LXXXIII.—*Kjeldahl's Method for the Determination of Nitrogen.*

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KJELDAHL'S method for the determination of nitrogen has, in some one or other of its modifications, superseded the well-known soda-lime process of Varrentrap and Will in most agricultural laboratories, both in England and on the Continent, and to a very large extent in America. For the analysis of feeding stuffs and fertilisers,

it has many advantages over the soda-lime process, not being liable to the errors arising from variations of temperature in combustion, and obviating the necessity for reducing the substance to the fine state of division required when that method is used.

Kjeldahl's process, as originally devised, had the disadvantage of being very slow, but the modification introduced by Gunning has not only rendered it speedy, but also increased the accuracy, whilst Jodlbauer's modification has enabled it also to replace the Ruffe process for the determination of total nitrogen in fertilisers containing nitrates as well as organic ammoniacal nitrogen.

The method, however, has not found its way into the laboratory of the organic chemist—at any rate in England—and I determined, with the help of one of my assistants, Mr. E. H. Roberts, to try it on a number of representative organic compounds of varied constitution.*

It will be convenient at the outset to describe the particular modifications of the process which experience has led me to adopt.

The original Kjeldahl process consisted in the oxidation of the substance by boiling with strong sulphuric acid, completing the action by the addition of potassium permanganate (*Zeit. Anal. Chem.*, **22**, 366). The use of potassium permanganate cannot be recommended, unless the quantity used is very moderate; it has a tendency to cause low results, probably by oxidizing the ammonia. On the other hand, however, the use of a drop of mercury, or a little mercuric oxide (Wilfarth, *Chem. Centr.*, **3**, 16, 17, 113; and Arnold *loc. cit.*, 337) is a great advantage, as it greatly expedites the oxidation of carbonaceous matter. The greatest advance made on the process, however, was the introduction by Gunning (*Zeit. Anal. Chem.*, **28**, 188) of the use of potassium sulphate as an addition to the sulphuric acid after the action of the acid on the material had continued for a short time. This reduced the time of oxidation in the case of refractory substances from some hours to about half an hour, or even less. The effect is to form a strong acid solution of potassium bisulphate, which boils at a considerably higher temperature than the sulphuric acid, thereby very greatly facilitating the oxidation. When the Gunning modification is employed, the use of mercury can be dispensed with; although a little mercury renders the process even still more rapid. This process with the

* At the time these experiments were undertaken, the author was unaware of the work of Dafert (*Landwirth-Versuchs.*, **34**), and of the later work of Arnold and Wedermeyer (*Zeit. Anal. Chem.*, **31**, 525) in the same direction. On the whole, the experience of these authors is similar in most directions to that now recorded, although the majority of the representative compounds selected for their experiments were different from those used by the present author.

addition of mercury, which I now describe, I shall refer to as the "Kjeldahl-Gunning-Arnold" process.

In the analysis of feeding stuffs or fertilisers (not containing nitrates), any quantity, varying according to circumstances, from 0.5 gram to 5 grams, or even more, is introduced into a round-bottomed flask of hard Jena glass, and treated with about 20 c.c. of strong sulphuric acid, with the addition of a single drop of mercury. The flask is closed with a loosely fitting balloon stopper (*see Fig.*), and heated over a gas flame, gently at first, until the initial vigorous action has ceased; the heat is then gradually increased, so that the liquid boils briskly, and in about 15 minutes 10 grams of potassium sulphate are added, and the boiling continued until the contents of the flask are clear and colourless; this generally occurs within about half an hour, or, in the case of very carbonaceous substances, within an hour. The sulphuric acid condenses on the internal projection of the balloon stopper, and falls

back, so that there is little loss of acid except as sulphurous acid. The contents of the digesting flask are then washed out into a spacious distilling flask, also of Jena glass, which is connected by a doubly perforated cork with any convenient condensing apparatus; the second perforation bearing a tapped funnel through which an excess of sodium hydrate solution is added, and also a little sodium sulphide to decompose any nitrogen compounds of mercury that may be formed; if mercury is not used, the sodium sulphide may of course be dispensed with. Some zinc foil is put in the flask to prevent bumping, and the products of distillation are collected in a measured quantity of standard acid, the ammonia which distils over being determined by titration in the usual way. I find it best to allow the steam charged with ammonia to pass directly into the acid, contained in a flask standing in a tank of running water, using as a means of communication between the distilling flask and the receiving flask a block-tin tube bent in the form of an arch; this rises perpendicularly from the cork of the distilling flask to a height of 15 or 18 inches before turning over. With the apparatus arranged in this way, I find that there is no danger whatever of the passing over of soda spray with the steam, and the use of any of the various forms of "spray trap" is unnecessary. The other end

of the tube is united by a cork to a pear-shaped adapter having a large expansion, and terminating in a narrow end which dips into an ordinary Erlemeyer flask in which the acid is contained. The pear-shaped expansion allows for the variations of pressure during distillation, and is sufficient to avoid any regurgitation of the acid into the distilling flask.

