ANION-EXCHANGE CHROMATOGRAPHY OF DICARBOXYLIC ACIDS IN SULPHATE MEDIUM

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SUMMARY

Complex mixtures of dicarboxylic acids are separated by anion-exchange chromatography in sodium sulphate and determined automatically by chromic acid oxidation. With strongly polar species, the anion-exchange affinity increases with decreasing ionic size and with increasing strength of the parent acid. This acid strength effect is more important in sulphate than in phosphate medium. With non-polar species, hydrophobic contributions exert a predominant effect upon the retention volume.

INTRODUCTION

Anion-exchange chromatography coupled with automatic analysis of the eluate is an excellent tool in separations of complex mixtures of monocarboxylic acids[1]. The most generally applicable analysis system is based on chromic acid oxidation and subsequent colorimetric determination of either the chromic acid consumption or of the formation of green Cr(III) ions. The latter gives the most reliable results provided that quantities not less than about 1 mg of the species to be separated are present. An analysis system which permits the determination of microgram amounts by recording the chromic acid consumption has been developed but is sensitive towards various disturbances and therefore not quite satisfactory in routine work[2].

In most eluents, several important dicarboxylic acids give a serious tailing when milligram amounts are applied to the column[3,4]. Recently, it was observed that several polyhydroxy (aldaric) acids could be separated in neutral sodium sulphate medium without serious tailing[5]. The present work was carried out to study this method in more detail and to evaluate its possibilities in separations of various types of dicarboxylic acids.

EXPERIMENTAL

All experiments were carried out in jacketed columns of dimensions 4 × 1120 mm which were filled with a strongly basic anion-exchange resin (Dowex 1 X8, particle size 15–18 μm) in its sulphate form. The column was preconditioned with the eluent unless the same eluent was used in consecutive runs. The acids were applied to the column in aqueous solution as their sodium salts (pH 8) and eluted with sodium

sulphate solution. Unless otherwise stated, the applied amounts were about 2 mg and the linear flow rate was 7.5 cm/min (calculated for an empty column).

The eluate was analysed automatically by chromic acid oxidation with determination of the formation of Cr(III) ions[1]. When acids that are not readily oxidizable were present (succinic, glutaric, methylsuccinic and 3,3-dimethylglutaric acids) silver sulphate (10 g/l) was added to the chromic acid solution[4].

The adjusted retention volumes, V_R' (peak elution volume *minus* interstitial volume), were determined from runs with single acids and checked with acid mixtures. Calculated in column volumes, the adjusted retention volume gives the volume distribution coefficient, $D_V = V_R'/X$, for species which give symmetric elution curves, whereas with tailing species the true D_V value is somewhat higher.

INFLUENCE OF TEMPERATURE

Chromatographic runs in 0.03 and 0.04 M sodium sulphate solution showed that the temperature had an influence similar to that previously observed with phosphate solutions[4]. The width of the peaks which appeared early on the chromatogram were only slightly diminished when the temperature was increased from 30° to 80°, whereas for compounds such as fumaric and suberic acids, which exhibited large retention volumes, the peak width decreased markedly.

With galactaric acid, the retention volume increased when the temperature was raised, indicating that the overall reaction is endothermic (Fig. 1). A similar behaviour was previously observed for several monocarboxylic polyhydroxy acids in sodium acetate medium[6]. The positions of malic, D-glucaric, L(+)-tartaric, itaconic and oxalic acids were virtually unchanged when the temperature was raised from 30° to 80°. Among the unsubstituted saturated acids, glutaric and adipic acids showed positive values of dD_V/dt. The retention volume of pimelic acid was unchanged whereas that of suberic acid decreased markedly at an increased temperature indicating that the overall reaction was exothermic; similarly, fumarate anions exhibited a large negative temperature coefficient.

Considering the disadvantages and advantages by working at high and low
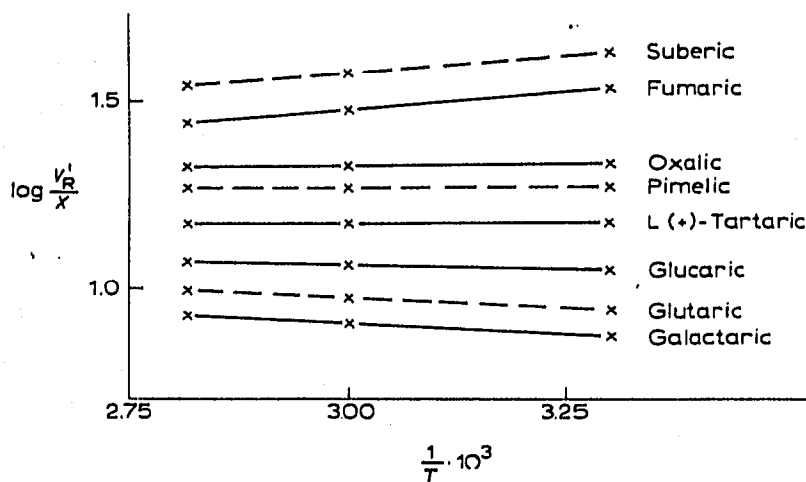

