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CHROMATOGRAPHY OF AROMATIC ACIDS, ALDEHYDES AND PHENOLS ON PELLICULAR POLYAMIDE IN AQUEOUS SOLUTION

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SUMMARY

The retention volumes of a large number of aromatic compounds in acetic acid and dilute hydrochloric acid were determined on two pellicular polyamide resins. With carboxylic acids, ion exchange is an important sorption mechanism in dilute eluents. Hydrogen bonding of the hydroxyl proton to the carbonyl group in the polyamide is the dominant sorption mechanism for non-electrolytes that contain hydroxyl groups. Intramolecular hydrogen bonding decreases the sorption of *ortho*-substituted solutes containing hydroxyl groups. Several solutes were separated within a few minutes but greater separation factors were required than in experiments with small, uniform beads.

INTRODUCTION

Polyamides have previously been applied to the separation of aromatic compounds¹, but very little work has been carried out using aqueous solutions. In many practical analyses, the use of an aqueous medium is a great advantage. The purpose of this work was to study separations in aqueous solutions on two pellicular polyamides chemically linked to an inert core and to compare the retention data with those obtained in a previous study of polyvinylpyrrolidone (PVP) under similar conditions².

EXPERIMENTAL

One of the pellicular polyamides (Pellidon, Reeve Angel, London, Great Britain) was made from adipic acid and hexamethylenediamine. The amount of polyamide on the beads was 1.5% (calculated from the nitrogen content). The other polyamide, prepared from caprolactam (Perisorb PA-6) was a development product kindly supplied by E. Merck, Darmstadt, G.F.R. The amount of polyamide was 8%.

The test substances (0.01–1.5 μg) were applied to jacketed glass columns (1000 \times 2.2 mm) filled by dry packing and aqueous solutions of acetic or hydrochloric acid were used as eluent. The absorbance was recorded at 254 nm with a Chromatronix Model 220 monitor. The flow-rate was 1.2 ml/min, corresponding to

TABLE I

ADJUSTED RETENTION VOLUMES (CALCULATED IN COLUMN VOLUMES) RECORDED IN HYDROCHLORIC ACID AND ACETIC ACID

Compound	Perisorb PA-6		Pellidon	
	0.001 M HCl	0.1 M HAc	0.001 M HCl	0.1 M HAc
Benzene	0.50	0.48	0.09	0.09
Naphthalene	8.96	8.29	2.87	2.55
Phenol	0.84	0.78	0.16	0.17
1,2-Dihydroxybenzene	0.77	0.71	0.13	0.14
1,3-Dihydroxybenzene	1.50	1.35	0.30	0.27
1,4-Dihydroxybenzene	0.93	0.88	0.17	0.14
1,3,5-Trihydroxybenzene	2.53	2.36	0.50	0.50
2-Hydroxytoluene	1.53	1.42	0.37	0.32
3-Hydroxytoluene	1.64	1.49	0.38	0.35
4-Hydroxytoluene	1.57	1.42	0.39	0.35
2,4-Dimethyl-1-hydroxybenzene	2.93	2.73	0.87	0.74
1,2-Dimethyl-4-hydroxybenzene	2.96	2.86	0.82	0.72
1,3-Dimethyl-2-hydroxybenzene	2.01	1.86	0.52	0.44
1-Hydroxy-2,4,5-trimethylbenzene	5.92	5.61	1.78	1.56
1-Ethyl-2-hydroxybenzene	2.58	2.67	0.78	0.72
1-Ethyl-3-hydroxybenzene	2.88	2.69	0.81	0.74
1-Ethyl-4-hydroxybenzene	2.72	2.54	0.77	0.70
2-Methoxyphenol	0.61	0.58	0.09	0.09
3-Methoxyphenol	1.43	1.30	0.32	0.27
4-Methoxyphenol	0.95	0.87	0.19	0.16
2,6-Dimethoxyphenol	0.48	0.45	0.07	0.06
2-Methoxy-4-propylphenol	3.53	3.24	1.06	0.91
4-Allyl-2-methoxyphenol	2.48	2.36	0.75	0.64
Methoxybenzene	0.67	0.61	0.14	0.13
1,2-Dimethoxybenzene	0.47	0.44	0.08	0.07
1,3-Dimethoxybenzene	1.06	0.93	0.26	0.24
1,4-Dimethoxybenzene	0.86	0.84	0.18	0.16
1,2,3-Trimethoxybenzene	0.42	0.44	0.05	0.05
1,3,5-Trimethoxybenzene	2.00	1.75	0.49	0.43
Benzoic acid	2.0	2.4	0.6	0.9
2-Hydroxybenzoic acid	> 40	> 40	18	> 40
3-Hydroxybenzoic acid	5.0	5.6	1.5	2.1
4-Hydroxybenzoic acid	5.4	4.8	1.3	1.4
2,5-Dihydroxybenzoic acid	> 50	> 50	18	> 50
4-Hydroxy-3-methoxybenzoic acid	4.1	3.8	0.9	1.1
Benzaldehyde	0.44	0.42	0.05	0.02
2-Hydroxybenzaldehyde	0.79	0.75	0.15	0.14
3-Hydroxybenzaldehyde	1.35	1.22	0.26	0.23
4-Hydroxybenzaldehyde	1.78	1.54	0.36	0.29
2,4-Dihydroxybenzaldehyde	3.37	3.12	0.85	0.70
4-Hydroxy-3-methoxybenzaldehyde	1.38	1.22	0.27	0.23
1,3-Dihydroxynaphthalene	43.8	39.8	13.5	12.2
2,7-Dihydroxynaphthalene	41.0	32.8	9.66	8.8
2,6-Di- <i>tert.</i> -butylphenol	> 50	> 50	> 50	> 50
Benzoquinone	0.10	0.08	0.00	0.00
Benzyl alcohol	0.31	0.30	0.02	0.03
2-Phenylethanol	0.33	0.36	0.02	0.03
3-Phenyl-1-propanol	0.63	0.64	0.10	0.10

