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A study of the utility of planimetric determinations in the quantification of non-aminated organic acids separated by thin-layer chromatography

Planimetric determination is a simple method for the quantitative analysis of substances separated by thin-layer chromatography (TLC). The relationship between the spot area, F, and the amount of substance, M, is for the most part a straight line, where the formula $F = a \log M + b$ can be applied to the medium concentration sector and the formula $\sqrt{F} = a \log M + b$ to the sector of higher concentration[1,2]. NYBOM[3] and BONDIVENNE *et al.*[4] reported on the relationship between F and M, F and $\log M$, $\sqrt{F}$ and M and $\sqrt{F}$ and $\log M$ for different organic substances separated by TLC.

Method

We analysed the relationship between F and M, F and $\log M$ and $\sqrt{F}$ and $\log M$ for several non-aminated organic acids separated by two-dimensional TLC (*trans*-aconitic, fumaric, glycolic, hippuric, L-lactic, D,L-malic, pyrrolidonecarboxylic and succinic acids). Usually we placed 0.1–1.0 μmole on the plates (the minimum amounted to about 10 μg, the maximum to about 180 μg). The acids were applied to the cellulose plates (DC-Fertigplatten Cellulose, ohne Fluoreszenzindikator, 20 × 20 cm, Schichtdicke 0.1 mm*) by means of a micro-pipette** and were then chromatographed two-dimensionally in the solvents of GOEBELL AND KLINGENBERG[5] [first direction: 95 % ethanol–25 % NH_4OH–water (8:2:1); second direction: isobutanol–5 M formic acid (2:3)].

The development time in the basic solvent amounted to about 10 h, in the acidic solvent to about 7 h (development in a refrigerator at 15°). The mean R_F values of the single acids are town in Table I.

After developing and drying, the plates were evenly sprayed with aniline–xylose

TABLE I

MEAN R_F VALUES

Acid	*Basic solvent*	*Acidic solvent*
Fumaric	31.4	83.3
Glycolic	50.3	44.4
Hippuric	66.8	81.5
L-Lactic	58.3	67.0
D,L-Malic	23.9	30.1
Pyrrolidonecarboxylic	46.7	45.6
Succinic	27.2	69.8
trans-Aconitic	9.3	78.5

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reagent (1 g xylose in 3 ml water + 1 ml aniline and methanol to 100 ml), dried in air for about 5 min and heated in an oven at 140° for 5 min. The visible acid spots were marked immediately with a lead pencil and then copied on millimetre tracing paper. The areas were determined by counting the millimetre squares under magnification. The analysis was carried through in two series. In series 1 more indicator was sprayed on the plates than in series 2.

