

THE COLORIMETRIC DETERMINATION OF PHOSPHORUS

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By

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Bachelor of Science

Oklahoma Agricultural and Mechanical College

1940

Submitted to the Department of Chemical Engineering

Oklahoma Agricultural and Mechanical College

In Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

1942

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TO VENORA,  
My Wife and Sweetheart

## PREFACE

With the recent advent of very accurate and sensitive photoelectric equipment colorimetry has taken its proper place along with gravimetric and volumetric methods of chemical analysis. Colorimetry has very distinct advantages over other methods when dealing with small quantities of material or when estimating substances that represent only a very small fraction of the parent material. It is not at all unusual to find colorimetric procedures that will detect as little as one billionth of an ounce of the element or compound in question—a quantity no balance in existence could possibly weigh and one that only the most refined volumetric procedure could hope to cope with.

Phosphorus is an element that lends itself well to such colorimetric methods of analysis. The element was discovered by the Alchemist Brand, of Hasburg, Germany in the year 1669 while he was searching for the philosophers stone. Owing to its peculiar properties and the secrecy which was maintained about its preparation it remained a very rare and costly substance until the demand for it in the manufacture of matches brought about its production on a large scale. The determination of this element is required in a large number of instances since it is widely distributed in nature as the phosphate. As calcium phosphate it occurs in all fertile soils and the lack of it causes plants to be stunted. Calcium phosphate is also the chief mineral constituent of the bones of animals and is a necessary requisite to normal healthy growth. The chemist is especially concerned in the determin-

ation of phosphate for the evaluation of materials used in manufacturing phosphoric acid and phosphate fertilizers. In this case, however, the phosphorus content is usually quite high and a volumetric or gravimetric method for its determination is suitable. Another special concern of the chemist is the determination of phosphorus in such materials as soil or iron and steel. In these materials the phosphorus content is quite low--rarely exceeding one half of one percent. Phosphorus is present in all kinds of iron and steel and usually in the alloys used in steel making. Generally, it is an undesirable constituent likely to cause brittleness and the permissible quantities are rigidly defined in steel specifications. In most steels the phosphorus content lies between 0.01 and 0.1 percent and that of wrought iron or pig iron between 0.1 and 0.3 percent. Such small quantities as these are most readily and accurately detected by colorimetric procedures.

Weldon Wiley Shelton

Stillwater, Minn.

June, 1942

## ACKNOWLEDGEMENT

The author owes a great debt of gratitude, which cannot be adequately acknowledged, to Dr. Horace J. Harper, who, by extending financial aid thru a laboratory assistantship, made a year of graduate work and this research possible. Thanks are also due to Mr. Charles O. Geyer, Chief Chemist of the Inland Steel Company, for furnishing a number of analyzed steel samples.

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## THE COLORIMETRIC DETERMINATION OF PHOSPHORUS

### Introduction

A critical study of several colorimetric methods recommended for the determination of small quantities of phosphorus was undertaken to develop a rapid procedure of general applicability which might be used instead of the somewhat long and tedious volumetric and gravimetric methods now in general use. The first investigations were carried out under the direction of the Oklahoma Agricultural and Mechanical College Agronomy Department to determine the feasibility of using some such method for the estimation of phosphorus in soil. The original procedure developed has now been refined and modified in certain details so as to apply to the determination of phosphorus in iron and steel, forage or grain, phosphate fertilizers and phosphate rocks. Although it has not been tried, it is believed that the method might be suitable for the determination of phosphorus in phosphate baking powders, blood, serum, plasma and other biological materials.

Once a reaction has been discovered which gives a colored solution with a given element or compound, the entire problem of using this reaction for a quantitative measure of the substance resolves itself into obtaining consistent color

values for a given amount of the substance in solutions which may vary considerably in chemical composition. This basic problem may be broken down into the following principal subproblems:

- (1) Establishing the ideal conditions for color development,
- (2) Determining the tolerable limits of foreign substances which may adversely influence the desired color,
- (3) Developing a procedure which will insure the proper color values under all conditions, or to specify limiting condition,
- (4) To make accurate comparisons of the colors of unknowns with solutions of known concentration.

For this method of analysis a small amount of sodium or ammonium molybdate is added to a phosphate solution of specified pH. Then on the addition of certain reducing agents the solution changes from colorless to blue, the intensity of color being proportional to the amount of phosphorus present in the solution. The exact chemical reaction involved in producing the blue color is not known. Presumably there is no fixed stoichiometric reaction since different reducing agents produce different colors or intensities of color while the same reducing agent gives variable color intensities at various pH values.

Bucholz is credited with the discovery of this reaction in 1833 when he trititated molybdic oxide and powdered molybdenum metal together in water suspension. In 1867 Osmond(22) applied this reaction to the colorimetric determination of phosphorus by reduction of the

phosphomolybdate with stannous chloride. Deniges (6) and Troug and Meyer (30) made improvements of the method. Subsequent investigations have yielded a variety of data and opinions as to the nature of this so called "molybdenum blue". According to Schrieker and Dawson (26), the name, "molybdenum blue", properly applies only to that complex oxide or series of oxides of molybdenum lying between  $\text{Mo}_2\text{O}_5$  and  $\text{MoO}_3$ . They also state that whether it is a single oxide or series of oxides has not been fully established, nor does the evidence justify the assignment of a more specific composition than that expressed in the empirical formula  $\text{Mo}_n\text{O}_{3n-1}$  for the unstable anhydrous oxide and  $(\text{Mo}_n\text{O}_{3n-1})_2\text{H}_2\text{PO}_4$  for its stable phosphoric acid complex.

Regardless of the nature of the complex its relative stability in combination with certain substances such as phosphoric, arsenic and silicic acids, and its instability in their absence has led to its wide use in colorimetry for the determination of these substances.

Berenblum and Chaim (5) believe the mechanism of this reaction to be catalytic, an assumption originally proposed by Feigl (1922). This seems to be incompatible, however, because a fixed amount of phosphorus gives a limited color density. No such limit should exist unless, as suggested by Krumholz (1933) the catalyst is made ineffective during the course of the reaction. Berenblum offers the following experiment in support of this theory. The blue compound produced by reduction of phosphomolybdate with stannous chloride was destroyed by titration with potassium permanganate. On the addition of more stannous chloride the

original intensity of blue color appeared. From this he summarizes that the theory of catalytic action has been confirmed and concludes that definite end points are reached because the product of the reaction inactivates the catalyst. If this view is held the definition of a catalyst must be very broad indeed. A much more reasonable mechanism is to assume that the phosphorus combines with the molybdate and that the phosphomolybdate complex formed has a lower oxidation-reduction potential than the molybdate itself. An equilibrium ratio of the amount of molybdate combined with the phosphorus, which would shift with a change in pH, would account for the variable color densities produced by the same reducing agent at different pH values.

