JOHN PHILLIPS STREET, 1869–1938

JOHN PHILLIPS STREET

John Phillips Street was born January 30, 1869, in Beverly, N. J. He graduated from Rutgers College as a Bachelor of Science in chemistry and earned the Phi Beta Kappa key.

He entered the service of the New Jersey Agricultural Experiment Station at New Brunswick. In 1907, he went to the Connecticut Agricultural Experiment Station at New Haven, taking the place of A. L. Winton, who had entered the service of the Bureau of Chemistry of the United States Department of Agriculture.

During the war Street received a commission in the army in connection with food work. While in France he was reported as having been killed in action but this report was subsequently found to be erroneous, the unfortunate person being another John Street. Street retired from the army with the rank of major and went back to Connecticut. Shortly after this he resigned and went to Indianapolis to take charge of inspection work in that state for the National Canners Association, holding this post for three years. He then moved to Rochester as Executive Secretary of the New York State Canners Association.

He died in Rochester on September 22, 1938. He is survived by his mother, his wife, son, two grandchildren, his sister, and two brothers.

Street was an extremely valuable member of the Association of Official Agricultural Chemists, but did not attend the meetings for about the last twenty years of his life, during which period he was not doing official agricultural work, and for this reason was unknown to many of the younger members of the present generation. He attended his first meeting of the Association in 1890 (7th annual meeting), and with only one or two exceptions attended every subsequent meeting through the 34th meeting in 1917. His first work for the Association was a comparison of results on total phosphoric acid by magnesia mixtures of different composition (U. S. Dept. Agr., Div. Chem. Bull. 47, p. 62), presented at the 12th annual meeting in 1895.

At the 1896 meeting Street presented a Report on Nitrogen (U. S. Dept. Agr., Div. of Chem. Bull. 49, pp. 12–24), in which he first described the well-known Ulsch-Street method for determining nitric nitrogen. At the 1897 meeting as Reporter on Nitrogen Street presented his second Report on Nitrogen (U. S. Dept. Agr., Div. Chem. Bull. 51, pp. 15–26), and the Ulsch-Street method was made official (p. 29).

Street was appointed a member of the Abstract Committee for 1898, 1899, 1900, and 1901. He was elected Vice President for the 1906 meeting and President for the 1907 meeting. He was Chairman of Committee A on Recommendations of Referees at the 1905 meeting. He presented a paper on "The Detection of Peat in Commercial Fertilizers" (U. S. Dept. Agr., Bur. Chem. Bull. 105, pp. 83–85). In 1907 he was also Associate Referee on Cattle Feeds.

Street's Presidential Address for the 1907 meeting at the Jamestown Exposition, Norfolk, Va., is published in Bur. Chem. Bull. 116, pp. 28–34. In 1908 he presented a report as Referee on the Determination of Acidity in Cattle Feeds (Bur. Chem. Bull. 122, pp. 160–163).

He was Associate Referee on Vegetables and a member of Subcommittee A on Recommendations of Referees for the year 1909–1910. At the 1910 meeting he presented his report as associate referee on this subject (Bur. Chem. Bull. 137, pp. 122–134) and in association with C. B. Morison read a paper on "Ginger Extract" (*Ibid.*, pp. 76–79). He was appointed Chairman of the Appropriations Committee for 1911 and 1912, and Chairman of Subcommittee A on Recommendations of Referees for the year 1910–1911. He made reports as Chairman of this Subcommittee for the years 1912, 1913, and 1914. At the 1912 meeting he was also Chairman of the Committee on Participation in the Eighth International Congress of Applied Chemistry. He continued as Associate Referee on Vegetables during 1911 and 1912.

He was a member of the Special Committee on Editing Methods of Analysis

for the years 1913, 1914, 1915, and 1916, and a member of the first Board of Editors of *The Journal* of the Association for Vols. 1 and 2 (1915–1917). He was also a member of the Committee on Cooperation with Other Committees on Food Definitions for 1914, 1915, and 1916.

At the time Street went to Connecticut the food reports of the Connecticut Agricultural Experiment Station were among the few which food chemists desired to obtain and retain for their files. The reputation of these reports after Street came to Connecticut suffered no diminution. He began a systematic study of materials on the market, proprietary and otherwise, intended to be used by persons suffering from diabetes, and the published analyses attained an international reputation. This was before the days of insulin and long before the arrival of the present dietetic methods of treating persons afflicted with this disease. While at New Haven he also conceived the idea of publishing a book upon these proprietary and secret remedies, the analyses of which were scattered in various reports of persons engaged officially in examining such products. This compilation was published by the American Medical Association and was of inestimable value to those who were desirous of obtaining accurate knowledge of certain specific articles of this character.

No one person is competent to write concerning the character of another person. His family, his business subordinates, his business superiors, his neighbors, his social friends, and his intellectual friends all see a different side of his character.

My first meeting with Street was at the 1906 meeting of the official chemists at which time I was introduced to him by Winton. So indelibly was his appearance impressed upon my mind that I can now see him as he sat on the platform as vice president during the presidential address of Hopkins.

We subsequently became well acquainted. We visited each other's laboratories, but not each other's homes. We roomed together at many meetings of the A.O.A.C. and also met frequently at the meetings of the New England Food Control Officials of which for many years Street was the Secretary. We met at a few meetings of the National Association of Food and Drug Commissioners and of the American Chemical Society.

At the New Orleans meeting of the American Public Health Association he told many humorous stories of his experiences in France during the war and endeared himself to many of my New England friends engaged in public health work.

After I had attended a few meetings of the Association of Official Agricultural Chemists Street made me a member of the "Old Guard," which name he gave to a group of members of the Association, of which there are but few left. The "Old Guard" met on one evening of each Association meeting and reminisced. This group included Wiley, Frear, Trowbridge, Patrick, Mitchell, Hortvet, Doolittle, Ladd, Ross, Brackett, Bigelow, and others who have passed on. An evening with this group was an event to be remembered. A recent letter received from Mrs. Street states that she often heard Jack speak of the "Old Guard."

Street had many fine personal characteristics. Perhaps that for which I most admired him was that he was no gossip. I never heard him make a disagreeable statement about a man behind his back; if he had anything to say about a man, he said it to his face. He had a keen sense of humor. After returning from the war he remarked to a friend that he certainly enjoyed the obituaries resulting from the erroneous report that he had been killed. I heard him testify in New York City in the so-called Collier case. In cross examination he was asked, "Where is the mind?" He replied, "Ask a phrenologist."

It was men like Street that were largely responsible for making the Association of Official Agricultural Chemists what it is today.

Old friend, you have passed away. I well remember your voice, your aquiline countenance, your black mustache, your black hair with the one gray hair of which you were so justly proud.

HERMANN C. LYTHGOE

MONDAY—MORNING SESSION

REPORT ON ALCOHOLIC BEVERAGES

By J. W. SALE (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The analysis of alcoholic beverages was studied extensively during the past year, reports being submitted on fourteen topics. The general subjects given attention were malt, malt extract, malt adjuncts; beer; wines; distilled spirits, including whiskey and brandy; and cordials and liqueurs.

Methods for the analysis of beer and for the determination of carbon dioxide, volatile acids, sulfur dioxide, aldehydes, methanol, benzaldehyde, volatile esters, and the synthetic flavor, gamma undecalactone, in beer, wines, or distilled liquors were subjected to collaborative study. Methods for carbon dioxide and aldehydes were recommended for adoption as tentative methods. Study of the saponification of esters with lead acetate was discontinued, as it was found that lead acetate is ineffective in saponifying esters in acid form as they exist in wines. All the other subjects mentioned were recommended for further study next year, although in many instances the results obtained, when collaborative work was conducted, were gratifying. In one case, sulfur dioxide in alcoholic and carbonated beverages, it was found that the preparation of the samples for collaborative analyses presents a special problem, as the content of sulfur dioxide was found to change between the time of preparation and analysis of the samples.

The specific recommendations made by the Associate Referees follow.

RECOMMENDATIONS[1]

It is recommended—

(1) That the tentative pressure air method for the determination of CO_2 in beer (see p. 208) be adopted as tentative and further studied collaboratively with the object of making it official.

(2) That the Associate Referee on Beer study collaboratively the following tentative methods relating to beer (Chap. XIV):[2] (a) Extract in original wort; (b) real degree of fermentation; (c) total acid; (d) dextrin; (e) direct polarization; (f) pasteurization; and (g) chlorides; and that he also include hydrogen-ion concentration in this study.

(3) That methods for the determination of heavy metals (Fe, Cu, Pb), As, and fluorine be studied.

(4) That the viscometric method for the determination of the proteolytic activity of malt outlined by the associate referee last year (*This Journal*, **21**, 160) and the edestin titration method (*Wochschr. Brau.*, **53**, 297) be further studied.

(5) That the vacuum method for the determination of moisture in

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 65 (1939).
[2] *Methods of Analysis*, *A.O.A.C.*, 1935.

flour (p. 206, 2)[2] be studied as to its applicability to the determination of moisture in malt adjuncts (p. 161, 53).[2]

(6) That a special study be made of methods for the determination of fat that will be applicable to corn grits and brewers' rice and flakes.

(7) That a study of the method for determining the extract in malt adjuncts be made and that consideration be given to the suggestions to use a portion of the malt in the boiling operations.

(8) That special study be made of the diastatic activity of malt.

(9) That the study of methods for the detection of adulteration of distilled spirits be continued.

(10) That the collaborative study of sulfur dioxide in beer and ale be continued and that the study be extended to include a collaborative study of sulfur dioxide in wines.

(11) That further collaborative work be done on the tentative method for the determination of benzaldehyde (p. 183, 55), volatile esters (p. 181, 46) and gamma-undecalactone (p. 181, 47) in cordials.

(12) That the study of saponification of esters with lead acetate be dropped.

(13) That the distillation procedures for volatile acids in wines (p. 166, 23, 24) be studied further with a view to eliminating chance errors and that the modification described in the associate referee's report or some other modification of the Peynaud procedure be tested further.

(14) That the sulfite method for the determination of aldehydes in whiskey and other potable spirits be made tentative and that work on it be continued with a view to making it official.

(15) That study on the determination of total sulfur in wines be conducted.

(16) That the evaporation method described in the associate referee's report, and A.O.A.C. Method II (p. 167, 24)[2] for volatile acids in wines, be further studied to determine their applicability to distilled spirits, and that the cause of the slight loss resulting from the use of Method II be investigated.

(17) That the procedure for the quantitative determination of methanol in distilled spirits by the use of the neutral wedge photometer described in the associate referee's report be subjected to collaborative study for possible further improvement and simplification.

REPORT ON DIASTATIC ACTIVITY OF MALT

By CHRISTIAN RASK* (Albert Schwill & Co., Chicago, Ill.),
Associate Referee

The method for the determination of diastatic power of malt adopted as tentative by this Association and also as the official method of the

* Presented by J. A. LeClerc.

American Society of Brewing Chemists[1] is subject to much criticism because of the wide variations in results obtained in collaborative determinations.

According to Sallans and Anderson[2] the chief cause for the variations is the use of Fehling's solution for the titration of the converted starch solution. The method does not define in sufficient details the quantity of solution to be added for the preliminary boiling, neither does it specify the total boiling time, and it is doubtful whether it is at all possible to standardize a manipulation that would enable the individual analysts to perform the titration in identical manner.

Anderson and Sallans[3] developed a procedure based upon the present method of analysis with one exception—it depends upon the reduction of alkaline ferricyanide for the measurement of the converted starch. In a recent paper Laufer, Schwarz, and Laufer[4] recommended certain modifications of this method: the use of 1 cc. of malt infusion for malts of both low and high diastatic power, and a new basis for the calculation of maltose units and degrees Lintner.

During the past year the Associate Referee has studied the ferricyanide method outlined by Anderson and Sallans and the revised method developed by Laufer, Schwarz, and Laufer, and finds that more accurate results can be obtained with these than is possible with the methods based upon the titration with Fehling's solution.

Because the American Society of Brewing Chemists and also the Malt Analysis Standardization Committee of the American Association of Cereal Chemists have appointed special committees for the purpose of studying the numerous methods developed during recent years, no independent collaborative work has been done under the auspices of this Association.

The Associate Referee is in close cooperation with these two organizations, and in consideration of their current studies it is recommended that the present tentative method of this Association be retained as such for another year. It is expected that a method can be recommended at the next annual meeting and that it may be adopted as official before the publication of the fifth edition of *Methods of Analysis, A.O.A.C.*

No formal report on proteolytic activity of malt was given by the associate referee.

No report on malt extract in malt was given by the associate referee.

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[1] *A.S.B.C. Official Methods*, 1937.
[2] *Cereal Chem.*, **14**, 708 (1937).
[3] *Can. J. Research*, **15**, 70 (1937).
[4] *Am. Brewer*, June, 1938.

REPORT ON MALT ADJUNCTS

By F. P. SIEBEL, JR.* (J. E. Siebel Sons' Company, Chicago, Ill.),
Associate Referee

Methods for the analysis of malt adjuncts have been studied in great detail by the American Society of Brewing Chemists, and the Associate Referee has been in close contact with this work. Under the general heading of Methods for Analysis of Raw and Processed Cereals, the A.S.B.C. has adopted procedures with reference to Sampling, Physical Characteristics, Moisture, Oil, and Extract.

In the interest of avoiding needless duplication of efforts, close cooperation between the two organizations appears to be of great advantage to both. It is therefore recommended that the above identified methods of the A.S.B.C. receive consideration for adoption by the A.O.A.C. in the near future.

While extensive collaborative work has already been conducted on these methods by the A.S.B.C., all requirements demanded by the A.O.A.C. will also be fulfilled. To this end, further collaboration is planned, not only among members of the A.S.B.C., but also among the collaborators on alcoholic beverages of the A.O.A.C. It is hoped that the progress of this work will be such that the methods can be recommended for adoption by the A.O.A.C. as tentative, and be included in the next printing of *Methods of Analysis*.

It is also anticipated that in the near future, further studies pertaining to malt adjuncts will suggest themselves. In particular it appears to be desirable to make a further study of the influence that differences of manufacture or processing of products such as grits and flakes have on the readiness with which their oil content yields to quantitative estimation, and on the advantages or disadvantages of substituting enzyme preparations for the ground malt in the determination of extract.

REPORT ON BEER

By HUGO W. ROHDE* (Jos. Schlitz Brewing Company, Milwaukee, Wis.),
Associate Referee

In June, 1938, letters were addressed to thirty-three individuals, inviting them to participate in collaborative work on beer. Eighteen responded favorably, and to these people were sent five bottles of one beer and mimeographed copies of the methods of analysis to be used in making the determinations. Fourteen persons submitted the results of their findings, and these results are incorporated in this paper. To the

* Presented by J. A. LeClerc.

following collaborators who participated the Associate Referee wishes to express his appreciation.

Arthur E. Burhenn, Baltimore, Md.
D. Frederick Burnett, Newark, N. J.
N. J. Menard, Washington, D. C.
Morris A. Pozen, New York City
J. Bernard Robb, Richmond, Va.
Jos. Schlitz Brewing Company, Milwaukee, Wis.
Schwarz Laboratories, New York City
E. A. Siebel and Company, Chicago, Ill.
Stephl Laboratories, Milwaukee, Wis.
Wahl-Henius Institute, Chicago, Ill.
J. E. Siebel Sons' Company, Chicago, Ill.
Wallerstein Laboratories, New York City
J. B. Wilson, Washington, D. C.

Some of the collaborators submitted data of determinations made in duplicate and triplicate, and a number did not complete all the determinations outlined owing to lack of the necessary apparatus.

COMPARISON OF RESULTS

Close agreement, within the error of experiment, is shown in the data submitted by laboratories in which beer analysis is a daily routine. The following comments relate to the results obtained, and they follow the order given on the sheets sent to the collaborators.

Apparent Extract.—This was obtained by taking the specific gravity of the decarbonated beer at 20°/20° C., either with the Reischauer pycnometer, or with the Boot vacuum jacketed pycnometer (the latter type preferred) and obtaining the corresponding values from the Plato tables. Seventeen results ranged between 3.26 per cent and 3.54 per cent, averaging 3.40 per cent. Twelve results were within 0.05 per cent (plus or minus) of the average.

Alcohol (Per Cent by Weight).—Seventeen results ranged from 3.54 to 3.85 per cent by weight, the average being 3.72 per cent. Data from nine brewing laboratories varied from 3.64 to 3.80 per cent, averaging 3.71 per cent.

Real Extract.—The results of seventeen determinations ranged from 4.51 to 5.29 per cent. If the three lowest figures are omitted, the average is 5.15 per cent. Nine determinations were within 0.05 per cent (plus or minus) of the average.

Extract of Original Wort.—This is the concentration of the malt liquor before fermentation. The original formula, according to which this calculation was made, was devised by Carl Balling several generations ago, and is still considered to be quite accurate. It is based upon the amount of alcohol formed in fermentation, the unfermented extract present in the beer, the carbon dioxide produced, and the yeast formed during the fermentation of the wort. Later investigations have shown that the value

of the yeast multiplication is too high. The original extract of the wort can readily be calculated from the values of the alcoholic content and the real extract of the beer, namely:

2 Alcohol + Extract ± Correction.

For the average American beer the product of the alcohol by weight times 1.93 plus the real extract gives the original extract of the wort. If the latter computation is used in connection with the beers here given, the values of the original extract varies but 0.05 per cent in most of the cases from the values calculated according to the original formula.

The minimum for the original extract was 11.7 per cent; the maximum, 12.5 per cent; and the average 12.1 per cent.

Real and Apparent Degrees of Fermentation.—These values are computed for the real and apparent extracts and the original extract of the wort. The data in these tables show fairly close results.

Real Extract: 55.0–62.1 per cent, average 58.2 per cent.

Apparent Extract: 70.7–72.6 per cent, average 71.8 per cent.

Ash.—Seventeen results ranged from 0.13 per cent to 0.17 per cent. If the only high value is omitted, the average of sixteen determinations is 0.14 per cent.

Phosphoric Acid (P_2O_5).—Twelve data were submitted, varying from 0.026 per cent to 0.050 per cent; eleven data were between 0.045 per cent and 0.050 per cent, averaging 0.047 per cent. This determination was made according to the official A.O.A.C. method. One collaborator commented on this method, stating that the results obtained were too low, and that he preferred the gravimetric method.

Reducing Sugars.—The official method was used, and fourteen determinations varied from 1.19 per cent to 1.38 per cent, the average being 1.30 per cent when the two lowest figures are omitted.

Dextrin.—Twelve determinations for dextrin gave a minimum of 2.44 per cent, and a maximum of 2.84 per cent, averaging 2.66 per cent.

Protein.—With one exception, the figures of eleven protein determinations ranged between 0.33 per cent and 0.39 per cent, averaging 0.34 per cent.

Iodine Reaction.—This determination is made in order to ascertain whether or not the carbohydrates of the malt-wort have been properly converted. The test is subjective, and depends largely upon the observer. Five collaborators reported no reaction with iodine, and six reported an erythrodextrin (reddish coloration) reaction. From a technical viewpoint only a blue or violet coloration is objectionable.

Carbon Dioxide.—A method for the determination of carbon dioxide in beer has been developed by Philip P. Gray of the Wallerstein Laboratories. This method was submitted to the collaborators and six made eleven determinations. The minimum found was 0.42 per cent of CO_2 by weight, the maximum, 0.51 per cent, and the average, 0.46 per cent. This

Collaborative results on beer

	1		2		3	4		5	6	7	8	9	10	11	12	13	14
Specific gravity (20°/20° C.)*	1.01363	—	1.01292	1.01322	1.01330	1.01280	1.0130	1.01373	1.01336	1.01346	1.01336	1.01330	1.01330	1.01352	1.01358	1.01347	1.01332
Apparent extract (%)	3.48	3.43	3.30	3.38	3.40	3.26	3.37	3.54	3.41	3.44	3.42	3.39	3.40	3.45	3.44	3.50	3.40
Alcohol (% by weight)	3.58	3.57	3.85	3.84	3.78	3.85	3.83	3.54	3.64	3.73	3.80	3.75	3.78	3.66	3.70	3.65	3.68
Alcohol (% by volume)	4.58	4.56	4.92	4.91	4.85	4.94	4.90	4.45	4.67	4.79	4.88	4.80	4.78	4.70	4.65	4.60	4.63
Real extract	5.07	5.18	5.27	5.29	4.89	4.51	4.53	5.23	5.13	5.12	5.13	5.12	5.14	5.13	5.17	5.10	5.07
Reducing sugars, maltose (per 100 ml.)*	1.33	—	1.27	1.27	1.25	—	—	1.30	1.33	1.20	1.31	1.28	1.31	1.32	1.34	1.19	1.38
Dextrin (%)	2.72	2.68	—	—	2.46	—	—	2.71	—	2.74	2.84	2.73	2.62	2.44	2.65	2.60	2.82
Protein (N×6.25) (%)	0.51	—	—	—	0.34	—	—	—	0.34	0.33	0.39	0.34	0.34	0.32	0.35	0.35	0.34
Ash (%)	0.14	0.14	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.14	0.17	0.14	0.14	0.13	0.14	0.14	0.14
Phosphoric acid (P_2O_5) (%)	0.046	0.044	0.047	0.047	0.048	—	—	—	0.045	0.050	0.026	0.050	—	0.046	0.048	0.046	—
Total acidity as lactic acid (%)	0.15	0.15	—	—	0.13	—	—	0.14	0.10	0.14	0.11	0.13	0.14	0.15	0.14	0.13	0.13
Carbon dioxide (% by weight)	0.42 0.44	0.47 0.51	—	—	—	—	—	—	0.48	0.47, 0.45, 0.47	—	0.46	0.49	0.46	0.47, 0.50	—	—
Carbon dioxide (% by volume)	2.17 2.26	2.40 2.66	—	—	—	—	—	—	2.45	2.42, 2.32, 2.42	—	2.35	2.51	2.35	2.42, 2.55	—	—
pH value	4.40	4.40	—	—	4.24	—	—	—	4.40	4.35	4.55	4.40	4.45	4.43	4.44	4.50	4.42
Iodine reaction	0	0	—	—	0	—	—	Erythro	Erythro	0	0	Erythro	0	0	Erythro	Erythro	Erythro
Sulfur dioxide (SO_2) (mg. per l.)	4.10	4.10	—	—	5.48	—	—	—	3.4	7.6	3.3	5.0	—	14.9	5.8	—	—
Iron (Fe) (p. p. m.)	0.20	0.20	—	—	0	—	—	—	0	<0.5	0.55	0.6	<0.2	0.3	0.25	0.25	—
Color (Lovibond S. 52, 1/2" Cell)	—	—	—	—	—	—	—	—	3.7	4.3	3.0	4.5	4.3	4.25	3.75	4.3	4.25
Calculated data:																	
Original extract (%)	11.9	11.9	11.7	11.7	12.1	11.9	11.9	12.1	12.2	12.3	12.4	12.4	12.5	12.2	12.4	12.1	12.2
Real Degree of Fermentation (%)	57.4	56.4	55.0	55.6	59.5	62.1	61.9	56.7	58.0	58.5	58.6	58.6	58.8	58.0	57.9	57.8	58.4
Apparent degree of fermentation (%)	70.8	71.2	71.8	71.1	71.9	72.6	71.7	70.7	72.0	72.0	72.4	72.6	72.8	71.7	72.1	71.0	72.1

* Official method.

corresponds to 2.17–2.66 per cent by volume. The average is 2.38 per cent by volume. It happens occasionally that the closure on the bottle is not perfectly tight, and a loss of gas results. The bottles sent out for analysis were carefully examined in this respect.

Color.—The determination of color is a subjective matter. Tests have shown that the glasses of the Lovibond tinctometer are not absolutely correct. The color ranged from 3.0 to 4.5. If the lowest value is omitted, the average is 4.17.

Acidity.—The total acidity of thirteen determinations reported ranged from 0.10 per cent to 0.15 per cent, averaging 0.13 per cent. Omitting the two lowest results, eleven determinations averaged 0.14 per cent.

Hydrogen Ion Concentration or pH Value.—Twelve collaborators reported results varying from 4.24 to 4.55, and averaging 4.35.

Sulfur Dioxide (SO_2).—Seven determinations averaged 5 mg. of SO_2 per liter, ranging from 3.4 to 7.49, the highest figure being excluded. One collaborator stated that collecting 70 cc. of distillate from 200 cc. of beer is insufficient for this determination.

Iron.—Iron dissolved in beer and present in more than appreciable quantities will impair its durability. The colorimetric determination made by eleven analysts gave 0–0.6 p.p.m.

CONCLUSIONS

The fifteen determinations outlined in this work cover practically all the analytical tests required for judging the quality of a beer. In addition, a microscopic and bacteriological examination and a determination of dissolved air may be necessary. The practice of using antiseptics is no longer resorted to as modern brewery equipment and proper supervision have made this practice obsolete.

Methods of Analysis, A.O.A.C., 1935, in Chapter XIV on "Malt Beverages, Sirups and Extracts, and Brewing Material" contains: Par. 11, Volatile Acids; 14, Direct Polarization; 15, Glycerin; 27, Chlorides; and 28, Methyl Alcohol. These may all be omitted in future editions.

The American Society of Brewing Chemists has been actively engaged for several years in formulating methods for the analysis of beer. At the May, 1939, meeting, no doubt action will be taken on the adoption of certain methods for analysis that are now being studied. This being the case, the Associate Referee will refrain from making suggestions as to recommendations[1] in connection with the analysis of beer at this time.

No report on heavy metals in beer was given by the associate referee.

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 65 (1939).

REPORT ON CARBON DIOXIDE IN BEER

By P. P. GRAY (Wallerstein Laboratories, New York City),
Associate Referee

The tentative A.O.A.C. method for the determination of carbon dioxide in beer, *Methods of Analysis, A.O.A.C.*, 1935, 151, is somewhat time-consuming. A shorter method, suitable for laboratories equipped with the necessary apparatus, is the pressure-air method recently described by Gray and Stone.[1]

Last year it was decided to compare the two methods, and accordingly collaborative work by seven different laboratories was conducted. Two samples, representing beer of two different air contents, were sent to the collaborators. The air content of Sample No. 1 varied from 1.5 to 3.0 cc. per bottle, while the No. 2 beer contained from 6 to 10 cc. per bottle. The CO_2 results are given in Table 1.

TABLE 1.—*Collaborative results on CO_2*
(Results expressed as per cent)

COLLABORATOR	1	2	3	4	5	6	7	GRAND AVERAGE
Sample No. 1								
A. Chemical	0.482	0.471	0.43	0.423	0.504	0.45	0.478	
Method	0.485	0.443	0.45	0.383*		0.46	0.480	
	0.483	0.436	0.46	0.467		0.46	0.477	
Average:	0.483	0.450	0.45	0.445	0.504	0.457	0.478	0.467
B. Pressure	0.46	0.473	0.47	0.491	0.45	0.46	0.475	
Method	0.47	0.503	0.44	0.481	0.45	0.46	0.479	
	0.47	0.474	0.42	0.466		0.45		
Average:	0.47	0.483	0.44	0.479	0.45	0.457	0.477	0.465
Sample No. 2								
A. Chemical	0.365	0.336	0.34	0.302	0.335	0.34	0.359	
Method	0.366	0.350	0.34	0.280	0.361	0.34	0.360	
	0.368	0.345	0.34	0.311		0.35	0.358	
Average:	0.366	0.344	0.34	0.298	0.348	0.343	0.359	0.343
B. Pressure	0.37	0.314	0.35	0.341	0.32	0.34	0.357	
Method	0.36	0.368	0.33	0.343	0.34	0.34	0.356	
	0.36	0.368	0.35	0.344		0.34	0.334	
Average:	0.36	0.350	0.34	0.343	0.33	0.34	0.349	0.345

* Not included in averages.