a nominal linear flow-rate of 100 cm/min (in the empty part of the column). Variations within the range 50–100 cm/min had no detectable influence on the peak elution volume. The sharpest peaks were recorded at the lowest flow-rate. The reproducibility of the retention volumes of the non-electrolytes was within $\pm 2\%$ when the columns had been used continuously for 2 weeks. For benzoic acids, the results were affected by small variations in eluent composition, and the reproducibility was poor at the lowest eluent concentration, with deviations from the mean of up to $\pm 5\%$.

RESULTS AND DISCUSSION

Influence of the composition of the eluent

The adjusted retention volumes of all solutes in 1 mM hydrochloric acid at 30 °C are listed in Table I. All values were much higher with Perisorb than with Pellidon because of its higher content of polyamide. However, when the distribution coefficients are calculated on the weight of polyamide, the order is reversed. Experiments with Perisorb in 0.02 M hydrochloric acid showed that the elution volume of non-electrolytes was unaffected, whereas the carboxylic acids appeared earlier at the higher hydrochloric acid concentration (Table II). The stronger hydroxybenzoic acids with a hydroxyl group at the C-2 position were more affected than the weaker acids, which exhibit a low degree of dissociation in 1 mM hydrochloric acid. Evidently, ion exchange is an important sorption mechanism in the applied medium unless the pH is kept so low that the solutes are undissociated.

TABLE II

ADJUSTED RETENTION VOLUMES (CALCULATED IN COLUMN VOLUMES) IN HYDROCHLORIC ACID ON PERISORB PA-6

Compound	0.001 M HCl	0.02 M HCl	1 M NaCl in 0.001 M HCl
Benzene	0.50	0.49	0.78
Phenol	0.84	0.84	1.25
Benzoic acid	2.0	1.47	2.02
2-Hydroxybenzoic acid	>40	3.17	1.96
3-Hydroxybenzoic acid	5.0	3.88	5.32
2,5-Dihydroxybenzoic acid	>50	6.48	3.56

In order to confirm this conclusion, 1 M sodium chloride solution in 1 mM hydrochloric acid was used as eluent. The results included in Table II show that benzene and phenol exhibited an increased retention, which can be attributed to salting-out effects. For 2-hydroxybenzoic and 2,5-dihydroxybenzoic acids, which are dissociated to a high degree, the retention decreased markedly. This result confirms that for benzoic acids with a hydroxyl group in the *ortho*-position, ion exchange is of great importance. Benzoic and 3-hydroxybenzoic acids were only slightly affected by the presence of sodium chloride. Evidently, the salting-out and the effect of an increased concentration of chloride ions, which compete for the ion-exchange sites, were equally important under the conditions used.