Many colorimetric procedures using a great variety of reducing agents have been proposed for the determination of phosphorus in biological material. Information concerning several of these methods may be obtained from "Colorimetric Methods of Analysis" by Snell and Snell (29) or from "Photometric Chemical Analysis" by Yoe (35). Apparently the methods now in most general use in the United States are those proposed by Fiske and Subbarow (10) and the Briggs (6) modification of the Bell-Doisy (2) method. The method of Kuttner and Cohen (17) has gained wide recognition in Europe. Fiske and Subbarow carried out the reduction in strong sulfuric acid (0.5N) and used a solution of 1-amino-2-naphthol-4-sulfonic acid as the reducing agent in a sulfite-bisulfite mixture. The principal advantages claimed were, (1) rapidity of color development, (2) proportionality between color

density and the amount of phosphorus present over a wide range, and (3) relative freedom from interference by such substances as ammonium and ferric iron salts, nitrates, silicates and chlorides.

Yoe gives four colorimetric methods suitable for the determination of phosphorus in steel. In one of these, Riegler's method (24), the usual gravimetric procedure is followed up to and including the precipitation as ammonium phosphomolybdate--after washing, the precipitate is dissolved and the blue color developed using hydrazine sulfate as reducing agent. In this procedure all the molybdenum present is reduced and not just that in combination with the phosphorus. Consequently very thorough washing of the precipitate is essential. In the other three methods given the colors are developed in the presence of the dissolved iron but the colors produced by these methods are yellows or yellowish reds which do not lend themselves to accurate comparison because relatively large changes in the portion of the spectra absorbed are necessary to appreciably affect either visual or photoelectric colorimeters.

#### INFLUENCE OF FOREIGN SUBSTANCES ON COLOR DEVELOPMENT

Many substances have been found to interfere with proper color development in the various methods proposed. The maximum tolerable limits of such substances varies with the acidity of the solution, the amount of phosphorus present, the amount of molybdate present, the reducing agent used and probably with the type of acid used. Table 1 shows Allens' (1) values of permissible concentrations for several com-

TABLE 1

Allen's values of Permissible Concentrations of Some Interfering Substances When Using Amidol as Reducing Agent in Perchloric Acid Solutions

Substance	<u>Permissible Molar Concentrations in Presence of-</u>	
	<u>0.4 mg. of Phosphorus</u>	<u>0.01 mg. of Phosphorus</u>
$(\text{NH}_4)_2\text{SO}_4$	0.4	0.75
NaCl	0.5	0.6
$\text{NaNO}_3$	0.5	0.8
$\text{CCl}_3\text{COOH}$	0.25	0.5
NaF	0.01	0.02
$\text{K}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$	0.002	0.004
$\text{FeCl}_3$	0.0013	0.0013
$\text{C}_2\text{H}_5\text{OH}$	1.8	1.8

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pounds at two concentrations of phosphorus when using ascorbic acid as the reducing agent in perchloric acid solutions. He does not give the error produced by greater quantities than those given in the table nor does he state whether positive or negative errors result from such substances being present. Although by no means comprehensive this table may be considered indicative of the types of substances likely to interfere in the methods proposed. Usually such interfering substances may be classified into five types, namely:

- (1) Substances which cause a change in pH, thereby shifting the equilibrium between total and easily reducible molybdate, e.g. acids, alkalis and buffers.
- (2) Substances which form complexes with the molybdenum thereby preventing the formation of phosphomolybdate with the molybdenum thus tied up, e.g. fluorides, citrates, tartrates, oxalates, etc;
- (3) Substances which destroy, precipitate, form complexes with, or otherwise prevent the action of the reducing agent on the phosphomolybdate, e.g. oxidizing agents, etc;
- (4) Substances which give colored solutions under the conditions used for reduction of the phosphomolybdate e.g. chromates, etc;
- (5) Substances which form compounds similar to the phosphomolybdate thereby causing an increase in the molybdenum blue color, e.g. arsenic acid(arsenomolybdate), silicic acid(silicomolybdate), germanium(germanomolybdate) etc;

For the procedure recommended only substances of types four and five are likely to interfere. All organic substances being oxidized and all acids boiling below 200°C, the boiling point of perchloric acid, being volatilized in the digestion. Willard and Cake (33) found that silica was quickly dehydrated

when boiled in concentrated (70 to 72%) perchloric acid for a short period of time. Thus, silica may be completely eliminated and does not interfere with color development. Also it is not necessary to evaporate the solution to dryness to eliminate the silica as is necessary when using hydrochloric acid. King (15) first proposed the use of this acid in place of sulfuric acid in the Fiske and Subbarow method. Volk and Jones (32) have recommended it for the determination of phosphorus in soils. Hillebrand and Lundell (14) report that phosphoric acid was lost when digested with sulfuric acid at temperatures ranging from 200°C to 260°C in an open dish. The author has confirmed Giesekings (11) statement that no loss occurs on digesting a mixture of perchloric and phosphoric acids.

For the analysis of forage, grain and soil, none of the five types of substances listed above are likely to cause interferences. Most of the methods that have been proposed place a limit on the amount of ferric iron that may be in the solutions at the time of color development. The data of Table 2 shows that ferric iron has very little influence on the recovery of phosphorus by the recommended procedure. In rare cases enough arsenic might be present to seriously influence results when analyzing phosphate rocks and fertilizers although this would be unusual.

In the analysis of steel the only three elements likely to interfere are arsenic, vanadium and chromium, and the latter two only in the case of chrome or vanadium steels. Table 9 shows the percent phosphorus in several steel samples as found by the recommended procedure compared to that of other methods. The first nine samples are Bureau of Standards

TABLE 2

The Effect of Ferric Iron on the Recovery of Known Quantities of Phosphorus as Determined by the Recommended Procedure.

mg. of phosphorus added	mg. of Ferric Iron added	mg. of Phosphorus Recovered
0	0	.020
.10	0	.100
.20	0	.200
.40	0	.400
.50	0	.500
0	10	0
.04	10	.041
.10	10	.106
.20	10	.200
.40	10	.404
.50	10	.500
0	25	.002
.04	25	.040
.10	25	.111
.20	25	.203
.40	25	.404
.50	25	.504
0	50	.006
.04	50	.041
.10	50	.105
.20	50	.209
.40	50	.406
.50	50	.406
0	500	.013
.04	500	.052
.10	500	.113
.20	500	.215
.40	500	.420
.50	500	.520

samples and show the complete analysis as given by the Bureau. It will be observed that all these values agree closely except in the case of sample No. 2 which is high in arsenic, chromium and vanadium. This error has been traced to the vanadium and consequently the method is not applicable to vanadium steels without previous removal of this element. Arsenic, in the amounts found in any but special steels, does not interfere. One percent chromium in the steel samples produces a positive error amounting to about 0.002 percent phosphorus. Therefore, it is recommended that steels containing more than one percent chromium be analyzed by the volumetric procedure.

