time, the suspension was centrifuged at 6,000 rpm so as to separate the supernatant, and the polymer concentration was measured by refractometry via a calibration curve. Adsorption isotherms (9) were determined on 500mg of silica in 5ml solutions of PVP from 0.1% to 10%. For each kind of silica, the weight of adsorbed polymer per weight of silica was calculated ; This was then plotted as a function of polymer concentration in solution at equilibrium (Figure 1). These curves have a different adsorption limit, depending upon the bead pore size. The higher the porosity, the smaller the amount of coated polymer. As the specific surface area of these materials is given by the supplier, we have calculated the amount of the coated polymer per surface unit. The results are given in Table 1.

The same value (1mg/m^2) is obtained for 500 Å, 1,000 Å and 4,000 Å materials. Conversely, the value found for the 100 Å silica is smaller (0.8mg/m^2). These results are in good agreement with the chromatographic properties described below, and lead us to believe that a complete coverage of the silica surface is reached except in the case of the 100 Å material. Other measurements were achieved by thermogravimetry and elementary analysis of carbon and nitrogen and gave the same values with regards to experimental errors. (Table 2).

The coating stability was checked by measuring the amount of coated polymer via elementary analysis and thermogravimetry after several washings with pure water. No desorption could be detected. The same result was obtained by checking the coating before chromatography and after eluting more than 20 liters of water.

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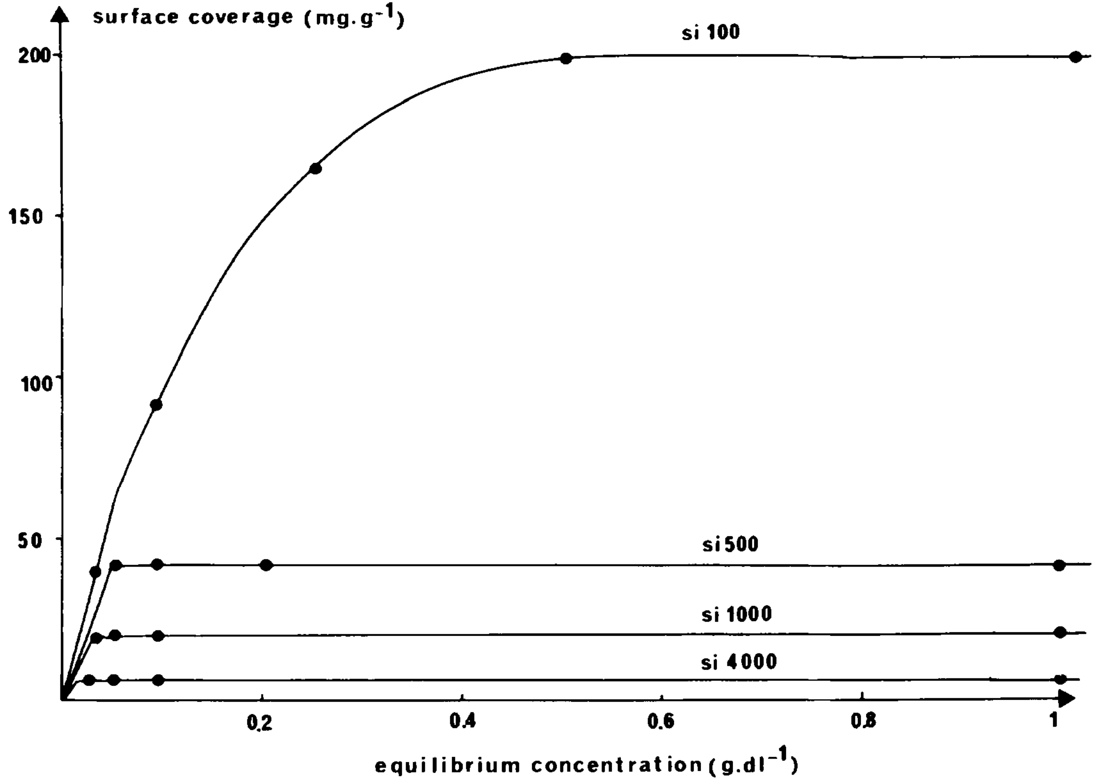

FIGURE 1

Adsorption isotherms for PVP K 15 on silica porous Lichrospher from H_2O.

