

CHROM. 18 265

## LIQUID CHROMATOGRAPHIC CHARACTERIZATION OF POROUS VINYLPIRIDINE POLYMER AS A WEAK ANION-EXCHANGE COLUMN PACKING

ATSUSHI SUGII\*, KUMIKO HARADA and NAOTAKE OGAWA

*Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862 (Japan)*

(First received August 27th, 1985; revised manuscript received October 22nd, 1985)

---

### SUMMARY

The retention behaviour of aromatic acids on a pyridine polymer column in ion-exchange high-performance liquid chromatography was investigated, and compared with that on a pyridinium polymer column. The effect of the eluent pH on the capacity factor,  $k'$ , of the acids on these polymer columns may be explained by the extent of dissociation of both the solute and the polymer. The relationship between the concentration of methanol in the eluent and  $k'$  showed that both polymer columns exhibit similar hydrophobic interactions with the solutes. On the pyridine polymer column, good resolution of structural isomers of aminobenzoic and hydroxybenzoic acids was obtained. It is suggested that undissociated pyridyl groups contribute to the solute retention.

---

### INTRODUCTION

In the analysis of aromatic acids by high-performance liquid chromatography (HPLC), a variety of separation modes such as ion-exchange, ion-exclusion, reversed-phase, normal-phase and ion-pair chromatographies are available<sup>1</sup>. We recently reported an evaluation of a porous vinylpyridine polymer cross-linked with divinylbenzene (pyridine polymer) for use as a column packing in HPLC<sup>2</sup>. So far, there have been no reports of the application of polymers containing pyridyl groups as column packings in ion-exchange HPLC, except for an investigation of a picolyl Kel-F column<sup>3</sup>.

This paper describes the separation of aromatic acids by ion-exchange chromatography on a pyridine polymer column. In general, the capacity of a weak anion-exchange resin is dependent on the pH of the eluent, and the retention of acidic compounds based on the ion-exchange mechanism is easily controlled by changing the eluent pH. Moreover, the interaction between the solute and the undissociated pyridine group is expected to influence significantly on the retention of the solute. The retention behaviours of the acids on the pyridine polymer have also been compared with those on a pyridinium polymer prepared by quaternization of the pyridine polymer with methyl bromide.

## EXPERIMENTAL

*Apparatus and materials*

An EYELA PLC-10 high-performance liquid chromatograph (Tokyo Rikakikai Co.) equipped with a UV spectrophotometric detector and a sample injector with a 10- $\mu$ l loop was used.

The chemicals used as chromatographic solutes and buffers were reagent grade from a variety of sources. Methanol and water used as the mobile phase were distilled from glass apparatus before use.

*Preparation of column packings*

Macroporous pyridine polymer (20% cross-linked with divinylbenzene, particle size 10–15  $\mu$ m) was prepared by the method described previously<sup>2</sup>. The pyridinium polymer was prepared as follows. A 5-g amount of the pyridine polymer (nitrogen content: 7.96%) suspended in 20 ml of methanol was placed in a 50-ml pressure bottle and cooled with a mixture of ice and salt. Methyl bromide (25 ml) was added to the cooled mixture and the mixture was heated at 60°C for 15 h. The product was filtered off and washed with methanol. The nitrogen and bromine contents of the quaternized polymer were 4.76% and 28.04%, respectively.

*Chromatography*

The columns (25  $\times$  0.26 cm I.D.) were made from stainless-steel tubing and packed with a methanol slurry of the packing materials. Unless stated otherwise, methanol–water (1:1) containing 0.05 *M* phosphate buffer and 0.05 *M* sodium nitrate was used as the eluent at a flow-rate of 0.5 ml/min. The eluent pH was measured after mixing the buffer and organic modifier. The column was maintained at 55°C by external circulation. The sample solutions (1 mg/ml) were prepared in methanol. The dead volume of the column was determined by using pyridine unretained solute.

## RESULTS AND DISCUSSION

In ion-exchange chromatography, the retention of solutes is affected by the nature and concentration of the salt added in the eluent owing to the competition between the solute and other ions. A comparison of the effects of monovalent anions in the eluent on the retention of nitrobenzoic acids is presented in Table I. The order of the capacity factor,  $k'$ , of the acids on the pyridine polymer was  $\text{H}_2\text{PO}_4^- > \text{Cl}^- > \text{NO}_3^- > \text{ClO}_4^-$ , and a similar behaviour was observed on the pyridinium polymer except that the values for  $\text{H}_2\text{PO}_4^-$  and  $\text{Cl}^-$  were nearly equal.