Fig. 1. Temperature-dependence of the adjusted retention volumes for various dicarboxylic acids in sodium sulphate. ×—× 0.04 M, pH 6.5; ×-·-× 0.04 M, pH 8.0.

temperature, respectively, and noting the instability of the resin at extremely high temperature[2], a temperature of 60° was chosen for the rest of the work reported in this paper.

INFLUENCE OF THE ELUENT CONCENTRATION AND pH

In the elution of monovalent ions with an eluting ion of the same charge, the distribution coefficient is inversely proportional to the concentration of the eluting ion[7]. This correlation has been found to give fair agreement in the elution of mono-carboxylic hydroxy acids with sodium acetate[8]. Provided that no abnormal changes in the activity coefficients occur, the same correlation should be valid for the elution of divalent anions with sulphate. To test its usefulness, log D_V determined at various eluent concentrations were plotted against $-\log[SO_4^{2-}]$.
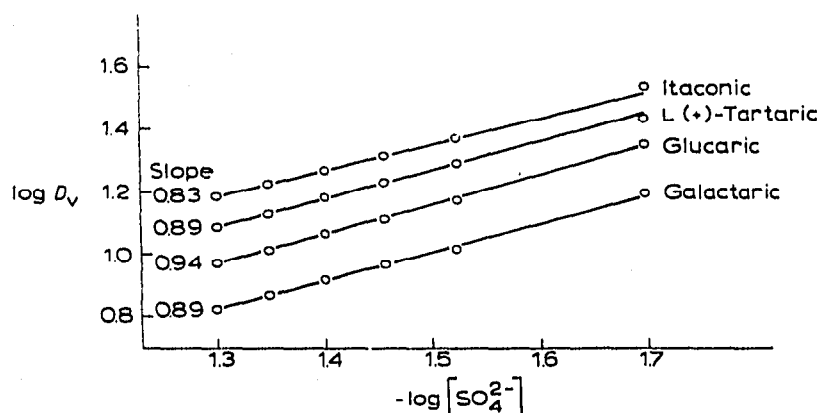

Fig. 2. Relationship between log D_V and $-\log [SO_4^{2-}]$.

The results given in Fig. 2 show that straight lines were obtained with all the investigated species. The slopes were somewhat lower than the theoretical slope ($+1$) even for species which are almost entirely present as divalent anions. Since electrolyte invasion can be disregarded within the studied interval, it is most likely that the variations in the activity coefficients have some influence. Itaconic acid, which is a weaker acid than the other species investigated, gave the largest deviation. The results can be used for predicting the position of elution peaks at different eluent concentrations from a chromatogram recorded at one concentration. Evidently, plots of the type shown in Fig. 2 can be useful even if a mixture of divalent and monovalent ions is involved.

Since the adjusted retention time is proportional to the D_V value, much time can be saved by working at high eluent concentration, but on the other hand the elution bands appear closer together as the concentration is increased. A chromatogram demonstrating the separation of ten different acids in 0.03 M sodium sulphate (pH 6.5) is shown in Fig. 3. Pimelic and tartronic acids overlapped seriously whereas the other species were easily separated. Under the applied working conditions, the elution of the last acid required about 12 h.

The last two peaks corresponding to fumaric and suberic acids, respectively, exhibited a marked tailing. As expected, the tailing decreased when the eluent concentration was increased. When the eluent concentrations were adjusted to give the same

