ANION EXCHANGE SEPARATION OF ORGANIC ACIDS IN ACETATE MEDIUM: INFLUENCE OF TEMPERATURE

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A decreased selectivity at elevated temperatures has been demonstrated by KRAUS, RARIDON AND HOLCOMB[1] in a study of the temperature coefficient of the $Br^- - Cl^-$ reaction with anion exchange resins. Similar results have been reported by many authors (*cf.* refs. 2 and 3) for monovalent cation exchanges.

Improved separations of various ionic species have, however, often been observed when chromatographic separations are carried out at an elevated temperature. The chief explanation for this is that the rate of diffusion inside the resin particles increases with increasing temperature and therefore less broadening of the elution curves occurs (*cf.* ref. 2).

In connection with our work on carbohydrates and on organic acids formed during the degradation of carbohydrates it has been found that in sugar separations by partition chromatography on ion exchange resins a great improvement is obtained when working at an elevated temperature[4], however, in separations of hydroxy acids by anion exchange chromatography in borate medium an increased temperature can result in serious complications[5]. Another technique used in our work is the separation of organic acids by chromatographic elution with sodium acetate solution[6]. The aim of this work is to investigate the influence of the temperature upon this separation.

EXPERIMENTAL

The experimental technique was the same as that used previously[5] with the exception that the eluant was preheated to the desired temperature in a separate column before entering the ion exchange column. This refinement of the technique is of importance only in runs at high flow rate. The anion exchanger was Dowex 1 X-8. Unless otherwise stated, the particle size was 40–60 μ. Different batches were used, but all results reported in each figure were obtained in runs on the same column. The eluate was analyzed in Technicon's Auto Analyzer using the dichromate method described in an earlier paper[7]. The aldobionic acids were prepared in earlier work[8] and all other chemicals were obtained from commercial sources.

The volume distribution coefficients (D_v) were calculated from the peak elution volumes by the conventional method[2].

RESULTS AND DISCUSSION

General

Anion exchange separations of carboxylic acids in sodium acetate medium are based upon simple ion exchange processes in which no consideration has to be given to the formation of covalent bonds or complexes. The volume distribution coefficients (D_v) of a monoprotic acid (HB) can be calculated from the following equation (*cf.* ref. 2):

$$\log D_v = -\log [A] + \log (\gamma_A/\gamma_B)_r - \log (\gamma_A/\gamma_B) + \log [A]_r + \log \rho_r$$

where [A] is the acetate concentration in the external solution (no subscript) and in the resin phase (subscript r), γ the activity coefficients, and ρ_r the mass of dry resin per cm^3 of the column.

Since acetate ions are present in large excess, $[A]_r$ can be considered independent of the external concentration and of the temperature over a large interval. Similarly, the changes in swelling are so small that ρ_r can be considered constant. In most systems the ratio between the activity coefficients in the external solution, independent of the temperature, can be assumed to approach unity, provided that the eluant concentration is low. At constant temperature, the ratio $(\gamma_A/\gamma_B)_r$ is determined by $[A]_r$ which is constant when [A] is varied within such limits that electrolyte invasion can be neglected. Since the resin phase behaves like a concentrated electrolyte solution of a complicated nature, this ratio can differ widely from unity[9] and can be dependent upon temperature. Hence, it would be expected that at constant temperature there would exist a linear relationship between log D_v and —log [A], the slope of which is equal to one.

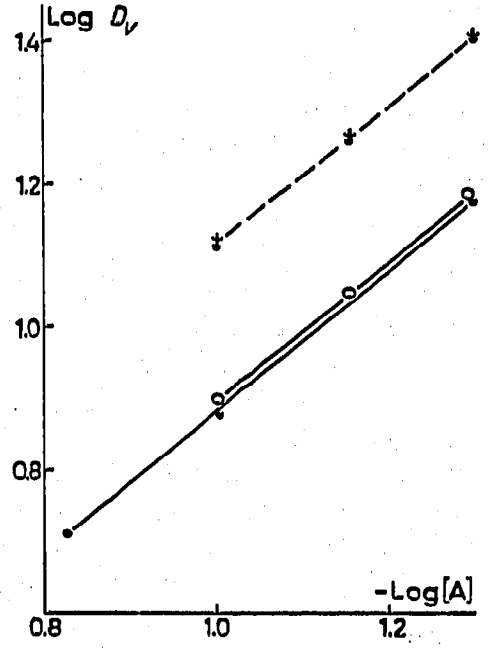

Fig. 1. Influence of the sodium acetate concentration (A moles/l) upon the volume distribution coefficient at various temperatures. – – – glyceric acid at 29° (●), and 80° (+). ——— gluconic acid at 29° (●), and 60° (○).

