Direct Titration of Ammonia in Kjeldahl Determinations with Nickelammoniumsulphate Solutions as Absorbent

JAKOB BLOM and BIRGIT SCHWARZ

The Laboratory, Tuborg Breweries, Copenhagen, Denmark

In the Kjeldahl method for the determination of nitrogen in organic substances two distillation procedures are in common use. In the classical method the ammonia is distilled into a measured volume of a standard strong acid, and the excess acid is titrated with a standard base. This procedure gives a sharp end-point but requires two standard solutions, the base being rather unstable. In order to be able to titrate the ammonia directly with standard acid Winkler[1] suggested to absorb the ammonia in an excess of an exceedingly weak acid, boric acid. This method eliminates the need for a standard solution of a base, and for the accurate measurement of the absorbent, and it simplifies the calculation of the analysis, but it requires an additional solution in the form of an indicator-boric-acid-solution by which the end-point is matched and adjusted.

More than a year ago we introduced the boric acid method in our routine work, but we found that determinations according to this method were less accurate than determinations according to the classical method. The advantage of the direct titration over the back titration method was however so evident, that we looked for another absorbent, which would make a direct titration of the ammonia possible. It later occured to one of us, that a solution of some salt, whose cation forms metal ammine complexes[2], might suit our purpose. In order to obtain a titration, where the attainment of the end-point is not slow, salts have to be selected, whose cations form ammine compounds in fast reactions and are not hydrolysed into metal hydroxides.

In a macro-Kjeldahl determination 1—3 mval of ammonia are distilled off with 120 to 150 ml of water. Our preliminary in-

Table 3. Different nitrogen fractions in normal and low-nitrogen T o r u l a.

g per 100 g dry matter	Normal N *Torula*	Low-N *Torula*	Decrease %
Total N	9.5	5.7	40
"Protein N"	6.1	4.1	33
N soluble in trichloracetic acid	3.4	1.6	53
NA-N	1.4	0.65	53.5
Other soluble N	2.0	0.95	52.5

decreased in *Torula* noticeably less than total-N. Other soluble N, again, has decreased in *Torula* as much as the nucleic acid-N. In *Ps. fluorescens* this N-fraction has not lowered at all. Accordingly, different nitrogen fractions of microorganisms do not change quantitatively in the same way as their nitrogen content lowers.

The *Pseudomonas* masses were prepared by Miss Ulla Winkler.

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vestigations were performed with 1 to 10 mval of cupric ion, zink ion, cobalt ion, cadmium ion and nickel ion. Under these conditions only 10 mval nickel ion in the form of nickelsulphate proved to be suitable. This method was adopted for routine work and used for several months. Using methyl red as indicator the green color of the nickel ion does not interfere with the detection of the end-point. If the titration is not carried out within about one hour after the distillation, the attainment of the end-point becomes rather slow, owing to the formation of minute amounts of a precipitate (nickel hydroxide ?).

Since the hydrolysis is depressed by ammonium ion, the same investigations were later on repeated with the addition of up to 20 mval of ammonium ion. Even with 20 mval of ammonium ion hydroxides are precipitated in solutions of cupric ion and zink ion. Suitable amounts of ammonium ion depress the hydrolysis of cobalt ion and cadmium ion, so that they might so far also be used. The amount of ammonium ion required is however greatest with cobalt ion, much smaller with cadmium ion, and smaller still with nickel ion. Moreover the red color of the cobalt ion interferes with the observation of the end-point using methyl red as indicator. Cadmium salt solutions are appreciably more acid than nickel salt solutions, so that methyl red cannot be used as indicator. We found that nickel ion was definitely best suited to our purpose. Of the different nickel salts we choose $NiSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$, since this salt is easily obtained chemically pure [3] and furthermore contains the two components in a proportion suitable for our purpose. In our routine work, where about 1 mval of ammonia is distilled off with about 130 ml of water 20 ml of 0.2 M nickelammoniumsulphate are used as absorbent. If the amount of ammonia is greater, proportionally more nickelammoniumsulphate solution has to be used. Under these conditions nickel hydroxide is not precipitated even on standing for hours. During distillation the pH is only raised from 5.1 to 7.6, and the volatility of the ammonia at room temperature is negligibly small; the receiver flasks may stand unstoppered for hours without measurable loss of ammonia. Nevertheless the ammonia may be titrated sharply with methyl red as indicator. With a mixture of methyl red and methylene blue the end-point is still more easily recognized. Contrary to the boric acid method a comparison solution is unnecessary.

Table 1. pH measurements with glass electrode. Total volume: 150 ml.

	H_2O	$NiSO_4 \cdot (NH_4)_2SO_4$ 0.2 *M* 20 ml	$CdSO_4$ 0.2 *M* $(NH_4)_2SO_4$ 0.4 *M* 20 ml
H_2O	—	5.05	4.80
NH_3, 1 mval	10.6	7.6	7.5
NH_4Cl, 1 mval	5.43	5.08	4.80
End-point of methyl red	5.45	5.08	5.20

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