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CHROMATOGRAPHY OF AROMATIC ACIDS AND ALDEHYDES AND PHENOLS ON CROSS-LINKED POLYVINYLPYRROLIDONE

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SUMMARY

Aromatic compounds containing hydroxyl and carboxyl groups are retained in aqueous medium by cross-linked polyvinylpyrrolidone due to hydrogen bonding, with the resin serving as a proton acceptor. Efficient separations were obtained in 0.001 *M* hydrochloric acid. With substances containing several proton-donating groups, improved results were obtained by using a more efficient eluent, *e.g.*, 5 *M* acetic acid.

INTRODUCTION

Cross-linked polyvinylpyrrolidone (PVP) has previously been used as a stationary phase in chromatographic separations of anthocyanins[1,2], anthocyanidinglucosides[3] and flavonoids[4] in aqueous media. The purpose of the present work was to study in further detail the applicability of PVP for the separation of various aromatic carboxylic acids and aldehydes and phenols and also to examine correlations between the retention data and the structures of the separated compounds.

EXPERIMENTAL

A commercial sample of cross-linked polyvinylpyrrolidone (Polyclar AT, General Aniline and Film Corp.) was sieved and the 60–75-μm fraction was treated with boiling 10% hydrochloric acid for 10 min. The particles were allowed to settle for 10 min and the supernatant was decanted. The sediment was suspended in distilled water and the washing repeated until the supernatant was clear.

Jacketted glass columns were packed with the resin by a technique commonly used in ion-exchange chromatography[5]. A 0.5-cm^3 Celite plug was placed at the bottom prior to packing. The eluent was passed through the column by means of a plunger pump and the eluate was analyzed by recording the absorbance at 280 nm (Chromatronix Model 220).

In a typical experiment, 5–50 μg of solute were eluted with 1 m*M* hydrochloric acid at 29° using a column of dimensions 790 × 4.3 mm and a flow-rate of 0.5 cm^3/min. This flow-rate corresponds to a nominal linear flow-rate of 3.5 cm/min. Experiments with phenol showed that variations in sample size within the range 4–400

