PHOTOELEOTRIC COLORIMETRY OF PHOSPHORUS IN SOIL EXTRACTS





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PHOTOELECTRIC COLORIMETRY OF PHOSPHORUS

IN SOIL EXTRACTS

by

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A Thesis

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I. INTRODUCTION

Of all the soil problems which have been attacked, the one which has received the most widespread attention is that of soil phosphorus in all its manifold phases and relationships. In any such field the value of the work done must stand in relation to the accuracy of the analytical methods by means of which the studies are carried out. Hence any improvement which can be made in the methods of study is of great importance.

The most widely used method for the determination of phosphate ion, at least so far as soil work is concerned, is the colorimetric ceruleomolybdate method of Deniges. Various modifications of the method have appeared from time to time.

The most accurate means by which color intensity measurement or comparison can be made is by the use of a photoelectric colorimeter. These have been used in soils work previous to this time, but most workers have used the balancing type of instrument which is very little more accurate than the ordinary colorimeter except in so far as ease of reading is concerned. An Evelyn photoelectric colorimeter (Evelyn, 1936) of the single cell type, equipped with light filters, recently became available and its advantages were immediately evident.

(1) The personal factor in reading the color intensity was eliminated.

(2) With the use of the instrument reproducible results were obtained, which were constant from day to day.

(3) The use of the light filters enabled the determination to be made in solutions, such as soil extracts, containing considerable amounts of foreign coloring matter with a high degree of accuracy.

(4) In a solution in which the color intensity is changing, it is manifestly very hard to obtain data on the reaction using an ordinary colorimeter or balancing type photoelectric colorimeter when the standard is also changing. With the Evelyn colorimeter both the standard and unknown are measured with the same constant standard, the percent transmission of light.

In the use of the Evelyn colorimeter (Evelyn --) the percent transmission of light is read directly on the galvanometer scale. These readings may be plotted directly on a graph to give a logarithmic curve, (concentration vs percent light transmission).

If Beer's law is followed, a straight line will be obtained when the concentration is plotted against the

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log of the percent light transmission. To avoid the necessity of referring to this graph, a constant K_1 may be calculated as follows.

Let G = corrected galvanometer reading (corrected for deviation of the galvanometer scale from linearity).

T =light transmission - ratio of light transmitted by sample and blank. Since G is proportional to the amount of light transmitted, $T = \frac{G}{100}$.

Since the laws of Lambert and Beer are usually applicable (if suitable filters are used)

C (concentration in sample solution)

$$= \frac{1}{K_{1}} \times \log_{10} \frac{1}{T}$$

$$= \frac{1}{K_{1}} \log \frac{100}{G} = \frac{2 - \log G}{K_{1}}$$

$$= \frac{L}{K_{1}}$$

where K_{l} is a constant depending on the nature of the reaction and filter used and $L = 2 - \log G$ -- the 'density' of the sample solution.

K_l is determined for the technique followed using standard solutions.

In finding the concentration in any sample, the value of L corresponding to the value of G found is read from a table. The concentration is given by $\frac{L}{K_1}$.

If the color intensity given by any reaction is reproducible, and Beer's law is followed, then K₁ will be constant from day to day, over the whole range of the galvanometer readings. Occasionally this value was checked using standard solutions.

It was therefore deemed worth while to make a study of the reaction with the photoelectric colorimeter, both in pure solutions and in solutions containing various substances which might interfere, particularly in colored soil extracts.

II. THE CERULEOMOLYBDATE METHOD FOR THE DETERMINATION OF PHOSPHORUS

A. The Ceruleomolybdate Reaction

1. Mechanism of the Reaction

The information available on this point is very limited, but the general outline is as follows.

The reduction of ammonium molybdate in neutral solution yields a blue compound, true molybdenum blue [Mo02.4Mo03] which is stable to heat and dilution.

If, however, the reduction is carried out in acid solution no color will be produced. But if phosphate or arsenate is present, a blue color develops, the intensity of which is proportional to the concentration of phosphate or arsenate. This is due to the formation of the phosphoconjugated ceruleomolybdate, molybdenyl phosphomolybdate, $[(MoO_3)_4.MoO_2]_2.H_3PO_4.4H_2O$ (sapphire blue) which has been isolated by Deniges (1920, 1927).

If the reduction takes place in very acid solution (5 N H_2SO_4 - Deniges 1927; 25 N H_2SO_4 - Zinzadze 1935) the true molybdenum blue compound is formed. On dilution the color is voided and reappears if phosphate or arsenate is present due to the formation of the blue phosphoconjugated ceruleomolybdate, as above.

Truog and Meyer (1929) consider that when the acid molybdate reagent is added to a solution containing phosphate, ammonium phosphomolybdate is formed but because of the high dilution, no precipitate appears. If now a reducing agent is added, the molybdenum in combination with the phosphate is reduced, but not that in the excess ammonium molybdate, if the acidity is high enough to prevent the reduction of ammonium molybdate itself. If the solution is too acid no color again appears probably because ammonium phosphomolybdate would not be formed at the high acidity, that is, the molybdate in combination as the free acid is not as easily reduced as when it is in the form of the ammonium salt.

Silicomolybdate may be formed at low acidities and is also reduced to a similar blue compound.

Comparative tests showed that the color intensities produced in solutions containing the same concentration of phosphate and arsenate were inversely proportional to the atomic weights of phosphorus and arsenic. This was considered as evidence that all the phosphorus and arsenic took part in the reaction under the conditions recommended for the test.

Wu (1920) shows that various heteropoly molybdic acids may be formed under different conditions, of which acidity is the most important factor. On reduction different color intensities are obtained.

Thus, there are three ways in which the colored complex could be developed:

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(1) Reduction of molybdate in the presence of phosphate under conditions of acidity such that the molybdate itself is not reduced.

(2) Addition of molybdenum blue to a solution containing phosphate.

(3) Oxidation of molybdenum dioxide in the presence of phosphate.

The first method is the one generally used.

Stannous chloride was originally used by Osmond (1887) for the reducing agent, but various other reducing agents have been used, phenylhydrazine (Taylor and Miller 1914), hydroquinone (Bell-Doisy 1922), 1.2.4.aminonaphtholsulphonic acid (Fiske and Subbarow 1925) and p-methylaminophenolsulphate (Tschopp and Tschopp 1932).

The second method was introduced by Deniges (1927) and later used by Zinzadze (1930, etc.)

Zinzadze (1935) tried the third method using potassium permanganate as the oxidant, but since heating was found to be necessary to develop the blue compound, there was no advantage in using the method.

Hein, Burawoy, and Schwedler (1936) showed that the reduction of the heteromolybdic acids by stannous chloride proceeds to an equilibrium.

Phosphomolybdic acid + stannous chloride =>

molybdenum blue + stannic chloride.

Addition of stannic chloride with the stannous chloride affects the equilibrium as expected, but if it is

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added afterwards there is no effect due to the colloidal condition of the molybdenum blue.

The data for the reaction obtained in this investigation would seem to indicate that two reactions are taking place.

(1) The reduction of the phosphomolybdate to the phosphoconjugated molybdenum blue, which is apparent by the rapid rise of the color to a maximum at about five minutes.

(2) A secondary reaction which reaches equilibrium with the first at about 15-20 minutes. Thereafter the color fades very slowly.

(3) This gradual fading may be due to the slow formation of colloidal particles since after from two hours to two days a visible colloidal precipitate develops in the solution.

It would seem that reactions (1) and (2) must be in equilibrium since the addition of more stannous chloride brings back the former maximum (within an hour) and also that the excess of stannous chloride was one of the factors influencing this equilibrium.

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2. Historical

The formation of a blue colored complex when ammonium molybdate is reduced in the presence of phosphate ion was first mentioned by Osmond in 1887. He reduced ammonium phosphomolybdate by stannous chloride in acid solution.

Taylor and Miller (1914) reported the first quantitative method. The precipitate of ammonium phosphomolybdate was dissolved and then reduced by hydrazine.

With the publication in 1920 of papers by Deniges, and by Bell and Doisy, the history of the development of the method is divided into two branches - one using stannous chloride, which is of particular interest in the present work, and the other using various organic compounds as reducing agents.

Bell and Doisy (1920) used hydroquinone to reduce an alkaline solution of ammonium phosphomolybdate. Briggs (1922) used an acid solution to prevent the rapid fading of the color which occurred in alkaline solution.

Fiske and Subbarow (1925) recommended the use of 1.2.4.aminonaphtholsulphonic acid as a reducing agent. The reaction was carried out in acid solution in the presence of sodium bisulphite. This method is extensively used in biological work.

Tschopp and Tschopp (1932) investigated the use of several organic reducing agents and recommended p-methyl-

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aminophenolsulphate (metol) introduced by Leiboff (1931).

The Stannous Chloride Reduction Method of Deniges and Its Modifications.

Deniges (1920) was the first to apply the ceruleomolybdate method to the quantitative estimation of phosphate ion without the preliminary separation of the phosphate as ammonium phosphomolybdate. His quantitative procedure was as follows:

To 5 ml. sample solution was added 1-10 drops Reagent A (1 vol. 10% ammonium molybdate + 1 vol. sulphuric acid) according to the phosphate content, the solution mixed and then 1-2 drops Reagent B (fresh stannous chloride solution - 0.1 g. tin dissolved in 2 ml. conc. hydrochloric acid with the addition of 1 drop 3% copper sulphate solution, and diluted to 10 ml.) added. The sensitivity was 0.13 parts per million phosphorus.

Florentin (1921) used Deniges method for phosphate determination in water. He modified the proportion of molybdate and sulphuric acid by using as his Reagent A 100 ml. 10% ammonium molybdate mixed with 300 ml. 20% sulphuric acid. The sensitivity of his method, 0.04 p.p.m. phosphorus, was greater than that of Deniges.

Atkins (1924) applied Florentin's modification to soil extracts and standardized the amounts of reagents used.

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To 100 ml. of diluted extract was added 2 ml. of Florentin's Reagent A, the solution mixed, and then 5 drops of Deniges' Reagent B. The color developed was compared with a similarly prepared standard. Good results were reported except that sometimes a green color was obtained which was attributed to the effect of ferric iron.

Von Wrangel (1926) used a method similar to Deniges original one. To 90 ml. extract (containing 0.01 to 0.04 mg. P205) was added 1.5 ml. Deniges Reagent A and after mixing 0.75 ml. Reagent B. The mixture was diluted to 100 ml. and the color comparison made 5 minutes after the addition of stannous chloride. Oxidizing substances interfered as well as arsenic and silica.

Parker and Fudge (1927) compared the method of Deniges (Atkins) with that of Fiske and Subbarow and conconcluded that both gave good results on soil extracts. The Deniges method was about five times more sensitive, the lower limit for the Fiske and Subbarow method being 1 p.p.m. phosphorus while the optimum for the Deniges was only 0.5 p.p.m.

Here again the history of the development of the method is divided:

- (1) The method of Kuttner and Cohen
- (2) Deniges reduced molybdenum blue method
- (3) Rapid methods (approximate)
- (4) The Truog and Meyer modification of the Deniges method.

