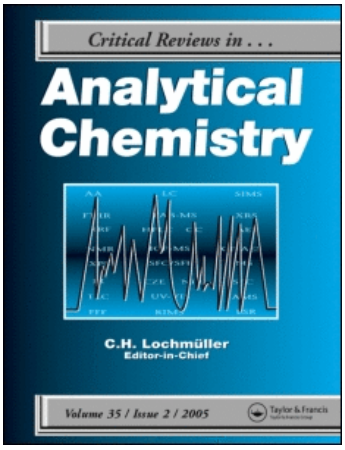

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MICRO-DETERMINATION OF NITROGEN

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I. INTRODUCTION

In his 1970 review of methods for the microdetermination of nitrogen, Schöniger[1] expressed the view that the further development of the old, well-established micromethods[2] and the quest for methods that will serve for the analysis of smaller samples were the areas of greatest importance, and the more recent literature continues to support this view. Automation or instrumentation of the old methods also continues to increase.[3]

Determinations of nitrogen are needed in a wide diversity of chemical applications and the nature of each application influences the selection of a method. In biological or agricultural applications, variants of the Kjeldahl method[4] seem to be first choice, whereas organic chemists for obvious reasons[3] prefer the CHN analyzer[5] or the modified Dumas technique.[6,7] Oil chemists have explored the method of catalytic cracking with hydrogenation followed by coulometric titration of ammonia.[8] Recent and interesting developments of physical techniques such as neutron[9,10] and proton[11] activation analysis may be widely applied in the future.

A comprehensive survey of each method is obviously beyond the capacity of this review (e.g., see Bradstreet's monograph[12] on the Kjeldahl method). However, recent developments in the various methods of nitrogen analysis will be briefly summarized and discussed in turn.

II. THE KJELDAHL METHOD

A. Introduction

The variants of the Kjeldahl[4] method are now so numerous that it seems necessary to define it so as to include any method which uses digestion with sulfuric acid to convert the organic nitrogen to ammonia.

The starting point for anyone wishing to apply (or review) the Kjeldahl method is Bradstreet's excellent and comprehensive monograph published in 1965.[12] In this review the details of the requirements for sulfuric acid, salt (e.g., potassium sulfate), catalysts, etc. and digestion time and temperature are discussed. Since then the application of automation and colorimetric determination of nitrogen has been extensive, and these will form the main topics here.

Also in 1965, two shorter reviews of the determination of nitrogen in biological materials appeared. One of these, that by Fleck and Munro,[13] although quite independent, was in close agreement with Bradstreet.[12] These authors extended their review, including some discussion of automation and colorimetric methods, in 1969.[14] The second review, by Jacobs,[15] was more general but did discuss closed-tube digestion and the ninhydrin method for the colorimetric determination of ammonia.

B. General Aspects

The conversion of nitrogen compounds to ammonia by sulfuric acid requires a suitably high temperature which is obtained (except in sealed-tube digestion) by the addition of salts, and also requires the appropriate amount of acid and a catalyst. Nitro compounds require prereduction.

The basic Kjeldahl method is summarized in the 11th edition of the Official Methods of Analysis of the Association of Anayltical Chemists,[4] and anyone wishing to determine nitrogen by a straightforward Kjeldahl method would be ill-advised to adopt any other procedure because of the weight of evidence in favor of each aspect of their methods (see, for example, References 12, 16, and 17). It is, however, interesting to note that the use of sodium sulfate is still permitted in the

macromethod despite its serious disadvantages and its omission from the official micromethod.

1. Temperature

The digestion of refractory compounds such as nicotinic acid requires a temperature close to 400°C.[12] Digestion is incomplete at 360°C or below, and nitrogen is lost at just over 400°C[12,14,18,19] (except in the special conditions of sealed-tube digestion, where temperatures up to 450°C are recommended).[15,20] The digestion temperature is controlled by the ratio of salt to acid, and a practical guide is given in Reference 4 and has been tabulated by Bradstreet.[12] For micromethods, a ratio of not more than 1g of potassium sulfate/cm^3 of sulfuric acid is usually suitable.[4,18] As Bradstreet pointed out some years ago,[21] a useful practical guide to the possibility of loss of nitrogen due to excessively high digestion temperature is that the digest solidifies on cooling in this situation when potassium sulfate has been used. If sodium sulfate is used, the digest tends to solidify on cooling at much lower ratios of salt to acid, thus making the cooled digest difficult to handle and eliminating a valuable guide to the possibility of loss of nitrogen.[12] Bradstreet has tabulated that in the presence of large amounts of carbon, additional sulfuric acid may be required.

