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CHROMATOGRAPHIC BEHAVIOUR OF AROMATIC COMPOUNDS ON NON-POLAR PHASES WITH ADSORBED QUATERNARY AMMONIUM SALTS

BO F. NILSSON and OLOF SAMUELSON*

Department of Engineering Chemistry, Chalmers University of Technology, S-412 96 Göteborg (Sweden)

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SUMMARY

Hexadecyltrimethylammonium (HDTMA) acetate and glycolate were strongly adsorbed from 1 m*M* solution on ODS-Hypersil (silica gel with octadecyl groups) and on Hitachi gel (porous styrene–divinylbenzene copolymer) in aqueous sodium acetate or glycolate solution. The presence of HDTMA favoured the sorption of aromatic carboxylate anions, while their retention was suppressed by increasing the concentration of the sodium salts. Under comparable conditions the presence of HDTMA had no significant effect on the sorption of the aromatic anions onto a commerical anion-exchange resin of the quaternary ammonium type (Dowex 1-X8), indicating that the ion pairing in the external solution was negligible. The results show that, in the presence of HDTMA, the non-ionic phases were converted into anion exchangers, and that ion exchange had a dominant effect on the retention of the benzoates and phenylacetates studied. Hydrophobic interactions contributed markedly to the sorption of the hydrophobic anions and phenols, while, for both types of solutes, hydrogen bonding between phenolic protons and the stationary phases was less important than with Dowex 1-X8.

INTRODUCTION

In a previous paper[1] the retention volumes were determined for phenols and aromatic carboxylate anions on a styrene–divinylbenzene anion exchanger containing quaternary ammonium groups (Dowex 1-X8), in solutions of sodium salts of simple aliphatic acids. Non-polar interactions of alkyl groups, and hydrogen bonding between phenolic hydroxyl protons and the aliphatic carboxylate anions in the resin, led to increased retention volumes of both ions and non-electrolytes, while intramolecular hydrogen bonding suppressed the hydrogen bonding with the resin. We here report on the sorption of the same solutes on non-polar stationary phases in aqueous sodium acetate and glycolate solutions containing hexadecyltrimethylammonium (HDTMA) ions. A porous copolymer, Hitachi gel 3011, with a chemical composition (styrene–divinylbenzene) similar to that of the matrix in the anion-exchange resin and a silica gel with covalently linked octadecyl groups (ODS-Hypersil) were used as column packings.

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Chromatography on non-polar phases in eluents containing ionic long-chain alkyl compounds has recently been reviewed[2,3]. Ion-pair chromatography and dynamic ion-exchange chromatography are among the names commonly used for related techniques. As shown below, ion-pair formation in the external solution is of little importance in the systems studied in the present work. Chromatography is inherently a dynamic process. We feel that the term "chromatography on sorbed ionic sites" would be better, and that the term "ion-pair chromatography" should be used only when ion pairing in the external solution is the predominant reason for the retention of the solutes.

EXPERIMENTAL

The jacketed stainless-steel columns (100 × 5 mm I.D.) were thermostatted by circulating water. ODS-Hypersil (5 μm; Shandon Southern, Runcorn, Great Britain) was packed according to the procedure recommended by Aslin[4]. The Hitachi gel (12 ± 5 μm; Hitachi, Tokyo, Japan) was kept in methanol. The solvent was displaced by water. The resin slurry was transferred to a reservoir attached to the column, and the column was packed by pumping distilled, boiled-out water through the system at 30 MPa.

Commercial hexadecyltrimethylammonium (HDTMA) chloride was converted into the acetate or glycolate by passing a 20% solution of it through a column packed with a polystyrene–divinylbenzene adsorbent, Amberlite XAD-2 (150 ± 30 μm; Rohm & Haas, Philadelphia, PA, U.S.A.). The column was then treated with ten bed volumes of 1 *M* sodium acetate (or glycolate) solution followed by five bed volumes of water. The acetate or glycolate form of HDTMA was eluted with five bed volumes of 92% (w/w) aqueous ethanol. Ethanol was removed by evaporation under reduced pressure and the sample dried under vacuum before weighing. The salts, which were free from chloride, were dissolved in water and stored as 10% (w/w) aqueous solutions.

