THE DETERMINATION OF ORGANIC NITROGEN: PAST AND PRESENT*

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It is the purpose of this review to present a picture, sufficiently comprehensive yet not too detailed, of the development of one of the most fruitful and important fields of organic analysis, the determination of nitrogen, as it has evolved from its crude inceptions down to the numerous specialized techniques of the present day. The plethora of available material and the fact that oxidized nitrogen occurs but rarely in organic materials as compared to the abundance of this element in its reduced condition, necessitates a sharp curtailment of the field. The bulk of the emphasis, therefore, has been placed upon the latter problem.

By way of background for what shall be a century of progress in this field, brief reference to the contributions of the old masters of analytical chemistry to the beginnings of ultimate organic analysis may not be out of order. Taking as a point of departure the pioneering investigations of Lavoisier of about 1784 (1) on the determination of the elementary composition of organic substances containing only carbon, hydrogen and oxygen—not accurate, it is true, by present-day standards yet none the less most significant because he exploited a method of analysis which was soon to be improved by others—we come to the contributions of Berthollet (2) with respect to nitrogen. In a sense, he applied to this determination the principle of dry distillation. The virtue of his method seemed to lie chiefly in the fact that a large sample was required, a fortuitous situation since the experimental error was large, doubtless no greater than was considered permissible in those days.

Following a brief desultory period of experimentation with gaseous oxygen under atmospheric pressure, attention turned to the use of solid oxidizing agents. Sometime near the beginning of the nineteenth century, Abildgaard had suggested (3) and

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later Berzelius (4) used this type of reagent; the one mangane-
sese dioxide, the other lead peroxide. The first satisfactory
values were obtained several years later by Gay-Lussac and
Thénard (5) who introduced the sample and oxidizing agent,
potassium chlorate, in pellet form into the combustion tube
fixed in a vertical position. The gaseous reaction products were
then analyzed. The presence of nitrogen in the sample made
necessary close control of the quality of chlorate added in order
to prevent its oxidation to the nitrate form. Although the re-
results which these investigaors obtained were better than others
before them had been able to get, the accuracy of their procedure
was still far from satisfactory.

It was at this time that Berzelius introduced the horizontal
combustion tube with movable burners, thus obviating the diffi-
culty of having particles of substance spatter into a cold por-
tion of the tube and so escape decomposition. Others subse-
sequently clung to the vertical tube; nevertheless the idea proved
superior and has remained in use to the present.

A suitable oxidizing agent was the next goal for it was ap-
parent that herein lay one of the limiting factors in the success-
ful determination of the ultimate composition of a compound.
Various metallic oxides and oxy-salts had been used up to this
time, though rather unsuccessfully except in the case of potas-
sium chlorate. By 1815 this search appears to have ended for
then Gay-Lussac (6) introduced the now familiar use of cupric
oxide as oxidizing agent, together with copper turnings for the
decomposition of oxides of nitrogen. Döbereiner (7) announced
the same discovery almost simultaneously and apparently inde-
pendently.

THE DUMAS METHOD

We now pass over a series of methods and modifications to
the work of a great coordinating genius, Justus von Liebig, and
his brilliant contemporary, Jean Baptiste André Dumas. It be-
came increasingly and painfully evident that no simple, accurate
method for organic elementary analysis was available, and that
this lack greatly crippled investigators in the field, for they had
no ultimate foundation upon which to rest their work. To Lie-
big, in particular, already on the threshold of fundamental dis-
coveries in organic chemistry, this exigency became so great
that he devoted a number of years to work on the problem, and was rewarded by a method which, though burdened with certain inescapable errors, nevertheless was reliable and accurate. His apparatus was characterized by extreme simplicity and was little more than a combination of parts well known at the time. In fact, Liebig said of it (8), "This apparatus involves nothing new, save its simplicity and the complete dependability which it affords." The first thing which he did was definitely to separate the nitrogen determination from those of carbon, hydrogen and oxygen. Almost like the search for the fabled philosopher's stone had been the quest after a universal method of embracing all organic compounds as well as all elements therein, and Liebig was the first to realize that, since each element has its own peculiarities, much more is gained by attacking them individually rather than collectively. It is true that methods of simultaneous analysis for several elements have been proposed from time to time, but even though very good results were claimed they have enjoyed only passing comment (9, 10). At this time, also, Liebig introduced his famous "Kaliapparat," for the purpose of absorbing the carbon dioxide evolved in the combustion of the sample. It was small enough to be weighed conveniently upon the analytical balance and is apparently the precursor of all later models.

Once the way was shown to a reliable method capable of reproducible results, and the limitations of this method clearly recognized, modifications and improvements were not slow of appearance. Of the mushroom crop which sprang forth, one in particular was accepted less reluctantly than the original; in fact, even the name by which the procedure was eventually known was that of modifier rather than originator. We refer to the contribution of Dumas (11).

