Colorimetric Determination of Phosphate in Sugar Products

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A fairly rapid colorimetric method for determining both inorganic orthophosphate and total phosphate in sugar products has been developed. The test is sufficiently accurate for quality control work and suited to series determinations in both colored and colorless products. This method is based on the observation that a yellow color proportional to the amount of orthophosphate present forms when an excess of ammonium molybdate is added to an acidified solution of ammonium vanadate and orthophosphate. The color is measured at a wave length of 420 mμ.

No adequate rapid method for the quantitative determination of the amount of inorganic and total phosphate present in sugar products is available in the literature. In this study, a photometric ammonium molybdivanadophosphate test was used to develop a method suitable for this purpose. Such a method has been used for phosphorus determinations in steel (*10*), iron ore (*3*), limestone (*2, 11*), phosphate rock (*1*), plants (*8*), and fertilizers (*4*). Kitson and Mellon (*7*) have made an extensive investigation of this method.

Apparatus

Photovolt Lumetron Photoelectric Colorimeter, Model 402-E. A 420-mμ Corning Glass filter was used. Standard colorimetric test tubes, 18 mm. in outside diameter, were employed as sample holders.

Palo multipurpose shaking machine.

Reagents

Standard Phosphate Solution. Dissolve an appropriate amount of pure potassium dihydrogen phosphate in distilled water so that 1 ml. of the solution contains 0.10 mg. of phosphorus pentoxide.

Ammonium Vanadate Solution Containing 0.20% of Vanadate. Cautiously add 2.35 grams of ammonium metavanadate, NH_4VO_3, reagent grade, to 500 ml. of boiling distilled water. Next add 100 ml. of dilute sulfuric acid (1 to 12). After cooling the solution, transfer it to a 1-liter volumetric flask and dilute to 1 liter with distilled water.

Ammonium Molybdate Solution Containing 5% of Molybdenum Trioxide (MoO_3). Dissolve an appropriate amount of pure ammonium molybdate, $(NH_4)_6MoO_{24}.6H_2O$, reagent grade in distilled water.

Concentrated nitric acid, concentrated sulfuric acid, and dilute sulfuric acid (1 to 1).

Procedure

Preparation of Calibration Curve. Add 0 to 15 ml. of standard phosphate solution with a buret, covering the range from 0 to 1.50 mg. of phosphorus pentoxide, into sixteen 100-ml. volumetric flasks. Dilute each flask to about 50 ml. with distilled water. Pipet 7 ml. of dilute sulfuric acid (1 to 1) into each flask and shake. Pipet 10 ml. of vanadate solution and then 20 ml. of molybdate solution with shaking into each flask and finally dilute to 100 ml. with water. After allowing the standard solutions to stand for 15 minutes, determine the absorbance at 420 mμ for each solution after setting the instrument to read zero absorbance using the reagent blank. Finally, plot the absorbance readings *vs.* milligrams of phosphorus pentoxide on rectangular graph paper and draw the best straight line through the points.

Determination of Total Phosphate (Both Inorganic and Organic). Pipet 10 ml. of distilled water containing 1 gram of sugar into a 100-ml. borosilicate glass volumetric flask. Digest the sample with 30 ml. of concentrated nitric acid and shake continuously over a low flame until the volume of solution is reduced to about 5 ml. (about 20 minutes). Cool, add 7 ml. of concentrated sulfuric acid, and heat over a low flame with continuous shaking until the solution turns pale yellow to colorless and sulfur trioxide fumes are evolved (about 10 minutes). If charring occurs at this point (very rare), it is the result of incomplete destruction of the organic matter present. The best procedure in this case is to repeat the digestion being careful to heat the nitric acid off more slowly, to use more nitric acid, or to use a smaller amount of sample initially. When sulfur trioxide fumes are evolved, cool the flask, cautiously add 25 ml. of water, and boil the solution for about 10 minutes with continuous shaking. Cool and dilute the solution to mark with water. Pipet 50 ml. of this solution and no additional acid into another 100-ml. volumetric flask and then prepare a reagent blank solution by adding 50 ml. of water and 7 ml. of dilute sulfuric acid (1 to 1) to a third 100-ml. volumetric flask. If the sample to be tested is very low in phosphate content, add a known amount of phosphate at this point with a standardized buret in order to bring the absorbance of the test solution into the more sensitive range of the calibration curve. Next, add the vanadate and molybdate reagents and obtain the absorbance of the test solution as described under "Preparation of Calibration Curve."

Determination of Inorganic Orthophosphate. Follow the same procedure as for total phosphate, but omit the acid digestion steps and add 7 ml. of dilute sulfuric acid (1 to 1) to the test solution before adding the vanadate and molybdate reagents to the flask.

