0.5% of its weight as carbon dioxide. The correction may be applied, if desired. The presence of toluol does not effect the accuracy of the method, as the toluol is not oxidized.

Summary.

The determination of ethyl alcohol by oxidation with sulfuric acid-dichromate solution has been carefully studied. It is considered that the best results are obtained when the alcohol is finally determined by distillation and titration of the acetic acid formed.

The preparation of the solution by distillation, even from a saturated salt solution, is tedious and troublesome and far from quantitative.

The alcohol solution may be saturated with ammonium sulfate and the alcohol carried over into concentrated sulfuric acid by a current of air at room temperature. The alcohol-sulfuric acid solution may then be mixed with a solution of potassium dichromate and the acetic acid distilled off at once. Results accurate within 1.5% of the amount used have been obtained by this method.

Necessary precautions and interfering substances are discussed.

AMES, IOWA.

[FROM THE N. Y. STATE DEPARTMENT OF HEALTH LABORATORIES, ALBANY, N. Y.]

THE EFFICIENCY OF THE AERATION METHOD FOR DISTILLING AMMONIA; IN ANSWER TO CERTAIN CRITICISMS.

BY PHILIP ADOLPH KOBER.

Received September 3, 1916.

From his "Comparative Study of Aeration and Heat Distillation in the Kjeldahl Method for the Determination of Nitrogen" Falk[1] concludes that "the aeration procedure in the ordinary Kjeldahl method for nitrogen very often gives inaccurate and therefore unreliable results, and should not be used."

This conclusion is based on his failure to remove all of the ammonia by aeration. Dillingham[2] has also recorded failure to obtain satisfactory results for the same reasons, while Bock and Benedict[3] and Greenwald[4] report great variations in the results of their determinations without accounting for them.

Accordingly, I have studied carefully the records of these observers in their published articles in the hope of discovering the sources of error, whether in the method or in the manner in which it was used.

Falk attempts to employ the results of Davis, as confirmatory data, in saying that:

[1] THIS JOURNAL, 38, 916 (1916).
[2] *Ibid.*, 36, 1310 (1914).
[3] *J. Biol. Chem.*, 20, 47 (1915).
[4] *Ibid.*, 21, 61 (1915).

"Davis found aeration to give unsatisfactory results in the determination in cottonseed meal and proposed to heat the solution during aeration."

But Davis[1] said that: "If the flask * * * were placed in an asbestos box * * * the solutions kept warm an hour and a half," and "the results checked with results obtained by ordinary Kjeldahl." Heat was only proposed if larger amounts of substance were used and then only for "fifteen minutes," and in conclusion, "the method described by Kober if modified as described will give good results with other nitrogen containing bodies."

The reason for this modification is apparent, as Davis states on page 56, that "the stream of air drawn by the ordinary glass pump was not strong enough to carry over the ammonia by the time the solution cooled," thus showing that he realized the necessity for strong aeration. However, it is quite unnecessary to heat, because a little longer aeration accomplishes the same result.

In the experiments of Gill and Grindley[2] mentioned by Falk, only the analysis of one substance failed to yield satisfactory results. When compared to a large number of accurate results which they obtained this is of little significance. These observers strongly recommended the aeration procedure for the distillation of ammonia in urea and urine estimations.

Finally, in citing the work of others Falk relies for most of his support on Dillingham,[3] but he overlooks the essential fact that to Dillingham's technic can be attributed all his unsatisfactory results. He used a flask of large diameter (800 cc. capacity), and thereby spread out and decreased the height of the liquid through which the ammonia should pass and almost destroyed the efficiency of his aeration. That the higher the column for a given liquid, the more efficient the aeration, is too obvious to need but passing mention. Dillingham's attention was called to this oversight two years ago, but no re-investigation, although promised, has thus far appeared. Whether any other defects in his technic are involved it is impossible to say.

To sum up, then, the only support in the literature found by Falk, was one instance with a defective pump (Davis), and another instance with an unsuitable flask (Dillingham).

