ON THE DETERMINATION OF SMALL QUANTITIES OF HYDROCYANIC ACID.

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In our work on cyanogenetic plants we found it necessary to estimate small quantities of hydrocyanic acid. The various titration methods as well as the silver gravimetric methods had to be excluded because we were working with plant distillates which usually contain reducing compounds. Furthermore, the quantities to be determined were often too small to permit the use of the above methods. We frequently had to deal with less than 0.5 mg. of hydrocyanic acid. Chapman[1] has shown that the picric acid colorimetric method of Waller[2] is not applicable to plant distillates which usually contain reducing substances other than hydrocyanic acid. This left two colorimetric methods to be examined, namely, the thiocyanate method and the Prussian blue method.

In the thiocyanate method of Francis and Connell[3] the hydrocyanic acid is distilled into a solution of potassium hydroxide, yellow ammonium sulfide is added and the solution is evaporated to dryness. The residue is dissolved in water, acidified with hydrochloric acid and the mixture is filtered to remove sulfur, after which the filtrate is further acidified and boiled to cause precipitation of the free sulfur. The process of boiling and filtering is repeated until all of the sulfur has been removed from the filtrate. Ferric chloride is then added to the filtrate and the color of ferric thiocyanate is obtained. After an examination of this method we found that it was not accurate enough for our work. Our two chief objections to this method are as follows:

In boiling an acid solution of a thiocyanate, some free thiocyanic acid is lost because it is volatile, the boiling point of the acid being 85°. This loss was shown by taking two equal quantities of potassium thiocyanate, diluting both portions with water, acidifying with hydrochloric acid and boiling one of them for ten minutes in an Erlenmeyer flask. The boiled solution was then cooled. Both the boiled and unboiled portions were tested by adding equal quantities of ferric chloride. The portion that had been boiled gave less color than the one not boiled. This test was repeated and we found that in every case boiling resulted in a loss of thiocyanic acid.

The second important objection to the thiocyanate method is based on the nature of the reaction between a thiocyanate and ferric chloride, $FeCl_3 + 3KCNS \rightleftarrows Fe(CNS)_3 + 3KCl$. The equilibrium of this reaction is so easily disturbed by the addition of ferric chloride, intensifying the color, or the addition of other salts, diminishing this color, that it is

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[1] *The Analyst*, **35**, 471 (1910); **36**, 269 (1911).

[2] *Proc. Royal Soc.*, (*B*) **82**, 574, 1910; *The Analyst*, **35**, 406 (1910).

[3] This Journal, **35**, 1624 (1913).

difficult to adjust conditions so as to obtain constant results. Changes of temperature also have a marked influence on the density of the color.

In recent years several papers have been published on the formation of Prussian blue from cyanides and the colorimetric estimation of the cyanide by comparison with a standard suspension of Prussian blue. The most careful attempts to arrive at a quantitative method seem to have been made by Berl and Delpy and by Lander and Walden.

Berl and Delpy[1] make the solution to be tested alkaline with potassium hydroxide, add a solution of ferrous sulfate, allow the mixture to stand at room temperature for at least ten minutes, shaking frequently, and finally boil for two to fifteen minutes. The resulting mixture is cooled and then acidified with hydrochloric acid. In the case of very dilute solutions of hydrocyanic acid, Berl and Delpy shake out the acidified solution with ether eight to ten times, and shake out the ether with a small quantity of potassium hydroxide solution, thus obtaining the hydrocyanic acid in a more concentrated form.

The method of Berl and Delpy has been improved by Lander and Walden.[2] These workers concentrate dilute alkaline solutions of hydrocyanic acid by boiling, the final concentrating being carried out in a test tube almost to dryness. The small quantity of liquid left is then cooled and ferrous sulfate solution added. The mixture is allowed to stand ten minutes, with frequent shaking, then acidified with hydrochloric acid and warmed gently.

Vorländer[3] investigated the formation of Prussian blue from cyanides. He adds a solution of ferrous sulfate to the alkaline solution of the cyanide and boils for one to two minutes. He then filters and acidifies the filtrate with hydrochloric acid, after which he adds a cold, freshly prepared, saturated solution of ferrous sulfate. He allows the mixture to stand until the maximum color is obtained.

Knight[4] adds to the alkaline distillate containing hydrocyanic acid, solid ferrous sulfate, then a solution of ferric chloride and boils for one minute. Hydrochloric acid in excess is then added to the hot solution. The precipitate of Prussian blue is filtered, washed with alcohol, and dissolved in a sufficient quantity of sodium hydroxide solution. This solution is acidified with acetic and hydrochloric acids, ferric chloride is then added and any brown color is removed by adding more hydrochloric acid. The mixture is concentrated to about one-half the original volume and the precipitate of Prussian blue, after filtration, is determined by weighing.

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[1] *Ber.*, 43, 1430 (1910).

[2] *The Analyst*, 36, 266 (1911).