The apparent  $\text{p}K_a$  value of the pyridine polymer determined in 50% aqueous methanol is nearly equal to the value (4.2) determined in water<sup>4</sup>, and aromatic carboxyl groups have  $\text{p}K_a$  values in the region of 2–5. Therefore, the  $k'$  values of the acids vary with the pH of the eluent by which dissociation of the functional groups on the polymer and acidic solute is affected. The relationships between eluent pH and  $k'$  for hydroxybenzoic and nitrobenzoic acids on pyridine and pyridinium polymers are shown in Fig. 1. Over the pH range examined, the degree of dissociation of 2-hydroxybenzoic acid and nitrobenzoic acids is approximately constant. Therefore, the decrease in  $k'$  values of these acids with increasing eluent pH is due to the decrease

TABLE I

EFFECT OF VARIOUS IONS ON THE CAPACITY FACTOR,  $k'$ , OF NITROBENZOIC ACIDS ON PYRIDINE POLYMER (I) AND PYRIDINIUM POLYMER (II) COLUMNS

Eluent: methanol-water (1:1) containing 0.05 M phosphate buffer and 0.025 M of the above ions (pH 5.52).

Compound	$k'$ on I				$k'$ on II			
	$H_2PO_4^-$	$Cl^-$	$NO_3^-$	$ClO_4^-$	$H_2PO_4^-$	$Cl^-$	$NO_3^-$	$ClO_4^-$
2-Nitrobenzoic acid	2.67	1.08	0.67	0.38	8.39	9.00	6.47	3.86
3-Nitrobenzoic acid	11.79	4.69	2.97	2.08	13.73	13.26	6.77	3.66
4-Nitrobenzoic acid	10.17	4.28	2.69	1.81	10.36	10.81	6.23	3.74

in dissociation of the pyridine polymer. As the pH decreased, the pH profiles of  $k'$  for 3- and 4-hydroxybenzoic acids tended to flatten out, due to a contribution from the undissociated acids. In the case of the pyridinium polymer which is completely dissociated in the pH range examined, the  $k'$  values for all the acids tested increase with eluent pH up to their maximum dissociation. The results shown in Fig. 1 may be accounted for in terms of the extent of dissociation of both the solute and the polymer.

Organic solvents in the eluent also affect not only the dissociation of functional groups but also the hydrophobic interactions between the solute and polymer. In the retention of organic compounds on a  $C_{18}$ -modified silica gel column<sup>5</sup>, porous polymer columns<sup>6</sup> and ion-exchange columns<sup>7-10</sup>, linear relationships between the logarithm of  $k'$  or the distribution coefficient and the concentration of organic modifier in binary water-organic eluents have been observed. Similar relationships were found for the pyridine and pyridinium polymer columns. The slopes of the lines obtained at 30-60% aqueous methanol are listed in Table II. The values for the acids having nearly equal  $pK_a$  values tend to increase with increasing hydrophobic nature of the