#### CHOICE OF REDUCING AGENT

Many reducing agents which have been proposed for the determination of phosphorus by the reduction of phosphomolybdate give inaccurate results in the presence of ferric iron. Troug and Meyer (30) recommend that solutions containing more than 6ppm (parts per million) of ferric iron be filtered thru a Jones reductor and the blue color then developed with stannous chloride. This reducing agent gives deep blue colors and would be ideal if the colors did not fade so rapidly. Also a precipitate of metastannic acid forms rapidly in solutions high in phosphorus. Kuttner and Cohen (17) have also recommended the use of stannous chloride, the principal advantages claimed being intensity of color produced and the almost instantaneous development of the color. They state that this reducing agent gives color densities twelve to fifteen times

TABLE 3

A Partial List of Reducing agents that have been proposed or tried for the reduction of phosphomolybdic acid

Reducing Agent	color produced	Reference
Amidol	Blue	(1)
1, 2, 4 Aminonaphtholsulfonic acid	Blue	(10)
Benzidine	Blue	
*Formaldehyde	Blue	
*Hydrazine Sulfate	Blue	(24,28)
Hydriodic Acid	?	(34)
*Hydrogen Sulfide	Yellowish-Red	(13)
*Hydroquinone	Blue in Alkali Green in acid	(2, 6)
*Hydroquinone in Bisulfite	Blue	(6)
*p-methylaminophenol sulfate(Elon)	Blue	
*Phenylhydrazine	Blue	
Quinol	Blue	(6)
*Sodium Thiosulfate	Blue	(19)
*Stannous Chloride	Blue	(3,8,16,17,22,30,36)
*Titanous Chloride	Blue	
Unstable Molybdenum Blue	Blue	(36)

\*Indicates reagents tried during this investigation.

the depth of those obtained by the Bell-Doisy or Fiske and Subbarrow methods. In the Briggs modification of the Bell-Doisy method hydroquinone is used as the reducing agent in a sulfite-bisulfite mixture.

Table 3 gives a list of reducing agents that have been proposed, the color produced, and if known, references to articles recommending their use. Those reagents tried during the course of this investigation are indicated by an asterisk. A comparison of the various available reducing agents indicated that hydrazine sulfate gave the most reproducible color intensities with solutions having varied chemical compositions. This reagent gives deep blue colors comparable to those formed in the presence of stannous chloride and does not have the objectionable features of the latter reagent. About the only objectionable feature of hydrazine sulfate is its inability to completely and rapidly reduce phosphomolybdic acid in the cold and therefore the solution must be boiled in order to obtain consistent color values within a reasonable length of time.

#### INFLUENCE OF pH AND MOLYBDATE CONCENTRATION

In slightly acid solutions many reducing agents, including hydrazine, will give the typical molybdenum blue color even when no phosphorus is present. As the pH value of the solution is decreased the molybdenum blue color decreases and finally disappears entirely. The pH at which complete disappearance of the blue color occurs depends on the amount of molybdate in the solution and the reducing agent used. In a solution

containing 0.07 to 0.08 percent sodium molybdate, the pH value at which no blue color is obtained using hydrazine (and in the absence of phosphorus) is about 1.0. If phosphorus is added to such a solution the blue color will appear and the intensity of color is proportional to the amount of phosphorus added. If the pH is now decreased the color density will remain constant over a short range and then begin to decrease, a pH value eventually being obtained at which no color will form even in the presence of phosphorus. These relations are shown in tables 4 and 5, and graphically in figure 1.

The color densities obtained using hydrazine sulfate were approximately the same for hydrochloric, sulfuric and perchloric acid solutions having the same pH value. Maximum color density appeared in a slightly higher pH range in the presence of sulfuric acid; no color was obtained at the lower pH values shown while colors did appear in solutions of similar pH containing hydrochloric or perchloric acid. No appreciable difference was observed between the hydrochloric and perchloric acid solutions. If much iron is present in the solutions to be analyzed undesirable yellows are obtained in the presence of hydrochloric acid; consequently perchloric acid is recommended in all cases where iron is likely to interfere. Hydrochloric acid may be substituted for perchloric acid in the analysis of forage after destruction of the organic matter by combustion.

If the solution contains considerably more or less than

TABLE 4

The Effect of Adding Varying Quantities of Different Acids on the Color Density of Molybdenum Blue Produced by the Reduction of Phosphomolybdate with Hydrazine Sulfate in the Presence of 0.2 mg. of Phosphorus and 5 ml. of 2%  $\text{Na}_2\text{MoO}_4$ --Final Volume 200 ml.

pH value of $\text{H}_2\text{SO}_4$ solution	Electrophotometer reading	pH value of HCl Solutions	Electrophotometer reading	pH value of $\text{HClO}_4$ Solutions	Electrophotometer reading
1.29	37.6	1.34	37.6	1.30	38.7
1.17	37.6	1.20	36.5	1.15	34.4
1.07	37.6	1.08	35.5	1.05	35.5
1.00	37.6	1.00	35.5	0.96	35.5
0.92	36.5	0.92	35.5	0.90	35.5
.87	27.7	0.86	35.5	0.84	35.5
.84	16.5	0.82	34.4	0.80	36.5
.75	*	0.74	22.9	0.72	32.1
.69	*	0.68	11.0	0.66	23.9

\* No Blue Color Developed in these Solutions

TABLE 5

The Effect of pH Value on the Reduction of Molybdic Acid  
(5 ml. of 2%  $\text{Na}_2\text{MoO}_4$  added) in the absence of Phosphorus  
at 150 ml. Volume---Final Dilution 200 ml.

pH value	1.40	1.21	1.12	0.98	0.90	0.82	0.60
Electro- photometer Reading	100+	75.1	21.2	11.0	3.0	2.9	3.0

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150 ml. Volume at Time of Color Development  
Final Dilution-200 ml.  
(Green Filter)

0.125 mg. of Phosphorus

No Phosphorus

### Normality of Solution

0.07 to 0.02 percent sodium molybdate the same type of curve as shown in Figure 1 is obtained but the entire curve is shifted to a higher or lower acidity. A family of curves such as this may be obtained by varying the concentration of molybdate. This indicates that the critical factor in obtaining consistent color values for a fixed amount of phosphorus is the acid-molybdate ratio rather than their independent concentrations. Apparently this fact has not been recognized by some investigators as considerable differences of opinion regarding the ideal acidity for proper color development is to be found in the literature.