It is noteworthy that in 1 mM hydrochloric acid, where ion exchange is an

important factor, 2-hydroxybenzoic acid was retained much more strongly than 3-hydroxybenzoic acid, and that this order was reversed at higher hydrochloric acid concentrations and in the presence of sodium chloride. In the latter media, the elution order was the same as for the other benzene derivatives containing polar substituents in *ortho*- and *meta*-positions (*cf.*, Table I).

The retention in 0.1 *M* acetic acid was recorded for all solutes (Table I) and it can be seen that the values were the same as in 1 *mM* hydrochloric acid or lower for all species except some of the carboxylic acids that were held more strongly in 0.1 *M* acetic acid although the pH was lower. This result can be ascribed to a lower ion-exchange affinity of acetate ions than that of chloride ions, as demonstrated with various types of anion exchangers. The high affinity of chloride ions for the polyamides was demonstrated in experiments with water as eluent. The adjusted retention volume on Pellidon (calculated in column volumes) was 3.5 for hydrochloric acid compared with 0.2 for acetic acid. The corresponding values for Perisorb were 16.8 and 0.4, respectively.

TABLE III

ADJUSTED RETENTION VOLUMES (CALCULATED IN COLUMN VOLUMES) IN ACETIC ACID ON PERISORB PA-6

Compound	0.001 <i>M</i> <i>HAc</i>	0.1 <i>M</i> <i>HAc</i>	1 <i>M</i> <i>HAc</i>
Benzene	0.50	0.48	0.48
Naphthalene	8.52	8.29	5.44
Phenol	0.85	0.78	0.67
1,3-Dihydroxybenzene	1.51	1.35	1.01
3-Hydroxytoluene	1.62	1.49	1.14
2-Methoxyphenol	0.61	0.58	0.49
Methoxybenzene	0.66	0.61	0.56
1,2-Dimethoxybenzene	0.46	0.44	0.40
Benzoic acid	16.1	2.36	1.07
2-Hydroxybenzoic acid		> 50	9.57
3-Hydroxybenzoic acid		5.6	2.21
4-Hydroxybenzoic acid		4.8	2.30
2,5-Dihydroxybenzoic acid		> 50	12.5
Benzaldehyde	0.44	0.42	0.37
1,3-Dihydroxynaphthalene		39.8	19.2
2,7-Dihydroxynaphthalene		32.8	15.1
Benzoquinone	0.09	0.08	0.08

The results in Table III show that the retention volume decreased with increasing acetic acid concentration. A decreased polarity of the mobile phase may contribute to decreased sorption. With benzene and benzene derivatives that lack phenolic hydroxyl and carboxylic acid groups, the effect was fairly small. The largest effect was obtained with the carboxylic acids. This can be partly explained by a decreased contribution from ion exchange. Another factor of importance with carboxylic acids and phenols is the competition between the hydroxyl proton of acetic acid and those of the solutes for the proton-accepting carbonyl groups in the polyamide. It is likely that, in addition, hydrogen bonding between acetic acid and the carboxylic acids and

phenols affects the sorption. The low concentration dependence of solutes that can act only as proton acceptors indicates that the competition with acetic acid for the proton-donating $-NH-$ groups and the formation of association complexes with acetic acid exert a modest influence upon the retention volume.

Effect of temperature

The influence of temperature on the retention volume of some aromatic compounds on polyamides in 0.001 *M* hydrochloric acid is shown in Table IV. It can be seen that the sorption of phenol and benzoic acid decreased markedly with increasing temperature. The enthalpy changes were more negative than those observed with PVP, for which the predominant exothermic reaction is the formation of a hydrogen bond between the hydroxyl proton and the stationary phase². In agreement with the results obtained with PVP, the compounds that contain two or more hydroxyl groups (*cf.* Table I) were retained much more strongly and exhibited more negative enthalpy changes than phenol and benzoic acid. These results indicate strongly that the principal mechanism for the sorption of phenols and benzoic acids on polyamides is the formation of hydrogen bonds between the carbonyl oxygen in the stationary phase and the hydroxyl protons in phenolic and carboxylic groups in the solutes.

