THE COLORIMETRIC DETERMINATION OF TOTAL PHOSPHOROUS IN PLANT SOLUTIONS.*

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INTRODUCTION.

A review of the literature reveals a number of methods for colorimetric determination of the phosphorous content of soil, blood and urine. The adaptation of these methods to plant analysis has been apparently neglected.

Gilbert (5) has used the coeruleo-molybdate method of Deniges (3) for the estimation of phosphates in plant extracts and has found this method fairly reliable. His determinations, however, involve only the quantity of phosphorous found in the expressed juice, and not the total quantity in the plant. Parker and Fudge, (6) have adapted the coeruleo-molybdate method of Deniges (3) to soils and soil extracts, they also have used the aminonaptholsulfonic acid (4) and hydroquinone methods (2). They claim these reagents may be used for plant analysis as well as for soil.

Since a reliable colorimetric method for total phosphorous in plant samples would hasten this determination materially, an attempt was made to adapt to the plant sample a colorimetric method which is commonly used for soils and biological material.

ANALYTICAL METHODS.

There are various reducing agents which give a more or less quantitative color value with ammonium-phospho-molybdate. Among these reducing agents are stannous chloride, aminonaphtholsulfonic acid, phenylhydrazine-hydrochloride and hydroquinone.

Previous experiments have cast some doubt upon the reliability of color development by the coeruleo-molybdate method due to a lack of stability, while phenylhydrazine-hydrochloride does not develop a color of sufficient intensity. Either the aminonaphtholsulfonic acid reagent or hydroquinone seemed to offer the best possibilities.

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Fiske and Subbarow, (4) outline the aminonaphtholsulfonic acid method as used for the determination of phosphorous in biological material. Arrhenius, (1) and Bell and Doisy, (2) have adapted the hydroquinone method to soils and biological material, respectively. These methods, by sufficient modification were adapted to plant solutions.

Preparation of Plant Solution.

One gram samples of finely ground corn plants, dried at 65° C, were digested by the usual sulfuric-nitric acid wet digestion method. The clear solutions were filtered into 250 cc. volumetric flasks to remove dehydrated silica and made up to volume. Duplicate 50 cc. aliquots were used for the usual ammonium-molybdate volumetric determination.

Table I.

Sample No.	Milligrams Phosphorous			NO_3
	Volumetric	Colorimetric		
		Salt Std.	Sample Std.	
1	.296	.194	Standard	—
2	.286	.235	.384	—
3	.354	.401	.371	+ +
4	.360	.369	.392	±
5	.323	.408	.254	±
6	.283	.333	.263	—
7	.313	.276	.255	+ +

Aminonaphtholsulfonic Acid Method.

A 25 cc. aliquot of the plant solution was tranferred to a 100 cc. volumetric flask and enough water added to bring the total to about 70 cc. The reagents of Fiske and Subbarow (4) were now added. These consist of 10 cc. of 2.5% ammonium molybdate and 4 cc. of 0.25% aminonaphtholsulfonic acid. The flasks were gently shaken and permitted to stand five minutes after which they were made up to volume and compared against a standard in the colorimeter.

The comparative values for phosphorous as determined volumetricaly and colorimetricaly are presented in Table I. In the first column of colorimetric values, the determinations were made against a standard consisting of a pure salt made up to contain 0.4 mgm. of phosphorous in 100 cc. Since the

results were so varied, the solutions were compared against one of the samples as a standard and the results are shown in the second column of colorimetric determinations.

Since the colorimetric values as determined by the comparison with a pure salt standard were rather inconsistent, it was thought that this might be due to an excess of sulfuric acid or traces of nitric acid in the wet digested plant solution. All of the samples have had approximately the same quantity of sulfuric acid added in the wet digestion process so one sample was selected as a standard. The use of a plant solution of known phosphorous quantity as a standard did not overcome the divergence between the volumetric and colorimetric values for phosphorous found in the same solution.

TABLE II.

SAMPLE NO.	MILLIGRAMS PHOSPHOROUS	
	Volumetric Double ppt.	Colorimetric Single ppt.
8	.199	.301
9	.199	.326
10	.285	.727
11	.341	.301
12	.330	.260
13	.374	.430

A diphenylamine test was made on the solutions for the traces of nitrate which might remain after wet digestion. From the data in Table I it is apparent that this is not the factor which inhibits full development of color. However, it is advisable to heat the plant solutions for sufficient time after clearing to drive off the last traces of nitric acid used in the wet digestion process. Fiske and Subbarow (4) report that nitrates will interfere with color development when analyzing for phosphorous in biological material.

As a further check on the value of aminonaphtholsulfonic acid as the reducing agent, a series of duplicate precipitations were made by the usual ammonium-molybdate method. One duplicate was redissolved after the first precipitation and the phosphate determined colorimetricaly, the other duplicate was double precipitated and the phosphate determined titrametricaly. The color development by aminonaphtholsulfonic acid is hardly proportional to the actual quantity of phosphorous present as indicated in Table II. This may be due to the

presence of ammonia used in dissolving the precipitate before adding the color producing reagents.

To determine the stability of the color produced by aminonaphtholsulfonic acid, readings were made at definite intervals over an hour and a quarter. Apparently the color was quite stable as shown in Table III.