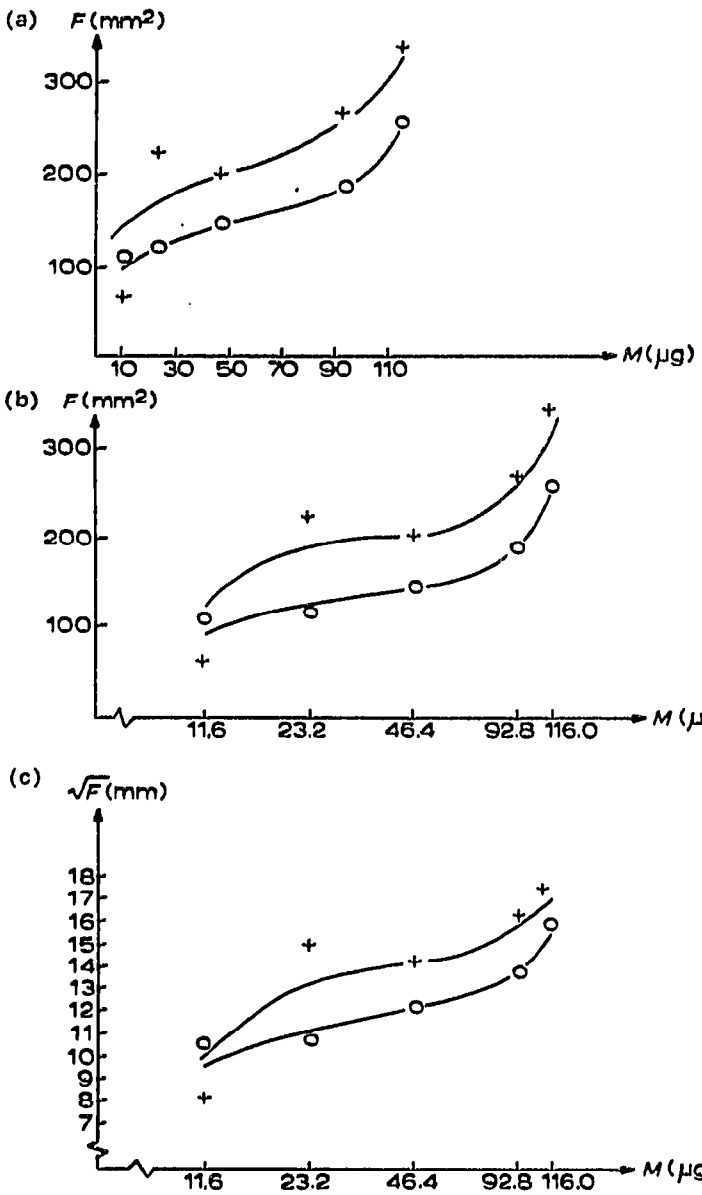

Fig. 1

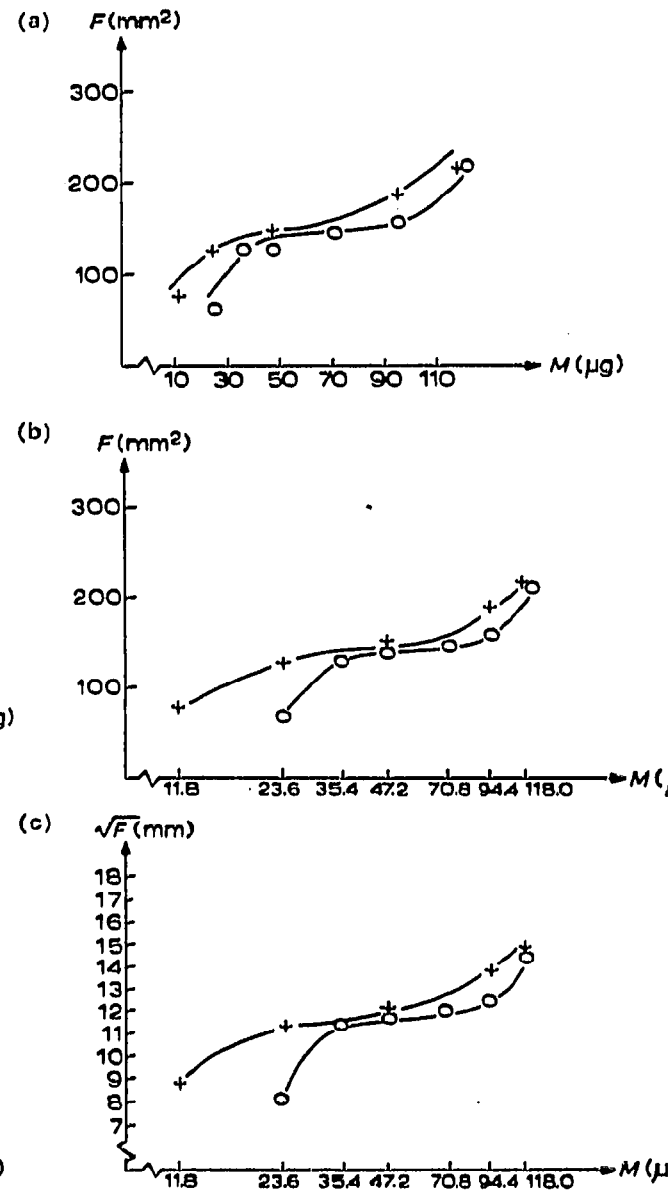

Fig. 2

Fig. 1. Fumaric acid: (a) relationship between F and M; (b) relationship between F and log M; (c) relationship between $\sqrt{F}$ and log M. For each graph: +, series 1; ○, series 2.

Fig. 2. Succinic acid: (a) relationship between F and M; (b) relationship between F and log M; (c) relationship between $\sqrt{F}$ and log M. + and ○ as in Fig. 1.

Results

Comparing the two series, we found that the curves obtained in series 1 showed bigger spot sizes for the same acid amounts than the curves obtained in series 2.

For all of the acids examined, except for *trans*-aconitic acid, the relationship between F and M was represented by a straight line in the range of 10/30–90/110 μg (Fig. 3a). For smaller amounts the curve showed an upward convex form and for larger amounts a downward convex form (Fig. 1a, series 2; Fig. 2a, series 2). In the extreme case, the straight line became short, and gave a curve which was first upward convex and then downward convex, with one turning-point (Fig. 1a, series 1; Fig. 2a, series 1).

The relationship between F and log M resulted in a straight line for glycolic and D,L-malic acids (Fig. 3b); a downward convex curve — at least for higher concentrations — for fumaric (Fig. 1b, series 2), hippuric, L-lactic and pyrrolidonecar-