Discussion and Results

Absorption Behavior of Colored Ammonium Molybdivanadophosphate Complex. The colored complex was found to follow the Beer-Lambert law for the range 0 to 1.50 mg. of phosphorus pentoxide at a wave length of 420 mμ. This wave length was used because it is close to the wave length of maximum spread between a solution containing 0.5 mg. of phosphorus pentoxide

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per 100 ml. of solution and a blank solution. The full color intensity is developed in 15 minutes and then remains stable for at least 1 hour. Because this method was designed as a rapid test, this point was not studied further. The color is not very sensitive to temperature variations, as the absorbances of test solutions were measured at room temperature which varied to the extent of 4° with negligible error.

Stability of Reagents. All the reagents used in this method remain stable for at least 3 months.

Diverse Ions. Silicate, pyrophosphate, sulfate, chloride, nitrate, calcium, ferrous, ferric, magnesium, potassium, and sodium ions do not interfere with the method. To test for diverse ions, 1 mg. of each of the above ions was added to a solution containing 0.5 mg. of phosphorus pentoxide. It was observed that 1 mg. of each of these ions was equivalent to not more than 0.01 mg. of phosphorus pentoxide. These ions were added in the sodium or chloride form, except for ferrous which was added in the sulfate form.

However, silicate ion interferes seriously with the method if an acidity of 0.5*N*, which Kitson and Mellon found to be the optimum acidity, is employed. At an acidity of 0.5*N* 1 mg. of silicon dioxide added as sodium metasilicate is equivalent to about 0.7 mg. of phosphorus pentoxide. In the method described an acidity of 1.2*N* was used, thereby eliminating silicate interference. The presence of up to 20 grams of sucrose per 100 ml. of solution does not cause any interference with color development.

Acid Concentration. An acidity of 1.2*N* was employed. Sulfuric, hydrochloric, nitric, or perchloric acids may be used to obtain the proper acidity. In view of these properties, it was decided to use sulfuric and nitric acids as a suitable digestion medium rather than the more dangerous perchloric and nitric acids. Ma and McKinley (*9*) have found that digestion with a mixture of sulfuric and nitric acid is sufficient for the decomposition of various types of organic phosphate compounds. Hardin (*6*) has reported that the sulfuric-nitric acid digestion adequately effects complete solution and ionization of the phosphorus content in fertilizer.

Acid digestion rather than ashing is preferred as a method for destroying all organic matter in test samples because the former procedure converts all phosphates to orthophosphate at the same time as it removes all organic matter. Acid digestion is also more suited to series determinations, in that as many as five digestions may be carried out simultaneously, with very little attention on the part of the analyst, if a Palo multipurpose shaking machine is employed. The ashing method, on the other hand, converts some of the phosphates present to meta- or pyrophosphate, rather than orthophosphate. This leads to low total phosphate results, since only ortho-

Table I. Extent of Hydrolysis of Some Sugar Phosphates to Inorganic Orthophosphate after 15 Minutes' Standing at Room Temperature and on Acidity of 1.20*N*

Compound	% Hydrolyzed Range	% Hydrolyzed Average
Glucose-1-phosphate	5.5–6.2	5.8
Fructose-6-phosphate	6.0–6.5	6.4
Fructose 1,6-phosphate	9.0–9.8	9.5

Table II. Precision of Method for Total Phosphate and Inorganic Orthophosphate

Product[a]	Total Phosphate: Av. P_2O_5 content, mg.[b]	Total Phosphate: Av. dev., mg.	Inorganic Orthophosphate: Av. P_2O_5 content, mg.[b]	Inorganic Orthophosphate: Av. dev., mg.
Philippine raw sugar				
A	0.063	0.011	0.083[c]	0.015
B	0.107	0.008	0.190[c]	0.012
C	0.159	0.007	0.300[c]	0.010
D	0.210	0.004	0.295[c]	0.012
E	0.110	0.009	0.120[c]	0.015
F	0.104	0.010	0.132[c]	0.014
Cuban raw sugar				
A	0.026	0.012	...	...
B	0.038	0.014	0.061[c]	0.019
C	0.024	0.012	0.016[c]	0.016
D	0.052	0.011	0.080[c]	0.018
E	0.071	0.009	0.083[c]	0.023
Puerto Rican raw sugar				
A	0.044	0.013	0.068[c]	0.019
B	0.054	0.010	0.010[c]	0.008
Peruvian raw sugar	0.050	0.012	0.012[c]	0.008
Affination magma				
A	0.087	0.013	0.010	0.007
B	0.221	0.007	0.021	0.008
Affination sirup				
A	0.172	0.010	0.105	0.015
B	0.257	0.006	0.172	0.013
Phosphoric acid treated molasses, A	1.108	0.006	0.926	0.012
Phosphoric acid treated molasses, B	1.004	0.007	0.819	0.014
High remelt sirup				
A	0.496	0.013	0.298	0.017
B	0.434	0.010	0.276	0.019
Low remelt sirup				
A	0.778	0.012	0.479	0.013
B	0.852	0.010	0.506	0.012
Final molasses				
A	1.025	0.006	0.674	0.012
B	1.184	0.007	0.703	0.015
Brown sugar				
A	0.364	0.010	0.288	0.014
B	0.316	0.011	0.262	0.016
Granulated sugar				
A	...	...	0.058[d]	0.006
B	...	...	0.071[d]	0.005
C	...	...	0.120[e]	0.003
Partial invert sirup				
A	...	...	0.046[d]	0.004
B	...	...	0.051[d]	0.005
C	...	...	0.102[e]	0.003