TABLE 1

Porosity (in Å)	Surface area (in m^2/g)	amount of coated polymer (in mg/g)	in mg/m2)
100	256	200	≈ 0,8
500	40	41	≈ 1
1,000	19	20	≈ 1
4,000	5	5	1

TABLE 2

Porosity (Å)	Amount of coated polymer (in mg/g)		elementary analysis
	isotherms	thermogravimetry	
100	200	170	164
500	41	40	43
1,000	20	23	24
4,000	5	--	9

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Chromatographic experiments

We first tried to fill chromatographic columns with coated silicas by the slurry technique using pure water as solvent. The resultant efficiency was generally poor and did not exceed 3,000 plates per foot. After several trials in other solvents, the best efficiency (≈ 4,000 plates per foot) was obtained with a chloroform-methanol (75:25 v/v) mixture. Unfortunately, the coating is not very stable in this solvent and is partially removed from silica. The columns packed under these conditions still present some adsorption effects.

We obtained efficient PVP-coated silica columns, by the following method. Unmodified silica was packed in chloroform-methanol mixture under a pressure of 300 bars, then reconditioned in pure water via THF, methanol and methanol-water (50:50). The plate count decreased from 4,000 to 3,000 plates per foot. The silica coating was obtained by eluting a PVP solution, through the column at a level of 10% in water during 24 hours, with a flow rate of 0.5 ml/mn. No decrease was observed in the efficiency. Some measurements were performed by elementary analysis and thermogravimetry and it was demonstrated that the amount of adsorbed polymer per surface unit is the same either by this method or by the adsorption isotherm method.

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The chromatographic experiments were performed at 30°C. At a flow rate of 1ml/mn, the pressure did not exceed 500 PSI per column for pure water. The polymer samples were run with injection volumes of 15µl at concentrations about 0.5%. The columns were characterized by plotting the logarithm of molecular weight of various polymer samples versus the partition coefficient, Kd defined by :

$$Kd = (V_e - V_o)/(V_t - V_o)$$

where V_e is the elution volume, V_o the void volume and V_t, the total volume of solvent.

DISCUSSION

We studied the chromatographic properties of our columns by injecting two sets of standards : dextrans and polyethylene oxides (PEO). As these two sets of compounds have different polydispersities (about 1 for PEO and about 2 for dextrans), we characterized their elution volumes by their "peak molecular weight" $M = \sqrt{(\bar{M}n.\bar{M}w)}$, which is the most representative parameter of the peak apex (10). In order to use Benoit's universal calibration, intrinsic viscosities [η] were measured by Ubbelohde viscometry.

The ability of the PVP coating to prevent adsorption of samples on silica surface is shown in Figure 2 : a classical universal

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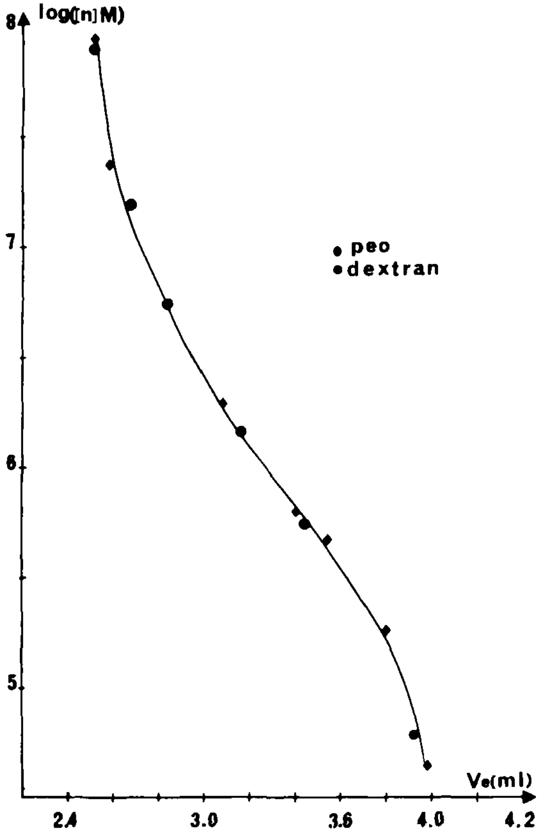

