CHARACTERIZATION OF VINYLPYRIDINE POLYMER COLUMNS FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Four spherical 2- or 4-vinylpyridine polymers (10–15 μm particle diameter), cross-linked with divinylbenzene and having different surface properties, are proposed as packing materials in high-performance liquid chromatography. The retention behaviour of various compounds on columns packed with these polymers was compared with that on a styrene–divinylbenzene copolymer column using methanol as the mobile phase. Interaction between polyhydric phenols and vinylpyridine polymers occurred more effectively in 4-vinylpyridine polymer than 2-vinylpyridine polymer, and porous 4-vinylpyridine polymer with 20% cross-linking provided the best peak resolution in the separation of the phenols. The retentions of acidic and basic compounds were correlated with the extent of acidity or basicity of the compounds. The effect of solvent strength on the retention of the selected compounds was also investigated by using methanol containing water or *n*-hexane.

INTRODUCTION

Vinylpyridine (VP) polymers cross-linked with divinylbenzene (DVB) have been widely studied as column packings in gas chromatography[1–3], ion-exchange or chelating resins[4,5], adsorbents for acidic compounds[6–8] and supports in affinity chromatography[9]. However, little has been reported on their application in high-performance liquid chromatography (HPLC). A gel-type 2-methyl-5-vinylpyridine–DVB copolymer was proposed by Freeman *et al.*[10], and its properties and chromatographic applications were studied.

This paper reports on the applicability of spherical 2- or 4-vinylpyridine–DVB copolymers which have sufficient hardness and with particles carefully classified to 10–15 μm in diameter as packing materials in HPLC. The polymers have high polarity compared with the commercially available porous styrene–DVB copolymer beads, and characteristic separations in HPLC are expected. In addition, the weak basicity of VP polymers based on the pyridyl group would also affect the retention of acidic or basic compounds. The retention behaviour of hydrocarbons, phenols, basic compounds and substituted aromatic compounds on the VP polymers has been compared with that on styrene–DVB polymer.

In a chromatographic system using methanol as the mobile phase, VP polymers showed a tendency to hold strongly hydrophilic compounds, and the retention of polyhydric phenols is particularly interesting. The influence of the surface properties and chemical structure of the polymers on the retention of solutes was also investigated.

EXPERIMENTAL

Materials

All reagents were of commercial reagent grade. 4-Vinylpyridine (4VP) and 2-vinylpyridine (2VP) were purified by distillation before use. Commercially available DVB solution (50–55%) was purified by washing with 5% sodium hydroxide solution to remove the stabilizer. Other reagents were uswed without further purification. Hitachi gel 3010 (H-3010), a macroporous styrene–DVB copolymer (20–25 μm particle size), was obtained from Hitachi.

Apparatus

A Hitachi 633A high-performance liquid chromatograph was used together with a KLC-200 variable-wavelength UV detector (Kyowa Seimitsu) or an SF-1107 refractive index detector (Atto). The specific surface areas and pore volumes of the polymers were measured with a Sorptomatic 1810 apparatus (Carlo Erba).

Preparation of microparticles of VP polymers

Polymerizations were carried out in a round-bottomed, three-necked flask under a nitrogen atmosphere. Toluene or isooctane solution containing 2VP or 4VP, DVB and benzoyl peroxide (2% of the monomers) was suspended in an aqueous solution containing 1% of hydroxyethylcellulose, 10% of sodium chloride and 0.04% of sodium hydroxide with rapid stirring (23–24 Hz) at 30°C. The volume of the aqueous phase used was three times that of the organic phase. As the temperature of the mixture rose slowly to 70°C, the stirring rate was gradually decreased to 4–5 Hz, then maintained these for 6 h. The resulting beads were washed with hot water until they were free from the adhering stabilizer. Finally, the beads were classified by a continuous solvent elutriation method[11] with methanol at 35°C, and the fraction of particle diameter 10–15 μm was collected.

Chromatography

The columns (50 × 0.26 cm I.D.) were made from stainless-steel tubing and packed with a methanol slurry of packing materials through a 10-ml stainless-steel packer at the rate of 2 ml/min for 1 h until the back-pressure became constant (at 140–180 kg/cm^2 for VP polymers). The solutes were dissolved in methanol at a concentration of approximately 2 mg/ml and 3–5-μl amounts of these were injected with a 10-μl syringe. Unless stated otherwise, methanol was employed as the eluent at a flow rate of 1 ml/min and the chromatographic procedure was performed at room temperature. The column dead volume was determined by using sodium nitrate, which was not retained.