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(1) Kuttner and Cohen (1927) developed a modification of the stannous chloride method, particularly for use on biological materials. They changed the final concentration of reagents considerably, although the evidence on which their claims were based is rather inconclusive.

The final concentration of sulphuric acid was increased from 0.4 N to 1.0 N, although the curve given shows more sensitivity at the lower acid concentration. The concentration of molybdate was 0.75% and stannous chloride 0.02% as compared with 0.05 and 0.005% respectively, used by Atkins. The color intensity was stated to reach a maximum at 15 seconds and was stable up to 2 hours.

Kuttner and Lichenstein (1930) showed that the amount of stannous chloride could be varied to quite an extent(0.01-0.03%) without appreciably influencing the results.

Bodansky (1932, 1937) found a considerable departure from Beer's law, 20% of the difference between the phosphate content of the known and unknown, and he worked out an elaborate correction table. The method was accurate only within the range 1-9 p.p.m. phosphorus, thus being much less sensitive than the Atkins' method.

Farber and Youngberg (1932) investigated the method for the determination of phosphate in natural waters. Silica up to 3,500 p.p.m. did not interfere on account of the high acidity. The limits of sensitivity were 0.3-10 p.p.m. phosphorus.

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(2) In 1927, Deniges used the reduced compound true molybdenum blue (MoO2.4MoO3) for the determination of phosphates. The blue reagent becomes colorless at the acidity and dilution used in the test solution and develops a blue color when phosphate is present in the solution, the depth of color being proportional to the amount of phosphate present.

The reagent was prepared by reducing sulphomolybdic acid (1 vol. 10% ammonium molybdate + equal vol. conc. sulphuric acid), diluted four times, for one hour by copper turnings, on a steam bath.

A 5 ml. sample was boiled 5 seconds with 0.2 ml. reagent, cooled, and the color compared with similarly prepared standards after a time of 10 minutes.

The advantage claimed for this method was that the blue color produced was stable for from 2 to 3 days or longer, although the necessity for heating was a disadvantage.

Zinzadze (1930, 1931, 1932) has a series of papers on the molybdenum blue method. Since the excess of reducing agent used in most of the reduction methods renders the color unstable, Zinzadze used a modification of the Deniges molybdenum blue reagent cited above. He reduced a solution of pure molybdic acid in sulphuric acid with molybdenum powder instead of copper and standardized the reagent against standard potassium permanganate solution.

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The blue color produced with phosphates and arsenates was quite stable (7-10 days) and was relatively free from the influence of extraneous substances. The color intensity was proportional to the phosphate concentration over the range 0.04-5.0 p.p.m. phosphorus. In 1935 he introduced further modifications to eliminate the effect of arsenates (up to a concentration of 1400 p.p.m.), nitrates (1000 p.p.m.) and ferric iron (1000 p.p.m. as Fe_2O_3). This he did by reduction with sodium bisulphite. The color was found to be stable for from 2 to 3 days.

The final method was as follows:

Reagents: I. 4.011 MoO₃ (anhy.) in 25 N sulphuric acid (1010 ml. 25 N H2SO₄ + 40.11 g. MoO₃ boiled until dissolved and diluted to 1 litre).

II. Reduced reagent. To 500 ml. I is added 1.78 g. molybdenum powder, the mixture boiled 15 minutes, cooled, decanted, and diluted to 500 ml. The reagents are standardized against 0.1 N potassium permanganate and mixed so that 5 ml. is equivalent to 5 ml. 0.1 N potassium permanganate.

Procedure: To 15 ml. sample (containing 0.01-0.3 mg. P_2O_5) (If the solution contains less than 2 mg. Fe_2O_3 , 10 mg. NO_3 and no arsenate, the determination may be made directly), add 5 drops **a**-dinitrophenol and neutralize. Add 5 ml. 1 N sulphuric acid and 5 ml. 8% sodium bisulphite, make to 30 ml. and shake. Allow to stand overnight (or

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hegt one hour on the steambath). Then add 5 ml. 5 times diluted reagent and heat 30 minutes more on the steambath. After cooling dilute to 50 ml. and make the color comparison.

(3) Bray, Spurway, Dahlberg and Brown, and Morgan use various modifications of the ceruleomolybdate method in their rapid methods for the determination of available soil phosphorus.

Bray (1929) used a 0.4% ammonium molybdate solution dissolved in dilute hydrochloric acid (1:15). One part of soil was shaken with 3 parts of solution in a test tube. After settling 5 minutes the suspension was stirred with a tin rod to develop the color which was compared after 10 to 20 seconds.

Spurway (1929) used a 2.5% ammonium molybdate solution dissolved in dilute nitric acid (1+3). The solution was allowed to percolate through the soil and a drop of the percolate was stirred with a tin rod.

Dahlberg and Brown (1932) mixed 5 ml. of sodium acetate soil extract with 5 ml. molybdate reagent (0.83% ammonium molybdate in 1.7 N hydrochloric acid) and stirred with a tin rod.

Morgan (1935) added one drop molybdate solution (2.5% sodium molybdate in 10% acetic acid) and 2 drops stannous oxalate (0.005-0.01 g. in 25 ml. (10% sodium

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acetate + 30 ml. acetic acid per litre)) to 10 drops of sodium acetate soil extract. The color was compared after one minute.

In each of these and other similar methods not cited, the color intensity is compared with a standard color chart and at best the results are only very approximate.

(4) Truog and Meyer (1929) were the first to make a thorough systematic study of the ceruleomolybdate reaction and its limitations. They avoided the necessity of preparing a fresh stannous chloride solution daily by dissolving $SnCl_2.2H_2O$ (C.P.) in 10% hydrochloric acid and protecting the solution from the air by floating a layer of mineral oil on the top. The solution was thus preserved indefinitely.

It was found that a concentration of 0.1% ammonium molybdate in the final solution was satisfactory for the development of a maximum blue color from ammonium phosphomolybdate.

At low acidities ammonium molybdate itself gave a blue color on reduction with stannous chloride but when the acidity was increased to 0.35 N in sulphuric acid only very slight traces of blue color were formed from this source. Thus in a standard phosphate solution, the color was too intense at low acidities, due to the reduction of some of the excess ammonium molybdate. At high acidities

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the color was too weak due probably to the incomplete formation of ammonium phosphomolybdate. The color development was shown to be satisfactory at an acidity about 0.35 to 0.40 N in sulphuric acid.

This acidity, 0.40 N sulphuric acid, was found to be sufficient to eliminate interference from silica up to a concentration of 700 p.p.m., preventing entirely the formation of any silicomolybdate which would form at the lower acid concentrations and be reduced to a blue color. The relation of color intensity to phosphate concentration was not independent of the acid concentration but since the same concentration is used in sample and standard, this was of no consequence in a determination. It was necessary to thoroughly mix the acid molybdate with the solution before the stannous chloride was added in order to prevent the reduction of any molybdate which might not have come in contact with the acid solution.

The color developed immediately and was stable for 10 minutes. Addition of another drop of stannous chloride brought back the original color.

Ferric iron was found to interfere above from 4 to 6 p.p.m. while ferrous was without effect. Reduction of ferric iron with cadmium in a Jones reductor was recommended. Titanium over 20 p.p.m. interfered markedly. Aluminium, manganese, calcium, magnesium had no effect at least up to 1000 p.p.m. while nitrates could be present up to 100 p.p.m., a nitrate content above this causing rapid fading.

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Improvements: 1. Introduced stable stannous chloride solution. 2. Eliminated silica interference. 3. Doubled amount of ammonium molybdate (0.05 to 0.1% in final solution) increased acidity from 0.27 to 0.40 N sulphuric acid. This made the test more sensitive especially to small amounts of phosphate.

Reagents: A. 25 g. ammonium molybdate are dissolved in 200 ml. water (60°C.) and filtered. This is added slowly to a cold solution of 280 ml. concentrated sulphuric acid (phosphorus and arsenic free) diluted to 800 ml. After cooling, the solution is diluted to 1000 ml. (this is a 2.5% solution of ammonium molybdate in 10 N sulphuric acid).

B. 25 g. stannous chloride hydrate (SnCl₂.2H₂O) are dissolved in 1000 ml. 10% hydrochloric acid and the solution covered with a 5 mm. layer of mineral oil and stored in a siphon bottle.

Procedure: To the sample solution, which must not be colored or turbid or very acid or alkaline, diluted to 95 ml. is added 4 ml. Reagent A. After shaking, 6 drops Reagent B are added, the mixture shaken and diluted to 100 ml. The color intensity is compared with standards prepared in a similar manner within 10 minutes.

Blank tests using all reagents must give no color. Organic materials may be ignited with magnesium nitrate. The sensitivity of the method is 0.5-0.1 p.p.m. phosphorus with an extreme minimum of 0.02 p.p.m. Chapman (1931) studied the reaction with the use of glass color standards. A Hellige comparator was used with 10 color discs corresponding to phosphate concentrations of from 0.235 to 2.02 p.p.m. so that the accuracy of his readings was not very great.

The effect of temperature on the rate of the development of the color was investigated and the data show that between 5 and 10 minutes after the addition of the reducing agent, the effect is not appreciable for ordinary variations in temperature. Virtually no difference was observed between 24.5° and 29.5°C. although a diminution of from 5 to 10 percent was noted when the temperature was decreased to 19.5°C.

Stannous chloride solution protected by mineral oil was found to become gradually oxidized, a 2.5% solution being found to be about 80% oxidized after 10 months.

The reaction was found to be quite reproducible, at least with sufficient accuracy for ordinary work.

Chapman (1932) studied the effect of varying amounts of stannous chloride. Up to 0.60 mgm. SnCl₂.2H₂O per 100 ml., the color was proportional to the amount of reducing agent added. Additional quantities above this produced definite increases in color intensity but around 7.4 mgm., corresponding to the amount used by Truog and Meyer, considerable variations from this amount effected but minor variations in the intensity of color produced.

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Since ferric iron is usually eliminated by reduction, the effect of ferrous iron was studied. It was found that the color was diminished if the solution was allowed to stand between the final making up to volume and the addition of the reducing agent. The addition of stannous chloride within about a minute was recommended.

The effect of various salts was next studied. Nitric acid above 140 p.p.m. gradually reduced the color intensity but potassium nitrate, sodium nitrate and ammonium nitrate did not interfere up to 1400 p.p.m. (NO₃) and then the color was slightly intensified. Calcium nitrate was without effect up to a concentration of 12,040 p.p.m., Potassium sulphate, magnesium sulphate and ammonium sulphate were found to exert no effect up to 2,400 p.p.m. (SO₄). Chlorides above 350-1700 p.p.m. decreased the color intensity and like nitrates induced rapid fading. Vanadium up to 26 p.p.m. was found not to interfere.

Zinzadze (1935) used a stannous chloride reduction method as well as the molybdenum blue method. A stannous chloride solution (0.08% in $SnCl_2.2H_2O$) and 1% in gum arabic was introduced. This was claimed to stabilize the color to a certain extent but his stannous chloride reagent was unstable. The molybdate reagent was made up from molybdic acid (instead of ammonium molybdate) and the concentration of the final dilution was 0.35 N in sulphuric acid and only contained molybdate the equivalent of 0.053%

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instead of 0.1% as in the Truog and Meyer method. Ferric iron above 28 p.p.m. up to 700 p.p.m. was reduced with sodium bisulphite. The ordinary range of the method was from 0.1 to 3.0 p.p.m. phosphorus in the final dilution. Comparison with the standard was made from 20 minutes to 6 hours after the addition of the reducing agent.

