fore obtainable in the presence of volatile substances other than alcohol and water. The new method is not only more accurate, but is much less time consuming than previous methods.

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ANALYSIS OF GLYCEROPHOSPHATES.* I. DISCUSSION OF ASSAY METHODS FOR FERRIC GLYCEROPHOSPHATE AND MANGANESE GLYCEROPHOSPHATE.

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A.—FERRIC GLYCEROPHOSPHATE.

Ferric glycerophosphate is customarily assayed by reducing the ferric ion with hydriodic acid, the iodine liberated being determined by titration with standard sodium thiosulfate (1). This reduction proceeds but slowly and is reversible. To force the reaction to completion it is necessary to keep the concentration of ferric ion and of hydriodic acid as high as possible. Thus a distinct excess of mineral acid such as hydrochloric acid is required to prevent the hydrolysis of ferric chloride to ferric basic chloride. However, the mineral acid concentration must not be too high since it tends to form complexes with the ferric ion. This excess of acid serves also to increase the concentration of hydriodic acid, the source of which is usually potassium iodide. Oxygen must be absent from the solution since it reacts slowly with hydriodic acid to form iodine, thus giving high results. The reaction is far from instantaneous, various authors recommending reaction periods of from 5–60 minutes. The reaction velocity increases with increasing temperature, temperatures of as high as 60° C. having been recommended.

With solutions of pure ferric salts the speed of reaction is not a problem, Oakley and Krantz (2) having found that the reaction is rapid. With phosphates present Kolthoff (3) obtained low results unless the concentration of mineral acid was sufficiently high. Glycerophosphates behave in a similar fashion, reducing the reaction velocity by forming complexes with the ferric ion.

The National Formulary V recognized this fact by prescribing in the assay procedure for ferric glycerophosphate a reaction period of 30 minutes at a temperature of 40° C. Since, however, it provided no means of removal of oxygen from the solution, this procedure gave results high by as much as two per cent.

In the newly published National Formulary VI this assay procedure has been improved by eliminating oxygen, by increasing the concentration of potassium iodide and of ferric glycerophosphate and by decreasing the concentration of hydrochloric acid. These changes all serve to promote a more rapid reaction with less error from air oxidation. However, even under these revised conditions the reaction is far from instantaneous and the minimum five-minute reaction period

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recommended by N. F. VI is insufficient to allow complete reaction except at elevated temperatures.

The effect of time and of temperature upon the completion of reaction of ferric glycerophosphate and hydriodic acid under the conditions imposed by N. F. VI is illustrated in Table I. The results are expressed in percentage completion of reaction, taking as 100 per cent the constant value obtained with reaction times in excess of 20 minutes at 40° C. That this is a valid procedure was demonstrated by preparing a standard solution of ferric sulphate from pure electrolytic iron, adding glycerophosphoric acid to make a synthetic ferric glycerophosphate and analyzing by the N. F. VI procedure. It was found that reaction times of 15 minutes or more at temperatures of 40° C. and above gave quantitative recovery of the iron present.

TABLE I.—EFFECT OF TEMPERATURE AND TIME UPON THE COMPLETION OF REACTION OF FERRIC GLYCEROPHOSPHATE AND HYDRIODIC ACID, N. F. VI PROCEDURE.

	Extent of Reaction, Per Cent.		
Time, Minutes.	Temperature ° C. 20	30	40
5	75.6	97.8	99.5
10	90.0	98.5	100.0
20	97.2	99.0	100.0

From Table I it may be seen that the reaction is incomplete in five minutes, even at 40° C. Complete reaction is not obtained even in 20 minutes at 30° C. It is evident that with an average laboratory temperature of 25° C. that the N. F. VI procedure, involving a minimum reaction time of 5 minutes, might lead to results 10 to 15 per cent low. That the reaction is incomplete in this time is indicated by the fact that the end-points are not permanent and that successive analyses are not reproducible.

In view of these findings it would seem desirable that the N. F. VI procedure for the assay of ferric glycerophosphate be modified slightly to include a minimum reaction time at a minimum temperature to insure completeness of reaction. The procedure suggested is:

"Dissolve about 1 Gm. of Ferric Glycerophosphate, dried to constant weight at 110° C. and accurately weighed, in 25 cc. of distilled water in a glass-stoppered flask. Add 3 cc. of hydrochloric acid, 1 Gm. of sodium bicarbonate in small portions, and 6 Gm. of potassium iodide; securely stopper the flask, and allow the mixture to stand 20 minutes at a temperature of 35–40° C. Cool to 20° C., add 50 cc. of distilled water, and titrate the liberated iodine with tenth-normal sodium thiosulphate, using starch T.S. as the indicator.