Dumas' improvements were twofold: the substitution of an inert but readily absorbable gas for air in the combustion tube and the use of potassium hydroxide solution, rather than mercury, as the confining liquid. The first modification eliminated an error inherent in Liebig's method but introduced not only the necessity of working with air-free carbon dioxide but also of expelling air from all parts of the apparatus before beginning a combustion. The second modification seems to have withstood best the test of time for it has suffered less by way of change than perhaps any other feature of the procedure.
Dumas produced carbon dioxide by heating lead carbonate in an extended portion of the combustion tube, a practice which was superseded later by the introduction of the separate gas generator (12). The Kipp apparatus has been found especially suited for this task if precautions are taken to produce air-free carbon dioxide, such as the procedure of Bernthsen (13) whereby one evacuates the water-covered marble chips, or that of Kreusler (14) who recommended the use of fused sodium carbonate and sulfuric acid. Numerous other procedures also are available (15, 16). More recently Pregl (17) recommended a preliminary etching of the marble chips with hydrochloric acid, followed by a careful rinsing with water.

As much effort has been spent upon the preparation of suitable reduced copper for the purpose of decomposing the oxides or nitrogen as securing pure carbon dioxide. Dumas reduced his copper with hydrogen, an unsatisfactory practice as subsequently became evident when it was observed (18) that copper thus prepared emitted, upon strong heating, a gas not absorbed by the potassium hydroxide solution. An explanation for this circumstance was eventually found in the fact that a copper hydride is formed during reduction (19) under these conditions. Further investigation (20, 21) showed that this difficulty could be corrected by igniting the copper strongly and then cooling it, all in a current of pure carbon dioxide. An entirely different approach to a solution of this problem is seen in the suggestion that a silver spiral be substituted for the copper because of the claim that it need be neither reduced nor dried, is more efficient than the latter in decomposing oxides of nitrogen, and serves to hold back halogens, if present (22, 23).

Finally, the technique of measuring the nitrogen evolved in the combustion, like all the other features of this determination, has undergone many changes, although the attainment of that development has not, apparently, been so difficult. The history of the convenient azotometer of Schiff (24) traces its course from the inverted bell jars of Lavoisier and of Gay-Lussac and Thénard, through the graduated cylinders of Liebig and Dumas, respectively. The whole picture of the development of ultimate organic analysis reflects a fine example of the scientific evolution of a group of procedures brought about by careful, tireless workers, over a period of many decades, by means of which the
crude seed, sown with prophetic vision, brings forth an hundredfold of perfect fruit.

THE KJELDAHL METHOD

In a discussion of the wet oxidation method for the determination of nitrogen one again finds material for ample development of the central theme of this review, progress and evolution, for the rise of this procedure embraces fully three-quarters of a century before, as well as the half-century after, the publication of Kjeldahl's original communication. Originally developed to meet the needs of the chemist in the brewing industry, yet it was not to be exclusively his tool for very soon it found entrée into other fields of technical, as well as scientific, analysis.

Essentially, the Kjeldahl method consists in decomposing the material under examination with strong sulfuric acid in the presence of oxidizing agents, accelerators or catalysts, used either singly or in combination. Following complete decomposition, the digestion mixture is made strongly alkaline, whereupon the ammonia is distilled off and subsequently determined by some suitable means.

The technique of effecting decomposition of an organic substance with a sulfuric acid-catalyst mixture as outlined above, appears to have been first employed by Berzelius (4) when he investigated the decomposition products from various animal and vegetable substances after digestion with a sulfuric acid-lead peroxide mixture. He, however, concerned himself only with the products of distillation from acid solution.

Sulfuric acid-digestion, followed by precipitation of the resulting ammonia as the chloroplatinate, first enters the picture in 1845 as a method for the determination of urea (25, 26). Inasmuch as it was applied to the examination of urine and employed no catalyst, this work of Heintz and Ragsky presages, in a sense, the important modern researches of Folin and coworkers. And in this connection it may be timely, too, to recall that Grete in 1878 (27) offered the suggestion that the decomposition of hair, wool, leather and similar substances by the now obsolete Will-Varrentrup soda-lime method (28) could be expedited by giving the material under examination a preliminary treatment with sulfuric acid.
It may also be of interest to make mention of the fact that Kjeldahl had apparently received the inspiration to try an acid medium from the researches of Wanklyn and associates (29) on the use of strongly alkaline permanganate solutions for the liberation of so-called "albuminoid ammonia". Reasoning that the tendency to split off ammonia would be much greater in acid than in alkaline medium, he tried first dilute and finally concentrated sulfuric acid and was rewarded in the second instance by a yield of ammonia which compared favorably with that obtained by the soda-lime method (28). For a series of pure compounds the results were found to agree well with the calculated values, a fact which gave the new procedure further weight. Because of its extreme simplicity and rapidity, together with the high degree of accuracy obtainable in the analysis of protein and related materials, Kjeldahl's method was not long in receiving recognition. Indeed, the germ of an idea suggested by the original communication exactly fifty years ago has brought forth reports of an unprecedented number of investigations, extensions, and modifications. Obviously, the only rational method of reviewing them is by classification, selection of typical examples, and drawing of summarized conclusions. To this end, the subject will be considered with as much brevity as is consistent with clarity of presentation under the topic headings of Digestion, Distillation of Ammonia, Determination of Ammonia, and, finally, Scope of Application of the Method.