In Table I are shown the proportions of the reagents used in the various methods. The sensitivity (approximate) and time of color comparison are also indicated. TABLE I. Concentration of Reagents Used in the Various Methods

| 2 | Concentration of Reagents at Final Dilution | | | | | | | | | |
|---|--|---|---|---|--|--|--|--|--|--|
| Method | Acid Normality in H ₂ S04 | Ammonium Molybdate % | Reducing Agent % | Time of Reading after addn. of reducing agent: min. | Sensitivity p.p.m. P | | | | | |
| Bell-Doisy Briggs Fiske & Subbarow Tschopp & Tschopp | - 0.5 0.1 | 0.25 0.25 0.25 0.25 0.01% aminaphthol sulphonic acid 0.5 0.01% me | | 5 min. to 1 hour 5-60 15-30 10- | 10-0.1 10-0.1 -1.0 17-1.7 | | | | | |
| Deniges 0.45 Florentin 0.34 Atkins 0.27 Von Wrangel 0.27 Parker & Fudge 0.27 Kuttner & Cohen 1.0 | | 0.125 0.06 0.05 0.075 0.05 0.71 Na2Mo04 -do- | Stannous chloride 0.015 0.0075 0.005 0.015 0.006 0.021 0.03 | 10 10 5 5-60 15 sec. to 2 hours 0-2 hrs. | $ \begin{array}{r} -0.13 \\ -0.04 \\ 2.0-0.04 \\ 0.15-0.04 \\ -0.8 \\ 10-0.3 \\ 9-1 \\ \end{array} $ | | | | | |
| Deniges (Mo blue) Zinzadze (-do-) " (SnCl2) Truog & Meyer | 0.18 0.35 0.35 0.40 | 0.05 0.053 0.053 0.10 | - 0.008 0.0075 | 30 30 5-2 hrs. 5-10 | -0.05 5-0.04 5-0.04 0.5-0.02 | | | | | |
| -do- modified for this investig- ation | 0.40 | 0.10 | 0.0125 | 5 | 0.5-0.02 | | | | | |
| Bray Spurway Dahlberg & Brown Morgan | 0.2 N HCl 4.5 N HNO3 0.8 N HCl 3% Acetic acid | 0.10 2.5 0.4 0.2 Na ₂ MoO ₄ | Sn Sn 0.003% Stannous oxalate | 10 1 min. | 1 | | | | | |

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3. Experimental

From a consideration of the foregoing it is evident that the Truog and Meyer modification of the Deniges ceruleomolybdate method offers the best possibilities. It is the most sensitive method and since the phosphate concentration in some soil extracts is very low, maximum sensitivity is desirable. The method is rapid and does not require heating like the Zinzadze method, which in the acid solution might possibly hydrolyze some of the organic phosphorus compounds of the soil extract.

a. Characteristics of the Reaction

(1) Reagents: 1. Ammonium molybdate-sulphuric acid (2.5% ammonium molybdate in 10 N sulphuric acid) according to Truog and Meyer (1929).

2. 25 g. SnCl₂.2H₂O are dissolved in 100 ml. concentrated hydrochlorid acid, with warming. When solution is complete, it is made up to 1 litre, covered with a 5 mm. layer of mineral oil, and stored in a siphon bottle.

3. Standard phosphate solution, according to Truog and Meyer. The Dilute Standard contains 5 p.p.m. phosphorus.

(2) Absorption Curve: A blue solution was prepared according to the method of Truog and Meyer and the approximate absorption curve determined with the colorimeter by using various light filters.

The results are given in Table 2 and are plotted in Fig. I. The maximum absorption occurs in the red at about 660 millimicrons. Accordingly the 660 filter (which has its maximum absorption at 660, limits from 635 to 720 millimicrons) was considered to be the correct one to use for the reaction.

| BLUG | Absorption Curve |
|--|-------------------------------|
| Wavelength of Filter; millimicrons | Percent Light Transmission |
| 720 | 85.0 |
| 690 | 73.0 |
| 660 | 73.0 |
| 635 | 74.5 |
| 620 | 76.0 |
| 600 | 78.0 |
| 580 | 81.2 |
| 565 | 84.5 |
| 540 | 87.0 |
| 520 | 89.0 |
| 490 | 90.5 |
| 4 40 | 93.0 |
| | |

TABLE 2. Data for Phosphoconjugated Molybdenum



Figure I. Phosphoconjugated Molybdenum Blue Absorption Curve.

(3) Procedure for use with the Evelyn Photoelectric Colorimeter.

The final technique adopted was as follows. Acid molybdate reagent was added to the test solution (4 ml. per 100) and made to volume. A 10 ml. aliquot was transferred to a colorimeter tube and the galvanometer adjusted to read 100, using the 660 filter. A matched set of twelve tubes was used, so that if the solution was colorless the blank setting was constant. If the test solution was colored this method eliminated the color due to the blank so that the final reading gave only the color developed. One drop of stannous chloride was then added, the solution well mixed and the reading again taken at the proper time interval.

This simple procedure was found to eliminate interference in even highly colored solutions.

The slight increase in volume due to the addition of the stannous chloride is the same in the standard and unknown, as is also the small blank reading given (99.0) and so may be neglected.

(4) Effect of Stannous Chloride.

Since it was observed that the addition of more stannous chloride then the prescribed volume intensified the color by a measurable amount, it was decided to investigate this point further. The sensitivity of the used by Chapman that it was felt that his results might not be applicable.

The effect of varying amounts of stannous chloride on the color intensity developed was determined at 3 different concentrations of phosphate covering the ordinary range, 0.1, 0.25, and 0.50 p.p.m. phosphorus, and in a blank. A soil extract was also used in order to be certain that a parallel reaction took place.

The phosphate solution was diluted to about 95 ml., 4 ml. acid molybdate added, the mixture shaken and diluted to 100 ml. 10 ml. portions were measured into the colorimeter tubes and the blank readings checked. The amounts of stannous chloride solution as shown in the table were then added by means of a graduated pipette (1 ml.) and the color intensity read after 5 minutes, the maximum intensity having been shown to be developed at this point. (See next section, "Rate of Reaction".) It was noted that the color was slightly more stable with the larger amounts of stannous chloride. The results are shown in Table 3 and are plotted in Fig. 2.

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| kl. stannous | Percent Transmission of Light | | | | | | | | | | |
|------------------------|-------------------------------|-----------|-------|--------------|---------|--|--|--|--|--|--|
| chloride per 10 ml. | p. p. m | • Phospho | rus | Blank | Soil | | | | | | |
| solution | 0.50 | 0.25 | 0.10 | DWATTK | Extract | | | | | | |
| 0.01 | | | 76.0 | 99.5 | 73.0 | | | | | | |
| 0.02 | 23.0 | 49.0 | 75.5 | 99 .5 | 73.0 | | | | | | |
| 0.031 | 22.7 | 49.0 | 75.0 | 99.1 | 72.7 | | | | | | |
| 0.04 | 22.7 | 48.0 | 74.8 | 99.0 | 72.2 | | | | | | |
| 0.05 ² | 22.7 | 47.75 | 74.6 | 99.0 | 72.0 | | | | | | |
| 0.06 | 21.7 | 47.25 | 74.4 | 98.75 | 71.9 | | | | | | |
| 0.07 | 21.2 | 46.5 | 73.5 | 98 .0 | 71.4 | | | | | | |
| 0.08 | 20.75 | 46.0 | 73.0 | 96.75 | 70.7 | | | | | | |
| 0.09 | 21.0 | 46.0 | 72.75 | 96.5 | 70.0 | | | | | | |
| 0.10 | 20.0 | 45.0 | 72.5 | 97.0 | 69.2 | | | | | | |

TABLE 3. Effect of Stannous Chloride on the Color Intensity

Corresponds to the amount used by Truog and Meyer.
 Corresponds to the amount used in this investigation.

It is evident that the effect of variations in the amount of stannous chloride is not very great near the concentration recommended by Truog and Meyer or that used in this work.

Apparently the higher concentrations of stannous chloride are able to reduce the ammonium molybdate itself, since the blank readings become appreciable above about


Figure 2. Effect of Stannous Chloride on the Color Intensity 0.08 ml. stannous chloride per 10 ml. solution. The effect in the soil extract, a 1-5 water extract of a Macdonald College soil, was similar to the pure phosphate solutions.

(5) Rate of Reaction.

Solutions containing three different concentrations of phosphate were made up and the color intensity observed at different time periods following the addition of stannous chloride. A soil water extract was also used.

The results are given in Table 4 and are plotted in Fig. 3. The values given are the average of four determinations.

| Concentration | Percent Light Transmission at Time Intervals | | | | | | | |
|---------------------------|--|------|-------------|-----------|------------------------|--------------|--------------|------|
| of solution, p.p.m. P. | 2 | 5 | 8 | Min 11 | ites 20 | 3 0 | 40 | 50 |
| 0.1 | 74.5 | 75.0 | 75.0 | 75.0 | 77.0 | 77.5 | | 78.0 |
| 0.25 | 49.0 | 48.6 | 48.4 | 49.1 | 52.1 | 52.9 | 53 .5 | 53.9 |
| 0.5 | 23.9 | 23.5 | 25.1 | 26.0 | 26.5 | 27.0 | 27.5 | 28.0 |
| Soil Extract | 72.8 | 72.0 | 71.9 | 71.9 | 7 3 •7 5 | 75 .7 | 75.85 | 76.0 |

Table 4. Rate of Development of Color Intensity

It is seen that the maximum color development occurs between 5-8 minutes, depending on the concentration. The color fades faster at the higher concentrations and reaches its maximum somewhat sooner.

Since most determinations are made within the range 0.1-0.25 p.p.m. phosphorus, the results indicate a reading time of from 5 to 8 minutes.



(6) Effect of Time of Standing of the Solution after the Addition of Acid Molybdate until the Addition of Stannous Chloride.

Since ferrous ions are known to interfere if the solution is allowed to stand between the addition of the two reagents (Chapman 1932), it was thought advisable to determine this effect on pure solutions and on soil extracts.

100 ml. of the solutions containing acid molybdate were made up and 10 ml. portions removed at the stated time intervals and the color intensity measured about 5 minutes after the addition of stannous chloride. The results obtained are given in Table 5.

| | Percent Light Transmission at Intervals of | | | | | | |
|---|--|---------|---------------|----------|--------|---------|--|
| Solution | 5 min. | 15 min. | 60 min. | 120 min. | 5 hrs. | 24 hrs. | |
| 0.25 p.p.m. P. | 46.5 | 46.5 | 47.0 | 46.7 | 46.0 | 48.5 | |
| 0.11 p.p.m. P. | 70.7 | 70.7 | 70.5 | 71.0 | 71.0 | 72.0 | |
| 0.2 p.p.m. P.+ 5 p.p.m. ferric iron | 56.4 | 56.5 | 56.4 | 56.7 | | | |
| Soil water extract | 70.6 | 70.6 | 70 . 7 | 70.8 | 71.0 | 75.5 | |

TABLE 5. Effect of time of standing between addition of the acid molybdate and stannous chloride reagents

The data show that the solutions were stable for several hours, that ferric iron is without effect and that the soil extract is also stable.