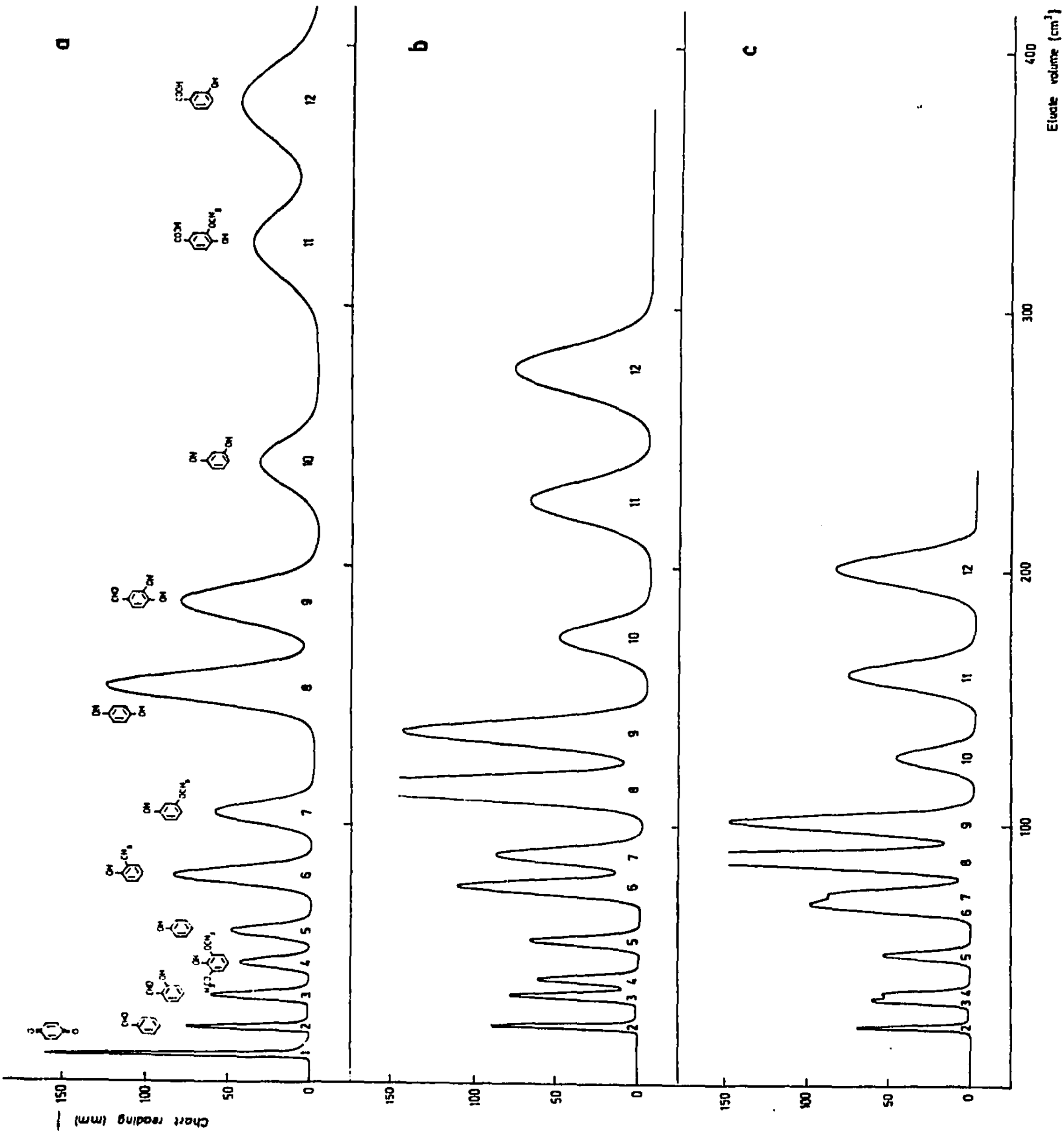

Fig. 1. Influence of temperature on the elution behaviour of twelve aromatic compounds. (a) 29°; (b) 50°; (c) 69°.

μg influenced the peak elution volume by less than 2%. Changes in the flow-rate within the range 2–6 cm/min had no effect on the peak positions. A comparison between different eluents was made on a smaller column (see Fig. 3).

Chromatography in 1 mM hydrochloric acid

The volume distribution coefficients, D_v, were calculated from the peak elution volumes[5] determined in a large number of experiments with single solutes and with mixtures containing several solutes, including phenol as a marker. The results obtained with 1 mM hydrochloric acid as the eluent are listed in Table I together with the retentions relative to phenol (r). Solutes which differ in their distribution coefficients by 20% are well separated. A chromatogram demonstrating the separation of twelve aromatic compounds at 29° is shown in Fig. 1a; the last compound was eluted in 12 h. The chromatograms in Figs. 1b and 1c show the separation of the same compounds at 50 and 69°. The first compound (benzoquinone) is unstable at elevated temperatures and was therefore not included in these experiments. Fig. 1 shows that those compounds which are held strongly by the resin at 29° appear much earlier in the eluate at the higher temperatures. At 69°, the last compound was eluted within 7 h. The separation factors of the last five compounds on the chromatograms were not appreciably affected by the change in temperature, whereas the peak widths decreased markedly. These results clearly show that increased temperatures yield more rapid and efficient separations of the more strongly held compounds. For the compounds that exhibited low retention volumes, the temperature coefficients were smaller.

Correlation between the structure of the solutes and the retention data

Hrazdina[3] suggested that the sorption of anthocyanidinglucosides in aqueous ethanol by PVP is due to hydrogen bonding, but no experimental evidence was presented to support this hypothesis. Evidence for the binding of proton donors, such as water and glacial acetic acid, by soluble poly-(1-vinyl-2-pyrrolidone) was presented in a recent paper by Rothschild[6].

