A NOTE ON THE FACTORS AFFECTING R_F VALUES ON CITRATE BUFFERED PAPER CHROMATOGRAMS

BY E. G. C. CLARKE AND A. E. HAWKINS*

From the Department of Physiology, Royal Veterinary College, London, N.W.1

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Experiments have been made to determine the effect of changes in experimental conditions on the speed of running of citrate/butanol systems on paper chromatograms.

PAPER chromatography is of the greatest value for the provisional identification of unknown nitrogenous bases in toxicological analysis. Among the systems widely used for this purpose are those using buffered paper such as the citrate-butanol method of Curry and Powell (1954). Such systems, however, suffer from the disadvantage that the reproducibility of the R_F value is poor, particularly from one laboratory to another (McKee, 1957) and attempts to improve it by use of freshly prepared solutions, by equilibration, and by control of other variable factors are of doubtful value (Goldbaum and Kazyak, 1956) and have the disadvantage that they entail sacrifice of the three outstanding advantages of paper chromatography: speed, simplicity and cheapness.

In the course of some thousands of runs made with the citrate-butanol system it was noticed that certain factors had a considerable influence on the speed of running while others made little difference. A series of experiments was made to confirm these observations. It is thought that the results obtained are worth recording.

EXPERIMENTAL

For these experiments, unless otherwise stated, the solvent consisted of the organic rich layer left after shaking butanol with its own volume of 2 per cent aqueous citric acid. Sheets of No. 1 Whatman filter paper (14 in. × 6 in.) were dipped in a 5 per cent solution of sodium dihydrogen citrate, blotted, dried for 1 hr. at 25° in a fume cupboard with the draught on and stored in a folder until required. The ascending method was used, the tank being 11 in. × $8\frac{1}{2}$ in. × $15\frac{1}{2}$ in. high. In the equilibration experiments a magnetic device was used to hold the sheets above the solvent until it was required to lower them. The alkaloids used were cocaine, (–)-hyoscyamine, morphine, strychnine and coniine. For the major factors confirmatory experiments were made using brucine, hyoscine, antazoline, diphenhydramine, nikethamide, amylocaine, and naphthazoline.

RESULTS AND DISCUSSION

Equilibration is undesirable because in addition to being time consuming it makes most substances run faster. For example, without equilibration the R_F values were cocaine 0·42, hyoscyamine 0·37, morphine 0·14,

* Now at Unilever Research Laboratories, Colworth House, Sharnbrook, Bedford.

strychnine 0·28, coniine 0·60 and after equilibration for 18 hr. 0·66, 0·60, 0·22, 0·52, 0·64 respectively. The R_F values of some 500 alkaloids (Clarke, 1962) were found to show the following distribution. R_F values are given first with the number of alkaloids in parentheses. 0 (13); 0–0·1 (43); 0·11–0·20 (47); 0·21–0·3 (49); 0·31–0·4 (47); 0·41–0·5 (61); 0·51–0·6 (68); 0·61–0·7 (96); 0·71–0·8 (49); 0·81–0·9 (22); 0·91–1·0 (13). Thus any procedure which increases the speed of running tends to crowd still further the upper half of the scale. This results in loss of resolution, and increases the difficulty of identification by R_F value.

Fresh solvent solutions gave faster and more erratic running than those which had been used for some time. With freshly prepared solvent the values were cocaine, 0·53; hyoscyamine, 0·50; morphine, 0·23; strychnine, 0·43; coniine, 0·63, but with an old solution these were 0·43, 0·38, 0·14, 0·29, 0·60. This was further investigated by using mixtures made by adding different volumes of water to a 0·48 per cent solution of citric acid in butanol, and it was found that there was a direct connection between the speed of running and the water content of the solvent. The results with the 5 alkaloids in the order given above were:

Water added, per cent	S.G.	R_F values
4	0·821	0·18, 0·18, 0·09, 0·10, 0·53
8	0·830	0·33, 0·31, 0·12, 0·23, 0·57
12	0·838	0·43, 0·35, 0·14, 0·26, 0·60
16	0·846	0·41, 0·38, 0·14, 0·26, 0·64
Saturated with water layer	—	0·54, 0·43, 0·17, 0·34, 0·64
Saturated with water layer equilibrated overnight..	—	0·69, 0·64, 0·25, 0·59, 0·64

There is little change between a water content of 12 and 16 per cent. The measurement of specific gravity forms a reliable guide to the composition of the solution and any drop may be corrected by adding water.