This has been demonstrated earlier in experiments with various hydroxy acids in sodium acetate solution carried out at room temperature[6,8]. As can be seen from Fig. 1 the same relationship holds true at elevated temperatures. With glycerate ions, which exhibit an ion exchange affinity close to that of acetate ions, no influence of temperature is detected. With gluconate ions, which are held less firmly than acetate ions $[(\gamma_A/\gamma_B)_r < 1]$, an increase in temperature results in a larger distribution coefficient.

A number of other anions, both those which are held less firmly than acetate ions and those which are held more firmly, were studied. From the D_v values determined at 28° and 80° (or in some experiments 60° to avoid serious decomposition) the temperature coefficient was calculated. The results of experiments carried out at two different acetate concentrations are given in Table I. It can be seen that ions

TABLE I

DISTRIBUTION COEFFICIENTS AND TEMPERATURE COEFFICIENTS (dD_v/dt) OF VARIOUS ORGANIC ACIDS

Acid	*Eluant (M)*	D_v *at 28°*	dD_v/dt
Lactobionic	0.05	7.35	0.01
Maltobionic	0.05	8.17	0.01
Cellobionic	0.05	8.26	0.01
Gluconic	0.05	15.1	0.01
Glyceric	0.05	25.5	0
Glyceric	0.1	12.6	0
Lactic	0.1	14.3	0.02
Glycolic	0.1	15.7	—0.01
Formic	0.1	26.2	—0.07
Pyruvic	0.1	40.4	—0.14

with low distribution coefficients exhibit an increased distribution coefficient at high temperature whereas those which are firmly held by the resin show a lowered ion exchange affinity. Hence, in most systems, the selectivity is lower at high temperature. Most interesting is the behavior of lactate ions which in the affinity series take an intermediate position. From the distribution coefficient it might be expected that lactate ions would have a negative temperature coefficient, but determinations carried out with two batches of resin showed that the temperature coefficient is positive. This means that the separation of lactic and glyceric acids is improved at elevated temperature whereas that of lactic and glycolic acids is jeopardized. Both conclusions have been verified in chromatographic runs with these acids.

From the observation that with most ions the selectivity decreases with an increased temperature it can be concluded that, with these anions, heat is evolved on the uptake of a preferred anion. No simple correlation exists, however, between the temperature coefficient and the distribution coefficient which means that there is no generally valid correlation between the selectivity coefficient and the heat of ion exchange.

The influence of temperature upon the shape of the elution curves also deserves some comments. The result of two typical runs with glyceric, formic and pyruvic acids are reproduced in Fig. 2. It is seen that a clear-cut separation was obtained

both at 28° and at 80°. In these runs very fine and carefully fractionated resin particles were used. It is seen that at the flow rate used in these experiments a sharpening of the elution curves occurred at the elevated temperature. This can be explained not only by the lower peak elution volumes, but also by a decrease in the height of a theoretical plate due to more rapid diffusion (*cf.* ref. 2). With less regular resin particles no sharpening of the curves was observed. This is explained by the fact that the non-uniform flow over the cross section of the column has a predominant influence upon the broadening of the elution curves.