RESULTS AND DISCUSSION

Characterization of polymers

Four kinds of spherical 2VP and 4VP polymers having different surface properties were prepared. Table I summarizes the composition and properties of the polymer beads used. H-3010 was used as a control and its properties are also listed. Although all the VP polymers were polymerized in the presence of diluents (toluene or isooctane), 4VP-30-T and 2VP-20-T were transparent beads under the microscope, and they had no measurable surface area or pore volume by the BET method. On the other hand, 4VP-20-T and 2VP-20-I were opaque, and they showed the characteristic surface properties corresponding to a porous structure.

The degree of swelling of a polymer in solvents is an important consideration when gradient elution is employed. The swelling was determined by placing a known amount of the dried polymer in a graduated cylinder, measuring its volume, adding an excess of solvent and measuring the volume again after the polymer had ceased to swell. The swelling was expressed as relative swelling, defined as the ratio of the swelling volume in a particular solvent to that in methanol, because methanol is mainly used as the mobile phase in this experiment. The values of the relative swelling of 4VP-20-T in aqueous methanol (10–99%), ethanol, isopropanol and acetone were nearly equal to unity, but those in acetonitrile, benzene and *n*-hexane were 0.94, 0.93 and 0.89, respectively.

Evaluation of VP polymer columns

The efficiency of columns packed with the proposed polymers was determined with some aromatic compounds by comparison with that of H-3010 and the results are given in Table II. The interaction between various solutes and the polymers was expressed as capacity factor (k') and the results are shown in Table III. Although 2VP-20-T had no measurable surface area or pore volume in the dry state, it showed a similar retention behaviour to 2VP-20-I and 4-VP-20-T, which have a porous structure.

TABLE I

CHARACTERIZATION OF VINYLPYRIDINE POLYMERS AND H-3010

4VP = 4-Vinylpyridine; 2VP = 2-vinylpyridine; DVB = divinylbenzene solution.

Polymer	Composition of monomers (g)			Diluent (g)		Elemental analysis: N (%)	Specific surface area[*] (m^2/g)	Pore volume (cm^3/g)	Average pore diameter (nm)
	4VP	2VP	DVB	Toluene	Isooctane				
4VP-20-T	40.0	—	26.7	40.0	—	7.96	65.5	0.169	5.2
4VP-30-T	26.7	—	40.0	40.0	—	5.44	0	—	—
2VP-20-T	—	40.0	26.7	40.0	—	8.03	0	—	—
2VP-20-I	—	40.0	26.7	—	40.0	8.59	14.0	0.095	13.6
H-3010[**]	—	—	—	—	—	—	288	—	4.0[***]

[*] Determined by BET method.
[**] Ref. 12.
[***] Most frequent pore diameter.

TABLE II

HEIGHTS EQUIVALENT TO A THEORETICAL PLATE (HETP) OF THE COLUMNS

Sample	HETP (mm)				
	4VP-20-T	4VP-30-T	2VP-20-T	2VP-20-I	H-3010
Benzene	0.72	0.84	0.58	0.91	0.82
Aniline	0.76	1.82	0.59	1.18	0.95
Phenol	0.70	2.27	0.71	1.24	0.93

TABLE III

CAPACITY FACTORS OF VARIOUS COMPOUNDS ON COLUMNS PACKED WITH THE POLYMERS

Detection: UV (254 nm).