The aromatic compounds were dissolved in distilled, boiled-out water and applied to the column, which was preconditioned with the eluent. Sparingly soluble acids were added as their sodium salts. To determine the retention volume, single compounds were chromatographed and the UV-absorbance was measured at 254 and 280 nm. The reported retention data were obtained with well separated mixtures of three to six compounds. The sample solutions (20 μl) were introduced through a sample loop. The nominal linear (empty tube) flow-rate was 5.1 cm min^{-1}.

Repeated runs under identical conditions showed that the retention volumes on both columns were reproducible within ± 1% for compounds eluted within less than 50 bed volumes. For compounds which appeared very late as broad peaks, the reproducibility was within ±2%. In the experiments referred to in Tables II and III, the loaded amounts were 0.20 μmol of each compound. Variations between 0.10 and 0.40 μmol had no significant effect on the retention volume.

The retention volumes are reported as $\bar{v}/X$, where $\bar{v}$ is the peak elution volume and X the bed volume. Some compression of the column packings occurred during prolonged use. This was disregarded when calculating the bed volume. The space at the top of the columns was filled with a thin layer of glass beads. The difference between ln $\bar{v}/X$ for a substituted compound, *e.g.*, methylphenol, and the parent compound (phenol) in the same medium is denoted by Δ. The breakthrough curves of HDTMA were determined for solutions of sodium glycolate and sodium acetate. The curves were recorded with a refractive index detector, and the results checked by colorimetric tests based on addition of potassium permanganate at pH 3 and extraction with chloroform[5].

RESULTS AND DISCUSSION

Influence of the HDTMA concentration

Like the experiments with Dowex 1-X8 reported previously[1], those with the stationary phases reported here were made at pH 7, which means that the aromatic carboxylic acids are almost completely ionized, while the phenols can be considered as non-electrolytes. The comparison between the results obtained in these investigations is facilitated by the fact that the distribution coefficients on Dowex 1-X8 were only slightly affected when 1 m*M* HDTMA was present. For 4-hydroxyphenol the distribution coefficient decreased by 1% compared to a blank without HDTMA, while an increase of 3% was recorded for 4-hydroxy-3-methoxybenzoate and 1% for 4-hydroxy-3,5-dimethoxyphenylacetate. Evidently, ion-pair formation in the eluent has no decisive influence on the sorption in this system. The experiments were made at 50°C, and, in agreement with results reported for other solutes, the distribution coefficients were higher than those at 60°C reported in the previous paper[1].

As shown in Fig. 1, the retention volumes for aromatic carboxylate anions in 0.5 *M* sodium acetate were much higher when HDTMA was present than in its absence. On ODS-Hypersil the highest values were obtained at a concentration of 0.1 mmol HDTMA per litre of eluent, while a slight, but significant, decrease was observed at the ten-fold concentration (*cf.*, ref. 6). For the Hitachi gel an asymptote was approached in 0.25 m*M* HDTMA solution. No further change was obtained by an increase to 1 m*M* HDTMA.

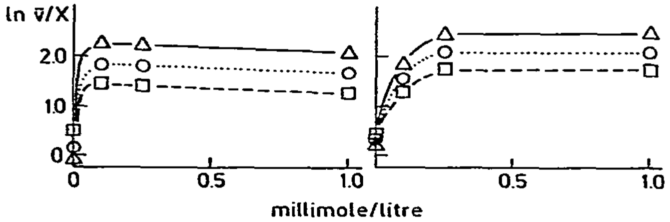

Fig. 1. Relationships between ln $\bar{v}/X$ and concentration of HDTMA in 0.5 *M* sodium acetate at pH 7 and 50°C on ODS-Hypersil (left) and Hitachi gel (right). △, 4-Hydroxybenzoate; ○, 4-hydroxy-3-methoxybenzoate; □, 4-hydroxy-3,5-dimethoxybenzoate.