[a] 0.500-gram samples used for all analyses unless otherwise indicated.
[b] Average of three analyses.
[c] 1.000-gram samples used.
[d] 10.000-gram samples used because sample colorless initially.
[e] 20.000-gram samples used because sample colorless initially.

phosphates are determined by the photometric method.

In testing for total phosphate, 7 ml. of concentrated sulfuric acid were used because it provides a suitable volume of digestion medium for a 1-gram sample of sugar and results in an acidity (1.2*N*) which eliminates silicate interference.

When testing for inorganic phosphate in the presence of organic phosphates as in the case for sugar products, the acid concentration of the test solution, along with its temperature and standing time, is of the utmost importance. Gee, Domingues, and Deitz (*5*) used an acidity of about 0.25*N* for their inorganic phosphate test. Spencer and Meade's method for inorganic phosphate (*12*) employs solutions of about 0.38*N*. The molybdivanadophosphate inorganic test can also be carried out at an acidity of 0.25*N* with good accuracy, provided silicates are absent. The calibration curves obtained using 0.25*N* and 1.2*N* acidity almost coincide.

To obtain an estimate of the extent of hydrolysis of sugar phosphates to inorganic phosphate using 1.2*N* acidity, the author selected the three compounds listed in Table I as representative sugar phosphates. Employing the currently described method, he then determined the inorganic phosphate content of each of these compounds four times using acidity of 0.25*N* and of 1.20*N*. From the literature it appears that negligible hydrolysis of sugar phosphates occurs at 0.25*N*. Therefore, the difference between the inorganic phosphate content found at these two acidities was taken as a measure of the extent of hydrolysis of these compounds due to the higher acidity in Table I.

Correction for Initial Yellow Color of Some Sugar Solutions. If a colored solution such as a raw sugar is to be tested for inorganic phosphate, consideration must be given to the fact that raw sugar solutions themselves are initially yellow in color. This can be accomplished by measuring the absorbance of 100 ml. of the raw sugar solution containing 7 ml. of dilute (1 to 1) sulfuric acid after setting the colorimeter at zero absorbance using distilled water at the same acidity. If this absorbance value is subtracted from the absorbance value obtained by adding vanadate and molybdate to a similar raw sugar solution, the corrected absorbance value is obtained.

Precision of Total Phosphate and Inorganic Orthophosphate Methods. To evaluate the precision of the total phosphate method 28 samples of various sugar products were analyzed in triplicate. As can be readily calculated from Table II, the standard deviation of total phosphate method is ± 0.010 mg. The precision of the inorganic orthophosphate method was evaluated by analyzing 27 samples of various colored sugar products and six colorless products. The standard deviation for colored products was found to be ± 0.015 mg., while that for colorless samples was calculated to be ± 0.005 mg.

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GOSSYPOL EXTRACTANTS

Oral Toxicity to Poultry of a Commercial Octylamine

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SEVERAL PROCESSES ARE BEING investigated in the Cottonseed Products Research Laboratory for removing gossypol from cottonseed meats with the aid of aliphatic amines during the ordinary extraction of oil from meats. Primary *n*-octylamine is the preferred amine so far. In all of the processes varying amounts of residue amine remain in the meal. The amounts are estimated to range from less than 0.1% up to about 0.4% of the weight of the meal.

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Knowledge of the general level of toxicity of *n*-octylamine was sought in order to determine whether complete removal of the residual amine from meal was necessary. If such were the case, the favored process would have to be substantially changed and a definite reorientation of research plans would be necessary.

This is a report on a preliminary investigation into the toxicity of a commercial primary *n*-octylamine, Armeen 8D (Armour & Co.) (*3, 4*). This amine has been used in much of the process development so far.

A considerable amount of toxicological data on aliphatic amines appears in the literature. With one exception (*6*), however, none of it refers specifically to octylamine. Most acute toxicological data appear to have been summarized in the "Handbook of Toxicology" (*7*). Some data from this handbook which are useful to the present investigation are reproduced in Table I.

Acute oral toxicity tests have been carried out on several acetate salts of aliphatic amines (*2*). The compounds tested were Armour's Armac C, Armac T, Armac TD, and Armac HT. The descriptions of these compounds have been given (*2*). They are manufactured from amines containing from 8 to 18 carbon atoms.