The distribution coefficients listed in Table I show that benzene, benzoquinone and benzaldehyde, which lack proton-donating groups, are held rather weakly by the resin, which suggests that the aromatic nucleus has a weak affinity for the resin. In this context, it is worth mentioning that the solubility of benzene is higher in the presence of water-soluble PVP than in pure water[7]. Experiments with nitrobenzene and nitrobenzaldehydes showed that the distribution coefficients are hardly affected by the introduction of nitro groups, which, in other systems, has been shown to greatly influence the sorption[8]. On the other hand, naphthalene is retained much more effectively than benzene, which indicates that the effect of aromatic nuclei is greater in polycyclic aromatic systems. The low distribution coefficients of benzoquinone, benzaldehyde, mono- and dimethoxybenzenes and 1,2,3-trimethoxybenzene, which all are proton acceptors, indicate that the ability of the resin to form association compounds by proton donation is of little or no importance.

Table I shows that, with the exception of naphthalene and 1,3,5-trimethoxybenzene, all solutes that are strongly held by the resin contain carboxyl groups attached directly to an aromatic ring or phenolic hydroxyls. These compounds have a strong ability to form hydrogen bonds by donating a proton. An increase in the number of these functional groups results in an increase in the sorption, which strongly indicates

TABLE I

VOLUME DISTRIBUTION COEFFICIENTS, D_v, IN 1 mM HYDROCHLORIC ACID AT 29°

Substance	D_v	r
Phenol	4.96	1.00
1,2-Dihydroxybenzene	7.60	1.53
1,3-Dihydroxybenzene	21.5	4.33
1,4-Dihydroxybenzene	13.5	2.73
1,3,5-Trihydroxybenzene	>50	>10
2-Hydroxytoluene	6.83	1.38
3-Hydroxytoluene	7.06	1.42
4-Hydroxytoluene	6.66	1.34
2,5-Dihydroxytoluene	31.7	6.38
2,4-Dimethyl-1-hydroxybenzene	9.16	1.85
1,2-Dimethyl-4-hydroxybenzene	9.74	1.96
1,3-Dimethyl-2-hydroxybenzene	6.68	1.35
1-Hydroxy-2,4,5-trimethylbenzene	13.1	2.65
1-Ethyl-2-hydroxybenzene	8.30	1.67
1-Ethyl-3-hydroxybenzene	8.42	1.70
1-Ethyl-4-hydroxybenzene	7.78	1.57
2-Methoxyphenol	4.26	0.86
3-Methoxyphenol	9.11	1.84
4-Methoxyphenol	6.47	1.30
2,6-Dimethoxyphenol	3.85	0.78
2-Methoxy-4-propylphenol	7.19	1.45
4-Allyl-2-methoxyphenol	7.21	1.45
Methoxybenzene	2.40	0.48
1,2-Dimethoxybenzene	2.50	0.50
1,3-Dimethoxybenzene	3.85	0.78
1,4-Dimethoxybenzene	3.24	0.65
1,2,3-Trimethoxybenzene	2.05	0.41
1,3,5-Trimethoxybenzene	7.21	1.45
Benzoic acid	6.36	1.28
2-Hydroxybenzoic acid	37.2 (t)*	7.50
3-Hydroxybenzoic acid	34.0	6.86
4-Hydroxybenzoic acid	32.9	6.63
2,5-Dihydroxybenzoic acid	>50	>10
3,4,5-Trihydroxybenzoic acid	>50	>10
4-Hydroxy-3-methoxybenzoic acid	29.0	5.84
3,5-Di-*tert*.-butyl-4-hydroxybenzoic acid	>50	>10
Benzaldehyde	1.55	0.31
2-Hydroxybenzaldehyde	2.69	0.54
3-Hydroxybenzaldehyde	6.04	1.22
4-Hydroxybenzaldehyde	6.54	1.32
2,4-Dihydroxybenzaldehyde	14.7	2.97
3,4-Dihydroxybenzaldehyde	16.5	3.33
4-Hydroxy-3-methoxybenzaldehyde	6.51	1.31
3-Hydroxy-4-methoxybenzaldehyde	6.32	1.27
4-Hydroxy-3-methoxypenylacetaldehyde	6.53	1.32
4-Methoxybenzaldehyde	2.21	0.45
1,3-Dihydroxynaphthalene	>50	>10
2,7-Dihydroxynaphthalene	>50	>10
Chlorogenic acid	54.7	11.0
1,2,3-Triketohydrindene	2.07	0.42