Sample	Capacity factor (k')			
	4VP-20-T	2VP-20-T	2VP-20-I	H-3010
Benzene	1.32	1.22	1.20	0.90
Toluene	1.34	1.35	1.17	1.20
Xylene	1.35	1.44	1.17	1.56
Naphthalene	2.37	2.48	1.57	3.33
Diphenyl	2.32	2.70	1.56	4.67
Anthracene	5.39	5.40	2.45	11.88
Phenanthrene	5.47	5.28	2.42	9.98
Triphenylene	18.00	12.07	4.35	38.07
Chlorobenzene	1.46	1.44	1.26	1.25
o-Dichlorobenzene	1.67	1.82	1.36	1.63
Nitrobenzene	1.79	1.58	1.33	1.27
Benzonitrile	1.35	1.11	1.11	0.86
Benzyl alcohol	1.26	1.07	1.09	0.27
p-Benzoquinone	1.35	1.11	1.11	0.65
Aniline	1.59	1.07	1.27	0.43
o-Phenylenediamine	1.55	0.92	1.21	0.38
m-Phenylenediamine	1.49	0.75	1.17	0.33
p-Phenylenediamine	0.81	0.52	0.84	1.23
Pyridine	0.85	0.56	0.86	0.47
α-Picoline	0.80	0.56	0.83	0.57
γ-Picoline	0.82	0.57	0.86	0.60
α-Aminopyridine	0.94	0.72	0.92	1.53
Quinoline	1.13	1.06	1.00	1.37
Quinaldine	1.07	1.08	0.95	1.47
Phenol	2.48	1.56	1.69	0.28
Hydroquinone	4.15	1.53	2.19	0.08
Resorcinol	6.24	1.93	2.81	0.06
Pyrogallol	10.41	1.82	3.36	0.04
o-Cresol	2.51	1.61	1.73	0.37
α-Napthol	3.00	3.83	3.13	0.97
β-Naphthol	2.33	3.27	2.65	0.96
Methyl benzoate	1.35	1.34	1.16	1.42
Methyl salicylate	1.59	1.67	1.31	1.71

In a chromatographic system using H-3010 and a polar solvent such as methanol, the order of elution of solutes would follow the hydrophobic strength of the solutes. Therefore, compounds of high hydrophobicity were strongly retained on this polymer and those of low hydrophobicity with hydrophilic groups were weakly retained. On the other hand, 4VP or 2VP polymers have lower hydrophobicities than H-3010, and tend to retain hydrophilic compounds more strongly than H-3010. Dieter and Walton[13] reported that $\pi-\pi$ interactions between aromatic compounds and the benzene rings of the polystyrene chains contributes to the retention. With VP polymers cross-linked with DVB, and also H-3010, the $\pi-\pi$ interaction would affect to the separation of aromatic compounds.

Pulverized VP polymers cross-linked with DVB, prepared by solution copolymerization, were introduced as adsorbents for phenols by Kawabata and co-workers[6,8]. They found that the adsorption of phenols from aqueous solution was influenced by both hydrophobic and acid–base interactions, and the formation of hydrogen bonds and/or associated ion pairs would contribute to the acid–base interaction.

As shown in Table III, the k' values of phenols on 4VP-20-T increase with increase of the number of hydroxy groups in phenols, but this tendency was reversed on H-3010. The acid–base interaction would be able to take part mainly in the retention of phenols on the 4VP-20-T column. In contrast, the k' values of phenols on 2VP-20-T and 2VP-20-I were scarcely influenced by the number of phenolic hydroxy groups. Kawabata and Ohira[6] also showed that adsorption behaviour of phenol on the 4VP polymer differs from that on the 2VP polymer, owing to the difference in the position of the nitrogen atom of the pyridine groups (2- or 4-position). With 2VP polymers, the results obtained in our work can be explained in terms of the steric hindrance of the molecular interaction between the hydroxy groups and the nitrogen atom in the pyridine moiety, which lies close to the polymer chain. Among the VP polymers tested, no apparent differences in the retention of most of the compounds except for phenols are observed. With phenols, 4VP-20-T gave the best peak resolution.

The relationship between the pK_a and log k' values of the ionic compounds determined with a 4VP-20-T column by using methanol as the mobile phase is shown in Fig. 1. The k' values of acidic compounds varied inversely with the pk_a values. The pK_a values[14] cited are those in aqueous solution, but may give an indication of acidity in methanol.

Nitrophenols and polyhydric phenols are held more strongly on the column than is predicted from their acidity. Benzoic acid, which has a higher acidity than phenols, could not be eluted from a 4VP-20-T column with methanol.

The main interactions expected between aromatic amines and VP polymers are hydrophobic and $\pi-\pi$ interactions based on benzene and pyridine rings on the polymers. The relationship between the pK_a and log k' values (Fig. 1) gives a good correlation. This result supports the suggestion that the basicity of the solutes is also related to their retention. Aliphatic amines with high basicity are hardly retained on the polymers.

