

CIRCULAR PAPER CHROMATOGRAPHY

Part IV. Separation of Organic Acids

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SUMMARY

The application of circular paper chromatographic technique to the separation of organic acids has been studied.

R_f and R values (with reference to malic and lactic acids) of some organic acids in various solvent mixtures have been reported.

The effects of the "aged" solvent mixture and different solvents used for prior equilibration of the paper on R_f values have been studied.

The suitability of this technique to the identification of organic acids present in natural products has been indicated.

The chromatographic separation of organic acids on filter-paper sheets using uni-dimensional and two-dimensional technique was studied by Lugg and Overell (1948) employing various solvent mixtures of which *n*-butanol-formic acid-water (containing 3.34 moles of the acid) was found to be very satisfactory. *n*-Pentanol-formic acid-water system was also reported to yield good results by Buch, Montgomery and Porter (1952) who, however, maintained that the R_f values of the organic acids were quite close and as such 'R values' expressed as the ratio of the distance moved by the unknown compound to the distance moved by a known pure substance might be preferred to the usual R_f values. In view of the successful application of the circular filter-paper chromatographic technique for the separation of amino acids in these laboratories by Giri and Rao (1952), it appeared desirable to investigate the separation of organic acids by this technique.

MATERIALS AND METHODS

Whatman No. 1 filter-paper was used in all the experiments. All the solvents were distilled and checked for their purity. The test acids were prepared in 0.5% solutions in ethyl alcohol (99%). The circular filter-papers of 24 cm. in diameter were used. 4-6 μ l. of the acid containing 20-30 μ g. were spotted at the centre of the filter circles. The procedure for running the chromatograms was the same as described before (Giri, *et al.*).

The chromatograms after development with the solvent were dried at room temperature for about 48 hours and then at 50–60° for 2 hours. However, it was found advantageous to dry by passing hot air at 45–50° for $\frac{1}{2}$ hour after preliminary drying at room temperature: the chromatograms were then sprayed with 0.04% brom-cresol-green. The reagent was prepared by dissolving the dye in 95% ethyl alcohol and adjusting the pH to 5.5 (glass electrode) by adding a few drops of N. sodium hydroxide.

RESULTS

Fig. 1 is a chromatogram of organic acids separated from a mixture.

R_f and R values in Table I are obtained from individual chromatograms which have been repeatedly confirmed. Under the experimental conditions employed, the width of the bands usually were found to be 2–3 mm.

TABLE I
*R_f and R Values of Organic Acids**

Acid	R_f	R_{malic}^\dagger	$R_{\text{lactic}}^\dagger$	Lugg's figures (F)
Glycollic	0.23	0.29	0.25	..
Oxalic	0.27	0.34	0.28	..
Tartaric	0.59	0.75	0.63	0.21–0.22
Citric	0.72	0.90	0.76	0.37–0.39
Malic	0.79	1.00	0.84	0.44–0.45
Maleic	0.80	1.01	0.85	..
Malonic	0.90	1.13	0.95	0.63
Tricarballic	0.93	1.17	0.98	0.67
Lactic	0.95	1.19	1.00	0.67
Succinic	0.96	1.21	1.02	0.74–0.75
Fumaric	0.99	1.24	1.04	0.88–0.90
Adipic	0.99	1.25	1.05	0.90

* Solvent mixture used: *n*-Butanol–Formic acid–Water (15:10.5:15).

† R_{malic} and R_{lactic} are chosen in consonance with Buch, Montgomery and Porter (1952).

The R_f values of the acids given in Table II have been calculated from chromatograms run with various solvent mixtures. The values obtained by measuring the distances both from the outer and the inner edges of the bands (together with the ranges) are given.

TABLE II
R_f Values with Different Solvent Mixtures

Mixture	Molar concentration after 10 hrs. keeping	Oxalic	Tartaric	Citric	Malic	Malonic	Succinic	Adipic
1 <i>n</i> -Butanol—Formic—Water 10 2 5	1.34	0.21-0.27	0.50-0.59	0.60-0.72	0.77-0.79	0.88-0.90	0.93-0.95	0.98-0.99
2 <i>n</i> -Butanol—Formic—Water 15 10.5 15	2.97	0.22-0.27	0.57-0.59	0.70-0.72	0.77-0.80	0.89-0.91	0.94-0.96	0.99
3 <i>n</i> -Butanol—Acetic—Water 4 1 5	1.26	0.17-0.21	0.44-0.46	0.60-0.63	0.71-0.75	0.80-0.83	0.91-0.94	0.97-0.98
4 Iso-propyl—Formic—Water alcohol 10 2 2	2.28	..	0.70-0.72	0.78-0.79	0.82-0.84	0.89-0.91	0.93-0.94	0.97-0.98
5 Iso-Butanol—Formic—Water 15 10.5 15	2.07	0.38-0.43	0.67-0.69	0.79-0.81	0.87-0.88	0.94-0.96	0.96-0.98	0.99
6 Iso-amyl—Formic—Water alcohol 15 10.5 15	2.01	0.13-0.19	0.36-0.39	0.46-0.50	0.61-0.64	0.80-0.84	0.99-0.92	0.98-0.99

The effect of the solvent mixture which had been kept for varying periods has been indicated in Table III. Lugg and Overell (1948) mention in this connection that partial esterification of the formic acid with butanol takes place on keeping the solvent mixture, which appears to exert some effect on the chromatograms.