Considering Falk's results independently, the following points are to be noted:

1. Disagreement in the results obtained by aeration.

2. With one or two exceptions, the amount of ammonia undistilled was nearly proportional to the total amount present originally.

[1] THIS JOURNAL, 31, 56 (1909).

[2] *Ibid.*, 31, 1249 (1909).

[3] *Ibid.*, 36, 1310 (1914).

3. That the amount of aeration varied as much as 39%, and even 30%, in a series of distillations "testing before and after the run."

4. That the speed of aeration during a run was never tested, and is therefore unknown.

It is clear from these four points that the main cause of the discrepancies in the results was poor aeration.

That the aeration was weak at the Harriman laboratory, due to inadequate water supply for the overcrowded rooms, was found by the writer personally and is shown by the fact that Falk's collaborator in this paper, Sugiura, under more favorable conditions in former years, when less water was used for other purposes, did get good checks for the aeration procedure.[1]

It is a very simple matter to test out the efficiency of aeration by adding Nessler's solution to the Kjeldahl residue, as was recommended in a previous paper,[2] but this seems not to have been considered by Falk, even though with steam distillation an increase in titration alone is no test for ammonia. Dissolved alkali from the glass condenser, if used, will alone account for such a phenomenon.

To re-check the cardinal points the following demonstration was made: An ordinary Kjeldahl distillation was run with an extra absorption bottle.

After aeration Nessler's reagent was added to the Kjeldahl flask and Graves' reagent to the extra absorption bottle. The negative result with the residue in the Kjeldahl flask leaves no question that all the ammonia had been aerated over, and since there was no ammonia in the extra absorption bottle it was proved conclusively that all the ammonia was in the first absorption bottle and was completely absorbed.

Where there is an abundance of air supply, as in institutes particularly equipped with air compressors and vacuum systems, and in all laboratories where the "micro" form of apparatus is used, which requires much less air, no such aeration difficulty is found. That in many laboratories water suction pumps are inefficient was brought out by Pennington[3] and accounts for the failure of Boussingault in 1850 to apply successfully the aeration method using only 56 liters an hour, while Folin employing 600–700 liters an hour was the first to demonstrate successfully that ammonia can be distilled by aeration from weakly alkaline solutions and absorbed quantitatively.

The positive results for urea estimations by Henriques[4] and Gammeltoft are not mentioned by Falk. He also fails to cite the work of Potter[5] and Snyder who state that "as to accuracy and ease Kober's statements have

[1] THIS JOURNAL, **35**, 1603 (1913).

[2] Kober and Graves, *Ibid.*, **35**, 1601 (1913).

[3] THIS JOURNAL, **32**, 561 (1909).

[4] *Skand. Archiv. Phys.*, **25**, 166 (1911); *Bohr-Gedachnesschrift.*

[5] *J. Ind. Eng. Chem.*, **17**, 226 (1915).

been confirmed." Furthermore, a large number of other workers who have used aeration in one form or another for distillation of ammonia have been completely overlooked.

It is of interest to note that while Falk was completing his article, Van Slyke[1] and Cullen, at the Rockefeller Institute, published a paper showing that the aeration of ammonia even in "micro" amounts is so thoroughly established that it follows a definite law, namely, that of a mono-molecular reaction, and given certain factors the time necessary for complete distillation could be calculated. Furthermore, even for very small amounts of ammonia Van Slyke and Cullen conclude that "*the accuracy attainable is limited only by that of the measurements and standard solutions.*"

It might be assumed that this statement applies to the micro and not to the macro process under discussion, but it would be difficult to explain, if ammonia in micro amounts can be aerated over and absorbed, why larger amounts should not be distilled over if aeration is used in proportion.

In regard to the publications of my collaborators and myself, Falk gives the impression that only comparatively few satisfactory results were obtained, ignoring the fact that the aeration method has been used by us for regular work exclusively for eight years and has been checked by many assistants. This work comprises several thousand[2] duplicate and triplicate analyses which agree closely, but only protocols of which have been published. Thus it is evident that the failure of aeration results to agree indicates defective technic.

