A New Method for Ultra Micro Determination of Ammonia or Organic Nitrogen

By Yoshimi OKADA and Hidesaburo HANAFUSA

(Received July 7, 1954)

The micro-Kjeldahl method is useful for rapid nitrogen analysis of various organic substances of low nitrogen content. However, it would be convenient to determine much smaller amounts of nitrogen in cases where the nitrogen content of the sample is very low or the amount of sample available is limited. For this purpose we replaced titration in this method by colorimetry with ninhydrin[1] and could determine 30 μg. of nitrogen by this modification.

This modified method is the same as the usual micro-Kjeldahl method except that the sample for single determination should contain 20–100 μg. nitrogen and the time of distillation is shortened to 1.5 minutes and 5 cc. of 1/100 N-H_2SO_4 is placed in an adapting flask. After distillation, the solution in the flask (usually 15–20 cc.) is diluted to 25 cc. with a citrate buffer (*p*H 5.0) and 1 cc. aliquot is taken to determine ammonia colorimetrically[1]. A standard curve was prepared previously with the solution of NH_4Cl (1–10 μg./cc.).

This method is particularly useful for the determination of amide-N of proteins, as the trouble of titration caused by octylalcohol, an anti-foaming reagent, can be eliminated completely. Some results by this method are shown in Table I.

TABLE I
EXAMPLES OF NITROGEN DETERMINATION BY AUTHORS' METHOD

Total-N

Sample	Found %	Calculated %
Acetanilide (354 μg.)	10.39	10.37
,, (425 μg.)	10.43	

Amide-NH_3

Sample	Found %	Reported %
Lysozyme (2.03 mg.)	1.88	1.7–2.0[2]
		1.87[3]
		2.08[4]
Taka-amylase A (2.87 mg.)	1.43	1.47[5]

By further modification of our method, by combining the diffusion method according to Conway[6,7], it would be possible to determine extremely small amounts of nitrogen. Experiments on this possibility are now in progress.

The authors wish to express their thanks to Prof. S. Akabori for his kind advice.

Laboratory of Biochemistry, Faculty of Science, Osaka University

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