

REPORT ON MICROANALYTICAL DETERMINATION OF PHOSPHORUS

A preliminary study of methods for determining phosphorus in micro quantities was conducted during the year. The purpose of the study was to decide if any method was to be preferred on the basis of accuracy or precision, or both, and also to determine which procedures are most commonly used.

Two samples, diethyl octadecyl phosphate and alpha-casein, were sent to each collaborator with the request that four analyses be performed on each by the procedure normally used in his laboratory. Collaborators were also requested to report all analyses and to supply general information about the method used. Forms were provided for this purpose.

Sample 1, diethyl octadecyl phosphate, was a purified, stable, non-hygroscopic compound; its theoretical phosphorus content was 7.62 per cent.

Sample 2, alpha-casein, although not a pure compound of known composition, was used to test the interlaboratory precision on a material of low phosphorus content similar to that which would probably be obtained from plant and animal sources. The alpha-casein sample had to be analyzed in the air-dry state because, when it was oven-dried, it developed too high a static charge for convenient handling of micro samples. Each collaborator was asked to determine the moisture content of the alpha-casein by drying it for 3 hours *in vacuo* at 70–80°C.; to calculate the results on the moisture-free basis; and to report the per cent moisture found.

RESULTS

Eleven collaborators reported 42 and 39 phosphorus values, respectively, for the two samples; eight collaborators reported moisture values for the alpha-casein sample. A statistical summary of the results, plus some general information on the procedure, is presented in Table 1. In Table 1, n is the number of analyses; \bar{x} is the analysts' mean; s is the standard deviation; and $\bar{\bar{x}}$ and s_m are the mean and standard deviation of the \bar{x} values. Only 10 of the 11 means for Sample 1, diethyl octadecyl phosphate, were used; collaborator 64 stated that his results were low because he was unable to remove the ammonium phosphomolybdate quantitatively from the precipitation flask. The values for Sample 1 ranged from 7.40 to 7.66 per cent with a mean of 7.52 or 0.10 per cent less than the theoretical

TABLE 1.—Results and summary of phosphorus determinations

COLLABORATOR NO.	DIETHYL OCTADECYL PHOSPHATE ^a			ALPHA-CASHEIN			MOISTURE	METHOD	
	n	\bar{x}	s ^b	n	\bar{x}	s ^b		SAMPLE DIGESTION	P DETERMINATION
0	6	7.62	0.104	4	0.893	0.009	per cent 7.47 7.3 ^b	HNO ₃ (Carius)	Spectrophotometric
25	4	7.66	0.021	4	0.890	0.035	5.5	H ₂ SO ₄ -HNO ₃	Gravimetric
29	4	7.47	0.034	4	0.900	0.015	4.42	H ₂ SO ₄ -HNO ₃	Gravimetric
31	4	7.48	0.074	4	1.070	0.056	7.40	H ₂ SO ₄ -H ₂ O ₂	Gravimetric
37	4	7.58	0.125	4	0.938	0.025	—	H ₂ SO ₄ -HNO ₃	Gravimetric
49	4	7.40	0.104	4	0.820	0.040	7.28	H ₂ SO ₄ -HNO ₃	Gravimetric
51	3	7.58	0.021	3	0.950	0.000	9.66 ^b	H ₂ SO ₄ -HNO ₃	Spectrophotometric
57	4	7.49	0.045	4	0.905	0.006	7.39	HClO ₄ (60%)	Gravimetric
64	2	7.01 ^c	0.070	1	0.91	—	4.86	H ₂ SO ₄ -HClO ₄	Spectrophotometric
74	3	7.47	0.006	3	0.863	0.016	—	H ₂ SO ₄ -HClO ₄ -HNO ₃	Gravimetric
80	4	7.44	0.076	4	0.935	0.010	—	H ₂ SO ₄ -HClO ₄ -Na ₂ MoO ₄	Spectrophotometric
\bar{x}		7.52	(0.061)		0.915	(0.021)			
s _m		0.085			0.063				
Gravim.	6	7.53	0.095	7	0.925	0.076			
Spectro.	4	7.51	0.080	4	0.898	0.031			

^a 7.62% P. ^b Determined by Karl Fischer titration. ^c Value not included in calculations (see Discussion).

value of 7.62 per cent. The inter-laboratory standard deviation was 0.085 per cent, which is considered quite good. However, when the ten values were arranged in order of magnitude, it was noted that they roughly fell into two groups: one 7.58 to 7.66 per cent, which brackets the theoretical value, and the other 7.40 to 7.49 per cent. No values fell in between these two groups. It is difficult to determine the reason for these two groups because of the small number of values, but the sample digestion may be responsible. Nitric acid was the chief oxidant used for all 4 values in the higher group; in the lower group, perchloric acid was used for 3 of the 6 values, hydrogen peroxide was used for 1 value, and nitric acid was used for the other 2.

The eleven values for Sample 2, alpha-casein, ranged from 1.07 to 0.82 per cent with a mean of 0.915 per cent and an interlaboratory standard deviation of 0.063 per cent. The distribution of the means for Sample 2 was fairly normal and there was no indication that nitric acid was a better oxidant than perchloric acid. With the exception of one high and one low value, the interlaboratory agreement for this low phosphorus sample was very good.

The phosphorus was determined either gravimetrically as the ammonium phosphomolybdate or spectrophotometrically (three analysts used the molybdenum blue color reaction and one, Collaborator 80, used the yellow phosphovanadic molybdenum complex). The gravimetric method has the advantage of the very favorable gravimetric factor of 0.01452, but the precipitation conditions must be rather carefully controlled and a period of several hours is required for complete precipitation. The high sensitivity of the spectrophotometric method partially overcomes the advantage of the favorable gravimetric factor, particularly when the sample contains very small amounts of phosphorus. The spectrophotometric procedure is more applicable than the gravimetric when several determinations are made at one time. As shown at the bottom of Table 1, there is no marked difference between the accuracy or precision of the results obtained by the two methods.

Table 1 also shows the moisture values obtained for alpha-casein. The collaborators were asked to determine the moisture content by drying the sample at 70–80°C. for 3 hours *in vacuo*. This time was apparently not long enough for some of the drying systems used. In the Referee's laboratory, drying for 5 hours raised the moisture value by only 0.03 per cent over that obtained after 3 hours, yet drying for 1 hour gave a figure only 0.11 per cent less than that for 3 hours.

SUMMARY

All eleven collaborators used a wet digestion procedure to decompose the samples. The more frequently used sulfuric-nitric acid combination seemed to be preferable for the diethyl octadecyl phosphate sample. The

gravimetric and spectrophotometric methods gave comparable results for both samples. Intra- and interlaboratory precisions were generally good and the accuracy of the results obtained for Sample 1 was acceptable.

It is recommended* that the study of microchemical methods for phosphorus be continued.

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* For report of Subcommittee C and action of the Association, see *This Journal*, 39, 74 (1956).