With soil extracts containing large amounts of organic material, it was found advisable to proceed as soon as convenient since it was observed that solutions which had stood for a period had become cloudy rendering the determination somewhat doubtful.

(7) Calibration Curve (and Beer's Law).

Data were obtained for a calibration curve using the procedure outlined above. A suitable aliquot of standard phosphate solution was diluted and made to volume after addition of the acid molybdate reagent. The blank reading was taken on a 10 ml. aliquot in a colorimeter tube, 1 drop (0.05 ml.) stannous chloride reagent was added and the reading made at the maximum color intensity (5 minutes).

The readings given in Table 6 are the average of three determinations.

| Conc. Phosphate, | Percent Ligh G | t Transmission | L = 2-log G | $K_1 = \frac{1}{C}$ |
|---------------------|-------------------|----------------|----------------|---------------------|
| p.p.m. P C | June 15 | July 6 | | |
| 0.50 | - | 22.7 | •643 | 1.29 |
| 0.45 | - | 26.5 | .577 | 1.29 |
| 0.375 | - | 32.25 | .491 | 1.31 |
| 0.25 | 47.0 | 47.0 | • 328 | 1.31 |
| 0.20 | 54.2 | 54.4 | .2650 | 1.32 |
| 0.15 | 63.9 | 63•9 | .1945 | 1.30 |
| 0.125 | 68.0 | 68.2 | .1667 | 1.33 |
| 0.10 | 73.2 | 73.9 | .1337 | 1.33 |
| 0.075 | 80.1 | 80.0 | •0968 | 1.29 |
| 0.05 | 85.2 | 85.9 | .0667 | 1.33 |
| 0.025 | 92.0 | 92.0 | .0362 | 1.44 |

TABLE 6. Calibration Curve Data

L = (2-log G), where G = percent light transmission (galvanometer reading) and

 $K_1 = \frac{1}{K}$, where C = concentration of phosphate(p.p.m. phosphorus) are also tabulated in Table 6.

The constant values for K_1 show that the reaction follows Beer's Law. Omitting the three extreme readings, 0.5, 0.45 and 0.025 p.p.m., $K_1 = 1.315$ (±0.017). The reading at 0.025 p.p.m. is probably not significant since a small error in the reading would result in a relatively large error in the value of K_1 .



The results are plotted in Fig. 4. In the graph also the concentration of phosphorus is plotted against percent light transmission. The straight line relation again shows that the reaction follows Beer's Law.

The readings on July 6 were made using all different reagents from those used on June 15. All values agree within the experimental error so that it is shown that the results are reproducible. Acid molybdate and stannous chloride reagents which had been standing on the laboratory desk for 1 month 4 days still gave the correct reading with standard phosphate solutions.

Since it has been variously stated that Beer's law is not applicable when the method is applied to soil extracts (Ward, 1933, Chapman 1932, Warren and Pugh 1930) the following experiment was carried out.

A large volume of a soil water extract low in phosphate was prepared. 40 ml. aliquots were placed in each of a series of 50 ml. volumetric flasks and known amounts of the standard phosphate solution were added to each. The solutions were made up to volume after adding 2 ml. of acid molybdate, and the color was developed and measured.

The results are given in Table 7.

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| Added Phosphate | Readings | ۱ ۸ ۳ | Found | Increase | Recovery |
|--------------------|------------------------|---------------------|-------|----------|----------|
| 0 | 03.7 94.0 93.5 | 93.75 | 0.020 | - | |
| 0.025 | 87.0 87.0 87.0 | 87.0 | 0.045 | 0.025 | 100.0 |
| 0.050 | 81.0 81.0 80.7 | 80.9 | 0.070 | 0.050 | 100.0 |
| 0.100 | 69.7 70.0 70.0 | 69.9 | 0.120 | 0.100 | 100.0 |
| 0.100 | 69.7 70.0 69.5 | 69.75 | 0.120 | 0.100 | 100.0 |
| 0.150 | 60.5 60.0 | 60.25 | 0.167 | 0.147 | 98.0 |
| 0.200 | 52.0 51.7 51.7 | 51.8 | 0.218 | 0.198 | 99.0 |
| 0.300 | 38.5 37.75 37.75 | 38.0 | 0.320 | 0.300 | 100.0 |
| 0.400 | 28.0 28.0 27.85 | 28.0 | 0.425 | 0.405 | 101.1 |

TABLE 7. Beer's Law in Soil Extracts

That Beer's law is followed is very well shown.

A reason other investigators have not found Beer's law to hold is suggested from an inspection of Fig. 3. It is seen that the color intensity fades faster in the more concentrated solutions and since very little attention has usually been paid to the fading which occurs before 10 minutes (or longer), the readings for the more concentrated solutions would tend to be too low, with the result that the values obtained would show a deviation from Beer's law.

Further, since most reactions follow Beer's law when monochromatic light of the correct wave length is used, the use of light filters also renders it more likely that the values obtained will follow Beer's law.

b. The Effect of Interfering Substances

(1) Ferric Iron

Truog and Meyer (1929) and Chapman (1932) state that ferric iron interferes above 4-6 p.p.m. but are very indefinite on the point.

A solution of ferric chloride in 1 N hydrochloric acid containing 500 p.p.m. iron was diluted to 50 p.p.m. and measured amounts were added to portions of a phosphate solution containing approximately 0.2 p.p.m. phosphorus.

The results are tabulated in Table 8.

The results indicate that ferric iron does not interfere seriously up to about 10 p.p.m. although a small decrease (2%) is noted before this. A high concentration of iron caused the solutions to fade very rapidly.

The greenish tints mentioned by Warren and Pugh (1930) were not observed.

| Ferric Iron | | Percent | p.p.m. P | Deviation | | |
|-------------|----|-------------------|--------------|----------------|------|--|
| p.p.m. | Fe | Transmission | ission Found | | % | |
| 0 | | 55.25 | 0.195 | | | |
| l | | -55.5 | 0.194 | -0.001 | -0.5 | |
| 2 | | 55.9 | 0.191 | -0.004 | -2.0 | |
| 3 | | 56.1 | 0.191 | -0.004 | -2.0 | |
| 4 | | 56.7 | 0.187 | -0.008 | -4.1 | |
| 5 | | 56.4 | 0.189 | -0.006 | -3.1 | |
| 6 | | 56.5 | 0.188 | -0.007 | -3.6 | |
| 7 | | 56.0 | 0.191 | _0 `004 | -2.0 | |
| 8 | | 56.2 | 0.190 | -0.005 | -2.6 | |
| 9 | | 56.7 | 0.187 | -0.008 | -4.1 | |
| 15 | | 55.2 ¹ | 0.195 | 0 | 0 | |
| 20 | | 54.5 ² | 0.198 | +0.003 | +1.5 | |
| 30 | | 54.0 ² | 0.201 | +0.006 | +3.1 | |
| 40 | | 55.0 ² | 0.196 | +0.001 | +0.5 | |

TABLE 8. Effect of Ferric Iron

1 Faded rapidly.

² Faded very rapidly with maximum at about 4 minutes.

(2) Ferrous Iron.

Ferric iron is usually eliminated by reducing it to ferrous iron, which is generally considered not to interfere (Lonstein 1926, Truog and Meyer 1929). Chapman (1932) shows that ferrous ions may reduce the color development if the solution is allowed to stand between the addition of the acid molybdate and reducing reagents. His data were very incomplete so that it was necessary to obtain further data in order to define the extent of the interference. Other workers have not mentioned the point.

A solution of ferrous ammonium sulphate in 0.18N sulphuric acid containing 50 p.p.m. iron was prepared.

For each determination, 25 ml. of a phosphate solution containing approximately 0.4 p.p.m. phosphorus was placed in a 50 ml. volumetric flask, a known amount of ferrous iron solution added and the mixture diluted to volume. For the comparison solutions a similar amount of 0.18 N sulphuric acid was added to the phosphate solution in order to have the same acidity in each. 10 ml. aliquots were placed in colorimeter tubes, 0.4 ml. acid molybdate reagent added, and the blank reading taken. The stannous chloride was added at the desired time interval after the molybdate and the color intensity determined as usual. The results are summarized in Table 9.

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| | | | | ···· |
|---|--|--|--|---|
| Solution | Time Interval Between Addn. of Molybdate & stannous chloride | Light Transmission % | p•p•m• Phosphorus Found | D eviation p.p.m. % |
| Blank, l p.p.m. Fe | 1 min 2 ^H 5 ^H | 99.0 99.0 99.0 | | |
| Phosphate soln. + 1 ml. 0.18N sulphuric acid | | 56.0 | 0.191 | |
| -do- + 1 p.p.m. Fe | 15 sec 30 " 45 " 1 min 2 " 5 " | 56.0 56.8 56.5 57.0 58.0 61.0 | 0.191 0.186 0.187 0.185 0.180 0.162 | 0.0 0 0.005 2.6 0.004 2.1 0.006 3.4 0.011 5.8 0.029 15.2 |
| Phosphate soln. + 2 ml. 0.18 N sulphuric acid | | 56.0 | 0.191 | |
| -do- + 2 p.p.m. Fe | 30 sec 1 min 2 " 5 " | 57.0 57.5 59.0 63.0 | 0.185 0.182 0.173 0.152 | 0.006 3.4 0.009 4.7 0.018 9.4 0.039 20.5 |
| Phosphate soln. + 5 ml. 0.18 N sulphuric acid | l min 2 " 5 " | 57.25 57.5 57.25 | 0.183 | |
| -do- + 5 p.p.m. Fe | 15 sec 1 min 2 " 5 " | 57.2 60.8 64.0 66.75 | 0.182 0.163 0.147 0.132 | 0 0 0.020 11.0 0.036 19.9 0.051 28.2 |
| | 1 | 1 | 1 | 1 |

Even 1 p.p.m. ferrous iron is sufficient to interfere with the color development unless the stannous chloride is added immediately after the acid molybdate. A delay of only 30 seconds gives a reduction of from 2 to 3 percent in the color produced. At 15 seconds no effect could be detected. It is, therefore, evident that it is necessary to add the reagents one immediately (less than 15 seconds) after the other if ferrous iron is present in the sample being determined.

(3) Organic Acids

It is known that citric acid even in fairly small amounts inhibits the normal reaction. In citric acid extracts the acid is usually destroyed by ignition (Lonstein 1926; Belgrave 1928, etc.). Nemec, Lanik and Koppova (1930) oxidized the citric acid by means of potassium permanganate in acid solution. The acetone formed did not interfere with the colorimetric determination. Warner and Pugh (1930) used sodium permanganate, having found that this reagent contained less phosphorus.