Digestion.—A Kjeldahl digestion mixture consists, in general, of representatives of two classes of substances: (1) strong mineral acids, and (2) catalysts or accelerators. There may also be added a third kind of substance, inert in itself, which dissolves in the acid and serves to raise the boiling point of the mixture, thus increasing the reaction velocity. Potassium sulfate, first employed by Gunning (31), or the less expensive sodium salt (32), is the most familiar example of this group. Its indiscriminate use, however, may lead, it is said (33, 34), to losses of ammonia during digestion when the composition of the residue approximates that of the acid salt. Kjeldahl himself suggested phosphorus pentoxide, an ingredient which has since been used by others either as such (35-37) or in the form of the acid as a substitute, wholly or in part (38-41), for the sulfuric
acid. The desirability of using phosphoric acid has not, however, gone unchallenged (42).

Rapid digestion methods involving phosphoric-sulfuric acid mixtures containing hydrogen peroxide as oxidizing agent have been investigated by Wieninger and Lindemann (43) who found that these methods, although quite satisfactory, nevertheless yield results appreciably lower than those obtained by the older, slower Kjeldahl method. They offer the further objections that these new methods are very hard on glassware and require comparatively expensive reagents, so that the great saving in time of digestion (10 to 20 minutes as against 55 to 60 minutes) is still of problematic value at present. These discrepancies may well be due to the extremely drastic treatment, rather than specifically to the presence of phosphoric acid. Finally, a proposal which suggests a page from the days when Carius fusions were popular merits mention. It is to the effect that the Kjeldahl digestion of substances not readily attacked be carried out at 330°C with fuming sulfuric acid in a sealed tube (44). Although this interesting suggestion is probably worthy of development, yet the experimental technique and hazards involved would preclude its use except under unusual circumstances.

Passing now to a discussion of catalysts, accelerators, and oxidizing agents used to increase the reaction velocity we find than an unusually large number of substances has been tried for this purpose. The accompanying table lists there, together with significant references and remarks on their efficacy.

### Table I

Selected List of Catalysts and Accelerators Available for Use in a Kjeldahl Digestion