In the absence of quaternary ammonium ions, 4-hydroxybenzoate was, on both stationary phases, held less strongly than the other anions studied. This was expected since this anion is more hydrophilic than the other anions. 4-Hydroxy-3-methoxybenzoate, with the phenolic proton linked to the vicinal methoxyl group by hydrogen bonding, is less hydrophilic. Accordingly, this anion exhibited a larger retention volume on the non-ionic stationary phases. More effective hydrogen bonding, and shielding of the hydroxyl group by two adjacent methoxyl groups, explain the strong sorption of 4-hydroxy-3,5-dimethoxybenzoate.

The elution order was reversed when HDTMA was present in the eluent, and was the same as the order previously established for conventional anion-exchange resins in acetate and glycolate media. The results indicate that, in the presence of HDTMA, the stationary phases constitute anion exchangers[7,8] with an ability to bind the carboxylate ions as counter ions.

The effect of the HDTMA concentration was less dramatic for the non-ionized phenolic compounds than for the anions (Fig. 2). A large increase in the retention volume was observed for 4-hydroxyphenol, which is held by hydrogen bonding to acetate counter ions in anion exchangers[1]. The smallest effect of HDTMA, and at the same time the largest adsorption on both stationary phases in the presence and absence of quaternary ammonium ions, was observed for 2-methoxyphenol. Intramolecular hydrogen bonding, resulting in increased hydrophobic interactions and a decreased ability to enter into hydrogen bonding with the stationary phases, explains both the high retention volumes and the small influence of HDTMA. The larger retention volume of 4-hydroxy-3-methoxyphenol compared to 4-hydroxyphenol, in the absence of HDTMA, is also attributed to intramolecular hydrogen bonding leading to increased hydrophobic interactions. The reverse of this order, in the presence of HDTMA, is explained by a less effective hydrogen bonding to the stationary phase for 4-hydroxy-3-methoxyphenol compared with 4-hydroxyphenol.

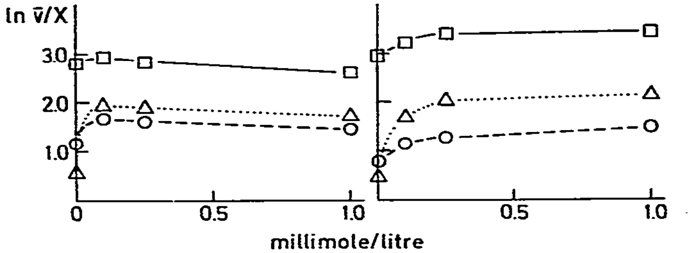

Fig. 2. Relationship between ln $\bar{v}/X$ and concentration of HDTMA in 0.5 M sodium acetate at pH 7 and 50°C on ODS-Hypersil (left) and Hitachi gel (right). □, 2-Methoxyphenol; △, 4-hydroxyphenol; ○, 4-hydroxy-3-methoxyphenol.

Adsorption of HDTMA

The adsorption of HDTMA, under the conditions used for the determinations of the retention data for the aromatic compounds, was calculated from the breakthrough curves recorded on the same columns after displacement of HDTMA. Gradient elution with methanol, 0–100%, was used for the displacement from the ODS-Hypersil column, while ethanol, 0–30% (w/w), was used for the Hitachi gel. The columns were then conditioned with sodium acetate (or glycolate) solution free from HDTMA. The breakthrough curves were recorded at influent compositions identical to the eluent compositions in most experiments with aromatic compounds.

Fig. 3 shows the breakthrough curves for ODS-Hypersil at an influent concentration of 1 mM HDTMA. The adsorption on both phases, calculated from the curves, is given in Table I, which shows that HDTMA was retained more strongly than any of the aromatic compounds studied in the present work. Very sharp breakthrough curves were obtained, indicating that the adsorption equilibrium was ap-

