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# Anion Exchange Chromatography of Organic Acids in Zinc Acetate Medium

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Zinc acetate solution is a useful eluent in the separation of various organic acids by means of anion exchange resins. The eluate concentration is determined automatically by two methods simultaneously: chromic acid oxidation and the carbazole reaction.

Ion exchange chromatography in the presence of complex forming organic anions is a well-known and widely applied technique in metal separations. In this case the ligand is present in large excess compared with the metal ions. Very little is known, however, about the possibilities of using complex formation with metal ions in the separations of anions on anion exchange resins.

Some theoretical aspects were discussed in an earlier paper [1] and a few applications of copper acetate solution as eluent in the separations of some organic anions were also presented.[1,2] To extend the application of this method and to adapt it to our cellulose research, a study of the behavior of various aldonic and uronic acids was carried out. Galactonic, mannonic, gluconic, arabinonic, and galaheptonic acids were eluted very early indicating that strong non-adsorbable complexes were formed. With Dowex 1 X-8 the volume distribution coefficients ($D_v$) of these acids in 0.05 M copper acetate were within the interval 0.9—1.6. Galacturonic acid ($D_v$ = 5.4), mannuronic acid ($D_v$ = 9.3), and glucuronic acids ($D_v$ = 10.4) were eluted much later. Hence the conditions are extremely favorable for a group separation of aldonic acids from uronic acids with the simultaneous separation of galacturonic and glucuronic acids from each other. The recovery of uronic acids was not satisfactory, however, since uronic acids were oxidized with the simultaneous formation of copper (I) oxide. It is likely that this complication can be eliminated by rapid elution at very low temperature, but it seemed more attractive to abandon this method and use some other complexing agent. The results obtained with zinc acetate will be discussed in this paper.

## EXPERIMENTAL

All the acids used were obtained from commercial sources except erythronic acid which was synthesized [3] and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose which was prepared from gum arabic [4] and identified as described previously.[5] The aqueous solutions of the acids to be investigated were neutralized at room temperature with potassium hydroxide and kept at pH 8 to split the lactones.[6] The separations were carried out with the acetate form of a strongly basic resin (Dowex 1 X-8). The column was washed with a small amount of water before and after loading it with the solutes to be separated. Standard techniques were employed. A jacketed column and a plunger pump in stainless steel for feeding the eluent onto the column were used.[7] The temperature was kept at 28° by circulating water from a thermostat.

Two different batches of resin delivered from the manufacturer on different occasions were used. One of these (A) was fractionated to obtain the particle size 40—60 μ and used in a bed of dimensions 10 × 880 mm. The eluate from this column was collected in a time actuated fraction collector and analyzed by chromic acid oxidation using the Technicon AutoAnalyzer.[8]

Of the second batch of resin (B) the fraction 13—17 μ was used in a resin bed of dimensions 6 × 880 mm. The eluate was analyzed automatically by two simultaneous methods: chromic acid oxidation with determination of the green Cr(III)-complexes, and the carbazole reaction. The monitor consisted of a peristaltic pump, reaction coils of teflon, a multichannel photometer (LKB-Produkter) and a recorder. Details of this analysing system are being published elsewhere.[5]

## RESULTS AND DISCUSSION

The chromatogram reproduced in Fig. 1 demonstrates the separation of six acids using a resin with the particle size 40—60 μ. It is seen that a clean-cut separation was obtained in this run in which the eluate was collected in a fraction collector and analyzed by chromic acid oxidation.