<table>
<thead>
<tr>
<th>Compound</th>
<th>Author</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>KMnO₄</td>
<td>Kjeldahl (30)</td>
<td>Added as a final step to insure oxidation.</td>
</tr>
<tr>
<td></td>
<td>Salkowski (45)</td>
<td>Ordinarily unnecessary, and objectionable in the presence of substances of high halogen content.</td>
</tr>
<tr>
<td></td>
<td>Phelps (46)</td>
<td>May cause loss of nitrogen.</td>
</tr>
<tr>
<td></td>
<td>Dowell and Friedemann (47)</td>
<td>Use discontinued.</td>
</tr>
<tr>
<td>Compound</td>
<td>Author</td>
<td>Remarks</td>
</tr>
<tr>
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</tr>
<tr>
<td>PtCl₄</td>
<td>Ulsch (48)</td>
<td>Use satisfactory except in excessive amounts.</td>
</tr>
<tr>
<td></td>
<td>Anderson (49)</td>
<td>Yields low results with partly hydrolyzed proteins.</td>
</tr>
<tr>
<td>Pt</td>
<td>Sivan and Raju (50)</td>
<td>Effective in the form of a spiral.</td>
</tr>
<tr>
<td>HgO</td>
<td>Wilfarth (51)</td>
<td>Action is fast but ammonia is held back.</td>
</tr>
<tr>
<td></td>
<td>Phelps and Daudt (52)</td>
<td>Superior to CuSO₄, alum, ZnCl₂, MnCl₂, MnO₂, WO₃, MoO₃, TiO₂, or V₂O₅.</td>
</tr>
<tr>
<td>CuO</td>
<td>Wilfarth (51)</td>
<td>Not as efficient as Hg.</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>Wilfarth (51)</td>
<td>Not recommended</td>
</tr>
<tr>
<td>Bi₂O₃</td>
<td>Wilfarth (51)</td>
<td>Not recommended</td>
</tr>
<tr>
<td>SnO₂</td>
<td>Wilfarth (51)</td>
<td>Not recommended</td>
</tr>
<tr>
<td>Pb (PbO₂, Pb₂O₃)</td>
<td>Wilfarth (51)</td>
<td>Not recommended</td>
</tr>
<tr>
<td>P₂O₅, Hg</td>
<td>Argutinsky (54)</td>
<td>Good results reported</td>
</tr>
<tr>
<td>CuSO₄, Hg, K₂SO₄</td>
<td>Trescott (55)</td>
<td>Efficiency of mixture confirmed</td>
</tr>
<tr>
<td></td>
<td>Emmett (56)</td>
<td>Adoption by A. O. A. C. recommended</td>
</tr>
<tr>
<td>CuSO₄, HgO</td>
<td>Arnold and Wedemeyer (57)</td>
<td>Time of digestion is shortened.</td>
</tr>
<tr>
<td></td>
<td>Bredig and Brown (58)</td>
<td>Mixture is more effective than either ingredient alone.</td>
</tr>
<tr>
<td>K₂S₂O₈</td>
<td>Dakin (59)</td>
<td>By its decomposition enhances the effect of the H₂SO₄.</td>
</tr>
<tr>
<td></td>
<td>Milbauer (60)</td>
<td>Efficiency confirmed.</td>
</tr>
<tr>
<td></td>
<td>Pittarelli (61)</td>
<td>Suitable in clinical analyses.</td>
</tr>
<tr>
<td></td>
<td>Wong (62)</td>
<td>Material saving in time.</td>
</tr>
<tr>
<td>V₂O₅</td>
<td>Oefele (63)</td>
<td>Recommended as &quot;oxygen carrier.&quot;</td>
</tr>
<tr>
<td></td>
<td>Marino and Gonnelli (64)</td>
<td>Shortens digestion period.</td>
</tr>
<tr>
<td>Compound</td>
<td>Author</td>
<td>Remarks</td>
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</tr>
<tr>
<td>S$_2$O$_3$</td>
<td>Eckert (66)</td>
<td>Sulfur is added to fuming H$_2$SO$_4$ in analysis of nitro-compounds.</td>
</tr>
<tr>
<td>SO$_3$</td>
<td>Nolte (90)</td>
<td>Aids in the decomposition of alkaloids.</td>
</tr>
<tr>
<td>Na$_2$S$_2$O$_3$</td>
<td>Dhommée (67)</td>
<td>Applied to urine analysis</td>
</tr>
<tr>
<td>WO$_3$</td>
<td>Margosches and Lang (65)</td>
<td>Yields good results in conjunction with CuO.</td>
</tr>
<tr>
<td>CeO$_2$</td>
<td>Margosches and Lang (65)</td>
<td>Moderately successful results obtained.</td>
</tr>
<tr>
<td>MoO$_3$</td>
<td>Phelps and Daudt (52)</td>
<td>Not recommended</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>Phelps and Daudt (52)</td>
<td>Not recommended</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>Kleeman (70)</td>
<td>Combined with K$_2$SO$_4$ and Hg, it shortens digestion time and causes no loss of NH$_3$</td>
</tr>
<tr>
<td>Skutil (71)</td>
<td></td>
<td>Procedure highly recommended.</td>
</tr>
<tr>
<td>Huess (72)</td>
<td></td>
<td>Well adapted to brewery chemistry.</td>
</tr>
<tr>
<td>Saccardi (73)</td>
<td></td>
<td>Satisfactory for leather, flour and cheese.</td>
</tr>
<tr>
<td>Wieninger and Lindemann (43)</td>
<td></td>
<td>Recommended, with reservations.</td>
</tr>
<tr>
<td>HgI$_2$</td>
<td>Sborowsky and Sborowsky (76)</td>
<td>Superior to Hg.</td>
</tr>
<tr>
<td>Hässig (77)</td>
<td></td>
<td>Superiority over Hg denied.</td>
</tr>
<tr>
<td>Richards (78)</td>
<td></td>
<td>Saving of time effected.</td>
</tr>
<tr>
<td>Fe, CuO</td>
<td>Kürschner (79)</td>
<td>Good results obtained with very resistant compounds.</td>
</tr>
<tr>
<td>BaO$_3$</td>
<td>Provvedi (80)</td>
<td>Less efficient than H$_2$O$_2$ on flour.</td>
</tr>
<tr>
<td>Cd</td>
<td>Saiko-Pittner (81)</td>
<td>Very efficient action on pyramidone.</td>
</tr>
<tr>
<td>Se</td>
<td>Lauro (82)</td>
<td>Satisfactory for flour analysis.</td>
</tr>
<tr>
<td>Compound</td>
<td>Author</td>
<td>Remarks</td>
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</tr>
<tr>
<td>SeOCl₂</td>
<td>Rich (85)</td>
<td>A mixture of SeOCl₂, Cu, and Na₂SO₄ is very effective.</td>
</tr>
<tr>
<td></td>
<td>West and Brandon (88)</td>
<td>A H₂SO₄-SeOCl₂ mixture is suitable for micro-analyses.</td>
</tr>
<tr>
<td></td>
<td>Osborn and Krasnitz (89)</td>
<td>Precipitated Se is more suitable than SeOCl₂.</td>
</tr>
<tr>
<td>KClO₄</td>
<td>Frey, Jenkins and Joslin (93)</td>
<td>About equal to HgO and CuSO₄ in leather analysis.</td>
</tr>
<tr>
<td>HClO₄</td>
<td>Mears and Hussy (68) Shortens time of digestion.</td>
<td></td>
</tr>
<tr>
<td>MnO₂</td>
<td>Parker (69)</td>
<td>Well adapted to leather analysis.</td>
</tr>
<tr>
<td></td>
<td>Stock (94)</td>
<td>Suitable for carbonization of all substances.</td>
</tr>
<tr>
<td></td>
<td>Thuau and de Korsak (95)</td>
<td>Applied successfully after preliminary treatment with H₂SO₄ alone.</td>
</tr>
<tr>
<td>K₂Cr₂O₇</td>
<td>Krüger (96)</td>
<td>Used in decomposition of organic substances.</td>
</tr>
<tr>
<td>Cl₂CCOOH</td>
<td>Grigaut and Thiery (74)</td>
<td>Aids in the decomposition of urine samples.</td>
</tr>
<tr>
<td></td>
<td>Lublin (75)</td>
<td>Used in conjunction with H₂O₂.</td>
</tr>
<tr>
<td>(COOH)₂</td>
<td>Nolte (90)</td>
<td>Oxalic acid is an aid in the decomposition of alkaloids.</td>
</tr>
<tr>
<td>C₁₂H₂₂O₁₁</td>
<td>Bouyer (91)</td>
<td>An aid in the decomposition of flour.</td>
</tr>
<tr>
<td></td>
<td>Nolte (92)</td>
<td>Value of sucrose depends largely upon nature of sample.</td>
</tr>
</tbody>
</table>
That the liberation of the resulting ammonia may be quantitative, it becomes necessary in some cases to destroy heavy metal-ammonium complexes formed during digestion. This is particularly true when mercury compounds have been used in this operation. Potassium sulfide, the precipitant most used today, was introduced by Wilfarth (51). Following this came sodium thiosulfate (98), now recommended in the current edition of “Methods of Analysis” of the Association of Official Agricultural Chemists as an alternate to sodium sulfide, then monosodium phosphate (99), potassium xanthogenate (100), and finally potassium arsenate (101), because its sponsor had found reason to criticize the action of sodium phosphate. Be that as it may, the first two of this list have been found to be satisfactory, provided a slight excess of the sulfide or a generous excess of the thiosulfate is used (102).