Owing to the simplicity of the pressure method, most brewing laboratories prefer it to the chemical method. As a result, few of the collaborating laboratories have had much experience with the chemical method. This fact probably accounts for some of the variations apparent in the tabulated results.

[1] *Ind. Eng. Chem. Anal.* Ed., 10, 15 (1938).

Where the method had been used to any extent much better agreement was found to be the rule, for example in the results obtained in the Associate Referee's laboratories (Column 1). However, the results of the individual collaborators show a generally good agreement, and only minor variations are shown in the results of all the collaborators. In general sufficiently good agreement is apparent in the pressure method results to warrant recommendations that this method be adopted as a tentative method.

The complete directions for the pressure-air method, which was adopted as tentative, follow. Inadvertently the first part of the method was omitted when it was published in *This Journal*, **22**, 73 (1939).

CARBON DIOXIDE IN BEER

Pressure Air Method

APPARATUS

Piercing apparatus.—A gas tight packing box and fastening for adjustment over the crown of the bottle, which holds a hollow spike. (A suitable apparatus may be obtained from a number of manufacturers.) With a can a metal frame, the top of which is pressed or screwed down and locked over the can top, holds a hollow spike surrounded by a compressible rubber sealing plug. The spike leads to an accurate pressure gage and an outlet valve.

One apparatus, adjustable for bottles and cans, may be used.

Absorption buret.—The buret as shown in Figure 1 consists of a graduated tube (0–6 cc. graduated in 0.1 cc. divisions and 6–25 cc. graduated in 0.2 cc. divisions), having a bulb, and closed at each end by stop cocks, The upper end is connected by rubber tubing to the outlet valve of the piercing apparatus and the lower end is connected by a length of rubber tubing to a leveling bulb.

DETERMINATION

If the sample is in a bottle, make a scratch mark at the beer level; if the sample is in a can, weigh the can with the contents. Submerge the container in a water bath at 25° C. long enough to bring the temperature of the beer to 25° C. Connect the piercing apparatus to the bottle or can. Fill the absorption buret with 15% NaOH solution and allow the solution to run up to stopcock B. Fill the upper capillary of the absorption bulb with hexyl alcohol and the remainder of the system between B and the tip of the spike with water in order to displace any air. With outlet valve A closed, drive the spike through the crown or can top and thoroughly shake and tap the bottle or can. Make pressure reading on the gage. Again shake and take pressure readings. Use the pressure reading that shows no change in consecutive readings.

Open stopcocks B and C of the absorption buret and then outlet valve A. Allow the gas, together with foam, to flow over into the absorption buret. Swirl contents of the buret to permit absorption of CO_2. When one-half to three-fourths of the alkali solution in the absorption buret has been displaced, shut off all the stopcocks and shake to permit complete absorption of CO_2. Set the buret in a vertical position, open the bottom stopcock, C, and allow alkali to flow back into the bulb. Open stopcocks B and A and repeat the above operation, tapping the bottle or can to accelerate evolution of CO_2. Close the upper stopcocks A and B and shake thoroughly to absorb the last traces of CO_2. Bring the leveling bulb to such a position that the height of the solution in the leveling bulb and buret are the same and

read the unabsorbed gas, which is reported as "air." Repeat the operations until consecutive readings as to "air" are the same.

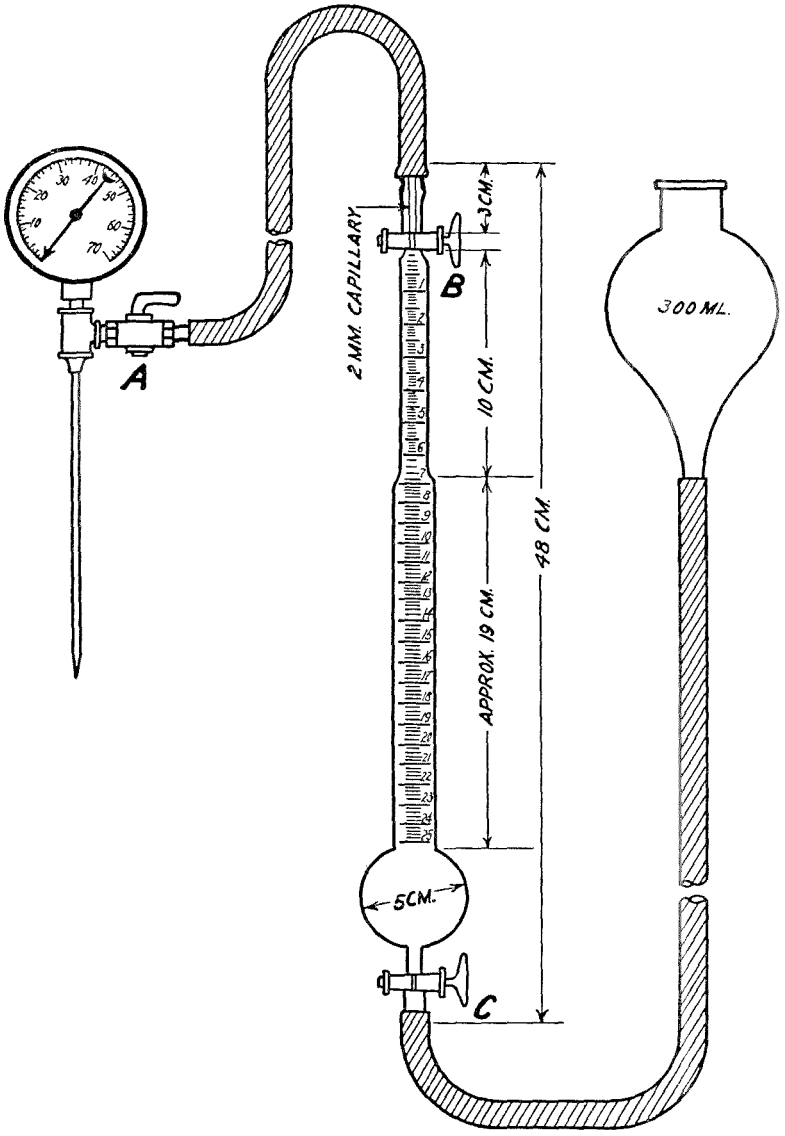

FIG. 1.—ABSORPTION BURET

Disconnect the bottle or can and determine the head-space volume as follows: If the sample is a bottle, fill with water to the top and pour off into a graduated cylinder to the scratch mark. The number of cc. of water thus poured off represents head space in cc.

If the sample is a can, weigh empty can after pouring out all remaining beer. The difference represents the weight of beer, which divided by the sp. gr. of the beer will give volume of beer in cc. Fill the empty can with water and weigh. Weight of water in grams is also the volume in cc., so that the difference between volume of water and volume of beer represents head space in cc.

Calculate CO_2 by weight by the following formula:

$$\%CO_2 = \left[P - \left(\frac{\text{cc. of air}}{\text{cc. of head space}} \times 14.7 \right) \right] \times 0.00965, \text{ in which}$$

P = absolute pressure in pounds per sq. in. at 25° C. = (ordinary gage pressure +14.7). (For routine work 15 may conveniently be substituted for 14.7.)

It is recommended that the pressure-air method for the determination of carbon dioxide in beer be adopted as a tentative method and that study of the method be continued.

REPORT ON WINES

By B. G. HARTMANN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

No work was done on total sulfur in wines.

Sufficient work was done on the saponfication of esters with lead acetate to show that acid ethyl tartrate is not saponified by treatment with lead acetate solution. The reaction in the case of diethyl tartrate proceeds rapidly, but the second ethyl group remains untouched even on continued heating. Since the esters in wines exist in the acid form, it is evident that for the purpose of their saponification treatment with lead acetate is ineffective.

RECOMMENDATIONS[1]

It is recommended—

(1) That the study on the determination of total sulfur be continued.

(2) That the study of the saponification of esters with lead acetate be dropped.

REPORT ON VOLATILE ACIDS IN WINE

By M. A. JOSLYN, (University of California, Berkeley, Calif.), *Associate Referee*

In the first report by this Associate Referee, *This Journal*, 21, 166 (1938), it was pointed out that the distillation methods used at present yield results that are reproducible by different chemists to not better than ±0.006 gram of acetic acid per 100 cc. on the average (corresponding to a titration error of 0.1 cc. of 0.1 *N* base per 10 cc.

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 65 (1939).

aliquot of wine). Unexplainably large departures from this mode often occurred, particularly with dry red wine of fairly high volatile acidity.

To obtain more information as to sources of these variations the following six samples were sent out for analysis on September 1, 1937:

SAMPLE	DESCRIPTION	SP. GR. 20/20	ALCOHOL	TOTAL ACIDITY AS TARTARIC
A	Solution of acetic acid in water	—	—	0.121*
B	Synthetic wine containing per 100 cc., approx. 0.10 g. of acetic acid, 5 g. of dextrose, 0.4 g. of malic acid, 0.3 g. of cream of tartar, and 13% of alcohol	1.200	12.6	0.648
C	Dry white wine	1.190	14.5	0.706
D	Dry red wine	1.309	14.8	0.750
E	Port type	1.466	20.3	0.735
F	Sherry type	1.084	21.0	0.550

* 0.097 gram of acetic acid per 100 cc.

The wines were thoroughly mixed and then filled into 6 oz. crown-capped bottles. The bottles were filled completely to avoid changes in the volatile acidity, particularly in the artificial wines. Samples were sent to the same thirty collaborators, but results were obtained only from the twelve mentioned. The date of the analysis is given because it was found that Sample D became infected with acid-producing organisms.

COLLABORATORS

Steward Berkshire, Deputy Commissioner of Internal Revenue, U. S. Treasury Department, Washington, Reported October 2, 1937.

Lyman Cash, Chemist, B. Cribari and Sons, Madrone, Calif. Reported September 17, 1937.

J. M. Curtis and Son, Wine Analysts for California Department of Health, Bureau of Food and Drug Inspection (through Milton P. Duffy, chief). Reported October 6, 1937.

Edwin N. Davis, Junior Chemist, Food Research Division, U. S. Bureau of Chemistry and Soils. Reported November 29, 1937.

Ray Dunn, Laboratory Assistant, Fruit Products Division, University of California (now with State Department of Chemistry at Sacramento, Calif.). Analyzed in October.

Robert A. Greene, Director, Arizona State Laboratory, Tucson. Reported October 13, 1937.

R. F. Love, Field Chemist, Internal Revenue Service, U. S. Treasury Department, San Francisco. Reported September 18, 1937.

Anna E. Mix, Beverage Section, Food Division, U. S. Food and Drug Administration. Analyzed November 1937.

J. Bernard Robb, Chemical Director, Virginia Alcoholic Beverage Control Board, Richmond. Reported October 11, 1937.

Bertha Schwartz, Schenley Products Company, New York City. Reported October 1, 1937.

T. E. Twining, The Twining Laboratories, Fresno, Calif. Reported September 15, 1937.

TABLE 1.—*Results (expressed as gram of acetic acid per 100 cc.) on volatile acidity of acetic acid solutions and wines obtained by several specified methods*

COLLABORATOR	A (ACETIC ACID)				B (SYNTHETIC WINE)				C (DRY WHITE)			
	1	2	3	4	1	2	3	4	1	2	3	4
S. Berkshire	0.090	0.084	0.084	0.088	0.095	0.090	0.096	0.096	0.095	0.090	0.093	0.090
L. Cash	0.080	0.087	0.084	0.087	0.084	0.096	0.096	0.096	0.085	0.102	0.102	0.099
J. Curtis	0.091	0.090	0.090	0.092	0.094	0.096	0.093	0.096	0.096	0.084	0.084	0.089
E. N. Davis	0.079	0.084	—	0.092	—	0.092	—	0.096	0.062	0.085	—	0.093
R. Dunn	—	0.092	0.092	0.092	—	0.094	0.098	0.095	—	0.092	0.091	0.093
R. A. Greene	0.085	0.076	—	0.085	0.085	0.085	—	0.085	0.085	0.079	—	0.085
R. F. Love	0.089	0.090	0.084	0.087	0.090	0.096	0.090	0.093	0.094	0.091	0.087	0.090
Anna E. Mix	0.085	0.083	0.085	0.079	0.090	0.096	0.085	0.079	0.092	0.090	0.087	0.090
J. B. Robb	0.089	0.093	0.090	0.084	0.092	0.135*	0.090	0.093	0.096	0.099	0.097	0.084
Bertha Schwartz	0.061	0.070	0.079	0.066	0.061	0.097	0.102	0.072	0.068	0.076	0.074	0.061
T. E. Twining	0.085	0.081	0.091	0.077	0.092	0.099	0.098	0.097	0.091	0.085	0.087	0.074
J. L. Weinberg	0.088	0.086	0.091	0.086	0.090	0.091	0.091	0.094	0.082	0.083	0.086	0.086
M. A. Joslyn*	—	—	0.089	—	—	—	0.084		—	—	0.084	—
Average	0.084	0.0845	0.087	0.0845	0.087	0.093	0.094	0.091	0.086	0.088	0.089	0.086
Maximum	0.091	0.093	0.092	0.092	0.095	0.097	0.102	0.097	0.096	0.102	0.102	0.099
Minimum	0.061	0.070	0.084	0.066	0.061	0.085	0.085	0.072	0.062	0.076	0.084	0.061

* Not averaged.

COLLABORATOR	D (DRY RED)				E (PORT)				F (SHERRY)			
	1	2	3	4	1	2	3	4	1	2	3	4
S. Berkshire	0.124	0.118	0.120	0.120	0.083	0.078	0.084	0.084	0.079	0.081	0.081	0.082
L. Cash	0.112	0.141	0.126	0.126	0.074	0.087	0.081	0.081	0.074	0.081	0.084	0.084
J. Curtis	0.127	0.117	0.117	0.117	0.081	0.075	0.057	0.075	0.080	0.078	0.054	0.078
E. N. Davis	0.092	0.120	—	0.123	0.069	0.075	—	0.081	0.058	0.078	—	0.079
R. Dunn	—	0.123	0.122	0.123	—	0.082	0.084	0.079	—	0.079	0.081	0.078
R. A. Greene	0.148	0.120	—	0.169	0.100	0.073	—	0.076	0.082	0.076	—	0.076
R. F. Love	0.121	0.120	0.120	0.108	0.077	0.084	0.081	0.078	0.072	0.075	0.073	0.077
Anna E. Mix	0.119	0.119	0.115	0.117	0.077	0.072	0.078	0.078	0.070	0.076	0.078	0.083
J. B. Robb	0.126	0.192	0.117	0.123	0.077	0.114	0.078	0.078	0.073	0.075	0.078	0.078
Bertha Schwartz	0.070	0.072	0.100	0.076	0.054	0.069	0.074	0.059	0.051	0.071	0.077	0.066
T. E. Twining	0.117	0.128	0.134	0.126	0.078	0.083	0.091	0.079	0.079	0.084	0.092	0.081
J. L. Weinberg	0.104	0.103	0.108	0.108	0.074	0.074	0.074	0.077	0.084	0.086	0.091	0.086
M. A. Joslyn*	—	—	—	—	—	—	0.082	—	—	0.072	—	—
Average	0.114	0.123	0.118	0.120	0.077	0.081	0.078	0.077	0.073	0.078	0.077	0.079
Maximum	0.148	0.192	0.134	0.169	0.100	0.114	0.091	0.084	0.084	0.086	0.092	0.086
Minimum	0.092	0.072	0.100	0.108	0.054	0.069	0.057	0.059	0.051	0.075	0.054	0.066

Jesse L. Weinberg, Chemist, Polak's Frutal Works, Inc., Long Island City. Reported October 26, 1937.

It was requested that the volatile acidity of the samples be determined by the following methods:

1. Method I, Official A.O.A.C. procedure (50 cc. of wine steam distilled). *Methods of Analysis*, 1935, p. 166.

2. Method II, Official A.O.A.C. procedure (10 cc. steam distilled). *Methods of Analysis*, 1935, p. 167.

3. With apparatus used in Method II, but preferably with a vertical condenser. Adjust the flow of water in condenser so that distillate comes over hot (about 70° C.), collect 100 cc. of distillate, and titrate hot.

4. As in Method II but collect 100 cc. of distillate, bring to boil, and titrate hot.

The results obtained are summarized in Table 1, as are also more recent analyses (October 1938) obtained by the Associate Referee. At this date the total titratable acidity in A expressed as acetic acid was 0.093 gram per 100 cc., a slight decrease from the previous value of 0.097. Sample D was gassy and had increased to 0.380 gram of acetic per 100 cc. There is considerable variation in the results reported, particularly with the dry red wine. The results reported by Bertha Schwartz are particularly low, and this collaborator obtained higher values throughout by Method 3. Twining obtained higher results by Method 3 and considerably lower results by Method 4, although this trend is not shown in the results obtained by others. There is too much variation in the results to show any definite trend in favor of any method. The most erratic results were obtained with the dry red wine. Changes in volatile acidity during the time that elapsed between shipment of samples and analysis probably does not account for this variation. No significant changes in volatile acidity of the samples of dry red wine occurred during a period of two months. However, the highest acidity was reported by Collaborators Greene and Robb. The latter analyzed the samples early in October, and although Davis reported late in November he stored his samples at 35° F.

From the data available it is difficult to determine whether the variations in results are due to erratic errors or whether systematic errors were coming in. The Associate Referee's results indicate that when the conditions of distillation are properly controlled, results may be readily duplicated to within a titration error of ± 0.02 cc. Thus Ray Dunn obtained the results shown in Table 2. He used the C. H. McCharles still (also known as B-K-H still), which consists of a large Sellier tube sealed into an Erlenmeyer flask and in which the 10 cc. of wine is steam distilled over an electric plate.

A comparison of the modified Fessler apparatus, the McCharles still, and the large Sellier tube official apparatus was made by Dunn to determine effect of variation in type of still. However his results, Table 3,

TABLE 2.—*Results obtained by Ray Dunn*

WINE	VOLUME DISTILLED (CC.)						TOTAL TITRATION, CC. 0.1 *N* NaOH
	50	10	10	10	10	10	
	CC. 0.1 *N* NaOH REQUIRED FOR EACH ALIQUOT						
A	1.36	0.07	0.05	0.03	0.03	0.02	1.55
	1.31	0.10	0.05	0.03	0.02		1.51
B	1.28	0.12	0.05	0.06	0.03	0.02	1.56
	1.35	0.10	0.04	0.04	0.04	0.02	1.58
	1.28	0.10	0.05	0.05	0.03	0.02	1.53
C	1.28	0.08	0.06	0.07	0.04	0.05	1.58
	1.28	0.06	0.08	0.07	0.05	0.03	1.57
D	1.67	0.10	0.10	0.08	0.05	0.05	2.05
	1.69	0.09	0.09	0.09	0.04	0.05	2.05
E	1.10	0.08	0.04	0.05	0.03	0.03	1.33
	1.17	0.06	0.05	0.05	0.03	0.03	1.39
F	1.05	0.08	0.06	0.05	0.04	0.03	1.31
	1.05	0.08	0.05	0.05	0.04	0.04	1.31

do not indicate that variation in the type of apparatus used had any significant effect.

TABLE 3.—*Effect of variation in type of apparatus*
(Results by Dunn)

WINE	FESSLER	MCCHARLES	LARGE SELLIER
A	0.092	0.092	0.092
B	0.095	0.094	0.098
C	0.093	0.094	0.091
D	0.123	0.123	0.123
E	0.079	0.082	0.084
F	0.078	0.079	0.081

Several of the collaborators experimented with various phases of the determination, and the results of these experiments together with pertinent comments are given as follows:

E. N. Davis.—In addition to the methods listed, results were obtained by Method 5, in which a Braun-Knecht-Heimann apparatus was used with 20 cc. of sample, the end point being taken where 10 cc. distillate is required to neutralize two drops of 0.1 *N* alkali. The results obtained with this method, expressed as gram of acetic acid per 100 cc., are as follows:
A—0.087, B—0.088, C—0.074, D—0.119, E—0.076 and F—0.068.

Davis commented on Method 2 as follows:

It was found that in bringing over the distillate at 60° C. or above, a considerable amount of the distillate was not condensed, and therefore a considerable part of the volatile acids was lost. Further, under the usual circumstances in the laboratory, e.g., varying water pressure, the temperature in the condenser could not be held constant with the set-up specified so that the use of this method was impractical.

The following additional comments were added:

1. In order to check on the efficiency of various methods of CO_2 removal from the titration, a sample of sparkling wine was used with Method 2 and various modifications, i.e., (a) incipient boiling, then cooling before sample is taken, (A.O.A.C.); (b) sample heated in the Sellier tube in boiling water bath until alcohol began to be evaported (90° C.)—this was before the tube was connected to the distillation trap and condenser; (c) sample taken after part of CO_2 was removed by pouring back and forth from beakers; (d) with as little removal of CO_2 as possible before the sample was distilled.

Results:	a	b	c	d
Standard alkali (cc.)	1.48 and 1.53	1.53	1.48 and 1.50	1.48

Modification (b) is, in my opinion, the most practical and time-saving method. Although it would seem that CO_2 does not interfere seriously, the close checks should not be taken at their face value as I know that under certain conditions the fading end point due to CO_2 in the distillate may give a high value.

2. More than the 100 cc. of water in the jacket called for in A.O.A.C. Method II is necessary as the sample may be superheated when the water level is lowered. This higher temperature forces over "fixed" acids. 150 cc. of water usually suffices.

3. Neither paraffin nor other foam-reducers are adequate with some wines, especially young dry wines with high protein content. If the distillation trap is large enough (2½" d. bulb) and the trap outlet is large enough so that the condensed distillate may run back, no foam reducer is usually necessary although it may be necessary to cut down the rate of distillation with the worst wines. I have not attempted to determine whether the rate of distillation is a function of the end point, although I have seen some evidence to that effect.

Regular evolution of water vapor into the wine is important with badly foaming wines and a few grains of granulated Zn in the water jacket has proved satisfactory in preventing bumping.

4. The total titration requires so little alkali (2 cc. of 0.1 *N* is the legal limit) that serious percentage errors may occur. The use of a 20 cc. sample as in the B-K-H apparatus will reduce the error by one-half if it is remembered that the definition of the end point must be changed. It might also be worth while to define the end point in terms of size of sample taken when a 0.02 *N* alkali solution is used. For a 10 cc. sample, it would probably be "tritrate each succeeding 10 cc. of distillate until *5 drops* of 0.02 *N* alkali are required tor each the neutral point." The use of 0.02 *N* alkali would probably give a more sensitive end point in addition to using five times the amount of alkali.

It might also be worth while to check the recovery of volatile acids at different concentrations, especially between 0.1 and 0.015 per cent. This could be done by adding known amounts of acetic acid (or a mixture of appropriate volatile acids and esters) to *wines* (not water) of known volatile acid content. This should throw some light on the absolute efficiency of the methods.

S. Berkshire.—Considerable difficulty was experienced with Method 3, due to the

difficulty of controlling the temperature of the water in the condenser. Of the methods used, No. 4 seemed preferable.

R. F. Love.—Methods 1, 2, 3, and 4 are in accordance with your letter. In addition to the four methods requested we determined the volatile acids in accordance with the method usually followed in this laboratory—listed as Method 5 in the report. (The results obtained from Method 5, expressed as gram of acetic acid per 100 cc., are as follows: A—0.090, B—0.093, C—0.093, D—0.123, E—0.084, F—0.078.)

Method 1: We are of the opinion that this method is open to criticism due to the fact that steam distillation is discontinued before all the volatile acids are removed, although repeated titrations of 15 cc. portions of the distillate tend to introduce a compensating error.

Method 2: It is believed that this method is satisfactory although a small error may be introduced by the titration of several small portions of the distillate. We believe that it is preferable to collect 100 cc. of distillate and titrate it in the case of ordinary wines such as those submitted. We believe it unnecessary to bring this small sample to boiling before steam distilling it as the amount of CO_2 in the 10 cc. sample is negligible. In the case of sour wines our experience indicates that approximately 200 cc. of sample should be distilled before titration.

Method 3: Considerable difficulty was experienced in adjusting the flow of water in the condenser so that the distillate came over hot (about 70° C.). In running wines according to this method we believe it is almost impossible, using the ordinary condenser, to prevent the distillate from becoming too hot, with the resultant loss of volatile acids.

Method 4: It is believed that boiling the distillate will cause more or less loss of volatile acids, thus introducing a small error in the final figures.

Method 5: This method is the same as Method 2 except that the sample of wine is not brought to the boiling point prior to distillation, and 100 cc. of the distillate is collected and titrated. In the case of sour wines and vinegars a 5 cc. sample is distilled to 200 cc. and titrated. Our experience shows that the amount of distillate collected is sufficient to ensure the removal of the volatile acids from the sample.

J. Bernard Robb.—Following are the volatile acid results, expressed as gram of acetic acid per 100 cc., on the samples of wine sent us recently:

Samples	1	2 Round Bulb (Official)	2 Long Bulb (Virginia)	3	4	Va.
A	0.089	0.093	0.087	0.090	0.084	0.087
B	.0924	.135	.093	.090	.093	.093
C	.096	.099	.097	.084	.096	.096
D	.126	.192	.126	.117	.123	.123
E	.0768	.114	.078	.078	.078	.081
F	.0726	.075	.072	.078	.078	.081

In Method 2 we found we could not get accurate results on all analyses with the round bulb as shown in the official method, so substituted our bulb, which is a long bulb with a break in the main tube about midway. The results in the column marked "Virginia" were determined by our regular method and the long bulb, distilled fast with perpendicular condensers. The results in Column 2 (Round Bulb), slow for reasons stated, should be discarded; that is, the results on B, D, and E.

Bertha Schwartz.—My observations of the results lead me to suggest that greater control should have been used relative to the atmospheric conditions of the experiments. In Methods 2, 3, and 4 where only 10 cc. of the wine is used and its volatile

acidity is very low, the results varied somewhat with the temperature of the liquid when pipetting. The lower the atmospheric temperature the greater the amount of volatile acidity found by these three methods.

TABLE 4.—*Effect of alcohol on rate of distillation of acetic acid*

VOLUME DISTILLED	ALCOHOL CONTENT (% BY VOLUME)				
	0	5	10	15	20
	PER CENT OF TOTAL ACID PRESENT DISTILLED OVER				
cc.					
10	7.3	4.2	3.0	2.5	2.1
20	10.5	9.1	7.2	6.2	5.1
30	15.7	14.2	12.3	10.8	9.0
40	20.6	18.2	17.3	15.9	13.7
50	25.0	23.6	22.0	20.8	18.8
60	29.1	28.0	26.7	25.6	23.8
70	33.1	32.0	31.1	30.0	28.5
80	36.7	35.9	35.1	34.3	32.8
90	40.3	39.5	38.7	38.3	37.0
100	43.6	43.0	42.3	42.0	40.8
150	57.6	57.4	56.7	57.2	57.0

TABLE 5.—*Effect of sugar on rate of distillation of acetic acid*

VOLUME DISTILLED	PER CENT OF SUGAR			
	0	2	5	10
	PER CENT OF TOTAL ACID PRESENT DISTILLED OVER			
cc.				
10	7.3	5.4	5.9	5.3
20	10.5	10.7	11.8	11.2
30	15.7	15.7	17.3	17.0
40	20.6	20.6	22.7	21.9
50	25.0	25.1	27.5	26.5
60	29.1	29.4	32.1	30.7
70	33.1	33.4	36.3	35.1
80	36.7	37.1	40.6	39.1
90	40.3	40.6	44.5	43.1
100	43.6	43.8	48.0	46.2

In addition it seems to me that the Sellier distillation apparatus should not be used for wines containing such low volatile acid content. The variations achieved are too great even in duplicate analyses.