The data of tables 4 and 5 show that when using a solution about 0.07 percent in sodium molybdate the pH should be between 0.8 and 1.0 for proper color development in the presence of perchloric acid. For the practical chemist a more reasonable method of stating this is to say the solution should be about 0.12 to 0.3 normal. This is shown in Figure 1 where the normality of the solutions (and the ml. of 70-72% perchloric acid necessary to produce this normality) at the time of color development is plotted against the readings of the electrophotometer used for measuring the color intensity. On this instrument the greater the reading the more intense the color value.

Five ml. of a two percent solution of sodium molybdate in the presence of 0.6 mg. of phosphorus will give a deep blue color- too deep to be compared visually and near the upper limit of the usual type of photoelectric colorimeter. Therefore this quantity of molybdate is sufficient to combine with any

amount of phosphorus that can be conveniently compared by the method proposed. Six ml. of a two percent solution of sodium molybdate will not produce a blue color in the presence of hydrazine sulfate at a total volume of 150 ml. if the pH of the solution is below one. Consequently, the molybdate may be added from a pipette or burette, keeping the volume as near five ml. as is convenient in routine analysis.

When hydrazine sulfate is added to a solution of proper pH, containing no phosphorus, a pale yellow color is produced on boiling. This yellow color is probably due to an intermediate reduction product of molybdenum as the depth of this color is increased appreciably by an increase in molybdate while very little increase in color is produced by the addition of more hydrazine sulfate. This intermediate reduction compound does not combine with phosphoric acid. If the molybdate solution is first boiled with the hydrazine sulfate, and the phosphate added later, no blue color will appear while if the phosphate is boiled with the reducing agent and the molybdate solution then added, the usual blue color appears immediately. This yellow color will interfere with the determination of small quantities of phosphorus (0.01mg or less) if color comparisons are made visually, because various shades of green are obtained before the blue color produced by increasing quantities of phosphate will mask the effect of the yellow color. When using a photoelectric colorimeter the yellow color does not interfere since this type of instrument measures the quantity of light absorbed by the solution and not the shade or intensity of color which may be present.

## THE COLORIMETER AND ITS CALIBRATION

During the course of these investigations two makes of photoelectric colorimeters have been used. In the earlier work a Cenco-Sheard-Sanford Photolometer was used and later an AF Model Fisher Electrophotometer. The latter instrument is superior in many respects to the older Cenco apparatus; consequently the Fisher instrument has been used whenever possible. All data obtained using the Cenco instrument has been revised in terms of the Fisher instrument so as to make the tables and graphs comparable.

The standard phosphate solutions used to calibrate the electrophotometer were made from recrystallized potassium dihydrogen phosphate. To these solutions was added 2½ ml. of concentrated perchloric acid (70-72%  $\text{HClO}_4$ ), 5 ml. of a 2 percent solution of sodium molybdate ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ) and 3 ml. of a 5 percent solution of hydrazine sulfate. The solutions were boiled at a volume of 150 ml. for one minute, allowed to cool overnight, diluted to 200 ml. and the electrophotometer readings obtained approximately eighteen hours after color development. The readings found using various filters are given in table 6 and these readings are plotted against the phosphorus content of the solutions in Figure 2. The scale of the electrophotometer has logarithmic graduations and the straight lines obtained in Figure 2 indicates that the molybdenum blue solutions obey the Lambert-Beer law.\* It will be observed that the point representing zero phosphorus

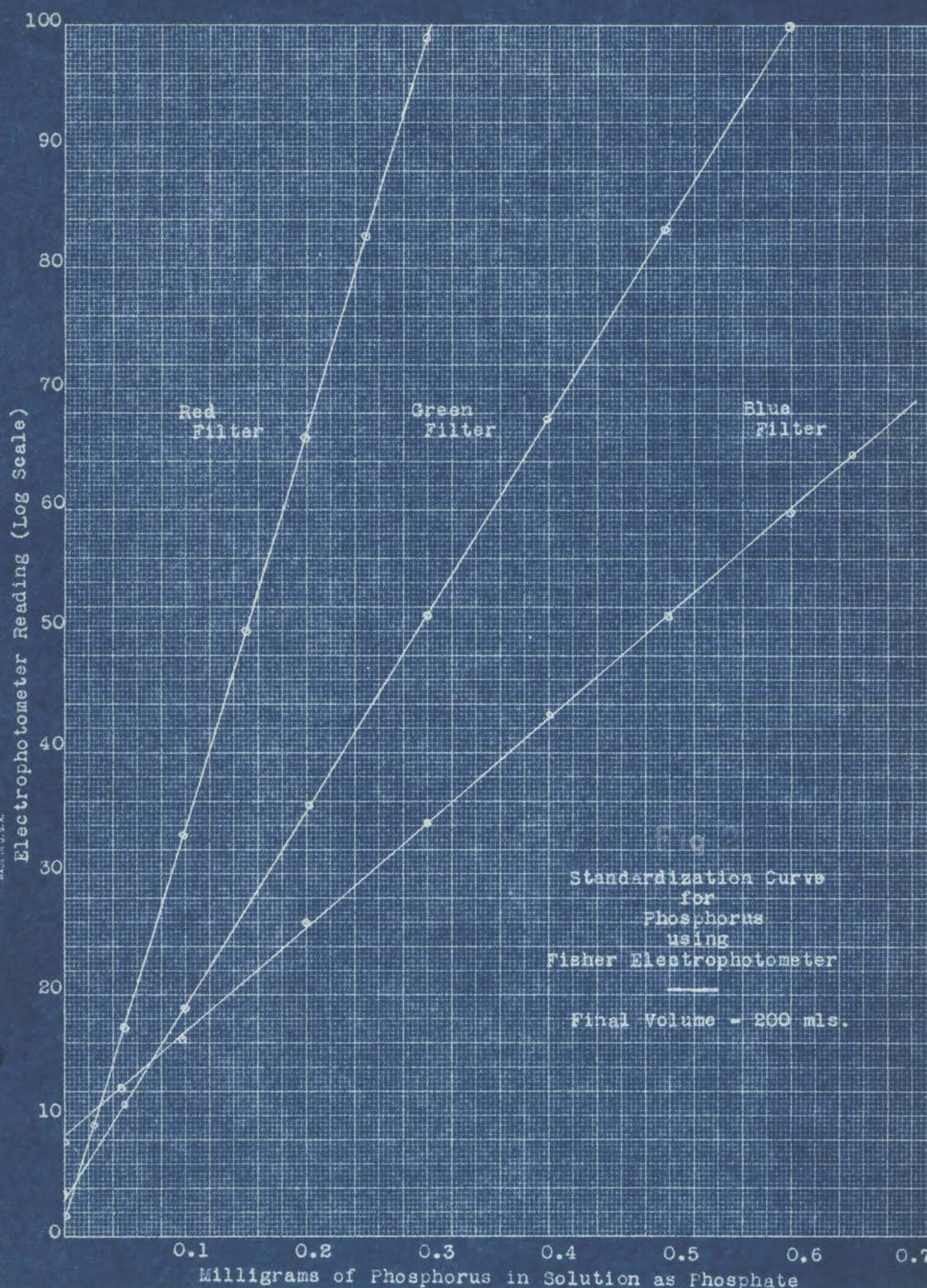
\*See any good text on Physical Chemistry for a statement of the Lambert-Beer Law