2. Catalyst

The continued use of catalysts other than mercury is surprising, and that of selenium is especially so because there is considerable evidence available that loss of nitrogen is a hazard when selenium is used.[12-14,22,23]

Certainly mercury is not the perfect catalyst because it forms a mercury-ammonium complex which must be decomposed before the determination of ammonia, either colorimetrically or by distillation.[12,22] However, investigations of recovery, etc., while demonstrating deficiencies in other catalysts, have shown that the best or full recoveries of nitrogen are obtained when mercury has been used as a catalyst.[22-25] The mercury-ammonium complex may be decomposed using sodium thiosulfate,[22] zinc dust,[22,23] or, in the automated methods, tartrate[26] or EDTA[27,28] (see below). The author has a personal preference for zinc dust,[13,29] although the "Official Manual Method"[4] specifies thiosulfate.

Three possible solutions have been proposed to the problems of environmental pollution that arise in connection with the disposal of mercury.[30] The first arose from an investigation of the optimal amount of mercury required, which led to evidence that this is about one sixth of that commonly used.[31] With this smaller amount of mercury catalyst together with potassium sulfate and sulfuric acid, good recovery of nitrogen in the digestion of nicotinic acid was obtained.

The second proposal is to recover the mercury from the mixture after digestion, and a method for precipitation and collection has been described.[32] It is important, however, to check the efficacy of this (e.g., by using atomic absorption spectroscopy to estimate the quantitiy of mercury in the discharged effluent) because under certain conditions precipitation may be incomplete.

The third suggestion, on which little new evidence is as yet available, is that copper be reevaluated as a catalyst.[30] In 1955, Quackenbush, Bates and Ethenedge reported not only that the recovery of nitrogen was greater when mercury was used as catalyst than when copper was used, but also that the precision was better with mercury.[24] However, Rexroad,[30] when attempting to evade the pollution problem with mercury, found that a mixture using a copper catalyst gave 99% recovery of nitrogen. He later reported[31] that 5 mg of cupric oxide was just as effective a catalyst in terms of recovery of nitrogen as 160 mg of mercuric oxide, which was approximately one sixth the quantity (1,000 mg) of mercuric oxide usually employed and recommended in the "Official (macro) Method."[4] On analyzing the results of a collaborative study of the determination of the protein content of milk by the Kjeldahl method, Strang and Sherbon[33] found no difference in results between laboratories which could be associated with the use of mercury or copper catalysts. In addition, Odland[34] found the use of a copper catalyst to be satisfactory for the analysis of feeds although he provided no evidence for its success with nicotinic acid.

Thus, we may anticipate some renewed interest in, and further studies of, copper as a catalyst in the Kjeldahl method.

3. Time of Digestion

The official methods[4] give the appropriate guidance, which is usually expressed as a recommended period of brisk boiling of the concentrated sulfuric acid after elimination of water. In the micromethod not more than 1 hr is required to

convert the nitrogen of even very refractory substances to ammonia, assuming the presence of the appropriate amount of potassium sulfate and a mercury catalyst. The macromethod requires 2 hr, or 30 min after clearing.

The rapid clearing associated with selenium and the consequent inadequate digestion time has been suggested as one possible reason for the observation of poor recovery of nitrogen when selenium is used as catalyst.[12]

4. Oxidizing Agents

Bradstreet[12] has reviewed in some depth the use of oxidizing agents and concluded that they are inadvisable. However, the use of small amounts (usually 3%) of perchloric acid,[35-37] notably in some of the automated procedures[38,39] is common, (see below) and hydrogen peroxide continues to be used.[40-43]

The risk of loss of nitrogen when perchloric acid is used has been known since at least 1942.[44] It is difficult to decide whether, in the automated method, it is the excessive heating of a thin film of the liquid digest (see below) or the presence of perchlorid acid that is the main cause of variable recovery of nitrogen with various substances,[38] although calibration problems remain even when perchloric acid is omitted and mercury is used as catalyst.[43,45]

The use of the oxidizing agent hydrogen peroxide does not seem to be so clearly associated with loss of nitrogen during digestion, and the possible reasons for this have been discussed by Bradstreet.[12] Certainly there is a report[40] that a mixture of sulfuric acid and hydrogen peroxide alone gave slightly better performance in the analysis of grasses than a more conventional approach. The author has confirmed the observation that the addition of small amounts of hydrogen peroxide can eliminate the frothing problems associated with the manual digestion of substances of relatively high carbon content such as food and feces and can eliminate the need for additional acid without leading to reduced recovery of nitrogen even from ammonium sulfate standards.[14,29]

It is apparent, however, that oxidizing agents should be employed in the Kjeldahl digest only when specially indicated, and then only sparingly and with care.

C. Compounds Containing -NO- Groups

Substances containing nitrogen-oxygen bonds are not easily converted to ammonia during straightforward Kjeldahl digestion.[12] The Official Methods of the AOAC[4] recommends pretreatment with salicylic acid, chromium metal, and hydrochloric acid, (Comprehensive Nitrogen Method, CNM[17]), or a nickel/aluminum catalyst (Raney catalyst powder) before proceeding with the conventional sulfuric acid digestion.