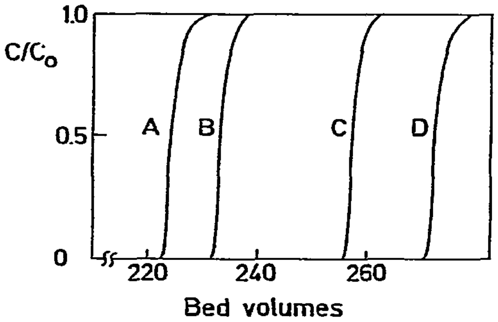

Fig. 3. Breakthrough curves for 1 mM HDTMA in acetate and glycolate solutions on ODS-Hypersil at pH 7 and 50°C. A, 0.3 M sodium glycolate; B, 0.5 M sodium glycolate; C, 0.3 M sodium acetate; D, 0.5 M sodium acetate.

proached rapidly. As can be seen, in 0.5 M sodium acetate the amount of HDTMA per ml of the bed volume was 272 times the amount present in 1 ml of the external solution. The adsorbed amount decreased only slightly when the eluent concentration was lowered by a factor of 10. Hence, the number of quaternary ammonium ions calculated per ml of the bed (which is equal to the ion-exchange capacity) was only slightly dependent on the concentration in the external solution. The capacity approaches that of conventional strongly basic anion-exchange resins of the styrene–divinylbenzene type having a low proportion of divinylbenzene. The adsorption increased slightly with increasing concentration of acetate or glycolate in the external solution. While the aromatic compounds (in the presence of HDTMA) were retained more strongly on the Hitachi gel than on ODS-Hypersil, the opposite was found for HDTMA in both acetate and glycolate solutions.

Influence of eluent concentration

Previously published reports on the effect of salt addition to eluents containing amines[9] or quaternary ammonium ions[10] show that electrolytes suppressed the retention of aromatic carboxylate anions. At high concentrations the effect became negligible[9]. For all aromatic carboxylate anions studied on ODS-Hypersil and the Hitachi gel in the present work (Tables II and III), the retention volume in aqueous solution

TABLE I

ADSORPTION OF HDTMA ON ODS-HYPERSIL AND HITACHI GEL FROM AQUEOUS SOLUTIONS CONTAINING 1 mM HDTMA

External solution	*Adsorbed amount (μmol per ml bed volume)*	
	ODS-Hypersil	*Hitachi gel*
Sodium acetate, 0.3 M	257	180
Sodium acetate, 0.5 M	272*	185
Sodium glycolate, 0.3 M	224	159
Sodium glycolate, 0.5 M	233	167

* In 0.1 mM HDTMA the adsorbed amount was 255 μmol ml^{-1}.

TABLE II

RETENTION DATA ON ODS-HYPERSIL FOR PHENOLS, BENZOATES AND PHENYLACETATES AT 50°C AND pH 7.0 IN ACETATE AND GLYCOLATE SOLUTIONS WITH ADDITIONS OF HDTMA (1 m*M*)

Parent compounds and substituents	*Acetate, 0.3 M*		*Acetate, 0.5 M*		*Glycolate, 0.3 M*		*Glycolate, 0.5 M*		*Acetate, 0.25 M**		*Acetate, 0.5 M**	
	$\bar{v}/X$	Δ	$\bar{v}/X$	Δ	$\bar{v}/X$	Δ	$\bar{v}/X$	Δ	$\bar{v}/X$	Δ	$\bar{v}/X$	Δ
Phenol	16.7		19.2		14.5		16.0		4.34		4.37	
4-Methyl	44.0	0.96	47.7	0.91	37.2	0.95	40.5	0.93	9.40	0.77	9.37	0.76
4-Hydroxy	4.9	−1.23	5.5	−1.25	3.6	−1.40	3.8	−1.44	1.37	−1.15	1.40	−1.14
4-Methoxy	13.3	−0.23	14.5	−0.28	11.4	−0.24	12.6	−0.24	3.08	−0.34	3.11	−0.34
2-Methoxy	12.5	−0.29	13.5	−0.35	12.0	−0.19	12.6	−0.24	3.24	−0.29	3.25	−0.30
4-Hydroxy-3-methoxy	3.8	−1.48	4.2	−1.51	3.1	−1.54	3.4	−1.56	1.21	−1.28	1.23	−1.27
Benzoate	31.1		22.5		46.1		35.3		8.82		5.34	
4-Methyl	84	0.98	64	1.04	119	0.95	96	1.00	21.6	0.89	12.7	0.86
4-Hydroxy	10.3	−1.11	7.9	−1.06	11.1	−1.42	8.4	−1.44	3.17	−1.02	2.03	−0.97
4-Methoxy	35.4	0.13	26.9	0.17	54	0.15	41.8	0.17	8.95	0.01	5.42	0.01
4-Hydroxy-3-methoxy	7.1	−1.48	5.3	−1.45	9.5	−1.57	7.2	−1.59	2.32	−1.34	1.60	−1.21
4-Hydroxy-3,5-dimethoxy	4.7	−1.90	3.5	−1.86	6.6	−1.95	5.0	−1.95	1.68	−1.66	1.23	−1.47
Phenylacetate	15.2		11.0		23.3		17.7		5.59		3.49	
4-Hydroxy	8.3	−0.61	5.9	−0.62	9.4	−0.91	7.0	−0.92	2.65	−0.75	1.82	−0.65
4-Methoxy	16.5	0.08	11.7	0.06	25.2	0.08	18.8	0.06	5.13	−0.09	3.27	−0.07
4-Hydroxy-3-methoxy	5.4	−1.03	3.9	−1.05	7.1	−1.20	5.3	−1.20	2.03	−1.01	1.44	−0.89
4-Hydroxy-3,5-dimethoxy	3.6	−1.44	2.8	−1.39	5.1	−1.51	3.9	−1.50	1.50	−1.32	1.11	−1.15