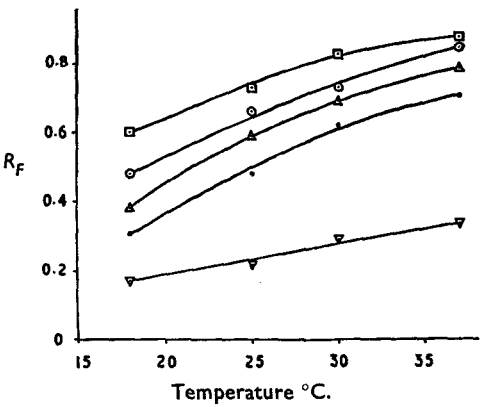

FIG. 1. R_F values plotted against temperature. ⊡ coniine, ⊙ cocaine, △ (−)-hyoscyamine, ● strychnine, ▽ morphine.

Increase of temperature, as is well known, increases the R_F values. The order of magnitude for this system is shown in Fig. 1.

The *time factor* was investigated by using fluorescent alkaloids and an ultra-violet lamp to determine the approximate R_F values at various stages during a run. There is a slow but steady increase of R_F value with time (Fig. 2). This is borne out by the higher values obtained after all-night runs.

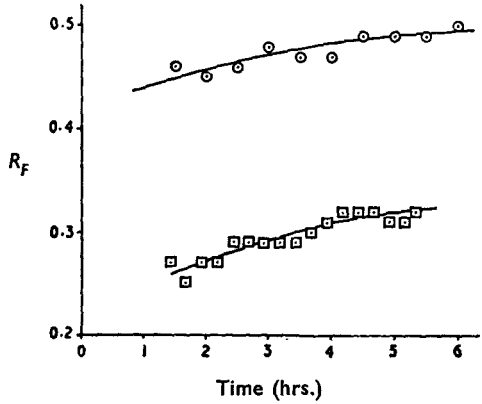

FIG. 2. R_F values plotted against time. ⊙ quinine, ⊡ proflavine.

The number of sheets in the tank had a profound effect on the R_F values. With a single sheet the values were cocaine, 0·57; hyoscyamine, 0·52; morphine, 0·21; strychnine 0·45; coniine, 0·65, but with four sheets the values were 0·42, 0·38, 0·15, 0·28, 0·62.

Tank size had a similar effect. For example, with a single sheet in a normal tank the R_F values for the same 5 alkaloids were 0·54, 0·48, 0·19, 0·41, 0·63, but with a single sheet in a small tank ($5\frac{1}{2}$ in. × $3\frac{1}{2}$ in. × $12\frac{1}{2}$ in. high) they were 0·39, 0·34, 0·14, 0·26, 0·58. If the system has been equilibrated the size of the tank and the number of sheets in it have no effect.

The above factors have the most influence on R_F values. No appreciable change in R_F value was caused by varying the concentration of the alkaloid solution, by the lateral position of the spot on the paper provided it was more than 1 cm. from the paper edge, nor by the distance of the starting line above the surface of the liquid. "Mixed spots" of alkaloids ran at the same speed as individual spots, while saturating the solvent with butyl citrate had no effect. Wet spots ran faster than dry ones and these again ran slightly faster than those dried with hot air. Paper kindly prepared for us by Dr. A. S. Curry gave similar results to paper prepared in this laboratory.

It may be concluded that reasonable reproducibility of R_F values may be obtained without loss of either resolution or simplicity if allowance is made for the factors outlined. It is worth noting how much less the R_F value of coniine varies with experimental conditions than do the R_F values of the other alkaloids used. This emphasises the danger of expressing the R_F value of one alkaloid in terms of the R_F value of another.

As the solvent should not be saturated with water it may be prepared conveniently by dissolving 4·8 g. of citric acid in a mixture of 160 ml. of water and 840 ml. of butanol, the specific gravity being kept between 0·838–0·846 by the addition of water.

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