be determined as soluble copper complexes by the method described by Woiwod[4].

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Quantitative Paper Chromatography of Amino-acids

INDEPENDENT observations made in this Laboratory, during recent months, serve to confirm and to extend the findings recently reported by Fowden and Penney[1], concerning the factors causing loss in the quantitative estimation of amino-acids by paper chromatography. Since our experiments have been carried out under somewhat different conditions from those of these authors, and further, since we are not in complete agreement on all points, we feel it is desirable to record our findings.

The chief differences in our experimental conditions are the use of: (1) Whatman No. 54 filter paper instead of Whatman No. 4; (2) phenol/water (4 : 1), that is, unsaturated, and with the absence of ammonia; (3) lutidine/water (3 : 1) as a second experimental solvent; (4) an initial temperature of 95–100° for drying chromatograms, the heating current being switched off from the commencement of drying.

Effect of using washed filter paper upon amino-acid recovery. In agreement with Fowden and Penney, we have found that the blanks on untreated filter paper are high and variable, and that treatment of the paper with dilute alkali reduces the blanks very considerably. However, we have not found a simple alkali-washing to be sufficient to give consistent blanks and good recoveries. The use of a more thorough treatment gives a paper with improved properties, as is indicated in Table 1.

Table 1

Amino-acid chromatographed	Quantity applied (μgm.)	Recovery on paper (per cent) Washed with alkali	Boiled with alkali
Glycine	10	74, 86	96, 96, 97·5
Leucine	10	73, 63	84, 84, 100

The specially treated paper was boiled with four successive portions of 1 per cent alkali and then with water until free from alkali. The necessity for such treatment would seem to indicate the presence in the paper of ninhydrin-reacting materials other than ammonia[2].

Effect of heat in the absence of solvent on the recovery of amino-acids from filter paper. In the range of temperature investigated by Fowden and Penney, heat has practically no effect on the recovery of amino-acids. Our work has shown, however, that at higher temperatures (150°) considerable losses occur.

Table 2

Amino-acid	Quantity applied (μgm.)	Average recovery (in μgm.) on drying for 15 min. at 50°	100°	150°
Alanine	10	10·7 (9)*	10·1 (8)	7·5 (8)
Glycine	10	9·7 (14)	9·6 (14)	7·2 (11)
Leucine	15	14·8 (5)	14·9 (6)	12·2 (6)

* Figures in brackets indicate the number of determinations averaged.

Effect of heat in the presence of solvent on amino-acid recovery. Filter paper was spotted with alanine, glycine and leucine, treated with aqueous phenol and dried for 15 min. At 150° C. losses were considerable; at 100° they were still appreciable, except in the case of glycine; while at 50° the recovery was satisfactory.

Table 3

Amino-acid	Quantity applied (μgm.)	Average recovery (in μgm.) at 50°	100°	150°
Alanine	10	10·7 (2)*	8·5 (5)	4·5 (3)
Glycine	10	10·2 (4)	9·5 (6)	6·6 (4)
Leucine	15	14·5 (6)	12·1 (9)	6·3 (3)

* Figures in brackets indicate the number of determinations averaged.

Recoveries of amino-acids from one-dimensional chromatograms. These are shown in Table 4.

Table 4

Amino-acid chromatographed	Quantity applied (μgm.)	Solvent	Recovery in (μgm.)
Alanine	10	phenol	9·3, 9·9
Alanine	10	lutidine	8·5, 9·8
Glycine	10	phenol	8·7, 9·6, 10·2, 9·6, 9·6, 9·75
Glycine	10	lutidine	9·6, 10·7, 10·9
Leucine	15	phenol	14·4, 14·7, 12·7, 12·6, 12·6, 15

It is to be noted that the results do not indicate that the behaviour of glycine differs notably from that of other amino-acids.

In all cases amino-acid contents of papers were determined by the method of Moore and Stein.

The above data are recorded with the permission of the South African Council for Scientific and Industrial Research.

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S.A. Council for Scientific and Industrial Research,
Pretoria. July 18.

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History of Chromatography

ALL those interested in the history of chromatography, including the authors of the present communication, have hitherto accepted the claim that M. S. Tswett was the discoverer of this extremely fruitful method of analysis. A detailed survey of the early literature of adsorption in petroleum technology recently initiated by one of us (H. W.)[1] strongly suggests that, despite the powerful influence which his experiments have undoubtedly had on later generations, Tswett has, in fact, received considerably more credit than is his due.

So early as 1897, D. T. Day[2], pursuing investigations of the methods by which natural petroleum is formed and transformed, showed that if crude petroleum is forced upwards through a column of powdered limestone some fractionation occurs and the oil which first emerges is thinner and lighter in colour than that which follows. A similar commercial process had, indeed, been devised some ten years previously for the petroleum industry by C. Engler and M. Boehm for the bleaching of oil and the pro-