[3] *Ber.*, 46, 181 (1913).

[4] *J. Ind. Eng. Chem.*, 6, 909 (1914).

In a method involving the quantitative estimation of a substance by means of colorimetry, it is obvious that a given quantity of the substance should always produce a given density of color in a given volume of solution. It is also necessary that the shade of color should always be the same. For instance, a blue solution cannot be compared accurately with a blue-green solution. Our aim in this work has been to obtain a constant shade as well as density of color. We have studied the following conditions which influence the formation of Prussian blue and the shade of the suspension.

Influence of Concentration on the Formation of Prussian Blue.

Berl and Delpy found that they obtained less Prussian blue from given quantities of hydrocyanic acid in a dilute than in a concentrated solution. These authors, therefore, concentrated dilute solutions of hydrocyanic acid by shaking out with ether as previously mentioned. They state that in this manner nearly all of the hydrocyanic acid can be obtained. In our attempts to concentrate the hydrocyanic acid by means of ether, we found that we could not obtain nearly quantitative results. These low results are due partly to the loss of hydrocyanic acid which will occur by evaporation of the ether. This evaporation cannot be prevented at room temperature, since the acid solution has to be shaken out eight to ten times with ether. Moreover, the volume of alkali needed to extract the hydrocyanic acid from the ether will be so great that the maximum quantity of Prussian blue can scarcely be expected, as shown below.

Lander and Walden, finding also less precipitate of Prussian blue in dilute than in concentrated solutions of hydrocyanic acid, boiled the alkaline solution down almost to dryness. They state that this could be done without the loss of hydrocyanic acid.

The influence of concentration is indeed a very important factor. We made a series of experiments, using 1 mg. of potassium cyanide in each case and concentrations ranging from less than 1 cc. to 10 cc. In a dilution of 10 cc. less than one-half as much Prussian blue was obtained as when the volume was only 1 cc. We found that the maximum color was obtained only when the volume of solution to be tested was not greater than 1.5 cc., while in greater volumes the density of the color decreased with the increasing volume. Where the quantity of potassium cyanide was more than 1 mg. the volume of the solution to be tested could be somewhat increased without loss.

Hence, working with the methods of Berl and Delpy or Knight, where larger volumes than those mentioned above are used, the maximum quantity of Prussian blue would not be obtained.

To test the method of concentrating used by Lander and Walden, we evaporated 25 cc. of an alkaline solution of a cyanide to 1 cc. in a distilling flask and lost almost one-third of the cyanide. When the final evaporation was performed in a test tube the loss was less but still considerable. Hence, it appears that the method of Lander and Walden also cannot give the maximum quantity of Prussian blue, since these authors evaporate over a free flame. It may be mentioned in this connection that evaporation in an open dish on a steam bath resulted in still greater losses than those mentioned above.

After numerous failures we found that alkaline solutions of hydrocyanic acid can be concentrated without appreciable loss by distilling under diminished pressure, as described below.

Influence of Salts on the Formation and Precipitation of Prussian Blue.

Vorländer states that the presence of salts delays or prevents the formation of Prussian blue. We tried the addition of salts, because in our early experiments we often obtained what seemed to be a colloidal form of Prussian blue. Since salts are known to precipitate colloids we tried the effect of adding sodium chloride and found that a clearer blue color was obtained than without the addition of this salt. We also tried the effect of several other halogen salts. Finally, we found that the presence of potassium fluoride had a remarkable effect on the formation and color of the Prussian blue. On acidifying in the final stage of the test the color appears at once and is very brilliant. The absence of a green shade makes it particularly suitable for comparison with a standard. If the acid is added very gradually the iron hydroxides dissolve and a colorless liquid with a white precipitate is obtained. On the addition of more acid the blue color appears. This is explained by the fact that ferric salts produce a complex salt with potassium fluoride,[1] K_3FeF_6. When an excess of acid is added this complex salt is decomposed and the ferric ions needed for the formation of Prussian blue are furnished. Contrary to the statement of Vorländer, our experiments show that certain salts, such as sodium chloride and particularly potassium fluoride, hasten the formation of Prussian blue.

Influence of Acids on the Formation of Prussian Blue and on the Color of the Suspension.

Previous workers all seem to have used hydrochloric acid in the final stage of the test for a cyanide to precipitate the Prussian blue. We found that an excess of this acid tends to make the color of the suspension green, owing to the formation of ferric chloride. The mixture of the yellow ferric chloride solution with the Prussian blue produces the green shade. To avoid the formation of the green shade we tried the effect of acids other than hydrochloric acid. We obtained good results with sulfuric and nitric acids in different concentrations. Hence, these acids are recommended instead of hydrochloric acid.

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[1] Greef, *Ber.*, 46, 2511 (1913).