TABLE 6

Electrophotometer Readings of Standard Phosphate Solutions Using Hydrazine Sulfate as Reducing Agent in the Presence of 5 ml. of 2% Sodium Molybdate and  $2\frac{1}{2}$  ml. of 70-72% Perchloric Acid--  
Final Volume 200 ml.

Mg. of Phosphorus in Standard Solutions	Electrophotometer Readings		
	Red Filter	Green Filter	Blue Filter
.000	1.0	2.9	7.8
.025	8.6	7.0	10.0
.050	17.1	11.0	11.9
.100	33.2	19.1	16.1
.150	49.9	28.0	21.1
.200	66.0	35.7	26.0
.250	82.5	43.4	30.5
.300	99.0	51.2	34.2
.350	off scale	59.7	38.4
.400		67.6	43.2
.450		75.6	47.3
.500		83.0	51.3
.550		92.0	55.4
.600		100	59.9

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content does not occur at the zero reading on the electro-photometer. This is because the machine has been balanced against plain water and the blanks have a pale yellow color due to the intermediately reduced molybdate. If the machine is balanced against this blank solution then the straight line starts from zero reading at zero phosphorus content, but it has been deemed wise to balance the machine against water and use the blank as a check against the reagents. This also permits the use of one standardization curve for all types of analyses. In the analysis of steel the blanks will have a reading slightly higher than that for the blank of the standard samples. It is then necessary to subtract the blank reading from the readings of the unknowns to obtain the true value of the phosphorus content of steel samples. This difference is only about 0.01mg. of phosphorus when using a steel of zero phosphorus content as a blank.

Table 7 shows the relation between the filters used and the wave length of light transmitted. The green filter has been found most useful and all the data showing electro-photometer readings was obtained using this filter.

#### REAGENTS REQUIRED

- (1) Perchloric acid, -70-72%  $\text{HClO}_4$
- (2) Sodium molybdate, -20gr of the C. P. salt ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ) in 600 ml of distilled water. After all is dissolved add 400 ml of concentrated (70-72%) perchloric acid.
- (3) Hydrazine Sulfate, -30 grams of the salt in a little water. Neutralize to litmus with concentrated ammonium hydroxide- after all is in solution dilute to 1000 ml.
- (4) Beta dinitrophenol, -0.1% in 25% ethyl alcohol.

## RECOMMENDED PROCEDURE FOR PHOSPHORUS IN STEEL

Weigh exactly one gram of steel filings or turnings into a 200 ml. calibrated Erlenmeyer flask. Add 50 ml. of 4 N nitric acid and heat until all has dissolved and very little oxide of nitrogen is visible in the neck of the flask. Add 10 ml. of 1.5 percent  $\text{KMnO}_4$  solution and boil for 2 or 3 minutes. If no precipitate appears, add more permanganate and boil again. (For pig iron as much as 30 ml. or more may be necessary) Dissolve the precipitated  $\text{MnO}_2$  by adding a little ferrous ammonium sulfate solution. After complete solution of the precipitate, add, from a pipette, 9 ml. of concentrated perchloric acid and evaporate until all the nitric acid is gone as is evidenced by the appearance of dense fumes of perchloric acid. Be sure that all nitric acid has volatilized but avoid excessive loss of perchloric acid. Cover the flask with a small watch glass, lower the temperature for about fifteen minutes to dehydrate the silica. If the temperature of the hot plate is properly regulated no perchloric acid will be lost. Remove the flask from the hot plate, cool until the solution is a crystalline mass, dilute to 200ml., stopper, and shake thoroughly. If much silica is present it should be removed at this point by filtration. Pipette 100 ml. of the solution which should contain 0.5 mg. or less of phosphorus into another 200 ml. calibrated Erlenmeyer flask. Add 5 ml. of the sodium molybdate reagent, 30 ml. of the hydrazine sulfate, place the flask on a hot plate, bring to boiling, and allow to boil about two or three minutes after the blue color begins to appear.

It is worthy of note that boiling as

long as thirty minutes after color development does not change the results obtained but two or three minutes boiling is sufficient. Remove the flask from the hot plate, cool to room temperature, add distilled water to the calibration mark, mix thoroughly, and obtain a light absorption reading with a photoelectric colorimeter. The quantity of phosphorus in the sample is calculated by reference to the calibration curve for the electro-photoometer.

If the above procedure is followed in detail the solution will have a pH value within the range for proper color development. Theoretically 4.2 ml. of 70 percent perchloric acid is necessary to dissolve one gram of iron. Thus after the complete volatilization of the nitric acid there is approximately 4 ml. of free perchloric acid left in the solution. Since only half of this solution is taken for the analysis, and 1½ ml of 70 percent perchloric acid are in the 5 ml of sodium molybdate reagent added, the total acidity of the solution at the time of color development lies between 1½ and 3½ ml. From Figure one it will be seen that these extremes are within the proper range of acidity.

#### PROCEDURE FOR FREE IRON

Follow the same procedure recommended for steel with the following exceptions.

- (1) after diluting to 200ml. and filtering off the silica pipette 25 ml of the solution into another calibrated Erlenmeyer flask for color development.

- (3) bring volume to about 120-30 mls with water
- (4) use only 20ml. of the hydrazine sulfate solution

#### PROCEDURE FOR SOILS

Weigh 2 grams of 100-mesh soil into a 50 ml. pyrex beaker. Add 4 ml. of concentrated perchloric acid and cover the beaker with a small watch glass. Digest on a gas or an electric hot plate covered with an asbestos pad for 30 minutes, or until the residue is white. The solution should boil very gently. When digestion is complete, remove the beaker from the hot plate, allow to cool a few minutes, wash off the cover glass into the beaker, and transfer the contents to a 200ml. calibrated Erlenmeyer flask using a funnel with a short stem. Add distilled water to the calibration mark, stopper the flask, shake thoroughly, and filter to remove the insoluble residue. Pipette 50ml. of the filtrate into a 200ml. calibrated Erlenmeyer flask and proceed with the color development as recommended for steel but using only 5ml. of hydrazine sulfate. If a blue color does not appear within one or two minutes after the solution starts to boil more hydrazine sulfate solution may be added to hasten the reaction.