* Sodium acetate dissolved in 20% (w/w) methanol to a final concentration of 0.25 or 0.5 *M*.

with 1 m*M* HDTMA decreased markedly when the acetate or glycolate concentration was increased from 0.3 to 0.5 *M*. This is consistent with the observation that HDTMA cations adsorbed onto the stationary phases convert the latter to anion exchangers with acetate or glycolate counter ions. According to the law of mass action, the increased eluent concentration should in an idealized system lead to a decrease in the distribution coefficients for the aromatic anions by 40%.

The observed decrease in $\bar{v}/X$ was much less and varied between 20 and 32%. If it is assumed that the relative interstitial volume in the column was 0.4, the calculated decrease in the distribution coefficients would be 20–33%. The low values can only in part be ascribed to the increased ion-exchange capacity of the stationary phase resulting from the increased adsorption of HDTMA. On both stationary phases and in both media, 4-methylbenzoate exhibited the largest deviation from the calculated value. This shows that hydrophobic interactions exerted a great effect on the uptake of the aromatic carboxylate anions. Large deviations from ideality have previously been found in ion exchange of aromatic carboxylate ions on conventional ion-exchange resins[1]. As expected, an increased eluent concentration led to a large decrease in the retention volume also in the experiments with methanol present.

For all the phenolic compounds, the retention increased with increasing concentration of acetate or glycolate in the aqueous eluent. The effect was smaller than the salting out observed in experiments with an anion exchanger with a styrene–divinylbenzene matrix. It can be assumed that for this resin the salting-out parameter reflects the activity coefficients in the external solution. Hence, the smaller effect

TABLE III

RETENTION DATA ON HITACHI GEL FOR PHENOLS, BENZOATES AND PHENYLACETATES AT 50°C AND pH 7.0 IN ACETATE AND GLYCOLATE SOLUTIONS WITH ADDITIONS OF HDTMA (1 m*M*)