Vorländer and Knight both filter off the iron hydroxides from the alkaline solution of sodium ferrocyanide and then acidify *before* adding the iron salt. This procedure may lead to a loss of hydrocyanic acid, since ferrocyanic acid oxidizes rapidly according to the following equation:

$$7H_4Fe(CN)_6 + O_2 = 24HCN + Fe_4[Fe(CN)_6]_3 + 2H_2O$$

Influence of Ferric Iron on the Formation of Prussian Blue and on the Color of the Suspension.

It is to be noticed that Berl and Delpy, Lander and Walden, as well as Vorländer, used only ferrous sulfate for the formation of small quantities of Prussian blue. In the ordinary laboratory test for a cyanide, ferric chloride is also added, which changes the color to a greenish shade and makes accurate colorimetric determinations impossible. The addition of a ferric salt is not necessary, since enough of the ferrous salt is oxidized during the operation to furnish the ferric ions needed. According to our experience it is very important that there should not be a large excess of ferric salts present. Therefore, to prevent the oxidation of too much of the ferrous hydroxide to ferric, we remove most of the air from the solutions of the cyanide by means of a water vacuum pump.

In this connection we may quote Vorländer's statement.[1]

> "Die Meinung ist verbreitet, dass zum Zustandekommen des Berlinerblaus aus Ferrocyankalium die Gegenwart von Ferro- und Ferrisalz nützlich sei. Ich habe hierfür nicht das geringste Zeichen finden können."

Here one might obtain the misleading impression that Prussian blue is formed in the utter absence of a ferric salt. Vorländer probably means to say that the addition of ferric salt is unnecessary, as enough of the ferrous salt is oxidized by the action of atmospheric oxygen to furnish the ferric ions needed.

Influence of Heat on the Formation of Prussian Blue.

It is interesting to note that other workers apply heat in the test for a cyanide. Sometimes the alkaline mixture is heated, and sometimes heat is applied after adding the acid. Others recommend that the mixture be heated, both before and after the addition of acid. In testing by our method heat is not necessary if potassium fluoride is used. In using sodium chloride instead of potassium fluoride, or in the absence of these salts, gentle heat hastens the formation of Prussian blue.

Description of the Method.

As a result of our various experiments, we recommend the following procedure: Before concentrating the hydrocyanic acid solution, as in the case of a distillate, the portion to be tested should contain a slight excess of free sodium hydroxide. We used 0.02 to 0.1 g. This solution is then concentrated in a round bottom flask of 200 cc. capacity by using

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[1] *Ber.*, 46, 188 (1913).

a vacuum pump and condenser. The heat is supplied by immersing the flask in a water bath kept below 70°. To avoid any loss by spattering the flask is fitted to the condenser by means of an adapter such as is used in the Kjeldahl method for the determination of nitrogen. We concentrate until less than 1 cc. of liquid remains in the flask. Two-tenths to one-half cubic centimeter of 3% freshly prepared ferrous sulfate solution and about 0.05 g. of potassium fluoride are then added. The flask is exhausted at once by means of a water vacuum pump. The contents are mixed by rotating the flask. After 5 to 10 min. the flask is detached from the pump and the mixture acidified with 30% nitric acid. The blue color appears at once. Where only traces of hydrocyanic acid are present it is sometimes necessary to warm to about 50° in a water bath before the color appears. The suspension is then diluted to a volume that would give a color density convenient to compare with a suspension of Prussian blue made from a known weight of potassium cyanide. As a standard we used a suspension of Prussian blue made from 1 mg. of potassium cyanide. Such a suspension diluted to 25 cc. gave a color of convenient density. For comparison we used a Duboscq colorimeter.

If the cyanide solution to be tested was sufficiently concentrated so that further evaporation was unnecessary, the test could be made in a test tube. We kept the air out by means of a stopper and rotated the tube only enough to mix the reagents, allowing the mixture to stand 5 to 10 min. before acidifying. Much shaking must be avoided to prevent excessive oxidation of the ferrous hydroxide.

We ascertained the accuracy of our method by diluting 1 cc. of a standard solution of potassium cyanide to 25 cc. and evaporating under diminished pressure to 1 cc. When the residue was tested as described above we obtained the same quantity of Prussian blue as that formed by applying the test to 1 cc. of the undiluted standard solution.

The quantities of reagents mentioned are suitable for 1 to 2 mg. of potassium cyanide. If less than 1 mg. of potassium cyanide is present, the quantities of the reagents should be reduced accordingly. A large excess of reagents must be avoided in order to obtain the maximum density of color. When the analysis is carried out as described the maximum error should not exceed more than 1 part in 20. Thus in a plant giving 20 mg. of potassium cyanide per 100 g. of plant the results might vary by 0.001% if 100 g. of material are used for analysis.

Application as a Qualitative Test.

This method also proved to be a very delicate qualitative test for the presence of a cyanide. Owing to the possibility of obtaining a pure blue color we were able to detect with certainty 0.00002 g. of potassium cyanide, which represents less than 0.00001 g. of hydrocyanic acid.

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.