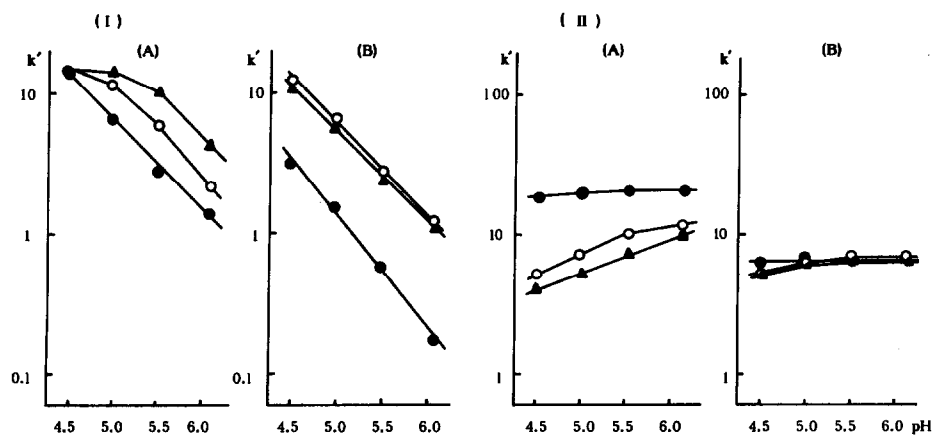


Fig. 1. Effect of eluent pH on the capacity factor,  $k'$ , of hydroxybenzoic acids (A) and nitrobenzoic acids (B). Column: I = pyridine polymer; II = pyridinium polymer. ● = 2-, ○ = 3- and ▲ = 4-substituted benzoic acid. Eluent: methanol-water (1:1) containing 0.05 M phosphate buffer and 0.05 M sodium nitrate.

TABLE II

SLOPE,  $a$ , AND CORRELATION FACTOR,  $r$ , FOR THE RELATIONSHIP BETWEEN THE CONTENT OF METHANOL IN THE ELUENT,  $c$ , AND  $\log k'$  ON PYRIDINE POLYMER (I) AND PYRIDINIUM POLYMER (II) COLUMNS

$\log k' = ac + b$ , where  $c =$  percentage of methanol in water.

Compound	$pK_a$	$\log p^*$	I		II	
			$a$	$r$	$a$	$r$
3-Hydroxybenzoic acid	4.08	1.50	-0.009	-0.998	-0.007	-0.995
4-Chlorobenzoic acid	3.99	2.75	-0.016	-0.998	-0.017	-0.998

\*  $\log p$  are hydrophobicity values<sup>15</sup>.

compounds. A similar behaviour was previously found by Sherma and Rieman<sup>9</sup> for the retention of ketones on a Dowex 50-X8 column with aqueous solutions of various organic solvents as eluents. The slope obtained on the pyridine polymer is similar to that on the pyridinium polymer. Although the polymers have different functional

TABLE III

CAPACITY FACTOR,  $k'$ , OF AROMATIC ACIDS ON PYRIDINE POLYMER (I) AND PYRIDINIUM POLYMER (II) COLUMNS

Eluent: methanol-water (1:1) containing 0.05  $M$  phosphate buffer and 0.05  $M$  sodium nitrate (pH 5.52).

Compound	$pK_a$	$k'$	
		I	II
2-Aminobenzoic acid	2.05, 4.95	8.08	6.55
3-Aminobenzoic acid	3.07, 4.74	2.06	4.64
4-Aminobenzoic acid	2.38, 4.89	4.78	4.06
2-Hydroxybenzoic acid	3.00, 13.40	2.75	20.27
3-Hydroxybenzoic acid	4.08, 9.93	5.94	9.89
4-Hydroxybenzoic acid	4.58, 9.23	10.11	7.19
2-Nitrobenzoic acid	2.17	0.56	6.36
3-Nitrobenzoic acid	3.49	2.67	6.66
4-Nitrobenzoic acid	3.44	2.33	6.23
2-Chlorobenzoic acid	2.94	1.11	4.05
3-Chlorobenzoic acid	3.82	4.69	5.96
4-Chlorobenzoic acid	3.99	5.25	5.13
1,2-Benzenedicarboxylic acid	2.95, 5.41	2.52	19.47
1,3-Benzenedicarboxylic acid	3.62, 4.60	2.25	35.23
1,4-Benzenedicarboxylic acid	3.54, 4.46	1.97	29.03
2-Methylbenzoic acid	3.91	3.84	2.09
3-Methylbenzoic acid	4.27	5.28	2.71
4-Methylbenzoic acid	4.37	5.31	2.55
Benzoic acid	4.21	3.31	3.17
Nicotinic acid	4.82, 12.00	0.25	1.30
Isonicotinic acid	4.84, 12.33	0.17	1.34
4-Methylbenzenesulphonic acid	1.70	0.39	10.07

groups, they have the same polymer backbone, it is this which mainly contributes to the hydrophobic interaction.

Under the chromatographic conditions in which the pyridyl groups of the polymer are slightly dissociated, the retention behaviour of various acids was investigated and the results are summarized in Table III together with those on the pyridinium polymer. Benzenedicarboxylic acids and some aromatic acids having low  $pK_a$  values such as 2-hydroxybenzoic, 2-nitrobenzoic and 4-methylbenzenesulphonic acids were strongly retained on the pyridinium polymer, but only weakly retained on the pyridine polymer. From these results, it is presumed that the contribution of ion exchange to the retention on the pyridine polymer is small. Among the structural isomers tested, the acids having low  $pK_a$  values were eluted more rapidly on the pyridine polymer. However, this was not the case on the pyridinium polymer.