The Raney catalyst powder method[4] is not necessarily applicable to all nitrogen compounds: Aminopyrene and antipyrene, for example, yield only three quarters of their nitrogen as ammonia with this method.[46] Rexroad[31] has confirmed that the Raney catalyst powder method may give results that are low (by 0.2 to 5%) when applied to the determination of nitrogen in feed and fertilizers.

Of the methods so far proposed, the "Comprehensive Nitrogen Method" (CNM)[4] seems to be the most satisfactory.[47-49] For example, recoveries as low as 87% have been reported[48] when salicylic acid is used, and chloride is a possible source of low nitrogen results.[48] Sucrose has been used as a reducing agent in the pretreatment of nitro compounds, but if the initial temperature is allowed to rise above 200°C, low recoveries of nitrogen are obtained.[50]

A fully automated method, the Missouri Automated Nitrogen Method,[26,42] has been applied to the determination of nitrogen, including nitrate-nitrogen in fertilizers. This method includes a prereduction step with chromous ions in the presence of a titanium catalyst and yields results that compare well with those obtained by the CNM.[42]

D. Evaluation of Manual Methods

Two fairly recent comparisons[51,52] of the Dumas method (Coleman Analyzer) and the Kjeldahl method as applied to samples of feed and fertilizer agree that the Kjeldahl method is slightly more precise but is also the less accurate, tending to give slightly lower results. Several claims that satisfactory results may be obtained with apparently unsatisfactory variants of the Kjeldahl methods (e.g., the use of copper or selenium as catalyst or of low quantities of potassium sulfate) [33,34,36,53] may be attributed to the very small proportions in which compounds refractory to digestion are present in most biological materials.

The analysis of the results of a collaborative study of the determination of the protein content of milk by the Kjeldahl method, in which more than half of the laboratories participating used a mercury catalyst, could identify no effect that was related to the catalyst employed, although between-laboratory variations were significant. The overall coefficient of variation in this study was 2.75. It is perhaps ironical that a brewer's recommended method[53] seems not to be fully up-to-date in its retention of a copper-selenium catalyst, even though Kjeldahl's method was designed originally for the determination of nitrogen at various stages in the brewing process.

E. Automation

Until recently the Kjeldahl method had been automated (or mechanized) in only two ways. The simpler was to carry out the digestion in flasks, as in the manual method, but to automate the determination of ammonia in the digest;[14,27-29] this will be referred to as the semiautomated method. The full procedure, including digestion, has also been automated;[26,38,54] this will be referred to as the fully automated method. Both methods use Technicon AutoAnalyzer equipment* (or recent variants) in which the analysis is carried out in an air- or gas-segmented continuously moving stream of liquid.

A third automated method has recently been announced by a commercial firm and evaluation is at present (early 1974) underway. This new approach to the macro-Kjeldahl method could be classed as discrete analysis in contrast with the now well-established continuous flow automated methods mentioned above. In essence the new method is the well-tried manual method carried out mechanically in a specially designed free-standing apparatus. This contains six Kjeldahl flasks to each of which in turn is added sample, digestion mixture, then mechanically, sulfuric acid and hydrogen peroxide. Digestion is effected by gas burners, and the temperature is claimed to reach 410°C. After digestion the flask is rapidly air-cooled before dilution with water, addition of alkali, and steam distillation. The ammonia is titrated continuously with sulfuric acid using photoelectric monitoring. The six flasks are mounted so that each moves successively through the process. The suggested sample size is 0.5 to 1g of protein, and accuracy is claimed to be as good as for Kjeldahl methods. The analysis time is 12 min and 20 samples per hour can be handled. The equipment costs approximately £10,000 in the UK.**

F. Automation of Digestion

In the continuous-flow fully automated method, digestion takes place in a glass helix[54] which is kept rotating at constant speed and causes the liquid segments to move from one end to the other. Heating takes place in three stages, and the temperature attained is best monitored by checking the electric current that flows.[55] It has been postulated that digestion is rapid because it can take place at areas at the periphery of each liquid segment where the temperature is high and the fluid film is very thin.[38] Such conditions could obviously give rise to loss of nitrogen and may be responsible for the unsatisfactory results obtained by some workers[28] and the requirement found by others[45] that the procedure be standardized with material of known nitrogen content of composition similar to the unknown. Several investigators have shown that the relationship of time and temperature during digestion varies with the substance being studied,[38,56] Apart from the initial study by Ferrari,[54] the use of ammonium sulfate as the sole nitrogen standard has been uniformly rejected until the appearance of a recent study.[37] Since selenium was used as catalyst and perchloric acid was present in this study, these observations contrast with those of Marten and Catanzarrow,[38] who did find different optimal digestion conditions for different substances. The proposal that ammonium sulfate be used as the sole standard should be treated with caution and carefully investigated by potential users of this method. This is especially so because of the results of studies by such groups as those of Gehrke, Wall, and Absheer,[43] Uhl, Lancaster, and Vojnovich,[45] and others[57] who found ammonium sulphate to be quite unsuitable as the sole standard.