Benzene, benzaldehyde and methoxybenzene, which lack proton-donating groups, exhibited very low retention volumes on PVP. The enthalpy changes were small and positive, indicating that the sorption should be ascribed to an increase in entropy. Similar effects have been observed with other sorbents in aqueous medium and have been attributed to hydrophobic interactions or more specifically to the influence of hydrophobic solutes on the water structure³. This water structure-enforced sorption should be of importance also with polyamides as stationary phases. It can be seen from Table IV that with Perisorb, ΔH° for benzene was virtually zero. Its degree of sorption was fairly high on this polyamide, which must be ascribed to an increase in entropy. In contrast, benzaldehyde and methoxybenzene exhibited negative ΔH° values. Evidently, sorption mechanisms other than the water structure-enforced sorption have an influence. Other attraction forces that might be of importance with the polyamides are those between the $-NH-$ group and the π -electrons in the aromatic nuclei. With methoxybenzene and benzaldehyde, hydrogen bonding between the oxygen and the proton-donating $-NH-$ group may contribute to the negative ΔH° . It is likely that such interactions contribute to the differences between PVP and polyamide.

With Pellidon, the retention volumes of several compounds were so low that the experimental errors involved in the determination of ΔH° were larger than with Perisorb and PVP. The observation that the sorption of benzene decreases with increasing temperature on Pellidon whereas it increases with PVP and is virtually unaffected on Perisorb cannot be ascribed to experimental error. These observations suggest that the relative importance of the water structure-enforced interactions decreases in the order PVP > Perisorb > Pellidon. The different behaviour of these three phases with aromatic hydrocarbons was confirmed by the enthalpy changes observed for naphthalene, which, at 323 °K, were -0.9 with PVP, -3.7 with Perisorb and -4.6 with Pellidon. The large differences between PVP and the polyamides may be ascribed to interactions between the strongly electron-attracting $-NH-$ group in the polyamide and the π -electrons of the solute.

TABLE IV
ADJUSTED RETENTION VOLUMES (V'_R) (CALCULATED IN COLUMN VOLUMES) AND THERMODYNAMIC FUNCTIONS FOR AROMATIC COMPOUNDS ON PERISORB PA-6 AND PELLIDON

Compound	Temperature (°K)	Perisorb PA-6		Pellidon			
		V'_R	ΔH° (kcal/mole)	$\Delta \Delta S^\circ$ (cal/mole-deg)	V'_R	ΔH° (kcal/mole)	$\Delta \Delta S^\circ$ (cal/mole-deg)
Benzene	303	0.48			0.10		
	323	0.47	0.0	6.8	0.08	-2.7	5.5
	343	0.48			0.06		
Naphthalene	303	8.43			2.97		
	323	6.16	-3.7	0.5	1.89	-4.6	6.0
	343	4.21			1.23		
Benzaldehyde	303	0.44			0.06		
	323	0.37	-1.5	1.7	0.04	-5.9	-5.8
	343	0.33			0.02		
Methoxybenzene	303	0.66			0.15		
	323	0.59	-1.5	2.6	0.11	-4.1	2.0
	343	0.50			0.07		

1,3,5-Trimethoxybenzene	303	1.84	-3.2	-1.2	0.46	-4.0	4.1
	323	1.37			0.29		
	343	0.99			0.21		
Phenol	303	0.82	-2.4	0	0.17	-4.7	0
	323	0.67			0.12		
	343	0.52			0.07		
Benzoic acid	303	1.97	-2.1	2.5	0.61	-2.6	9.3
	323	1.64			0.48		
	343	1.31			0.37		
1,3-Dihydroxybenzene	303	1.44	-3.9	-3.9	0.29	-5.5	-1.8
	323	0.98			0.17		
	343	0.68			0.10		
4-Hydroxy-3-methoxybenzoic acid	303	3.63	-4.6	-4.5	0.90	-5.0	2.1
	323	2.23			0.53		
	343	1.48			0.34		
3-Hydroxybenzoic acid	303	4.89	-4.3	-2.8	1.44	-4.3	5.3
	323	3.18			0.91		
	343	2.13			0.62		

* ΔAS° calculated with phenol as reference substance (see ref. 2).

Relationship between the structure of the solutes and the retention data

Of the solutes studied, benzoquinone was only slightly retarded, which confirms that the effect of the aromatic ring is considerable and indicates that hydrogen bonding between the $-NH-$ groups in the stationary phase and oxygen in the solutes is a small effect in aqueous medium. The low retention volumes recorded for benzaldehyde and methoxybenzene support this conclusion.