Summary.

To insure accurate results in distilling ammonia by aeration it is necessary to use:

1. A sufficient volume of air.

2. As high a column but as low a volume of liquid as is convenient.

3. A saturated solution of pure sodium hydroxide in adequate excess.[3] An impure alkali containing or producing sulfite is liable to cause error because of the sulfur dioxide evolved and carried over into the standard acid before the acid of the Kjeldahl mixture is completely neutralized.

4. Potassium hydroxide must not be used because the difficultly soluble potassium sulfate which separates may carry down ammonia by occlusion or as a double salt. Errors of 10% may be caused in this way but potassium sulfate as ordinarily used is not sufficient to produce appreciable error.

[1] *J. Biol. Chem.*, **24**, 117 (1916).

[2] Levene and Kober, *Am. J. Physiol.*, **23**, 328 (1909); Saccharine Report, U. S. Dept. of Agriculture, Referee Board of Consulting Scientific Experts, from the Herter Lab. The statements in these publications that the total nitrogen estimations were made according to the Kjeldahl-Gunning process are not quite correct; they were practically all distilled according to the aeration method.

[3] Kober and Graves, THIS JOURNAL, **35**, 1600 (1913).

5. The complete removal of the ammonia from the Kjeldahl mixture should be tested with Nessler's solution. The precaution of Van Slyke to run the aeration slowly or at half speed for the first minute or two may be an advantage.

Addendum.

After sending in this paper for publication I learn that recently a number of other investigators have tried the aeration method and found it accurate. I. K. Phelps and H. W. Daudt, from the Bureau of Chemistry, Washington, D. C., reported favorably on the method at the Urbana meeting of the Society. B. S. Davisson, E. R. Allen and B. M. Stubblefield[1] were able, with a powerful aeration, to remove and absorb small amounts of ammonia from large volumes of solution accurately, using only magnesium hydroxide as an alkali.

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[CONTRIBUTION FROM THE SPRECKLES AGRICULTURAL RESEARCH LABORATORY.]

PRESENCE OF NITRITES AND AMMONIA IN DISEASED PLANTS, ITS SIGNIFICANCE WITH REGARD TO CROP ROTATION AND SOIL DEPLETION.[2]

BY P. A. BONCQUET.

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The present study was made independently from the discussion now at large concerning the presence of nitrites in plants.[3] It is not the intention of the writer to decide the above contentions, but only to make known some facts which may throw new light on the controversy.

Nitrites in Diseased Beets.—During previous work *Bacillus morulans*, Boncquet,[4] was found to be an inhabitant of the sieve tubes of sugar beets affected with Curly Leaf.[5] Moreover it was established that the organism was not confined to the disease of beets called Curly Leaf, but that it was connected with a great variety of irregular foliage types representing various forms of leaf wrinkling, curling and distortions.[4] The same organisms were also isolated in cultures from the interior of leaves of beets

[1] *J. Ind. Eng. Chem.*, **8**, 896 (1916).

[2] The writer is greatly indebted to the Spreckles Sugar Company for the splendid facilities placed at his disposal in the realization of this investigation.

[3] Klein, *Bot. Centbl. Beihefte, I Abt.*, n 1, **30**, 141–166 (1915); *E. S. R.*, **33**, 627; Oso and Sekine, *Ibid., I Abt.*, **32**, 146–147 (1914); *E. S. R.*, **33**, 627; Maze, *Compt. rend. soc. Biol.*, [Paris] **78**, 98–102 (1915); *E. S. R.*, **34**, 627.

[4] Boncquet, *Baccillus morulans n. sp.* A bacterial organism which inhabits the sieve tubes of sugar beets and related plants. Its characters and significance. A thesis presented for the degree of Doctor of Philosophy at the University of California.

[5] Ralph E. Smith and P. A. Boncquet, "New Light on Curly Top of the Sugar Beet," *Phytopathology*, **5**, 103–107 (1915).