"Each cc. of tenth-normal sodium thiosulfate is equivalent to 0.0311 Gm. of $Fe_2(C_3H_5(OH)_2PO_4)$ and to 0.005584 Gm. of Fe."

TABLE II.—TYPICAL ANALYSES OF SEVERAL COMMERCIAL SAMPLES OF FERRIC GLYCEROPHOSPHATE, MODIFIED N. F. VI PROCEDURE.

Sample.	Trial 1, Per Cent Fe.	Trial 2, Per Cent Fe.
A	16.05	16.04
B	17.80	17.82
C	17.38	17.39
D	17.02	17.02

This procedure differs from the present official one only in the time and temperature of reaction. This slight change is sufficient to insure closely reproducible results as exemplified by the series of check analyses on several samples of commer-

cial ferric glycerophosphate given in Table II. It will be seen that the results agree within the limit of experimental error in volumetric analysis.

B.—MANGANESE GLYCEROPHOSPHATE.

Soluble manganese glycerophosphate N. F. V was assayed officially by solution in water, precipitation as manganous sulfide and ignition to manganous-manganic oxide. Manganese glycerophosphate N. F. VI is the insoluble variety of the salt. The N. F. VI method for its assay is the same as that of N. F. V for the soluble salt with the exception that the former is first dissolved in hydrochloric acid and the latter in water.

Because of the large excess of hydrochloric acid employed to dissolve the salt the new N. F. VI procedure is unworkable. N. F. VI specifies that 0.5 Gm. of the salt be dissolved in 95 cc. of water plus 5 cc. of hydrochloric acid, that to this solution 20 cc. of ammonia T.S. and 10 cc. of ammonium sulfide T.S. be added to precipitate pink colloidal manganous sulfide and that the solution be boiled until the sulfide is transformed to the green, readily filterable form. This transformation does not occur. Instead, as boiling proceeds, hydrogen sulfide is volatilized until the pink colloidal manganous sulfide redissolves. The large amount of hydrochloric acid used to dissolve the sample, when neutralized with the ammonia T.S., forms an ammonium chloride-ammonium hydroxide buffer of which the p_H value is too low to allow the transformation of pink to green manganous sulfide. To make the N. F. VI procedure workable it is merely necessary to use a much smaller quantity of hydrochloric acid. If the N. F. VI procedure be changed to read as follows this trouble is avoided:

"Wet about 0.5 Gm. of manganese glycerophosphate, dried to constant weight at 110° C. and accurately weighed, with about 5 cc. of distilled water, add hydrochloric acid drop by drop from a micro pipette until solution is complete (not over 0.5 cc.), add 95 cc. of distilled water; add 20 cc. ammonia T.S. and 10 cc. of ammonium sulfide T.S., and heat a few minutes until the precipitate has become green. Allow" etc., as in N. F. VI.

Using this procedure the transformation to green manganous sulfide occurs before the solution even reaches the boiling point and the analysis may be completed.

Even with the inclusion of this step the N. F. VI method is none too satisfactory on account of the variability of the composition of the ignition product, manganous-manganic oxide. The composition of this material is dependent upon the temperature of ignition and upon the degree of access of air during the ignition (4). Even with precise control of all conditions Raikow and Tischkow (5) were not able to reproduce the weight of the precipitate within 0.5 per cent. It has been the writer's experience that two analyses run simultaneously will give results in close agreement with each other but may or may not check the results of two similar analyses run at a different time or by a different analyst, the differences often exceeding two per cent. Since the N. F. VI assay minimum is 98 per cent the error in the analysis may be larger than the impurity limit allowed. It would seem that a better method should be sought.

In the presence of organic matter the determination of manganese is limited to gravimetric methods unless preliminary separation as the sulfide is utilized. In addition to the sulfide method manganese may be determined gravimetrically by

precipitation as manganous ammonium phosphate followed by ignition to pyrophosphate or by weighing as sulfate at 450–500° C. after preliminary separation as sulfide. Other methods are of minor importance. The pyrophosphate method appears the most practical one for manganese glycerophosphate.

In order to determine the applicability of this method a stock solution of manganous sulfate was prepared. Exactly 25.00-cc. aliquots of this solution were evaporated to dryness and ignited to $MnSO_4$ at 450–500° C. in order to determine the concentration of manganese in the solution. The same size aliquots were then subjected to analysis by the sulfide and by the pyrophosphate method both with and without the addition of glycerophosphoric acid. The results obtained in these analyses are shown in Table III. The results are reported as grams of manganese per 25.00 cc. of solution.

TABLE III.—ANALYSES OF A STOCK SOLUTION OF MANGANOUS SULFATE FOR MANGANESE.