Parent compounds and substituents	*Acetate, 0.3 M*		*Acetate, 0.5 M*		*Glycolate, 0.3 M*		*Glycolate, 0.5 M*	
	$\bar{v}/X$	Δ	$\bar{v}/X$	Δ	$\bar{v}/X$	Δ	$\bar{v}/X$	Δ
Phenol	32.0		34.5		25.0		26.0	
4-Methyl	83	0.95	90	0.96	65	0.95	67	0.95
4-Hydroxy	7.9	−1.40	8.5	−1.40	5.1	−1.58	5.4	−1.57
4-Methoxy	27.5	−0.16	29.4	−0.16	20.8	−0.18	22.7	−0.14
2-Methoxy	28.8	−0.11	31.6	−0.09	25.7	0.03	26.9	0.03
4-Hydroxy-3-methoxy	3.9	−2.12	4.4	−2.06	4.7	−1.68	5.0	−1.66
Benzoate	46.5		32.8		52		37.9	
4-Methyl	122	0.96	88	0.99	129	0.91	99	0.96
4-Hydroxy	16.4	−1.04	11.6	−1.04	14.6	−1.27	10.4	−1.28
4-Methoxy	56	0.18	38.9	0.17	62	0.17	45.5	0.19
4-Hydroxy-3-methoxy	11.9	−1.36	8.2	−1.39	12.6	−1.42	9.2	−1.41
4-Hydroxy-3,5-dimethoxy	8.1	−1.74	5.6	−1.76	9.1	−1.74	6.7	−1.74
Phenylacetate	25.1		17.3		28.4		20.3	
4-Hydroxy	13.4	−0.63	9.5	−0.60	12.9	−0.79	9.2	−0.79
4-Methoxy	28.5	0.13	19.9	0.14	32.7	0.14	23.8	0.16
4-Hydroxy-3-methoxy	9.3	−0.99	6.4	−1.00	9.8	−1.07	7.1	−1.05
4-Hydroxy-3,5-dimethoxy	6.4	−1.37	4.4	−1.37	7.3	−1.36	5.5	−1.31

obtained in the systems studied in the present work indicates that the effect of the altered activity coefficients in the external solution on the distribution coefficients is suppressed by changes in the stationary phase.

In the experiments with 20% methanol in the eluent, an increase in the sodium acetate concentration from 0.25 to 0.5 *M*, had no significant influence on the retention volume of the phenolic compounds.

Experiments with the Hitachi gel in aqueous 1 m*M* HDTMA (pH 7) in the absence of sodium acetate or sodium glycolate showed that carboxylate ions were held very strongly by the resin. Hence, 4-hydroxy-3,5-dimethoxybenzoate, which exhibited very low retention volumes in the salt solutions, remained in the column after an elution volume corresponding to 120 column volumes. In contrast, the non-electrolytes appeared earlier in the eluate when no sodium salts were present. Compared to the retention volume in 0.3 *M* sodium acetate, the retention of 2-methoxyphenol was lowered by 22%. The corresponding values for 4-hydroxyphenol and 4-hydroxy-3-methoxyphenol were 61 and 59%, respectively. Hence, chromatography at a very low concentration of the eluting anion can be useful for separations of carboxylate anions from non-electrolytes.

Comparison between acetate and glycolate

Table I shows that HDTMA was held more strongly in sodium acetate than in sodium glycolate by both stationary phases studied in the present work. Hence, the ion-exchange capacity of the column packings will be higher in acetate medium, which will favour the uptake of the aromatic carboxylate ions. It can also be predicted that the sorption of the alkyl ammonium ion will depend on the activity coefficients of the counter ions. An analogous phenomenon is the adsorption of dyes onto cotton in aqueous solution. For dyes containing sulphonic acid groups the sorption is favoured by counter ions, which are held strongly by cation exchangers of the sulphonic acid type[11,12], *i.e.*, by counter ions with low activity coefficients in the gel phase. This suggests that glycolate ions present in the stationary phases exhibited larger activity coefficients than acetate ions. With regard to the activity coefficients of the competing counter ions, it can therefore be predicted that the aromatic carboxylate ions should be retained more strongly in glycolate solution than in acetate.

As shown in Table II, all aromatic carboxylate anions were held more strongly on ODS-Hypersil in glycolate than in acetate solution of the same concentration. The same was, with two exceptions, found also for the Hitachi gel. These results indicate that the direct effect of the activity coefficients of the eluent anion in the stationary phase on the ion-exchange equilibrium was more important than the indirect effect due to their influence on the sorption of the quaternary ammonium ion.