**Distillation of Ammonia.**—Distillation and absorption problems have received their measure of attention, too, the attack upon them being more from the mechanical rather than the chemical standpoint. Space does not permit a description of the many spray traps that have been devised to correct the tendency, because of violent ebullition or foaming, of alkali to be carried over into the condensing system, nor yet a discussion of the evolution of the distillation apparatus from that in vogue in Kjeldahl’s time to the highly efficient, multiple-unit commercial type of the present day. Suffice it to state, however, that these difficulties may be practically eliminated by the use of suitable block tin condensers and distilling traps, and such remedial measures for bumping and foaming as zinc, or graphite (102), or even, as is still the practice of some, to follow the Biblical admonition of pouring oil—in this case paraffin—upon troubled waters (175).

It was not long after the appearance of the Kjeldahl method that the suggestion was made that steam distillation (103) be used for the recovery of the ammonia. This practice has met with a certain measure of success (104-106) although some investigators (40) have reported that it causes small amounts of alkali to be entrained and carried over by the spray. In microanalysis, especially, has this technique been widely used (17, 40, 107-111).
Aeration methods have found not a little favor among analysts, particularly in physiological and biological fields (112, 113). A comparison of results (104) obtained on the one hand by aeration and on the other by distillation has shown, in several instances, that at least as much as one per cent of the nitrogen obtainable by the latter mode of recovery was not carried over by the former. It was found, however, that steam distillation served to bring these low results up to the values obtained by heat distillation, and that a large excess of strong alkali materially aided in increasing the yields by aeration.

Determination of Ammonia.—There now remains the analytical determination of the ammonia. Here again many procedures are available and all of them have found a measure, but by no means the same measure, of success. Of antiquarian interest only is the extravagant precipitation as the double platinum salt (26). If not wholly obsolete, the iodometric determination of excess acid proposed by Kjeldahl has enjoyed a very limited use not only because of its cost, but also because of the extreme simplicity of the competitive acidimetric procedure which came into use soon after Kjeldahl’s original communication appeared.

References to indicators pertinent to this titration are indeed numerous. A discussion of them has been omitted in order to make room for less common means of attaining the same end. A variant of the acid-base procedure, proposed by Sors (114), consists in carefully neutralizing the digestion mixture, adding a measured excess of standard alkali solution, boiling to expel ammonia, and determining by titration the decrease in alkalinity of the solution because of this evolved gas. Satisfactory results are claimed, distillation is avoided, but still the method has not been much used. The thought suggests itself, although it has not been verified experimentally, that the presence of such large quantities of salts in solution would make for a sluggish end-point.

A happy union of two old neighbors, so to speak, was brought about when Nessler’s reagent was applied to Kjeldahl distillates, for the history of this reagent can be traced back nearly as far as that of the Kjeldahl digestion. As early as 1839 the fact was announced (115) that a dark brown precipitate is formed when mercuric iodide is treated with ammonium hydroxide, but no analytical use was made of this reaction until
Nessler described it (116) as an invaluable one for characterizing ammonia. Following this, it was widely accepted as a delicate qualitative and quantitative reagent for extremely small amounts of ammonia (117, 118). Eventually it was applied to biological materials (112), and to micro-analysis in general (40, 119).

The reaction between formaldehyde and ammonium salts, studied extensively by Schiff (120), has given rise to still another important method for the determination of ammonia. If we treat a neutral ammonium salt with an excess of strong, neutralized formaldehyde solution, the products of reaction are the free acid and, probably, hexamethylenetetramine. Since one equivalent of acid combines with one of ammonia, the titration of this liberated acid is equivalent to the amount of ammonia in the original salt. This fact was first utilized by Ronchese (121), and was subsequently employed by others for the determination of ammonia in urine (122), as well as quaternary ammonium bases and ammoniacal solutions (123). Its first application to the analysis of a Kjeldahl distillate was apparently in 1910. Its use has spread widely since then (124-129).