TABLE I *(continued)*

Substance	D_v	r
3,3′,4′,5,7-Pentahydroxyflavone-3-L-rhamnoside	>50	>10
5,5-Dimethyl-1,3-cyclohexanedione	1.51	0.30
Nitrobenzene	1.79	0.36
3-Nitrophenol	8.20	1.65
4-Nitrophenol	9.07	1.83
2-Nitrobenzaldehyde	1.60	0.32
4-Nitrobenzaldehyde	1.25	0.25
2,4,6-Trinitrophenol	>50	>10
3-Nitrobenzoic acid	>50	>10
Formic acid	0.76	0.15
Acetic acid	0.68	0.14
Acetone	0.48	0.10
Benzene	1.62	0.33
Naphthalene	18.1	3.65
Benzoquinone	0.60	0.12
Benzyl alcohol	1.36	0.27
2-Phenylethanol	1.39	0.27
3-Phenyl-1-propanol	1.88	0.37

* t = tailing.

that the formation of hydrogen bonds with the proton-accepting groups in PVP is the most important sorption mechanism. The few exceptions to this rule probably result from either steric factors or intramolecular hydrogen bonding. The importance of these factors is reflected in the retention order between the solutes containing the same number of proton-donating groups. For example, among the solutes containing one phenolic hydroxyl group, only 2-hydroxybenzaldehyde ($r = 0.54$), 2-methoxyphenol ($r = 0.86$) and 2,6-dimethoxyphenol ($r = 0.78$) are held less strongly than phenol ($r = 1.00$). These compounds contain oxygen atoms at suitable distances for internal hydrogen bond formation[9]. The internal bonds, which compete with the formation of hydrogen bonds with the resin, are stronger with 2-hydroxybenzaldehyde than with 2-methoxyphenol[9]. In other phenolic compounds, which lack the ability to form intramolecular hydrogen bonds, the presence of aldehyde plus methoxy groups actually enhances the sorption. The low retention of 2,6-dimethoxyphenol is due to the presence of two oxygen atoms, which can serve as intramolecular proton acceptors. Moreover, steric hindrance of the methoxy groups would decrease the tendency of the hydroxyl group to form a hydrogen bond with proton acceptors in the resin.

Other observations lend support to the conclusion that intramolecular hydrogen bonds tend to decrease the interactions with PVP. For example, the D_v value of 4-hydroxybenzoic acid is lowered by the introduction of a methoxyl group at C-3. Likewise, a methoxyl group *vicinal* to the hydroxyl group in hydroxybenzaldehyde has a small effect. Similarly, the low D_v value recorded with 1,2-dihydroxybenzene, compared with those of its isomers, can be attributed to internal hydrogen bonds.

The introduction of methyl groups in the aromatic ring of phenol resulted in a marked increase in the distribution coefficients. The isomeric compounds studied differed only slightly in their chromatographic behaviour, with the exception of 1,3-dimethyl-2-hydroxybenzene, which, as expected for steric reasons, had a much lower distribution coefficient than its isomers. With the other methylated compounds, a plot of log D_v against the number of methyl groups could be represented satisfactorily by a straight line, demonstrating that each methyl group gave a constant incremental change in the free energy of the sorption. The fact that the positions of the methyl groups generally have little influence upon the peak position is, of course, a disadvantage from an analytical point of view. On the other hand, the positions of more polar groups, such as methoxyl and hydroxyl groups, exert a strong influence upon the distribution coefficients. Many isomeric compounds can therefore be easily separated.

The introduction of aliphatic side-chains containing several carbon atoms was not studied systematically. The large D_v values of 2-methoxy-4-propylphenol and 4-allyl-2-methoxyphenol compared with that of 2-methoxyphenol suggest that non-polar interactions exerted by aliphatic substituents[10,11] contribute significantly to the sorption on to PVP. Moreover, 3,5-di-*tert.*-butyl-4-hydroxybenzoic acid is held more strongly than benzoic acid, even though the hydroxyl group in the butyl-substituted acid is surrounded by bulky substituents that prevent the phenolic hydroxyl group from forming hydrogen bonds. The higher retention value is probably due to non-polar interactions involving the aliphatic substituents.

