Application to Microchemical Analysis.

The test for hydrocyanic acid was applied microchemically to sections of cherry bark and bitter almond with marked success. Sections or small parts of the material were treated with the reagents in a test tube according to our method.[1]

Conclusions.

1. Dilute alkaline solutions of a cyanide can be concentrated under diminished pressure without appreciable loss of cyanide.
2. The maximum quantity of Prussian blue can be obtained from a cyanide only when the volume of the solution to be tested is sufficiently small, as has been indicated by Berl and Delpy and by Lander and Walden.
3. In the test for a cyanide it is better to acidify with nitric or sulfuric acid than with hydrochloric, since an excess of the latter tends to produce a green color.
4. Any considerable excess of ferric salts should be avoided in testing for a cyanide.
5. Application of heat is not necessary in testing for a cyanide by the method described.
6. The presence of certain salts, particularly potassium fluoride, in the liquid to be tested, has proved to be of great advantage.
7. The method furnishes a very delicate qualitative test for the presence of a cyanide.
8. The method is suitable for the estimation of very small quantities of a cyanide in distillates.
9. The test as described herein can be applied microchemically to sections of cyanogenetic plants.

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A COMPARATIVE STUDY OF METHODS FOR THE QUANTITATIVE DETERMINATION OF SULFUR IN PEPTONE

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As a preliminary step to making quantitative determinations of the amount of sulfur in culture media, both before and after inoculation with sewage or water samples, and before and after incubation, a comparative study was made of those methods for the determination of sulfur in organic compounds which a search of the literature showed as having given the

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[1] Further work is in progress on the application of this method to microchemical analysis.

best results in the hands of other investigators. Witte peptone was used as the sulfur-containing material and at least three determinations were made in each case, using two-gram portions of sample.

Methods for Total Sulfur.

The following methods for total sulfur were tried:

Liebig Method.[1]—A slight modification of this method was also experimented with, using less heat and larger quantities of potassium nitrate in the fusions.

Osborne Method.[2]

A. O. A. C. Method.[3]

Liebig-Koch Method.[4]—After two trials of the method as described in the above reference, it was modified as follows:

The solution of the material, after the nitric acid treatment, was introduced into a nickel crucible containing eight grams of potassium hydroxide and about one-half gram of potassium nitrate and then evaporated over an alcohol lamp while a jet of air was allowed to impinge on the surface of the liquid. This resulted in a homogeneous mixture of the material under examination with the hydroxide and nitrate, thus precluding the tendency to burn which the peptone displayed in earlier determinations. Another device, trivial, but nevertheless worthy of note, was used in connection with the transference of the material, which had been treated with nitric acid, to the nickel crucible. A number of particles difficultly soluble in hot water clung tenaciously to the sides of the beaker. Obviously a rubber-tipped rod could not be used to loosen them because of the danger of minute particles of the rubber becoming detached which might contribute appreciable amounts of sulfur. Therefore, a small piece of filter paper on the end of a glass rod was employed for scrubbing. This effectively loosened all particles. The piece of filter paper with any adhering material was introduced into the nickel crucible and was, of course, destroyed during the alkali-nitrate fusion.

It was deemed advisable to ascertain whether any loss of sulfur in volatile form might take place during the treatment with nitric acid in the Liebig-Koch method.

For this purpose, a 250 cc. ether wash bottle was modified by fusing on a small separatory funnel. The lower end of the entry tube was drawn out and bent into a shepherd's crook. To the other tube, which emerged from the stopper of the wash bottle, was fused a piece of glass tubing which was bent so as to nearly reach to the bottom of a Fritz Friedrich's gas

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[1] As described in "Organic Analysis," Sherman, 1912 edition, p. 298.

[2] THIS JOURNAL, 24, 142 (1902).

[3] U. S. Dept. of Agr., Bur. Chem., *Bull.* 107, Revised, p. 23.

[4] *Chem. Zentr.*, 1886, p. 894.

wash bottle, the connection being made by means of a soft-rolled cork stopper. The glass tube which was fused into the stopper of the Fritz Friedrichs wash bottle was connected with a suction pump.