In addition to the composition of the digest, the critical factors in this automated digestion procedure seem to be

*Technicon Instrument Corporation, Tarrytown, New York, U.S.A.

**Agents – Foss Electric (UK) Limited, The Chantry, Bishopthorpe, York YO2 1DF.

a. Suitable preparation of specimen for reproducible sampling.

b. Temperature at various stages of digestion.

c. Time of digestion.

d. Conditions during the addition of sample.

e. Resampling for the colorimetric determination of ammonia.

These critical factors have been investigated by a large number of workers.

a. Preparation of specimen – A method for the direct analysis of solid material has been described which is suitable for fertilizers, etc.[43] With suspensions of material in liquid, care is obviously required because variable settling in the sample container can occur before sampling. It is obviously best to sample from stable solutions, and solutions of high viscosity and very strong acids or organic solvents which might damage the plastic sample tubing must be avoided.

b. Digestion temperature – The temperatures of the three independent heating segments of the digestor are governed by the electric power consumed, but some workers[28,55] have specified the current used, others[38] the power, and still others the temperature.[42,56] It appears to be more reliable to assess digestion temperature from the power consumption rather than to measure it directly.[55]

It appears that the temperature settings of the digestor are critical. In a collaborative study,[55] all laboratories except one used the same temperature settings for digestion, and the one using a different setting reported two results that were rejected from the final performance assessment. In their early work, Marten and Catanzarrow clearly demonstrated that increasing the temperature of digestion led to the loss of nitrogen.[38] Temperatures greater than 410°C have been used in the automatic digestion method,[28,38,56] and it may be that an optimum can be achieved at which the rate of loss of nitrogen from all substances is approximately the same,[38] thus permitting the use of an ammonium sulfate standard.[37,38] However, successful digestions at temperatures just below 400°C have also been reported.[42,57] One of these methods, the Missouri Automated Nitrogen Method,[42] employed hydrogen peroxide and sulfuric acid, and the other[57] gave the best results with potassium sulfate and mercuric oxide. The addition of potassium sulfate gave smoother boiling in the helix[57] but could give rise to problems at the resampling stage after digestion.[28]

c. The time of digestion is governed by the rate of rotation of the helix and is related to the temperature and the rate of flow of digestion acid[43] The digestion time is shorter than in micro-Kjeldahl methods, and it is evident that there are optimum conditons of time, temperature, and flow for most substances[38,43,45,56-58]

d. In practice some difficulties may be encountered as the sample and acid are introduced to the digestor. These may be overcome by a suitable modification.[57]

e. Practical difficulties may also be encountered at the stage of dilution and resampling on completion of digestion. Useful modifications include preheating of the diluent water and the addition of a fiberglass sleeve to prevent irregular dripping of condensate into the digest.[42]

G. Automated Methods for the Analysis of Nitrates

The fully automated method (Missouri Automated Nitrogen Method) has been adapted to the determination of the nitrogen contents of fertilizers containing nitrate.[26,42] Prereduction of the material with chromium powder in hydrochloric acid is necessary, followed by digestion with sulfuric acid and hydrogen peroxide.[42] After the digestion step, some precipitation of chromium with tartrate (see the following section on colorimetric determination of ammonia) is eliminated by adjusting the concentration of tartrate to the optimum value, 0.2 *M*.[42] With this method up to 30 samples/hr or 150 samples per working day could be handled with good agreement with the official Comprehensive Nitrogen Method.[42]

Finally, there remains one source of analytical error in continuous-flow or Technicon AutoAnalyzer methods. There is now a considerable accumulation of evidence that the cam which drives the sampler in Technicon systems can be a source of analytical error.[28,59] Davidson, Mathieson, and Boyne[28] seem to be the first to have demonstrated this for the nitrogen method, but others have found that it may apply widely.[59,60] A simple electronic timer substituted for the cam can eliminate this source of error.[60]

H. Colorimetric Determination of Ammonia

In both the fully automated and semiautomated procedures the ammonia in the digest is

determined colorimetrically, almost invariably by a variant of the Berthelot reaction. Because of the large amount of recent work on this phenate/hypochlorite or indophenol reaction of ammonia and its wide application, it is appropriate to review it in some depth.