The adsorptions on to cross-linked dextran (Sephadex) of phenols and benzoic acids monosubstituted in the *meta*- or *para*-position can be correlated by the Hammett equation[12]. A plot of log r against Hammett's substituent constant[13] is shown in Fig. 2. It can be seen that for the compounds which contain one phenolic hydroxyl group as the only proton-donating group, a straight-line relationship exists for the compounds which contain methyl, ethyl, methoxyl, nitro and aldehyde groups. The retentions of the difunctional solutes (dihydroxybenzenes and monohydroxybenzoic acids) can also be correlated by the Hammett equation. The slope of this line and its intercept on the ordinate are larger than those for the monofunctional species. This can be explained if it is assumed that both hydroxyl groups are involved in the formation of hydrogen bonds.

It is interesting to note that acetic and formic acids, which have the ability to

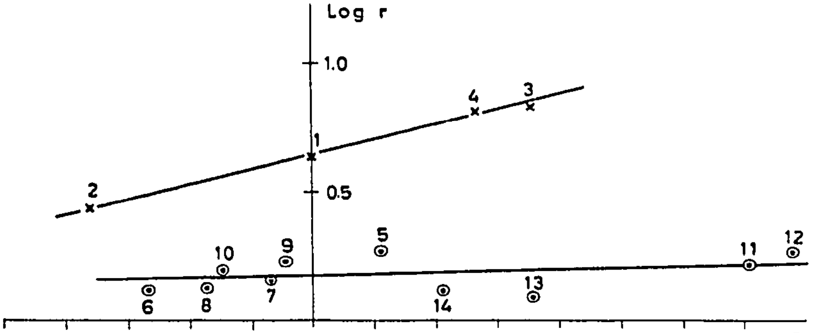

Fig. 2. Correlation between Hammett's substituent constant (σ) and log r for monosubstituted phenols. 1, 1,3-Dihydroxybenzene; 2, 1,4-dihydroxybenzene; 3, 3-hydroxybenzoic acid; 4, 4-hydroxybenzoic acid; 5, 3-methoxyphenol; 6, 4-methoxyphenol; 7, 3-hydroxytoluene; 8, 4-hydroxytoluene; 9, 1-ethyl-3-hydroxybenzene; 10, 1-ethyl-4-hydroxybenzene; 11, 3-nitrophenol; 12, 4-nitrophenol; 13, 3-hydroxybenzaldehyde; 14, 4-hydroxybenzaldehyde.

serve as proton donors, are only slightly retarded by the resin. In dilute aqueous solution, the formation of dimers is slight[14] and cannot explain this behaviour. The results suggest that water–solute and water–resin interactions are more important in these systems than the formation of hydrogen bonds between the acids and the resin.

From a practical point of view, it is important that the phenyl-substituted aliphatic alcohols are eluted much earlier than the phenols. The observations that the relative retentions of benzyl alcohol ($r = 0.27$) and 2-phenylethanol ($r = 0.27$) were lower than that of benzene ($r = 0.33$) and that 3-phenyl-1-propanol ($r = 0.37$) exhibited a low affinity for the resin strongly indicates that for alcohols of this type hydrogen bond formation with the resin is of little importance. The higher retention of the highest homologue may be attributed to non-polar interactions.

TABLE II

VOLUME DISTRIBUTION COEFFICIENTS, D_v, AND THERMODYNAMIC FUNCTIONS OF THE SORPTION OF AROMATIC COMPOUNDS ON TO PVP AT VARIOUS TEMPERATURES

Compound	*T* (°K)	D_v	$\Delta H°$ (kcal·mole^{-1})	$\Delta\Delta S°$ (cal·mole^{-1}·deg^{-1})
Benzene	303	1.66	1.9	5.3
	323	1.91	0.9	3.1
	343	2.04	0.5	3.5
Naphthalene	303	18.4	−0.5	2.3
	323	17.3	−0.9	1.9
	343	15.2	−2.1	0
Benzaldehyde	303	1.56	1.2	3.0
	323	1.72	0.6	2.2
	343	1.77	0.2	2.3
Methoxybenzene	303	2.37	1.1	3.3
	323	2.52	0.3	1.9
	343	2.57	0.2	2.9
1,3,5-Trimethoxybenzene	303	7.36	−1.4	−2.7
	323	6.33	−1.6	−2.1
	343	5.45	−1.7	−0.9
Phenol	303	4.96	−0.4	0
	323	4.70	−0.7	0
	343	4.30	−1.2	0
Benzoic acid	303	6.38	−0.5	0
	323	6.00	−0.9	−0.3
	343	5.35	−1.6	−0.9
1,4-Dihydroxybenzene	303	13.6	−3.0	−6.6
	323	9.99	−3.3	−6.5
	343	7.38	−3.6	−5.8
4-Hydroxy-3-methoxybenzoic acid	303	29.7	−3.8	−7.9
	323	20.2	−4.0	−7.4
	343	13.8	−4.1	−6.1
3-Hydroxybenzoic acid	303	34.3	−3.1	−5.3
	323	24.7	−3.5	−5.2
	343	17.5	−4.1	−5.5