The exceptions obtained for the Hitachi gel were 4-hydroxyphenylacetate, with virtually the same retention in both eluents, and 4-hydroxybenzoate, which exhibited a slightly higher retention in acetate than in glycolate. It is noteworthy that for ODS-Hypersil the smallest differences between glycolate and acetate were observed for 4-hydroxy-substituted anions. Moreover, the most hydrophilic anions, *i.e.*, those with 4-hydroxy or 4-hydroxy-3-methoxy groups, were held more strongly by Dowex 1-X8 in acetate than in glycolate solution, while also with this resin the more hydrophobic anions exhibited higher distribution coefficients in glycolate[1]. The results indicate that the same interactions have an influence on the retention of the aromatic carboxylate anions in these systems but that their relative importance differs from one stationary phase to another.

In the presence of HDTMA all phenols were held more strongly on ODS-Hypersil in acetate solution than in glycolate. The same holds true for Dowex 1-X8 studied previously[1], and with the exception of 4-hydroxy-3-methoxyphenol, also for the Hitachi gel. One factor which would favour the sorption in acetate medium is that the acetate counter ions will render the stationary phase more hydrophobic than glycolate ions. Hydrophobic interactions are of importance even for the sorption of the more hydrophilic aromatic compounds. The results indicate, however, that other interactions, such as hydrogen bonding of the solutes to the stationary phase, are also affected by the counter ions.

Influence of substituents on retention data

Tables II and III show that in aqueous media the substituent parameter Δ for a given compound was affected only slightly when the concentration of acetate or glycolate was increased from 0.3 to 0.5 *M*. This is true both for ODS-Hypersil and for the Hitachi gel. Similarly, the Δ values were hardly affected when the eluent was changed from 0.5 to 1.0 *M* sodium acetate on Dowex 1-X8[1]. Very similar Δ values were obtained for 4-methylphenol and 4-methylbenzoate in all media and on both stationary phases. In contrast, there were large differences between the Δ values for the three compounds containing a 4-hydroxyl substituent. For these compounds, less negative values were found in acetate than in glycolate. The results show that several types of interactions are of importance for the retention volume of the solutes.

In aqueous media, the sorption of ions and non-electrolytes on ion-exchange resins increases when hydrophobic substituents are introduced in the solutes. Accordingly, Tables II and III show that strongly positive Δ values were obtained for 4-methylphenol and 4-methylbenzoate on both stationary phases. The Δ values were larger than those reported for Dowex 1-X8 at 60°C in acetate and glycolate media[1]. Experiments at 50°C showed that the decrease in temperature from 60°C had no significant effect on the Δ values. Evidently, the change in free energy related to the transfer of a methyl substituent from the external solution to the stationary phase depends not only on the properties of the external solution but also on the stationary phase, and is larger (more negative) for ODS-Hypersil and Hitachi gel than for Dowex 1-X8. As expected, Δ was suppressed by the presence of 20 % methanol in the eluent. In aqueous solutions the corresponding ethyl derivatives were held so strongly that it was impossible to determine their retention volumes with sufficient accuracy.

For anion-exchange resins with various carboxylate counter ions, the Δ values resulting from the introduction of a phenolic hydroxyl group in the 4-position were positive for phenol, benzoate and phenylacetate while, as can be seen from Tables II and III, these substituents gave rise to strongly negative Δ values both for ODS-Hypersil and for the Hitachi gel. For the anion exchanger the enhanced sorption is ascribed to hydrogen bonding between the phenolic hydroxyl proton and the acetate or glycolate counter ions present at high concentrations in the resin phase. For the systems studied in the present work, the positive contributions of hydrogen bonding to the distribution coefficients are less, due to a lower number of acetate or glycolate counter ions per unit volume of the column than in Dowex 1-X8. Possibly, the steric conditions are also more favourable for hydrogen bonding in the elastic ion-exchange resin than in the more rigid phases used in the present work. Moreover, the negative contributions to Δ resulting from a decreased hydrophobicity of the solutes will be larger for ODS-Hypersil and the Hitachi gel. These factors together may explain why negative Δ values were obtained on ODS-Hypersil and the Hitachi gel.

For the species containing a methoxyl group in the *para* position, the hydrophobic interactions should be less than for the methylated compounds[13]. Moreover, 4-methoxyphenol is a weaker acid than phenol. Hence, the methoxyl group will decrease the hydrogen bonding of the phenolic proton to the stationary phase. This can explain the negative Δ values observed on both phases in both media (and also in the experiments with methanol present). In agreement with the experiments with Dowex 1-X8, positive Δ values were obtained for 4-methoxybenzoate and 4-methoxyphenylacetate.