retention volumes as those recorded in sodium phosphate at pH 7, the tailing observed (with milligram quantities applied to the column) was less than in phosphate medium. Previous studies of non-polar monocarboxylic hydroxy acids indicated that non-polar interaction forces contribute both to the tailing and to the relative retention of the carboxylate anions[9]. The results mentioned above indicate that the same holds true for the dicarboxylic acids.
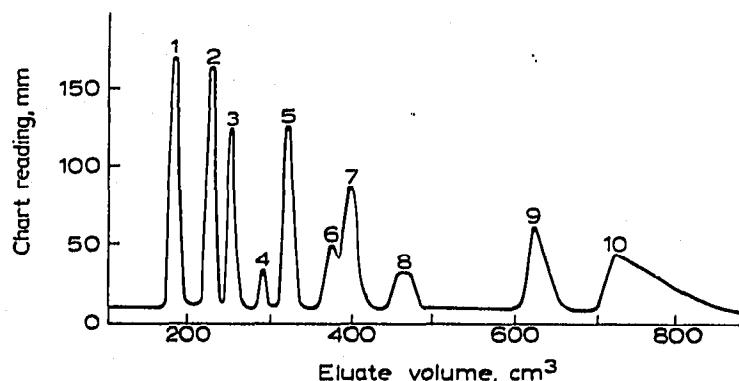

Fig. 3. Separation of 2.0 mg galactaric (1), 2.0 mg malic (2), 2.0 mg glucaric (3), 2.0 mg citraconic (4), 2.0 mg L(+)-tartaric (5), 4.0 mg pimelic (6), 2.0 mg tartronic (7), 4.0 mg oxalic (8), 2.0 mg fumaric (9) and 4.0 mg suberic acid (10). Eluent: 0.03 M sodium sulphate, pH 6.5.

The adjusted retention volumes recorded for various dicarboxylic acids in sodium sulphate solutions at 60° are listed in Table I. Determinations were carried out both in solutions without any additions (pH 6.5) and with additions of sodium hydroxide or ammonia to obtain pH 8.

Unfortunately, reliable dissociation constants for the second proton (K_2) are not available for most species at the temperature used in our experiments, but from available data it can be concluded that the stronger acids (high K_2) were present almost entirely as divalent anions at pH 6.5. With these species, it could be predicted that the increased pH should not affect the retention volume. This was confirmed by the results given in Table I. With other species, it could be assumed that besides divalent anions, monovalent anions would be present, but for the species investigated in both media, their effect on the retention volume was found to be negligible or almost negligible.

As mentioned previously, several polyhydroxy acids were investigated in sulphate medium in a previous study[5]. The investigation was carried out with 0.04 M sodium sulphate (pH 6.5) at 30°. It was found desirable to include some results from that study in the present discussion of the ion-exchange affinities (Table II), and to make sure that no interfering amounts of monovalent ions were present the retention volumes were redetermined under the same conditions and also determined at pH 8. The retention volumes of the investigated species (2-deoxy-*erythro*-pentaric, 2-deoxy-*lyxo*-hexaric, glucaric and mannaric acids) agreed very well with those reported previously, and even with the deoxy acids no increase in the retention volume was observed at the higher pH.

TABLE I

ADJUSTED RETENTION VOLUMES, CALCULATED IN COLUMN VOLUMES, IN SODIUM SULPHATE AT 60°

Acid	Adjusted retention volumes		
	pH 6.5		pH 8.0
	0.03 M	0.04 M	0.03 M
Oxalic	28.8	21.8	
Malonic	14.6		14.8
Succinic	12.1		12.1
Glutaric	12.6		12.7
Adipic	14.8		15.1
Pimelic	22.8		24.8
Suberic	43.4		
Azelaic	96.1		
Methylmalonic	15.2		15.0
Methylsuccinic	12.1		12.2
3,3-Dimethylglutaric	13.8		13.7
Tartronic	24.8	18.7	24.7
Malic	13.4	9.6	13.5
L(+)-Tartaric (threaric)	19.6	14.8	19.8
meso-Tartaric (erythraric)	17.8	13.5	18.1
2-Deoxy-erythro-pentaric	13.6	10.3	
Ribaric		14.0	
2-Deoxy-lyxo-hexaric	10.3	7.6	
Allaric		11.8	
Glucaric	15.2	11.5	
Galactaric	11.0	8.1	
Mannaric	21.0	17.1	
Talaric		9.8	
Fumaric	38.2	30.6	39.3
Maleic	18.7		20.2
Citraconic	17.9		18.4
Itaconic	23.3	18.2	23.8
α-Ketoglutaric	39.5		

TABLE II

INFLUENCE OF HYDROXYL GROUPS ON THE RELATIVE RETENTION (r) IN SULPHATE AND PHOSPHATE (pH 7.0) MEDIA

The results calculated for the first six acids refer to experiments in 0.03 M sodium sulphate at 60° and 0.4 M sodium phosphate (pH 7.0) at 80°. The other values were calculated from data in ref. 5 and refer to 0.04 M sodium sulphate at 30° and 0.3 M sodium phosphate at 80°.