T. E. Twinning.—Variations in results are mostly due to bringing sample to boiling before test is made. Even a few seconds of boiling will account for an appreciable loss of acetic acid on a dilute acetic acid solution. If alcohol is present, this loss will be smaller. Carbon dioxide may best be disposed of by subjecting the wine to vacuum for few minutes.

TABLE 6.—*Effect of pH on rate of distillation of acetic acid*

VOLUME DISTILLED	*p*H			
	2.15	2.73	3.65	4.40
	PER CENT OF ACID PRESENT DISTILLED OVER			
cc.				
10	5.8	5.8	5.5	4.0
20	11.3	11.3	10.6	7.3
30	16.5	16.8	15.6	11.2
40	21.6	21.8	20.1	14.4
50	26.2	26.1	24.5	17.5
60	30.7	30.4	28.6	20.7
70	34.8	34.5	32.5	23.6
80	38.7	38.4	36.1	26.5
90	42.4	42.1	39.7	29.0
100	45.7	45.3	42.8	31.7

J. L. Weinberg.—In the course of the determinations by A.O.A.C. Method II and its modifications, the writer experienced difficulty due to foaming during the distillations of the samples. Substitution of a Kjeldahl-Clark connecting bulb for the Kjeldahl bulb solved this problem.

A preliminary investigation of the effect of several factors on the rate of distillation of acetic acid from solution was made. Acetic acid solutions, containing approximately 0.08 gram of acetic acid per 100 cc., were steam distilled at a constant and reproducible rate, the solutions in the distilling flask being maintained at a constant volume of 100 cc. The results (Table 4–6) indicate that although alcohol reduced the initial rate of distillation of acetic acid, it had but little effect on rate of distillation of volatile acid, but that the *p*H had a very noticeable effect, the rate of distillation decreasing markedly with increase in *p*H.

A complete investigation of the four points stressed in the previous report was not made, but some interesting results were obtained in connection with a modified Peynaud[1] procedure. Peynaud had suggested the use of barium hydroxide to remove sulfurous acid from wines. His procedure is essentially as follows:

Neutralize 25 cc. of wine from which the CO_2 has been removed by vacuumization to phenolphthalein by a saturated solution of $Ba(OH)_2$, store 15 minutes, and keep pink by additions every 5 minutes of more $Ba(OH)_2$. Make up to 50 cc., filter rapidly a 40 cc. filtrate corresponding to 20 cc. of wine, add 15 cc. of a solution of tartaric acid 30 grams/liter, distil, and recover 50 cc. of distillate, which corresponds to 80 per cent of the volatile acids in the sample of wine.

To make this method more amenable to the existing conditions it was altered as follows:

Pipet 50 cc. of wine from which the CO_2 has been removed by vacuumization into a 100 cc. volumetric flask and neutralize to phenolphthalein (if white) or to

[1] *Ann. fals.*, 30, 1–7 (1937).

natural pigment (if red) by a saturated solution of $Ba(OH)_2$. Allow the mixture to stand for 15 minutes and maintain at the phenolphthalein end point by the addition of more $Ba(OH)_2$ if necessary. Then make up to 100 cc. and filter. Transfer 20 cc. of filtrate into the inner Sellier tube of a Hortvet type apparatus, add 1 cc. of H_2SO_4 (1+3) and distil over 100 cc.

This procedure accomplishes two purposes: It removes the sulfur dioxide and it maintains the solution to be distilled at a distinctly acid point, a *p*H of about 1.0. Some results obtained with this method are given in Table 7. The distillations were made in a modified Fessler still, and no particular attention was given to rates of distillation.

TABLE 7.—*Comparative determinations of volatile acidity before and after defecation with barium hydroxide*

SAMPLE	DESCRIPTION	DIRECT DISTILLATION				MODIFIED PEYNAUD PROCEDURE			
		CC. 0.1045 *N* BASE			ACIDITY	CC. 0.1045 *N* BASE			ACIDITY
A	Acetic acid	1.40	1.42	1.36	0.088	1.25	1.25	1.25	0.078
B	Synthetic wine	1.33	1.32	1.35	0.084	1.25	1.15	1.20	0.076
C	Dry white	1.37	1.23	1.29	0.084	1.50	1.45	—	0.094
D	Dry red	6.08	5.32*	6.0	0.380	5.30	5.27	—	0.330
E	Port	1.30	1.28	1.17	0.082	1.55	1.53	1.54	0.096
F	Sherry	1.17	1.12	1.16	0.072	1.34	1.32	1.20	0.084
G	Sauterne type	1.30	1.44*	1.33	0.085	0.70	0.73	0.70	0.044
H	Sauterne type	1.43	1.45	1.50	0.092	0.68	0.67	0.65	0.042
I	Sauterne type	1.98	2.30*	1.92	0.123	0.71	0.67	0.65	0.042
J	Sauterne type	1.17	1.32	1.27	0.079	0.83	0.80	0.77	0.050
K	Dry white	2.00	2.13	2.07	0.130	1.75	1.85	1.80	0.113
L	Dry red	1.58	1.52	1.50	0.096	1.48	1.42	1.50	0.092

* Values not averaged in calculating acidity.

Under these conditions an occasional erratic result is to be expected. It was found that more readily reproducible results were obtained with the modified Peynaud procedure. It is significant that in the sulfited wines, G to K, considerably lower results were obtained by this procedure, while appreciably higher results were found with fortified wines. The analysis of these results, both in regard to recovery of volatile acids and increase in volatility of fixed acids such as lactic, remains to be done.

It is recommended[1] that the distillation procedures be studied further with a view to eliminating chance errors, and that the modification given, or some other modification, of the Peynaud procedure be tested further.

REPORT ON SULFUR DIOXIDE IN BEERS AND WINES

By L. V. TAYLOR, JR.* (American Can Co., Maywood, Ill.),
Associate Referee

Last year, following a study of sulfur dioxide methods for alcoholic and carbonated beverages (Taylor, Beardsley, and Lueck, *This Journal*,

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 65 (1939).
* Presented by J. W. Sale.

20, 610 (1937)), the Associate Referee recommended that studies on these methods be continued and that collaborative work be conducted.

Accordingly a series of 12 ounce samples of beer and ale was prepared for collaborative studies. Four codes of beer and four codes of ale, each code consisting of two samples, were submitted to collaborators who had expressed a willingness to cooperate in the project. The accompanying directions suggested that one sample of each code be analyzed volumetrically and gravimetrically by the Monier-Williams method and that its duplicate be analyzed by the tentative A.O.A.C. method for beer.

The analysis of each code at the time the samples were prepared indicated that all ale and beer codes represented in the series contained sulfur dioxide in the order of 20 and 30 p.p.m., respectively, as determined by the Monier-Williams method. Prior to submitting the samples to the collaborators, and approximately one month after their preparation, representative samples of each code were again analyzed. These results indicated that no apparent change had occurred in the sulfur dioxide content during the storage period and also that the samples would be suitable for use in comparing the two methods.

Of those receiving samples, only three reported on the work, namely, H. W. Edwards, Department of Agriculture, Lansing, Mich.; J. B. Thompson, North Dakota Regulatory Department, Bismarck, N. D.; and R. A. Osborn, U. S. Food and Drug Administration, Washington, D. C. Analyses were also made at the American Can Company Laboratories by C. L. Beardsley and E. D. Sallee.

The results reported by the collaborators range from 5 to 33 p.p.m. of sulfur dioxide in the beer samples and from 5 to 21 p.p.m. in the ale samples. Because of these wide variations it is impossible to evaluate the results with respect to the procedures, but they indicate that the wide discrepancy might have been due to the chemical changes occurring in the samples before they were analyzed. Following receipt of the collaborative results, the Associate Referee analyzed additional samples of each lot, and these results further indicated that changes had occurred in the sulfur dioxide content of both products.

Although a comparison of the two methods is not possible from the collaborative results, the Associate Referee considers that the suggestions and comments of the collaborators will be of value in future work on the subject and in preparing collaborative samples of products containing sulfur dioxide. In general, the collaborators experienced difficulty from foaming of the beer when using the tentative A.O.A.C. method. Trouble was experienced with blanks in the Monier-Williams method, and in addition the rapid decomposition of the hydrogen peroxide reagent was also pointed out.

The Associate Referee recommends[1] that the collaborative study of sulfur dioxide in beer and ale be continued and that sulfur dioxide in

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 65 (1939).

wine also be studied. Preparation of samples for collaborative analyses appears to present a special problem.

REPORT ON VOLATILE ACIDS IN DISTILLED SPIRITS

By G. F. BEYER (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.), *Associate Referee*

When official Method I[1] for the determination of volatile acids in wine was applied to whiskey, the results were found to be a trifle high when compared with those obtained by official Method II and the evaporation method. When Method I was used in connection with a solution containing 72 grams of acetic acid per 100 liters of 50 per cent alcohol, the correct result was obtained on the distillate, but the residue required 0.3 ml. of 0.05 *N* sodium hydroxide for neutralization. When Method II was applied to the same solution, the result obtained was 1.5 grams low. The Sellier tube used was 8.5 inches long and 1.0 inch in diameter.

Volatile acids in whiskey were also determined by evaporating just to dryness, then adding 10–15 ml. of water, and again evaporating to dryness. The residue was dissolved in about 25 or 30 ml. of neutral alcohol (50 per cent) and diluted to any convenient volume, and the fixed acids were titrated with 0.1 *N* sodium hydroxide. The difference between this figure and that obtained for the total acids represents the volatile acids.

A number of determinations showed that this method of determining volatile acids in distilled spirits compared very favorably with Method II, and that it is likely to give nearer the correct result than either of the other two methods.

RECOMMENDATIONS[2]

It is recommended that the evaporation method and A.O.A.C. Method II be further studied with the view to adopting the former or both as official for distilled spirits. It is further recommended that the cause of the slight loss resulting from the use of Method II be investigated.

REPORT ON ALDEHYDES IN WHISKEY AND OTHER POTABLE SPIRITS

By PETER VALAER (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.), *Associate Referee*

For some time there has been a demand for an improved A.O.A.C. method for the determination of aldehydes in distilled spirits by those who are opposed to colorimetric methods if there is a gravimetric or

[1] *Methods of Analysis, A.O.A.C.*, 1935, 166.
[2] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 65 (1939).

volumetric method available. Some analysts find it difficult to produce uniform results by the present A.O.A.C. method (sulfite-fuchsin), principally on account of the instability of the sensitive reagents required. Others have found that there is too wide a variation in the aldehyde content of spirits. For example, when the same barrel of whiskey is analyzed at three or six months' intervals, the difference in results seems to be due to errors in analysis, rather than to changes that actually occur in the barrel during aging.

It was decided to select a method based on the original Ripper principle,[1] which had given good results in the Associate Referee's laboratory.

For collaboration purposes there were prepared three large quantities of whiskey, rum, and brandy containing different amounts of aldehydes. Sealed one-half pint samples and the method of analysis to be used were sent or given to sixteen collaborators. Their results are shown in the table.

The method was published in *This Journal*, **22**, 73 (1939).

DISCUSSION OF RESULTS

The procedure selected is based on sound chemical principles; it is relatively easy and speedy of manipulation and is also one that has given some measure of satisfaction to the users.

In reply to a request for suggestions for improving the method submitted several collaborators offered some interesting and helpful ones. No one was positively opposed to the method; and only one collaborator preferred the present method because he was familiar with it and had designed special equipment for it. It may be well, therefore, to retain the present method as an alternative method.

Several collaborators stated that they would prefer this proposed method to the present A.O.A.C. method provided it produced satisfactory results. It was believed by others that the method would give better results than those submitted when the analysts had had more experience in using it. One chemist stated that it is entirely satisfactory from the standpoint of laboratory operation, but that the reason for standardizing the iodine and bisulfite solutions was not apparent. If exactly 25 cc. of bisulfite solution and 30 cc. of iodine solution are added to each sample and to several controls, the aldehyde is equivalent to the difference in the volume of the thiosulfate solution required for the sample and for the controls. He makes reference to the work of Joslyn and Comar.[2]

Another chemist makes the following most serious objection:

> The sodium bisulfite method shows more aldehydes present than there actually are, and the magnitude of the error increases as the aldehyde concentration diminishes. The error becomes very high in the range of aldehyde concentrations we normally find present in whiskey.

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[1] *Monatsh.*, 21, 1079 (1900).
[2] *Ind. Eng. Chem. Anal. Ed.*, 10, 364 (1938).

Still another chemist writes as follows:

We feel that the fuchsin-sulfurous acid method for aldehydes is quite satisfactory for rapid determinations. However, its limit of error is very great, due to the small amounts of distillate used in the determination. This makes it almost useless for research work of any comparative nature. Thus, you can readily understand that it was with a great deal of interest that the present collaborative work was followed.

It would be very difficult at the present moment to state definitely the cause for the above description. Possibly the work of other collaborators in conjunction with our own will give us a clue.

There are several suggestions which could be used to render the proposed test more accurate. The distillate receiver should be kept in an ice bath to prevent the volatilization of some of the aldehydes during the distillation process. Thus the condensation of aldehydes from the distillate would be independent of the atmospheric conditions of the room.

Collaborative results on aldehydes in whiskey and other potable spirits
(grams per 100 liters)

COLLABORATOR	WHISKEY	RUM	BRANDY
G. F. Beyer	3.85	8.60	43.6
	3.40	8.25	44.6
		8.80	45.1
Loren Burritt	3.00	7.26	43.12
	3.08	7.04	40.40
	3.08		40.00
Peter Valaer	3.30	7.70	40.30
	3.74	7.40	42.90
	3.74	7.90	42.90
C. L. Tucker	3.36	8.40	47.10
		8.10	46.00
A. C. Garland	3.04	8.32	44.00
—. Edwards	3.52	8.58	46.86
	4.40	9.02	47.52
—. Whiting	3.96	9.90	49.94
	3.96	9.68	
Geo. Hamill	3.41	8.40	42.4
	3.28	8.07	42.1
	3.39	8.73	44.2
C. T. Carson	5.52	10.93*	44.97
M. C. Brockman	2.71	8.47	45.60
	2.86	9.28	48.80
Bertha Schwartz	3.41	8.23	32.4*
A. Herman	2.64	8.82	46.52
	2.66	8.77	45.27
Bernard Robb	4.02	8.80	42.03
	3.74	8.36	40.55
John B. Wilson	3.10	8.40	44.1
	3.20	8.40	44.7
Averages	3.46	8.43	44.2

* Averages do not include the lowest brandy or highest rum.

A further standardization of the method could include the designation of the temperature of the sample at the time of pipetting. If the temperature is kept at about 20° C. or lower there will be no loss of the lower-boiling point constituents in the pipetting.

While all the comments could not be included without making this report unduly voluminous, the general trend of opinion may be deduced from the foregoing discussion.

It is recommended[1] that this work be continued for another year in order that improvements based upon the suggestions and experiences obtained may be made.

REPORT ON DETECTION OF ADULTERATION OF DISTILLED SPIRITS

By S. T. SCHICKTANZ (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.), *Associate Referee*

No definite report will be given at this time, but the Associate Referee should like to discuss briefly the problems encountered during the year.

One of these problems relates to the potentiometric titration of the acids in alcoholic beverages. Two values that were obtained by this method are significant. They are the initial *p*H and the shape or contour of the titration curve, both of which are dependent to a large degree on two factors, temperature and dielectric constant. The *p*H usually increases with a decrease in dielectric constant; or, in other words, the *p*H increases with an increase in concentration of alcohol. To make the values obtained significant and relative, it is necessary to make the titration at an agreed temperature and at a definite dielectric constant. However, the same results may be obtained by making determinations at any temperature and any concentration of ethyl alcohol, and correcting back to a standard basis by means of correction charts. Work has been done on these correction charts during the past year, but due to errors, presumably experimental, in the results of various collaborators, it seemed advisable to obtain more data before presenting these tables.

Another interesting procedure is the chromatographic method of analysis. It has been shown by several German investigators that it is possible to identify coal tar dyes used in the coloration of wines. Much more work must be done, however, to make this method applicable to alcoholic beverages in general. In conjunction with this problem, it is necessary to use such methods as spectrophotometric and ultraviolet absorption.

A new method for the determination of fusel oils is also being investigated. All that can be said for this method at the present time is that it requires approximately three hours for an analysis. The results are in-

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 65 (1939).

dependent of the presence of esters and aldehydes, and the yields are better than 83 per cent of the original fusel oils present.

It is suggested that work on these problems be continued and that results and recommendations be submitted to the committee for approval in the near future.

The report of the Associate Referee on Wood Alcohol in Brandy is included in his paper, entitled "Application of the Neutral Wedge Photometer to the Quantitative Determination of Methanol in Distilled Spirits," published in *This Journal*, 22, 151 (1939).

REPORT ON CORDIALS AND LIQUEURS

By JOHN B. WILSON (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

An apricot cordial and a peach cordial were prepared by the Associate Referee for analysis as a means of combining the collaborative study of the determinations of esters, benzaldehyde, and gamma-undecalactone.

In each case 1 kg. of the dried fruit was ground in a food chopper, placed in a 1 gallon bottle, and macerated with 2 liters of dilute alcohol (1+1) for 4 days, during which period the contents of the bottles were shaken vigorously several times a day. The liquid portions were then strained through cheese-cloth and reserved in large bottles. Second and third extracts were made in a similar manner, and the maceration was continued for a week. The three extracts of each fruit were mixed and made up to about 7 liters with water.

Determinations of volatile acids and esters were made upon the steam distillates from 300 cc. of each of these mixtures. After these determinations had been made the solutions were rendered alkaline and evaporated as described under the A.O.A.C. tentative qualitative test for gamma-undecalactone, and the lactones were separated. Before the test was made the lactone residue was dried over sulfuric acid and weighed. The odor of the residue was noted and the qualitative test made. The results are given in Table 1.

TABLE 1.—*Analysis of extracts of dried apricots and peaches, with alcohol (1+1)*

EXTRACT	APRICOT	PEACH
Quantity distilled (cc.)	300	300
Dried fruit represented (grams)	43	43
Volatile acid (acetic) per liter (gram)	0.26	0.12
Volatile esters (ethyl acetate) per liter (gram)	0.009	0.012
Residue, lactone separation per liter (gram)	0.015	0.015
Gamma-undecalactone crystals	None	None
Gamma-undecalactone odor	None	None

The next step in making the cordials was that of preparing an artificial flavor that would contribute the correct proportions of esters and gamma-undecalactone. A quantity of oil of cognac was obtained, and its ester content was determined in terms of ethyl acetate. It was found to contain no gamma-undecalactone. The saponification value of a commercial preparation labeled "Peach Aldehyde" was also determined in terms of ethyl acetate, and gamma-undecalactone was shown to be present both by the odor of the residue from the extraction for lactones and by a qualitative test.

About 10 grams of the gamma-undecalactone (peach aldehyde) and 21 grams of oil of cognac were then dissolved in 95 per cent alcohol and diluted to 2 liters. A portion (25 cc.) of the solution was added to 225 cc. of water and steam distilled. The distillate, when neutralized and saponified, was found to contain 132 mg. of esters as ethyl acetate, equivalent to 5.36 grams of ethyl acetate per liter of solution.

The two cordials were then prepared from the fruit extracts and synthetic solution, with the addition of sugar sirup, water, alcohol, and oil of bitter almonds in the proportions given in Table 2.

TABLE 2.—*Ingredients used in making apricot and peach cordials*

	APRICOT	PEACH
Fruit extract (liters)	7.0	6.7
Sirup containing 3.2 kg. sucrose (liters)	4.0	4.0
Artificial flavor solution (liter)	0.8	1.0
Alcohol 95% by volume (liters)	2.2	2.3
Oil of bitter almonds (grams)	2.08	1.25
Total volume (liters)	14.0	14.0

About 1 liter of each cordial was submitted to eight collaborators with instructions to make the determinations listed below.

1. *Alcohol by volume:* Use Method II, p. 170, 5.[1]
2. *Total Solids:* Use 37(c), p. 180.[1]
3. *Benzaldehyde:* Use 100 cc. of sample, following 56, p. 182, except to use two 10 cc. portions of alcohol 30% by volume for the final washing of the precipitate instead of 10% alcohol.
4. *Esters as ethyl acetate:* Determine in duplicate on 300 cc. of sample using 46, p. 181.[1]
5. *Gamma-undecalactone:* Unite the duplicate solutions from the determination of esters and treat as directed in 47, p. 181. Use a tared beaker for the evaporation of the final ether solution containing gamma-undecalactone, dry in a desiccator over H_2SO_4, and report the weight of the residue. If your laboratory is not equipped for the microscopic examination of the crystals of hydrazino-gamma-undecalactone, transfer them to a small vial and mail to the Associate Referee, who will arrange for their examination.

The reports of the collaborators are incorporated in Table 3.

[1] *Methods of Analysis, A.O.A.C.*, 1935.

TABLE 3.—*Analysis of apricot and peach cordials*

COLLABORATOR	BATTISTA	BURRITT	CHRISTENSEN	EDWARDS	SMITH	VALAER	WHITING	WILSON	PRESENT
				Apricot Cordial					
Determination									
Alcohol by volume (%)	41.30								
	41.28	37.66	36.76	36.84	35.99	37.66	37.12	37.56	
Solids per 100 cc. (grams)		24.91	26.44	24.7[1]	23.04[2]	24.91	24.6[1]	23.78	
Benzaldehyde per 100 cc. (mg.)		203	18.1	14.1	13.7	181	13.3	15.8	14.8
Esters as ethyl acetate per 100 cc. (mg.)	24.2	28.2	32.3	26.0	26.4 28.9	26.7	27.4	26.4	31.1
Gamma-undecalactone per liter (mg.)	6.0	30.8	23.7	14.6	44.0	33.5	7.0	249.3	357
Qualitative	+	+	−	+	+	+		+	
				Peach Cordial					
Alcohol by volume (%)	41.44 41.46	39.50	37.76	38.56	38.14	39.95	38.60	39.24	
Solids per 100 cc. (grams)		24.8	27.37	24.3[1]	22.30[2]	24.8	24.4[1]	23.55 23.19	
Benzaldehyde per 100 cc. (mg.)		159.0	9.2	11.0	9.1	132.0	7.1	10.0	8.9
Esters as ethyl acetate per 100 cc. (mg.)	26.4 26.56	33.7	56.0 38.4	34.5	lost	35	35.9	35.2 35.5	38.8
Gamma-undecalactone per liter (grams)	6.1	37.4	36.6	17.3	lost	38.5	13.8	185.7	286
Qualitative	+	+	+	+		+		+	

[1] Determinations were made by diluting the residue from the alcohol determination to 50 cc. and taking the immersion refractometer reading.
[2] Calculated from the sp. gr. of the dealcoholized sample.

Additional results on solids were submitted by some of the collaborators as given in Table 4.

TABLE 4.—*Additional results on solids in cordials*

METHOD USED	APRICOT CORDIAL	PEACH CORDIAL
	g./100 cc.	*g./100 cc.*
Wilson: Dried in vacuo on dry asbestos 18 hrs. at 70° C.		
Sample 3 cc.	25.02	24.79
Sample 5 cc.	24.94	24.53
Burritt: Dried in air oven at 100° C. to constant weight	25.07	25.52
Valaer:	25.37	25.95
Smith: Calculated by the formula $\frac{\text{Sp. gr. sample} - \text{sp. gr. distillate}}{0.00386}$	22.84	22.11
From sp. gr. of dealcoholized sample	23.04	22.30

Dilute alcohol, 30 per cent by volume, was chosen to wash the precipitated benzaldehyde phenylhydrazone because it had been noted that in preliminary experiments somewhat high results were obtained when the precipitate was washed with 10 per cent alcohol, as directed in *Methods of Analysis, A.O.A.C.* In these cases a strong odor of esters and gamma-undecalactone was noted, but when the drying was continued for 24 hours or more the precipitate gradually approached the proper amount and the odor was less noticeable. When pure benzaldehyde phenylhydrazone was dried at 70°C. in a vacuum, practically no loss of weight was found after 24 hours.

Another experiment indicates the possibility of an increased percentage of alcohol as a precipitation medium to prevent inclusion of flavoring ingredients by the precipitated phenylhydrazone. A stock solution of benzaldehyde was prepared, and precipitations were made with the phenylhydrazine reagent in several strengths of alcohol. The data are given in Table 5.

TABLE 5.—*Precipitation of benzaldehyde phenylhydrazone in various strengths of alcohol*

ALCOHOL	BENZALDEHYDE PHENYLHYDRAZONE	BENZALDEHYDE
per cent	*gram*	*gram*
10	0.0860	0.0465
20	0.0858	0.0464
30	0.0837	0.0453
40	0.0824	0.0446

A further experiment was performed with the same stock solution in which the benzaldehyde phenylhydrazone was precipitated as usual in a

medium of 10 per cent alcohol and the precipitate washed with other strengths of alcohol.

TABLE 6.—*Recovery of benzaldehyde phenylhydrazone when washed with stronger alcohol*

ALCOHOL FOR WASHING	BENZALDEHYDE PHENYLHYDRAZONE	BENZALDEHYDE
per cent	*gram*	*gram*
30	0.0852	0.0461
	0.0861	0.0466
40	0.0836	0.0452
	0.0800	0.0433

These experiments indicate that the precipitate of benzaldehyde phenylhydrazone may be washed with alcohol, 30 per cent by volume, with comparative safety but that stronger alcohol should not be used. Further work needs to be done to establish whether or not alcohol as strong as 30 per cent by volume may be used as a medium for precipitation of benzaldehyde phenylhydrazone without material loss.

DISCUSSION OF RESULTS

No estimate of the alcohol or solids content of the samples could be made owing to the method of manufacture. A fair agreement was attained by the various collaborators on these two determinations, but better agreement would be desirable.

Except for those of two collaborators, the results for benzaldehyde are remarkably good, the average for the apricot cordial being 15 mg. per 100 cc. as compared with 14.8 mg. calculated. The average for the peach cordial is slightly high, 11.6 mg. per 100 cc., against the calculated 8.9 mg. per 100 cc. It is hoped that slight modifications in the procedure will result in even better results next year.

While fair agreement is shown among collaborators for the ester content of the two samples, there is a discrepancy between the esters found and calculated. It appears to be due to the fact that the esters used were not completely soluble. A thin film of oily matter was found upon the remaining stock of cordials some time after the samples had been distributed. This accounts for the somewhat low results in a number of cases.

The majority of the collaborators succeeded in extracting the gamma-undecalactone from both samples, but the weight of material obtained does not show that this procedure can be used as a quantitative method in its present form. However, it is hoped that some modifications that have already been subject to trial will enable the Associate Referee to develop a quantitative procedure for this substance.

It is recommended[1] that further collaborative work be done on the determination of benzaldehyde, volatile esters, and gamma-undecalactone in cordials.

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 65 (1939).

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MacIntire (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Referee*

The 1937 report of the Referee carried five recommendations for continued work, and a recommendation that a study of methods for the determination of fluorine in soils be initiated. All of these recommendations have been in operation during the past year. The Referee has served only in an advisory capacity for the work that was already under way at the 1937 meeting. An imperative need had arisen, however, for a dependable technic for the recovery of fluorides from soils of known experimental history and for the determination of the fluorine content of crops grown thereon. The Referee and his associate, J. W. Hammond, therefore, pursued a study of the operations that would bring all forms of fluorides into solution for distillation by the Willard-Winter procedure. The resultant technic was transmitted to the Associate Referee, whose findings and conclusions will be embodied in his own report.