#### PROCEDURE FOR PHOSPHATE R ONS AND HIGH PHOSPHATE FERTILIZERS

Follow the same procedure recommended for soil with the following exceptions:

- (1) Use a one half gram sample
- (2) After filtering dilute to 1000 ml; pipette

off an appropriate aliquot, dilute to about 150-40 ml., add one ml. of perchloric acid and proceed with the color development. For pure  $\text{Ca}_3(\text{PO}_4)_2$  a 5 ml. aliquot is sufficient. Because of the small aliquot used proper precautions are necessary in weighing, diluting and removing the aliquot if accurate results are to be expected. This method checks the volumetric procedure of Hall (12) within one or two tenths of a percent.

#### PROCEDURE FOR FORAGE AND GRAIN

Place 1 gram of finely ground oven-dry forage or grain in a 200 ml. tall form pyrex beaker. Add 10 ml. of acid containing 4 parts of concentrated perchloric and 6 parts of concentrated nitric acid. Cover the beaker with a watch glass and digest on a steam plate or hot water bath for about 20 minutes. Transfer the beaker to a gas or an electric hot plate, set the watch glass ajar to allow nitric acid to escape, and heat gently until dense fumes of perchloric acid have evolved for about one minute. Replace the cover glass and continue heating at a simmering temperature until the solution is colorless and the residue is white. Remove the beaker from the hot plate, cool, transfer the solution and residue to a calibrated 200 ml. Erlenmeyer flask and add distilled water to the calibration mark. Stopper, mix thoroughly, and filter to remove silica. Transfer 20 ml. of the filtrate to a calibrated 200 ml. Erlenmeyer flask, and develop the molybdenum blue color as recommended for steel but using only 5 ml. of hydrazine sulfate.

If a larger sample of soil, forage or grain is needed for the determination of other elements more acid may be added to insure proper digestion. If this is done, a proper aliquot should be taken for the analysis and treated

in the following manner; Dilute the sample to about 100 to 125 ml., add 5 drops of Beta dinitrophenol indicator and titrate to the yellow of the indicator with 25% ammonium hydroxide. Then add the molybdate reagents and proceed as outlined previously.

#### STABILITY OF THE MOLYBDENUM BLUE COLOR

Molybdenum blue colors which are obtained by the action of hydrazine sulfate on phosphomolybdic acid are very stable. If the solutions are kept stoppered the electrophotometer readings will remain approximately constant indefinitely. In routine analysis it is convenient to develop the colors and allow the solutions to cool overnight before making the comparisons. A comparison of electrophotometer readings obtained at intervals of 2 and 16 hours after color development on two samples of soil is given in Table 8. These analyses were made in quadruplicate to show the variations that may be expected between individual analyses. The greatest variation between readings for each analysis was about one scale division which is equivalent to 0.006 mg. of phosphorus. A comparison of readings and the corresponding percent phosphorus found after 4, 24, and 200 hours on nine Bureau of Standards steel samples is given in Table 8A. It will be observed that no great change in the solutions occurred even after standing for more than a week, and the maximum variation with time is equivalent to about 0.002% phosphorus in the samples.

TABLE 8

The Effect of Time Interval Between Color Development and Electrophotometer Readings on the Stability of Molybdenum Blue produced using the method outlined for soil. Also the variation between Quadruplicate Determinations is shown for two samples of soil.

		Electrophotometer Readings	
Official Soil:		2 Hours after color	16 Hours after Color
Sample No.		Development	Development
4193	A :	38.6	39.0
	B :	37.6	37.9
	C :	38.0	37.9
	D :	37.9	38.0
5174	A :	23.9	23.9
	B :	24.4	24.4
	C :	24.8	24.8
	D::	24.4	24.4

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TABLE 8A

The Effect of Time Interval Between Color Development and Electrophotometer Readings on the Stability of Molybdenum Blue Color produced and the corresponding amount of Phosphorus found using the method outlined for steel.

No.	Bureau of Standards : Sample No.	Electrophotometer Readings and Percent Phosphorus Found					
		After 4 Hours		After 24 Hours		After 200 Hrs.	
		Read	%P	Read	%P	Read	%P
1A	65	26.1	.026	26.2	.026	27.0	.027
B		26.0	.026	26.3	.026	27.5	.028
2A	30B	35.1	.037	35.3	.037	36.5	.039
B		36.0	.038	36.2	.038	37.5	.040
3A	34A	30.4	.032	30.3	.032	31.5	.034
B		29.6	.031	29.7	.031	31.0	.033
4A	15B	32.7	.035	32.7	.035	33.5	.036
B		32.0	.034	32.1	.034	33.0	.035
5A	33B	36.0	.038	36.2	.038	37.0	.039
B		36.9	.039	37.1	.039	38.0	.040
6A	21B	57.3	.065	57.5	.065	58.8	.067
B		56.3	.064	56.3	.064	57.8	.066
7A	22B	70.0	.081	70.5	.082	72.5	.084
B		70.5	.082	70.5	.082	72.5	.084
8A	10D	76.0	.088	76.0	.088	78.0	.090
B		75.0	.087	76.0	.088	76.0	.088
9A	8B	82.0	.095	82.0	.095	82.5	.096
B		82.5	.096	82.5	.096	82.5	.096

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## EXPERIMENTAL RESULTS

The percent phosphorus found by the recommended procedure in 18 samples of steel is compared with the values given by the source of the samples in Table 9.

Those samples whose source is given as "laboratory reagent" were analyzed by the volumetric method outlined by Scott (27) using the details given in Hall (12). In all cases, except No. 2, which is high in Vanadium, the different procedures give results that agree within 0.003 percent or less. A redetermination of the phosphorus content on all the samples by the volumetric procedure of Scott agreed with the colorimetric values rather than those given by the source in all cases except sample number 2, the high value of which, as explained previously, is due to vanadium. It seems that any difference between the methods is due to incomplete oxidation of phosphorus to phosphate in the digestion as determinations by the different procedures on aliquots from the same digestion gave almost identical values of percent phosphorus in every case.

A comparison of the total phosphorus in 206 soil samples analyzed by the recommended procedure and by two volumetric methods is given in Table 10. The first 122 were analyzed by the perchloric acid digestion method recommended by Volk and Jones (32) and the last 84 by the magnesium nitrate digestion method (21). The average phosphorus content of the 206 soils as determined by the volumetric procedures is 204 ppm(parts per million) while

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the average by the recommended procedure is 203.5 ppm. Only 27 of the 206 soil samples had a wider variation than 20ppm. Of this number, 15 of the samples were higher and 12 were lower in phosphorus than the volumetric values. The different methods of digestion used in these analyses may account in part for the lack of agreement in some instances.