1. The Berthelot Reaction

The reaction seems first to have been mentioned in correspondence by Berthelot in 1859.[61] In this he stated that a mixture of phenol, ammonia, and calcium hypochlorite produced a blue color similar to that given by aniline with certain oxidizing agents. A listing of the numerous variants described between 1859 and the 1950s would contribute little to our understanding of the method except to indicate that it probably did not give uniformly satisfactory results. In the 1960s numerous applications of the method appeared. Examples include the development[62] and investigation[63] of an automated method of determining ammonia, applications to the determinations of urea and ammonia,[64-70] and estimation of ammonia in Kjeldahl digests[23,71,72] and in boiler feed water.[73] The method has found wide application in the estimation of blood urea following its conversion to ammonia (and carbon dioxide) by the action of urease and was shown to give more accurate and acceptable results than a similar method in which the Nessler reaction was used to determine the ammonia formed.[74]

Three reagents are commonly used:

1. Alkaline phenate (i.e., a solution of phenol in sodium hydroxide or sodium phosphate).
2. Alkaline hypochlorite.
3. A catalyst which may be nitroprusside, acetone, or a manganese salt. The catalyst may be omitted.

It seems to be generally accepted that the reaction consists of three steps: a fast reaction of hypochlorite with ammonia to form chloroamine, the slow condensation of chloroamine with phenol to form quinonechloroamine, and finally the fast condensation of quinonechloroamine with phenate ion to form indophenol.[42,63,69,73,75-77]

The mechanism of action of the catalysts is not clear, although there is agreement that the nitroprusside undergoes rearrangement to ferrous nitritopentacyanide before being effective.[69,77]

The following paragraphs summarize what is presently known about the optimal conditions for the Berthelot reaction.

Order of addition of reagents – Horn and Squire[68,69] found that maximum sensitivity was obtained by using nitroprusside as catalyst and adding the hypochlorite to the ammonia first. The reaction occurred at room temperature and high pH; the optimum pH is 12.5. The time of addition of the phenate is critical (2 min later) and the optimum pH is close to 11. The maximum color was developed after 30 min at 37°C. Although Bolleter, Bushman, and Tidwell[63] also added the hypochlorite reagent first, most other investigators[23,73,75-79] have found it best to add the phenate reagent first, and apparent progressive loss of ammonia has been found when, if the phenate was not added first, it was not added immediately after the hypochlorite.[67,76] When the phenate is added first, delay in addition of the hypochlorite is not critical.[78] This observation has been utilized in a study of the mechanism of the reaction after which a reaction-rate method for estimation of ammonia was devised.[77] The blue indophenol color, once formed, is stable for many hours at 37°C or below.[75,78]

Temperature and time – Many studies of the optimum conditions have been undertaken. In the presence of nitroprusside as catalyst, an increase in temperature leads to increased rate of reaction and chromogenicity. The maximum color is attained at 75° or 100°C but at the higher temperature the chromogenicity declines somewhat shortly after reaching its maximum,[76] although the rate of reaction is increased. Adequate sensitivity is attained at temperatures from room (20°) to 37°C, and the rate of color development is statisfactory (10 to 20 min[64]) when sufficient nitroprusside[76] or acetone[73] catalyst is present.

Concentration of reagents – The optima have been widely investigated. Examples include: without catalyst,[63] for nitroprusside catalyst,[75-77] for acetone catalyst,[71,73] and for automated methods.[26,27,42,43]

The optimum pH values for the two stages of the reaction are different, and this is especially so when the hypochlorite is added first.[68,69] Horn and Squires[68,69] found that the optimal pH for the production of chloroamine was 12.5 and that maximum color development after addition of phenate took place at pH 10 to 11 in agreement with Bolleter, Bushman, and Tidwell.[63] Bohley,[71] using an acetone catalyst, found the final optimum

pH to be 11. Others have found the amount of sodium hydroxide in the phenate reagent or final mixture to be critical.[26,63,73]

The amounts of phenate and hypochlorite are not quite so critical,[23,63,73,76,78] although insufficient hypochlorite causes a rapid fall off in color.[76]

The final concentrations of reagents, particularly phenol and alkali, in different reaction mixtures vary widely, and the reasons for the success of the different techniques are not immediately apparent.

An interesting continuous gradient method of determining the optimal concentrations of reagents in automated methods has been described by Gehrke's group.[27,42]

Catalysts – (a) Nitroprusside is widely used, although Gehrke, Kaiser, and Ussary[27] concluded that with it the reaction was rather sensitive to a variety of metal ions, including those of copper, zinc, and mercury, and they now use Mn as a catalyst.[26]

Some recommend the use of fresh nitroprusside,[75] while others suggest that time for conversion to nitritopentacyanide should be allowed.[69] The catalytic effect of nitroprusside has been adequately illustrated by Weichselbaum, Hagerty, and Mark[77] and a tenfold increase of sensitivity has been shown to result from the use of this catalyst.[76] (b) Acetone – The addition of acetone also increases the color development,[71,73] and although acetone has been used with nitroprusside,[66] nitroprusside alone produces a greater increase in sensitivity.[69] A possible advantage of the use of acetone as a catalyst is that the reaction may then be less sensitive to interference from metal ions such as mercury.[71] (c) Manganese (Mn^{2+}) – In 1944, Russell described a modification of the Berthelot reaction in which she used manganous ion as catalyst.[80] Gehrke, Killingley, and Wall[42] added manganous chloride at the stage of dilution of the digest in a fully automated method and found, by comparision with a method[81] not using manganous ion as catalyst, that the increase in sensitivity was about 17%.