Acid	Reference	$\log r_S$	$\log r_P$	$\Delta \log r$
Tartronic	Malonic	0.23	0.12	0.11
Malic	Succinic	0.05	−0.01	0.06
Threaric	Malic	0.16	0.08	0.08
Erythraric	Malic	0.12	0.01	0.11
Glucaric	Adipic	0.01	−0.15	0.16
Galactaric	Adipic	−0.13	−0.22	0.09
Arabinaric	2-Deoxy-erythro-pentaric	0.15	0.07	0.08
Ribaric	2-Deoxy-erythro-pentaric	0.11	0.07	0.04
Xylaric	2-Deoxy-threo-pentaric	0.11	0.01	0.10
Mannaric	2-Deoxy-arabino-hexaric	0.27	0.16	0.11
Glucaric	2-Deoxy-arabino- hexaric	0.06	0.01	0.05
Talaric	2-Deoxy-lyxo-hexaric	0.10	0.05	0.05
Galactaric	2-Deoxy-lyxo-hexaric	0.02	−0.01	0.03

INFLUENCE OF SOME STRUCTURAL FACTORS ON THE RETENTION VOLUME

It was mentioned previously that non-polar interaction forces[10] contribute to the anion-exchange affinity. Fig. 4, which is based on results from the present work and from an investigation of sodium phosphate as eluent[2], shows that for the unbranched saturated acids non-polar contributions are clearly reflected in the high retention volumes of pimelic ($n = 5$), suberic ($n = 6$) and azelaic ($n = 7$) acids when both sodium sulphate and sodium phosphate were used as eluents. For monocarboxylic acids, it has been shown that the non-polar contributions are less important with branched chains[2], and as expected, 3,3-dimethylglutaric acid takes an intermediate position between its unbranched isomer (pimelic acid) and glutaric acid. This holds true in both sulphate and phosphate medium. It is interesting to note that for higher unbranched homologues, the plots of the logarithm of the retention volume (which is closely related to the change in free energy, $-\Delta G^0$, of the ion-exchange process) against the number of methylene groups approximated to straight lines. The results indicate that the increment in $-\Delta G^0$ obtained by the introduction of one methylene group is approximately constant for the higher homologues. According to the Gibbs–Donnan equation, an increased ionic volume (or, expressed more precisely, an increased partial molar volume of the exchanger salt[11]) will tend to decrease the distribution coefficient, owing to the fact that the resin exhibits a large swelling pressure.
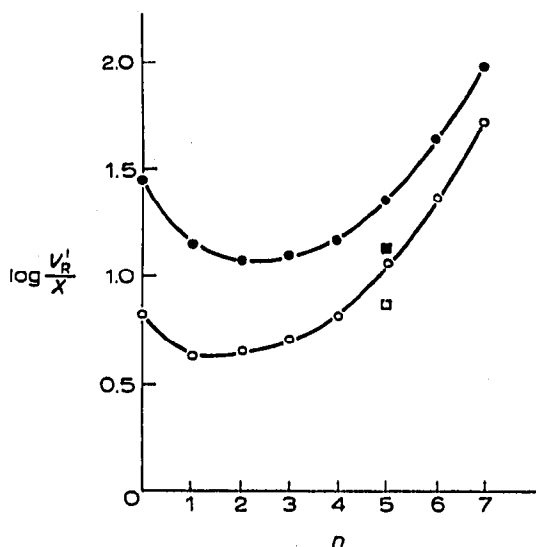

Fig. 4. Correlation between the adjusted retention volume, calculated in column volumes, (V_R'/X), and number of carbon atoms (n) between the carboxylic groups. ●, HOOC–$(CH_2)_n$–COOH in 0.03 M sodium sulphate; ■, 3,3-dimethylglutaric acid in 0.03 M sodium sulphate. Open symbols [○, □] refer to values determined in 0.4 M sodium phosphate at pH 7.0 (data from ref. 2).

The results show that with the higher homologues the influence of the pressure–volume term is small compared to the non-polar interactions exerted by the methylene groups.

The slopes of the curves within the higher range are almost the same in the two media, indicating that the non-polar contributions to the ion-exchange affinity are approximately the same under the applied working conditions. On the other hand, the introduction of two methyl groups in glutaric acid has a greater influence on the relative retention in sodium phosphate.

The non-polar contributions are changed very little when one methyl group is introduced into the lower homologues. Moreover, competing factors may exert an influence in the opposite direction[12]. This offers an explanation of the small changes in the D_V values of malonic and succinic acids by the introduction of a methyl group.