The method that was developed from the determination carried out by Hammond is here given, with tables of experimental data and observations relating thereto.

USE OF PEROXIDES OF CALCIUM AND MAGNESIUM IN DETERMINATION OF FLUORINE CONTENT OF SOILS, SILICEOUS MATERIALS, AND ORGANICS*

By W. H. MacIntire and J. W. Hammond

The following directions were submitted to the collaborators:

I. SOILS

Sampling.—Follow the procedure given in *Methods of Analysis, A.O.A.C.*, 1935, 1, and then grind 10 grams of the air-dried soil to 325-mesh in an agate mortar; mix thoroughly and preserve in a suitable stoppered container.

Distillation Apparatus.—Use either that prescribed by Willard and Winter, *This Journal*, 16, 105 (1933), or the multiple-unit setup recommended by Reynolds, Kershaw, and Jacob, *Ibid.*, 19, 156 (1936).

Determination.—For soils of high fluorine content, use a 0.5 gram charge; for those low in fluorine, use 1 gram. Mix the charge intimately with three times its weight of precipitated CaO_2 in either a nickel or platinum crucible; char thoroughly and then heat at approximately 900° C. for 30 minutes. Cool, and carefully brush the incinerated contents of the crucible into the distillation flask. Wash the walls of the flask with 5 cc. of water; add 3 drops of phenolphthalein; neutralize with 60% perchloric acid and then add 15 cc. additional. Bring to 135° ± 5° C. and maintain the distillation temperature and volume during the collection of a distillate of 200–250 cc. while passing a balanced current of steam through the suspension.

For soils of low fluorine content, concentrate the entire distillate by evaporation prior to titration; for those of high fluorine content, titrate an aliquot of 5–50

* A study conducted at The University of Tennessee Agricultural Experiment Station under auspices of The Tennessee Valley Authority.

cc. Titrate the concentrate or the aliquot with 0.01 *N* thorium nitrate in a 50% ethyl alcohol solution, using 2 drops of a 0.05% aqueous solution of sodium alizarin sulfonate as indicator.

II. ORGANICS AND NON-SILICEOUS MATERIALS

Sampling.—From a thoroughly mixed air-dried sample take an appropriate subsample, as governed by the supposed content of fluorine.

Preparation of subsample.—Grind to less than 1 sq. cm. Mix well and either heat overnight in an oven at 105° C., or dry over H_2SO_4 under reduced pressure for 24 hours, and preserve in a reagent bottle.

Distillation apparatus.—Use that prescribed for "Soils."

Procedure.—Weigh a charge to furnish approximately 1 mg. of fluorine. (This may require as much as 25 grams.) To a 10 gram charge or less, add one-half its weight of MgO_2 and mix in a 200 cc. evaporating dish of either nickel or platinum. When a larger charge is used, add 5 grams of MgO_2 in aqueous suspension; dry the wetted mixture at 105° C. for 24 hours; char slowly; and then incinerate in either an electric furnace or an incinerator at 500°–600° C. (Incineration is complete in 4–6 hours; in some instances, however, 12 hours may be required.)

Transfer the ash to the distillation flask. Neutralize with $HClO_4$ and add an excess of 15 cc. Bring to a temperature of 135° ± 5° C. and collect 200–250 cc. of distillate while maintaining that temperature during the passage of a balanced current of steam. Evaporate the distillate to a volume of 10–20 cc. and dilute with an equal volume of alcohol; add 3 drops of 0.05% sodium alizarin sulfonate; adjust reaction with NaOH and HCl, and then titrate with 0.01 *N* $Th(NO_3)_4$; or follow the zirconium-alizarin colorimetric procedure prescribed by Winter, *This Journal*, 19, 362 (1936).

EXPERIMENTAL

The removal of fluorine from the perchloric acid digestions by means of a balanced current of steam, as used by MacIntire, Shaw, and Hardin[1] in the dissolving of phosphates, was compared with removal from boiling

TABLE 1.—*Effect of a balanced current of steam upon the recovery of fluorine by distillation*

MATERIAL	CHARGE	FLUORINE RECOVERY FROM DISTILLATION VOLUME MAINTAINED	
		BY ADDITIONS OF WATER	BY CURRENT OF STEAM
	grams	*per cent*	*per cent*
Rock phosphate[a]	0.5	3.58	3.64
W.D. triple phosphate	1.0	1.80	1.85
Tenn. brown rock phosphate	0.5	3.39	3.40
		p.p.m.	*p.p.m.*
Soil used in pot experiment No. 2	1.0	110	110

[a] Bureau of Standards sample No. 56.

digestions kept to volume by water replacement. The distillations (Table 1) show that substantially identical results were obtained by the two

[1] *Ind. Eng. Chem.*, 10, 143 (1938).

TABLE 2.—*Effect of variable prior treatment of soils, as influencing recovery of fluorine by steam distillations*

SOIL AND CHARGE	FLUORINE ADMIXTURE	TREATMENT PRIOR TO DRYING AND INCINERATION	HEAT TREATMENT		FLUORINE RECOVERY	
			TEMPERATURE	TIME		
grams	*grams*		*°C.*	*hrs.*	*grams*	*per cent*
0.3185	0.00933[b]	None	500	2	0.00873	94.5
0.3478	0.00468[c]	None	500	2	0.00402	88.8
0.3621	0.01222[b]	Wetted with 5 cc. of 5% solution of sucrose	500	2	0.00974	79.8
Red Clay[a] 0.6386	0.01543[b]	Wetted with 2 cc. of 5% solution of sodium acetate	500	2	0.01542	99.9
0.8250	0.0065[b]	Wetted with 0.75 gram of $Mg(NO_3)_2$ in solution	500	2	0.0023	34.0
0.8241	0.0065[c]	Mixed with 2 grams of MgO_2	500	2	0.00278	42.5
1.0000	0.01894[b]	Mixed with 3 grams of CaO_2	900	½	0.01898	100.2
0.5000	0.01762[c]	Mixed with 1.5 grams of CaO_2	900	½	0.01742	99.0
0.5000	0.01450[c]	Mixed with 1.5 grams of CaO_2, wetted, and dried at 105° C.	900	½	0.0145	100.0
0.4569	0.01750[b]	None	500	2	0.01749	99.8
0.3489	0.00986[b]	Wetted with 5 cc. of 10% suspension of $MgCO_3$	500	2	0.00526	53.4
Hartsells 1.0000	0.01105[c]	Wetted with 0.75 gram of $Mg(NO_3)_2$ in solution	500	2	0.00994	90.0
fine sandy 1.0000	0.01105[c]	Mixed with NH_4Cl and $MgCO_3$[e]	800	2	0.0020	18.1
loam[d] 1.0000	0.01105[c]	Mixed with NH_4Cl and $CaCO_3$[e]	800	2	0.0022	19.9
1.0000	0.01105[c]	Mixed with NH_4Cl and $BaCO_3$[e]	800	2	0.0048	43.4
0.5000	0.0211[c]	Mixed with 0.5 gram CaO_2	900	½	0.0211	100.0
1.0000	0.0211[c]	Mixed with 3 grams CaO_2	900	½	0.0211	100.0

[a] Red clay subsoil, laboratory No. 6562, air-dried, and ground to 325-mesh.
[b] Added as CaF_2.
[c] Added as $BaSiF_6$.
[d] Used in T.V.A. pot experiments.
[e] One gram of chloride and 8 grams of carbonate.

procedures. Apparently, however, activation and the "sweeping" of the current of steam decreased by one-half the time requisite for complete removal of fluorine. The passage of steam also eliminates bumping, frequently encountered and especially with highly siliceous materials.

Effect of elevation of temperature was also injected into the comparisons. Variations in the range of 135°–145°C. showed no definite influence upon the effectiveness of the fluorine recoveries.

Influence of variable treatment prior to steam-current distillation

Table 2 shows the recoveries obtained from a red clay and an experimental soil that were fortified by variant additions of calcium fluoride and barium silicofluoride and then subjected to ignitions with different calcic and magnesic compounds. Liberation of fluorine by perchloric acid digestion was greatly repressed by the several magnesic materials—nitrate, carbonate+ammonium chloride, and peroxide—that were used in the ignitions precedent to distillation.

Apparently the generated magnesic oxide induced silicate combinations from which fluorine is not liberated by the perchloric acid. A similar repressive effect was induced by the 8 to 1 mixtures of the carbonates of calcium and barium with ammonium chloride. The opposite effect—complete recovery of the added fluorine—was obtained by the use of calcium peroxide in the ignitions that preceded distillations.

The peroxide ignitions imparted a splendid physical condition to the soil residues and probably induced formation of calcium fluoride from which ready release of fluorine is effected by the perchloric acid digestion.

Calcium peroxide incinerations vs. sodium-potassium carbonate fusions

Eleven experimental soils that had received treatments of different fluorides, over a period of 8 years, were subjected to ignition with calcium peroxide in comparison with sodium-potassium carbonate fusion as treatments precedent to perchloric acid distillation. The fusion procedure was that prescribed in Bureau of Standards Research Paper 110.

The results (Table 3) demonstrate that the calcium peroxide ignitions induced the highest recoveries from the soils that had been treated with fluorides years previously. In some cases fluorine recovery from the fusions was less than that from the soil given no treatment immediately prior to the perchloric acid distillation. Such disparities may be accounted for by the fact that the carbonate fusions developed siliceous colloidal material that repelled evolution of fluorine during the perchloric acid digestion. During such digestion the charges that had been ignited with calcium peroxide maintained a physical state much superior to that which characterized the carbonate-fusion charges during their digestion with perchloric acid.

TABLE 3.—*Calcium peroxide incinerations vs. sodium-potassium carbonate fusions as prior treatments for the perchloric acid distillation of fluorine from soils*

SOIL[a]	ANALYTICAL CHARGE	PRIOR CARBONATE FUSION[b] OR PEROXIDE INCINERATION[c]	FLUORINE RECOVERY[d]
Pot experiment	*grams*		*p.p.m.*
	0.5	None	169
No. 1	2.0	K_2–Na_2CO_3	100
	1.0	CaO_2	205
	0.5	None	96
No. 2	2.0	K_2–Na_2CO_3	98
	1.0	CaO_2	185
	0.5	None	80
No. 3	2.0	K_2–Na_2CO_3	177
	1.0	CaO_2	315
	0.5	None	100
No. 4	2.0	K_2–Na_2CO_3	117
	1.0	CaO_2	154
	0.5	None	65
No. 5	2.0	K_2–Na_2CO_3	95
	1.0	CaO_2	138
	0.5	None	108
No. 6	2.0	K_2–Na_2CO_3	281
	1.0	CaO_2	488
		Lysimeter	
	0.5	None	84
No. 6569	2.0	K_2–Na_2CO_3	70
	1.0	CaO_2	132
	0.5	None	166
No. 6570	2.0	K_2–Na_2CO_3	338
	1.0	CaO_2	372
	0.5	None	676
No. 6571	2.0	K_2–Na_2CO_3	388
	1.0	CaO_2	743
	0.5	None	187
No. 6572	2.0	K_2–Na_2CO_3	376
	1.0	CaO_2	472
	0.5	None	225
No. 6573	2.0	K_2–Na_2CO_3	209
	1.0	CaO_2	402

[a] Soils were from lysimeters and T.V.A. pot experiments. Last four lysimeter soils had been treated with $BaSiF_6$.
[b] The procedure outlined in Bureau of Standards Research Paper 110.
[c] 0.5 gram of CaO_2 was mixed with 0.5 gram of soil; incineration for 30 minutes at 900° C.
[d] Averages of two to four determinations per sample.

Calcium peroxide vs. magnesium peroxide for organics

The data of Table 2 demonstrate the adaptability of calcium peroxide, and the inadmissibility of magnesium peroxide, as oxidants and for incinerations of soils and siliceous materials. Comparisons were therefore made to determine the comparative values of the several calcic and magnesic materials of Table 4 in the pre-treatment requisite for determination of the fluorine content of organics by perchloric acid distillation.

TABLE 4.—*Comparative recoveries of fluorine from organic material*[a] *by incineration with basic compounds and distillation with perchloric acid*

FLUORINE ADMIXTURE[b]	ADMIXED BASIC COMPOUND		INCINERATION		FLUORINE RECOVERY
	COMPOUND	CHARGE	TEMPERATURE	TIME	
grams		*grams*	*°C.*	*hours*	*per cent*
0.00905	$CaCO_3$	1.0	550	12	94.0
0.0181	CaO_2	1.5	Loss due to explosion		
0.0226	$Mg(NO_3)_2$	1.0	500	2	79.0
0.0452	MgO	3.0	550	6	96.0
0.0226	MgO_2 and	2.0			
	$NaC_2H_3O_2$	1.0	600	4	81.5
0.0181	MgO_2	2.0	600	4	100.1
0.0452	MgO_2	3.0	750	1	96.0
0.0226	MgO_2	2.0	600	4	100.0
0.0091	MgO_2	2.0	500[c]	6	100.1

[a] Constant charge of 5 grams of millet.
[b] Fluorine enrichment by admixture of sodium fluoride.
[c] Ashed over a low flame; other incinerations were made in a muffle furnace.

Complete recovery of the fluorine supplied by admixed sodium fluoride was obtained only by the use of magnesium peroxide. Ideal incinerations and complete fluorine recovery were obtained when a mixture of 5 grams of millet and 2 grams of magnesium oxide were incinerated for periods of 4–6 hours in the range of 500°–600° C. The reaction between the peroxide of calcium and the organic matter was, however, so violent that it was deemed inadvisable to try to adjust workable proportions, particularly since the peroxide of magnesium proved such an effective and workable oxidant.

For materials of low organic and high siliceous content, peroxide of calcium is therefore advocated to the exclusion of magnesium peroxide. For materials of high organic and low siliceous content the peroxide of magnesium is preferred.

It is recommended[1] that the proposed procedure be subjected to further collaborative study by the Associate Referee.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 53 (1939).

REPORT ON HYDROGEN-ION CONCENTRATION OF SOILS OF ARID AND SEMI-ARID REGIONS

By W. T. McGeorge (College of Agriculture, University of Arizona, Tucson, Ariz.), *Associate Referee*

In cooperation with the members of the Western Society of Soil Science a study of several methods of determining *p*H of arid soils is now underway. It involves different soil-water ratios using both distilled water and tap water. It has been found that when alkali soils are diluted with water for a *p*H determination the values obtained are much higher than the *p*H of the soil at field moisture content. However, if tap water, which is fairly well buffered, is used for the dilution, the value obtained more closely approaches that in the field than does the value obtained with distilled water. For this reason the use of tap water is being studied.

While there is nothing to report on this work at present, it is hoped that this data will be ready by the next annual meeting. It is recommended[1] that the variation of type of water used and its ratio to soils be studied further.

No report on hydrogen-ion concentration of soils of the humid regions was given by the associate referee.

REPORT ON LIMING MATERIALS

By W. M. Shaw (University of Tennessee Agricultural Experimental Station, Knoxville, Tenn.), *Associate Referee*

The Associate Referee studied the reactivity of limestone in boiling ammonium chloride solution. Special consideration was given to dolomitic limestones from different regions, since this material is being used extensively to condition and to supply nutrient magnesium in mixed fertilizers. The experimental procedure and some of the results on the reactivities of dolomitic limestones of varying degree of fineness will be followed by a more detailed presentation.

PROCEDURE FOR THE DETERMINATION OF REACTIVITY OF LIMESTONES

CHEMICAL PRINCIPLE

A suspension of calcic or dolomitic limestone in a solution of ammonium chloride will react according to the equations—

1. $CaCO_3 + 2NH_4Cl \rightleftarrows CaCl_2 + (NH_4)_2CO_3$.
2. $Ca \cdot Mg(CO_3)_2 + 4NH_4Cl \rightleftarrows CaCl_2 + MgCl_2 + 2(NH_4)_2CO_3$.

When the suspension is heated the $(NH_4)_2CO_3$ is volatilized. The distillate is condensed and trapped in standard acid to afford a measure of the rate of reaction. Calcic and dolomitic limestones show marked differences in reactivity and in the

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 53 (1939).

effect of crystallinity and other physical properties. Reactivity will be affected also by the factors of mass, fineness, concentration of reagent, temperature of the suspension, agitation, and rate of removal of volatile by-products. In a study of any

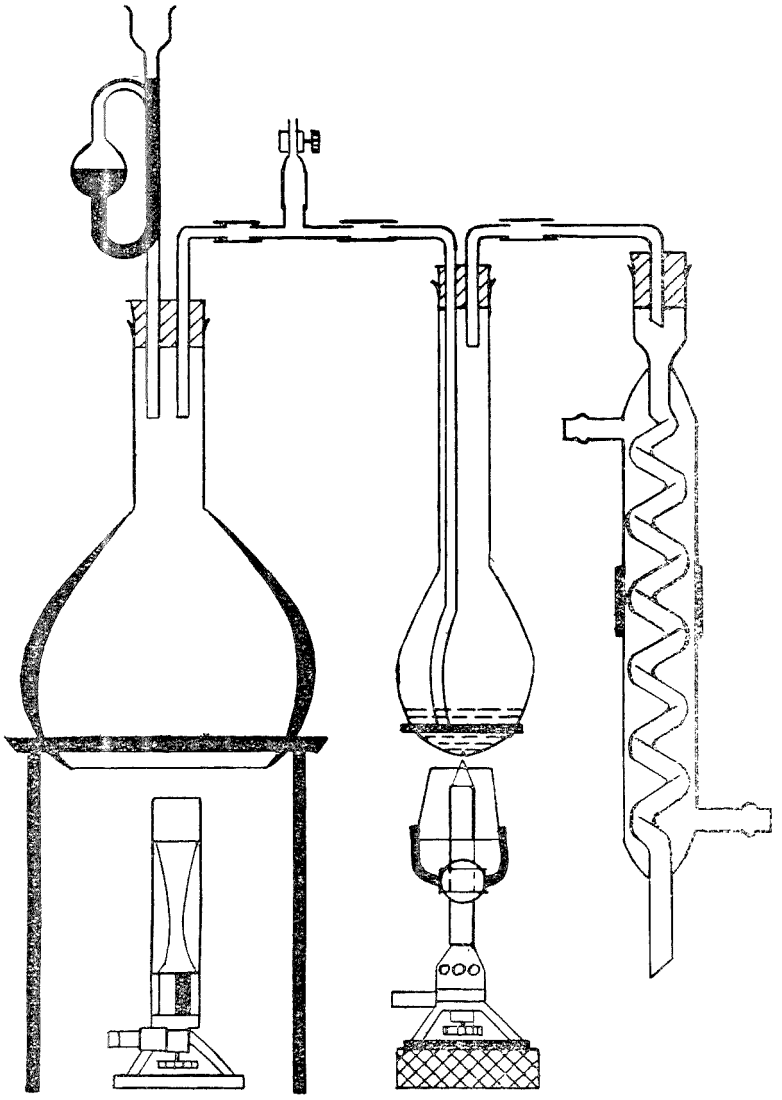

FIG. 1.—AMMONIUM CHLORIDE STEAM DISTILLATION APPARATUS FOR THE EVALUATION OF LIMESTONES

one of those factors, it is essential to maintain all other variables constant. This objective was sought in proposing the apparatus and the procedure herein described.

APPARATUS

(1) The apparatus is shown in Figure 1. The steam generator consists of a 2 liter Pyrex boiling flask, provided with a mercury pressure gage and steam outlet. (2) The reaction is effected in a 500 cc. long-necked Kjeldahl flask provided with a 2 holed rubber stopper carrying 7 mm. glass inlet and outlet tubing; the inlet tube should be so bent as to give a spoon-like outline inside the flask, with an opening constricted to 3 mm. and resting about 7 mm. from bottom of flask slightly off center to the left. (3) The 14 inch condenser is of the spiral type. (4) Graduated beakers, 150 cc. capacity. (5) One Bunsen burner and one Fisher burner. (6) A "T" tube between the steam generator and the digestion flask to allow opening and closing of the steam line.

PROCEDURE

Heat the steam generator to boiling and maintain that temperature. Regulate the heat to vaporize 100 cc. of water every 5.5 minutes. Introduce a 0.25 gram charge of either limestone or dolomite into the reaction flask. Measure the required amount of 0.1 *N* HCl into a graduated beaker, add 2 drops of 0.1% methyl orange indicator, and place the beaker under the condenser. Deliver into the reaction flask 100 cc. of 2 *N* NH_4Cl(C.P.), washing down the adhering material. Connect the reaction flask with the condenser and steam line and bring the steam generator to vigorous boiling by means of the Fisher burner, simultaneously closing the steam line and applying heat to the reaction flask. Maintain the flame under the reaction flask one-inch high and one inch from the bottom of the flask. Collect successive 100 cc. distillates. Titrate the distillate to a change from golden yellow to orange yellow. This titration times 2 expresses the per cent $CaCO_3$ equivalence of sample for that distillation period.

RELATIVE AVAILABILITY OF LIMESTONE SEPARATES

In applying the above procedure to 100-mesh limestone, complete decomposition was effected during the digestion period requisite for a single distillate of 100 cc. If this value is used as 100 per cent availability, a relative value can be assigned any dolomite.

The experimental results on the availability of different separates of a number of typical dolomites are presented in Table 1. It will be observed that in the 20–40-mesh separate four of the six dolomites show an availability of 15±per cent. The other two are known to be exceptional. The one of low availability was an exceptionally hard rock, and the one of

TABLE 1.—*Availability of dolomitic limestone separates and surface reactivity constants for various dolomites*

SOURCE OF DOLOMITE	AVAILABILITY (PER CENT OF CHARGE)					SURFACE REACTIVITY K
	−20 +40	−40 +60	−60 +80	−80 +100	−100 +140	
Kelley Island Lime Co.	16.0	24.2	32.8	37.4	49.0	25
Ladd Lime Co.	14.0	20.6	26.6	34.4	40.4	20
Warner Co., Sample A	12.6	18.2	21.0	25.2	29.0	14
Warner Co., Sample B	15.6	23.6	30.6	36.4	45.0	23
Standard Lime Co.	14.1	22.6	30.8	36.8	45.0	23

much higher value contained calcium carbonate in excess of the dolomitic ratio. The results of each of the normal dolomites show a fairly constant progression in availability with each decrease in particle size. To establish a more fundamental relationship between particle size and availability, the computed surface area of each dolomite separate was plotted against degree of availability (Figure 2). The figures on the base line show the coincidence between each separate and its computed surface area.

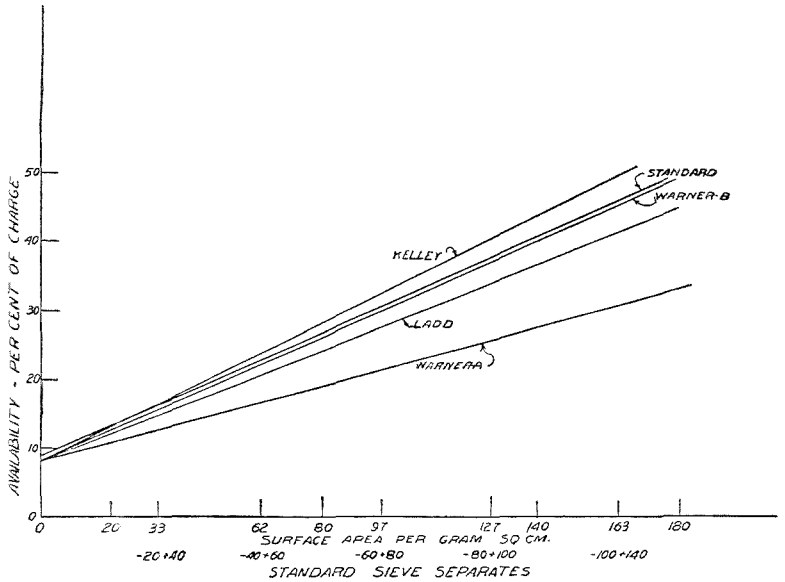

FIG. 2.—AVAILABILITY OF DOLOMITIC LIMESTONES AS AFFECTED BY SURFACE AREA OF SEPARATES

It should be noted that the graphs of Figure 2 were computed on the assumption of spherical particles, although such uniformity in shape does not obtain. Moreover, the mean of diameter taken was the mean of the openings of the respective pairs of sieves, on the assumption of an even distribution of extremes. This assumption, however, may not be justified for all separates. Nevertheless, distinct and close correlation between the variables was found for each dolomite, as shown by the straight lines in Figure 3. These straight lines indicate that for *this experimental range,* at least, the availability by the proposed method is directly proportional to the surface area of the separates. The relationship between surface and availability may be expressed by the straight line equation: $Y = A + BX$, in which Y is the availability in per cent of charge, X is the surface area per gram of dolomitic separate in terms of square centimeters, A, the

point at which the extension of the experimental lines intersects the Y-axis, which in the experimental range has a common value of 8, and B, which is a characteristic constant of each dolomite. This B value of any unknown dolomite may be determined empirically for one separate or preferably for two separates and the resultant Y values substituted in the formula, $Y=8+BX$. For simplicity, making K equal 100B, a surface reactivity constant of each dolomite can be expressed as percentage calcium carbonate equivalence of charge per 100 square centimeter surface per gram material. This K value, determined by averaging the several experimental points for each rock of Table 1, is given in the last column of that table. The evaluation of this constant presents a most sensitive method for detecting heterogeneous composition of a given sample. For example, the Mascot Knox dolomite, which contains about 10 per cent calcium carbonate over the dolomitic ratio, gives the following computed K values for the respective experimental points in Table 1: −49, 34, 33, and 26. Such divergence in K values of the individual experimental points establishes the presence of a carbonate more soluble than the dolomite proper, usually either calcite or aragonite.

Taking 23 as the average K value for normal dolomites, the writers recalculated the availability of the several separates by inserting the surface area of the respective separates in place of X, thus $Y=8+.23X$. The following values were then obtained: 15.6, 23.3, and 33.8 for the 20 to 40-, 40 to 60- and 60 to 100-mesh separates, respectively. These mean values are in fair agreement with Taylor and Pierre's[1] experimental data obtained by determination of the carbonate decomposition during a single season, which were 12, 21, and 33 for the respective separates. The writer's outdoor experiments[2] with Mascot dolomite and Cumberland silt loam showed for one season a mean carbonate decomposition about 10 per cent above the laboratory figures for the same dolomite. It is recognized that the reaction between a soil and a dolomite will depend on degree of soil acidity and other factors. The above soil experiments are cited merely to indicate the correlation of the present laboratory data with those obtained from one season's soil contact.

The same procedure may be applied to field samples of limestone to express availability in terms of 100-mesh calcic limestone. Such valuation will reflect the factors of fineness, and also physical and chemical characteristics of the rock.

It is recommended[3] that the ammonium chloride steam distillation procedure for the evaluation of limestone availability be studied further in relation to soil-carbonate reactions in pot experiments.

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[1] *J. Am. Soc. Agron.*, **27**, 764 (1935).
[2] *Ibid.*, 22, 272 (1930).
[3] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 53 (1939).

REPORT ON THE AMMONIUM CHLORIDE-DISTILLATION PROCEDURE FOR THE DETERMINATION OF EXCHANGEABLE BASES IN SOILS

By W. M. SHAW (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.)

