

A Century of Kjeldahl (1883–1983)

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Johan Gustav Christoffer Thorsager Kjeldahl (1849–1900) first presented his method for the determination of nitrogen in organic compounds at a meeting of the Danish Chemical Society in Copenhagen on the 7 March 1883 and it was subsequently published in *Zeitschrift für Analytische Chemie* in the same year¹. One hundred years later it seems appropriate that this significant advance in analytical chemistry should not pass unnoticed. We use his name not only to refer to the analytical method, but also the apparatus used and even the determinand itself—Kjeldahl Nitrogen. Few scientists have been so honoured.

Kjeldahl developed his method for the determination of nitrogen at the Carlsberg Foundation in Denmark, where he was director of the chemical laboratory from 1876 until his death in 1900. The Carlsberg Foundation is a research and philanthropic organisation set up by the Danish brewer, J. C. Jacobsen. Research for the brewing industry was seriously hampered at the time by the lack of a convenient method for nitrogen in proteinaceous materials. The only methods available of proven reliability were the Dumas² and Will and Varren-trap³ combustion methods which were extremely laborious, time-consuming and required a high level of analytical laboratory skill for acceptable results. Kjeldahl set himself the task of devising a more convenient method by which much larger numbers of analyses could be carried out and he recognised that a solution to the problem would be of value in many other fields as well as the brewing industry.

His starting point will be familiar to many analysts who have been involved in water analysis, namely Wanklyn's albuminoid ammonia method, i.e. distillation from alkaline permanganate to release ammonia from organic nitrogen compounds⁴. Kjeldahl refers to Wanklyn's attempts to use the method to determine nitrogen in proteins with disappointingly low and erratic results. He found that boiling with acidified permanganate, i.e. in dilute sulphuric acid, followed by making alkaline and distilling, gave improved recoveries of nitrogen, but was still not satisfactory. His next step was to substitute concentrated sulphuric acid for the dilute acid and the results were much more encouraging. Kjeldahl's initial method was then to heat the sample with concentrated sulphuric acid

near its boiling point, followed by the addition of dry powdered potassium permanganate to complete the oxidation. He found that the use of fuming sulphuric acid or the addition of phosphoric anhydride speeded up the digestion process and it is perhaps significant that Kjeldahl recommended the use of a sulphuric acid/phosphoric anhydride mixture for materials in which the nitrogen is very firmly bound. It seems likely that the phosphoric anhydride served to raise the boiling point of the digest, just as potassium or sodium sulphate does in later versions of Kjeldahl's method.

Ammonia in the digest was recovered by making alkaline and distilling into excess standard acid in the usual way, but Kjeldahl's method of measuring the excess acid is of interest. He did not back-titrate with standard alkali, but added a mixture of potassium iodide and iodate and titrated the liberated iodine with standard sodium thiosulphate. This method was old in Kjeldahl's day and is based on the fact that the amount of iodine liberated by the action of iodate on iodide is dependent on the amount of free acid present and can be used to standardise solutions of strong mineral acids with reference to potassium iodate as the primary standard. It may seem a cumbersome method, but in practice is not so, and Kjeldahl was well aware from his other work on carbohydrates and the diastatic activity of malt that the starch-iodine reaction affords a very precise end-point in iodometric titrations. This enabled him to work with relatively dilute standard solutions and to obtain adequate precision with smaller samples than he would have needed with the acid-base indicators available in his day. He recommends no more than 0.7 g sample for cereal products.

The direct titration of ammonia after absorption in boric acid, which has become the usual manual method for the determination of ammonia is by no means new, having been introduced by Winkler in 1913⁵.

Kjeldahl's method immediately attracted attention from analytical chemists elsewhere and numerous modifications and improvements followed. From the brief description given above, it will be clear that his method as first published differed in several important respects from the procedures most analysts now regard as the Kjeldahl method. There is no mention of any metal catalyst or of the use of potassium or sodium sulphate to raise the boiling point of the digest, though, as noted earlier, the phosphoric anhydride would probably have had a similar effect. Potassium permanganate is no longer used, although recently there has developed some interest in digestion procedures which use other strong oxidising agents, especially hydrogen peroxide.

It seems therefore of interest to trace the major steps which led to the method (or methods) we now know as the Kjeldahl method. The first significant advance came in 1885 with the discovery by H. Wilfarth of the accelerating action of certain metals on the course of the digestion⁶. Wilfarth studied the effects of iron, mercury, manganese, bismuth, zinc, lead and copper. He found that mercury, copper and iron had marked catalytic effects, the level of the effect being in the order stated, i.e. Mercury > Copper > Iron. Wilfarth's finding that mercury is the best catalyst for the Kjeldahl digestion has been amply confirmed by later work.