The statement that hydrogen bonding between the carbonyl group in the polyamide and phenolic and carboxylic hydroxyl groups is the major sorption mechanism for solutes that contain these groups is supported by the observation that the introduction of electronegative substituents (aldehyde and methoxyl groups in *meta*- and *para*-positions) resulted in a marked increase in the retention volume of both phenol and benzoic acid. In agreement with the results obtained with PVP, the introduction of these groups in *ortho*-positions in phenol had an opposite effect, which can be explained by the formation of intramolecular hydrogen bonds. With 2,6-dimethoxyphenol, in which two proton-accepting groups can participate in intramolecular hydrogen bonding, the retention volume was even lower than that for benzene. With this compound, the steric conditions are unfavourable for hydrogen bonding with the polyamide. Similarly 1,3-dimethyl-2-hydroxybenzene is held less strongly than its isomers. All of these observations show that with phenols, hydrogen bonding of the hydroxyl proton is a very important sorption mechanism and that intramolecular hydrogen bonding of the hydroxyl proton decreases the sorption markedly. The lower retention of 1,2-dihydroxybenzene compared with that of the isomers supports this conclusion.

It can therefore be predicted that benzoic acid and especially the hydroxybenzoic acids should be held more strongly than phenol. The results in Table I confirm this conclusion. Again, the compound with the phenolic hydroxyl group in the *ortho*-position was held less strongly than the isomers (under conditions where dissociation of the acid is negligible).

The aromatic alcohols exhibited much lower retention volumes than phenol. This result was to be expected, as the introduction of methylene groups between the hydroxyl group and the aromatic ring decreases the electron-attracting power of the aromatic ring and therefore also the strength of the hydrogen bond between the hydroxyl group and proton-accepting groups in the adsorbents. 3-Phenyl-1-propanol is retained more strongly than benzyl alcohol and 2-phenylethanol on both PVP and the polyamides. Non-polar contributions from the three adjacent methylene groups are responsible for the increased sorption.

The introduction of methyl groups on the aromatic ring in phenol resulted in a larger increase in the retention relative to phenol on both polyamide phases than on PVP. Except for 1,3-dimethyl-2-hydroxybenzene, which, for steric reasons, is held less strongly than its isomers, the position of the non-polar substituent had little effect in comparison with the effect of the position of polar substituents. In principle, owing to induction, the introduction of a methyl group will lead to an increased electron density in the aromatic ring, which will decrease the ability of the phenolic hydroxyl group to donate its proton but increase the ability of the compound to serve as an electron donor and for this reason interact with electron-attracting groups in the stationary phase ($-NH-$ in polyamides). Moreover, the presence of methyl groups will increase different types of non-polar interactions, including dispersion effects

and water structure-enforced interactions. It is worth mentioning that in non-polar solvents, hydroxytoluenes are held less strongly than phenol by Pellidon, indicating that under these conditions the hydrogen bonding of the phenolic hydroxyl group to the carbonyl oxygen in the stationary phase is the predominant mechanism¹. Evidently, the contributions of other interactions are much greater in aqueous medium.

The importance of non-polar substituents in aqueous solution is illustrated by the effect of the introduction of ethyl groups in phenol and of 4-propyl or 4-allyl groups in 2-methoxyphenol. The increase in the relative retention was much greater than that observed with PVP. As expected from these results 2,6-di-*tert.*-butylphenol could not be eluted with a reasonable amount of the aqueous eluent.

Analytical applications

The great advantage of pellicular stationary phases is that elution can be carried out much more rapidly than with crushed resin particles and, in certain separations, more rapidly than with beads of small particle size. The chromatograms shown in Fig. 1 show that four solutes were well separated in 18 min on Perisorb with 0.1 *M* acetic acid as eluent. The fifth compound overlapped but the separation was sufficiently good for a quantitative estimate to be made. With 1 *mM* hydrochloric acid, the last two compounds overlapped seriously.

The results show that pellicular polyamides can be used advantageously in the rapid separation of solutes that exhibit large separation factors, including certain isomers. Of the compounds included in the run referred to in Fig. 1, the separation factor for benzoic acid–4-hydroxy-3-methoxybenzoic acid was 1.60 in 0.1 *M* acetic acid. The overlapping was negligible. For the last two compounds, which overlapped to an appreciable extent, the separation factor was 1.48 and the corresponding value in hydrochloric acid was 1.28. The separation factors required for efficient separations on the pellicular polyamides are thus much greater than those required with columns filled with resin beads of a small and uniform particle size⁴. This can be ascribed to the large particle diameter (about 40 μm) of the pellicular materials and the mixing of the mobile phase in the relatively large space between the beads.