There is but little difficulty in obtaining sulphuric acid, sodium hydroxide, and sodium sulphide practically free from nitrogen compounds, or so free as to introduce practically no error into the determination. A blank experiment should, however, always be made when new stocks of material are bought, and the result, including the slight error caused by the action of steam or standard acid on the glass, should not give an error (constant for the particular materials in use) amounting to more than the equivalent of 0.0003 to 0.0005 gram of nitrogen, or from 0.2 c.c. to 0.4 c.c. of decinormal sodium hydroxide solution. The smaller margin of alkalinity will generally be reached in the distillate, even when distilled water is boiled over. In my own laboratory, the error is determined as a matter of routine at least once a week, but it is rare to find it exceeding the higher limit of 0.0005 gram.

When nitrates are present in addition to organic or ammoniacal nitrogen, Jodlbauer's modification (*Chem. Centr.*, 3, 17, 433) suffices to determine accurately the total nitrogen. This process consists in previously adding to the sulphuric acid used for oxidation, either phenol or, preferably, salicylic acid—generally about 2 grams for a determination. While the contents of the flask are still cold, from 1 to 2 grams of zinc dust are added (as well as a drop of mercury) and allowed to dissolve before the flask is heated. The process is then continued exactly as before, when the whole of the nitrogen is obtained as ammonia. There is no difficulty whatever in determining the nitrogen in potassium or sodium nitrate in this manner; but I find that when ammonia salts are present as well as potassium or sodium nitrate, the results are invariably too low, unless the sulphuric acid containing the salicylic acid is poured quickly into the flask out of a beaker, so that the nitrate shall be completely covered by the acid before the lapse of an appreciable interval of time; this prevents the formation of the lower oxides of nitrogen, and consequent loss. When even ammonium nitrate is treated in this way, the whole of the nitrogen is retained in solution. I allude to this detail, because I have nowhere seen attention drawn to it, and because I think there is a probability of large errors occurring in the analysis of compound fertilisers, including mixtures of ammonia salts and alkali nitrates, if the acid is allowed to flow on to the sample from a pipette in the usual way. I have little doubt that

the observance of the simple precaution mentioned above will remove a difficulty which has hitherto been productive of discrepancies in the analysis of such materials. The process, as I have described it, may be called the Kjeldahl-Gunning-Arnold-Jodlbauer process.

The degree of accuracy possessed by these methods will be to some extent gathered from the results of their application to the various compounds now to be mentioned. It should be said that in calculating the percentages of nitrogen the atomic weights used are $O = 16$; $C = 12$; $N = 14.046$; $H = 1.0024$; $S = 32.06$. The strengths of the standard solutions of acid and of alkali used in titrating, were determined by reference to pure ammonium sulphate.

Kjeldahl-Gunning-Arnold Method.

Compound.	Formula.	Percentage of nitrogen calculated.	Percentage of nitrogen found.
Uric acid	$C_5H_4N_4O_3$	33.40	33.31, 33.31
Asparagine	$C_5H_5(NH_2)(CO \cdot NH_2)(CO \cdot OH)$	18.71	18.69, 18.69
Caffeine	$C_8H_{10}N_4O_2$	28.93	28.94
Indigotin	$C_{16}H_{10}N_2O_2$	10.72	10.57, 10.51
Atropine	$C_{17}H_{23}NO_3$	4.86	4.83
Morphine (anhyd.)	$C_{17}H_{19}NO_3$	4.63	4.58, 4.62
Quinine sulphate (anhydrous)	$(C_{20}H_{24}N_2O_2)_2H_2SO_4$	7.53	7.54
Strychnine	$C_{21}H_{19}N_2O_4$	8.41	8.39, 8.42
Aniline	$C_6H_5 \cdot NH_2$	15.09	14.95, 14.95
Sulphanilic acid (para-amidobenzenesulphonic acid)	$C_6H_4 \cdot NH_2 \cdot SO_3H$	8.11	8.08, 8.09
Diphenylamine	$(C_6H_5)_2NH$	8.31	8.33, 8.33
β -Naphthylamine	$C_{10}H_7NH_2$	9.82	9.83, 9.84
Acetanilide	$C_6H_5 \cdot NH \cdot C_2H_5O$	10.40	10.42
Orthobenzoic sulphide	$C_6H_4 < \begin{smallmatrix} CO \\ SO_2 \end{smallmatrix} > NH$	7.67	7.64
Parasulphamidobenzoic acid	$C_6H_4(HSO_2NH_2) \cdot COOH$	6.98	6.97
Fyridine	C_5H_5N	17.77	17.45, 17.48
Benzidine (diamidophenyl)	$C_{12}H_8(NH_2)_2$	15.26	15.24
Aldehyde-ammonia	$CH_3 \cdot CH(OH) \cdot NH_2$	23.00	23.03, 23.12
Nitrosodimethylaniline	$C_6H_5 < \begin{smallmatrix} NO \\ N(CH_3)_2 \end{smallmatrix} >$	18.71	18.52, 18.74, 18.59
Potassium cyanide (? pure)	KCN	21.55	21.27, 21.27
Ethyl cyanide (propionitrile) (? pure)	$C_2H_5 \cdot CN$	25.51	25.13, 25.21
Potassium ferrocyanide	$K_4(FeC_6N_6)$	19.93	19.91, 20.00
Potassium ferricyanide*	$K_3(FeC_6N_6)$	25.56	24.84, 24.81, 24.93

* Addition of zinc dust gave nitrogen, 25.26; addition of sodium thiosulphate gave nitrogen, 25.41, 25.42, 25.39, 25.49.

