Quantitative Paper Chromatography of Fatty Acids

II. Some General Considerations on Direct Photometry

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Theoretical considerations together with experimental results indicate that letting the scanning window cover most of the width of the spots will render no significant bias to a chromatographic assay according to Kaufmann-Seher when the spot contents are kept below the 20 μg limit for the validity of Beer's law for every spot area element.

It is recommended that the length of the scanning window should cover a little less than the largest visible width of the spot.

Theoretical considerations [1] have lead to a description of the shape of chromatographic spots as ellipses with Gauss-distributions of the molecules along any of the elliptic axes.

When scanning the radioactivity content of chromatographic spots one can only expect the result to be influenced by the distribution of the molecules throughout the thickness of the paper and that has even experimentally been shown [2] to be of no significance in connection with fatty acids separated according to Kaufmann et al.[3] The result of a photometric scanning, however, will also be influenced by the distribution of the molecules along one of the dimensions parallel to the surface of the paper. This is due to the fact that the light-extinction values are not directly proportional to the amount of substance like the corresponding radioactivity countings. In quantitative paper chromatography according to Kaufmann-Seher [3,4] light transmission follows Beer's law within certain limits (Fig. 2 in a preceding paper *) and it is thus the logarithm of a modification of the electrical impulses coming from each area element of the photo cell and corresponding to a homogeneously coloured area element of the paper that should be summed in order to get true values. The way quantitative photo-scanning is done in practice (Seher [4]), the scanning window covers the whole width of the spot and this leads to direct automatic addition of the said electrical impulses along a dimension (u_1) parallel to the window and perpendicular to the scanning direction. Even though one takes the logarithm of each galvanometer reading along the scanning direction,

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* Acta Chem. Scand. 17 (1963) 139.

the final curve will thus only partly represent the corresponding marginal distribution of the copper-complex molecules along the dimension (u_2).

These considerations are illustrated by the integrations shown in Table 1 which have been done numerically on the assumption of circular spots with the same Gauss-distribution in all directions. I represents the ideal case with two-dimensional scanning of small homogeneously coloured area elements. II represents one-dimensional section-wise scanning the way it is done in practice. III is like II but without taking the logarithm of each galvanometer reading. The integration limits are measured in standard deviation units. Except for the * marked values the results in each row are given in units which make the value for A = 1 be unity. In all cases varying the factor A varies the total amount of colour, the latter depending partly on the amount of copper soap present, partly on the colouring agent. A comparison with experimental results showed that with dithio-oxamide as the colouring agent A = 3 was equivalent to a spot content of about 20 μg fatty acid. When a potassium ferro-cyanide solution as originally suggested by Kaufmann and co-workers [3] was used as the colouring agent about 20 μg fatty acid were equivalent to A = 0.3.

A further investigation of the influence of the width of the scanning area as indicated by the difference between IIa and IIb is shown in Fig. 1. Here the results of scannings with two different window lengths are compared with the true values based on radioactivity countings. Besides variation in total spot content the shown chromatogram also covers the case where one of the spots differs from the others in shape. The colouring agent was dithio-oxamide.

Table 1.