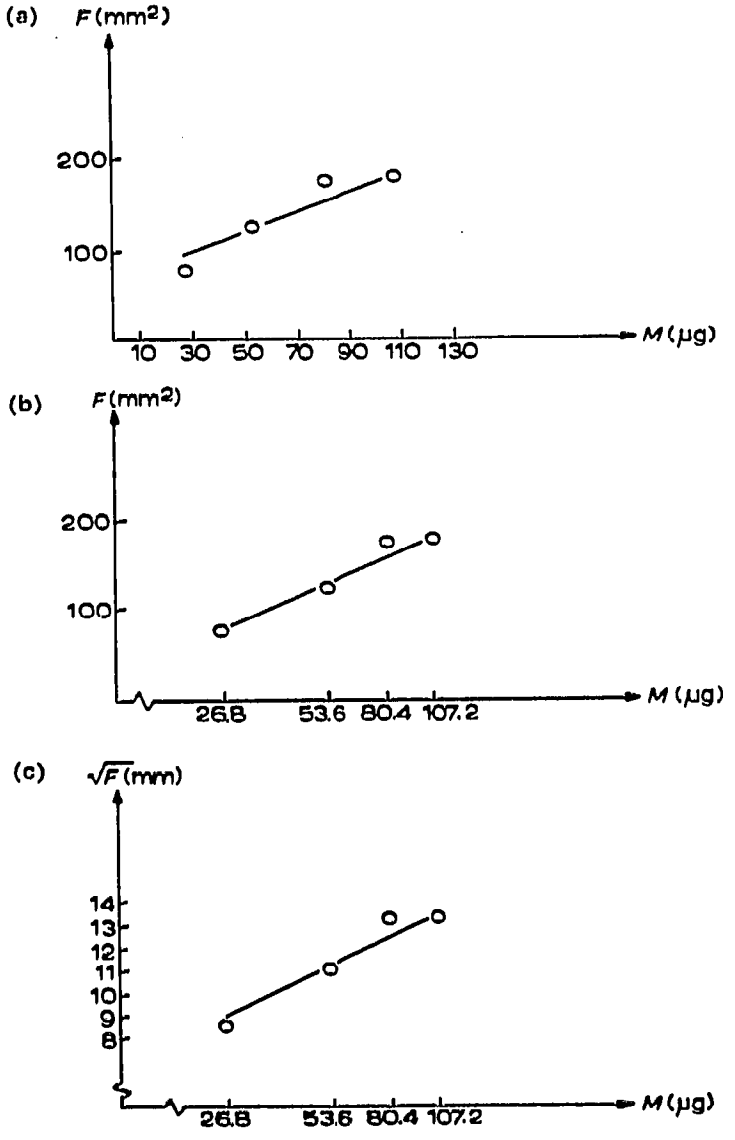

Fig. 3. D,L-Malic acid: (a) relationship between F and M; (b) relationship between F and log M; (c) relationship between $\sqrt{F}$ and log M.

boxylic acids; a first upward convex curve and then downward convex curve again for fumaric (Fig. 1b, series 1) and succinic (Fig. 2b, series 1 and 2) acids.

The relationship between $\sqrt{F}$ and log M had no other aspects (Figs. 1c, 2c, 3c).

For *trans*-aconitic acid the combination of F and M did not show any relationship in any of the analysed modifications. A statistic analysis of the results was not possible because of the low number of determinations.

Discussion

NYBOM[3] examined the relationship between F and M for malic and citric acids at different thicknesses of the cellulose layers. When F was regarded as a function of log M, straight lines resulted for the two acids when the cellulose layer was 0.2 mm, whereas downward convex curves resulted from a thickness of 1.0 mm. On the contrary, the relationship between $\sqrt{F}$ and log M showed straight lines for the two acids with a cellulose layer of 1.0 mm and upward convex curves at a thickness of 0.2 mm.

We worked with cellulose plates of 0.1 mm thickness. Therefore, generalizing the results of NYBOM[3], we had most probably to expect straight lines for the relationship between F and log M, but upward convex curves, however, for the relationship between $\sqrt{F}$ and log M. In fact we found a straight line for malic acid, when F was regarded as a function of log M (Fig. 3b); but on the contrary, no upward convex curve resulted from the relationship between $\sqrt{F}$ and log M, as this was a straight line too. Also, in the case of the other acids, curves resulted in a way which was different from what we expected from the results of NYBOM[3].

More interesting, in this context, appear to us the results of BONDIVENNE *et al.*[4], who, for different organic substances, found the relationships between F and M, $\sqrt{F}$ and log M and $\sqrt{F}$ and M to be curves which were first upward convex, then straight and finally downward convex. We observed such curves most clearly with fumaric and succinic acids (Figs. 1 and 2), but it is possible that this curve form is also valid for the other acids examined (except *trans*-aconitic) but only parts of the total curve were shown because of the concentration sector chosen. We found the statement of NYBOM justified that the relationship between spot size and amount of substance will vary from substance to substance[3].

When F (or $\sqrt{F}$) is related to M (or log M), the accuracy with which two different amounts of substance can be distinguished increases with the steepness of the corresponding curve. For our results this means that in the medium concentration sector (M about 30/50–70/90 μg), which is the most interesting, for several acids there is no accuracy in the differentiation of two different amounts (fumaric, glycolic and succinic acids), or only a low accuracy (D,L-malic and hippuric acids). Only for L-lactic and pyrrolidonecarboxylic acids does there exist high accuracy in differentiating two different amounts.

It can also be said that the planimetric determination of substances separated by TLC has important sources of error. The accidental errors caused by the fact that the spot form is influenced by the attendent substances chromatographed, and by difficulties in outlining the spots, are considerable[6]. Additionally, in two-dimensional chromatography, a substantial systematic error results from the fact that a standard solution cannot be chromatographed at the same time and on the same plate. BONDIVENNE *et al.*[4] conclude that a general method for the quantitative TLC determina-

tion of substances by direct measurement of the spot size is hardly possible. On the basis of our analysis we must assume the same for the non-aminated organic acids.

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