[CONTRIBUTION FROM CENTRAL ILLINOIS TESTING LABORATORIES]

DIRECT NESSLERIZATION OF KJELDAHL DIGESTIONS

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The method outlined here is the outgrowth of a study started in the summer of 1922 as one step in the simplification of the methods of Folin-Wu and of Benedict for blood analysis to make them more suitable as routine tests for use by technicians of limited basic training. The work was started in the pathological laboratory of the St. John's Hospital, Springfield, Illinois, and continued at the present location since that time.

The Nesslerization of ammonia in the presence of considerable quantities of inorganic salts is an old problem. The published methods to date seem to be confined to limiting the amount or the nature of the salts present. It seems strange that so little apparent effort has been made to attack the problem by the use of protective colloids. Folin and Denis[1] have developed a method for the direct Nesslerization of micro Kjeldahl digestions by the substitution of phosphoric acid for a large part of the sulfuric acid and omitting the alkali sulfate generally used. This digestion mixture has the serious fault of attacking the glassware, leaving a considerable amount of insoluble sediment; moreover, only low concentrations of ammonia can be Nesslerized, limiting the method to micro determinations.

By the use of a protective colloid to prevent precipitation of the color in the Nesslerized solutions as here described it is possible to Nesslerize directly any of the commonly used Kjeldahl digestion mixtures. Moreover, it is thus possible to Nesslerize a much higher concentration of ammonia in the presence of relatively large amounts of alkali sulfates than by the usual methods even in the absence of any extraneous salts. A number of colloids have been tried for this purpose but by far the most satisfactory one found to date is a specially prepared gum arabic solution. By the use of this material it is possible to successfully Nesslerize concentrations of ammonia as high as 20 mg. of nitrogen per 100 cc. of final solution and this in the presence of from 5 to 8 g. of anhydrous sodium or potassium sulfate. Such solutions develop their maximum color rapidly and do not start to precipitate for a considerable time; in fact, aliquot portions of Kjeldahl digestions containing in the Nesslerized solution 5 mg. of ammonia nitrogen per 100 cc. have been retained without noticeable precipitation for as long as two weeks. Satisfactory Nesslerizations have even been carried out in solutions saturated with sodium sulfate.

The method has been tried in comparison with the usual Kjeldahl methods both macro and micro on the following materials: blood, blood

[1] Folin and Denis, *J. Biol. Chem.*, **26**, 473 (1916).

filtrates, urine, milk, grain feeds, tankage, and cottonseed meal. The following table shows some of the comparative results.

TABLE I

COMPARATIVE RESULTS

Sample	Distillation and titration	Direct Nesslerization	Aeration and Nesslerization
Blood	2.95	2.97	....
Blood	2.62	2.58	....
Urine	1.22	1.24	....
Urine	0.835	0.840	....
Milk	.634	.632	....
Breast milk	.227	.228	....
Breast milk	.218	.218	....
Blood N. P. N.	.0316	.0316	0.0310
Blood N. P. N.	.0298	.0299	.0297
Blood N. P. N.	.0424	.0426	.0420
Cottonseed meal	7.02	7.11, 6.94 Av. 7.05	....
Cottonseed meal	6.98	7.07, 6.90 Av. 7.03	....
Tankage	9.68	9.78, 9.56 Av. 9.69	....
Tankage	9.52	9.63, 9.44 Av. 9.55	....
Mixed dairy feed	1.69	1.70, 1.66 Av. 1.67	....

In the above comparative determinations, digestions were made in the usual manner, the digested solutions were made up to a definite volume and proper aliquots used for each method. In some of the digestions copper was used and in some mercury. For products of low nitrogen content the result is calculated from one careful reading at the colorimeter. For materials of high nitrogen content the result is calculated from the average of five distinct readings; the results calculated from the highest and the lowest readings are also reported. A study of the comparative results will show only the differences to be expected due to the inherent inaccuracy of colorimetric determinations.

Since one of the principal advantages of this method is the saving of time, we have found it expedient to use it in combination with the sulfuric-perchloric acid decomposition method of Mears and Hussey.[2] This we have found very satisfactory for many materials although the results are usually somewhat low for urine and for this reason we prefer to omit the perchloric acid in this case. For micro determinations we have found the following digesting mixture to be very convenient: concentrated sulfuric acid 70 cc., water 50 cc., 20% perchloric acid 20 cc., anhydrous sodium sulfate 15 g. and copper sulfate 1 g.

[2] Mears and Hussey, *J. Ind. Eng. Chem.*, 13, 1054 (1921).

For micro analysis blood, urine, milk and similar liquid materials are suitably diluted and such aliquots as will normally give from 2 to 5 mg. of ammonia nitrogen are taken and digested with 1 or 2 cc. of the above digestion mixture in the usual manner, heating for at least two minutes after the solutions are colorless. If desired, particularly for urine, the perchloric acid may be omitted. The mixture after digestion is cooled, transferred to a volumetric flask and 5 cc. of colloid solution is then added for each 100 cc. of final volume; two or three drops of Nessler solution are added to serve as indicator and the solution neutralized with 10% sodium hydroxide solution. The solution is then diluted and the Nessler solution, to which the colloid has been added, is run in to the amount of from 10 to 25 cc., according to the nitrogen content of the solution, for each 100 cc. of final solution. No care need be taken as to the manner of addition of the Nessler solution except to note its volume, which must be the same as for the preparation of the standard for comparison. After the addition of the Nessler solution, the flask is filled to the mark, shaken and compared with Nesslerized standard prepared in the same manner.