The introduction of hydroxyl groups into the hydrocarbon part of non-polar acids has been found to markedly decrease the non-polar contributions to the D_V values[9]. The increased size and hydration tend to decrease the D_V value as well. On the other hand, the introduction of hydroxyl groups close to the carboxylic groups gives rise to a marked increase in strength of the parent acid and would for this reason give a positive contribution to D_V[13]. In phosphate medium, both galactaric and glucaric acids exhibit lower D_V values than adipic acid, which shows that the effect of the increased acid strength was less than that of the competing factors. In sulphate medium, the same holds true for galactaric acid, whereas for glucaric acid, which is a stronger acid[14], the acid strength contributions predominate.

Mannaric acid, which exhibits a larger dissociation constant (K_2) of the second proton[14] than the diastereomers studied, is held much more strongly not only in sulphate medium but also in phosphate[5]. This gives additional support to the hypothesis that the parent acid strength is a factor of great importance for the anion-exchange affinity.

Among the diastereomeric tetraric acids, it can be predicted that threaric acid, which is the strongest acid, should be held more strongly than erythraric acid. The results obtained in both sulphate and phosphate are in agreement with this prediction. Moreover, malic acid would be expected to take an intermediate position between the unsubstituted (succinic) and the dihydroxysuccinic acids. This elution order holds true in sulphate medium, but in sodium phosphate, malic and succinic acids exhibit almost identical distribution coefficients[2]. With a batch of the same resin different to that used in the previous work, the elution order was the same as that observed in sulphate medium although the difference between the distribution coefficients was smaller in phosphate than in sulphate. The results indicate that the acid strength is a more important factor in sulphate than in phosphate medium.

As in phosphate medium, oxalate ions are held very firmly in sodium sulphate. A comparatively small effect of the pressure-volume term offers an explanation but most likely other factors have an influence as well. From the results in Fig. 4 and from experience gained in studies of monocarboxylic acids, it can be predicted that the non-polar contributions are negligible both for oxalate and malonate ions. The larger volume of the higher homologue will tend to lower its distribution coefficient but this effect will be almost equal in the sulphate resin to that in the phosphate resin. As can be seen from Fig. 4, the difference between the logarithm of the retention volume for oxalic acid and malonic acid is much larger in sodium sulphate than in phosphate. Results already mentioned indicate that the acid strength effect is more important in the former medium. If this rule holds true, it is reasonable to assume that the fact that oxalate is held much more strongly than malonate is explained at least in part by the higher K_2 value of the parent acid.

To test the hypothesis that the contributions which arise from the acid strength of the parent acid are more important in sulphate than in phosphate medium, the logarithm of the retention volume of various hydroxy acids was calculated relative

to that of corresponding deoxy acids lacking hydroxyl groups in one or both α-positions (log r). Calculations were made both for experiments in sodium sulphate (r_S) and sodium phosphate (r_P). The results given in Table II include those obtained with all the dicarboxylic hydroxy acids investigated for which data are available for the corresponding 2-deoxy acid. In both media, log r is largely independent of both the eluent concentration and the temperature for the range studied.

It is seen that in sulphate medium, log r is positive for all species except for galactaric acid relative to adipic acid. This negative value, as well as the negative values obtained in phosphate medium for glucaric and galactaric acids relative to adipic acid, reflect the influence of the non-polar contributions to $-\Delta G^0$ already mentioned. For two pairs of acids, the observed values of log r_P were —0.01, i.e. practically zero, which means that the acid strength contributions are offset by the competing factors, probable size and hydration contributions connected with the introduction of the hydroxyl group.

The last column in Table II shows that $\Delta \log r$, i.e. log r_S — log r_P, is a positive quantity for all investigated species. Since a shielded methylene group, i.e. one between two polar groups, will not contribute much to the non-polar interactions, it is reasonable to assume that other contributions to $-\Delta G^0$ are larger in sulphate than in phosphate medium. Since all the acids studied are stronger than the corresponding reference acid, it can be concluded that the acid strength factor has a great influence on the ion-exchange affinities of dicarboxylate anions and that a higher acid strength of the parent acid gives a larger contribution to the retention volume in sulphate than in phosphate medium.

In phosphate medium, it was observed that the presence of carbon–carbon double bonds gave large contributions to the anion-exchange affinity of the divalent anions[2]. Their high retention volumes compared to those of the corresponding saturated compounds show that the same holds true in sulphate medium. The order between maleate and fumarate ions is the same in both media and reflects the order of the dissociation constants for the second proton. In both media, itaconic acid takes an intermediate position whereas the retention volumes for citraconic (methylmaleic) acid are very close to those of maleic acid.

ACKNOWLEDGEMENTS

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