Approximately one year after the above determinations were made, 60 of the samples were reanalyzed by another individual. The average phosphorus content found by this analyst varied less than one thousandth of one percent from values obtained formerly. During the past two years several thousand samples of soil, forage and grain have been analyzed for phosphorus by this method in the Oklahoma Agricultural and Mechanical College Agronomy Department laboratory and the method has proved accurate even in the hands of unskilled analysts.

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TABLE 9

Recovery of Phosphorus in Steel Samples by the Recommended Procedure as compared to Values Given by the Sample Source--Also shows known Values other Elements present in the Samples.

No.	Source: % P : % P:Diff.:			OTHER VALUES GIVEN BY THE SOURCE											Type of Steel
	No.	by	by	%	%	%	%	%	%	%	%	%	%	%	
		Col.	source												
		method		C	Mn	S	Si	Cu	Ni	Cr	V	Mo	As		
1	65	.025	.022	.003	.238	.746	.040	.412	.060	.044	.049	.003	.004	.011	Acid Electric Hearth
2	30B	.037	.024	.013	.292	.499	.031	.212	.130	.282	1.03	.208	.014	.022	Chrom- Vanadium
3	34A	.031	.028	.003	.762	.501	.026	.276	.222	.232	.275	.007	.003	.009	Acid Open Hearth
4	15B	.034	.032	.002	.101	.559	.038	.292	.145	.083	.064	.005	.003	.008	Basic Open Hearth
5	33B	.038	.037	.001	.336	.700	.032	.223	.114	3.48	.029	.005	.003	.016	Nickel Acid Open
6	21B	.064	.064	.000	.605	.564	.036	.162	.031	.062	.020	.005	.006	.009	Hearth
7	22B	.082	.084	.002	.674	.935	.042	.123	.009	.003	.003	.005	-	-	Bessemer
8	10D	.088	.088	.000	.419	.915	.030	.063	.010	.002	.006	.006	.003	.005	Bessemer
9	8D	.096	.099	.003	.078	.482	.083	.018	.013	.004	.007	.003	.003	.007	Bessemer

TABLE 9 (continued)

No.	Source of Sample	Found by	Diff. by Col. method	OTHER VALUES GIVEN BY THE SOURCE												Type of Steel
				%	%	%	%	%	%	%	%	%	%	%	%	
				C	Mn	S	Si	Cu	Ni	Cr	V	Mo	As			
10	4	1.000	1.008	.000	.080	.025	-	-	-	-	-	-	-	-		
11	3	1.013	1.013	.000	.770	.032	-	-	-	-	-	-	-	-		
12	5	1.026	1.024	.002	.35	.75	.036	-	-	-	-	-	-	-		
13	6	1.041	1.039	.002	.33	.32	.036	-	-	-	-	-	-	-		
14	2	1.037	1.039	.002	.10	.92	.260	-	-	-	-	-	-	-		
15	1	1.037	1.066	.001	.10	.37	.026	-	-	-	-	-	-	-		
16	7	1.203	1.210	.002	-	1.76	.027	0.73	-	-	-	-	-	18 Iron		
Laboratory Reagent																
17	1	1.000	1.003	.000	-	-	-	-	-	-	-	-	-	-		
18	2	1.004	1.004	.000	-	-	-	-	-	-	-	-	-	-		
19	3	1.252	1.264	.002	-	-	-	-	-	-	-	-	-	-		

\*These values determined volumetrically by Procedure outlined in Scott(27)

TABLE 10

Recovery of Phosphorus in Soil Samples by the Recommended Procedure  
as Compared to Values Found by Volumetric Procedures.

NUMBER	OFFICIAL SAMPLE NO.	P.P.M. COLORIMETRIC	P.P.M. VOLUMETRIC	DIFFERENCE COL. - VOL.
1	1655	227	235	-8
2	56	231	224	7
3	57	138	150	-12
4	58	153	170	-17
5	59	190	204	-14
6	60	112	144	-32
7	5811	212	230	-18
8	12	118	240	-22
9	13	100	90	10
10	14	82	100	-18
11	15	88	88	0
12	16	520	533	-13
13	17	310	323	-13
14	18	228	235	-7
15	19	233	249	-16
16	20	245	263	-18
17	21	272	288	-16
18	22	136	124	12
19	23	84	78	6
20	24	66	63	3
21	25	73	75	-2
22	26	74	70	4
23	27	199	232	-33
24	28	124	105	19
25	29	66	88	-22
26	30	54	90	-36
27	31	91	85	6
28	32	180	180	0
29	34	149	145	4
30	35	86	78	8
31	36	92	88	4
32	37	87	85	2
33	40	74	68	6
34	41	66	60	6
35	42	79	60	19
40	43	130	115	15
41	44	104	93	11
42	45	281	173	8
43	46	160	145	15
44	47	84	66	18
45	48	83	82	1

NUMBER	OFFICIAL SAMPLE NO.	PPM COLORIMETRIC	PPM VOLUMETRIC	DIFFERENCE COL - VOL.
46	5849	110	86	24
47	50	96	99	-3
48	51	66	75	-9
49	52	66	75	-9
50	53	158	170	-12
51	54	272	270	2
52	55	224	225	-1
53	56	127	125	2
54	57	143	138	5
55	58	146	148	-2
56	59	236	233	3
57	60	234	240	-7
58	61	223	225	-2
59	62	241	235	6
60	63	182	185	-3
61	64	155	163	-8
62	65	122	130	-8
63	66	120	125	-5
64	67	94	103	-9
65	68	109	115	-6
66	69	225	232	-7
67	70	185	170	15
68	71	124	120	4
69	72	117	100	17
70	73	106	85	21
71	74	87	70	17
72	75	92	80	12
73	76	259	260	-1
74	77	183	165	18
75	78	153	150	3
76	79	124	110	14
77	80	128	130	-2
78	81	158	165	-7
79	82	225	225	0
80	83	307	310	-3
81	84	239	250	-11
82	85	171	180	-9
83	87	212	230	-18
84	88	203	220	-17
85	89	416	430	-14
86	90	806	793	13
87	91	94	100	-6
88	97	231	218	13
89	98	376	375	1
90	99	322	308	14