Interfering substances – (a) Metallic and other ions. Gehrke, Kaiser, and Ussary[27] list the ions that were found to interfere in a study of an automated indophenol method. They found that mercury, in the range from 0 to 35 ppm, enhanced the color,[27] but others[23,71,82] have found mercury to inhibit color development when present in the amounts usual in Kjeldahl digestion. Copper and selenium also interfere.[27,75,82]

In order to avoid this interference of ions in the estimation of nitrogen in biological materials, Bohley[71] added EDTA to the phenol reagent and used acetone as catalyst. Other workers[82] have found that decreasing the amount of mercury can give adequate digestion with no mercury interference, despite the omission of EDTA.

After a careful study Gehrke, Kaiser, and Ussary[27] concluded that EDTA was unsuitable as a complexing agent for the Berthelot reaction because increasing its concentration led to a decrease of the sensitivity of the reaction. However, satisfactory results when using EDTA have been claimed by other workers.[56,71,79,83] In an early manual micromethod, Mann[23] used zinc dust in an attempt to eliminate the effects of the mercury catalyst.

An alternative complexing agent is tartrate. In an early study, Lubochinsky and Zalta[75] used tartrate to complex their copper catalyst and stated that mercury must not be used. Subsequently, several groups of workers have used tartrate either alone[26,42,43,55,58,84] or in combination with EDTA.[45]

Tartrate has been used successfully with a manganese catalyst,[26,27,42,43] although the concentration of tartrate has to be modified in order to avoid precipitation with certain salts.[42] (b) A list of nonprotein nitrogen substances which can influence the indophenol reaction[85] is available. This is of little significance, however, in the present context.[20]

Finally, an interesting modification of the method of carrying out the Berthelot reaction has been described.[81] In this, the reaction is carried out in boric acid solution, as had been previously described;[63] chloramine-T is used as the oxidant, an aqueous solution of phenol is used instead of alkaline phenate, no catalyst is added, and alkali is added only after heating (at 60°C for 16 min). Good precision (standard deviation = 0.0013 ppm at 0.20 ppm) and sensitivity (absorbance = 0.60/ppm/cm at 625 nm) are claimed. The aqueous phenol reagent is said to be more stable than alkaline phenate, and chloramine-T solution is also stable and has the advantage of being simply made up in well-defined concentration, unlike chlorine water or hypochlorite.

2. Ninhydrin Reaction

The reaction of ammonia with ninhydrin has been used in the determination of nitrogen in sulfuric acid digests of biological material.[15,20,86,87] The color reaction is essentially similar to that given by amino acids,[88] and specific discussion of the ammonia reaction seems to be rare. Factors influencing the production of the ninhydrin color have been explored in detail.[88-91]

A possible advantage of using the ninhydrin reaction to estimate ammonia in Kjeldahl digests is the lack of interference from the catalysts selenium or mercury; copper and zinc, among other ions, do not inhibit the reaction when it is carried out in the presence of 0.4 *M* sodium citrate at pH 5.0.[86,87] Citrate also minimizes the interference by neutral salts.[86] It is surprising that the reaction has not been widely applied to automated Kjeldahl nitrogen methods, although perhaps cost has been a determining factor. In comparison with a conventional open-tube method, a sealed-tube sulfuric acid digestion method followed by ammonia determination by the ninhydrin reaction gave good results.[20] Also, following open-tube Kjeldahl digestion with a mixture of copper(II) sulfate and potassium sulfate and hydrogen peroxide, good recoveries were obtained using ninhydrin with a citrate buffer.[86] This method gave more satisfactory accuracy and reproducibility than a Nessler method.[86]

3. Nessler Method

In previous reviews,[13,14] it was concluded that the presence of salts and the pH, among other variables, could influence the color produced in such a variable fashion as to make the direct application of the Nessler method unreliable. Certainly Berthelot methods have been found to be more accurate and reliable for the determination of urea after treatment with urease to yield ammonia.[74] Following Kjeldahl digestion a ninhydrin method of determining the ammonia produced has been found to be superior to a Nessler method.[86]

The critical aspects of the Nessler method have been listed as:[92] (1) type of Nessler's reagent; (2) digestion method and composition; (3) temperature of solution during Nesslerization; (4) pH of solution; (5) amount of sodium sulfate; (6) time interval after addition of Nessler reagent before measuring absorption; and (7) rate of mixing during addition of NaOH. In an almost simultaneous study[93] these factors were confirmed, and it is apparent that turbidity is a problem because clear instructions for a micro-Kjeldahl type distillation have been given to avoid this difficulty[92,94] and to obtain the most accurate results.[94] The accuracy of a direct Nessler method was found to be 5%.[95] The addition of tartrate was found to decrease the interference from metal ions.[93]

It should be noted that although the Nessler method continues to be described in the Official Methods of Analysis,[95] it is designed to be applied in the Kjeldahl method to distillates of ammonia and not directly to the digest.