Fig. 2 shows a chromatogram recorded automatically using both chromic acid oxidation and the color reaction with carbazole. In this run the particle size of the resin was 13—17 μ. The seven acids included in this run were well separated. It is seen that with this column length there was a slight overlapping of the elution curves corresponding to galactonic and arabinonic acids. This overlapping does not seriously interfere with the evaluation of the chro-

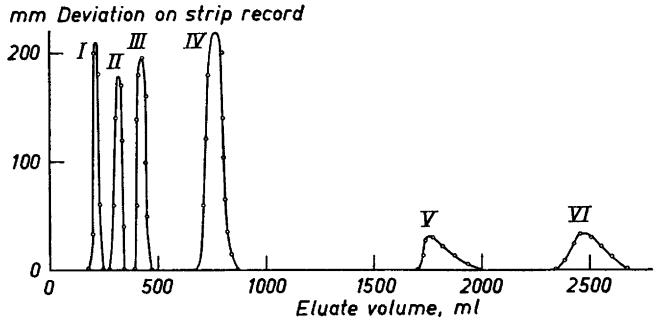


*Fig. 1.* Separation of 10 mg galactonic (I), 12 mg lactic (II), 15 mg galacturonic (III), 40 mg glucuronic (IV), 20 mg formic (V), and 30 mg pyruvic (VI) acid by elution with 0.05 M zinc acetate solution. Flow rate: 2.6 ml $cm^{-2}$ $min^{-1}$.

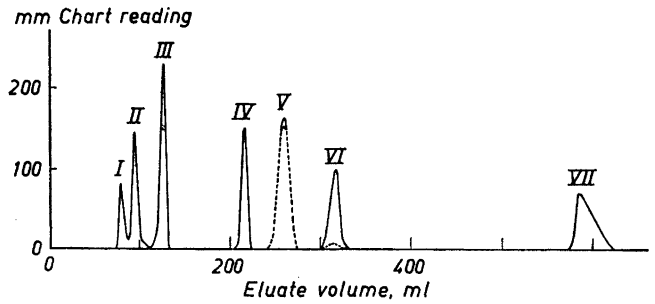


*Fig. 2.* Separation of 0.6 mg galactonic (I), 0.8 mg arabinonic (II), 1.5 mg glycolic (III), 3.0 mg levulinic (IV), 1.6 mg glucuronic (V), 2.0 mg glyoxylic (VI), and 3 mg formic (VII) acid by elution with 0.05 M zinc acetate. Automatic analysis by chromic acid oxidation (full line) and carbazole (broken line). Flow rate: 4.1 ml $cm^{-2}$ $min^{-1}$.

matogram for quantitative purposes and can be eliminated if desired, by increasing the column length. The time required for the separation of these seven acids was about 9 h.

The application of an automatic analysing system with the recording of the color developed in two different reactions facilitates the identification of the separated acids. All acids were recorded as well reproducible elution curves in the chromic acid channel. In repeated runs the peak elution volumes were reproducible with deviations from the mean values of less than 1 %. A first identification was obtained, as usual, from the peak elution volumes. Carbazole which is the common reagent for uronic acids gave a positive reaction not only with uronic and biouronic acids, but also with metasaccharinic, lactic, glyoxylic, and pyruvic acids. With the other acids studied no color reaction with carbazole was recorded. The intensity of the color developed with carbazole is different even with various uronic acids and the ratio between the area recorded by the carbazole channel and that recorded by the chromic acid channel can, therefore, serve as additional identification.[5]

The peak elution volumes were determined from a great number of runs in 0.05 M zinc acetate with single acids and with mixtures of acids. From these determinations the volume distribution coefficients ($D_v$) were calculated as usual.[7] The results obtained with both batches of resin are given in Table 1.

It is seen that some of the acids exhibited a very similar elution behavior and could not be separated from each other in this medium whereas other acids had distribution coefficients which differed greatly from one another. The order of elution was reversed with some of the species compared with elution with sodium acetate solution.[9] This shows that complex formation with zinc ions is an important factor. With most acids the distribution coefficients were much lower than in sodium acetate solution of the same acetate concentration indicating that nonadsorbable complexes were formed. The change in position was greatest with some of the acids which appeared early in the eluate which suggests that complex formation exerts the greatest influence with these species. It should be emphasized however, that the order of elution is deter-

*Table 1.* Volume distribution coefficients in 0.05 M zinc acetate with two batches of Dowex 1 X-8 (A and B).