Effect of degree of cross-linking on capacity factor

Table IV shows the capacity factors of four compounds on 4VP-20-T and 4VP-30-T, which have 20 and 30% of DVB as cross-linker, respectively. Naphtha-
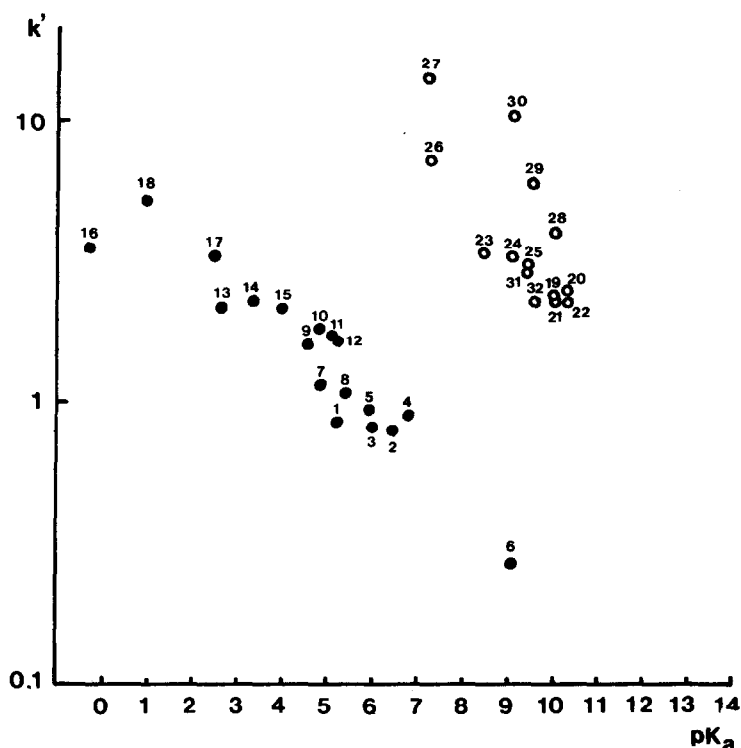
Fig. 1. Relationship between pK_a and capacity factor (k') on a 4VP-20-T column. Mobile phase: methanol. Detection: UV (254 nm). ●, Basic compounds; ○, acidic compounds. 1 = Pyridine; 2 = α-picoline; 3 = γ-picoline; 4 = α-aminopyridine; 5 = β-aminopyridine; 6 = γ-aminopyridine; 7 = quinoline; 8 = quinaldine; 9 = aniline; 10 = N-methylaniline; 11 = N-ethylaniline; 12 = N,N-dimethylaniline; 13 = o-chloroaniline; 14 = m-chloroaniline; 15 = p-chloroaniline; 16 = o-nitroaniline; 17 = m-nitroaniline; 18 = p-nitroaniline; 19 = phenol; 20 = o-cresol; 21 = m-cresol; 22 = p-cresol; 23 = o-chlorophenol; 24 = m-chlorophenol; 25 = p-chlorophenol; 26 = o-nitrophenol; 27 = p-nitrophenol; 28 = hydroquinone; 29 = resorcinol; 30 = pyrogallol; 31 = α-naphthol; 32 = β-naphthol.

lene, which was more hydrophobic than the others, was held more strongly on 4VP-30-T than 4VP-20-T. Aniline, phenol and hydroquinone, which were less hydrophobic, showed the reverse retention behaviour on these polymers. These results may be due to the increase in the hydrophobicity of the polymers due to the increase

TABLE IV

EFFECT OF DEGREE OF CROSS-LINKING OF 4VP POLYMERS ON CAPACITY FACTORS OF SELECTED COMPOUNDS

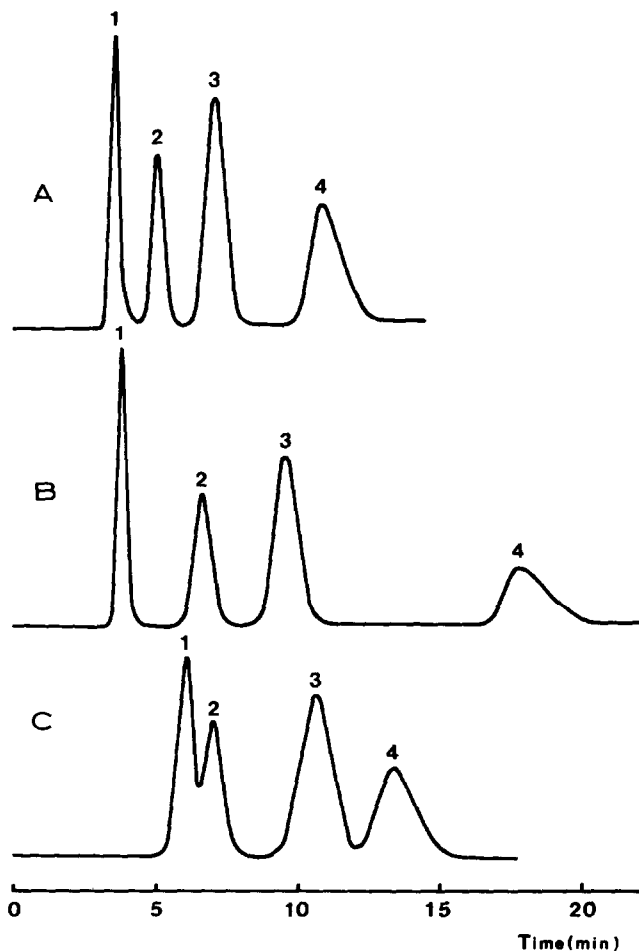| Degree of cross-linking (%)	Polymer	Capacity factor (k')			
		Naphthalene	Phenol	Aniline	Hydroquinone
20	4VP-20-T	2.37	2.48	1.59	4.15
30	4VP-30-T	3.10	2.18	0.90	2.64