It is considered that non-ionic interactions between the undissociated acids and the pyridyl groups on the polymer contribute to the retention of the acids. Accordingly, it is necessary to take into account that both ionic and non-ionic interactions are involved in retention of the acids on the polymers. The interactions were therefore analyzed by a dual binding model, which has been employed previously to explain the retention of compounds in ion-pair chromatography<sup>11,12</sup> and to interpret the silanophilic and hydrophobic interactions of silica-bonded hydrocarbonaceous stationary phases<sup>6,13,14</sup>. Accordingly to Bij *et al.*<sup>13</sup>, this model can be expressed by the equations

$$\frac{A}{k'_0 - k'} = \frac{1}{k_{20}K_A} + \frac{A}{k_{20}} \quad (1)$$

$$k' = k_1 + k_2 \quad (2)$$

$$k'_0 = k_1 + k_{20} \quad (3)$$

where  $k'_0$  and  $k'$  are the measured capacity factors in the absence and presence of a competing agent (CA) for ionic interaction,  $k_1$  and  $k_2$  are non-ionic and ionic ca-

TABLE IV

CONTRIBUTION OF NON-IONIC  $k_1$ , AND IONIC  $k_2$ , CAPACITY FACTORS TO THE RETENTION OF COMPOUNDS ON PYRIDINE POLYMER (I) AND PYRIDINIUM POLYMER (II) COLUMNS

Eluent: methanol-water (1:1) containing 0.05 M phosphate buffer and 0.05 M or without sodium nitrate (pH 5.52).

Compound	I		II			
	$k_1$	$k_2$	$k_1$	$k_2$		
		Without		0.05 M	Without	
4-Nitrobenzoic acid	2.47	9.20	-0.14	4.99	9.75	1.24
4-Hydroxybenzoic acid	10.07	2.82	0.04	3.19	39.95	4.00
4-Aminobenzoic acid	4.73	0.52	0.05	3.33	4.56	0.73

capacity factors,  $k_{20}$  is the ionic capacity factor in the absence of CA,  $K_A$  is the binding constant of CA and  $A$  is the concentration of CA. Under constant chromatographic conditions, except for the concentration of CA,  $k_1$  is constant independently of the concentration of CA.

Sodium nitrate was used as CA and its concentration was varied from 0 to 0.005, 0.025 and 0.05 *M*. The concentration of the phosphate buffer used to adjust the eluent pH was constant. The value of  $k'_0$  was the capacity factor measured in the absence of sodium nitrate. The  $k'$  values measured at various concentrations of sodium nitrate were employed in eqn. 1. An excellent linear relationship was obtained between  $A/(k'_0 - k')$  and  $A$ . The reciprocal of the slope of this line represents the value of  $k_{20}$ . The value of  $k_1$  was obtained by substitution of  $k_{20}$  into eqn. 3. The value of  $k_2$  in the presence of various concentrations of sodium nitrate was calculated from eqn. 2. The data obtained are listed in Table IV. On the pyridine polymer column, the  $k_1$  values of 4-hydroxybenzoic and 4-aminobenzoic acids were greater than  $k_2$  in the absence of CA, but in the case of 4-nitrobenzoic acid the opposite was found. When CA is present,  $k_2$  decreases, therefore it is clear that these compounds (shown in Table IV) are mainly retained by non-ionic interaction. Despite the presence of CA, strong ionic interaction contribute to the solute retention on the pyridinium polymer. These results suggest that the solute retention on the pyridine polymer is due predominantly to non-ionic interactions and in very small part only to ionic interactions under the chromatographic conditions shown in Table III. However, it is almost certain that ionic interactions do exist in this chromatographic system, for the dissociation of the pyridyl groups affected the retention of the acids. Considerable ionic interaction must be involved in solute retention on the pyridinium polymer under the same conditions.