A study of the influence of temperature upon the distribution coefficients was carried out with some of the solutes. The results given in Table II include the calculated change in enthalpy (ΔH^0) and the last column shows the change in entropy ($\Delta\Delta S^0$) during the sorption relative to that of phenol at the same temperature. The calculations were made using the equation

$$\Delta\Delta S^0 = R \ln D_v/D_{v(p)} + (\Delta H^0 - \Delta H_p^0)/T$$

where the subscript p refers to the reference substance (phenol) and R and T are the gas constant and thermodynamic temperature, respectively.

It can be seen that no generally valid correlation exists between the distribution coefficients and the change in enthalpy (ΔH^0). One observation made with all of the investigated species is that ΔH^0 becomes more negative (or less positive in the case of benzene, methoxybenzene and benzaldehyde) with an increase in temperature. Moreover, the sorption of all solutes containing hydroxyl or carboxyl groups linked to the aromatic ring exhibited a decrease in enthalpy which was much larger with those species which contain two phenolic hydroxyl groups or one hydroxyl and one carboxyl group than with those which contain only one proton-donating group. These observations lend support to the conclusion that hydrogen bonding is the dominant sorption mechanism with these solutes.

In contrast to the species that contain proton-donating groups, benzene, methoxybenzene and benzaldehyde exhibited positive ΔH^0 values. Evidently, the sorption of these solutes is connected with a gain in entropy, *i.e.*, with an increased disorder. Similar effects have been observed in many other systems in which hydrophobic solutes are adsorbed from aqueous solutions, and possible explanations have been discussed[11]. With naphthalene, the sorption was exothermic but the ΔH^0 values were about the same as found with phenol, although the distribution coefficients were about three times higher. This suggests that an increased entropy was obtained with naphthalene as well, *i.e.*, that the sorption mechanism exhibits similarities to that valid for benzene. Likewise, the sorption of 1,3,5-trimethoxybenzene was exothermic but with this solute the $\Delta\Delta S^0$ values were distinctly negative. With all solutes that contain two proton-donating groups, $\Delta\Delta S^0$ is negative, which means that with these species the sorption results in a higher degree of order than in the case of phenol, whereas with the solutes that lack proton-donating groups, except for 1,3,5-trimethoxybenzene, the opposite holds true. This was expected with regard to the sorption mechanisms postulated above and suggests that with the difunctional species both proton-donating groups have the ability to form hydrogen bonds.

Influence of the eluent composition

For most of the experiments, 1 mM hydrochloric acid (pH about 3) was employed as the eluent. This medium is preferred to pure water because some carboxylic acids exhibited a broadening of elution peaks in pure water. A comparison between results obtained in these media and those in acetic and formic acid media is given in Table III. The two carboxylic acids studied were held less strongly in pure water than in the dilute acidic eluents. This can be attributed to an increased dissociation of the carboxylic acids at the higher pH. In 0.1 M sodium hydroxide solution the sorption of 3-hydroxybenzoic acid was very small (D_v = 0.56). Chlorogenic acid was decomposed.