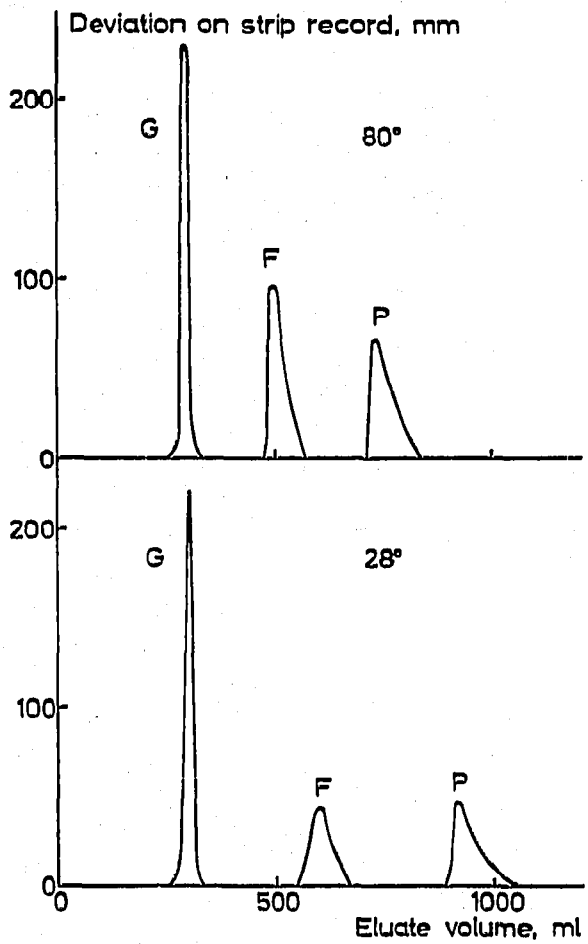

Fig. 2. Separation of 11 mg glyceric acid (G), 21 mg formic acid (F) and 30 mg pyruvic acid (P). Eluant: 0.1 *M* sodium acetate. Column: 6 × 805 mm Dowex 1 X-8, 18–23 μ. Flow rate: 3.3 ml cm^{-2} min^{-1}.

The experiments referred to in Fig. 2 were carried out with a batch of resin other than those used in Fig. 1 and Table I. It is seen that with both resins the position of the curve corresponding to glycerate ions is only slightly affected by the temperature, whereas those corresponding to formate and pyruvate appear earlier in the chromatogram at the elevated temperature. When only these acids are involved, the decreased selectivity at high temperature has no detrimental effect upon the separation.

Another observation worth mentioning is that with the resins used in this

work the separations of lactic and glycolic acids were not as good as those obtained under comparable conditions with another batch of the same resin used in previous work (*cf.* ref. 6). A comparison between the chromatograms showed that this is explained by less favorable distribution coefficients with the new resin. On the other hand glyceric acid could not be separated quantitatively from lactic acid with the resin used previously, whereas with the resin used in the present work an effective separation could be obtained independent of the temperature. The different results obtained with the two batches of resin can be fully explained by differing selectivity coefficients, *i.e.*, by the influence of the resin structure upon the equilibrium uptake of various ionic species.

Epimerization of aldonic acids

In experiments with aldonic acids at high temperatures serious complications occurred. In runs at high flow-rate these complications were less important and hardly detectable in the chromatogram. A comparison between the chromatogram obtained with gluconic acid at 28° (Fig. 3A) and that from a run at high speed at 65° (Fig. 3B)