Ward (1933) criticized these methods since he found that the reagents contained so much phosphorus that the blank was of the same order of magnitude as the determinations. Accordingly, he used the ignition method. Citric acid also interferes with the precipitation of phosphate as ammonium phosphomolybdate and ignition is the usual method of removing it when this method is used. Arrhenius (1928) proposed a method for the

direct determination in citric acid extracts, using a modification of the Bell-Doisy-Briggs procedure. An aliquot of 2% citric acid extract was made to a final

concentration of reagents, 0.63 N sulphuric acid, 0.25% in ammonium molybdate, and 0.006% in hydroquinone (also 0.2% in sodium sulphite). The mixture was allowed to stand on a water bath 6 hours at 50°C. After 24 hours the color was compared with a standard. This is the only method so far developed for the direct determination of inorganic phosphate in citric acid extracts.

Riehm (1932) and Fort (1933) showed that silica interfered and that if the concentration of citric acid was more than 0.2% in the final solution, low results were obtained.

Davies and Davies (1932) demonstrated that malic and citric acids form molybdate complexes, by means of molecular rotation data. Citric, oxalic, tartaric, malic, lactic and glycollic acids were found to interfere with the Bell-Doisy and Fiske-Subbarow methods at quite low concentrations.

In order to obtain some information of the type and concentration of compounds interfering with the reaction, the following experiments were carried out as shown in Table 10.

The reagents were added to a phosphate solution containing 0.20 p.p.m. phosphorus and the concentration of acid shown (concentration in final dilution given).

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| Acid | Acid conc. | Phosphorus (p.p.m.) found at time Intervals after addition of stannous chloride of | | | | |
|--|--------------------|--|----------------|----------------|--|--|
| | % | 5 min. | 10 min. | 20 min. | | |
| Citric | 0.9 0.1 0.02 | 0 0.03 0 | 0.04 | 0.05 | | |
| Tartaric | 0.2 | 0.052 | | 0.065 | | |
| Acetic, | 0.2 | 0.200 | 0.200 | 0.180 | | |
| Glycollic, | 0.2 | 0.175 | 0.162 | 0.150 | | |
| CH2OHCOOH Oxalic, COOH.COOH | 0.2 | 0 | 0 | 0 | | |
| Trichloracetic CCl ₃ COOH | 0.2 2.5 | 0.200 0.168 | 0.196 0.172 | 0.180 0.162 | | |
| Propionic, CH_CH_COOH | 0.2 | 0.200 | | | | |
| Lactic, CH ₃ CHOHCOOH Hydraacrylic, CH ₂ OHCH ₂ COOH | 0.02 0.4 0.2 | 0.200 0.10 0.200 | 0.125 0.193 | 0.125 0.180 | | |
| Malonic COOHCH2COOH | 0.2 | 0.200 | 0.200 | 0.185 | | |
| Hydroxy malonic COOHCHOHCOOH | 0.2 | 0 | | | | |

TABLE 10. Effect of Organic Acids

The data show that the **a**-hydroxy acids (and oxalic) affect the reaction markedly. The possibility of these occurring in the extracts of normal soils in concentrations sufficient to affect the reaction is remote, however. (4) Organic Matter (Soil Extracts).

It has been generally assumed that more than small amounts of organic matter would interfere with the reaction (Harper, 1933; Ward, 1933). In previous work it has been necessary to have colorless extracts, since even a trace of yellow color renders the determination very uncertain or impossible when an ordinary colorimeter is used. (Parker and Fudge, 1927; Truog and Meyer, 1929). Therefore the effect of organic matter on the reaction has not been investigated. A large number of our soil extracts, particularly water extracts, are quite yellow in color and contain appreciable amounts of soluble organic matter.

Accordingly, the recovery of known amounts of phosphate added to a number of these extracts was determined.

A number of water extracts, containing large amounts of organic matter, were prepared by shaking the soil and water (1-5) on a mechanical shaker for an hour and then filtering through a Buchner funnel with suction, filtering through the soil mat to obtain a clear solution. Inorganic phosphate was then determined directly. To another portion a known amount of phosphate was added and the recovery determined. The results are given in Table 11 a. The extracts (a), (b), (c), were all quite yellow in color.

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| Soln. | Added phosphate p.p.m. P | Phosphate found p.p.m. P | Increase due to added phosphate p.p.m. P | Recovery of added phosphate |
|-------|--------------------------------|---|---|-----------------------------------|
| (a) | - 0.10 | 0.098 0.099 0.197 | 0.0985 | 99.3 |
| (b) | 0.0195 0.039 0.078 | 0.055 0.075 0.094 0.132 | 0.020 0.039 0.077 | 102.0 100.0 98.9 |
| (c) | - 0.05 0.10 0.20 | 0.057 0.057 0.107 0.152 0.252 | 0.050 0.095 0.195 | 100.0 95.0 97.5 |

TABLE 11 a. Effect of Organic Matter (in colored soil extracts).

In Table 11 b is shown the effect when different aliquots are taken for analysis.

| TABLE | 11 | b. | Effe | ct of | Organi | o Matter |
|-------|----|----|------|--------|---------|-------------|
| | | | (in | colore | ed soil | . extracts) |

| Solution | Aliquot used Made up to 50 ml. | Phosphate found p.p.m. P | Conc. in Extract p.p.m. P |
|----------|--------------------------------------|-----------------------------|------------------------------|
| (a) | 10 ml. | 0.098 | 0.490 |
| | 10 ml. | 0.099 | 0.495 |
| | 20 ml. | 0.202 | 0.505 |
| (d) | 20 ml. | 0.023 | 0.057 |
| | 40 ml. | 0.043 | 0.054 |
| (e) | 20 ml. | 0.030 | 0.075 |
| | 40 ml. | 0.062 | 0.077 |

The data show that there was no interference due

B. <u>Application to the Determination of Phosphorus</u> in Soil Extracts

1. Historical

Atkins (1924) first applied the ceruleomolybdate method to soil extracts and it has been widely used since then. The stannous chloride reduction method has been used almost exclusively because of its greater sensitivity than the Fiske and Subbarow and Bell-Doisy methods. It is also more rapid as well as more sensitive than the Zinzadze modification.

Since Atkins, many modifications of the method and manner of preparation of the solutions for analysis have been introduced, with the object of eliminating various interfering substances and conditions.

Lonstein (1926) first mentioned the effect of silica and ferric iron. In citric acid soil extracts, he eliminated them by evaporation of an aliquot of the solution to dryness and ignition of the residue with calcium acetate to render the silica and iron insoluble. Reduction of ferric iron to ferrous was also mentioned.

Parker and Fudge (1927) determined total phosphorus in the extracts by gentle ignition with magnesium nitrate and determined inorganic phosphorus directly.

Deniges (1928) reduced ferric iron in diluted nitric acid extracts, high in iron content, by reducing his molybdenum blue reagent directly in the solution to be tested. The copper reduced the ammonium molybdate to molybdenum blue which reduced the iron. The excess copper then rereduced the molybdenum blue.

Belgrave (1928) was unable to obtain satisfactory results by this method so he introduced the use of zinc and hydrochloric acid, and claimed that good results were then obtained.

Truog and Meyer (1929) advocated the reduction of iron over 4-6 p.p.m. by means of a Jones reductor using metallic cadmium.

Warren and Pugh (1930) compared the Atkins and the Fiske and Subbarow methods on ignited (by Lonstein's method) hydrochloric acid and citric acid soil extracts. They obtained good agreement with the gravimetric except in some heavy soils high in iron. The effect noticed was probably due to the fact that the color comparisons were made at any time up to half an hour and the iron caused the determinations to fade faster than the standards so that low results would have been obtained.

They proposed the method of oxidizing organic matter, and citric acid also, with sodium permanganate in hydrochloric acid solution. Iron was precipitated with potassium ferrocyanide. The excess ferrocyanide was removed by neutralizing the solution with ammonium hydroxide and adding manganous sulphate. They found that the manganous ferrocyanide was quantitatively insoluble at neut-

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rality and did not redissolve when the solution was acidified to pH 3 to dissolve the precipitated phosphate. Silica was found not to interfere and good results were obtained compared with the gravimetric method.

Nemec, Lanik, and Koppova (1932) found that Warren and Pugh's method gave lower values than Nemec's procedure (oxidation with potassium permanganate) and the gravimetric method, and ascribed this to co-precipitation of phosphate with the ferrocyanide precipitate.

Ward (1933) criticized the above oxidation methods on account of the impurity of the reagents and of the difficulty of filtering off the colloidal ferrocyanide precipitate in Warren and Pugh's method. Accordingly, ignition of the citric acid extracts was practised.

His Hawaiian soils were high in titanium and since titanium above 20 p.p.m. interferes with the Deniges reaction (Truog and Meyer 1929), this method could not be used. Precipitation of titanium and iron by sodium hydroxide was impracticable because of occlusion of phosphate in the precipitate. Cupferron was valueless since it was very difficult to destroy the excess reagent. Finally, iron was separated electrolytically, using a mercury cathode in which to collect the deposited iron. The Zinzadze method was used for the determination since it was found not to be affected by titanium up to 150 p.p.m.

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A small deviation from Beer's law was found in soil extracts, so that it was necessary to have a standard of very nearly the same concentration as the unknown.

Hochensmith et al (1933) oxidized organic matter in their potassium carbonate soil extracts by boiling with bromine water. The excess bromine was destroyed with sodium sulphite.

Tischer (1934) recommended the use of potassium cyanide to eliminate ferric iron.

Zinzadze (1935) recommended boiling the solution with sodium bisulphite in acid solution to reduce iron above 28 p.p.m. up to 700 p.p.m. iron.

In the following determinations it was found that iron was seldom present in concentrations above l p.p.m. at the dilution at which the determination was made, so that it was unnecessary to eliminate this element. Also, with the exception of one possible case, organic matter did not interfere.

2. Experimental

a. Description of the Soils

The soils used were picked at random from a number of samples at hand. The podsol samples are all surface layers. A list of the soils is given in Table 12.

-50-

| Soil | No. | |
|------|-----|---|
| 1 | | Brown Forest Soil, Surface Soil, R. Mason, Iron Hill (Brome Lake) |
| 2 | | Muck soil, subsoil, Sherrington-Ste.Clothilde. |
| 3 | | Calciferous Moraine Loam soil, Chateauguay. |
| 4 | | Podsol soil, Chartier, Garthby, untreated. |
| 5 | | Podsol soil, Chartier, Garthby, 500 lbs. super- phosphate per acre. |
| 6 | | Podsol soil, Coates, East Angus, 300 lbs.super- phosphate per acre. |
| 7 | | Podsol soil, Coates, East Angus, 500 lbs. super- phosphate per acre. |
| 8 | | Podsol soil, Coates, East Angus, 700 lbs. super- phosphate per acre. |
| 9 | | Podsol soil, McKay, Sawyerville, untreated. |
| 10 | | Podsol soil, McKay, Sawyerville, 500 lbs. super- phosphate per acre. |

b. Total Phosphorus in Solutions

Since it was desired to determine both inorganic and organic phosphorus in the extracts, a direct determination was made on the extract for inorganic phosphorus, and total phosphorus was determined by ignition with magnesium nitrate as recommended by Parker and Fudge (1927).

To test the method and technique determinations were carried out on several different soil extracts with and without the addition of known amounts of phosphate.