TABLE III

VOLUME DISTRIBUTION COEFFICIENTS, D_v, RECORDED AT 26° IN DIFFERENT ELUENTS

Substance	*1 mM HCl, pH 3*	*Distilled water*	*0.1 M acetic acid, pH 2.9*	*5 M acetic acid, pH 1.7*	*0.05 M formic acid pH 2.4*
Phenol	4.96	4.93	4.95	3.16	4.77
1,2-Dihydroxybenzene	7.78	7.64	7.56	3.91	7.61
1,3-Dihydroxybenzene	23.0	22.0	21.8	7.11	22.4
1,4-Dihydroxybenzene	14.2	13.9	13.5	4.74	13.9
4-Hydroxytoluene	6.54	6.27	6.33	3.44	6.43
3-Methoxyphenol	9.05	9.02	8.86	3.35	9.14
2-Hydroxybenzaldehyde	2.58	2.74	2.76	1.56	2.69
3-Hydroxybenzoic acid	36.6	20.9 (f)*	35.9	7.71	36.1
Chlorogenic acid	54.7	28.0 (t)*	55.0	4.17	54.4
3,3′,4′,5,7-Pentahydroxyflavone-3-L-rhamnoside	>50	>50	>50	12.5	>50
2,7-Dihydroxynaphthalene	>50			41.5	
1,3-Dihydroxynaphthalene	>50			~67	

* t = tailing; f = fronting.

Phenolic compounds lacking carboxylic acid groups were also eluted rapidly in 0.1 *M* sodium hydroxide solution. The following D_v values were obtained: phenol, 0.88; 1,3-dihydroxybenzene, 0.73; 1,4-dihydroxybenzene, 0.68; 4-methylphenol, 0.79; 3-methoxyphenol, 0.92; 2,7-dihydroxynaphthalene, 0.75. These results show that the completely ionized species which no longer contain protons that can bind to the active sites in the resin are only slightly retarded. This further confirms that interactions other than hydrogen bond formation between the resin and the aromatic compounds are weak. In this respect, the cross-linked PVP resin exhibits a behaviour different from that of soluble PVP, which was found to display a strong binding affinity towards, for instance, sodium benzoate[15]. On the other hand, the D_v values in 0.1 *M* sodium hydroxide solution of benzene (1.72) and naphthalene (15.2) differed only slightly from the values recorded at pH 3. These results confirm that the interactions between the resin and these solutes are of a different type than those obtained with proton-donor solutes.

Alkaline solutions can be used to strip off solutes which are held very strongly in acid medium and to recover these compounds as a group. However, it should be emphasized that many phenolic compounds are easily oxidized and that with these species air should be excluded.

The D_v values of the phenolic compounds in pure water were only slightly lower than those recorded in 1 m*M* hydrochloric acid, except for 2-hydroxybenzaldehyde, which was held more strongly in pure water, probably owing to a weakening of the intramolecular hydrogen bond at the higher pH.

The use of acetic and formic acids as eluents was expected to decrease the D_v values, as these carboxylic acids can be retained by PVP through hydrogen bond formation, thereby blocking sorption sites in the resin. At the lowest concentrations studied this blocking effect was small because, as shown in Table I, the sorption of these acids in dilute solutions is very slight. However, in 5 *M* acetic acid, the distribution coefficients of the aromatic compounds decreased markedly. The ratio between the D_v value in 0.1 M acetic acid and that in 5 M acid varies for each solute (the range of variation is between 1.6 and 16), indicating that factors other than the blocking effect have an appreciable influence. Among these factors are hydrogen bonding between acetic acid and the solutes, which seems to be most important with the carboxylic acids, and the decrease in polarity of the solvent.

The use of 5 M acetic acid as the eluent has advantages over 1 mM hydrochloric acid in the separation of solutes that are held very strongly in the latter medium. Fig. 3 shows a separation of four solutes, which exhibit D_v values above 50 in 1 mM hydrochloric acid. Even the isomeric dihydroxynaphthalenes are easily separated on this small column. Stepwise elution or a gradient elution with an increasing concentration of acetic acid may also be useful in such systems. However, for some solutes the separation factors are less favourable than in dilute hydrochloric acid.

