ANALYSIS OF GLYCEROPHOSPHATES. II. DETERMINATION OF SMALL AMOUNTS OF ORTHOPHOSPHATES IN GLYCEROPHOSPHATES.*

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Present methods for the detection of traces of phosphates in glycerophosphates consist in the observation of the time required for the first formation of a precipitate of yellow ammonium phosphomolybdate. Such methods are evidently qualitative limit tests and not quantitative. It has been the experience of this laboratory over a period of years that the time of formation of such a complex precipitate as ammonium phosphomolybdate is dependent upon so many factors, some of which are beyond precise reproduction, that it is unreliable even for a qualitative limit test.

The presence or absence of a particle of dust or an air bubble, the manner of addition of the reagent, the rate of agitation—such factors as these may determine whether or not precipitation occurs within a given time. It is impossible to reproduce such conditions precisely. Thus it is not surprising that, as we have found, certain samples of glycerophosphates containing several tenths of a per cent orthophosphate pass the official N. F. VI limit tests while other samples containing but a few hundredths per cent fail to pass. Such a condition naturally leads to confusion in the trade. The need, therefore, for a sensitive quantitative routine method is apparent.

The literature concerning this analysis is fragmentary. One method proposed is that given in the "British Pharmaceutical Codex," 1934, page 982. It involves precipitation of the glycerophosphate together with the phosphate as the calcium salts from a hot solution neutral to thymol blue. The phosphate, present at this p_H value as the secondary salt, is converted into insoluble tricalcium phosphate and probably calcium primary phosphate. The latter may be determined alkalimetrically. This method, although simple and rapid, is hardly more accurate than 0.05 per cent (as P_2O_5) at its best.

On account of the small amounts of orthophosphates usually present in glycerophosphates it seemed best in developing a method of analysis to attempt to utilize the ammonium phosphomolybdate method as a quantitative procedure. Orthophosphates are precipitated quantitatively as yellow ammonium phosphomolybdate, $(NH_4)_3PO_4.12MoO_3$ from a warm nitric acid solution containing molybdic acid and ammonium nitrate. The method is ideally suited for minute amounts of orthophosphates since the precipitate is of constant composition and contains but 1.64 per cent phosphorus.

Unfortunately, unless conditions are adjusted carefully, precipitation of the ammonium phosphomolybdate may be incomplete, the precipitate may be of incorrect composition or it may be contaminated with molybdic oxide. The correct concentration of nitric acid, ammonium nitrate, molybdic acid and the proper temperature are rather easily obtained when the solution contains little except the small amount of phosphate being determined. However, many substances, among them glycerophosphates, decidedly retard the formation of the

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precipitate. In order to obtain complete precipitation in the presence of such substances it is necessary to employ a huge excess of precipitating reagent. This is particularly dangerous when glycerophosphoric acid is present, since it is hydrolyzed slowly into glycerol and orthophosphoric acid in acid solution.

The establishment of a procedure for the quantitative determination of small amounts of orthophosphate in glycerophosphate therefore involves adjusting the conditions to obtain complete precipitation of ammonium phosphomolybdate while preventing hydrolysis of the glycerophosphoric acid. This requires use of the minimum temperature, minimum time of standing and minimum concentration of nitric acid.

In order to ascertain the optimum conditions for the precipitation, a sample of sodium glycerophosphate was freed from phosphates by repeated fractional crystallization. To portions of this sample small known quantities of potassium monophosphate were added. Such knowns were treated with ammonium molybdate reagent, the yellow precipitate being determined gravimetrically. The concentration of molybdic acid and ammonium nitrate were so adjusted that complete precipitation occurred with the minimum concentration of nitric acid, the minimum temperature and the minimum precipitation period. After numerous trials a definite procedure was adopted. The method is as follows:

Precipitating Reagent:

Solution A. 100 Gm. molybdic anhydride A. R.
120 cc. ammonium hydroxide, concentrated
300 cc. distilled water
Dissolve the molybdic anhydride by warming. Add 380 cc. distilled water. Cool.

Solution B. 300 cc. nitric acid, concentrated
20 Gm. ammonium nitrate, A. R.
900 cc. distilled water.

Add solution A to solution B with constant stirring. Allow to stand at least 24 hours. Filter through a fine quantitative paper immediately prior to use.