The next step came from J. W. Gunning in 1889 who found that the addition of potassium sulphate brought about a substantial increase in the rate of diges-

tion, which he attributed to the increase in the boiling point of the digestion mixture⁷.

The role of C. Arnold in the development of the Kjeldahl method has been the subject of some controversy. Arnold reported an apparently synergistic effect between mercury and copper as catalysts in 1886, but others have failed to confirm his findings⁸. His main contribution, published in 1892 in collaboration with K. Wedermeyer, seems to have been the combination of copper and mercury catalysts due to Wilfarth with Gunning's use of potassium sulphate⁹.

Both Gunning and Arnold became closely associated with the Kjeldahl method in the analytical literature in the early years of this century, especially in the U.S.A. where the method was referred to as the Kjeldahl/Gunning/Arnold method or even the Gunning/Arnold method. H. B. Vickery published an extensive reappraisal of Arnold's contribution in 1946¹⁰ and concluded that there was little justification for associating Arnold's name with the method, whilst Wilfarth's contribution in discovering the catalytic effect of certain heavy metals had been overlooked. The method in the form in which it has come to be widely used should perhaps be called the Kjeldahl/Wilfarth/Gunning method, but it is no longer fashionable to refer to analytical methods by the names of their originators. Few analysts today will have even heard of either Wilfarth, Gunning or Arnold; only Kjeldahl is remembered.

By the end of the 19th century the main features of the method most of us would now recognise as the Kjeldahl method had been established, but since then numerous variants have appeared in the literature. Bradstreet in his book on the Kjeldahl method published in 1965¹¹ lists well over 1000 references and the flow shows no sign of drying up yet. In spite of, or perhaps because of, this welter of published information, analysts still seem to find difficulty in agreeing on a standard procedure. In my opinion those wishing to find the criteria by which the Kjeldahl digestion process may be optimised will find more confusion than enlightenment in much of the published literature on the method.

Opinions still differ amongst analysts about the value of selenium as a catalyst. First introduced by M. F. Lauro in 1931¹², selenium was observed to have a marked catalytic effect, as judged by the disappearance of colour in the digest, possibly superior to mercury in this respect, but many analysts have reported low recoveries of nitrogen when large amounts of selenium are used or when digestion times are prolonged. Despite this selenium is still recommended as a catalyst and the painstaking researches of Ogg, Coe and Willitts in the U.S.A. in the late 1940s are often overlooked¹³.

It has often seemed to me that many analysts who have used or published papers on the Kjeldahl method have failed to appreciate the importance of the composition of the digest at the end of the digestion process as a source of error. The boiling point of the mixture is largely determined by the sulphate/acid ratio, and this ratio and the metal catalyst used are the main factors which determine the power of the method to recover nitrogen from refractory compounds, for example, heterocyclic nitrogen compounds. The boiling point must be high enough to ensure that heterocyclic nitrogen is completely converted to ammonia but not so high that ammonia is lost by volatilisation. To maintain the sulphate/acid ratio and therefore the boiling point at an optimum level, any loss

of sulphuric acid during the process must be known and, if necessary, due allowance made to the volume of acid when starting the procedure.

P. A. W. Self seems to have been the first to realise this and the possible errors by loss of ammonia if too much acid is used up during the digestion¹⁴. In his paper of 1912 he points out that the main cause of loss of acid is by oxidation of organic matter in the sample and he describes a simple method for calculating this loss for carbohydrate, protein and fat by assuming the final products to be carbon dioxide, water and ammonia. Oxygen required for these conversions is supplied by the sulphuric acid which is reduced to sulphur dioxide. Self showed that these assumptions were confirmed experimentally at least sufficiently closely for practical purposes and his figures are, in my opinion, worth repeating and are as follows.

1g carbohydrate consumes 4.0ml concentrated sulphuric acid.

1g protein consumes 4.9ml concentrated sulphuric acid.

1g fat consumes 9.7ml concentrated sulphuric acid.

His results also showed that ammonia will almost certainly be lost if the digest is allowed to approach the composition of the acid sulphate salt.

Self's work, as judged from the subsequent published literature, seems to have attracted little attention until the 1950's when his approach was extended by Middleton and Stuckey¹⁵ in this country and by Bradstreet¹⁶ in the U.S.A. as a basis for standardising the Kjeldahl method for the widest possible range of substances. Middleton and Stuckey recommended a sulphate/acid ratio of 1 (by weight) to 2 (by volume) whilst the American official methods use a rather higher ratio of 1 to 1.3^{17,18}.