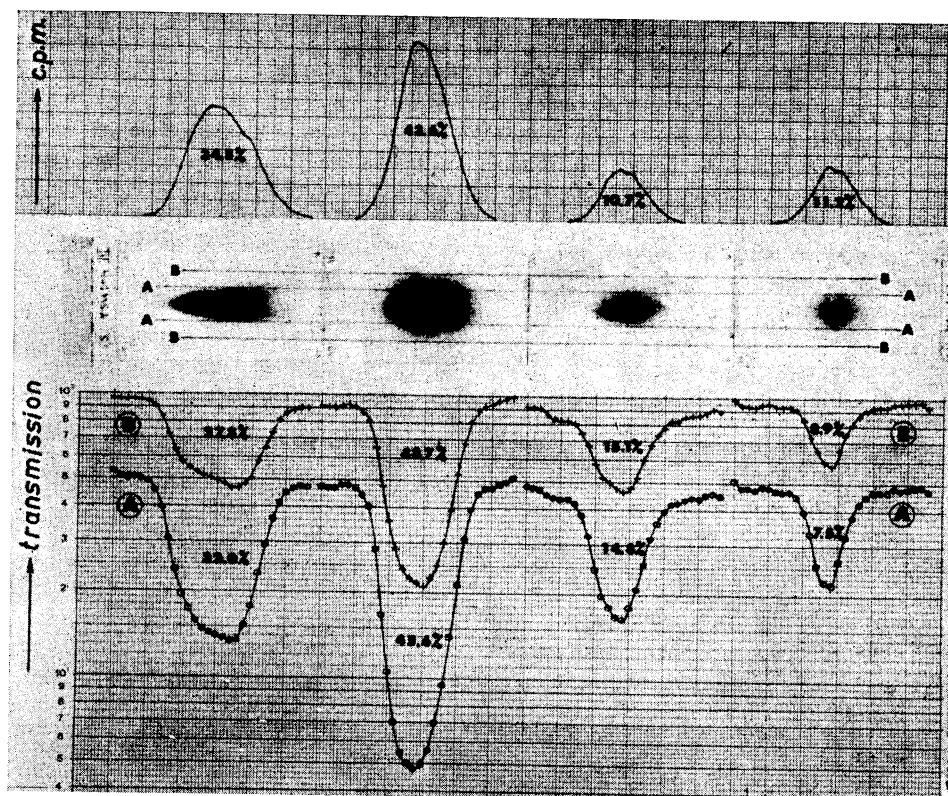|	A = 0.3	A = 1	A = 3	A = 10
I $\quad \int_{-3}^{3}\int_{-3}^{3} \log 10^{1-A(1/2\pi)\exp[-\frac{1}{2}(u_1^2+u_2^2)]} \, du_1 \, du_2$	0.30	1	3.0	10
II $a \quad \int_{-3}^{3} \log \int_{-2}^{2} 10^{1-A(1/2\pi)\exp[-\frac{1}{2}(u_1^2+u_2^2)]} \, du_1 \, du_2$	0.30	1	2.9	8.3
II $b \quad \int_{-3}^{3} \log \int_{-3}^{3} 10^{1-A(1/2\pi)\exp[-\frac{1}{2}(u_1^2+u_2^2)]} \, du_1 \, du_2$	0.22 * 0.31	0.7 * 1	2.0 * 2.8	5.2 * 7.3
III $\quad \int_{-3}^{3}\int_{-3}^{3} 10^{1-A(1/2\pi)\exp[-\frac{1}{2}(u_1^2+u_2^2)]} \, du_1 du_2$	0.32	1	2.6	5.6

* Standardized as II$_a$.

Fig. 1. Quantitative photometric assays with two different lengths of the scanning window compared to the true values found by radioactivity measurements.

DISCUSSION

As long as the spot contents are kept below the 20 μg limit for the validity of Beer's law, a section-wise scanning as illustrated by IIa in Table 1 will render no significant bias to the chromatographic assay. When A = 3, which corresponds to the stated 20 μg limit when the copper soaps are coloured by dithio-oxamide, Table 1 shows that the relative error induced will be only about 3 %. Table 1 also shows that the length of the scanning window should be no more than about 4 standard deviation units which means that it should cover a little less than the largest visible width of the spot. This is seen by comparing IIa with the lowest row in IIb.

The result of the scanning of the first spot in Fig. 1 is in agreement with a comparison between IIa and the * marked values. It is seen that widening the scanning area around a single spot means finding a lower spot content in that spot. Fig. 1 shows that such a specific widening can occur in practice when the spots vary in shape and thereby in the size of their respective stan-

dard deviations. Even though the length of the scanning window is the same during the whole scanning of such a chromatogram, the width of the scanning area measured in standard deviation units will vary from spot to spot. The results from Fig. 1 again together with the results from II in Table 1 show also that when spot shapes vary it is better to let the scanning limits cut across the wide spots than to let too much uncoloured area fringe the narrow spots.

Section-wise scanning without taking the logarithm of each galvanometer reading as illustrated by III in Table 1 is one of the easiest things to do. This can be recommended at least if potassium ferro-cyanide is used as the colouring agent.

REFERENCES

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