Two grams of peptone were placed in the ether wash bottle, which was mounted on a boiling water bath and connected to the Fritz Friedrichs wash bottle, into which 250 cc. of 12% potassium hydroxide had been introduced. Suction was applied and 20 cc. of nitric acid (sp. gr. 1.4) was introduced through the separatory funnel. The suction was so regulated that about two bubbles of air per second were drawn through the apparatus. Thus, any volatile compounds of sulfur .which might be liberated during the process would be drawn into the potassium hydroxide solution and held there.

It required two weeks, running eight to ten hours a day, to completely distil all of the nitric acid from the ether wash bottle into the Fritz Friedrichs wash bottle.

The material in the ether wash bottle was then dissolved in hot water and transferred to a Jena glass beaker, acidified with hydrochloric acid, evaporated to dryness on a water bath, 20 cc. of distilled water added, again evaporated to dryness, heated for 4 hours in an air bath at 110° to dehydrate the silica, dissolved in water, 5 cc. of hydrochloric acid added, filtered, and the sulfur existing as sulfuric acid, precipitated and weighed as barium sulfate.

For comparison, the amount of sulfur existing as sulfuric acid after treatment of peptone with nitric acid in open beakers was determined.

For this purpose, 2 g. portions of peptone were placed in Jena glass beakers, 20 cc. of nitric acid (sp. gr. 1.4) were added to each, and digestion was carried on for two hours on a water bath; each solution was then evaporated to dryness, 20 cc. of distilled water added, again evaporated to dryness, heated for 4 hours in an air bath at 110°, dissolved in water, 5 cc. of hydrochloric acid added, filtered, and the sulfur existing as sulfuric acid precipitated and weighed as barium sulfate.

The close agreement between the results obtained in the closed system and in the open beakers, while not proving that there is no loss of volatile sulfur compounds, does prove that the length of time of the digestion with nitric acid above two hours has no effect, as the nitric acid was in contact with the peptone for two hours in the one case and for two weeks in the other.

To ascertain whether any loss had taken place during the nitric acid treatment in the closed system, the amount of sulfur in the solution contained in the Fritz Friedrichs wash bottle was determined.

For this purpose, the solution—which was calculated to contain 30 g. of potassium nitrate, resulting from the neutralization of the 18.3 g. of nitric acid distilled over from the ether wash bottle plus 14 g. of potassium hydroxide remaining from the 30 g. originally introduced in the form of 250 cc. of 12% potassium hydroxide, together with any potassium sulfide, potassium sulfite or potassium sulfate which might have been formed—was transferred to a large nickel crucible, evaporated to dryness and fused, in order that any sulfide or sulfite might be oxidized to sulfate. The fused mass was dissolved in water, an excess of hydrochloric acid added, evaporated to dryness, 20 cc. of distilled water added, again evaporated to dryness, heated for 4 hours in an air bath to 110° to dehydrate the silica, dissolved in water, 5 cc. of hydrochloric acid added, filtered, and precipitated with barium chloride in the usual manner.

The results showed that there was no formation of volatile sulfur compounds during the nitric acid digestion in the closed system; and since the results in the open beakers were in such close agreement with those obtained in the closed system in the matter of the percentage of sulfur revealed by nitric acid digestion, it is safe to assume that no loss of sulfur as volatile compounds occurs during nitric acid digestion in open beakers.

Petersen Method.[1]

Perhydrol Method.—It was thought that very complete and rapid oxidation of the sulfur-containing organic compounds might be obtained by substituting perhydrol for the 3% hydrogen peroxide used in the Petersen method. Without giving the details of the methods employed, suffice it to say that—dissolving the peptone in water and then boiling with perhydrol—dissolving the peptone in alkaline solution and then boiling with perhydrol—digesting the peptone with nitric acid (sp. gr. 1.4) and then boiling with perhydrol, were all tried without success.[2]

Parr Calorimetric Method, employing for each charge a mixture of 1 g. of peptone, 15 g. of sodium peroxide and 1 g. of potassium chlorate.