The work of the Associate Referee on Liming Materials during the past year was directed toward improvements in the technic and accuracy of the ammonium chloride-distillation procedure for the determination of base exchange values of soils. It was thought advisable to postpone collaborative work until the completion of the studies leading to such improvements. The results of those studies follow.

ACCELERATION OF THE AMMONIUM CHLORIDE-SOIL DIGESTION

The procedure of the 1937 report called for digestion of soils with ammonium chloride in 600 cc. covered beakers over a Bunsen burner until a special bromocresol green test paper gave a negative test for ammonium hydroxide in the issuing vapors. Under such circumstances a soil carrying a moderate supply of dolomitic limestone would require 4–5 hours for the complete dissolving of the carbonates. In related studies on the rates of decomposition of limestones and dolomites by ammonium chloride it was found, however, that the passage of steam through the digestion medium greatly expedited the decomposition process. The apparatus (see Figure 1, p. 238) was adopted for soil digestions with ammonium chloride. This assembly consists of a 2 liter boiling flask as a steam generator, a 500 cc. long-necked Kjeldahl digestion flask, and a 14 inch coiled-tube condenser with connections of 7 mm. glass tubing. The inlet tube extends to 7–8 mm. from the bottom of the Kjeldahl flask and is constricted to a 3 mm. opening.

Directions for the present technic are as follows:

> Weigh 10 gram charges of soil into the digestion flask, and wash the neck and sides of flask by an addition of 100 cc. of 2 *N* NH_4Cl solution. Connect the digestion flask with the steam generator and with the condenser. Bring the steam generator to boiling temperature and adjust the burner to give a vaporization rate of 100 cc. of water every 5.5 minutes. Place the receiver with 0.1 *N* acid under the condenser and begin digestion by closing the T-tubes and adjusting the auxiliary burner to give a flame 1 inch high and 1 inch below the bottom of the digestion flask. Collect each successive 100 cc. distillate and determine its titration value until the 0.1 *N* titer value is reduced to 1.0 cc.

The objective of this procedure was to effect complete disintegration of the carbonates of the soil *without decomposition* of the soil complex. It was therefore essential to determine how readily included calcic and dolomitic limestones are dissolved by the proposed procedure. Accordingly, 9 soils of wide range in texture, organic matter content, and degree of base saturation were selected. Each soil was subjected to the

digestion-distillation process in order to establish its inherent capacity to release ammonia from a boiling solution of ammonium chloride. Each soil was also supplemented with 0.25 gram of high-grade dolomite, and the progressive release of ammonia from these mixtures was compared with such release from the untreated soil. The results are presented in Table 1.

It will be observed that the ammonia released by the unsupplemented surface soils, Series A, shows a wide range, and digestion periods ranging from 11 to 33 minutes against a range of 5.5–22 minutes for subsoils. All the original differences in ammonia release from the surface soils were obliterated in the dolomite-supplemented surface soils and with completion effected by 12 or 13 distillation periods. The dolomite decomposition in the subsoil samples was effected more rapidly, during 9–10 periods. The consistent difference between the order of dolomite decomposition in surface soils and subsoils suggests the possibility that the dolomite is actually decomposed in a shorter time than that indicated in all cases, and that the prolonged release of ammonia from the surface soils is due to a difference between equilibrium between ammonia and organic matter and a corresponding equilibrium between ammonia and the mineral soil complex. It should be noted that the dolomite supplements were much larger than the amounts to be found ordinarily in soils. Moreover, in a soil sample ground to pass a 60 mesh, the dolomite would be much finer than the 60- to 80-mesh fineness taken in these experiments. These considerations point to the conclusion that a 2.5 per cent content of dolomite would be dissolved completely from a soil during a 1 hour digestion by the proposed procedure and that smaller proportions of dolomite would be dissolved in still less time.

The dissolving of calcic limestone by this procedure is so rapid that no problem is presented. This is shown by 3 c. of Table 1. The addition of 2.5 per cent calcic limestone of 60–80 mesh to a highly saturated Dewey silt loam required only one 5.5 minute digestion beyond the single digestion required for the unsupplemented soil.

These experiments established maximal limits of digestion periods for complete dissolving of mineral carbonates under extreme conditions. The actual time requirement for each sample can be established definitely by the ammonia release in successive distillates as above presented. The closed system steam-digestion requires only about one-fourth of the time required for the previously described beaker digestion. Digestion in the Kjeldahl flask has an additional advantage over beaker digestion in that the soil is readily removed from the flask by a jet of the wash solution, whereas both beaker and its cover-glass have to be policed free of adhering soil. This method of digestion also admits of more accurate recognition of the complete dissolving of dolomite present in soils.

TABLE 1.—*Decomposition of 60-80-mesh limestone and dolomite admixed with various soils, as determined by the ammonium chloride-steam distillation procedure*

REF. NO.	SOILS*	ADDITION†	0.1 *N* TITER OF SUCCESSIVE 100 CC. DISTILLATES													
			1	2	3	4	5	6	7	8	9	10	11	12	13	SUM
1A	Hartsells sandy loam	0	1.5	1.0												2.5
1B	Hartsells sandy loam	Dolomite	14.1	10.8	7.6	5.6	4.5	3.0	2.3	2.0	1.8	1.5	1.3	1.1		
2A	Clarksville silt loam	0	2.3	1.2	.9											4.4
2B	Clarksville silt loam	Dolomite	14.5	10.3	7.5	5.7	4.2	(4.0)	2.5	2.2	1.9	1.6	1.4	1.2	1.0	
3A	Dewey silt loam	0	6.0	2.0	1.5	1.2	1.0									11.7
3B	Dewey silt loam	Dolomite	17.5	10.2	8.3	6.4	5.2	4.2	3.2	2.4	2.0	1.5	1.2	1.0		
4A	Colbert silty clay loam	0	10.3	3.2	2.1	1.6	1.3	1.0								19.5
4B	Colbert silty clay loam No. 6562	Dolomite	20.6	11.7	8.9	7.6	5.7	4.9	3.9	3.1	2.3	1.8	1.3	1.2	1.0	
5A	Decatur silt loam‡	0	35.0	9.3	5.0	3.2	2.8	2.1	1.6	1.3	1.2	1.0				
6A	Cherokee clay subsoil No. 6557	0	.7													.7
6B	Cherokee clay subsoil No. 6557	Dolomite	13.0	12.5	8.7	6.9	4.7	2.8	1.8	1.2	1.0					
7A	Cherokee clay subsoil No. 6561	0	1.9	.7												2.6
7B	Cherokee clay subsoil No. 6561	Dolomite	15.1	12.9	9.0	6.2	4.0	2.4	1.5	1.2	1.0					
8A	Colbert silty clay subsoil	0	6.0	1.6	1.1	.9										9.6
8B	Colbert silty clay subsoil	Dolomite	18.6	12.0	11.4	7.0	5.2	3.6	2.5	1.8	1.3	1.0				
3C	Dewey silt loam	Limestone	42.5	10.4	3.0	1.6	1.2	1.0								

* 10 grams of air-dry soil.
† 0.25 gram of limestone or dolomite.
‡ This soil has a natural dolomite content of 2.19% as $CaCO_3$ equivalence

ACCURACY OF MAGNESIUM DETERMINATION BY THE BOILING AMMONIUM CHLORIDE SOLUTION

In the collaborative study of this procedure in 1937, the magnesium results were discordant and in general higher than those obtained by the Associate Referee. Some of the collaborators suggested that the discordance may be due to decomposition of magnesic minerals by the boiling ammonium chloride solution. To throw some light on this point the Associate Referee made duplicate and triplicate extractions of 8 soils and determined the magnesium content of each extract. The results (Table 2) seem to establish the precision of the magnesium determination. Most of the determinations agree within a few hundredths of 1 mg. equivalent per 100 grams of soils and only 3 of the 20 determinations were out of line as much as 0.2 cc. The higher values obtained by some collaborators in 1937, therefore, cannot be attributed to drastic action of the boiling ammonium chloride solution. The cause for erroneous magnesium values is

TABLE 2.—*Precision and extent of absorbed magnesium determination by extraction with boiling ammonium chloride solution*

SOIL	MAGNESIUM FROM BOILING EXTRACTION			1937 REPORT BY ASSOC. REF.
	1	2	3	
1937 A.O.A.C. No. 1	1.26	1.04	1.16	1.32
1937 A.O.A.C. No. 5	2.08	2.04	—	2.25
1937 A.O.A.C. No. 6	1.04	1.16	1.00	1.10
Cherokee clay subsoil No. 6562	0.66	0.74	—	—
Colbert silty clay loam X-10	2.62	2.66	2.55	—
Colbert silty clay subsoil	1.64	1.60	1.46	—
Becket sandy loam	5.28	5.32	—	—
Becket sandy subsoil	1.18	1.16	—	—

probably incomplete removal of the manganese, which is nearly always present in the extracts. Complete removal of this element can be accomplished by the addition of ammonium persulfate to a slightly ammoniacal solution at boiling heat. The hot ammonium chloride wash solution should be distinctly ammoniacal to preclude the dissolving of manganese during the washing of the hydrated oxide. Contamination of alumina is obviated by the addition of 3–4 cc. of molar citric acid before the precipitation of the magnesium.

EFFECTIVENESS OF ADDED MAGNESIUM OXIDE IN THE DISTILLATION OF ABSORBED AMMONIA IN SOILS

In the determination of absorbed ammonium ions as a measure of exchange capacity the ammonia release and distillation is usually induced by the addition of magnesium oxide to the soil-water suspensions. The released ammonia in successive 100 cc. distillates was determined as a

measure of the speed of ammonia evolution by the magnesium oxide displacement. The ammonia release from soils of high absorptive capacity was prolonged and quite incomplete in distillates of 200 cc. Six soils of high absorption capacity were distilled from aqueous suspensions of limited sodium hydroxide concentration in comparison with distillations in which magnesium oxide was used. The results are given in Table 3.

TABLE 3.—*Comparative efficiency of MgO and NaOH in the distillation of absorbed NH_4OH in soils*

SOIL	MgO OR NaOH	0.1 *N* TITER OF 100 CC. DISTILLATES						
		1	2	3	4	5	6	SUM 1-2
Dewey silt loam	MgO*	10.1	.3					10.3
	NaOH†	10.1	.2					10.3
Decatur silt loam	MgO	13.4	.5	.1	.1			13.4
	NaOH	14.3	.2	.1	.1			14.5
Colbert silty clay loam	MgO	14.3	1.0	.5	.3			15.3
	NaOH	18.5	.3	.1	.1			18.8
Colbert silty clay loam subsoil	MgO	30.9	2.6	1.4	.8	.6	.4	33.5
	NaOH	38.2	.6	.1	.1			38.8
Colbert silty clay loam X-10	MgO	35.2	2.2	1.2	1.0	.6	.5	37.4
	NaOH	44.6	.7	.2	.1	.1		45.3
Becket sandy loam	MgO	24.7	.4	.15	.15			25.1
	NaOH	25.4	.4	.20	.15			25.8

* 0.5 gram of MgO for each determination.
† 12.5 cc. normal NaOH determination.

It was anticipated that objection would be raised against the use of sodium hydroxide on the grounds that it decomposes soil organic matter. Sodium hydroxide effects a dispersion of organic matter, but the results of Table 3 show no vitiative effect on the absorbed ammonium determination, since the higher values obtained by the use of sodium hydroxide are not coincident with the high organic matter content of the soils. On the contrary, the greatest differences occur with soils of low organic matter content and subsoils. The minimal difference between the ammonia releases by magnesium oxide and sodium hydroxide was shown by the Becket sandy loam, whose absorption is due almost entirely to the organic matter content. Moreover, on soils with only moderate absorption capacity, 10 m.e. or less, concordance in ammonia distillations is shown for the two hydroxides. With soils of high inorganic colloid content, however, complete recovery of the absorbed ammonia is not effected by distillation with magnesium oxide.

EFFECT OF AMMONIA ADDITIONS TO THE AMMONIUM CHLORIDE SOIL SUSPENSION AND TO ALCOHOL WASH ON EXCHANGE CAPACITY VALUES

Neutralization of the wash alcohol with ammonium hydroxide is generally followed by investigators in determining exchange capacity by ammonia absorption. It was found difficult to maintain the alcohol at the neutral point and the quantities of ammonia required for neutralization appeared to be excessive. Furthermore, the addition of the slightest amount of water to such neutralized alcohol results in a *p*H value of 8 or 9. Usually about 2.5 cc. of normal ammonium hydroxide per liter of alcohol is required to raise the *p*H to 7.0, but the addition of only 0.1 cc. of 0.1 *N* sodium hydroxide to 100 cc. of alcohol brought a *p*H of 9.0. This problem is also linked up with the state of ammonia saturation of the soil after digestion. Because of the hydrolysis of the ammonium chloride the soil suspension after digestion is distinctly acid, *p*H of about 4.0. This acid condition is neutralized by the addition of 15 cc. of 0.1 *N* ammonium hydroxide to the soil suspension before filtering. It is probable that this constant addition of ammonia will not effect the same degree of neutralization of the various soil-ammonium chloride systems. Within a wide range of soils it is possible that some may not be completely neutralized, whereas others may be by the 15 cc. addition of 0.1 *N* ammonium hydroxide. To evaluate the effect of varying degrees of neutralization of the soil suspension subsequent to the digestion with the ammonium chloride, absorbed ammonia was determined on three typical soils under different conditions as to neutralization of the soil suspension and also the alcohol wash. The experimental results are given in Table 4.

In considering these data the maximal ammonia absorption was taken as the basis of comparison for the various treatments. In treatment A, in which the soil suspension was filtered without neutralization and washed with untreated alcohol, the ammonia retentions were from 52 to 84 per cent of the respective maxima. The greatest variation from maximum, as a result of withholding of ammonia from both stages of the procedure, is shown for the soils of high content of organic matter. The absorptive capacity of the Becket soil is due almost entirely to its organic matter content, and the greatest deficiency occurred in this soil. Treatment B, in which the soil suspensions were not neutralized, but in which the alcohol wash contained the equivalence of 5 cc. of 0.1 *N* ammonium hydroxide, caused an ammonia absorption practically equal to the ammonia carried by the alcohol wash. This indicates that the alcohol readily yields its ammonia content to the unsaturated soils. Treatment C, in which the soil suspensions were neutralized, but which were washed with plain alcohol, was not quite so effective as treatment B in the build-up of ammonia absorptions. This means that the absorption from 5 cc. of 1 *N* ammonia in the alcohol wash was greater than absorptions

TABLE 4.—*Effect of NH_4OH additions to soil suspension and to alcohol wash, upon exchange capacity value by the NH_4Cl method*

SOIL	SERIES	0.1 *N* NH_4OH ADDED TO—		EXCHANGE CAPACITY
		SUSPENSION	ALCOHOL	
		cc.	*cc.*	*m.e.*
Colbert silty clay subsoil	A	0	0	31.6
	B	0	5	35.5
	C	15	0	34.5
	D	15	5	36.2
	E	15	12.5	37.8
	F	25	5	36.8
Colbert silty clay loam No. 6561	A	0	0	25.2
	B	0	5	30.3
	C	15	0	29.8
	D	15	5	32.8
	E	15	12.5	33.9
	F	25	5	32.4
Becket sandy loam (very high organic matter content)	A	0	0	25.0
	B	0	5	33.6
	C	20	0	32.0
	D	20	5	39.7
	E	20	12.5	48.0
	F	30	5	45.8

from additions of 10–20 cc. to the soil suspensions. Treatment D, whereby both the soil suspensions and the alcohol wash were neutralized, registered ammonia absorptions close to the maximum for the two minerals soils against a disparity of about 8 m.e. for the Becket sandy loam, which is essentially an organic complex. Treatment F, in which an extra 10 cc. of ammonia was added to the soil suspensions and which otherwise was the same as treatment D, gave results almost identical to those of the soils to which no excess of ammonia was added, except for the Becket soil, which showed an appreciable increase in absorbed ammonia.

The results of these experiments indicate that (a) neutralization of the soil suspension is essential only to an approximate degree, (b) ammoniation of the alcohol wash has a balancing effect in removing excess of ammonia as well as in neutralizing absorbed hydrogen in the soil system, (c) increases in the ammonia content of the alcohol wash exerts only a slight effect on ammonia absorbed by mineral soils, such increase causing marked enhancement in ammonia absorption by soils of high organic matter content.

It is recommended[1] that studies of the function of ammonia in the alcohol wash used in the determination of base exchange capacity be continued.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 53 (1939).

REPORT ON LESS COMMON ELEMENTS IN SOILS

FLUORINE IN SOIL AND OTHER MATERIALS RELATED TO AGRICULTURE

By J. S. McHARGUE, *Associate Referee,* and W. S. HODGKISS
(Department of Chemistry, Kentucky Agricultural Experiment Station, Lexington, Ky.)

During the past year the Department of Chemistry of the Kentucky Agricultural Experiment Station has been interested in ascertaining the fluorine content of soil and other materials related to agriculture. The analytical results reported here were obtained by W. S. Hodgkiss, and collaborative work was accomplished through the assistance of W. H. MacIntire and J. W. Hammond of the Tennessee Experiment Station, who sent to this laboratory three samples of soil and two samples of bones for analysis of their fluorine content, and also a copy of the methods they use for the determination of the fluorine content of organics, siliceous materials, and soils.

These methods were followed in the determinations of fluorine in the samples submitted. Some difficulty was experienced in checking some of the results on fluorine sent to us later by Hammond, but with further experience, significant differences in the results obtained by the two collaborators were largely eliminated. Table 1 shows the results obtained by Hammond and Hodgkiss.

TABLE 1.—*Results obtained by Hammond and Hodgkiss*

	FLUORINE CONTENT	
	HAMMOND	HODGKISS
Tennessee Samples		
	p.p.m.	
Lysimeter Soil No. 1	110	83
Lysimeter Soil No. 2	743	411
Red Clay Subsoil No. 3	9	45
Steamed Bone A	920	977
Normal Bone B	404	428
Kentucky Samples		
Sandy loam soil, Ky. C-4165	0.59	0.68
Wheat, plot No. 1, above soil, grain	1.80	1.85
Wheat, plot No. 1, above soil, straw	0.72	0.84
Wheat, plot No. 12 P.C., above soil, grain	1.39	1.24
Wheat, plot No. 12 P.C., above soil, straw	0.72	0.61
Wheat, plot No. 13 R.P., above soil, grain	1.40	1.76
Wheat, plot No. 13 R.P., above soil, straw	0.84	0.63
Phosphatic Sand (B.P.L. 60%)	3.26%	3.28%

During the year this Department was also called upon to make fluorine determinations of about 25 samples of bones collected from horses ranging in age from suckling colts to animals five or six years old. The

fluorine content of the samples ranged from about 100 p.p.m. in the fat-free bones of the younger animals to approximately 300 p.p.m. in similar fat-free bones of the mature horses.

In the course of the analyses for fluorine in the bones of the horses it was observed that when a sample of raw bone was burned in a silica dish with a Bunsen burner until all the volatile matter was consumed and the

TABLE 2.—*Fluorine content of ashed whole bone as compared to fat-free bone*
(1 gram sample used)

LAB. NO.	KIND OF BONE	F FAT-FREE BONE ASH BASIS	F WHOLE BONE ASH BASIS
		p.p.m.	*p.p.m.*
C-10053	Rib	246	254
C-10054	Vertebrae	313	310
C-10055	Femur	276	318
C-10060	Rib	228	263
C-10061	Vertebrae	235	233
C-10062	Femur	241	246

incineration was continued in a muffle furnace at dull red heat until free of carbon, the bone ash thus obtained contained as much fluorine as did the ash in other portions of the same sample which were extracted with ether to remove the fat.

This observation suggested the following experiments: Fat-free bone samples were ashed without the addition of magnesium peroxide, which is

TABLE 3.—*Fluorine content of fat-free bones from the same animal ignited at 600° C. without and with the addition of magnesium peroxide*
(1 gram sample used)

LAB. NO.	KIND OF BONE	MgO_2 ADDED		FLUORINE
		gram		*p.p.m.*
C-10053	Ribs	0.0		148.2
C-10053	Ribs	0.5		146.0
C-10053	Ribs	1.0		148.0
			Av.	147.4
C-10054	Vertebrae	0.0		174.0
C-10054	Vertebrae	0.5		173.4
C-10054	Vertebrae	1.0		175.0
			Av.	174.1
C-10055	Femur	0.0		164.0
C-10055	Femur	0.5		163.5
C-10055	Femur	1.0		165.0
			Av.	164.2

a slight modification of the procedure suggested by MacIntire and Hammond for the determination of fluorine in materials containing organic matter.

The results (Table 3) indicate that it is not necessary to add magnesium peroxide in the determination of fluorine in fat-free bone material when the samples are burned for about 3 hours in an electric furnace at approximately 600° C.

The slight modifications in the method for the determination of fluorine in bones suggested by the results of the foregoing experiments probably apply only to bones. No other experiments were made to ascertain whether other materials containing organic matter can be burned directly without the loss of fluorine.

Other products that have required the determination of the fluorine content during the past year are the commercial mineral mixtures that are used as supplements to the feed for live stock.

TABLE 4.—*Fluorine content of mineral mixtures used for live stock*

SAMPLE	*per cent*
1	0.0326
2	0.0386
3	0.0430
4	0.0578
5	0.1157
6	0.1748
7	0.3550
8	0.5220
9	0.7147

Due to the rather high percentages of fluorine contained in certain of these mineral mixtures and also to the fact that a considerable number of farmers in Kentucky have experienced the loss of valuable live stock from their use, the Departments of Chemistry and Feed Control of the Kentucky Agricultural Experiment Station regard mineral mixtures containing as much fluorine as the majority of the above samples show as a potential source of danger to live stock. The importance of a reliable method for the determination of fluorine in products related to agriculture is therefore apparent.

It is recommended[1] that further study be made of the calcium peroxide method for the determination of fluorine in soil. It is suggested that comparative data be obtained on the fluorine content of soil by its determination by fusion with sodium or potassium hydroxide in a nickel crucible and the results obtained by the calcium peroxide procedure.

No report on selenium in soils was given by the associate referee.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 53 (1939).

The two papers on the "Decomposition of Dolomite Limestone in Soils when Used as Neutralizing Agents in Complete Fertilizers," presented by Dawson, Snyder, Leighty, and Reid, and by Collins and Speer, respectively, were published in *This Journal*, 22, 137, 142 (1939).

REPORT ON FERTILIZERS

By G. S. FRAPS (Agricultural and Mechanical College of Texas, College Station, Texas), *Referee*

The recommendations of the various associate referees will be presented in their reports, and therefore the Referee will do no more here than to express the appreciation of this Association for their services. There are, however, two subjects that should be discussed.

A recent method by one of the members of this Association requires the use of wide-necked graduated flasks of suitable size to take a two-holed rubber stopper. In response to a letter protesting the use of such inaccurate measuring devices, he replied that flasks of this kind are listed by chemical supply houses as "fertilizer flasks." The diameters of the necks of such flasks are far beyond the sizes adopted by the Bureau of Standards. The use of such flasks brings up the question whether a certain degree of accuracy should not be required in the weights, volumetric flasks, pipets, and burets, used in connection with A.O.A.C. methods of analysis. The Bureau of Standards has set limits of precision for measuring instruments and for weights. These limits may be too exacting for control work but if so, limits of error should be adopted so that the use of inaccurate measuring instruments would not be permitted by this Association. It is not difficult to test the calibration of volumetric flasks by means of a Morse buret calibrated by the Bureau of Standards, and pipets can be tested by weighing the water which they deliver. The testing of burets is somewhat more difficult. At this Station the pipets and measuring flasks have been tested for a number of years. Recently 21 of 24 graduated flasks manufactured by a national manufacturer, did not meet the requirements and were returned. Two or three years earlier another lot of 20 out of 24 was returned as not sufficiently accurate. It is not unusual to receive a few flasks and pipets that are not sufficiently accurate. Weights, though accurate when purchased, may deteriorate under the severe service of a control laboratory, and may need testing from time to time.

The Referee believes that this Association should consider this matter and specify the use of instruments of the proper degree of exactness for control work, which would at the same time outlaw the wide-necked flasks now known as fertilizer flasks and other inaccurate instruments. Therefore it is recommended that an associate referee be appointed to

test volumetric instruments and weights and to work in cooperation with the Bureau of Standards.

At the last meeting of the Association a method for estimating the available phosphoric acid in fertilizers was presented, and it has since been published in *The Journal* of this Association and in *Industrial and Engineering Chemistry*. The official ammonium citrate method for determining available phosphoric acid has been in use for over 50 years and is the basis for large transactions in phosphates. It is therefore necessary for this Association to examine very carefully and thoroughly any new method in order to see that it meets the requirements of the trade and does not bring about any unforeseen and undesirable consequences. The proposed new method is probably shorter than the one now in use, provided the determination of total phosphoric acid is not needed, since the new method requires only one determination of phosphoric acid instead of two. The new method is claimed to give the same results with superphosphate as does the present method, but the analyses given in the paper referred to are not altogether in accord with this claim, since one of them is 0.8 per cent lower than the official method, and other differences are shown. Analyses of 18 samples were made by the proposed method in the Texas Laboratory by S. E. Asbury. Of 49 determinations 12 (nearly 25 per cent) were discarded as too wide. The new method did not give entirely concordant results on the same samples. When the average results are compared, 7 come within 0.25 per cent of the official method, one is 0.25–.40 per cent too low, 4 are over 0.4 per cent too low, 2 are 0.25–0.4 too high, and 4 are over 0.4 per cent too high. The new proposed method thus does not give exactly the same results on superphosphate as the official method. It is necessary to consider seriously the question whether the different results secured by the new method with some superphosphates will offset the possible advantages of the method. There should also be some reason given for the discrepancies between the results of the two methods. The proposed method also needs modification so as to secure concordant results on the same samples. The *p*H of the solvent prepared by the method described is 4.4 and not 4.2, as stated in the description. This difference requires explanation.

The proposed new method also gives different results from the official method with other materials than superphosphate, and in some cases the differences are quite large. Much more of the phosphoric acid of tricalcium phosphate is found to be available by the new method than by the official method. What effect these differences would have on the fertilizer industry is a matter for consideration. The new method is worthy of study, but many comparisons with the official method among fertilizers are needed, together with much information regarding the reasons for the differences which will be found, before the Association will be in position

to consider seriously the question of replacing the official method by the new method.

RECOMMENDATIONS[1]

The Referee on Fertilizers makes only one recommendation in addition to those made by the associate referees, namely, that an Associate Referee on Testing Volumetric Apparatus and Weights be appointed, to work in cooperation with the Bureau of Standards and to recommend methods for discouraging the use of apparatus and weights that are too inaccurate.

REPORT ON PHOSPHORIC ACID

A. EFFECT OF METHOD OF FILTERING ON DETERMINATION OF WATER-SOLUBLE P_2O_5. II. B. VARIATION IN CITRATE-INSOLUBLE P_2O_5 WITH TIME INTERVAL BETWEEN WATER EXTRACTION AND CITRATE DIGESTION, II.

By WILLIAM H. ROSS, *Associate Referee*, and J. RICHARD ADAMS
(Bureau of Chemistry and Soils, Washington, D. C.)

In a laboratory study that was made last year by Adams and Ross[2] at the suggestion of the Referee on Fertilizers,[3] it was found that:—

(A) The kind of filter paper on which the sample is washed in the determination of water-soluble P_2O_5 has little, if any, effect on the results, and that washing with suction gives lower results than washing under gravity.

(B) The citrate-insoluble P_2O_5 in materials such as ammoniated superphosphate, which contain di- or tricalcium phosphate, increases with prolonged standing of the washed residue before digestion in citrate solution, but not in materials, such as ordinary superphosphate, which do not contain appreciable quantities of either di- or tricalcium phosphate.