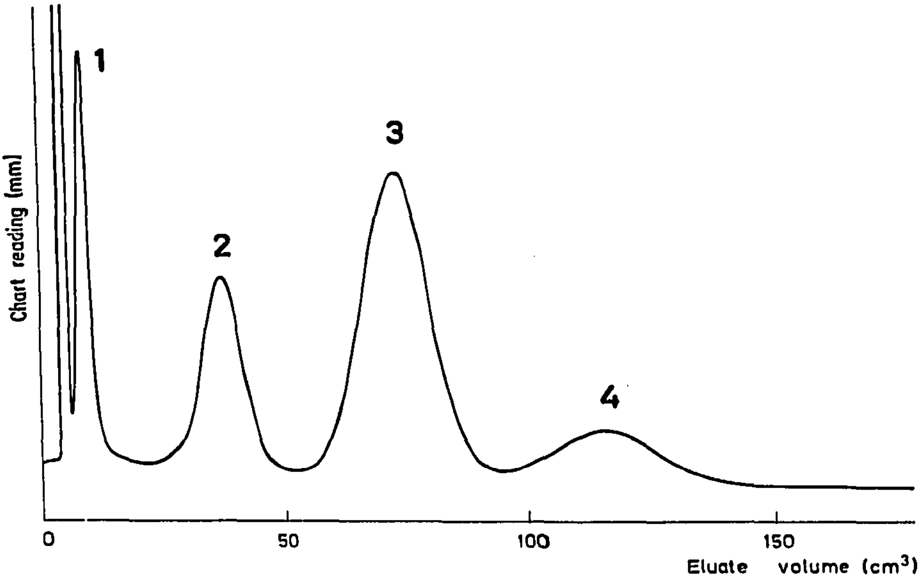

Fig. 3. Separation of (1) chlorogenic acid, (2) 3,4,5-trihydroxybenzoic acid, (3) 2,7-dihydroxynaphthalene and (4) 1,3-dihydroxynaphthalene in 5 M acetic acid at 29°. Column, 110 × 4 mm. Nominal linear flow-rate, 4.1 cm/min.

Another method of decreasing sorption involves the application of a mixed solvent as eluent. The retention data for aqueous ethanol are listed in Table IV and show that, with all solutes, the distribution coefficients decreased continuously as the proportion of ethanol in the eluent increased. As expected, this decrease was larger with some solutes than with others, as ethanol can serve both as a proton donor and a proton acceptor. For some pairs of species, the elution order was reversed in the ethanol-containing eluent. Furthermore, in some systems increased separation factors were obtained whereas in other systems the separation factors were less favourable.

The column efficiency, as reflected in the width of the peak corresponding to 1,4-dihydroxybenzene, was calculated in the usual manner[5]. The number of theoretical

TABLE IV

VOLUME DISTRIBUTION COEFFICIENTS, D_v, IN 0.1 *M* ACETIC ACID IN AQUEOUS ETHANOL OF DIFFERENT CONCENTRATIONS AT 26°

Substance	*Aqueous ethanol concentration (%, w/w)*			
	0	*10*	*30*	*50*
Phenol	4.76	4.57	3.07	1.77
1,2-Dihydroxybenzene	7.61	7.05	4.89	2.99
1,3-Dihydroxybenzene	22.5	18.6	9.95	5.31
1,4-Dihydroxybenzene	14.0	11.8	6.72	3.94
4-Hydroxytoluene	6.25	5.38	3.08	1.73
3-Methoxyphenol	8.82	7.34	3.71	1.97
2-Hydroxybenzaldehyde	2.64	2.49	1.90	1.36
3-Hydroxybenzoic acid	36.1	31.7	11.3	4.58
Chlorogenic acid	55.0	51.8	18.0	9.52
3,3',4',5,7-Pentahydroxyflavone-3-L-rhamnoside	>50	>50	47.3	14.6

plates in the column (175 × 4 mm) was nearly constant for those eluents in which this solute was retained most strongly (*e.g.*, distilled water, 253; 0.1 *M* acetic acid, 258; 0.05 *M* formic acid, 255), but was significantly lower for 5 *M* acetic acid (194) and for 0.1 *M* acetic acid–ethanol (10% ethanol, 212; 30% ethanol, 158; 50% ethanol, 135). The column did not suffer any appreciable changes in sorption capacity when applied in different solvents. For example, the decrease in the distribution coefficients in 1 m*M* hydrochloric acid after a column had been used continuously for 2 weeks with 10–50% ethanol was less than 2%.

The method is suitable for the quantitative analysis of complex mixtures of aromatic compounds. With compounds that were well separated, the peak areas were reproducible within ±2%.

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