Closely related to the above work is that of Sørensen (130), the so-called formol titration of amino acids. This method depends upon the fact that amino acids in general are neutral substances because of a mutual compensation of the amino and carboxyl groups. Upon treatment with formaldehyde, however, the amino group is changed to a neutral methylene imino group as shown below, causing the acidity of the carboxyl group to become available by titration.

\[
RCHNH\cdot COOH + HCHO = RCHN\cdot CH\cdot COOH + H_2O
\]

Since the reaction is reversible, an excess of formaldehyde is employed to drive imine formation far towards completion. Very wide application has been given this method, as for example in urine analysis (131), in studies on the proteolysis of brewery products (132), in nitrogen metabolism investigations (133), in determining the nitrogen content of bacteriological media, and as an index of the falsification of honey (134-136).

The formol titration method is rapid and convenient, the distillation involved in most determinations of basic nitrogen is eliminated, yet it is not a precision method. Difficulties (136)
hanging around concentration of solutions, concentration of indicators, and the small but unavoidable excess of alkali in the neutralized formaldehyde make duplication of results difficult. Very recently Levy (137) in a physical-chemical study of the equilibria involved has observed that two types of reaction, in which either one or two mols of formaldehyde per mol of amino acid are involved, may occur. Then, too, the fact that certain amino acids have a basic reaction is mentioned also as an additional complication. In short, for almost every variation in experimental conditions or nature of amino acids, there is a change in pH of the end point. Moreover, each acid in a mixture—they nearly always occur in mixtures—exhibits a different end-point when titrated under these conditions. The formol titration is thus the concurrence of many separate and complex phenomena and can hardly be regarded as a single definite operation.

As we have seen, the Kjeldahl method attained early popularity as a commercial analytical procedure because of its simplicity and adaptability to routine work. That it should become the subject of constant investigation with a view to further simplification, in the hope of effecting even small economies of time or materials, could hardly have been otherwise. In particular, the need for two standard solutions as in the ordinary acidimetric procedure has been circumvented in a number of ways. One of these, the formol titration, has already been discussed. Another, proposed by Neumann (138), is based on the observation (139) that, given a properly cooled water condenser, and a sufficiently large volume of liquid in the receiver, no ammonia is lost, even though an insufficient quantity of standard acid be present. All that is necessary is to measure out a slightly insufficient amount of standard acid solution, perform the distillation, and finish the titration in the presence of a suitable indicator. It seems a bit strange that no references pertinent to this method, other than the two cited, appear in the literature.

In contradistinction to this lukewarm reception stands that given the boric acid absorption method of Winkler (140). Its principle may best be given in the author's own words, "Boric acid is indeed such a weak acid, that its solution does not noticeably cause color changes of certain indicators. Ammonia is, however, completely fixed by it, provided that a suitable excess
of the acid be present." Distillation is carried to completion, whereupon the ammonia is titrated directly in the presence of congo red (141), bromphenol blue (142) or methyl red indicators (143). The method has been successfully applied to a large variety of subjects.

The iodometric titration of the excess of standard acid takes place according to the following equations:

$$5 \text{KI} + \text{KIO}_3 + 3 \text{H}_2\text{SO}_4 = 6 \text{I} + 3 \text{K}_2\text{SO}_4 + 3 \text{H}_2\text{O}$$
$$\text{I}_2 + 2 \text{Na}_2\text{S}_2\text{O}_3 = \text{Na}_2\text{S}_4\text{O}_6 + 2 \text{NaI}$$

This reaction, described in most of the older books on analytical chemistry, is not much used today for the reason that it is extremely sensitive towards carbon dioxide (144).

Less than three decades ago there appeared a second iodometric procedure (145, 146) for the determination of ammonia which has received a fair measure of recent recognition. It depends upon the fact that ammonia is oxidized quantitatively to nitrogen by an alkaline solution of hypobromite according to the following reaction:

$$2 \text{NH}_3 + 3 \text{NaBrO} = 3 \text{NaBr} + \text{N}_2 + 3 \text{H}_2\text{O}$$

A measured excess of the latter is added, the unused portion being subsequently determined iodometrically. Willard and Cake (147) were the first to apply this procedure to the Kjeldahl determination. A number of others have done likewise since (148-151). In a sense, oxidation of ammonia by hypobromite is a reaction with a dual role, for, besides carrying out the iodometric procedure as described, the nitrogen which is evolved may be determined gasometrically. This was done some time ago, in principle at least, on urea (152). The reaction has been widely utilized since (101, 151-154).

Scope of Application of the Method—The great bulk of those natural products, cereals, meat, milk, glue, tankage, or any of their manufactured derivatives, in which the nitrogen content is of vital significance, have a common fundamental characteristic, to wit: nitrogen is present there in the basic condition for the determination of which the Kjeldahl method is universally applicable.
But there exists a second large class of substances, prominent among which are nitro-, nitroso-, and azo-bodies, hydrazine, quinoline and pyridine derivatives, and inorganic nitrites and nitrates which are characterized by a very refractory behavior toward a Kjeldahl decomposition. To the numerous attempts made to subjugate these and so to universalize the method, some attention must be paid. It has been pointed out that the Kjeldahl treatment involves strenuous oxidation. It is quite logical to expect that little could be accomplished with substances unsatisfactorily decomposed merely by increasing the severity of treatment. A search of the literature reveals, indeed, that no such attempts were successful and that whatever has been done by way of extending the method has been accomplished by means of a preliminary reduction before oxidation.