NUMBER	OFFICIAL SAMPLE NO.	PPM CHLORIMETRIC	PPM VOLUMETRIC	DIFFERENCE CHL - VOL.
91	5901	150	148	2
92	02	289	305	-6
93	03	211	225	-14
94	04	82	79	3
95	05	73	80	-3
96	06	82	90	-8
97	07	65	63	2
98	08	58	65	-7
99	09	66	75	-7
100	5951	319	313	6
101	52	95	103	-13
102	53	416	415	1
103	54	474	463	11
104	55	306	323	-17
105	56	223	230	-7
106	57	212	225	-11
107	58	188	195	-17
108	59	441	435	3
109	61	444	458	-14
110	62	335	343	-8
111	63	260	275	-15
112	64	251	265	-14
113	65	336	333	3
114	66	348	355	-7
115	67	302	330	-28
116	68	52	65	-13
117	69	40	45	-5
118	70	41	35	6
119	71	57	70	-13
120	72	58	68	-10
121	73	65	80	-15
122	74	287	305	-18
123	3	250	260	-10
124	6	236	255	-49
125	8	274	160	114
126	9	124	133	-9
127	11	194	178	16
128	13	544	545	-1
129	23	276	283	-7
130	27	613	500	118
131	31	454	445	9
132	34	411	400	11
133	35	456	433	23
134	101	607	640	-33
135	1664	55	30	25

NUMBER	OFFICIAL SAMPLE NO.	PPM COLORIMETRIC	PPM VOLUMETRIC	DIFFERENCE	
				GR.	- VOL.
136	1692	40	30		10
137	3373	73	39		34
138	1872	55	40		15
139	5189	60	41		19
140	5115	48	50		-8
141	5112	50	57		-7
142	5116	55	65		-10
143	5113	70	77		-7
144	5122	70	85		-15
145	5143	70	89		-19
146	5134	73	93		-17
147	5135	88	93		-5
148	5208	92	94		-2
149	5142	89	98		-10
150	5201	100	110		-10
151	5186	102	110		-8
152	5114	110	113		-3
153	5151	127	127		0
154	5130	124	130		-6
155	5128	124	135		-11
156	5127	134	138		-4
157	5132	140	140		0
158	5148	149	142		7
159	5102	134	145		-11
160	5136	128	151		-23
161	5129	150	159		-9
162	5126	146	163		-17
163	5159	168	163		5
164	5227	164	165		1
165	5203	156	172		-16
166	5148	172	190		-18
167	5111	183	190		-7
168	5174	254	195		59
169	5147	203	195		8
170	5105	300	201		-1
171	5137	217	205		12
172	5106	217	211		6
173	5173	210	215		-5
174	5157	212	221		-9
175	5160	223	231		-8
176	5195	235	235		0
177	5164	240	243		-3
178	5177	229	245		-16
179	5104	266	248		18
180	5163	222	251		-29

NUMBER	OFFICIAL SAMPLE NO.	PPM COLORIMETRIC	PPM VOLUMETRIC	DIFFERENCE COL. - VOL.
181	5101	263	259	4
182	5107	242	262	-20
183	5108	251	275	-24
184	5243	263	276	-13
185	5193	292	285	7
186	5241	266	285	-19
187	5190	259	293	6
188	5242	303	295	8
189	5152	317	295	21
190	5172	353	300	33
191	5173	311	300	11
192	4950	239	314	-15
193	4972	311	320	-9
194	4973	333	345	-12
195	4978	328	350	-22
196	5181	357	353	4
197	1640	390	363	27
198	5224	419	373	46
199	1633	419	385	34
200	5192	427	412	15
201	5103	466	445	21
202	5233	483	467	16
203	3948X	523	495	28
204	476	859	838	21
205	3803	640	653	-13
206	7062	501	503	-2

## SUMMARY

A critical study of several recommended colorimetric methods for the determination of phosphorus was made in order to develop a method of general applicability. Of the many reducing agents proposed for the reduction of phosphomolybdic acid, it was found that hydrazine sulfate gave the most consistent color values with known quantities of phosphorus in solutions of varied chemical composition. This reducing agent gives molybdenum blue colors which do not change greatly even after standing for more than a week after color development. A method suitable for the colorimetric determination of phosphorus in iron and steel, forage and grain, phosphate fertilizers and phosphate rocks is proposed. When using standards, the method outlined will detect as little as one part of phosphorus in 100,000,000 parts of solution. The proposed method checks determinations of phosphorus by the usual volumetric procedures within 0.002 percent; i.e. If the volumetric method indicates that 0.100 percent phosphorus is present the procedure outlined will show a phosphorus content of  $0.100 \pm 0.002$  percent. The total phosphorus content of 306 soil samples was determined volumetrically and by the method outlined for soil with an average difference between the two methods of less than 0.0001 percent. Only 27 of the 306 analyses differed by more than 0.002 percent by the two methods. Eight Bureau of Standards steel samples analyzed by the method outlined for steel agreed, on the average, within less than 0.001 percent of the Bureau's values while the maximum difference was 0.003 percent. Vanadium and chromium in the amounts

present in vanadium and high chrome steels interferes and consequently the method is not applicable to such steels without previous removal of the interfering elements. Obtaining consistent color values by the proposed method is greatly influenced by the pH value of the solution and the concentration of the molybdate. It was found that the color density depended on the acid-molybdate ratio rather than on their independent concentrations. Large quantities of ferric iron, that adversely influence the color development in many of the methods previously recommended, has little influence on the colors obtained by the proposed method.

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## AUTOBIOGRAPHY

I, Weldon Riley Shelton, was born in Memphis, Tennessee near the muddy banks of the Mississippi river, on December 16, 1913. In the fall of 1918 my family and I moved to Shawnee, Oklahoma and six months later to Stillwater, Oklahoma where my father, William Riley Shelton, was connected with the Extension Division of the Oklahoma Agricultural and Mechanical College. At Stillwater the first eight years of school were completed. In 1922 we moved to Muskogee, Oklahoma where the ninth and tenth grades were completed at West and Central High Schools. In the fall of 1931 I entered the Oklahoma Military Academy at Claremore, Oklahoma, graduating from high school in the spring of 1933 and finishing two years of Junior College in the spring of 1935. I was married to Ida Venora McKee, of Muskogee, Oklahoma on December 22, 1934 at Henryetta, Oklahoma. From September 1935 until September 1937 was spent in the civil service at Washington, D. C. On September 6, 1937 I resigned and entered the Oklahoma Agricultural and Mechanical College, receiving the Bachelor of Science degree in Chemical Engineering on June 3, 1940. The next fifteen months were spent with the Research Department of the Phillips Petroleum Company working on the development of raw materials for synthetic rubber. In September of 1941 I returned to the Oklahoma Agricultural and Mechanical College as a research assistant in the Agronomy Department. Since that time I have been engaged in studies leading to the Master of Science degree in Chemical Engineering.

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