The separation of structural isomers of aminobenzoic and hydroxybenzoic acids is shown in Figs. 2 and 3, respectively. The good resolution of each isomer of the aminobenzoic acids on the pyridine polymer is due to the non-ionic interactions. The elution order of the hydroxybenzoic acids on this polymer differs from that on the pyridinium polymer. 2-Hydroxybenzoic acid was strongly retained on the latter,

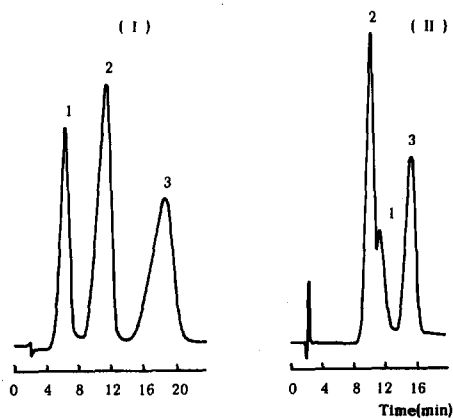


Fig. 2. Separation of aminobenzoic acids on pyridine polymer (I) and pyridinium polymer (II) columns. Peaks: 1 = 3-aminobenzoic acid; 2 = 4-aminobenzoic acid; 3 = 2-aminobenzoic acid. Other conditions as in Table III.

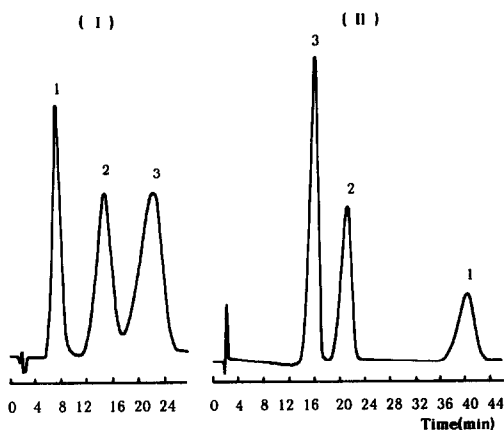


Fig. 3. Separation of hydroxybenzoic acids on the pyridine polymer (I) and the pyridinium polymer (II). Peaks: 1 = 2-hydroxybenzoic acid; 2 = 3-hydroxybenzoic acid; 3 = 4-hydroxybenzoic acid. Other conditions as in Table III.

but weakly on the pyridine polymer. An excellent separation of aromatic acids is obtained on the pyridine polymer column under conditions in which the pyridyl groups are slightly dissociated. The weak ionic interactions of the pyridine polymer result in faster elution of acids having low  $pK_a$  values, and non-ionic interactions contribute to the separation of the structural isomers.

#### ACKNOWLEDGEMENT

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education.

#### REFERENCES

- 1 R. Schwarzenbach, *J. Chromatogr.*, 251 (1982) 339.
- 2 A. Sugii, N. Ogawa, K. Harada and I. Sato, *J. Chromatogr.*, 294 (1984) 185.
- 3 M. Kruempelman and N. D. Danielson, *Anal. Chem.*, 57 (1985) 340.
- 4 A. Sugii, N. Ogawa, Y. Iinuma and H. Yamamura, *Talanta*, 28 (1981) 551.
- 5 W. R. Melander, B.-K. Chen and Cs. Horváth, *J. Chromatogr.*, 185 (1979) 99.
- 6 A. Nahum and Cs. Horváth, *J. Chromatogr.*, 203 (1981) 53.
- 7 K. P. Naikwadi, S. Rokushika and H. Hatano, *Anal. Chem.*, 56 (1984) 1525.
- 8 J. Sherma and W. Rieman, *Anal. Chim. Acta*, 18 (1958) 214.
- 9 J. Sherma and W. Rieman, *Anal. Chim. Acta*, 19 (1958) 134.
- 10 J. Sherma and W. Rieman, *Anal. Chim. Acta*, 20 (1959) 357.
- 11 A. Sokolowski and K.-G. Wahlund, *J. Chromatogr.*, 189 (1980) 299.
- 12 S. Eksborg and B. Ekqvist, *J. Chromatogr.*, 209 (1981) 161.
- 13 K. E. Bij, Cs. Horváth, W. R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.
- 14 E. Papp and G. Vigh, *J. Chromatogr.*, 259 (1983) 49.
- 15 T. Fujita, J. Iwasa and C. Hansch, *J. Am. Chem. Soc.*, 86 (1964) 5175.