At first rather mild reagents were used, for von Asboth (155) claimed to have obtained satisfactory results in the analysis of nitro- and cyano- compounds by adding to the mixture, to provide the necessary reducing environment, either sucrose or benzoic acid. Arnold, however, found that the claims for this mode of procedure were overstated (156).

In the same year Jodlbauer (157) introduced an important fundamental principle which is still in use today for the determination of nitrates by the Kjeldahl method, viz: the addition of easily nitrated phenols to the sulfuric acid which fixes the nitrogen in a form lending itself to ready and complete reduction. The value of this modification is especially significant in view of the wide occurrence and application of nitrates in fertilizers, etc. The reducing agent used here consisted of several grams of zinc dust with a small amount of platinum chloride solution as catalyst.

Several years later Förster (97) employed a phenol-sulfuric acid mixture of five to six per cent phenol content, with sodium thiosulfate. Following this, after a lapse of twenty-seven years, came the use of salicylic acid (158) in sulfuric acid solution as the nitrate-fixing agent. This reagent now enjoys an "official" status. A suggestion (159) that resorcinol or phloroglucinol be used instead of phenol itself has apparently received little notice.

Zinc in acid solution is by far the most widely used reductant for the resulting nitro-group. In addition, zinc and iron filings
(160), stannous chloride in the presence of metallic tin (161), iron powder (162), elementary sulfur alone (66), and sodium hydrosulfite (163, 164) have found application.

Reduction methods of an entirely similar nature also serve to bring within range of the Kjeldahl decomposition aromatic nitro-, nitroso-, hydrazine, and azo-substances, although low results have often been recorded in recalcitrant cases (35, 106, 158, 160, 161, 163, 165,). Very recently Friedrich (166) developed a micro method of universal applicability, involving concentrated hydriodic acid (d. 1.7) as reducing agent, followed by a Kjeldahl digestion. With volatile substances, the reaction is carried out in a sealed tube under pressure.

Although it had been established that molecular structure, as well as position and nature of substituents, plays a significant role in determining the susceptibility of aromatic nitrogen compounds to a Kjeldahl decomposition, whether plain or modified, nevertheless, attempt at generalization have proved futile. This is also true of the sugar osazones (167).

It has been stated that accurate generalizations are futile; still, the mention of one such attempt by Fleury and Levatier (168) may be helpful in giving a concise, if crude, picture of the situation as it stands. By the application of their procedure they find that most substances are completely decomposed by one-hour’s digestion. Difficultly attacked substances, such as creatin, skatol, isatin, quinine, pipеразине, morphine, betaine, choline, atropine, tyrosine, and pyridine, require not more than one and one-half hours. The addition of benzoic acid makes analysis of the following possible: sodium nitrate, benzonitrile, the oxime of acetophenone, and pipеридине. Reduction with zinc extends the method to picric acid, m-dinitrobenzene, phenylhydrazine, mannose hydrazine, and glucosazine. A combination of benzoic acid and zinc opens the way to semicarbazids and semicarbazones. No good decomposition procedure was found for either antipyrine or pyramidone.

With this discussion of the extension of application of the Kjeldahl method there is concluded the historical sketch of the evolution of this great analytical tool. There remain a few words about micro-analytical and biological methods.

**Microchemical Methods Applied to Nitrogen Analysis.** It has become a matter of common knowledge that Fritz Pregl
was the originator of the new branch of analytical chemistry called micro-analysis. His contribution to combustion analysis was far greater than mere reduction of the scale of operations and time involved, as many suppose, for through his painstaking research he succeeded in a fuller refinement of the method and a more complete approach to theoretical accuracy than had previously been achieved. This two-fold advance has been of particularly great benefit to workers in biological chemistry, since the tedious necessity of collecting workable samples of vital materials is greatly lessened thereby. Historically, it is interesting to remember that the inception of micro-methods was due to just this difficulty encountered by Pregl in 1910. While engaged in his physiological researches at Innsbruck he was faced with the choice of spending many weary months purifying quantities of material, or devising analytical methods to fit the samples available. Inasmuch as he was a fine laboratory technician, he chose the latter expedient, continuing work after his transfer to Gratz, until, in 1914, he gave the first demonstration lecture in microanalysis before the Vienna Academy. But still the method did not suit him for it was not until 1917 that the first edition of his now famous book, “Die quantitative organische Mikroanalyse”, appeared. Even here progress did not stop, as the book was twice revised and enlarged, the third edition being released in 1929, only one year before his passing. Because he realized that successful micro-analyses are possible only through meticulous attention to details, Pregl took great pains to avoid being misunderstood. To this end he published no articles on the subject in the scientific journals, and made his book almost trivial in its attention to detail, so that those who sought information had of necessity to go to the source and delve deeply.