FIGURE 2

Universal calibration curve on 500 Å column : flow rate 1ml/mn.

calibration curve for PEO can be obtained on the 500 Å column, whereas these compounds are strongly retained on unmodified silica. Besides, the two universal calibration curves for dextrans and PEO are identical (Figure 2) and prove the absence of non-steric effects in the elutions. The same result was observed with the 1 000 Å and 4 000 Å columns.

On another hand, the 100 Å material presents a particular behaviour. A universal calibration can be obtained for dextrans but adsorption occurs with PEO. Excluded PEO samples, which are strongly adsorbed on unmodified silicas, are eluted here at the void volume on coated materials, while lower molecular weight samples, which penetrate into the pores, are retarded. This phenomenon, in connection with adsorption isotherm results, can be explained by incomplete surface coverage. The coating material has, in solution, too large a hydrodynamic volume ($\bar{M}w \simeq 10{,}000$) to enter completely into the pore structure during the coating procedure and

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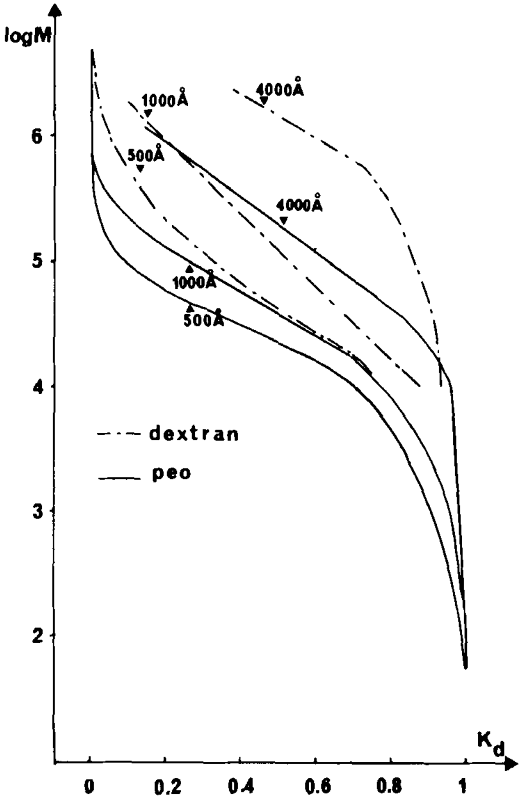

FIGURE 3

Calibration curves for PEO and dextran on 500, 1,000 and 4,000 Å columns. Solvent : H_2O. Flow rate : 1ml/mn.

TABLE 3

Porosity (Å)	Exclusion limit	
	Dextrans	PEO
500	6.10^5	2.10^5
1,000	3.10^6	5.10^5
4,000	10^7	2.10^6

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the surface coverage is probably complete outside the beads but partial within the pores. The use of a lower molecular weight PVP could probably overcome this drawback and we are studying this molecular weight parameter.

In Figure 3, we have plotted the calibration curves $\log\bar{M}w=f(Kd)$ for dextrans and PEO on 500 Å, 1,000 Å and 4,000 Å coated packings in pure water. These curves enhance the difference of behaviour for the two sets of standards since the hydrodynamic volume of PEO is larger at a given molecular weight than the dextran one. In addition these curves show that the coating procedure does not significantly affect the packing porosity and that PVP coated silicas have a very wide range of molecular weight selectivity. The different exclusion limits with dextrans and PEO are given in Table 3.

As an example, Figure 4 represents the separation of a PEO sample mixture on a 1000 Å column.