The Δ values observed for 2-methoxyphenol on ODS-Hypersil in acetate and glycolate were negative and very close to those found on Dowex 1-X8, while the values found on the Hitachi gel were less negative. The results are consistent with the observation that intramolecular hydrogen bonding suppresses the hydrogen bonding of the phenolic proton to the stationary phase, but tends to increase the contribution of hydrophobic interactions to the retention[14].

4-Hydroxy-3-methoxyphenol exhibited lower retention volumes than 4-hydroxyphenol on ODS-Hypersil and Hitachi gel in both acetate and glycolate. Similarly, the 4-hydroxy-3-methoxy-substituted carboxylate ions were held less strongly than those with a 4-hydroxy substituent. Hydrogen bonding between the phenolic proton and the methoxyl group may have an influence in this direction. The introduction of a second methoxyl group, vicinal to the hydroxyl group, led to an additional decrease in the retention volume. The same effect was found on Dowex 1-X8, and was explained by effective intramolecular hydrogen bonding and the steric shielding of the phenolic hydroxyl group.

Both in 0.3 and 0.5 *M* solutions of the sodium salts, all aromatic solutes were held more strongly on the Hitachi gel than on ODS-Hypersil. The retention of all aromatic compounds, except 4-hydroxy-2-methoxyphenol, was previously determined in 0.5 *M* sodium acetate and glycolate solutions at 60°C on Dowex 1-X8. All benzoates and phenylacetates were, despite the higher temperature, held more strongly on the anion exchanger than on the Hitachi gel. The largest difference was observed for the ions containing a phenolic hydroxyl group. Likewise, 4-hydroxyphenol exhibited a much higher retention on the anion exchanger, while for 4-methoxyphenol the difference was small. The other phenolic compounds were held more strongly on the Hitachi gel. This holds true both in acetate and in glycolate media. The results confirm that hydrogen bonding between the resin and the phenolic proton in phenols, and the presence of carboxylate anions, give much larger contributions to the observed retention volumes on Dowex 1-X8 than on the stationary phases studied in the present work.

Finally, the chromatogram shown in Fig. 4 illustrates the usefulness of the applied technique for practical separations. As can be seen, six closely related aromatic carboxylic acids were separated within about 20 min by isocratic elution with 0.25 *M* sodium acetate, 1 m*M* HDTMA, in the presence of 3% (v/v) methanol. A decrease in the methanol concentration to 2% (v/v) led to a less good resolution. At 10% (v/v) methanol, the first two peaks overlapped seriously. In addition to organic solvents, changes in pH, eluent concentration and of eluting anion offer great possibilities to optimize the conditions for separations of various acids and non-electrolytes. Under comparable conditions, a better resolution was obtained with ODS-Hypersil than with the Hitachi gel. On the other hand, the Hitachi gel is more stable and can be used over a wider pH range.

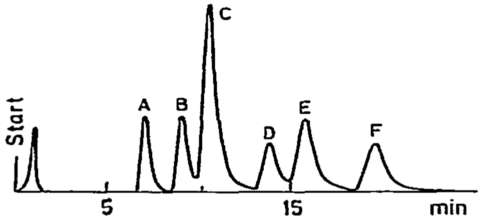

Fig. 4. Separation of benzoates and phenylacetates on ODS-Hypersil in 1 mM HDTMA and 0.25 M sodium acetate (3% (v/v) methanol present) at pH 7 and 50°C. Wavelength, 280 nm. A = 4-Hydroxy-3,5-dimethoxy-phenylacetate (40 nmol); B = 4-hydroxy-3,5-dimethoxybenzoate (8 nmol); C = 4-hydroxy-3-methoxyphenylacetate (40 nmol); D = 4-hydroxy-3-methoxybenzoate (8 nmol); E = 4-hydroxyphenylacetate (40 nmol); F = 4-hydroxybenzoate (48 nmol).

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