The higher sulphate/acid ratio of the American methods gives shorter digestion times and a shorter after-boil, 1 h compared with 2 h recommended by Middleton and Stuckey, but there may be an increased risk of localised overheating leading to loss of ammonia. Many of the early workers with the Kjeldahl method including Self were insistent that the digestion flask should not be exposed to direct heat above the level of liquid in it. A common device was to support the flask on an asbestos sheet with a circular hole in it only large enough to allow the gas flame to impinge on the flask below the liquid surface inside it.

Bradstreet subsequently (1965) published an extensive review¹¹ in book form of the Kjeldahl method and its many variants, which is a comprehensive source of literature references to the earlier work on the method, but in my opinion the most useful practical guide to optimising the older manual method is still the Middleton and Stuckey paper of 1951¹⁵.

Self¹⁴ stated that he was unable to find any reference in the literature (up to 1912) to the loss of sulphuric acid as a source of error in the Kjeldahl method and it may be of interest to speculate how far the early workers with the method were aware of this problem. It seems likely that Kjeldahl himself may have appreciated this source of error because he used a relatively small volume of sulphuric acid, 10ml and recommended that not more than 1g of cereal products be used. He usually used much smaller sample weights, and as noted earlier, was able to achieve a satisfactory precision by using the iodometric back-

titration. On the other hand a footnote in Gunning's paper of 1889⁷ says that only a larger flask is needed to digest larger quantities of organic matter, e.g. a whole egg or 100g of meat.

It is sometimes not appreciated that Kjeldahl nitrogen is a non-specific determinand and therefore cannot be defined independently of the method of analysis. The aim is to measure organic nitrogen and Kjeldahl nitrogen is often taken to mean organic nitrogen plus ammonia, excluding oxidised nitrogen (nitrate and nitrite) though modifications to the method designed to include the latter are well known. There are, of course, many different forms of organic combination of nitrogen and some of these will often be present together in the same sample. Some forms of organically combined nitrogen are not amenable to the Kjeldahl method without a preliminary reduction stage. Examples are nitro compounds and azo compounds, i.e. compounds in which nitrogen is linked directly to oxygen or another nitrogen atom.

It is therefore difficult to select a standard reference substance which can be used to check the performance of the method and the accuracy of the results on a routine basis. In proteins, probably the commonest sample type to which the Kjeldahl method is applied, nitrogen occurs both in easily recoverable forms and in heterocyclic structures in which it is well known not to be easily converted to ammonia.

Recovery tests on substances which break down easily to yield their nitrogen as ammonia are of limited value as an indication of the performance of a particular digestion procedure for a variety of nitrogen forms and sample types. Ideally a substance is required which is available in a pure enough state, is amenable to the Kjeldahl method, but is equally or more difficult to digest and convert to ammonia than any nitrogen forms likely to be present in samples of interest. In my experience, for general applications of the method, the nearest approach to this ideal standard substance is nicotinic acid. References to its use for this purpose appear in the 1940s by Belcher in this country and Ogg and Willits in the U.S.A. If it is known that the samples only contain nitrogen in easily recoverable forms, more easily digestible substances could be used as reference standards and attention given to optimising the process for other criteria, for example, speed of digestion.

Modern Kjeldahl systems are based on the use of heating-block digestion followed by automatic distillation and titration, and the emphasis is on sample throughput and speed of digestion. The after-boil period which is a feature of the older Kjeldahl methods seems often to be omitted. For the digestion of water and effluent samples in which the amount of organic matter is relatively small, methods are in use in which hydrogen peroxide is added to complete the digestion. The use of a strong oxidising agent is a reminder of Kjeldahl's original procedure in which he completed the digestion by adding solid potassium permanganate and stated that digestion was complete in 2 h for most cereal products.

In the water industry experience with some of these rapid block digestion methods using hydrogen peroxide on standard reference substances, even those which are easily digested such as glycine and other α -amino acids, has been disappointing. Such methods may be quite adequate for some purposes, but in

my opinion, need the back-up of carefully designed analytical quality control schemes if the results are to be of acceptable accuracy¹⁹.

The recovery of nitrogen from standard reference substances such as nicotinic acid is not always given the attention it deserves when changing from the traditional Kjeldahl apparatus to the modern high speed block digestors. A common approach seems to be to run the two methods in parallel for a short period and if no statistically significant differences between the two sets of results can be detected, to continue with the new method from then on. This is not, of course, an absolute test of bias, but only of bias between the two methods and may not cover all forms of combination of nitrogen or sample type. Unless the experimental design of the comparative tests is based on sound statistical practice, small but still important differences or bias may not be detected. There should be in addition regular analyses of standard reference substances either on their own or as standard addition to real samples. These additional check analyses should be much easier to fit into the laboratory routine with modern Kjeldahl systems than was the case with the older manual methods and apparatus.

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