4. Miscellaneous Colorimetric Methods

Two recent new colorimetric methods are of interest. In the first, ammonia, nitrate, and amino compounds are converted to nitrite, which is then used in a diazotization reaction with sulphanilamide and *N*-1-naphthylethylenediamine.[96] The peak absorption of the resultant azo dye is at 520 nm. There is also a description of the same method using an AutoAnalyzer for application to water and soil extracts.[97]

The second colorimetric method has been applied to the determination of ammonia in air and depends on the color produced by reacting *O*-(benzenesulfonamido)-*p*-benzoquinone in benzene with ammonia in dioxane.[98] This reaction is said to be three to four times more sensitive than the Nessler method.

5. Conclusion

The weight of evidence at present favors some form of the Berthelot reaction for a simple, reliable colorimetric method for determining ammonia after Kjeldahl digestion. It can be applied either to simple manual methods or to fully or semiautomated continuous-flow methods. Once suitable concentrations of the reagents have been selected, it may be necessary to use a catalyst in order to increase sensitivity. Nitroprusside is possibly the best catalyst, but the selection may require to be modified according to circumstances. Certainly care must be taken to avoid variation in chromogenicity due to the presence of metallic ions, either introduced as Kjeldahl catalysts or in the starting material.

It is apparent that modifications of the Berthelot reaction have now generally and advantageously replaced Nessler methods of determining ammonia.

Other colorimetric methods (e.g., ninhydrin) have not been sufficiently widely employed for comparative assessment. The main advantage of the ninhydrin method for ammonia estimation is its resistance to interference by metal ions when suitable reagents are used.

II. DUMAS AND RELATED METHODS

A. The Dumas Method

Nitrogen determination by micro-Dumas methods are now commonly carried out using automated techniques,[1,4] and the recent emphasis has been on simultaneous, automated CHN or CHNO analysis.[1]

A review giving a brief summary of important aspects of the Dumas method has been mentioned earlier.[15] Detailed comments on automated nitrogen analysis using the Coleman model 29A Nitrogen Analyzer have been published.[4] Important aspects are the loading of the combustion tube and the setting up of the digital azotometer. In comparison with an Official Kjeldahl Method,[4] the micro-Dumas automated method was slightly more accurate and precise when using a copper oxide-platinum catalyst and a combustion temperature of 850 to 900°C.[99] Combustion at 750°C in the Coleman Analyzer is inadequate for heterocyclic compounds, for which a modified method using platinized asbestos with cupric oxide and combustion at 850°C gives the lowest errors.[100]

In a study of the analysis of nitrogen-containing drugs using the Coleman Analyzer, it was concluded that it was essential to know the nature of the drug and to modify details of the technique appropriately in order to obtain satisfactory results (i.e., 98 to 102% of theoretical).[6]

Recently, Merz[101,102] has described a fully automatic method in which combustion is carried out at 850°C in a stream of oxygen. This method has a cycle time of 2.5 min; combustion takes place in 30 sec, and an electronic balance and electromagnetic charging valve are essential.[101]

Some simple steps to eliminate sources of error from the automatic azotometer (Azotomat) have been described.[103]

An automatic method in which pyrolysis can be programmed to suit the nature of the compound to be analyzed yields an accuracy of 0.3%.[7]

B. CHN and CHNO Analyzers

Since the description of a dynamic approach to CHN analysis by Walisch,[104] there have been several reviews of methods and equipment.[1-3] The review by Schöniger[1] lists equipment available and gives accompanying descriptive flow diagrams.

The fundamental principles of one dynamic approach to CHN analysis have been discussed,[105] and its application to compounds containing fluorine and phosphorus and some metals have been reviewed.[106] Many of the modern CHN or CHNO analyzers employ the gas chromatographic principle with electronic integration for the determination of the gaseous end products.[1,5,107]

As a result of collaborative studies[5,108] of CHN analysis in which the Hewlett-Packard 185, the Perkin-Elmer 240, or equivalent models were used, it was concluded that catalyst, combustion time, and temperature were critical aspects. In the second study,[5] for which electronic integration methods were a prerequisite for participation, it was found that the appropriate catalyst filling, high combustion temperature, and holding combustion for 30 sec were essential for good results.

A recent analyzer (Consolidated Electrodynamics 1102)[107] uses the gas chromatographic principle with electronic integration and combustion in a stream of helium and can achieve 40 CHN analyses or 23 CHNO analyses per day.