Parker and Fudge (6), evaporated the soil solutions with 1 cc. N–1 Mg $(No_3)_2$ and ignited the residue. They found that there was practicaly no loss of phosphorous when they used this procedure. When $Mg(No_3)_2$ was not used they found a phosphorous loss of about 10% in some cases. This procedure as outlined by Parker and Fudge was tried with the plant solutions. The heat necessary to remove the sulfuric acid is sufficient to volatize the phosphorous compounds. In every case where

TABLE III

Sample No.	Milligrams Phosphorous				
	Volumetric	Colorimetric			
		5 min.	20 min.	35 min.	75 min.
14	.441	.408	.398	.404	.414
15	.395	.333	.314	.325	.325
16	.437	.322	.343	.334	.349

the sulfuric acid was evaporated off, the color development with aminonaphtholsulfonic acid was not of sufficient intensity to secure readings in the colorimeter.

Aminonaphtholsulfonic acid does not appear to be a reliable reducing agent for the colorimetric determination of phosphorous in wet digested plant material. The wide divergence between the volumetric and colorimetric values found for phosphorous in Tables I and II demonstrates that the reagent is not adapted to this particular procedure.

Hydroquinone Method.

The method as outlined by Bell and Doisy (2) was substituted for the aminonaphtholsulfonic acid method in the determination of phosphorous colorimetricaly in the plant solution. The general procedure was the same as for the previous method, 25 cc. aliquots of the plant solution were used, 5 cc. of the molybdic acid reagent added and then 2 cc.

of the hydroquinone reagent. The solutions were permitted to stand about ten minutes, the sulfuric acid was then neutralized with the carbonate-sulfite solution and the color allowed to develop for about one hour.

As previously noted with the aminonaphtholsulfonic acid method, the color development was not as intense in the solution as when an equivalent quantity of pure phosphate salt was usep for a standard. The procedure seems to be more reliable if one of the plant solutions is analyzed volumetricaly and used as a

TABLE IV.

Sample No.	Milligrams Phosphorous				
	Volumetric	Colorimetric			
		S-19	S-23	S-27	S-31
17	.570	.556	.....	.....	.....
18	.563	.552	.....	.....	.....
19	.515	.515	.....	.....	.....
20	.482	.507	.....	.....	.....
21	.465	.462	.....	.....	.....
22	.424	.415	.432	.....	.....
23	.404	.389	.404	.....	.....
24	.387	.376	.375	.....	.....
25	.347	.369	.332	.405	.....
26	.323	.344	.309	.338	.....
27	.310	.....	.300	.310	.....
28	.283	.....	.....	.279	.....
29	.269	.....	.....	.252	.252
30	.252	.....	.....	.244	.218
31	.212	.....	.....	.227	.212
32	.202	.....	.....	.....	.208
33	.174	.....	.....	.....	.182

standard. Four different plant solutions ranging at intervals of about 0.1 mgm. of phosphorous were used as standards in Table IV. This eliminated to some extent the error due to light absorption when the colorimeter prisms are at widely different depths of solution. The columns designated by "S-19," "S-23," etc. represent the number of the sample used for the standard. In each case the samples were not compared with the selected standard when the solution under observation became too weak or too strong for efficient use of the colorimeter.

Comparison of the data in Table IV with Tables I and II indicates that hydroquinone gives more reliable results than aminonaphtholsulfonic acid when used with wet digested plant

material. It is to be noted especially, that the accuracy of the colorimetric determination is fairly good within a range of 0.05 mgm. above or below the standard. However as the standard used becomes more dilute the error in reading the colorimeter becomes larger. Also the dilute solutions seem to retard full color development. The data indicate that several standards of varying concentrations must be used if the samples under observation cover a wide range in phosphorous content.

The superiority of hydroquinone over aminonaphtholsulfonic acid may be explained on the basis of acidity concentration. Deniges (3) and Parker and Fudge (6) have shown that the acidity of the solutions under observation must be controlled

TABLE V.

SAMPLE NO.	MILLIGRAMS PHOSPHOROUS		
	Volumetric	Colorimetric	
		1 hour	5 hours
34	.320	.266	.277
35	.320	.257	.264
36	.387	.331	.320
37	.482	.392	.389

if comparative results are desired. The plant solutions in this instance are made up with sulfuric acid and there may be slight variations in acidity between samples due to volatization of the acid during digestion. The aminonaphtholsulfonic acid method does not neutralize this excess acidity. In fact the development of color by this method is dependent upon the 10 N sulfuric acid in the reagents. The reagent acidity is constant, but the sample acidity as previously mentioned, may vary to some extent.

With the hydroquinone method, color development depends upon the neutralization of the sulfuric acid present in the reagent with the carbonate-sulfite mixture. At the time of neutralization of reagent acidity, the carbonate-sulfite mixture is added in sufficient quantity to neutralize the sample acidity. This overcomes the differences in acidity between samples and causes a more comparative color development.

The color development by hydroquinone is remarkably stable as it changes but slightly over a period of several hours. This is a valuable characteristic since it enables the investigator

to make a large number of determinations at once without the possibility of the color fading or intensifying during the time of analysis. The data in Table V show that after a period of five hours the color had not materialy changed in the solutions.

CONCLUSIONS.

Phosphorous may be determined with fair degree of accuracy in plant material by a colorimetric determination on the wet-digested dry sample. Hydroquinone is preferable as a reducing agent.

The color development is neither proportionaly nor geometricaly progressive. For this reason the error cannot be corrected for by means of a table or curve. It may be minimized by using several standards, preferably at about 0.1 mgm. intervals.

The presence of sulfuric acid and other chemical substances in the plant solution prevents accurate determination of the colorimetic phosphorous values when compared with a pure salt standard. The results more closely agree with the volumetric analysis when a plant solution of known phosphorous quantity is used as a standard.

This method should not be considered as a reliable substitute for the usual volumetric determination. In cases where the number of samples to be handled is very large and a number of rapid and fairly accurate determinations are desired, the method can be used with the assurance of at least comparative results.

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