TABLE III

R_f Values with n-Butanol—Formic Acid—Water (15-10.5-15) on Keeping

Time	Molar concentration	Oxalic	Tartaric	Citric	Malic	Malonic	Succinic	Adipic
1st day ..	2.97	0.22-0.27	0.57-0.59	0.71-0.73	0.77-0.80	0.89-0.91	0.94-0.96	0.98-0.99
2nd day ..	1.99	0.16-0.19	0.56-0.60	0.70-0.72	0.78-0.80	0.89-0.92	0.94-0.96	0.98-0.99
3rd day ..	1.82	0.17-0.23	0.58-0.62	0.71-0.74	0.77-0.80	0.90-0.92	0.95-0.97	0.99
4th day ..	1.74	0.17-0.24	0.59-0.63	0.71-0.75	0.76-0.79	0.90-0.93	0.94-0.96	0.99

The effect of prior equilibration with (i) distilled water, (ii) aqueous phase (the aqueous portion of the mixture of solvents that separate on keeping) and (iii) the solvent mixture, for different intervals of time is given in Table IV.

TABLE IV

*R_f Values and Equilibration with Different Solvents**

	Tartaric	Citric	Malic	Malonic	Succinic	Adipic
1. 15 minutes over water	0.52-0.55	0.63-0.66	0.71-0.74	0.85-0.87	0.93-0.95	0.99
2. 1 hour over water	0.58-0.61	0.69-0.72	0.75-0.78	0.88-0.91	0.94-0.96	0.98-0.99
3. 15 minutes over water phase	0.58-0.62	0.69-0.72	0.76-0.79	0.87-0.89	0.92-0.94	0.99
4. 1 hour over solvent phase	0.64-0.67	0.74-0.76	0.80-0.82	0.89-0.91	0.94-0.96	0.98-0.99
5. Without equilibration	0.57-0.59	0.70-0.72	0.77-0.80	0.89-0.91	0.94-0.96	0.99

* Solvent mixture used for developing the chromatograms was *n*-Butanol — Formic Acid — Water (15:10.5:15).

All the *R_f* values presented in Tables (II-IV) have been calculated from chromatograms run with mixtures of acids and not individually.

DISCUSSION

The R_f values of amino acids separated by circular paper chromatography were found to be uniformly higher than those reported by other investigators who used filter-paper sheets for running the chromatograms (Rao and Giri, 1953). The same is true of organic acids also, as is evident from the R_f values given in Table I and those of Lugg and Overell (1948). In all these cases, the order of separation of acids, however, remains unchanged. It is for this reason that a number of fast moving acids may not be separated satisfactorily by this technique, thus limiting its applicability.

Of the various solvent mixtures tried, mixture No. 2 was found to give the best separation thus confirming the findings of Lugg and Overell (1948). Although a very clear separation with narrow bands (1–1.5 mm. wide) were obtained with solvent system No. 4 the R_f values were high, and contrary was the case with solvent system No. 6 which gave rise to lower R_f values and broader bands. However, a compromise was reached in solvent system No. 3. It was found easier to dry and remove most of the formic acid from chromatograms developed with all the solvents containing formic acid except the solvent No. 4 where considerable difficulty was encountered in the removal of last traces of formic acid.

Due to the progressive esterification of the *n*-butanol-formic acid-water system (No. 2), which attains equilibrium conditions by about 4 days, the chromatography of organic acids using these "aged" solutions was investigated (*vide* Table III). It could be seen that even though the R_f values are not much affected, the bands become somewhat diffused, and hence it was found desirable to employ more or less fresh solutions. However, in order to minimise the variations in R_f values, when fresh solutions are used, it was found necessary to employ solutions after keeping for at least 10 hours.

For obtaining good chromatograms, prior equilibration of the filter-paper was also found necessary, and best results were obtained if these were equilibrated for 15 minutes with distilled water, which was then replaced by the solvent mixture for developing the chromatogram. When the papers were equilibrated with solvents containing formic acid (Nos. 3 and 4) higher R_f values were obtained (*vide* Table IV) with some diffusion of the bands and unsatisfactory separation of the acids.

ORGANIC ACIDS PRESENT IN UNRIPE MANGO FRUIT (*Mangifera indica*)

The above technique was applied to the identification of organic acids in naturally occurring materials. The technique for carrying out the mixed

chromatograms was the same as the one described by Giri and Rao (1952) for amino acids. Unripe mangoes (sour variety, commonly used for pickles) collected locally from the trees were minced in a Waring Blender, and after clarification by centrifugation, removal of the proteins by 70% ethyl alcohol, and concentration (25 ml. corresponding to 55 gs. fruit), the extract was chromatographed. Oxalic, citric, malic and succinic acids were identified. From the intensity of the bands, it was found that citric acid appeared to be in high concentrations. In addition, there are two more acids present which were not identified; [R_f values are (i) 0.48-0.50; (ii) 0.69-0.72].

REFERENCES

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