Methods for Part of Sulfur Only.

Schultz Method, for loosely bound sulfur, described by Osborne.[3]

Nitric Acid-Potassium Chlorate Method.—The effect of a saturated solution of potassium chlorate in nitric acid was next tried upon peptone for the reason that it was believed that this reagent would give a measure of the *easily oxidized sulfur* present.

The method consisted in digesting 2 g. of peptone with 20 cc. of a saturated solution of potassium chlorate in nitric acid in a Jena glass beaker on a water bath for two hours, then evaporating to dryness, adding 5 cc. of hydrochloric acid and 20 cc. of water and evaporating to dryness, dehydrating, and determining the sulfur as barium sulfate.

Hydrochloric Acid-Potassium Chlorate Method. — Determinations were made using potassium chlorate and hydrochloric acid as the oxidizing agent.

Sulfur in Untreated Peptone.

The amount of sulfur precipitable as barium sulfate in a solution of peptone which had not been treated in any way was determined.

Results of Methods.

The average results for all of the foregoing methods appear in Table I.

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[1] *Z. anal. Chem.*, 42, 406.

[2] This work was repeated by Miss M. I. Henkel several months later with the same results.

[3] THIS JOURNAL, 24, 140 (1902).

TABLE I.

All determinations were made in triplicate.	Uncor. per cent. of S in peptone.	Per cent. moisture in peptone.[1]	Per cent. S contributed by reagents.	Per cent. S in peptone cor. for moisture and for sulfur contr. by reagents.
Liebig method (KOH, KNO_3 fusion)	0.9282	6.19	0.0040	0.9902
Liebig method. Less heat and more KNO_3	0.9067	6.19	0.0048	0.9665
Osborne method (Na_2O_2 fusion)	0.8888	6.19	0.0000	0.9479
A. O. A. C. method (Na_2O_2, Na_2CO_3 fusion)	0.9122	6.19	0.0056	0.9664
Liebig-Koch method (HNO_3 digestion and KOH, KNO_3 fusion)	0.9479	6.19	0.0041	1.0061
HNO_3 digestion in closed system	0.5005	6.19	0.0012	0.5323
HNO_3 digestion in open beakers	0.4999	6.19	0.0012	0.5318
Distillate from HNO_3 digestion in closed system	0.0041	6.19	0.0043	0.0000
Petersen method (digestion with H_2O_2 in alkaline solution)	0.2770	6.19	0.0072	0.2876
Perhydrol method (digestion with 30% H_2O_2 in alkaline solution)	0.2927	6.19	0.0089	0.3025
Modified perhydrol method (digestion with HNO_3 followed by digestion with 30% H_2O_2)	0.5667	6.19	0.0097	0.5938
Parr calorimeter	0.9142	6.19	0.0009	0.9736
Schultz method for loosely bound S	0.3610	6.19	0.0031	0.3813
HNO_3, $KClO_3$ meth. for easily oxid. S	0.4899	6.19	0.0007	0.5194
HCl, $KClO_3$ method	0.0723	6.19	0.0010	0.0761
Untreated peptone	0.0004	6.19	0.0000	0.0004
Alc. extr. of peptone, Liebig-Koch meth.	0.7013	12.30	0.0041	0.7950
Res. insol. in alc., Liebig-Koch meth.	0.9974	7.71	0.0041	1.0763

Summary.

1. Of the methods tried, the Liebig-Koch method for total sulfur gave the highest and most consistent results.

2. During the preliminary treatment with nitric acid in the Liebig-Koch method no sulfur is lost in volatile form.

3. The length of time beyond two hours during which nitric acid is allowed to act has no influence in the Liebig-Koch method.

4. Of the methods for determining a part only of the sulfur, the Schultz method for loosely bound sulfur, and the digestion with nitric acid and potassium chlorate for easily oxidized sulfur gave the most consistent and most valuable results.

ITHACA, N. Y.

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[1] Determined by drying at 70° for 30 hours in vacuum over $CaCl_2$.