Many water soluble polymers such as polyvinylalcohol, polyacrylamide and hydroxyethylcellulose, have been successfully eluted in pure water without non-steric effects. However, PVP samples seem to be retarded, because of dipolar interactions between coated PVP and PVP chains in solution, leading to an apparent increase of their elution volumes. This non-steric effect does not permit the accurate analysis of PVP samples.

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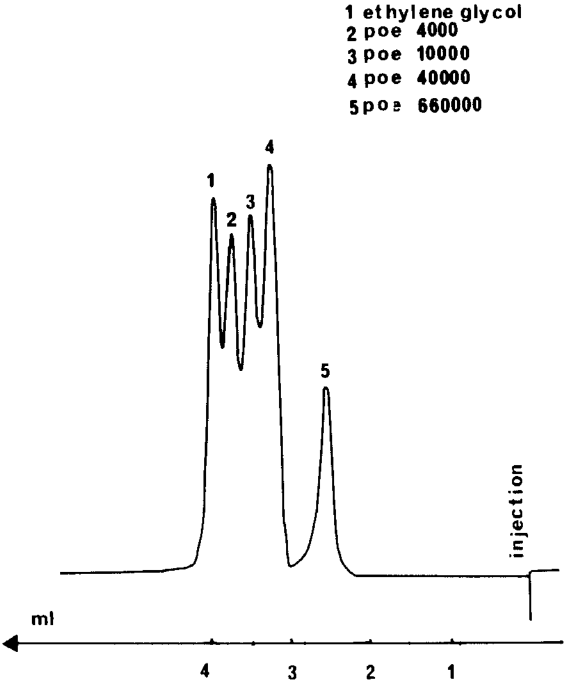

FIGURE 4

Separation of a mixture of PEO on SI 1,000 (30cm). Mobile phase : H_2O. Flow rate : 1ml/mn.

Finally, molecular size separation of charged polymers such as proteins or polymethacrylic acid were achieved with phosphate buffer as eluent. Figure 5 shows the separation of some proteins on the 500 Å column.

CONCLUSION

We have shown that the porous silica surface properties could be dramatically modified by coating it with a polymer such as poly-

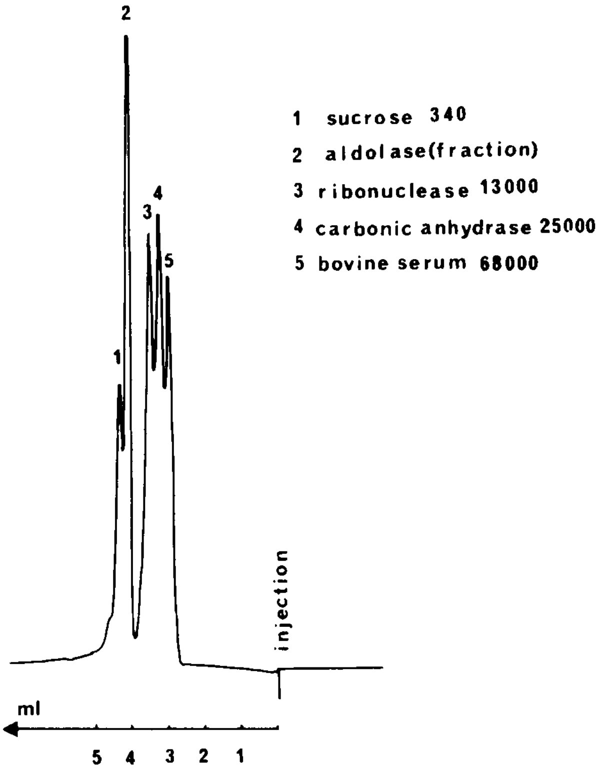

FIGURE 5

Separation of proteins on SI 500 (30cm). Mobile phase : phosphate buffer 0,1M. Flow rate : 0,5ml/mn.

vinylpyrrolidone using a very simple procedure. These PVP-coated silica packings are stable in water and may replace commercial bonded phases, that are chemically more difficult to obtain.

The direct application of these coated materials in aqueous steric exclusion chromatography is highly interesting and promising since the absence of non-steric effects enables the exact molecular weight characterization of many water soluble polymers.

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