This reagent must be stored in a cool place. At higher temperatures molybdic acid is precipitated, rendering the reagent insensitive. In case such precipitation occurs the reagent should be discarded. Procedure: Place in a 150-cc. beaker sufficient sample, usually 1 to 5 Gm., accurately weighed, to give no more than 0.15 Gm. yellow precipitate. Add 10 cc. distilled water. In the case of sodium, calcium and manganese salts add nitric acid drop by drop until acid to methyl orange and solution is complete; in the case of the ferric salt effect solution by warming on the steam-bath with constant stirring. If solution is incomplete filter, keeping the volume below 20 cc. In a second beaker warm to 55° C. *on the steam-bath* 100 cc. of freshly filtered precipitating reagent. Pour this solution into the beaker containing the sample. Stir, let stand fifteen to twenty minutes, filter through a Gooch crucible dried at 120–130° C., transferring the precipitate by means of a rubber policeman. Wash thoroughly with a solution of 5 cc. of nitric acid per 100 cc. of water. Dry to constant weight at 120–130° C.

$$\text{Percentage orthophosphate as } P_2O_5 = \frac{\text{wt. yellow precipitate} \times 0.0376 \times 100}{\text{wt. sample}}$$

This procedure is simple, rapid and readily applicable to routine control. It involves no more apparatus or technique than required in a simple gravimetric sulfate determination.

The results obtained by applying this procedure to samples of various glycerophosphates are given in Table I. Two types of determinations were made. The first involved addition of known amounts of phosphate, as potassium monophosphate to the sample of sodium glycerophosphate entirely free from phosphate; the second, addition of such known amounts to the filtrate after preliminary removal of any phosphate present originally.

TABLE I.—RECOVERY OF KNOWN AMOUNTS OF PHOSPHATE ADDED TO VARIOUS GLYCEROPHOSPHATES.

Salt.	Phosphate Added.	Phosphate Added, Per Cent P_2O_5.	Phosphate Recovered, Per Cent P_2O_5.	Error, Per Cent P_2O_5.
Sodium	Directly	0.056	0.058	+0.002
"	"	0.056	0.057	+0.001
"	To filtrate after pre-	0.010	0.009	−0.001
"	liminary separation	0.025	0.028	+0.003
"	of phosphate origi-	0.025	0.028	+0.003
Calcium	nally present	0.056	0.058	+0.002
"	"	0.056	0.061	+0.005
"	"	0.056	0.062	+0.006
Manganese	"	0.141	0.143	+0.002
"	"	0.141	0.145	+0.004
Iron	"	0.282	0.283	+0.001
"	"	0.113	0.114	+0.001

It is apparent that the method gives quantitative recovery with each of the four salts used, the errors ranging from 0.001–0.006 per cent as P_2O_5.

Establishment of the absence of hydrolysis of glycerophosphoric acid to glycerol and phosphoric acid under the conditions of precipitation was accomplished by reheating to 55° C. the filtrates from each of the above determinations and observing them for an additional 15 minutes. No further precipitation of ammonium phosphomolybdate was noticed in any case. On longer standing or at higher temperatures further precipitation occurred. The addition of as little as 0.0005 per cent phosphate as P_2O_5 resulted in immediate precipitation. This indicates that the amount of hydrolysis is too small to be considered.

In order to establish the reproducibility of the method a series of analyses was performed on various commercial samples of the different glycerophosphates. The results are given in Table II.

TABLE II.—DUPLICATE ANALYSES OF VARIOUS GLYCEROPHOSPHATES FOR SMALL AMOUNTS OF PHOSPHATE.

Salt.	Per Cent Phosphate as P_2O_5. Trial 1.	Trial 2.	Variation, Per Cent P_2O_5.
Sodium	0.004	0.003	0.001
"	0.017	0.017	0.000
"	0.095	0.095	0.000
Calcium	0.015	0.018	0.003
"	0.018	0.020	0.002
"	0.024	0.027	0.003
Manganese	0.026	0.028	0.002
"	0.203	0.209	0.006
Iron	0.336	0.338	0.002
"	0.162	0.159	0.003
"	0.094	0.093	0.001

It will be observed that the results are uniformly consistent, indicating that the method yields readily reproducible results.

CONCLUSION.

A rapid, simple precise method for the quantitative determination of orthophosphates in glycerophosphates, based upon their precipitation as ammonium phosphomolybdate, is described. It is accurate to ±0.003 per cent phosphate as P_2O_5. This is a distinct improvement over the customary tests depending upon the time of formation of ammonium phosphomolybdate which have been found to give erratic results.