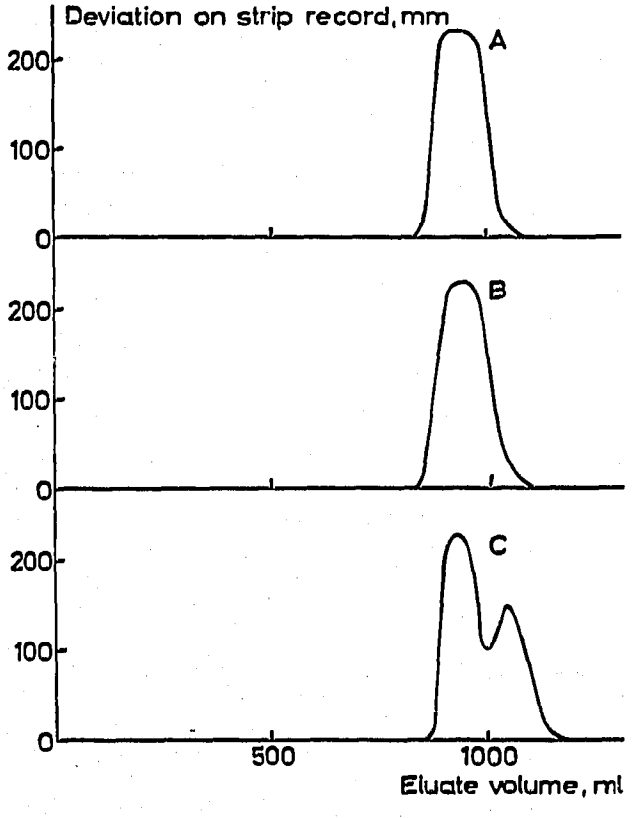

Fig. 3. Elution of gluconic acid (50 mg) with 0.1 *M* sodium acetate solution. Column: 15 × 900 mm. (A) 28°; 0.6 ml cm^{-2} min^{-1}. (B) 65°; 3.9 ml cm^{-2} min^{-1}. (C) 65°; 0.6 ml cm^{-2} min^{-1}.

shows that very small changes occurred. It can be concluded that the peak elution volume of gluconic acid is only slightly affected by changes in temperture. Experiments with mannonic and arabinonic acids showed that these acids behaved in a similar manner.

In experiments at elevated temperature and a low flow rate chromatograms with two overlapping elution bands were recorded. A typical example is given in Fig. 3C. The peak elution volume of the first band corresponded to that of gluconic acid whereas the second band, which was more or less well developed, depending upon working conditions (temperature and flow rate), had a position between that of gluconic and mannonic acids. Separate runs with mannonic acid on the same column showed that its peak elution volume was equal to 1250 ml. The eluate fractions corresponding to the second band in the chromatogram were rechromatographed at low temperature and gave a peak elution volume corresponding to that of mannonic acid. After reduction with sodium borohydride, mannose was identified by paper chromatography[10]. These experiments show that during runs at elevated temperature in acetate medium (pH about 8) gluconic acid is partially converted to mannonic acid. The position of the mannonic acid is displaced to the left because mannonic acid is successively formed during the passage of gluconic acid down the column.

The partial conversion (epimerization) of gluconic acid to mannonic acid in strongly alkaline medium after heating for several days has been established earlier[11], but as can be seen from the results presented above the epimerization is also of importance under comparatively mild conditions. In connection with this, it can be mentioned that an epimerization of gluconic acid was observed after heating an aqueous solution at pH 7 or slightly below 7 for 96 h on a steam bath. The ion exchange method was used to separate the acids.

The reverse reaction, *i.e.* the conversion of mannonic acid to gluconic acid, has also been established in experiments carried out in strongly alkaline solution[11]. This reaction seems to be slower, since no significant formation of gluconic acid was observable during the elution of mannonic acid with 0.1 *M* sodium acetate at elevated temperatures. Heating a solution of mannonic acid at pH 8 for 100 h on a steam bath, however, gave a chromatogram which exhibited bands corresponding to both gluconic and mannonic acids.

On the column which was used in the experiments reproduced in Fig. 1, arabinonic acid was found to give a peak elution volume of 1150 ml at 28°. The value obtained at 80° (1200 ml) differed only slightly from that obtained at the lower temperature. Both elution bands were sharp. By analogy with the behavior of gluconic acid, a conversion of arabinonic acid into ribonic acid was suspected. In separate experiments the elution behavior of ribonic acid was, however, shown to differ so slightly from that of arabinonic acid that these acids could not be distinguished from each other.

On the other hand an elution of these acids with 0.1 *M* sodium tetraborate solution resulted in a clear-cut separation (Fig. 4). This separation method was therefore employed to study the possible conversion of arabinonic acid into ribonic acid upon heating the solution in weakly alkaline medium. The results given in Fig. 5 show that this reaction occurs to a remarkable extent at pH 8.

An analogous experiment carried out with ribonic acid showed that the reverse reaction also occurred. The chromatogram showed two distinct elution bands, a major band corresponding to that of ribonic acid and a minor band corresponding to arabinonic acid. The presence of arabinonic acid in the eluate was further established by reduction with sodium borohydride. The sugar formed from the reduction was identified by paper chromatography.