The procedure was as follows:

To an aliquot of the solution in an evaporating dish (boiled in hydrochloric acid) was added 1 ml. N magnesium nitrate solution (phosphorus free), and the solution evaporated to dryness on a steambath and ignited at 550° C. in a muffle furnace until ashed. After cooling, 5 ml. of dilute hydrochloric acid (1-20) was added, the dish covered with a watch glass and heated on a steambath for about two hours, and then evaporated down to a volume of about 1 ml. This is then diluted to volume in a volumetric flask and an aliquot taken for the colorimetric determination.

Results are given in Table 13.

| ومعاليا متاريب بريد فيدخل فتراجع خالوه بالمستوي والتقار ومحدور والتجرب والمتحد والمتعاور والتقار والمتعاور وال | | | | |
|--|----------------------------|--------------------|-----------------------------|----------|
| Solution | Add ed Phosphate | Phosphate found | Increase due to added | Recovery |
| | p.p.m. P | p.p.m. P | phosphorus p.p.m. P | ka |
| | | | | |
| Standard Solution | 0.095 | 0.093 | 0.093 | 98.0 |
| | 0.095 | 0.095 | 0.095 | 100.0 |
| | 0.20 | 0.193 | 0.193 | 96.5 |
| | 0.20 | 0.190 | 0.190 | 95.0 |
| | 0.10 | 0.103 | 0.103 | 103.0 |
| | 0.10 | 0.095 | 0.095 | 95.0 |
| Water Extracts (a) | _ | 0.100 | _ | _ |
| | - | 0.102 | - | _ |
| | 0.200 | 0.296 | 0.195 | 97.5 |
| | 0.200 | 0.298 | 0.197 | 98.5 |
| (h) | _ | 0.195 | _ | |
| (5) | 0.100 | 0.300 | 0.105- | 105.0 |
| | 0.200 | 0.386 | 0.191 | 95.5 |
| haid Extrepts (s) | _ | 0.140 | _ | |
| (Oucher extra) | 1.00 | 1.10 | 0.96 | 96.0 |
| (duener exis.) | - | 0.146 | - | |
| | 1.00 | 1.13 | 0.98 | 98.0 |
| (b) | _ | 0.085 | - | |
| / | 0.200 | 0.279 | 0.194 | 97.0 |
| | - | 0.080 | | 101-0 |
| | 0.200 | 0.200 | 0.208 | I TO7.0 |

TABLE 13. Recovery of Total Phosphorus

The results show that the recovery is satisfactory, considering the low concentration of phosphorus in the solutions.

c. Water Extracts

After considerable experimentation, the following method of obtaining the water extracts was adopted as the most satisfactory.

30 g. of soil (20-mesh) were shaken with 150 ml. distilled water (soil-water ratio, 1-5) for one hour on an end over end shaking machine. The soil-water mixture was then poured on a Buchner funnel. The first few ml. of filtrate were poured back and the remainder filtered through the soil mat, a process requiring 5-15 minutes. To this fairly clear solution 1 ml. of N magnesium nitrate (phosphorus free) was added. After standing a few minutes, the solution was filtered. The magnesium nitrate was used since it was found to be free from phosphorus and had a high flocculating power.

Aliquots of the filtrate were then taken for the determination of inorganic and total phosphorus.

It was found that all the soils examined gave extracts sufficiently colored to prevent the determination being made with an ordinary colorimeter.

In order to be certain that all the inorganic phosphorus was being determined in the extracts and that no interfering substances were present, a known amount of phosphate was added to each solution and the recovery determined.

Iron was determined in the extracts by the aa'dipyridyl method (McFarlane 1936), the iron being reduced with sodium bisulphite.

The results are summarized in Table 14.

TABLE 14. Total and Inorganic Phosphorus in Water Extracts

| Soil No. | Inorg. Phosphorus | | | Recovery of added | Total Phosphorus | | | Fe |
|-------------|-------------------|-------|--------------|----------------------|------------------|----------------|----------------|--------|
| | al | b | Α v . | phosphate % | a | ď | Αν. | p.p.m. |
| 1 | 0.040 | 0.042 | 0.041 | 99.3 | 0.490 | 0.510 | 0.500 | 0.1 |
| 2 | 0.975 | 0.941 | 0.958 | 103.0 | 0 2.40 | (2. 48 | 0 2. 44 | 0.5 |
| 3 | 0.042 | 0.040 | 0.041 | 99.0 | 0.404 | 0.400 | 0.402 | 0.1 |
| 4 | 0.062 | 0.078 | 0.070 | 98.0 | 0.238 | 0.268 | 0.253 | 0.5 |
| 5 | 0.081 | 0.088 | 0.084 | 97.0 | 0.296 | 0.292 | 0.294 | 0.5 |
| 6 | 0.115 | 0.130 | 0.122 | 89.0 ² | 0.712 | 0.736 | 0.724 | 1.0 |
| 7 | 0.165 | 0.150 | 0.158 | 87.82 | 0.878 | Q.786 | 0.832 | 1.0 |
| 8 | 0.204 | 0.210 | 0.207 | 88.8 ² | 0.938 | 0.990 | 0.964 | 1.0 |
| 9 | 0.218 | 0.205 | 0.211 | 97.3 | 0.7 70 | 0.730 | 0.750 | 0.5 |
| 10 | 0.450 | 0.445 | 0.447 | 101.7 | 1.45 | 1.44 | 1.445 | 0.6 |

1 a and b represent duplicate extractions

² These extracts were brown in color and a slight cloudiness was observed on adding the acid molybdate, so that the readings are only approximate, as is evidenced by the slightly low recovery. Very good agreement between duplicate extractions was obtained, some of the duplicates of which were made on different days. Satisfactory recovery of added phosphorus is indicated except for the Coates soil (Nos. 6, 7, 8), the probable reason for which is given in the footnote. Iron was found to be quite low.

The interesting point is the large difference between total and inorganic phosphorus which is presumably due to organic phosphorus compounds.

In Table 15 the organic phosphorus is shown on the basis of the soil extracts.

| Soil | Phosphate e | Organic | | | |
|------|-------------------------|------------|-----------------------|--------------------------------------|--|
| No. | Inorganic Phosphorus | Total P | Organic Phosphorus | Phosphorus as percent of total | |
| 1 | 0.21 | 2.50 | 2.29 | 91.8 | |
| 2 | 4.79 | 12.20 | 7.41 | 60.7 | |
| 3 | 0.205 | 2.01 | 1.805 | 89.8 | |
| 4 | 0.350 | 1.26 | 0.91 | 72.2 | |
| 5 | 0.420 | 1.47 | 1.05 | 71.4 | |
| 6 | 0.610 | 3.62 | 3.01 | 83.1 | |
| 7 | 0.790 | 4.16 | 3.37 | 81.0 | |
| 8 | 1.035 | 4.82 | 3.785 | 78.5 | |
| 9 | 1.05 | 3.75 | 2.70 | 71.9 | |
| 10 | 2.23 | 7.22 | 4.99 | 69.0 | |

TABLE 15. Organic Phosphorus in Water Extracts

d. Acid Extracts

The Deniges method is in general use for the direct determination of inorganic phosphorus in acid extracts of soils (Truog, 1930; Lohse and Rhunke, 1933; Wrenshall and McKibbin, 1935).

In the past, however, there seems to have been no distinction made between the inorganic and total phosphorus content of dilute acid soil extracts. Some authors oxidize organic matter thus determining total phosphorus (Ward, 1933; Warren and Pugh, 1930), while others use the direct method, obtaining only inorganic phosphorus (Truog, 1930, etc.).

It is known that some fertile soils give very low values for inorganic phosphorus. In such cases it was thought possible that the active phosphorus might be present in organic combination and hence not determinable directly in acid extracts. In the present state of our knowledge the availability of the organic phosphorus is unknown.

Most investigators have assumed that dilute acid extracts very little organic phosphorus from the soil. In view of the large amounts removed in the water extracts, it seemed reasonable that dilute acid should also extract a portion of the organic phosphorus. Therefore both inorganic and total phosphorus were determined in the acid extracts cited below. The Quebec method (Wrenshall and McKibbin, 1935) for available soil phosphate was used for the extraction as follows.

2 g. 20 mesh soil was shaken for $\frac{1}{2}$ hour with 400 ml. Quebec solution on an end over end shaking machine. The mixture was filtered immediately. Suitable aliquots were taken for the direct determination of inorganic phosphorus and for total phosphorus (50 ml.) by ignition with magnesium nitrate as above.

A blank test conducted on the Quebec solution showed no phosphate. The results are shown in Table 16.

| Soil No. | Phosphate as p.p.m. Phosphorus of the Extract | | | | | | |
|-------------|---|----------|---------|------------------|---------------|--------|--|
| | Inorg | anic Pho | sphorus | Total Phosphorus | | | |
| | 8 | b | Av. | <u>a</u> | b | Av. | |
| ı | 0.034 | 0.035 | 0.0345 | 0.085 | 0.08 0 | 0.0825 | |
| 2 | 0.106 | 0.110 | 0.108 | 0.140 | 0.146 | 0.143 | |
| 3 | 0.072 | 0.073 | 0.0725 | 0.097 | 0.106 | 0.1015 | |
| 4 | 0.141 | 0.140 | 0.1405 | 0.135 | 0.145 | 0.140 | |
| 5 | 0.214 | 0.221 | 0.2175 | 0.227 | 0.226 | 0.2265 | |
| 6 | 0.066 | 0.065 | 0.0655 | 0.085 | 0.081 | 0.083 | |
| 7 | 0.078 | 0.081 | 0.0795 | 0.100 | 0.098 | 0.099 | |
| 8 | 0.164 | 0.165 | 0.1645 | 0.196 | 0.196 | 0.196 | |
| 9 | 0.280 | 0.254 | 0.267 | 0.277 | 0.262 | 0.2695 | |
| 10 | 0.437 | 0.435 | 0.436 | 0.444 | 0.429 | 0.4365 | |
| | | | l. | | | 1 | |

TABLE 16. Inorganic and Total Phosphorus in Acid Extracts

Recovery of phosphorus added to both direct and total determinations was quantitative in all cases. As with the water extracts, in no case did the iron content pass 1 p.p.m. iron.

The organic phosphorus expressed as p.p.m. of the soil is shown in Table 17.

| Soil No. | Phosphate | Percent Organic | | |
|-------------|-----------|--------------------|---------|-------------------------------------|
| | Inorganic | Total | Organic | Phosphorus of total extracted |
| 1 | 6.9 | 16.5 | 9.6 | 58.2 |
| 2 | 21.6 | 28.6 | 7.0 | 24.5 |
| 3 | 14.5 | 20.3 | 5.8 | 28.6 |
| 4 | 28.1 | 28.0 | nil | nil |
| 5 | 43.5 | 45.3 | 1.8 | 4.0 |
| 6 | 13.1 | 16.6 | 3.5 | 21.1 |
| 7 | 15.9 | 19.8 | 3.9 | 19.7 |
| 8 | 32.9 | 39.2 | 6.3 | 16.1 |
| 9 | 53.4 | 53.9 | 0.5 | 0.9 |
| 10 | 87.2 | 87.3 | 0.1 | 0.1 |
| | | | 1 | |

TABLE 17. Organic Phosphorus in Acid Extracts

The Chartier (4, 5) and McKay (9, 10) soils are the only ones showing no organic phosphorus in the extracts. In the other cases a very considerable proportion is present in the organic form. It is thus very evident that phosphorus determined after oxidation will not always represent inorganic phosphorus as has been heretofore generally assumed.