| Acid | Resin A | Resin B |
|---|---|---|
| Pyruvic | 33.8 | 31.6 |
| Formic | 24.9 | 23.1 |
| Glyoxylic | 14.1 | 12.0 |
| Glucuronic | 10.5 | 9.97 |
| Levulinic | 6.69 | 8.24 |
| Galacturonic | 5.67 | 5.39 |
| Glycolic | 4.88 | 4.58 |
| Lactic | 4.30 | 4.46 |
| Erythronic | 4.23 | 4.26 |
| Arabinonic | 3.29 | 3.26 |
| 6-*O*-(β-D-Glucopyranosyluronic acid)-D-galactose | 2.86 | |
| Gluconic | 2.64 | 2.82 |
| Galactonic | 2.64 | 2.74 |
| Metasaccharinic | 2.56 | |

mined not only by the stability constants of the complexes, but also by the selectivity coefficients of the anions present in the solution.[1]

The order of elution was the same with both batches of resin but the distribution coefficients varied to some extent. More interesting is the fact that with some acids the separation factors (the ratio between the distribution coefficients) were less favorable with resin B than with the older batch of resin. A typical example is the separation of lactic and glycolic acids. Resin B was identical with that used in recent separations of uronic acids in sodium acetate medium [5] and with this system as well, it was found that the separation factors were less favorable than with an older batch of the same resin. The fact that

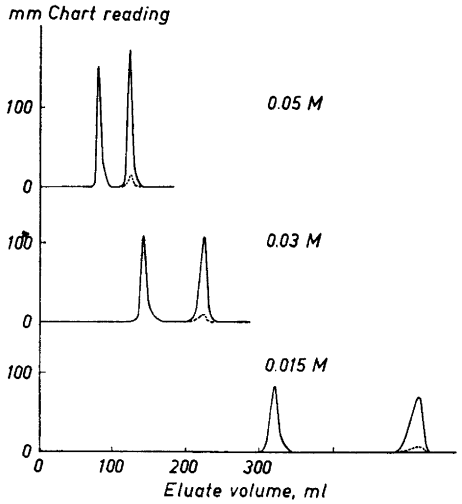


*Fig. 3.* Influence of the zinc acetate concentration upon the elution of 0.8 mg galactonic acid (first elution band) and 1.5 mg lactic acid (second elution band). Automatic analysis by chromic acid oxidation (full line) and carbazole (broken line). Flow rate: 4.1 ml $cm^{-2}$ $min^{-1}$.

more favorable results were achieved with some acids with resin B can be explained entirely by an improved column efficiency resulting from the use of finer resin particles.

A study of the influence of the eluent concentration upon the course of elution revealed that improved separations were obtained at low eluent concentration. The chromatograms reproduced in Fig. 3 show that galactonic and lactic acids were well separated from each other in 0.05 M zinc acetate. The elution was completed within 2 h. At a lowered eluent concentration the acids appeared later in the eluate. Despite the broadening of the curves observed at low concentration the distance between the elution curves increased. It is possible to take advantage of this fact in separating acids which exhibit a similar elution behavior. In these respects the elution with zinc acetate is quite similar to that with sodium acetate. The order of elution of these acids is independent of the eluent concentration.

Since the complex formation can be affected by changes in pH, work at constant pH is recommended. In all experiments reported in this paper the zinc acetate solution was acidified with acetic acid to obtain pH 4.6. An experiment with gluconic and glucuronic acids run at pH 6 showed, however, that with these solutes the change in pH had a negligible effect. In order to avoid complications due to changes in pH during the first part of the run, the column was washed with water before and after the sample solution was introduced into the column.

A practical application of this method in carbohydrate chemistry is the group separation of aldonic acids from galacturonic and glucuronic acids which is quantitative even when large amounts are chromatographed. With some acids the resolution is not as good as with sodium acetate solution, but in other systems there is a marked improvement. Lactic and glucuronic acids appear in the same elution band when sodium acetate is used as eluent [10] whereas the zinc acetate method gives a convenient and clean-cut separation. The same holds true for erythronic and levulinic acids. In the separation of the complicated mixtures of acids which arise in various branches of carbohydrate chemistry this method is a valuable complement to existing procedures.

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