A recommendation was adopted at the last meeting of this Association that the work be continued by collaborative study. The standard samples submitted to the collaborators in compliance with this recommendation were as follows:

STANDARD SAMPLES

1. Mixed fertilizer (6-12-6).
2. Mixed fertilizer (5-10-5).
3. A mixture of the principal components of an ammoniated superphosphate, fluorine free.
4. A mixture of the principal components of an ammoniated superphosphate, containing 2.4 per cent of fluorine.

The formulas of the mixed fertilizer samples were as follows:

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 51 (1939).
[2] *This Journal*, 21, 268 (1938).
[3] *Ibid.*, 20, 223 (1937).

MATERIAL	MIXED FERTILIZER (6-12-6) SAMPLE NO. 1	MIXED FERTILIZER (5-10-5) SAMPLE NO. 2
Ammoniated superphosphate (5.65% N; 11.33% avail. P_2O_5)	1420	—
Ordinary superphosphate (20.25% avail. P_2O_5)	—	989
Dicalcium phosphate (51.37% avail. P_2O_5)	154	—
Ammonium sulfate (20.9% N)	97	383
Sodium nitrate (16.5% N)	121	121
Potassium chloride (57.8% K_2O)	208	173
Tennessee rock phosphate	—	334
	2000	2000

The compositions of the assimilated ammoniated superphosphate samples were as follows:

MATERIAL	ASSIMILATED AMMONIATED SUPERPHOSPHATE SAMPLE NO. 3	ASSIMILATED AMMONIATED SUPERPHOSPHATE SAMPLE NO. 4
Monoammonium phosphate	100	100
Dicalcium phosphate	1100	1100
Tricalcium phosphate	500	500
Ammonium sulfate	200	200
Calcium sulfate dihydrate	100	—
Calcium fluoride	—	100
	2000	2000

Samples 1 and 4 contained fluorine and di- and tricalcium phosphates; Sample 2 contained fluorine but no di- or tricalcium phosphate; while Sample 3 contained di- and tricalcium phosphate but no fluorine.

The directions sent to the collaborators were as follows.

DIRECTIONS FOR ANALYSIS

A-1. Determine water-soluble P_2O_5 in each of the standard samples as directed in *Methods of Analysis*, *A.O.A.C.*, 1935, p. 21, **13**, using a 9 cm. Whatman filter No. 2 for the water extraction, and wash under gravity.

A-2. Repeat the determinations, using a 9 cm. Whatman filter No. 5, and wash under gravity.

A-3. Repeat the determinations, using a 9 cm. Whatman filter No. 5, and wash under suction.

A-4. Repeat the determinations, using a Shimer filter, and wash under suction.

B-1. Determine citrate-insoluble P_2O_5 in each of the standard samples as directed in *Methods of Analysis*, *A.O.A.C.*, 1935, for acidulated samples, p. 22, 16(a). In making these determinations, wash the sample by one of the procedures outlined above, and when the washing has been completed, immediately transfer the filter containing the residue to the citrate solution at 65° C.

B-2. Repeat the determinations, allowing the washed residue to stand on the filter paper for 2 hours before transferring it to the citrate solution.

B-3. Repeat the determinations, allowing the washed residue to stand on the filter paper for 4 hours before transferring it to the citrate solution.

B-4. Repeat the determinations, allowing the washed residue to stand on the filter paper for 18 hours before transferring it to the citrate solution.

NOTES

a. The filter pulp for the Shimer filter is most conveniently prepared from Schleicher and Schüll's No. 292 filter pulp disks. When these are not available, prepare the filter pulp by tearing from five to eight 9 cm. filters or their equivalent of sheet filters into shreds, place the pieces in a 500 cc. Erlenmeyer flask, add 250 cc. of hot water, close the flask with a rubber stopper, and, under the protection of a towel, shake vigorously until the paper is reduced to a pulp. The mat in the Shimer filter should be $\frac{1}{4}''$–$\frac{3}{8}''$ thick when compacted by suction. It is necessary to compact the filter mat by suction in order to make it sufficiently retentive to hold the residue, and to prevent disruption of its upper surface when the wash water or solution to be filtered is poured directly on the filter mat. A rubber stopper fastened to a glass rod may be used to compact the filter.[4]

b. The funnels in which the filters containing the washed residues are allowed to stand for a time before digestion in the citrate solution should be covered with a watch-glass and the tips of the funnels kept under water to prevent drying of the residues.

c. The results obtained for each sample by the different procedures outlined should not vary greatly, and it is therefore very important, if the true effect of each of the factors under investigation is to be determined, that no variation be made in any of the procedures other than the one specified.

METHODS OF FILTERING

The directions submitted to each collaborator for the analysis of the standard samples were accompanied by a questionnaire requesting information on the method of filtering and the kind of filter used (a) in the determination of water-soluble P_2O_5; (b) in filtering off the yellow ammonium phosphomolybdate precipitate obtained in the volumetric method of determining P_2O_5; and (c) in filtering off the residue from the citrate digestion.

The replies received indicate that water-soluble P_2O_5 is commonly determined by washing on filter paper under gravity. The method of filtering the phosphomolybdate precipitate, however, varies greatly in different laboratories. The preferred procedure seems to consist in filtering on a mat of asbestos or filter paper pulp in a Shimer or similar type filter. Filtering with suction on a filter mat was reported by C. A. Butt to have advantages over filter paper in the ordinary funnel in that it requires a smaller number of washings for complete removal of acidity, decreases danger of loss of precipitate, and avoids the necessity of pulping the filter paper before titration.

The relative efficiency of filter paper and of asbestos mats in filtering

[4] MacIntire, Jones, and Hardin, *This Journal*, **18**, 301 (1935).

ammonium phosphomolybdate precipitates was compared by one of the writers in the analysis of a standard solution of monopotassium phosphate by the volumetric method. The range of the results obtained with filter paper, filter pulp mats, and mats made from seven grades of asbestos, including recovered asbestos, did not exceed the limits of experimental error. This study also supported the claims of Butt as to the advantages of filter mats over filter paper in filtering the phosphomolybdate precipitates in routine work.

The method most commonly used by the collaborators for the recovery of the citrate-insoluble residues consists in filtering with suction on one or more layers of filter paper in a Büchner or glass funnel.

COLLABORATORS

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RESULTS OF ANALYSIS

Table 1 summarizes the results reported by the collaborators for water-soluble P_2O_5 in the standard samples by different methods of filtering. The results obtained for citrate-insoluble P_2O_5 in the samples when the time interval between washing and the citrate digestion was varied are given in Table 2.

INTERPRETATION OF RESULTS

The values given in Table 1 agree with the conclusions of last year's report[2] in showing (a) that the two types of filter paper used in determining water-soluble P_2O_5 have little or no effect on the results; and (b) that washing with suction gives lower results for water-soluble P_2O_5 than washing under gravity.

It is well known that the calcium phosphates, with the exception of the last in the series, hydrolyze in contact with water to form a less soluble phosphate and free phosphoric acid as illustrated in the following equation:

$$10CaHPO_4+2H_2O=[3Ca_3(PO_4)_2]\cdot Ca(OH)_2+4H_3PO_4. \qquad (1)$$

Slow washing promotes hydrolysis, and the higher results obtained for water-soluble P_2O_5 by gravity washing as compared with washing by suction are no doubt due to the greater proportion of free phosphoric acid formed during the slower washing procedure. Hydrolysis as a result of slow washing may also give rise, in the presence of fluorine, to an increase

TABLE 1.—*Effect of method of filtering on water-soluble P_2O_5 in standard phosphate samples*

COLLABORATOR	PER CENT WATER-SOLUBLE P_2O_5 BY FILTRATION UNDER—			
	GRAVITY		SUCTION	
	WHATMAN FILTER NO. 2	WHATMAN FILTER NO. 5	WHATMAN FILTER NO. 5	SHIMER FILTER
		Sample No. 1		
1	2.96	3.19	2.68	2.34
2	3.00	3.08	2.66	2.83
3	3.29	3.21	2.58	2.36
4	3.03	3.13	2.75	2.64
5	3.13	3.16	3.19	3.07
6	3.07	3.17	2.45	2.53
7	4.00	3.13	2.92	2.83
8	3.22	3.18	3.00	2.80
9	3.36	3.32	3.16	3.00
10	3.42	3.45	3.32	2.97
11	3.15	3.30	2.90	2.90
12	2.98	2.98	2.90	2.80
13	3.05	3.00	2.58	2.60
14	3.15	3.20	2.90	2.90
15	3.18	3.01	2.90	2.73
Mean	3.20	3.17	2.85	2.75
		Sample No. 2		
1	8.95	9.26	8.93	8.90
2	8.68	8.77	8.53	8.73
3	9.00	9.09	8.55	8.60
4	8.77	8.87	8.48	8.58
5	9.08	9.06	9.09	9.02
6	9.05	9.04	8.38	8.48
7	9.18	9.12	9.01	8.98
8	9.02	8.89	8.68	8.57
9	8.86	8.92	8.90	8.95
10	9.27	8.95	9.22	8.98
11	9.20	9.30	8.85	9.00
12	8.85	8.88	8.80	8.83
13	8.70	8.70	8.50	8.43
14	9.15	9.25	8.85	8.85
15	9.19	9.09	8.89	8.64
Mean	8.99	9.01	8.78	8.77

TABLE 1.—*Effect of method of filtering on water-soluble P_2O_5 in standard phosphate samples—Continued*

COLLABORATOR	PER CENT WATER-SOLUBLE P_2O_5 BY FILTRATION UNDER—			
	GRAVITY		SUCTION	
	WHATMAN FILTER NO. 2	WHATMAN FILTER NO. 5	WHATMAN FILTER NO. 5	SHIMER FILTER
	Sample No. 3			
1	3.51	3.70	3.38	3.28
2	3.30	3.36	3.09	3.19
3	3.12	3.15	2.80	2.93
4	3.57	3.62	3.49	3.34
5	3.70	3.75	3.67	3.70
6	3.65	3.65	2.78	2.78
7	3.81	3.72	3.47	3.50
8	3.65	3.58	3.45	3.30
9	3.48	3.60	3.54	3.30
10	3.50	3.45	3.77	3.52
11	3.80	3.80	3.50	3.55
12	3.53	3.45	3.48	3.38
13	3.20	3.85	3.08	3.05
14	3.80	3.80	3.40	3.45
15	3.29	3.03	3.31	3.21
Mean	3.52	3.57	3.35	3.30
	Sample No. 4			
1	4.07	4.24	3.78	3.60
2	4.11	4.22	3.70	3.82
3	4.06	4.13	3.50	3.60
4	4.13	4.30	3.79	3.85
5	4.20	4.24	4.23	4.17
6	4.40	4.42	3.42	3.38
7	4.40	4.00	3.90	4.06
8	4.23	4.19	3.97	3.90
9	4.40	4.56	4.08	4.12
10	4.15	4.60	4.17	3.92
11	4.40	4.50	3.90	3.90
12	4.20	4.28	3.95	3.98
13	3.95	3.95	3.73	3.68
14	4.25	4.25	3.85	3.90
15	4.20	3.98	4.06	3.84
Mean	4.21	4.26	3.87	3.85

in citrate-insoluble P_2O_5, as shown in the paper by Rader and Ross.[5] It would seem, therefore, that the determination of water-soluble P_2O_5 should be made as quickly as possible and that washing with suction should be recommended for samples that can not be rapidly washed under gravity.

[5] *This Journal*, 22, 400 (1939).

The results given in Table 2 are also in agreement with the conclusion reached last year, that prolonged standing of the washed residue before digestion in citrate solution causes an increase in citrate-insoluble P_2O_5 in the analysis of ammoniated mixtures of the ordinary type (Sample 1) but not in non-ammoniated mixtures (Sample 2).

In the ammoniation of fertilizer mixtures the monocalcium phosphate of the superphosphate in the mixture is changed into monoammonium

TABLE 2.—*Effect of varying the time interval between washing and citrate digestion on the citrate-insoluble P_2O_5 in the standard phosphate samples*

COLLABORATOR	PER CENT CITRATE-INSOLUBLE P_2O_5 WITH TIME INTERVALS BETWEEN WASHING AND CITRATE DIGESTION OF—			
	0 hours	*2 hours*	*4 hours*	*18 hours*
		Sample No. 1		
1	3.31	3.43	3.45	3.79
2	3.38	3.42	3.54	3.88
3	2.98	3.00	3.14	3.20
4	3.01	3.32	3.10	3.76
5	3.65	3.64	3.66	3.80
6	2.70	3.15	3.40	3.65
7	4.00	4.50	4.30	4.25
8	3.61	3.70	3.74	3.90
9	2.21	2.10	2.40	2.57
10	3.38	3.39	3.43	3.88
11	3.90	4.70	4.75	4.70
12	3.10	3.26	3.17	3.51
13	2.60	2.74	2.90	2.94
14	3.80	3.95	4.00	4.00
15	2.43	2.55	2.61	2.83
Mean	3.19	3.39	3.44	3.64
		Sample No. 2		
1	4.66	4.73	4.66	4.70
2	4.67	4.62	4.70	4.69
3	4.50	4.50	4.36	4.53
4	4.67	4.56	4.51	4.55
5	4.92	4.90	4.88	4.92
6	4.68	4.53	4.55	4.58
7	4.60	4.52	4.60	4.65
8	4.79	4.79	4.81	4.70
9	4.47	4.41	4.42	4.53
10	4.77	4.77	4.91	4.97
11	4.75	4.75	4.75	4.90
12	4.75	4.76	4.77	4.75
13	4.46	4.44	4.38	4.40
14	4.70	4.75	4.80	4.85
15	4.51	4.61	4.49	4.49
Mean	4.66	4.64	4.64	4.68

TABLE 2.—*Effect of varying the time interval between washing and citrate digestion on the citrate-insoluble P_2O_5 in the standard phosphate samples—Continued*

COLLABORATOR	PER CENT CITRATE-INSOLUBLE P_2O_5 WITH TIME INTERVALS BETWEEN WASHING AND CITRATE DIGESTION OF—			
	0 hours	*2 hours*	*4 hours*	*18 hours*
		Sample No. 3		
1	2.31	2.19	2.28	2.61
2	2.38	2.39	2.42	2.39
3	1.91	1.86	2.00	2.10
4	2.50	2.59	2.60	2.64
5	2.78	2.74	2.73	2.85
6	2.40	2.15	2.20	2.45
7	3.15	3.16	3.37	3.00
8	2.76	2.74	2.74	2.77
9	1.49	1.68	1.61	2.03
10	2.41	2.26	2.36	2.43
11	3.15	3.25	3.25	3.45
12	2.39	2.37	2.34	2.52
13	1.86	1.98	2.00	1.82
14	2.45	2.65	2.70	2.75
15	1.72	1.66	1.84	1.88
Mean	2.38	2.38	2.43	2.51
		Sample No. 4		
1	2.67	2.94	2.96	3.30
2	3.62	3.72	3.79	4.37
3	2.85	2.90	3.08	3.19
4	3.18	3.30	3.31	3.40
5	3.63	3.67	3.68	3.74
6	2.23	2.65	2.70	3.30
7	3.40	3.54	4.25	3.95
8	3.68	3.70	3.69	4.07
9	2.27	2.59	2.33	3.13
10	3.10	3.08	3.31	4.02
11	3.80	4.00	4.15	4.40
12	2.82	2.97	2.95	3.10
13	2.82	2.60	3.00	2.96
14	3.20	3.55	3.70	4.00
15	2.22	2.47	2.79	2.68
Mean	3.03	3.18	3.31	3.57

phosphate, and di- or di- and tricalcium phosphates.[6,7] Sample 3 contained these three compounds and calcium sulfate. Sample 4 differed from Sample 3 only in that the calcium sulfate was replaced with calcium fluoride. The results in Table 2 show that little, if any, increase in citrate-insoluble P_2O_5 took place in Sample 3 when its washed residue was allowed

[6] Keenen, F. G., *Ind. Eng. Chem.*, 22, 1378 (1930).
[7] White, Hardesty, and Ross, *Ibid.*, 27, 562 (1935).

to stand for 18 hours before digestion, whereas the increase in citrate-insoluble P_2O_5 that occurred under the same conditions in the analysis of Sample 4 was about the same as in the case of Sample 1. It may, therefore, be concluded that the formation of citrate-insoluble P_2O_5 on prolonged standing of the washed residue before digestion in citrate solution is limited to samples that contain both fluorine and di- or tricalcium phosphate and that the decrease in available P_2O_5 in such samples is due to the formation of a relatively insoluble fluorine-containing phosphate,[8,9] as explained in the report of last year.[2]

Samples 3 and 4 contained the same phosphatic materials in exactly the same proportions. The total P_2O_5 in each sample amounted to 40.18 per cent. The initial water-soluble and citrate-insoluble P_2O_5 in the samples must also have been the same if the influence of the calcium fluoride on the solubility of the phosphates in Sample 4 is assumed to be the same as that of the calcium sulfate in Sample 3. The results reported by the collaborators, however, show a higher value for both water-soluble and citrate-insoluble P_2O_5 in Sample 4 than in Sample 3. These results would be expected if the reactions that may have taken place during the storage and analysis of Sample 3 were accompanied in Sample 4 by the additional reaction represented in Equation 2.

$$9CaHPO_4 + CaF_2 = [3Ca_3(PO_4)_2] \cdot CaF_2 + 3H_3PO_4. \qquad (2)$$

Results obtained by Rader and Ross[5] in a study of the reversion of P_2O_5 during analysis not only conform with the reports of the collaborators, but they also show that an increase in citrate-insoluble P_2O_5 usually accompanies a prolonged digestion of the washed residue in citrate solution. They show, moreover, that little or no reversion occurs during analysis of cured mixtures when each of the different steps in the process is completed within one hour, and that the increased citrate-insoluble P_2O_5 found in Sample 4 as compared with Sample 3 occurred during storage of the sample before analysis rather than during the process of analysis.

CONCLUSIONS

Slow washing of fertilizer mixtures of the ordinary type gives higher results for water-soluble P_2O_5 than does rapid washing due to the formation of free phosphoric acid by hydrolysis of the citrate-soluble phosphates present. If fluorine is present, the increase in water-soluble P_2O_5 may be accompanied by an increase in citrate-insoluble P_2O_5.

Prolonged standing of the washed residue before digestion in citrate solution causes an increase in citrate-insoluble P_2O_5 in ammoniated mixtures of the ordinary type, and in mixtures containing fluorine and di- or tricalcium phosphates. Prolonged standing of the washed residue has little

[8] MacIntire, Hardin, Oldham, and Hammond, *Ibid.*, 29, 758 (1937).
[9] Ross, Rader, and Beesom, *This Journal*, 21, 258 (1938).

if any effect on the citrate solubility of the P_2O_5 in fluorine-free mixtures or in non-ammoniated mixtures of the ordinary type. Little or no reversion occurs in the analysis of cured phosphatic materials or mixtures when each of the different steps in the process is completed within a period of one hour.

RECOMMENDATIONS[10]

It is recommended—

(1) That the words, "Place 1 g of the sample on a 9 cm filter and wash with successive small portions of H_2O, allowing each portion to pass thru before adding more, until the filtrate measures about 250 cc," *Methods of Analysis, A.O.A.C.*, 1935, sec. **13**, p. 21, lines 1–3, be changed to read, "Place 1 g of the sample on a 9 cm filter and wash with successive small portions of H_2O until the filtrate measures about 250 cc. Allow each portion of the wash water to pass thru the filter before adding more, and wash with suction if the washing would not otherwise be complete within 1 hour."

(2) That the words, "Heat 100 cc of the NH_4 citrate soln to 65° in a 250 cc flask . . . Shake the flask every 5 min.," *Methods of Analysis, A.O.A.C.*, 1935, sec. **16(a)**, p. 22, lines 1–8, be changed to read, "After washing out the water-soluble P_2O_5, **13**, transfer the filter and residue, within a period not to exceed an hour, to a 250 cc flask containing 100 cc of the NH_4 citrate soln previously heated to 65° in a water bath. Close the flask tightly with a smooth rubber stopper and shake vigorously until the filter paper is reduced to a pulp, relieving the pressure by momentarily removing the stopper. Loosely stopper the flask to prevent evaporation and return it to the bath. Maintain the contents of the flask at exactly 65°, keeping the level of the H_2O in the bath above that of the citrate soln in the flask. Shake the flask every 5 min."

(3) That the Associate Referee give further consideration to the method proposed by MacIntire, Shaw, and Hardin[11] for the determination of available phosphoric acid.

REPORT ON NITROGEN*

By A. L. PRINCE (Agricultural Experiment Station, New Brunswick, N. J.) *Associate Referee*

The official method for determining water-insoluble nitrogen in organic materials is still open to a number of criticisms, despite the fact that certain minor changes have been made in an attempt to obtain more consistent results on all products. The recent changes, *This Journal*, 20, 252 (1937), consisted in specifying the grade and size of filter paper to be

[10] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 51 (1939).
[11] *This Journal*, 21, 113 (1938); *Ind. Eng. Chem. Anal. Ed.*, 10, 143 (1938).
* Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

used on all materials. On the basis of the data obtained, these points were favorably passed upon as editorial changes at the 1936 meeting of the A.O.A.C.

Differences in results with various products still persist chiefly because of minor variations in the manner in which the method is run. Renewed interest in this subject has arisen from two sources: (1) certain States have passed laws which require a guarantee of the water-insoluble nitrogen; (2) a demand has been created for material showing a high percentage of water-insoluble nitrogen, and consequently the producers desire a method which will give the highest results. Of course, the A.O.A.C. can not and should not devise methods to favor or give advantage to any particular product. Suggestions by those in the fertilizer industry or others as to changes in the methods are always acceptable and should receive careful consideration provided they make for the improvement of the methods in general.

The inherent differences occurring in various products often make it difficult to prescribe a definite procedure for a particular determination that will work exactly alike for all. One is faced with the problem of devising a special method for every product, or by being satisfied with the slight differences that occur when a universal method is adopted. The present policy of the A.O.A.C. tends toward the elimination of many methods that are essentially the same. Occasionally, when a widely used product is so radically different from the general run of material that the prescribed official method gives widely divergent results, exception to the above policy should be allowed and a special method prescribed. This was the situation in the case of the determination of water-insoluble nitrogen in cyanamid. A special method for determining water-insoluble nitrogen in cyanamid was devised and finally adopted as an official method last year.

However, the majority of organic nitrogen materials are similar enough in character to warrant the use of a general method for determining water-insoluble nitrogen, provided the directions are made specific. Other factors beside the type of material which may cause discrepancies in the results by different analysts are the quality and size of filter paper, the manner of manipulation, and the temperature of the wash water. The first of these factors has been taken care of, namely, the quality and size of the filter paper. The second factor, manner of manipulation, refers to the actual method of washing and is probably the largest contributing factor toward disagreement in results among analysts. Unless the conditions of washing are definitely specified, analysts will vary their manipulation in respect to washing and obtain disagreeing results.

A preliminary study of this phase of the problem has been made during the past year. In washing various materials for water-insoluble nitrogen, it should be borne in mind that no substance is entirely insoluble, and that

prolonged washing would continually bring small amounts of the material into solution. It is therefore necessary to arbitrarily select a point where washing may be considered complete for all practical purposes.

Five different methods of washing were tried out on three materials. ground fish, process tankage, and peanut meal. The total nitrogen content of these materials was 8.11, 6.73, and 7.39 per cent, respectively. Both one and two gram samples were used, and each size sample was leached to two different volumes, namely, 200 cc. and 250 cc.

The different methods of washing are described below:

1. *Regular Official Method.*—Place the material on an 11 cm. Whatman No. 2 filter paper, wet with alcohol, and wash with water at room temperature to the desired volume. In this case the washing was rapid, new additions of water being added as soon as each portion went dry.

2. *Regular Method (modified).*—Same as No. 1, with the exception that the washing was carried on slowly, by allowing the funnel to drain 5 minutes between washings.

3. *Beaker Method.*—Place the material in a 50 cc. beaker, wet with alcohol, add 20 cc. of water, and allow the mixture to stand 15 minutes, with occasional stirring. Transfer the supernatant liquid to an 11 cm. Whatman No. 2 filter paper and wash 4 or 5 times by decantation with water at room temperature. Finally transfer all the residue to the filter paper and complete the washing rapidly to the desired volume.

4. *Shaking Method.*—Place the material in a 200–250 cc. Erlenmeyer flask and wet with alcohol; add 50 cc. of water, and place in a shaking apparatus. Shake for 15 minutes, then proceed as directed in the regular method.

5. *Automatic Method.*—

Apparatus: A 200 cc. volumetric flask containing a 2-holed rubber stopper. In each hole of the rubber stopper place two glass tubes, one a little shorter than the other (roughly $1\frac{1}{2}''$, and $1\frac{1}{4}''$, respectively).

Method: Place the material on an 11 cm. Whatman No. 2 filter paper, wet with alcohol, and add 25 cc. of water. Immediately invert the volumetric flask containing water (25 cc. less than the required volume to which material is to be leached) over the paper with the tip of the longer tube just below the surface of the water in the paper. When the flask is empty, wash the paper with several small portions of water until the desired volume is reached.

The data obtained by these different methods for water-insoluble nitrogen in ground fish is reported in Table 1, and for process tankage and peanut meal, in Table 2. It will be noted that the automatic method gives relatively higher results than the other methods on all three materials, and also that there is a tendency for a greater divergence between individual determinations. Although this method is very rapid and is a great convenience as far as requiring attention, the results are too high and irregular.

The regular official method, specifying successive washing as soon as each portion goes dry, also runs higher in most cases than the slower filtration, beaker, or shaking methods. By this method, as well as the automatic method, excess water always remains on the material. The

TABLE 1.—*Per cent water-insoluble nitrogen in ground fish determined by different procedures*

METHOD	1 GRAM LEACHED TO 200 CC.	2 GRAMS LEACHED TO 200 CC.	1 GRAM LEACHED TO 250 CC.	2 GRAMS LEACHED TO 250 CC.
	per cent	*per cent*	*per cent*	*per cent*
Regular	6.02	5.94	5.73	5.81
	5.94	6.07	5.85	5.82
	6.06	5.98	5.72	5.89
Average	6.01	6.00	5.77	5.84
Regular (Modified)	5.85	5.88	5.77	5.73
	5.86	5.85	5.78	5.85
	5.86	5.89	5.80	5.78
Average	5.86	5.87	5.78	5.79
Beaker	5.94	5.90	5.80	5.81
	6.01	5.94	5.73	5.85
	5.99	6.00	5.80	
Average	5.98	5.95	5.78	5.83
Shaking	5.72	6.02	5.60	5.90
	5.77	6.02	5.81	5.83
	5.83	6.03	5.75	5.85
Average	5.77	6.03	5.73	5.86
Automatic	6.14	6.10	6.09	6.01
	6.23	5.64	6.02	5.90
	5.98	6.05	5.93	5.97
Average	6.12	5.93	6.02	5.96

data indicate that rapid washing on the funnel does not remove all the water-soluble nitrogen.

The regular method, modified to allow for slower filtration, checks quite closely with the beaker and shaking methods. The results by these three methods are quite comparable and the individual results are fairly consistent. However, the beaker method saves time in the filtration process, and allows at the start for thorough wetting and solubility of the material. B. F. Carpenter of the Virginia-Carolina Chemical Corporation also did considerable work on several types of material, using various methods of washing, and came to the conclusion that the beaker procedure was the best. It is a method that can be easily handled for rapid routine work.