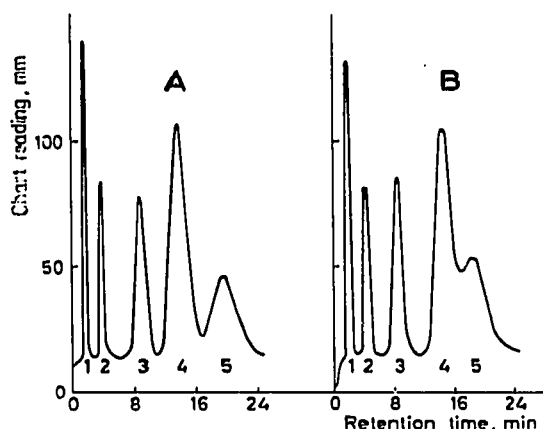


Fig. 1. Separation of (1) benzoquinone, (2) phenol, (3) benzoic acid, (4) 4-hydroxy-3-methoxybenzoic acid and (5) 3-hydroxybenzoic acid in (A) acetic acid and (B) hydrochloric acid on Perisorb PA-6 at 30 °C. Column: 1000 × 2.2 mm. Nominal linear flow-rate: 83 cm/min.

When the same compounds were chromatographed in 1 mM hydrochloric acid on Pellidon under otherwise unchanged conditions, the last compound was eluted after 12 min. The peaks corresponding to the three carboxylic acids exhibited appreciable overlapping. In 0.1 M acetic acid, four well separated peaks were recorded. The third peak, which exhibited a distorted front, contained both benzoic and 4-hydroxy-3-methoxybenzoic acids. The results confirm that large separation factors are required in order to achieve clean separations. Despite this limitation, the results show that many important separations can be carried out on pellicular polyamides in aqueous media.

As can be seen from Tables II and III, the retention volumes are affected by changes in the composition of the eluent. For reasons already mentioned, the largest effects are obtained with the carboxylic acids. Advantage can be taken of these effects so as to improve the separation factors in some systems and especially to speed up the elution of solutes held strongly in dilute acid. Stepwise or gradient elution with an increasing concentration of acetic acid can be used advantageously when solutes such as hydroxybenzoic acids and hydroxynaphthalenes are involved.

In analyses of solutions containing several carboxylic acids, it was found that serious overlapping occurred, *e.g.*, of 2-hydroxybenzoic and 2,5-dihydroxybenzoic acids in acetic acid (1–5 M). At the highest concentration these solutes were displaced very rapidly but the overlapping was worse than in 1 M solution. As 2-hydroxybenzoic acid appeared first, although it is the stronger acid, it was expected that it should be possible to achieve a clean separation in 1 M acetic acid containing hydrochloric acid to depress the dissociation of these comparatively strong acids. As ex-

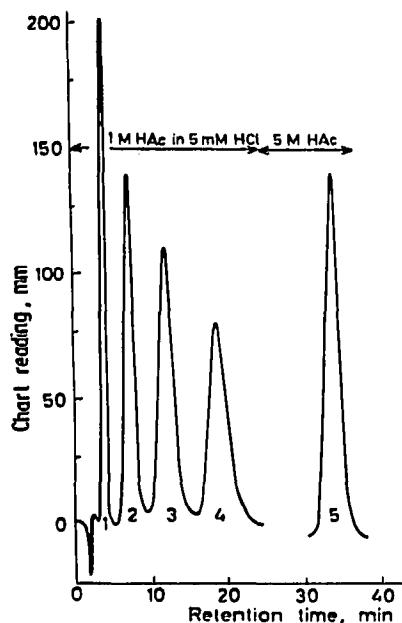


Fig. 2. Separation of (1) phenol, (2) 4-hydroxy-3-methoxybenzoic acid, (3) 2-hydroxybenzoic acid, (4) 2,5-dihydroxybenzoic acid and (5) 1,3-dihydroxynaphthalene on Perisorb PA-6 at 30 °C. Column: 1000 × 2.2 mm. Nominal linear flow-rate: 83 cm/min.

pected, both acids appeared earlier with this eluent and, as can be seen from Fig. 2, the acids were well separated and eluted within 40 min. After 35 min, the eluent was changed from 0.005 *M* hydrochloric acid in 1 *M* acetic acid to 5 *M* acetic acid in order to effect a rapid elution of 1,3-dihydroxynaphthalene, which was held much more strongly than the other four solutes in this run. By means of this precaution, the last solute was eluted completely within 60 min, whereas in a run without a change in the composition of the eluent, 90 min were required.

ACKNOWLEDGEMENT

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