There are several reports which confirm the general applicability of the new automatic analyzers.[109-111] Satisfactory analysis of petroleum compounds containing only 0.05 to 0.1% N[109] have been carried out. The Perkin-Elmer 240 can also be used for the determination of the CHN content of atmospheric aerosols.[110] Another analyzer (Hewlett-Packard 185) has achieved satisfactory results in the analysis of rock and soil samples,[111] although it was found that carbide C was not fully accounted for. The generally satisfactory state of CHN analysis might be inferred from a report which recommended that no further study of automatic determination of CHN be done until some new developments were made.[112]

C. Microcoulometric Methods

In 1966, Martin[113] described a rapid (10 min) and sensitive (0.2 ppm) method for the determination of nitrogen in petroleum samples. This was based on a much earlier method of catalytic hydrogenation over a nickel and magnesium oxide

catalyst[114] followed by the innovation of automatic microcoulometric titration of the ammonia produced.

Modification and development of this method followed. For example, Oita[8] used a modified sampling system and a higher temperature (900°C) to eliminate coking and employed a Dohrmann Instrument Company coulometer and titration cell to obtain good agreement (about 5%) with Kjeldahl methods and reasonable precision (about 4%) in the analysis of motor oils, gasolines, and water samples. A detailed description of the application to wastewater followed[115] in which agreement with a distillation and titration method and a Nessler method was obtained. More recently the hydrogenation and hydrocracking tube design has been modified;[116] this permits the determination of 1 ppm of nitrogen.

Other groups of workers have further increased the sensitivity of the method, to 0.1 to 0.02 ppm,[117] in applications to petroleum products and to water and other solvents.[118] In a detailed examination of the method[119] in which catalysts, "scrubbers," and high- and low-N-containing compounds were studied, it was concluded that the precision was 0.03 ppm or 3.0% and that there was good but not excellent agreement with a Kjeldahl method.

D. Conclusions

For the straightforward determination of nitrogen in many compounds, an automatic micro-Dumas method[4] will be the one of choice. If it is necessary to carry out CHN or CHNO analyses, one of the automatic analyzers[1,109-111] is capable of giving satisfactory results in most circumstances, even when halogens, phosphorus, and other elements are present.[106,120] There are few problems in using these analyzers, but in some cases, for example, the analysis of high boiling point petroleum products, difficulty may be experienced and the microcoulometric method for N may then have advantages.

III. MISCELLANEOUS METHODS

A. Nitrides

Although some nitrides may be susceptible to analysis by the Dumas method, an alternative approach has been proposed for the analysis of vanadium, uranium,[121] and silicon nitrides.[122] Vanadium and silicon nitrides can be dissolved by treatment with hydrofluoric acid, alone or with hydrochloric acid, in a polytetrafluoroethylene-lined closed vessel. After dissolution, which may take up to 4 days at 150°C in the case of vanadium, alkali is added, the mixture is steam-distilled, and ammonia is determined by titration.

B. Nitriles and Cyanide

The nitrogen contents of acidic aqueous solutions of nitriles or cyanide (e.g., KCN, acetonitrile, acrylonitrile) can be determined by alkaline oxidation with H_2O_2, after which the ammonia may be determined by distillation and titration.[123]

C. Photo-oxidation

Nitrogen in water has been determined by photo-oxidation using a 900-W high-pressure mercury-arc lamp to oxidize organic and other nitrogen compounds to nitrite and nitrate, which can then be determined by an automatic colorimetric method.[124] The method is claimed to be 100 times more sensitive than the Kjeldahl method.[125]

D. Emission Spectroscopy

Plant tissue has been analyzed using a "briquetting technique" with this method to yield results for 21 elements including N.[126]

E. Activation Analysis

Neutron activation analysis of N in feeds has been shown to agree well with results obtained by the Kjeldahl method.[9] In the analysis of grains using fast neutrons at 2×10^{11} n/sec irradiation for 10 min was followed by a 10-min cooling period before analyzing the resultant γ spectrum with a 512-channel analyzer. Corrections for ^{38}K interference were found to be necessary, and the final precision was 3% relative standard deviation.[10] In an earlier study also using fast neutrons, it was found that phosphorus and silicon interfered, but corrections could be made and the coefficient of variation was 0.9 to 1.73.[127] Neutron activation analysis has been applied to whole animals.[128]

Proton activation analysis has also been applied to the determination of nitrogen[11,129] as has γ activation.[130]

IV. CONCLUSIONS

The Kjeldahl method remains the method which will be used for the analysis of most samples

of biological origin, the advantage being the "wet ashing" technique. The advantages of automation are apparent when applied either in whole or to part of the analytical process, and analytical performance is satisfactory.

Automation of the micro-Dumas method and CHN analysis is common, and the results for a wide variety of materials are good or excellent. In a small number of special cases, alternative methods may have an advantage, and these have been mentioned.

Physical methods, especially activation analysis, may find increasing application, but, initially at least, proximity to a reactor or sources of fast neutrons, etc. will determine whether such methods are used.

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