The shaking method is also equally as effective with some materials such as with process tankage, but with certain materials, especially peanut meal, a colloidal suspension is obtained which prevents filtration. Consequently this method was eliminated in the peanut meal determinations.

TABLE 2.—*Water-insoluble nitrogen in process tankage and peanut meal by different procedures*

METHOD	PROCESS TANKAGE			PEANUT MEAL	
	1 GRAM LEACHED TO 200 CC.	2 GRAMS LEACHED TO 200 CC.	1 GRAM LEACHED TO 250 CC.	1 GRAM LEACHED TO 200 CC.	2 GRAMS LEACHED TO 200 CC.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Regular Official	5.98	5.86	6.04	2.65	2.43
Rapid Filtration	6.09	5.99		2.71	2.43
	6.10				
Average	6.02	5.93	6.04	2.68	2.43
Regular Official (Modified) Slow Filtration	5.86	5.57	5.72	2.55	2.73
	5.80	5.60		2.52	2.75
	5.80				
Average	5.82	5.59	5.72	2.54	2.74
Beaker	5.77	5.80	5.62	3.02	2.62
	5.80	5.52		2.91	2.76
	5.81				
Average	5.79	5.66	5.62	2.97	2.69
Shaking	5.80	5.63	5.81		
	5.77	5.70			
	5.80				
Average	5.79	5.67	5.81		
Automatic	6.82	6.30	6.28	3.10	2.60
	6.85	6.36		3.23	2.70
	6.80				
Average	6.82	6.33	6.28	3.17	2.65

The problem of the formation of channels in washing the material by the regular, modified, or automatic methods, has a significant bearing on the results, but is of less importance with the beaker or shaking methods. The size and angle of the glass funnel is also an important factor in the filtration of the material. Long-stemmed funnels of 60° angle and having a diameter of $2\frac{1}{2}$ inches in size allow for a more uniform rate of filtration.

The differences in results obtained when a one or two gram sample is used are not significant. Either weight when leached to the same volume appears to give comparable results except by the automatic method, where the results average lower when a 2 gram sample is used.

When the washing was carried on to a volume of 250 cc. rather than 200 cc., the results in general on ground fish and process tankage are slightly lower for water-insoluble nitrogen. In most cases the results are lower by between 0.1 and 0.2 of 1 per cent.

In the present official method for determining water-insoluble nitrogen,

Methods of Analysis, A.O.A.C., 1935, 28, **37,** the material is washed to 250 cc. From the above data it would seem that this stipulation should continue, and all raw materials, such as fish tankage, cottonseed meal, etc., should be leached to this volume. The question is often raised as to why the weight of the sample and volume leached to should be different in the official method described under par. **34(b),** page 27. Here, however, a preliminary determination is being made for the separation of different forms of nitrogen in a mixed fertilizer. The 2 gram charge taken and the subsequent 200 cc. volume obtained are very convenient quantities to work with for the remaining determinations. From the above data, and those obtained in 1937, *This Journal,* **20,** 250–252, the differences brought about by the extra 50 cc. of wash water are not appreciable. Furthermore, the amount of organic material in mixed fertilizers is relatively small, and consequently less washing would be required.

Another point that has been raised concerning the manipulative features of this determination is the temperature of the wash water. The present directions specify room temperature, but this terminology covers quite a range, especially during winter and summer months, and in different parts of the country. Some analysts have already found differences as great as 0.3 of 1 per cent on samples washed with water at 31° C. in comparison with water used at 20° C. Hence it would seem wise to limit the term "room temperature" to a definite range, such as between 20°–25° C.

Although the beaker method seemed to yield the best results in this preliminary study, collaborative work should be done to compare the method with other procedures. The exact directions for this method are as follows: Place 1 or 1.4 grams of the material in a 50 cc. beaker, wet with alcohol, add 20 cc. of water, and allow to stand 15 minutes, with occasional stirring. Transfer the supernatant liquid to an 11 cm. Whatman No. 2 filter paper, and wash 4 or 5 times by decantation with water at room temperature (20°–25° C.). Long-stemmed funnels $2\frac{1}{2}$ inches in diameter and having an angle of 60° should be used. Finally transfer all the residue to the filter paper and complete the washing until the filtrate measures 250 cc. Dry, and determine nitrogen in the residue as directed under **21** or **23.**

During the past year attention was called to the fact that considerable difficulty was encountered in securing concordant results in the determination of total nitrogen in certain fish products, especially Canadian dogfish meal, by the regular A.O.A.C. methods. Consistent results were obtained, however, when a small percentage of potassium persulfate was incorporated in the digestion materials of the regular Kjeldahl-Gunning method. Ten grams of the following mixture was used for each determination: K_2SO_4, 83.7%; $K_2S_2O_8$, 9.3%; HgO, 7.0%. This modified procedure was tried out on a number of organic materials for comparison with the

regular method. The following substances were analyzed by both procedures: Crab meal, cottonseed meal, castor pomace, process tankage, garbage tankage, chicken manure, sewage sludge, and ground fish. Unfortunately, it was impossible to obtain any Canadian dogfish meal. The results of this study are summarized in Table 3. The method using potassium persulfate gave slightly higher results in nearly all cases, but the difference between the methods was usually less than 0.1 of one per cent. No nitrogen was found in the potassium persulfate. The use of this reagent did cut down the time of digestion somewhat. However, the results do not indicate that a change in the official method is necessary for the ordinary fertilizer products. With special products, such as Canadian dogfish meal, meat scraps, and coconut meals, the use of potassium persulfate might be desirable, and further work should be done with these products.

TABLE 3.—*Comparison of the total nitrogen in various organic fertilizers by the official method and by the method using potassium persulfate*

MATERIAL	OFFICIAL METHOD	POTASSIUM PERSULFATE METHOD
	per cent	*per cent*
Crab Meal	10.42	10.46
Cottonseed Meal	6.40	6.44
Castor Pomace	5.19	4.97
Process Tankage	6.73	6.82
Garbage Tankage	3.32	3.28
Chicken Manure	2.63	2.71
Sewage Sludge	0.92	0.94
Ground Fish	8.11	8.20

In *Methods of Analysis, A.O.A.C.*, 1935, par. 19(g), p. 23, an error has been noted in the preparation of the sodium hydroxide solution. It reads as follows: "dissolve approximately 450 g of commerical NaOH, free from nitrates, in 1 liter of water. This solution should have a sp. gr. of 1.43–1.48." A solution containing 450 grams of sodium hydroxide per liter would have a sp. gr. of only 1.36. Although considerable latitude should be allowed in the concentration of this solution, it would be well to modify the last sentence of this paragraph to read: "A solution having a sp. gr. of 1.36 or higher may be used."

RECOMMENDATIONS[1]

It is recommended—

(1) That a collaborative study be made of the beaker method in comparison with other methods for the determination of water-insoluble nitrogen.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 51 (1939).

(2) That the use of potassium persulfate along with mercury as catalyst in the determination of total nitrogen be studied on such materials as Canadian fish meal, meat scraps, and coconut meals.

(3) That the last sentence in par. 19(g), p. 23, of the 1935 edition of *Methods of Analysis*, be deleted, and the following sentence be incorporated in its place: "A solution having a sp. gr. of 1.36 or higher may be used."

(4) That the reduced iron method for the determination of nitrate nitrogen in mixed fertilizers or nitrate salts described under Sec. 31, p. 26, of the 1935 edition of *Methods of Analysis*, be deleted (final action).

REPORT ON MAGNESIUM AND MANGANESE IN FERTILIZERS*

By JOHN B. SMITH, *Associate Referee*, and E. J. DESZYCK (Agricultural Experiment Station, Kingston, R. I.)

The study of methods for determining magnesium and manganese, *This Journal*, **20**, 252 (1937); **21**, 277 (1938), was continued. As in previous years, the writers are indebted to many collaborators for ideas, criticism, and analytical assistance.

MAGNESIA

Progress made during the year is reported under the topics outlined in previous reports. The emphasis has been on methods for acid-soluble magnesia shorter than the official method.

Collaborative Analyses

Seven methods were sent to collaborators, five for magnesia and two for manganese. As the same collaborators undertook work on both elements, description of the samples and presentation of results are combined in this section.

The procedures are as follows:

1. Acid-soluble Magnesia, Official. *Methods of Analysis, A.O.A.C.*, 1935, p. 34, 54, as modified in 1936, *This Journal*, **20**, 252 (1937).

2. The Bartlett-Tobey Method developed at the Maine Agricultural Experiment Station and modified in minor details by the writers. The detailed procedure follows:

Weigh 2.5 g. of fertilizer into a 250 cc. volumetric flask, add 30 cc. of HNO_3 and 10 cc. of HCl, and boil for 30 minutes. Cool, make to volume, mix, filter thru a dry filter paper, and transfer a 100 cc aliquot to a 400 cc. beaker. Add a few drops of methyl red. Add NH_4OH until the solution is yellow, then HCl until barely pink. Add 15 cc. of a saturated solution of NH_4 oxalate, adjust the solution to *p*H 5.0 (a faint pink color) by the addition of HCl (1+4), or NH_4OH (1+4), boil for a few minutes, cool, and again adjust the reaction to *p*H 5.0, adding more methyl red if

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* Contribution No. 551 of this Station.

necessary. Stir thoroughly and allow the solution to stand until the precipitate settles. Filter through a 11 cm. filter paper fine enough to retain Ca oxalate and wash 10 times with hot water. To the filtrate add 2 cc. of 10% HCl, evaporate to a volume of approximately 100 cc., and add 5 cc. of a 10% Na citrate solution and enough concentrated NH_4OH to make the solution alkaline. (Blue with bromothymol blue). If the fertilizer does not contain soluble phosphoric acid, add 5 cc. of a 10% solution of $(NH_4)_2HPO_4$. Stir vigorously until precipitation is completed. Add 15 cc. of concentrated NH_4OH and allow to stand at least 2 hours, stirring frequently, or allow to stand overnight. Transfer the precipitate to a small filter or filtering crucible. Wash, and ignite as directed under **II, 54.** If $Mn_2P_2O_7$ is present, correct for it as directed under **II, 54.**

3. A volumetric modification of the official method suggested by P. McG. Shuey. Briefly, this includes solution of a 4 gram sample in a Kjeldahl flask with HNO_3, H_2SO_4, and KNO_3, treatment of the entire sample with alcohol and water to precipitate $CaSO_4$, a single precipitation of $MgNH_4PO_4$, washing free from ammonia with 50% alcohol and titration of the precipitate with 0.1 *N* HCl and 0.1 *N* NaOH.

4. Magnesia insoluble in 4% citric acid titrated to *p*H4, with NH_4OH 90 minutes, 90°–95° C.

5. Water-soluble magnesia in magnesium sulfate and sulfate of potash-magnesia.

6. A volumetric periodate method for acid-soluble manganese, *This Journal*, **21**, 292 (1938).

7. A colorimetric modification of Method 6, suggested by H. D. Haskins and J. W. Kuzmeski of the Massachusetts Agricultural Experiment Station.

Collaborators submitting results were: C. A. Butt and C. M. Cartledge, International Agricultural Corporation; E. J. Deszyck, Rhode Island Agricultural Experiment Station; W. Y. Gary, Florida Agriculture Department; E. T. Hord, North Carolina Department of Agriculture; L. F. Rader, Jr., U. S. Department of Agriculture; P. McG. Shuey, Shuey and Co.; Oscar I. Struve, Eastern States Cooperative Milling Corporation. The amount of work requested was greater than usual, and full acknowledgment is made of the generous help received.

The samples are described in Table 1. The first is high in phosphate; the second contains considerable organic matter; and the third contains only inorganic materials including silica in sand. The results are shown in Tables 1 and 2.

Acid-Soluble Magnesia

Hoffman Method.—This method, now official, has received favorable comment for accuracy, but has been criticized by many for its length. One of the collaborators states the criticism very clearly in this paragraph: "From our standpoint, the time saved by the short-cut magnesia methods overshadows in importance the slight difference in the results obtained. It has been inconvenient, to say the least, to have to wait the larger portion of a week before magnesia tests could be completed, while fertilizer analyses were otherwise complete within a day or two after receipt of samples. For the present my sentiment is that the Hoffman method should be retained as the official method; but, if at all possible,

TABLE 1.—*Samples for collaborative analysis*

SAMPLE	INGREDIENTS	POUNDS	ADDED MgO (PER CENT)			
			KIESERITE	DOLOMITIC LIMESTONE	NON-CARRIER	TOTAL
1. 8-16-12	Sulfate of ammonia	360				
	Urea	120				
	Nitrate of potash	200				
	Tankage	140				
	Triple superphosphate	710				
	Muriate of potash	300				
	Dolomitic limestone	100				
	Kieserite	70	1.01	0.95	0.44	2.40
2. 5-8-7	Sulfate of ammonia	170				
	Urea	50				
	Tankage	515				
	Superphosphate	720				
	Muriate of potash	275				
	Dolomitic limestone	200				
	Kieserite	70	1.01	1.89	0.22	3.12
3. 7-6-6	Sulfate of ammonia	570				
	Nitrate of soda	120				
	Superphosphate	540				
	Muriate of potash	236				
	Dolomitic limestone	200				
	Kieserite	100				
	Quartz sand, 40-mesh	234	1.44	1.89	0.11	3.44

		Mn
4.	Kieserite	
5.	Sulfate of potash magnesia	*per cent*
6.*	Sample 1, but substitute 125 lbs. manganese sulfate, 24.94 per cent Mn, for 70 lbs. Kieserite, 55 lbs. dolomitic limestone	1.56
7.*	Sample 2, but substitute 85 lbs. manganese sulfate for 85 lbs. dolomitic limestone	1.06
8.*	Sample 3, but substitute 43 lbs. manganese sulfate for 43 lbs. sand	0.54

* The ingredients, other than manganese sulfate, supplied traces of manganese too small in amount to affect results for these samples.

work should be continued on the short-cut methods with a view to arriving at a procedure giving reasonably satisfactory results in materially less time, as an alternative procedure." The Associate Referee is in full accord with the idea, and believes that the objective can be accomplished without sacrifice of accuracy. The Hoffman method, as stated in previous reports, has an authoritative background, and has been advocated and adopted because it assures accuracy. Thus, it fills the need for a reference method, with which to compare other procedures, but justifiable adverse criticism of the method for routine work has been anticipated.

TABLE 2.—*Collaborators' results for MgO and Mn (per cent)*

COLLABORATORS	SAMPLES 1	2	3	1	2	3
	ACID-SOLUBLE MgO (PER CENT)					
	METHOD 1, OFFICIAL			METHOD 2, BARTLETT-TOBEY		
Butt, Cartledge	2.19	2.92	3.20	2.40	3.07	3.29
Deszyck	2.47	3.25	3.56	2.37	3.17	3.47
Gary	2.21	3.01	3.37	2.58	3.26	3.57
Hord	2.22	3.00	3.38	2.38	3.20	3.46
Rader	2.48	3.24	3.42	2.44	3.24	3.42
Shuey	—	—	—	2.41	3.26	3.39
Struve	2.32	3.00	3.37	2.48	3.28	3.41
Average	2.31	3.07	3.38	2.44	3.21	3.43
Recovery of MgO (%)	96	98	98	102	103	100
	METHOD 3, SHUEY			METHOD 4, ACID-CITRATE-INSOLUBLE		
Butt, Cartledge	2.48	3.24	3.46	0.60	1.60	1.41
Deszyck	2.40	3.21	3.54	0.36	1.79	1.37
Gary	—	—	—	—	—	—
Hord	2.25	3.05	3.36	0.78	1.56	1.14
Rader	2.46	3.35	3.67	0.66	1.41	1.13
Shuey	2.42	3.22	3.52			
Struve	2.45	3.20	3.55			
Average	2.41	3.21	3.52	0.60	1.59	1.26
Recovery of MgO (%)	100	103	102	45*	22*	37*

	WATER-SOLUBLE MgO	
	KIESERITE	SULFATE OF POTASH MAGNESIA
Butt, Cartledge	28.73	—
Deszyck	28.67	10.46
Hord	28.30	10.17
Rader	29.02	10.87
Shuey	28.61	—
Struve	28.67	10.26
Average	28.67	10.44

	SAMPLES 4	5	6	4	5	6
	ACID-SOLUBLE Mn (PER CENT)					
	VOLUMETRIC METHOD			COLORIMETRIC METHOD		
Deszyck	1.55	1.06	0.65	1.58	1.04	0.65
Gary	1.21†	1.04	0.68			
Hord	1.72	1.12	0.63			
Rader	1.63	1.04	0.63	1.75	1.04	0.67
Shuey	1.52	1.00	0.56			
Average	1.61	1.05	0.63	1.67	1.04	0.66

* Recovery of MgO added as dolomitic limestone, as citrate-soluble MgO, subtracting 0.08, 0.12, and 0.07 % citrate-insoluble MgO from non-carriers in Samples 1, 2, and 3, respectively, from results by analysis.
† Omitted from average. Gary reported 1.58 % Mn by a bismuthate method.

Bartlett-Tobey Method.—Several alternatives have been suggested. The first was an attempt by J. M. Bartlett and E. R. Tobey* to shorten the time needed for the orthodox procedure for separating calcium and magnesium as the oxalates. The authors of the method pointed out deviations from text-book versions of the method but found no significant deviations in results. This was a promising start, and the procedure has now been tried collaboratively for three years, either for acid-soluble magnesia or for the water-soluble fraction. It has been modified in minor details in this laboratory to meet possible criticisms. Extraction with 1 per cent hydrochloric acid has been changed to treatment with nitric acid and hydrochloric acid to destroy organic matter and increase the probability of dehydrating silica, but the procedure, as published in this report, follows the original suggestion quite closely. The principal deviations from the standard procedure are single precipitations of calcium oxalate and magnesium ammonium phosphate and shorter time allowances for formation of these precipitates. The single precipitation has precedent in methods of this Association, both for calcium oxalate and magnesium ammonium phosphate (*Methods of Analysis, A.O.A.C.*, 1935, II, 9; XII, 10, 11, 12; XVII, 24 and XXVI, 19, 20), although not under conditions exactly similar to those here described. Ross, *This Journal*, 11, 180 (1928), after thorough study of the precipitation of magnesium ammonium phosphate for the determination of phosphoric acid in fertilizers decided that a double precipitation in a routine method did not add sufficient accuracy to justify the increased time required.

Comparisons of the Bartlett-Tobey modification with the official method may be found in the two previous reports on this topic, cited previously, and in Table 2. In addition, 37 samples of commercial brands of fertilizer that had been analyzed at the Maine Agricultural Experiment Station and 33 samples from the North Carolina Department of Agriculture were supplied by the chemists of those institutions and analyzed at this Station. The results are summarized in Table 3. On the average the Bartlett-Tobey method gave values 0.05 per cent greater than those by the official method. Results by collaborators have been equally consistent by both methods. Average differences between two laboratories for individual samples by the official method have been about equal to the differences between the two methods at one laboratory. These differences are somewhat greater where the official method at one laboratory is compared with the Bartlett-Tobey method at another. From the results at hand, either method seems sufficiently accurate for control work, with the shorter method giving slightly higher results. This tendency does not appear to be correlated with the percentages of magnesia in the mixture or with the percentages of calcium oxide actually determined in the 70 fertilizers mentioned. Reprecipitation of calcium oxalate and magnesium

* Private communication.

TABLE 3.—*Summary of comparisons of the official method with the Bartlett-Tobey procedure*

		MgO *per cent*
12	Collaborative samples	
	Average by Bartlett-Tobey Method greater	0.05
	Mean deviation, Official Method	0.09
	Mean deviation, Bartlett-Tobey Method	0.08
37	Samples. Analysis at Maine Agr. Exp. Station and the Rhode Island Exp. Station	
	Average of differences for individual samples	
	Official Method, Maine vs. Rhode Island	0.08
	Official and Bartlett-Tobey Methods,	
	Maine vs. Rhode Island	0.13
	Rhode Island alone	0.09
	Average, all analyses, Bartlett-Tobey greater	0.04
33	Samples analyzed at North Carolina Department of Agriculture and Rhode Island Exp. Station	
	Average of differences for individual samples	
	Official Method, N.C. vs. R.I.	0.08
	Official and Bartlett-Tobey Methods	
	N.C. vs. R.I.	0.11
	Rhode Island alone	0.07
	Average, all analyses, Bartlett-Tobey greater	0.05

ammonium phosphate did not change the results by the Bartlett-Tobey method consistently at this laboratory. Hord, however, with results by the Bartlett-Tobey method in excellent agreement with the averages for those of other collaborators this year, reduced these results consistently by reprecipitation of the magnesium ammonium phosphate. His results were then in better agreement with averages for the official method than for the shorter procedure. Probably the higher results by the Bartlett-Tobey method are caused by mixtures of phosphates in the precipitate, including calcium, or by silica occlusions, and can be corrected by reprecipitation, but the added accuracy does not justify the loss of time. The magnitude of the changes that would result are of about the same magnitude as the variations from other apparently unavoidable errors.

Because of experience with the Bartlett-Tobey method, and confidence that it will fill the immediate need for a shorter method in laboratories where the number of magnesia determinations is increasing rapidly, the Associate Referee is recommending this method as a tentative method. This is done without prejudice to the other methods to be discussed below, but only because these other methods have not yet received thorough trial.

Shuey Volumetric Method.—P. McG. Shuey has suggested* a very

* Private communication.

promising adaptation of a modification of the official method combined with the acidimetric titration of magnesium ammonium phosphate discussed by Handy.[1] Briefly, the procedure includes solution and oxidation of a 4 gram sample in a Kjeldahl flask with sulfuric and nitric acids, and finishing with potassium nitrate if organic matter remains. The solution is transferred to a 200 cc. volumetric flask with 50 cc. of water, and calcium sulfate is precipitated during a 2 hour period by adding alcohol. More alcohol is added to complete the volume. The alcohol is evaporated from a filtered aliquot, and magnesium ammonium phosphate is precipitated in ammoniacal solution containing the citrate ion by stirring or standing. The precipitate is filtered on a Gooch pad, washed with alcohol (equal volumes of 95% alcohol and water), dissolved in 0.1 *N* hydrochloric acid, and the excess acid is titrated to the usual methyl orange color change.

This is the most rapid method that has been tried this year. The results (Table 2) are more consistent than those for the official method, and equal to those with the Bartlett-Tobey procedure. The method is decidedly promising, but needs more thorough trial next year to justify a decision concerning its ultimate usefulness. As with any single precipitation of magnesium ammonium phosphate, several types of contamination are possible. Some have greater effect on an ignition method, and others in titration. Several modifications are possible. The precipitate may be ignited as for other methods. The excess ammonia in the precipitate and filter may be driven off at 40° C. or by standing at room temperature. This volumetric modification may be used with the oxalate separation of calcium, allowing for simple determination of that element in the same aliquot. A great advantage of the volumetric method is the time saved in transfer of the final precipitate to a filter. Washing may be by decantation and the policing of the particles adhering to flask or beaker is avoided. The Associate Referee will gladly send copies of the detailed procedure to any who apply.

A third method is the application of the volumetric modification of the 8-hyroxy-quinoline procedure recommended after trial by W. B. Byers. This method is said to be very rapid and to allow for the complete separation of manganese, thus avoiding the correction for this element in the final precipitate. W. J. Gascoyne, after experience with a considerable number of samples, suggested a method somewhat like the Bartlettt-Tobey procedure but which separates calcium as the oxalate and phosphate at about the neutral point, in the presence of ammonium chloride, rather than adjusting carefully at a lower *p*H to avoid precipitation of calcium phosphate. Each of these suggestions deserves more careful attention than has been possible this year.

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[1] *J. Am. Chem. Soc.*, 22, 31 (1900).

Active Magnesia

Previous reports have discussed in detail this fraction, which is intended to measure the magnesia available to plants in a single cropping season. This year real progress was made in determining the average rate of decomposition of dolomite in the soil. Part of the work was a collaborative effort organized by the Sub-Committee on Fertilizer Reaction from the American Society of Agronomy, *This Journal*, 22, 137, 142 (1939). Independent studies were made at the Indiana Experiment Station. The fertilizers and dolomites used were made available to the Associate Referee for chemical studies to find a solvent that will correlate with the reactivity of the soil. Results from the pot tests were not ready in time for a satisfactory completion of the laboratory work but preliminary tests with the neutral ammonium citrate solution used for phosphoric acid and the *p*H 4.0 ammonium citrate solution, which has shown greater promise, *This Journal*, 21, 277 (1938), were tried in this laboratory. The acid citrate solution was also used by the collaborators for the samples reported in Table 2. The results (Table 4) show definitely that neutral ammonium citrate does not dissolve enough of the dolomites for the purpose. The acid citrate, although far from perfect shows a considerable degree of correlation, and is the most promising solvent tried. As presented to the collaborators, however, it is unsatisfactory, for it does not give concurring results. It must be rewritten to employ lower and accurately controlled temperatures, preferably 65°, to agree with the method for citrate-insoluble phosphoric acid and a shorter heating period, and to allow for the determination of the magnesia dissolved, rather than the insoluble portion. This can doubtless be done by varying *p*H and citrate concentration, and will be attempted next year.

If the solvent is to prove useful, it should apply to the dolomites alone as well as in mixtures. Otherwise it will be difficult to calculate fertilizer formulas to contain the desired amounts of active magnesia. J. W. Kuzmeski has supplied important information on this topic, *This Journal*, 22, 147 (1939). Treating 0.2 gram charges of nine dolomitic limestones as directed for the acid-citrate solution at *p*H 4.0 he finds considerable variations among different limestones of equal fineness and among different separates of the same limestone. Average percentage solubilities for magnesia were as follows: 60–80 mesh, 57; 80–100 mesh, 66; 100–200 mesh, 84; through 200 mesh 98.6; mill-run, 71. These results are similar in general to the solubility of the dolomites reported in Table 4.

Water-Soluble Magnesia

Laboratory studies of this fraction have been held in abeyance this year, for the problem is agronomic rather than chemical. An analytical procedure that was tried has worked with apparent satisfaction in Maine for several years. Agronomists, however, are not agreed on the necessity

TABLE 4.—*Average decomposition of dolomites in fertilizer mixtures in eight acid soils and the average solubility of these dolomite fertilizer mixtures in two citrate solutions*

MIXTURE	RECOVERY OF MgO		
	DECOMPOSITION BASED ON RESIDUAL CARBONATES IN THE SOIL	NEUTRAL NH_4-CITRATE 1 G. CHARGE, 1 HR., 65°C.	4% CITRATE MADE TO pH 4.0 WITH NH_4OH, 1 G. CHARGE, 90 MIN., 90–95°C.
	per cent	*per cent*	*per cent*
Dolomite A			
Mesh			
20– 40	24	2	43
40– 60	41	7	57
60– 80	55	7	57
80–100	62	10	60
100–200	75	10	74
Through 200	85	25	83
Composite	57	6	61
Dolomite B, Composite	63	9	70
Dolomite C, Composite	51	3	38

The fertilizers, dolomites, and pot tests are described by Emerson R. Collins and Paul R. Dawson in papers presented before this Association, *This Journal*, **22** 137, 142 (1939). The average decomposition of each dolomite used was calculated from a preliminary report made available to the Associate Referee.

for a separate determination of this portion. Many believe that a determination of active magnesia will suffice; others stress the importance of water-soluble magnesia for tobacco in the South East, potatoes in Maine, and for acute deficiencies elsewhere. Because of this uncertainty, it seems best to await a more definite demand before recommending official status for the method for water-soluble magnesia.