For analysis of feeds and other dry materials it is best to make the usual macro digestion using either copper sulfate or mercury for catalyst. In general copper is preferable as it is easier to get crystal clear Nesslerization when it is used than in the case of mercury. When mercury is used as a catalyst somewhat more protective colloid should be added to the solution before neutralization; also the solution should be diluted as much as possible before neutralizing. The turbidity due to mercuric oxide formed in neutralizing when a slight excess of alkali is added usually, however, entirely clears after Nesslerization. By the use of the colloid as described it is possible to add the solution to be Nesslerized to the diluted Nessler and colloid solutions and in this way it is unnecessary to make several standards, as an acid mixture corresponding to the digested mixture may be neutralized and the Nessler solution added and then the standard ammonia solution until it approximately matches the unknown. It is, of course, very necessary to use an accurate buret for the measurement of the concentrated standard solution.

For routine work we have found it desirable to use definite amounts and dilutions and obtain results from a prepared chart referred to colorimeter scale readings.

Preparation of Protective Colloid Solution

While many samples of gum arabic may be used without special preparation, all samples which we have examined give considerable color with Nessler solution as well as an appreciable reduction to free mercury upon standing for some time. All of the ammonia and most of the other interfering substances can be easily removed by treatment of the solution with Permutit powder.

Ten grams of powdered gum arabic are slowly added with vigorous stirring to 190 cc. of ammonia-free water and the mixture stirred until the gum is entirely dispersed. The solution is transferred to a flask and approximately 4 g. of Permutit powder as prepared for ammonia determinations is added and the mixture shaken at intervals for ten minutes. Upon standing for a few minutes the Permutit powder settles and the slightly turbid supernatant liquid is decanted. This liquid should at most give only a faint coloration with Nessler solution; if a test portion gives an appreciable coloration with Nessler solution, the treatment with a fresh portion of Permutit powder is repeated. The solution as prepared is somewhat turbid but the insoluble material will settle out on standing for some hours. The turbidity in the freshly prepared solution, however, usually does not interfere and the solution may be used at once. Some samples of gum arabic treated as above have considerable reducing action on the Nessler solution; such solutions may be oxidized by the addition of small amounts of sodium peroxide, allowing the solution to stand until the evolution of oxygen has ceased; but as there appears to be no satisfactory indication as to the proper amount of sodium peroxide to be added, resulting in solutions which are not uniformly satisfactory, we have found the following method of oxidation to be more satisfactory.

The colloid solution from which the ammonia has been removed by treatment with Permutit as described above is treated with about one-tenth its volume of Nessler solution, the mixture allowed to stand until clear and the clear solution decanted when it is ready for use.

This colloid solution may be added in amounts to suit the particular case in hand, but the addition of 3 cc. of this solution to 15 cc. of Nessler solution we have found suitable for nearly all cases met in practice. Apparently any of the various modifications of Nessler solution may be used, but we have found it desirable to follow Folin's directions with the exception of making more concentrated, so that after the addition of the colloid solution the final concentration of alkali and mercury will be that given by Folin and Wu.[3]

Standard ammonia solutions for the preparation of Nesslerized standards may be made by suitable dilution of a macro digestion of a material whose nitrogen content has been accurately determined by the usual distillation and titration procedure or from an accurately weighed portion of pure, dry ammonium sulfate. Care must be taken, however, that the standard and unknown solutions when Nesslerized contain as nearly as possible the same concentration of salts. This would seem to be particularly true when iron is present in appreciable amount, as the colloid will hold considerable ferric hydroxide in the colloidal state and this will undoubtably affect the readings, although to what extent we have not as yet determined.

[3] Folin and Wu, *J. Biol. Chem.*, **38**, 81 (1919).

Further work on the method is in progress but owing to the very limited time available for this work it was thought advisable to publish the progress at this time.

Summary

1. A protective colloid prepared from gum arabic has been utilized in the Nesslerization of ammonia in the presence of alkali sulfates.

2. By this method a higher concentration of ammonia nitrogen can be Nesslerized in an ordinary Kjeldahl digestion solution than by previous methods even in the absence of any extraneous salts.

3. The limit of accuracy for the colorimetric determination of nitrogen by this method appears to be the accuracy of the colorimeter readings.

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ISOXAZOLINE OXIDES. VIII

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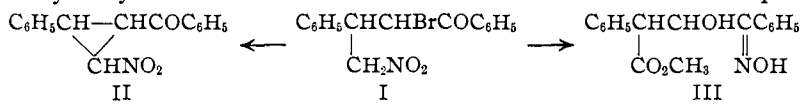
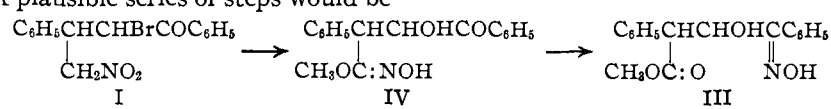
It has been shown that cold methyl alcoholic potassium acetate transforms certain α-bromo-γ-nitro ketones into cyclopropane derivatives and hydroxy oximido esters.[1] The oximido esters were unexpected



products. Their discovery led to an investigation of the mechanism by which substances so remotely related are formed from the nitro ketones. The mechanism has not been established completely, but a significant intermediate has been isolated, and the facts which have been accumulated provide a basis for a reasonable explanation.

The method of investigation was based on the fact that no compounds corresponding to the hydroxy oximido esters are formed from bromo-nitro ketones in which the nitro group is attached to a secondary carbon atom. This observation led to the inference that the oximido esters are formed by rearrangement of some hydroxamic acid derivative because the formation of such derivatives is peculiar to primary nitro compounds. A plausible series of steps would be



For the purpose of testing this hypothesis, methyl alcoholic potassium acetate was unsuitable because the solution steadily increases in acidity

[1] (a) Kohler and Shohan, THIS JOURNAL, **48**, 2425 (1926); (b) Kohler and Goodwin, *ibid.*, **49**, 219 (1927).