This factor should be taken into account when citric acid extracts (generally oxidized) are compared with the ordinary extraction methods in which inorganic phosphorus only is usually determined.

e. Total Phosphorus of Soils

Determination of total soil phosphorus by a colorimetric procedure is quite common.

Although a fairly large dilution factor is involved, Warren and Pugh (1930) believe that the method is just as accurate as the ammonium molybdate precipitation method, on account of the relatively large number of manipulations involved and the possibility of incomplete precipitation. At any rate, the saving in time is a large factor since a determination may be made much more rapidly and a much smaller sample may be used. Mather (1929) and Steenkamp (1930) have also published procedures for the colorimetric micro-determination of soil phosphorus.

The soil may be fused as in the ordinary methods and a direct determination made on a suitably diluted aliquot. A suggested procedure using the magnesium nitrate ignition method is as follows:

To 0.5-1 g. of finely ground soil (100 mesh), add 5 ml. 1 N magnesium nitrate solution (proportionally more if the soil contains much organic matter). Mix well and evaporate to dryness on a hot plate. Ignite at a low red heat until organic matter is completely destroyed. Add water to moisten, pulverize and add 10 ml. concentrated hydrochloric acid. Evaporate to dry-Again add 10 ml. concentrated hydrochloric acid, ness. heat on the steambath, finally bring to a boil and filter on a Buchner. Wash well with hot water and dilute to 1000 ml. Take a suitable aliquot (10 ml. for soils containing about 0.1% phosphorus) dilute to about 45 ml. with water, add 2 ml. acid molybdate reagent and dilute to 50 ml. after shaking. The phosphorus is then determined as previously described by the addition of a drop of stannous chloride reagent to 10 ml. in the colorimeter tube and taking the reading.

Total phosphorus was determined on a number of soils by the above method and by the precipitation method (A.O.A.C. I-30).

Unless the soils are very high in iron content, the dilution used will be sufficient to prevent interference by this element.

The results obtained by the two methods are given in Table 18.

-60-

| Soil No. | Total Phosphorus, percent of soil | | | | | |
|------------------------------|-----------------------------------|--------|----------------|---------|--|--|
| | Gravimetric | Av. | Colorimetr | ric Av. | | |
| Macdonal d College | 0.1024 | | 0.100 | | | |
| 7 | 0.1019 | 0.1021 | 0.100 | 0.100 | | |
| ± | 0.0780 | 0.0789 | 0.073 | 0.0725 | | |
| 2 | 0.0946 0.09 90 | 0.0968 | 0.094 0.099 | 0.0965 | | |
| 6 | 0.0782 0.0786 | 0.0784 | 0.078 0.077 | 0.0775 | | |

TABLE 18. Comparison of Total Soil Phosphorus by Precipitation and Colorimetric Methods

It is evident that either method gives results within the experimental error.

III. ORGANIC PHOSPHORUS IN SOILS

It has long been recognized that the soil phosphorus exists in both inorganic and organic combination. Repeated attempts have been made to estimate the quantities of each form which may be present.

A. Historical

Stewart (1910) has reviewed the early literature on the subject of soil organic phosphorus, so that a resume only of such work as relates to the problem at hand will be given here,

Mulder (1844) noted the presence of phosphorus in association with the soil organic material. A considerable number of early workers, notably Grandeau (1872). Eggertz (1889), Wiklund (1891), and later Snyder (1896 and 1897), and Ladd (1898 and 1899), considered that the phosphorus extracted from soil by dilute ammonia was mainly combined in organic form while that contained in the 'matiere noire' precipitated by dilute acids was thought to be certainly in organic combination. Pitsch (1881) and Van Bemmelen (1890) opposed these views. Pitsch showed the solubility of certain mineral phosphates in dilute ammonia, while Van Bemmelen believed that the phosphorus contained in the 'matiere noire' was simply adsorbed phosphorus in the form of a 'colloidalen Humato-Silicat-Komplex'.

Eggertz and Nilson (1889) demonstrated that ignition of a soil markedly increased the amount of phosphorus soluble in dilute acids (2% hydrochloric acid) and attributed this increase to the destruction of the organic matter which formerly held the phosphorus in organic combination.

Schmeeger (1893) confirmed this work of Eggertz and Nilson (using 12% hydrochloric acid) and showed further that heating the soil to 150-160° in an autoclave rendered an approximately equal increase in the dilute acid (12% hydrochloric acid) soluble phosphorus. This indicated the existence of the organic phosphorus as nuclein or some closely allied body, as lecithin was found only in traces. Later work (1897, 1897) substantiated these results.

Aso (1904) confirmed Schmoeger's work finding only 0.049% of lecithin in the soil, with the remainder of the organic phosphorus probably present as nuclein material.

Hopkins and Pettit (1908) proposed a calculation method. They found the mineral composition of the surface and subsoil of same soils to be constant, particularly the potassium content. Therefore they calculated the organic phosphorus from the difference between the phosphorus in the surface soil and subsoil and the ratio of the nitrogen content of the surface and subsoil, the excess phosphorus

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in the surface layer being assumed to be organic.

Stewart (1910) investigated the calculation method and proposed factors for the calculation of the organic phosphorus from the ratio of the nitrogen and carbon contents of the surface and subsoil. He compared the calculated values with results obtained by ammonia extraction, ignition and by the autoclaving procedure of Schmoeger, cited above, and concluded that calculation gave a conservative estimate of the organic phosphorus content of the soil.

Fraps (1911, 1908) made a very critical study of the methods proposed for estimating the organic phosphorus content of soils, and reached the conclusion that no satisfactory method had been advanced. He demonstrated the solubility of inorganic phosphates in ammonia (particularly iron and aluminium compounds such as wavellite and vivianite) and in addition showed that their solubility in acid was increased by ignition. Without actual testing, he considered that autoclaving in acid solution would also increase the acid solubility of some inorganic phosphates. Also he found that the phosphorus dissolved from ignited soils by ammonia was increased by previous treatment with dilute acid. So that the ammonia soluble phosphorus certainly was not all organic. The calculation method was based on too many assumptions to be more than speculative.
J. Stewart (1912) seems to have been the first to apply the magnesia mixture precipitation of Forbes (1910) to the determination of inorganic phosphorus in the alkali extracts of soils. The extraction of the precipitate with the acid-alcohol used by Forbes dissolved so much organic matter that the method was found to be inapplicable.

Shorey (1913) isolated nucleic acid from a number of soils and while his methods gave incomplete extraction, he concluded that a very considerable proportion of the organic phosphorus compounds were comprised of nucleic acids.

Korolev (1911) found a small solubility of organic phosphorus compounds in 12% hydrochloric acid, and that heating caused an increase in the solubility in dilute acids.

Potter and Benton (1916) proposed a method for determining the organic phosphorus content of soils, by the precipitation of the inorganic phosphorus in the alkali extract with magnesia mixture. The precipitate was washed with dilute nitric acid instead of the acid-alcohol recommended by Forbes. The phosphorus was then reprecipitated as the molybdate, dissolved, precipitated by magnesia mixture and then the phosphorus determined by weighing the ignited precipitate of a molybdate precipitation according to the method of Lorenz (1901). The organic phosphorus was given by the difference between the total and the inorganic phosphorus. It was found that N/5 hydrochloric acid did not remove any organic phosphorus, so the soil was first extracted with hydrochloric acid to remove bases, in order that the ammonia would extract the maximum amount of organic material.

Tests showed that centrifuging the ammonia extract removed the clay and other colloid, the phosphorus content of which would otherwise appear as organic phosphorus.

Schollenberger (1918) modified Potter and Benton's method somewhat and made a thorough study of the conditions required for the complete extraction of organic phosphorus and for the determination of inorganic phosphorus.

Known quantities of inorganic phosphate were added to the ammonia extract and a larger amount of magnesia was used to ensure complete precipitation. Extraction with 5% sodium hydroxide (for 2 hours) with preleaching of the soil with 1% hydrochloric acid was considered to be the best procedure. One ammonia extraction removed practically all the organic phosphorus and none could be demonstrated in the acid leachings by the method used. From the fact that added phosphorus is completely recovered and that tests showed clay and colloids to be thoroughly removed by centrifuging, it was concluded that the organic phosphorus indicated was really organic. Evidence was

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presented to show that the phosphorus was not simply inorganic phosphorus adsorbed on humus and organic colloids as believed by Gortner and Shaw (1917). It was concluded that the method gave satisfactory results, while data obtained by the ignition method and the Hopkins and Pettit's method were shown to be erroneous.

Auten (1922 and 1923) critically examined the proposals of Potter and Benton, and of Schollenberger, for determining the organic phosphorus, and concluded that they were sound in principle and gave reliable results. He studied the reactions of the soil organic phosphorus, and concluded that it does not exist to any extent as nucleic acid, phytin, lecithin or pyrimidine nucleotides. Because he was able to condense phosphate by digesting it with sugar and amino acid to form a synthetic "humus," he concluded that it was probably similarly incorporated in the humus of soil, "combined in a calcium magnesium or other metal salt of an organic amphoteric complex."

Parker and Fudge (1927) applied the ceruleomolybdate colorimetric reaction of phosphate ions of Deniges (1920) to the determination of organic phosphorus in soil extracts. Only the inorganic phosphate ion reacts with the reagent so that organic phosphorus may be calculated by subtracting the inorganic phosphorus from the total phosphorus in the solution. It was necessary to have clear and colorless solutions so the method could not be applied

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directly to ammonia extracts. The chief advantage of the method is its sensitivity to amall amounts and its rapidity.

Odynski (1936) revived the ignition method of Schmoeger. He considered the difference in phosphorus extracted by 2 N sulphuric acid from the soil before and after ignition to represent the total organic phosphorus of the soil.

From what has already been said, it is obvious that the results do not represent organic phosphorus.

Wrenshall (1936) has shown that 45-65% of the organic phosphorus indicated in a soil by the Potter and Benton method is recoverable as nucleotides.

B. Experimental and Discussion

From a consideration of the above it is evident that no satisfactory procedure has been developed. The Potter and Benton method is too time consuming to be practical in routine work.

Since considerable organic phosphorus was found in the water and dilute acid extracts of some soils studied, it was obvious that this point should be investigated further. Experiments showed that large amounts of organic phosphorus were extracted by acid from the soils at hand. Plimmer (1913) has shown that organic phosphorus compounds are quite stable to hydrolysis by acid or alkali at ordinary temperatures. Schollenberger (1918) has shown that 4% ammonium hydroxide does not decompose any organic phosphorus compound in the soil extract over a period of 4 days.