Not only is a small sample of advantage in biochemical investigations, but it also offers great possibilities in another and less explored field, namely the study of side reactions occurring in conjunction with a given main reaction. It has been said that side reactions are the curse and the hope of organic chemistry. The curse, because they lower the yield of desired product and make it difficult to obtain it pure; the hope, because suitable modification of conditions can make the erstwhile side reaction attain predominance, thus increasing the scope of every synthesis and beating back yet farther the frontiers of synthetic
organic chemistry. Obviously, a systematic, accurate method of characterizing small quantities of materials must be the foundation stone of any such investigation.

Concerning application of the principles of the Kjeldahl procedure to micro-analysis, much has been written, though little can be said in a review of this nature. The general tendencies in this application have been first, the use of all-glass apparatus for digestion and distillation in order to minimize the errors of transfer or those occasioned by leaching of rubber connections; second, the use of colorimetric methods for determining the evolved ammonia. Since each of these phases has been adequately treated in previous pages, they will not be discussed again.

One may well question, from the standpoint of sampling, the advisability of applying micro-analysis to agricultural products in general where fine pulverization is so often impossible. Always a difficult problem in this field, it becomes more acute when one considers the responsibility involved in selecting three or four grains of wheat as representative of a carload or a tiny fragment of tankage as a true average of the heterogeneous substances composing that product. Furthermore, in semi-solid materials, the difficulty of representative moisture content would be almost insurmountable. To conclude, then, micro-analysis is probably more valuable than macro-analysis where one is dealing with chemically pure compounds of unquestioned homogeneity because of economies of sample, reagents, apparatus, and time. But agricultural products as they ordinarily occur present the very antithesis of an ideal sample for this work, and, hence, the procedure must be applied with extreme caution, if at all.

Nitrogen Determination in Relation to Biochemistry. Because of the vital relationship that obtains between the nitrogen content of living cells, their products, and life itself, as exemplified by the familiar nitrogen cycle it is most natural and inevitable that biochemists and physiologists should have seized upon the analysis for nitrogen as a research method of the most profound significance and universal application. It is natural, also, that they should have developed their own methods for this purpose. They are accurate enough to indicate the trends in
which they were interested, but more convenient than the time-consuming precision methods referred to above. The formal titration of Sörensen is one such method; it has been discussed elsewhere. Again, the contributions of Folin and co-workers, appearing in the Journal of Biological Chemistry from about 1910 to date, have done much to further the practical estimation of ammonia, amines, amino acids, etc., in biological materials. These last cannot be discussed here, however, for we would lose the ocean for the water in the attempt.

The name of D. D. Van Slyke is another one well known among workers in this field, for to him goes the credit for devising the gasometric determination of amino nitrogen based on the familiar reaction of primary amines with nitrous acid

$$\text{RNH}_2 + \text{HNO}_3 = \text{ROH} + \text{H}_2\text{O} +\text{N}_2$$

in which a molecular equivalent of gaseous nitrogen is liberated for every mol of amine present. This was first introduced (169) in 1910 and has been widely used since in the study of proteins, urea, and enzymes. Since nitrous acid also reacts with urea, it is necessary that this material be carefully removed before analysis (170).

Urea is a particularly important substance because it is the chief end-product of nitrogen metabolism in the animal body. A method for its determination proposed by Marshall (171) in 1913 depends upon the fact that it is hydrolyzed quantitatively to ammonium carbonate by the enzyme urease. The ammonia may then be distilled off and determined iodometrically or in any of various other ways. Apparently the selection (172) of an active urease preparation is a matter of some concern for not all such extracts are of equal quality. Attention must also be given to the concentration of extract, since it has an effect upon the amount of hydrolysis obtained (173). All in all, the procedure is one of typical clinical accuracy, but serves very well for comparative purposes.

The determination of nitrogen in water and sewage is properly included here, again because of its animal origin and its relation to the history of the water under examination. Ordinarily a sample of water is examined quantitatively for so-called "albuminoid ammonia", free ammonia, nitrite, and nitrate nitrogen. Of these, the last two involve color reactions with or-
ganic dyes which need not be taken up here. Ammonia is determined by Nesslerization, with or without distillation, depending on the amount present. The first-named, or "albuminoid nitrogen", is defined as that nitrogen which is liberated as ammonia by digestion with alkaline permanganate solution after expulsion of any ammonia originally present. Its determination was first proposed by Wanklyn (174) in 1867 and, although only of qualitative significance, and incapable of precise definition, has nevertheless persisted these sixty-odd years as a measure of the loosely combined organic nitrogen content of waters and sewage.

CONCLUSION

And now we have indeed reached the end of this review of a century or more of progress of the analytical aspect of nitrogen in organic combination. From the pioneer flights of "pure" chemistry to the practical routine of technology, scientists are rendered invaluable aid by the practice of organic nitrogen analysis. The chemistry of nitrogen is inseparable from the clothes we wear and the food we eat. The chemistry of organic nitrogen is the chemistry of life, and he who understands it has gone a long way towards fathoming the mysteries of bodily economy. The analytical or research chemist who has in his mind some picture of the century-and-a-quarter panorama of evolution preceding even our present limited knowledge cannot but have a deeper appreciation for those who have gone before and for his own humble work in consequence. It is the hope of the authors that this review will prove a contribution to such a picture.
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