Glycerophosphoric Acid.	Gm. Manganese per 25.00 cc. $MnSO_3$ Method.	$Mn_2P_2O_7$ Method.	Mn_3O_4 Method.
Absent	0.1521	0.1525	0.1573
Absent	0.1534	0.1533	0.1582
Absent	0.1528	0.1522	0.1522
Absent	0.1525	0.1530	0.1521
Average	0.1527	0.1528	0.1549
Present		0.1525	0.1522
Present		0.1521	0.1517
Present		0.1528	0.1562
Present		0.1524	0.1567
Average		0.1525	0.1542

It is apparent that the sulfate and pyrophosphate methods furnish results in good agreement and that the results by the latter method are the same whether or not glycerophosphate is present. It is evident, too, that some of the analyses by the sulfide method are in good agreement with the others but some are not. These data are representative of the type encountered with this method and show the variability in the composition of manganous-manganic oxide. The results by the pyrophosphate method are encouraging for its use as an official method of assay of manganese glycerophosphate.

The precipitation of manganese ammonium phosphate must be carried out under carefully controlled conditions. When a hot acid solution of a manganese salt containing considerable phosphate and ammonium salts is neutralized dropwise with ammonia a flocculent precipitate of manganous phosphate first forms. This slowly changes into granular manganese ammonium phosphate. With glycerophosphates present this change is not always complete so that results with one precipitation are often low. This necessitates a double precipitation. Results with two precipitations are always good. The data in Table III were obtained in this manner. The exact procedure employed was as follows:

Wet with 5 cc. of distilled water about 0.5 Gm. magnanese glycerophosphate dried at 110° C. and accurately weighed. Dissolve with 2 cc. of hydrochloric acid. Dilute with 120 cc. distilled water, add 15 cc. ammonium secondary phosphate T.S. and 10 Gm. ammonium chloride. Heat to boiling. Add a few drops of methyl red T.S. Keeping the solution boiling throughout and

stirring constantly add ammonia T.S. drop by drop from a burette. As soon as precipitation commences adjust the rate of addition to about one drop per 5 seconds. Continue to add ammonia at this rate until the solution is neutral to methyl red, then add 2 cc. excess. Keep hot for thirty minutes, cool in ice and decant the clear mother liquor through a Gooch crucible previously ignited and weighed. Without washing the precipitate in the beaker redissolve it with 2 cc. of hydrochloric acid, add 125 cc. distilled water, 10 Gm. of ammonium chloride and 15 cc. of ammonium secondary phosphate T.S. Again heat to boiling, add methyl red T.S. and precipitate as before, adding ammonia T.S. to the boiling solution at the rate of one drop per 5 seconds until neutral and then 2 cc. excess. Let stand hot two hours, chill in ice and filter through the same Gooch as before. Wash thoroughly with water containing 1 cc. ammonia T.S. per 100 cc., dry in the oven a few minutes then ignite to constant weight at a bright red heat to $Mn_2P_2O_7$.

Each Gm. of $Mn_2P_2O_7$ is equivalent to 1.5849 Gm. of $MnC_3H_5(OH)_2PO_4$ or to 0.38695 Gm. of Mn.

Although the mechanics of precipitation are more exacting than in the sulfide method the increase in accuracy of the results is distinct. To illustrate the comparative results obtained by the two methods Table IV shows the results of several analyses by the two procedures on two samples of commercial manganese glycerophosphate. The results are expressed as per cent manganese.

TABLE IV.—MANGANESE CONTENT OF MANGANESE GLYCEROPHOSPHATE BY THE SULFIDE AND PYROPHOSPHATE METHODS.

	% Manganese.			% Manganese.	
Sample	$Mn_2P_2O_7$ Method.	Mn_3O_4 Method.	Sample	$Mn_2P_2O_7$ Method.	Mn_3O_4 Method.
A	23.8	24.4	B	25.1	25.2
	23.7	24.1		25.2	25.4
	23.7	24.1		25.1	25.7
	23.8	24.7		25.2	26.0

It is evident that the sulfide method gives values generally too high and varying over several tenths of a per cent while the results by the pyrophosphate method are readily reproducible to 0.1%.

CONCLUSION.

The assay method of the National Formulary VI for ferric glycerophosphate has been shown to give low results on account of the short reaction time allowed and the failure to specify a minimum temperature. A reaction period of 20 minutes at 35–40° C. prevents this difficulty.

The assay method of the National Formulary VI for manganese glycerophosphate is unworkable on account of the large excess of hydrochloric acid used to dissolve the sample. This difficulty is remedied by using 0.5 cc. instead of 5.0 cc. of hydrochloric acid. Even with this modification the method fails to give reliable results because of the variability of composition of manganous-manganic oxide. It has been shown that a double precipitation of manganese ammonium phosphate followed by ignition to manganese pyrophosphate gives much more reliable results.

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