So it seemed probable that organic phosphorus would not be hydrolyzed by fairly concentrated hydrochloric acid. Since the amount of inorganic phosphorus dissolved by ammonia from a soil after acid extraction is very small, it was proposed to determine organic phosphorus by the following procedure: First, extraction with 6N hydrochloric acid and washing, then extraction by 5% ammonium hydroxide.

Since our procedure for inorganic phosphorus allowed the determination to be made on colored extracts, inorganic phosphorus was determined directly in the acid extract and in the filtrate from acidification of the ammonia extract. Total phosphorus was determined on both extracts. In the ammonia extract the amount of organic material was so great that it had to be removed by hydrochloric acid precipitation. As shown in the experimental part, the 'humic acid' precipitate was not found to adsorb any appreciable quantity of inorganic phosphorus. The total inorganic phosphorus contained in the ammonia fraction was found to be almost negligible. Results showed that almost as much organic phosphorus was found

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in the acid extract as in the ammonia extract, and that the total phosphorus extracted was over 90 percent of the total contained in the soils examined.

Since Schollenberger (1918) has shown that no more organic phosphorus is obtained by further extraction with ammonia, and since 90 per cent or over of the total soil phosphorus was removed, the remainder in the silicious residue is probably undecomposed mineral phosphate.

The procedure finally adopted was as follows:

To a 1 gm. sample of finely ground soil (100 mesh) contained in a 50 ml. Erlenmeyer flask was added 25 ml. dilute hydrochloric acid (6 N - redistilled) and the contents mixed by rotating the flask . The flask was shaken several times at intervals and allowed to stand overnight. The mixture was then filtered through hardened filter paper, the residue washed thoroughly with distilled water, and the filtrate diluted to volume in a 100 ml. volumetric flask. With soils of a high organic matter content, the organic material sometimes had a tendency to peptize. In much cases the wash water was made very slightly acid with hydrochloric acid.

Inorganic phosphate (A) was determined in the acid extract by direct colorimetry on a suitable aliquot and total phosphorus (B) in another aliquot by ignition with magnesium nitrate as heretofore described. The difference was considered to be organically combined phosphorus (C).

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The residue from the acid extraction was transferred quantitatively to a beaker by washing from the hardened paper with distilled water. 20 ml. concentrated ammonium hydroxide were added and the mixture diluted to the mark in a 100 ml. volumetric flask. It was shaken several times at intervals and allowed to stand overnight. An aliquot of the supernatant liquid was pipetted off and centrifuged to remove suspended and colloidal material which might contain undissolved inorganic phosphorus.

Total phosphorus (E) was determined by magnesium nitrate fusion on one aliquot (10 ml.). Inorganic phosphate (D) could not be determined directly on this extract so the following method was used. An aliquot (10 ml.) was made slightly acid with 6 N hydrochloric acid, added slowly to avoid as much as possible the occlusion of inorganic phosphorus in the precipitate. After filtering and washing thoroughly with distilled water, the filtrate was diluted to volume (100 ml.). Phosphate was determined on this filtrate by direct colorimetry.

The difference (E-D) was taken to represent organic phosphorus (F).

The sum (C+F) of the organic phosphorus contained in the acid and ammonia extracts was taken as the total organic phosphorus content of the soil.

A number of soils were analyzed by the above procedure. The results obtained are given in Table 19.

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| | | - | Extrac | ts | | | |
|-----------|---------|----------------|----------------|----------------|----------------|----------------|------------------------|
| | | Phospl | norus as | mg. pl | iosphorus | s per g. | soil. |
| Soil | | Acid | Extract | | Ammonia | a Extrac | t |
| | | Inorg P | Total P | Org P | Inorg P | Total P | Org P |
| | | A | В | C | D | E | F |
| Brown Ear | rth | | | | | | |
| 1 | a⊥ b | 0.370 0.380 | 0.530 0.540 | 0.160 0.160 | 0.012 | 0.176 0.170 | 0.16 4 0.164 |
| Muck 2 | a b | 0.100 0.099 | 0.490 0.490 | 0.390 0.391 | 0.013 0.017 | 0.466 0.455 | 0.453 0.438 |
| Podsol 6 | a b | 0.317 0.317 | 0.562 | 0.245 | 0.009 | 0.150 0.150 | 0.141 |

TABLE 19. Phosphorus Distribution in Acid and Ammonia

1 a and b represent duplicate extractions.

In Table 20 is given the total organic phosphorus.

| Total Organic Phosphorus | | | | | | | | |
|--------------------------|---|--|--|--|--|--|--|--|
| Phosphorus per g. | % Organic of total s | % Organic of total | | | | | | |
| soil | Extracted | soil | | | | | | |
| mg. | Phosphorus | Phosphorus | | | | | | |
| 0.324 | 45.9 | 39.5 | | | | | | |
| 0.324 | 45.2 | | | | | | | |
| 0.843 0.829 | 88.2 87.8 | 85.9 | | | | | | |
| 0.386 0.386 | 54.2 54.2 | 49.5 | | | | | | |
| | Total 0: Phosphorus per g. soil Mg. 0.324 0.324 0.324 0.843 0.829 0.386 0.386 0.386 | Total Organic Phosphorus PhosphorusPhosphorus% Organic of total Extracted Phosphorus0.32445.90.32445.20.84388.20.82987.80.38654.20.38654.2 | | | | | | |

TABLE 20. Total Organic Phosphorus

These results show that a considerable part of the organic phosphorus in these soils is removed in the acid extract.

In Table 21 is given the total phosphorus in the soil, and the proportion removed by the acid and ammonia extractions.

| Soil | Total Phosphorus Extracted mg. P per g. soil | Total Phosphorus of Soil mg. P per g. soil | % Extraction |
|----------|---|---|-----------------|
| l a b | 0.706 0.716 | 0.789 | 91.1 |
| 2 a b | 0.956 0.945 | 0.970 | 98.0 |
| 3 a b | 0.712 0.712 | 0.780 | 91.2 |

TABLE 21. Proportion of Total Phosphorus Extracted

More drastic treatment, such as fusion, would be necessary to extract completely all the phosphorus in the soil. The completeness of extraction in soil 2, however, shows that cold acid and alkali very probably extract the whole of the organic phosphorus.

A determination of the total phosphorus by magnesium nitrate fusion on the washed residue of a Macdonald College soil (after acid and ammonia extraction) showed that about 6% of the total phosphorus of the soil remained in the silicious residue. This is in harmony with the data in Table 21. The silicious residue remaining after extraction was a whitish grey color. In order to be certain that no interfering substance was present in the extracts which would prevent the inorganic phosphorus from reacting quantitatively, known amounts of phosphorus were added to the extracts and a determination made. 99-100 per cent recovery was obtained in both the acid and ammonia extracts.

In the determination of inorganic phosphorus in the ammonia extract, it might be argued that the "humic acid" precipitate would adsorb some of the inorganic phosphate. To test this point, the precipitate on the filter was redissolved in 10 ml. 5% ammonium hydroxide, by warming and allowing to stand several hours. It was then reprecipitated by 6 N hydrochloric acid and filtered. The precipitate was washed with water and the filtrate diluted to 25 ml. Inorganic phosphorus was then determined directly on the filtrate. The results are given below in Table 22.

| Soil Extract | Reading (Galvanometer) | mg. Phosphorus per g. soil |
|--------------|---------------------------|-------------------------------|
| la | 98 | 0.003 |
| 2 a | 97 | 0.004 |
| 6 த | 98.5 | 0.002 |

TABLE 22. Inorganic Phosphorus adsorbed in 'Humic Acid' precipitate

Since the blank reading itself is 99, the amount of phosphorus present is very small. The values given above represent the maximum limit, and even then amount to less than 0.4% of the total phosphorus in the soil.

In another experiment, the total phosphorus contained in the 'humic acid' precipitate was determined, being the equivalent of 0.1 mg. phosphorus per g. soil. After dissolving and reprecipitating, the total phosphorus was again determined, this time being only 0.05 mg. phosphorus per g. soil. Thus 50% of the total phosphorus remained in the filtrate on reprecipitation. This was as would be expected if the phosphorus was present as an organic compound and not as adsorbed phosphate (Wrenshall 1936).

As a further test, a known amount of phosphorus was added to the ammonia solution before acidifying with hydrochloric acid. The procedure was then carried out as usual. 95 to 100 percent of the added phosphate was recovered (at a concentration of 0.2 p.p.m. phosphorus).

These results strongly indicate that the amount of adsorbed phosphate is small.

As a further test of the method, a sample of a brown forest soil was ignited at about 650° to completely destroy organic matter. It was then extracted with acid and ammonia as for the determination of total organic phosphorus. The results obtained are given in Table 23.

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| TABLE | 23. | Organic Pl | nosphorus | in | Igni | ited | Soil |
|-------|-----|------------|-----------|-----|------|------|-------|
| | | (Mg. | Phosphon | rus | per | g. | soil) |

| | 1 | Acid Extra | act | Аг | Total | | |
|---|------------|----------------|-------------------------------|------------|------------------------|-------------------------------|-----------------|
| | Total P | Inorganic P | Difference ='Organic' P | Total P | Inorga nic P | Difference ='Organic' P | 'Organic' P |
| a | 0.8375 | 0.8375 | + 0 0019 | 0.0400 | 0.0375 | + 0 0019 | + 0 0024 |
| Ъ | 0.8400 | 0.8375 | + 0.0012 | 0.0375 | 0.0375 | | ≁ 0.0024 |

In the extracts analyzed above, determinations of ferric iron indicated that the concentration would be of the order of 1 p.p.m. or less, at the final dilution at which the determinations were made - considerably below the concentration necessary to vitiate the results.

IV. SUMMARY

The ceruleomolybdate reaction has been studied and the procedure for the determination of phosphorus has been improved by the application of the photoelectric colorimeter. Reproducibility of the results has been obtained and a method capable of giving high accuracy has been developed.

The effect of stannous chloride and the rate of the reaction have been defined much more accurately than before. It has been shown that the maximum color intensity is developed at about 5 minutes after the addition of the reducing agent.

Both ferric and ferrous iron have been shown to interfere with the reaction; ferric iron causes rapid fading at concentrations exceeding 6-10 p.p.m.; ferrous iron diminishes the color development if the stannous chloride reagent is not added immediately after the acid molybdate reagent.

The use of light of a definite portion of the spectrum, approximating monochromatic light, has shown that Beer's law is followed by the reaction, both in pure solutions and in soil extracts.

It has been shown that the reaction is strictly applicable to the analysis of soil extracts of various kinds, yielding results of the most quantitative nature.

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The determination may be carried out even in quite highly colored extracts without any impairment of accuracy. The colorimetric method as applied is not a method of approximation but one in which the highest quantitative accuracy may be obtained, the results being fully as satisfactory as the ordinary gravimetric or volumetric methods.

Organic and inorganic phosphorus in solutions, whether colored or not, can be determined, and a practical method for the determination of the total organic phosphorus of the soil has been worked out. Previous to/this time no satisfactory method has been offered for general use.

The soil is extracted first with cold 6 N hydrochloric acid and second with 5 percent ammonium hydroxide. The acid extract contains a large proportion of organic phosphorus and the sum of the organic phosphorus in the acid and alkaline extracts gives the total organic phosphorus contained in the soil.

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