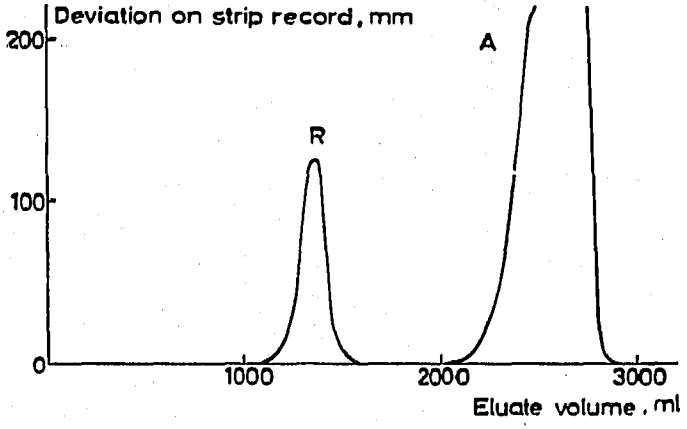

Fig. 4. Elution of ribonic (R; 26 mg) and arabinonic (A; 124 mg) acids with 0.1 *M* sodium tetraborate solution (28°). Column: 10 × 910 mm. Flow rate: 2.5 ml cm^{-2} min^{-1}.

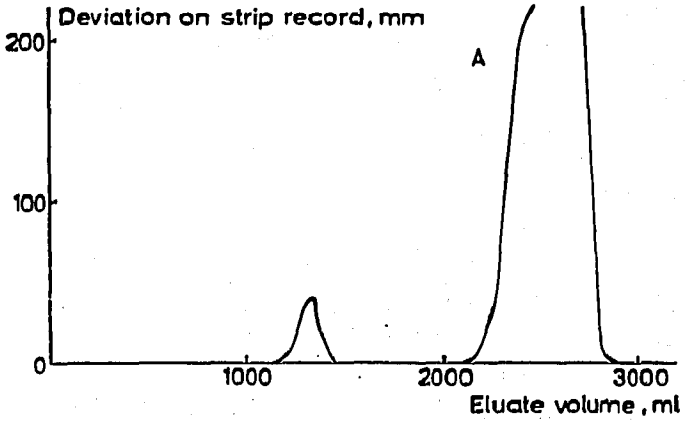

Fig. 5. Elution of arabinonic acid (A; 154 mg) after heating at pH 8 for 96 h on a steam bath. Eluant: 0.1 *M* sodium tetraborate solution (28°). Column: 10 × 910 mm. Flow rate: 2.5 ml cm^{-2} min^{-1}.

The same technique was used to study the epimerization of galactonic acid. On the borate column galactonic acid gave a peak elution volume of 4300 ml whereas the elution curve of talonic acid showed a maximum at 1770 ml. After heating at pH 8, under the same conditions as given in Fig. 5, the galactonic acid solution gave two distinct elution bands on the borate column. The position of the first band corresponded to that of talonic acid whereas the position of the main band was the same as that of galactonic acid. The acid present in the first band was reduced with sodium borohydride and gave a paper chromatogram which showed excellent agreement with that obtained after reduction of an authentic sample of talonic acid.

These results show that the aldonic acids epimerize very easily in alkaline medium. Heating of the solutions and addition of excess alkali should therefore be avoided when the lactones are saponified before separation of the acids.

Destruction of uronic acids

Elution with sodium acetate solution at room temperature is a useful technique in separations of various uronic acids[12]. Experiments carried out at elevated temperature showed that serious destruction occurred. In an experiment at 60°, on a column with the dimensions 15 × 950 mm, run at a flow rate of 1.7 ml·cm^{-2}·min^{-1}, only about 50 % of the added glucuronic acid was recovered in the eluate. At 80° the corresponding value was about 10 %. Some destruction products appeared ahead of the uronic acid band while some were held so strongly that they were not recorded on the chromatogram. Galacturonic acid exhibited similar behavior. With both acids the temperature had only a slight influence upon the peak elution volume of the remaining uronic acid. In a run carried out at a 28° the recovery of the uronic acids was complete.

ACKNOWLEDGEMENT

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SUMMARY

The elution of organic acids from anion exchange columns with sodium acetate solution at elevated temperatures was studied. In most systems very little can be gained by working at elevated temperatures when using a resin of low particle size. In some separations the decreased selectivity seriously affects the result.

With aldonic acids an interfering epimerization can be detected even at 60°, and with uronic acids serious destruction occurs at this temperature.

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