Compound.	Formula.	Percentage of nitrogen calculated.	Without reducing agent.	With zinc dust.	Salicylic acid and zinc dust.	Sugar and zinc dust.
Azobenzene	$C_6H_5 \cdot N_2 \cdot C_6H_5$	15.42	14.94	15.04	15.45	15.46
Amidoazobenzene	$C_6H_5 \cdot N_2 \cdot C_6H_4 \cdot NH_2$	21.37	14.85 12.19 10.66	14.91 21.28	15.45 —	—
Amidoazotoluene	$CH_3 \cdot C_6H_4 \cdot N_2 \cdot C_6H_3(CH_3) \cdot NH_2$	18.71	—	18.79	—	—
Carbazol	$NH(C_6H_4)_2$	8.41	8.25 8.29	8.25 8.29	— —	— —
Hydrazobenzene*	$C_6H_5 \cdot NH \cdot NH \cdot C_6H_5$	15.25	15.25	—	—	—
Hydroxylamine hydrochloride.	$NH_2 \cdot OH, HCl$	20.21	3.18 1.64	10.61 9.42	18.84 18.84	Salicylic acid, sugar and zinc. 20.24† 20.18 20.21
Acetaldoxime	$CH_3 \cdot CH \cdot NOH$	23.78	23.08 23.00	23.30	—	Sugar and zinc. 23.59
β -Naphthaquinone(α)oxime ...	$C_{10}H_6O(NOH)$	8.12	8.10 8.11	—	—	—
Sodium nitroprusside†	$Na_2(NO)(FeC_5N_5) \cdot 2H_2O$...	28.24	20.36	25.76	25.76	26.44

* Hydrazobenzene, the "symmetrical" diphenylhydrazine, gives the theoretical yield of nitrogen, but phenylhydrazine hydrochloride, $C_6H_5 \cdot NH \cdot NH_2 \cdot HCl$ ($N = 19.48$), whether by the Kjeldahl-Gunning-Arnold method ($N = 5.57$), the Jodlbauer method ($N = 8.55$), or the latter with the addition of sugar ($N = 16.65$), failed to give satisfactory results. Hydrazobenzene, however, is rapidly converted on treatment with sulphuric acid into benzidine, which accounts for the good results obtained. Glucosazone, $C_{13}H_{22}O_4N_4$ ($N = 15.68$), also failed by any of these modifications to give more than 14.24 per cent. of nitrogen.

The direct inapplicability of Kjeldahl's process or its modifications to hydrazines has been already pointed out by Dafert (*Landolt-Verzeichn.*, 34), who suggests first reducing hydrazine salts with sugar in presence of sodium acetate for some hours before adding sulphuric acid.

† Sugar and zinc, without salicylic acid, gave only $N = 18.72$ and 18.17.

‡ The sample of nitroprusside was pure and of normal constitution, for a Dumas combustion of it, kindly made for me by Professor Meldola, gave the full theoretical yield of nitrogen.

Kjeldahl-Gunning-Arnold-Jodlbauer Method.

Compound.	Formula.	Percentage of nitrogen calculated.	Percentage of nitrogen found.
Potassium nitrate	KNO_3	13.88	13.81, 13.93, 13.90
Ammonium nitrate	NH_4NO_3	35.07	35.08, 35.07, 35.13
Urea nitrate.	$\text{CO}(\text{NH}_2)_2, \text{HNO}_3$...	34.21	34.14, 34.14
Nitrobenzene*	$\text{C}_6\text{H}_5\text{NO}_2$	11.41	11.38, 11.35
α -Nitronaphthalene	$\text{C}_{10}\text{H}_7\text{NO}_2$	8.12	8.15, 8.15
<i>m</i> -Nitraniline†	$\text{C}_6\text{H}_4(\text{NO}_2) \cdot \text{NH}_2$	20.34	20.21, 20.24, 20.21
Dinitrobenzene	$\text{C}_6\text{H}_4(\text{NO}_2)_2$	16.71	16.54, 16.51
Pieric acid (trinitro-phenol)‡	$\text{C}_6\text{H}_2(\text{NO}_2)_3 \cdot \text{OH}$	18.39	18.39, 18.39, 18.35
Potassium parabromo-chloronitrophenol§ ..	$\text{C}_6\text{H}_2\text{ClBr}(\text{NO}_2) \cdot \text{OK}$.	4.83	4.85