Fig. 2. Separation of polyhydric phenols on a 4VP-20-T column. Mobile phase: (A) methanol; (B) methanol–*n*-hexane (80:20); (C) methanol–water (80:20). Detection: UV (254 nm). Peaks: 1 = phenol; 2 = hydroquinone; 3 = resorcinol; 4 = pyrogallol.

in the degree of cross-linking with DVB (from 20 to 30%) which causes a decrease in the pyridyl content.

Effect of solvent composition

The effect of solvent composition on the retention of naphthalene, resorcinol and *p*-phenylendiamine was examined by using methanol containing 10–20% of *n*-hexane or water as mobile phases. The addition of *n*-hexane to methanol causes a decrease in the polarity of the mobile phase, and brings about a decrease in the retention volume of naphthalene and increases in those of resorcinol and *p*-phenyl-enediamine. In addition, the HETP values of all the compounds decreased slightly. The addition of water, having a reverse polarity effect, causes an appreciable in⸱ · ⸱ise in the retention volumes of naphthalene and resorcinol and a slight decrease in that
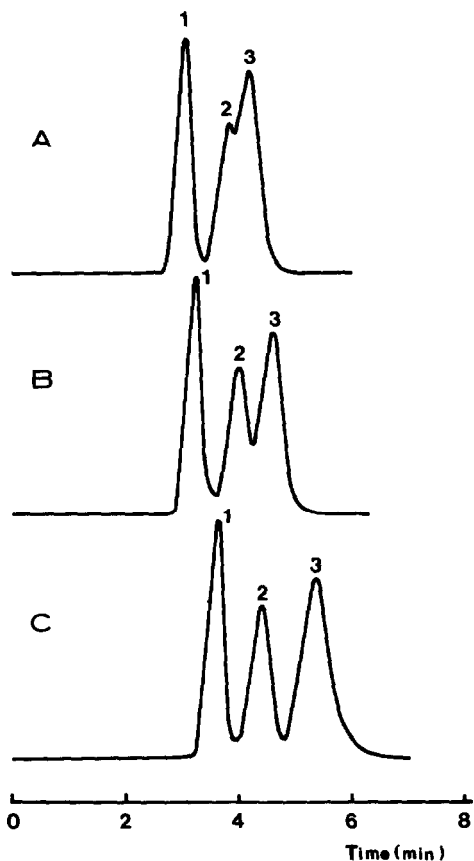
Fig. 3. Separation of aminophenols on a 4VP-20-T column. Mobile phase: (A) methanol; (B) methanol-*n*-hexane (90:10); (C) methanol-*n*-hexane (80:20). Peaks: 1 = *p*-aminophenol; 2 = *o*-aminophenol; 3 = *m*-aminophenol. Other conditions as in Fig. 2.

of *p*-phenylenediamine, whereas the HETP values of these compounds increased because of the increase in the viscosity of the mobile phase[15].

Fig. 2 shows the separation of four phenols using different solvent compositions. Although these compounds could be separated satisfactorily with only methanol, the peak resolution was improved markedly by the addition of *n*-hexane. As shown in Fig. 3, isomers of aminophenols were also separated perfectly with methanol containing 20% of *n*-hexane.

In conclusion, the VP polymers described here are promising column packing materials in HPLC, and a mobile phase such as a mixed solvent system can be used for the separation of a wide variety of compounds. In addition, VP polymers are weak anion-exchange resins, and applications in ion-exchange chromatography are now under investigation.

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