Water-Soluble Magnesia in Magnesium Sulfate, Kieserite, and Sulfate of Potash Magnesia

This topic is distinct from that discussed above. These materials are marketed on the basis of water-soluble magnesia content and a uniform method is necessary, but it is not intended for use with fertilizer mixtures. The procedure recommended last year, *This Journal*, **21**, 77 (1938), was tried by six collaborators, and the results (Table 2) show sufficient agreement to justify the procedure. As was true last year, there is a rather wide variation between the extremes but a reassuring tendency for a good proportion of the results to group about the means. The greatest divergence is for the sample of sulfate of potash magnesia this year.

Doubtless this method can be combined later with the method ultimately chosen as most rapid and satisfactory for measuring magnesia in

acid solution. After solution is accomplished the conditions are much the same, whether the solvent is acid or water.

ACID-SOLUBLE MANGANESE

The volumetric periodate method, published last year, *This Journal*, 21, 292 (1938), was tried by the collaborators. As stated last year, it was recommended by F. B. Carpenter and published originally by Willard and Thompson.[1] It should be applicable in the absence of chromium, cobalt, and cerium, with the precaution of removal of chlorides. The collaborative results (Table 2) show satisfactory agreement. W. Y. Gary reported a low result for Sample 1, but found the proper amount of manganese by the older bismuthate method, with which he was more familiar and which he prefers. The reason for this difficulty does not appear, and it was not experienced by the other analysts, nor by Gary for the samples with less manganese. The procedure seems to justify recommendation as a tentative method, and it may be modified later if experience shows this to be necessary.

One detail must be added to the description of the method as published last year. Preparation of the standard ferrous sulfate solution should read: "0.091 *N*. 25.3 grams of $FeSO_4 \cdot 7H_2O$ and 25 cc. of H_2SO_4 in 1 liter of solution. Standardize with the 0.0910 *N* $KMnO_4$." The sulfuric acid is necessary to stabilize the solution.

Colorimetric Periodate Method.—The volumetric method is generally applicable to all amounts of manganese in fertilizers and manganese carriers used as ingredients, and is necessary on that account. For lower percentages, such as usually occur in mixed fertilizers, it is probable that a colorimetric modification[2] is shorter and can be used to advantage.

As in many other instances, the Associate Referee is indebted to H. D. Haskins and J. W. Kuzmeski for independent work made available for his use. Space does not allow a complete account of the work, but the most pertinent details may be described briefly. Using the samples distributed last year, Nos. 3, 4, 5 in the last previous report, they tried several methods of solution. The one finally preferred is digestion of a 1 gram sample in a 200 cc. volumetric flask with 10 cc. of sulfuric acid and 30 cc. of nitric acid at the boilding point, and evaporation until white fumes appear. Five cc. of 85 per cent phosphoric acid is added with 30 cc. of water. The solution is brought to a boil and filtered. The filter is washed with water, 0.3 of potassium periodate is added for each 15 mg. of manganese, and the mixture is heated with stirring for 30 minutes. After cooling, the solution is diluted and made to a convenient, measured volume for comparison with standard potassium permanganate in a colorimeter. By this method, Kuzmeski reports 1.02, 1.02, and 1.01 per cent of manganese for Samples 3, 4, and 5, respectively. By the volumetric

[1] *Ind. Eng. Chem. Anal. Ed.*, 3, 309 (1931).
[2] *J. Am. Chem. Soc.*, 39, 2366 (1917).

method, two analysts at the Rhode Island Experiment Station find 1.03, 1.00 and 1.02 per cent manganese for these same samples.

The method was tested further and gave satisfactory results in this laboratory. As written for the collaborators, however, it was not entirely successful in the hands of the few who tried it. The results, reported in Table 2, show excellent agreement by two analysts, and especially good for Samples 3 and 4. Hord, however, reports an unsuccessful attempt. The principles of the procedure are sound, and it is only necessary to rewrite the method for more definite control of conditions to make it a valuable, modification of the volumetric method. This will be attempted next year.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for the determination of magnesia in water-soluble compounds, *This Journal*, **21**, 77 (1938), adopted as a tentative method last year, be adopted as official (first action), and that it be entitled, "Magnesia in Water-soluble Compounds Applicable to Sulfate of Potash Magnesia, Sulfate of Magnesia, and Kieserite."

(2) That the Bartlett-Tobey method for acid-soluble magnesia be adopted as a tentative method.

(3) That the Shuey volumetric method and other modifications of the present official method for acid-soluble magnesia be studied.

(4) That the study of methods for active magnesia in mixed fertilizers be continued.

(5) That the volumetric method for acid-soluble manganese in fertilizers and manganese salts, published in *This Journal*, **21**, 292 (1938), but with minor changes noted in this report, be adopted as a tentative method.

(6) That the colorimetric modification for acid-soluble manganese discussed in this report be further studied.

The paper, entitled "Effect of Particle Size on the Solubility of Magnesium in Dolomite and Magnesic Limestone in 4 per cent Citric Acid Solution Adjusted to *p*H 4.0 with Ammonium Hydroxide," by J. W. Kuzmeski, was published in *This Journal*, **22**, 147 (1939).

REPORT ON POTASH

By O. W. Ford (Purdue University Agricultural Experiment Station, West Lafayette, Ind.), *Associate Referee*

No collaborative work was carried out this year. At the 1937 meeting the General Referee on Fertilizers recommended: "That the Associate

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 51 (1939).

Referee on Potash be requested to ascertain whether any other state besides California forbids the use of the present official method for potash on account of provisions of the laws regarding water-soluble potash and to make any recommendations regarding this matter that seem advisable."

The following questionnaire was sent to the control officials of the 38 states having fertilizer control laws:

1. The Fertilizer Control Law of the State of ———— (permits, does not permit) the use of the present official method for potash.
2. The State of ———— (uses, does not use) the present official method for potash in fertilizer control work.
3. Do you regard the present official method for potash in the State of ———— as satisfactory (yes, no)?
4. Present-day fertilizers in the State of ———— (produce, do not produce) determinable amounts of water-insoluble residue, which will be weighed up as K_2PtCl_6 unless removed by filtration previous to the precipitation with H_2PtCl_6.
5. Please insert below any comment on the method, whether critical or otherwise.

A summary of the replies to Questions 1 and 2 of the questionnaire appears in Table 1.

TABLE 1.—*Use of the official method*

NUMBER OF STATES REQUIRING USE OF OFFICIAL METHOD	NUMBER OF STATES PERMITTING THE USE OF OFFICIAL METHOD	NUMBER OF STATES NOT PERMITTED TO USE OFFICIAL METHOD	NUMBER OF STATES USING OFFICIAL METHOD	NUMBER OF STATES NOT USING OFFICIAL METHOD
4	31	3	34	4*

* Two states are using either the perchloric acid or a modified perchloric acid method for potash.

A summary of the replies to Questions 3 and 4 of the questionnaire is given in Table 2.

TABLE 2.—*Comments on the official method*

NUMBER OF STATES REPORTING METHOD SATISFACTORY	NUMBER OF STATES REPORTING METHOD UNSATISFACTORY	NUMBER OF STATES NOT REPORTING WATER-INSOLUBLE RESIDUES	NUMBER OF STATES REPORTING WATER-INSOLUBLE RESIDUES	NUMBER OF STATES REPORTING CORRECTIONS FOR WATER-INSOLUBLE RESIDUES
23	6	23	12	10

OTHER COMMENTS ON THE OFFICIAL METHOD

One state reported that residues were found equivalent to potassium oxide ranging from 0.1 to 0.15 per cent when platinum dishes were used. It was necessary to filter before precipitating with potassium chloroplatinate to obtain satisfactory results.

Commercial chemists have reported water-insoluble residues equivalent to 0.23 per cent potassium oxide, and many report that they are

making corrections for these residues by dissolving out the potassium chloroplatinate and weighing back.

Allen and Gault, *This Journal*, 20, 101 (1937), state that they found more water-insoluble residue with the present official method than with the former method and that the residue increased with the increase of organic matter in the fertilizer. The residue was not affected by re-washing with the reagents used in the method.

More than a year ago, following the installation of natural gas in this laboratory but before final adjustments were made, water-insoluble residues were encountered that were larger than those reported by Kraybill and Thornton, *This Journal*, 18, 269 (1935). Their work, however, was completed prior to the installation of the natural gas. Since there was available no accurate means of measuring the temperature of ignition of the gas during the final burning off of the potash it was thought advisable to determine the effect on the amount of water-insoluble residues of ignition at different controlled temperatures in a muffle furnace.

EFFECT OF IGNITION TEMPERATURE ON WATER-INSOLUBLE RESIDUES

For this work, inspection sample L-4949, a 1–11–3 fertilizer with a high content of organic matter, was selected. A composite solution resulting from twelve 2.5 gram weighings from a portion of this sample ground to pass a 0.5 mm. sieve was used for all the determinations listed in Table 3. The water-insoluble residues were obtained as increased weight on a tared sintered glass filter after the potassium chloroplatinate had been filtered through it, weighed, and dissolved out and reweighed.

By using a sintered glass filter losses of weight occasioned when an asbestos pad is used were avoided, and many weighings and leachings out of the potassium chloroplatinate could be made before the filter would clog enough to slow up the filtration.

When the filter becomes clogged it can be cleared readily by treating it with aqua regia. With a filter of medium porosity (like a Jena BG-3), the speed of filtration is equal to that of a Gooch padded with asbestos. The one advantage of the sintered glass filter is that no increase of weight is obtained in a determination after the potassium chloroplatinate has been dissolved out unless water-insoluble residue is encountered. From the standpoint of additional work required this method of removing the residue is preferable to filtering before precipitation with potassium chloroplatinate.

Ohio analysts reported that they are removing the water-insoluble residue by filtration through an A. H. Thomas 16 G3 sintered glass filter.

It will be observed that at 550° C. noticeable residues were obtained and that at 650° and 750° correspondingly less residues were obtained, although one or two in answering the questionnaire indicated that they

TABLE 3.—*Effect of ignition temperature on water-insoluble residues in the determination of potash by the official method*
(Results expressed in percentage.)

SAMPLE NUMBER	AT 550° C.				AT 650° C.				AT 750° C.			
	K_2O	DIFFERENCE, ± FROM AVERAGE	K_2O CALCULATED FROM RESIDUE	DIFFERENCE, ± FROM AVERAGE	K_2O	DIFFERENCE, ± FROM AVERAGE	K_2O CALCULATED FROM RESIDUE	DIFFERENCE, ± FROM AVERAGE	K_2O	DIFFERENCE, ± FROM AVERAGE	K_2O CALCULATED FROM RESIDUE	DIFFERENCE, ± FROM AVERAGE
4949	3.22	−0.06	0.12	−0.17	3.02	−0.03	0.08	−0.01	2.95	+0.14	0.03	−0.01
4949	3.39	+0.11	0.30	+0.01	2.99	−0.06	0.03	−0.06	2.71	−0.14	0.02	−0.02
4949	3.22	−0.06	0.39	+0.10	3.10	+0.05	0.08	−0.01	2.95	+0.14	0.04	—
4949	3.45	+0.17	0.35	+0.14	3.08	+0.03	0.06	−0.03	3.02	+0.19	0.03	−0.01
4949	3.33	+0.05	0.25	−0.04	3.10	+0.05	0.08	−0.01	3.06	+0.25	0.08	+0.04
4949	3.31	+0.03	0.21	−0.08	3.02	−0.03	0.08	−0.01	2.96	+0.15	0.00	−0.04
4949	3.18	−0.10	0.28	−0.01	2.99	−0.06	0.08	−0.01	2.91	+0.10	0.00	−0.04
4949	3.20	−0.08	0.31	+0.02	3.10	+0.05	0.17	+0.08	2.98	+0.17	0.06	+0.02
4949	3.22	−0.06	0.30	+0.01	2.99	−0.05	0.08	−0.01	2.63	−0.18	0.04	—
4949	3.37	+0.09	0.30	+0.01	3.05	—	0.16	+0.07	3.01	+0.20	0.08	+0.04
4949	3.20	−0.08	0.24	−0.05	3.03	−0.02	0.08	−0.01	2.69	−0.12	0.01	−0.03
4949	3.25	−0.03	0.39	+0.10	3.07	+0.02	0.09	—	2.89	+0.08	0.11	+0.07
High	3.45	+0.17	0.39	+0.10	3.10	+0.05	0.17	+0.08	3.06	+0.25	0.11	+0.07
Low	3.18	−0.10	0.12	−0.17	2.99	−0.06	0.03	−0.06	2.63	−0.18	0.00	−0.04
Average	3.28		0.29		3.05		0.09		2.81		0.04	
Corrected for Residue	2.99				2.96				2.77			

would recommend heating above 750° C. to eliminate residue. It is possible that heating at 750° C. for a long period will volatilize some of the potash and give lower results. At least with the sample taken in this case the lowest results were obtained when the ignition was done at 750° C. It is recognized that any ignition carried out in an electric muffle will take longer and the dishes will be heated longer at the highest temperature of the ignition than will be the case when the ignition is finished off over a Meeker burner, even though the temperature of the Meeker may be as high as that of the muffle. Thus the muffle would afford a chance for volatilization of potash if any volatilizes at that temperature.

From Table 3 it will be seen that the most concordant results were obtained by ignition at 650°C. At this temperature the residues were not significant. In case the conditions of ignition cannot be sufficiently controlled to avoid residues the weight of the potassium chloroplatinate should be determined by weighing, dissolving out with water, and reweighing.

EFFECT OF FINENESS OF GRINDING ON UNIFORMITY OF RESULTS

Studies were also made relative to errors resulting from non-uniformity of the 2.5 gram samples weighed out for the official potash determination. One laboratory reported in the questionnaire that the potash salts were ground to pass through a 0.5 mm. sieve. Others have indicated at various times that that was the procedure they followed when they could not obtain good checks.

On several occasions during the past year the Associate Referee had difficulty in obtaining concordant results. On one occasion it was necessary to grind a portion of the reserve sample to pass a 0.5 mm. sieve. Two samples that gave exceptionally variable results were picked for investigation. From the reserve bottles of these two samples three 2-ounce portions were taken and ground to pass the 2-, 1-, and 0.5-mm. sieves, respectively. From each sample twelve 2.5 gram samples were weighed out for the determination of potash.

Table 4 lists the results obtained on inspection sample L-6289 (0–20–20), with ground tobacco as a conditioner, while Table 5 lists the results obtained on inspection sample L-5883, a muriate of potash (0–0–50) that had been cut with sand.

All bottles of fertilizer were full at the start, and all 12 samples weighed for determination were drawn from the bottles in the order listed in Tables 4 and 5.

The surprising thing about the results in Table 4 is that the coarsest ground portion produced both the highest and lowest potash values as well as the most erratic set of results, showing how slight is the chance to obtain a correct value on a product ground to pass only the 2-mm. sieve. As the degree of fineness was increased, more concordant results

TABLE 4.—*Effect of fineness of grinding on the uniformity of potash determinations*

SAMPLE NUMBER	2 MM.	DIFFERENCE ± FROM AVERAGE	1 MM.	DIFFERENCE ± FROM AVERAGE	0.5 MM.	DIFFERENCE ± FROM AVERAGE
		per cent		*per cent*		*per cent*
6289	21.16	+1.54	20.78	+0.62	19.76	−0.11
6289	20.70	+1.08	20.50	+0.34	19.72	−0.15
6289	19.99	+0.37	20.40	+0.24	19.70	−0.17
6289	20.70	+1.08	20.28	+0.12	19.62	−0.25
6289	19.71	+0.09	20.50	+0.34	19.74	−0.13
6289	19.96	+0.34	20.74	+0.58	20.08	+0.21
6289	20.24	+0.62	19.50	−0.66	20.30	+0.43
6289	19.62	—	20.25	+0.09	19.87	—
6289	18.06	−1.56	19.61	−0.55	19.44	−0.43
6289	17.88	−1.74	20.31	+0.15	19.84	−0.03
6289	16.86	−2.76	19.21	−0.95	20.24	+0.37
6289	20.54	+0.92	19.92	−0.24	20.08	+0.21
Average	19.62	—	20.16	—	19.87	—
Low	16.86	−2.76	19.21	−0.95	19.44	−0.43
High	21.16	+1.54	20.78	+0.62	20.30	+0.43
Difference between High and Low	4.30		1.57		0.86	
5883	49.18	+0.79	49.07	+1.64	47.89	—
5883	48.72	+0.33	48.49	+1.06	48.06	+0.17
5883	49.34	+0.95	48.45	+1.02	48.10	+0.21
5883	48.25	−0.14	48.53	+1.10	47.73	−0.16
5883	49.02	+0.63	48.58	+1.15	47.82	−0.07
5883	48.57	+0.18	48.30	+0.87	47.87	−0.02
5883	48.22	−0.17	48.53	+1.10	48.06	+0.17
5883	48.26	−0.13	48.29	+0.86	48.21	+0.32
5883	48.64	+0.25	47.27	−0.16	47.56	−0.33
5883	47.38	−1.01	48.65	+1.22	47.75	−0.14
5883	47.68	−0.71	48.61	+1.18	48.14	+0.25
5883	47.48	−0.89	48.39	+1.04	47.91	+0.02
Average	48.39	—	47.43	—	47.89	—
Low	47.38	−1.01	47.27	−0.16	47.56	−0.33
High	49.34	+0.95	49.07	+1.64	48.21	+0.32
Difference between High and Low	1.96		1.80		0.65	

were obtained. If these relationships hold for other types of samples it would be advisable to change the official method to permit grinding to pass a sieve finer than the 1 mm. now permitted, especially on those samples that did not give concordant results.

METHODS OF PLATINUM RECOVERY

Investigation of the methods of platinum recovery submitted by various control and commercial chemists resulted in the selection of four

for further investigation. Two of these specify zinc in the reduction and two specify aluminum. The methods and the remarks of their sponsors follow:

METHOD 1

A. *Pt from Alcoholic Washings*

(1) *Recovery and Reduction.*—Continually evaporate the washings in a fairly large porcelain dish on a steam bath, and keep rinsed down between additions with distilled water. (Evaporation of the alcohol completes the reduction. A filter paper added to the dish prevents excessive sticking of the Pt on the sides of the dish.)

(2) *Purification.*—Treat the residue in dish from (1) with 3 portions of HCl (1+3), or until traces of iron no longer appear, transfer to a small porcelain dish, and heat in a muffle at 650° C. Cool, extract with 2 portions of HNO_3 (1+4), wash, dry, and weigh.

(3) *Preparation of Solution.*—Dissolve the Pt from (2) in a porcelain dish on a steam bath with aqua regia (3 parts HCl to 1 part of HNO_3). After solution continue evaporation with additions of HCl three times for removal of excess HNO_3, then with additions of distilled water for three times for removal of excess HCl, but do not evaporate below ¼ volume. Filter the solution and make up to 1 gram of Pt for each 10 cc. of solution, from which dilutions can be made for fertilizers of low potash content (a 10 cc. portion is normally evaporated and tested for material insoluble in 80% alcohol). In case of blank on this portion reduce the solution at this point and prepare as directed in B.

B. *K_2PtCl_6 Salt Residue*

(1) *Reduction.*—Dissolve all K_2PtCl_6 residues in water, filter off, and reduce with HCl and sheet Al, checking traces of final reduction with HCl and KI (a 25 cc. portion of the clear supernatant liquid is acidified with HCl and KI, and if all Pt is reduced no red color appears and vice versa).

(2) *Purification.*—Destroy final traces of Al with excess HCl. Allow the Pt black to settle, decant off the supernatant liquid several times, and finally filter on a Whatman No. 2 in a Büchner funnel and wash several times with distilled water until a negative chloride test is obtained with $AgNO_3$. Transfer the Pt black to a small procelain dish, ash in a muffle at 650° C., cool, purify further by alternate extractions with HNO_3, H_2O, and HCl, and finally dry and weigh for solution.

(3) *Preparation of Solution.*—Proceed as directed in A(3), evaporating a 10 cc. portion and testing for material insoluble in 80% alcohol. In case of a blank at this point repeat the reduction and solution as in B.

Normal time required for preparation 4 days.

METHOD 2

Pt from Waste Solutions

Reduce the accumulated Pt waste solution with H_2 to Pt black. Generate H_2 by adding HCl and 20-mesh Zn to the waste solution. (Zn dust is frequently used as a starter.) Filter the Pt black and wash with water. After washing, boil the Pt black in HCl (1+1) for about 1 hour to remove excess metal and soluble impurities. Filter and again wash thoroughly with water. Burn off the filter paper and volatile impurities in a furnace at 750°–800° C. Redissolve the residue in aqua regia. Place the solution over a steam bath and remove all traces of HNO_3 by several additions of HCl, reducing the volume after each addition of HCl. When the solution is free from HNO_3, remove from the steam bath and filter. Test the solution for strength and adjust to the desired value. Time required for preparation 4–5 days.

METHOD 3

Pt from Scrap and/or Pt Black

(1) *Purification.*—Dissolve in aqua regia. Remove all HNO_3 by repeated evaporations just to dryness with HCl and water, using alternately, water last.

Make about a 10% solution with water and precipitate $(NH_4)_2PtCl_6$ with NH_4Cl. Allow to stand several hours, filter and wash the precipitate thoroughly with alcohol.

Ignite the precipitate—first at a low temperature, finally for 30 minutes at a very high heat. Wash by boiling in HCl, then in water, repeating several times.

Redissolve in aqua regia, remove HNO_3, dilute to a 10% solution, and neutralize with Na_2CO_3, using litmus for the end point. Filter off the precipitate containing impurities.

Heat the filtrate to boiling and reduce Pt with $NaCHO_2$. (Caution: Add the $NACHO_2$ a pinch at a time and stir well at each addition, because there is danger of excessive foaming and resultant loss of Pt.) Complete reduction is indicated when the solution clears to a water white.

Wash the Pt by boiling in HCl, then in water, and repeating several times.

Ignite first at a low heat, then for 30 minutes at high temperature. Weigh. Rewash, boiling in HCl then in water, repeating.

Redissolve in aqua regia, remove the HNO_3 as before and dilute as specified by A.O.A.C.

Recovery:—Combine the alcohol and NH_4Cl washes with $(NH)_2PtCl_6$ from the crucibles dissolved in water and add a small quantity of Zn dust, stirring frequently until reduction is complete. Allow to settle, decant the clear solution, and boil the Pt black in HCl, then in water, and repeating. Ignite and purify.

METHOD 4

Pt from Washings

Evaporate the alcoholic washings to a small bulk. Add 75–150 cc. of HCl (depending on the amount of Pt in the solution) and a piece of stick Al (10–20 grams). Continue until all Pt is reduced.

Digest precipitates of K_2PtC_6, together with asbestos, etc., with hot water. Decant the clear solution two or three times, using small portions of hot water, and then wash on the suction until all the K_2PtC_6 is free from the asbestos, etc. (Small amounts of asbestos in the solution will not interfere, in fact will aid subsequent filtration.) After all the platinic chloride is in solution, add Al and HCl as above, running the reduction until all Pt has been reduced. This can be tested as follows:

Pipet about 25 cc. of the clear solution into a 250 cc. beaker, add a few drops of HCl and a small amount of KI (in solution). If unreduced Pt is present, the solution will turn a reddish color; HNO_3 will give approximately the same color so it should not be present.

After all the Pt has been reduced, filter the Pt black on suction, using filter paper, and wash with hot water until clear. Burn the precipitate, together with the paper, in a silica dish, breaking up all large lumps with a Pt rod. After burning thoroughly digest the black in 50–200 cc. of HCl, the amount depending on the amount of Pt black, for 15–45 minutes. Then filter and wash with hot water on the suction. Again burn the precipitate and paper and digest with HCl. Follow this procedure until the HCl digestion is colorless. Add the HNO_3 and dissolve the Pt black. Standardize as directed in the official methods of the A.O.A.C.

NOTE: It is best to keep the washings and the water solution of the K_2PtCl_6 separate during the reduction of the Pt as filtering of the black will be much better.

COMMENT

In the determination of potash by the official method of the A.O.A.C., the $PtCl_6$ is usually made by reducing the platinum in both the alcohol washings and the hot solution of the K_2PtCl_6 precipitates by the use of zinc. This method seems to give an impure platinum black, which cannot be cleaned properly with hydrochloric acid, and it is necessary to rewash the black with nitric acid. Method 4, specifying metallic aluminum to reduce the platinum, has been found to be satisfactory without the use of nitric acid.

RECOMMENDATIONS[1]

It is recommended—

(1) That the study of the use of a factor weight or of factor weights in the determination of potash in fertilizers be continued.

(2) That the barium chloride method for the determination of potash, *Methods of Analysis, A.O.A.C.*, 1935, 31, 45, 46, and 47, be deleted (final action).

(3) That a study be made of the determination of potash by "dissolving out the potassium chloroplatinate and reweighing when the filtration is made on a glass sinter or asbestos padded Gooch," in place of "by filtration after ignition and solution," when platinum or silica dishes are used.

(4) That further study be made of the need for providing additional platinum solution concentrations.

(5) That a collaborative study of the four methods submitted or of other methods for the recovery of platinum be made with a view to recommending the adoption of one or more procedures.

(6) That a collaborative study be made of some modification of the present official method to prevent foaming during the boiling of the sample.

(7) That a collaborative study be made of degree of fineness of grinding with a view to elimination of the errors resulting from the non-uniformity of the 2.5 gram samples weighed out for the official potash determination.

(8) That the studies concerned with the solvent action of acid alcohol on potassium chloroplatinate be continued.

ACKNOWLEDGMENT

The writer wishes to express his gratitude to H. R. Kraybill for his counsel in connection with this report.

The paper, entitled "Filtering before Addition of Platinic Chloride in the Analysis of Fertilizers for Potash," by H. R. Allen, was published in *This Journal*, 22, 162 (1939).

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 51 (1939).

REPORT ON ACID- AND BASE-FORMING QUALITY OF FERTILIZERS

By L. E. HORAT (Department of Agricultural Chemistry, Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*

The referee work on this subject for the past year was concerned chiefly with the tentative method for the determination of acid- and base-forming quality of fertilizers, *Methods of Analysis, A.O.A.C.*, 1935, 34–35. In accordance with the recommendations of the Association, *This Journal*, **21**, 62 (1938), and in view of the development of a new mixed indicator by Pierre, Tully, and Ashburn,[1] a collaborative study of the tentative method was made with special reference to a comparison of the old and newly proposed indicators.

The use of 0.5 *N* sodium hydroxide solution in place of 1.0 *N* sodium hydroxide solution in the titration and the use of a filter paper cone to prevent loss by spattering, *This Journal*, **21**, 301 (1938), during the ignition were also included in this collaborative study.

The four fertilizer samples prepared for the collaborative work are listed in Table 1, together with pertinent analytical data. Sample C-1 is a high analysis superphosphate; Sample C-2 is a representative complete fertilizer made non-acid forming by the addition of limestone; and Sample C-3 is another complete fertilizer with its acidity uncorrected. Sample C-4 is the ordinary Tennessee Brown Rock phosphate. All the material was ground to pass a 40-mesh sieve to insure more uniform and representative analytical samples. In view of previous experience by the Associate Referee these samples furnish a thorough test of the tentative method and proposed modifications.

TABLE 1.—*Samples for collaborative analysis*

SAMPLE NUMBER	FERTILIZER ANALYSIS	INSOLUBLE P_2O_5	TOTAL *N*	WT. OF SAMPLE TO BE USED	TOTAL ACIDITY CORRECTION
		per cent	*per cent*	*gram*	*lbs. $CaCO_3$/ton*
C-1	0-44-0	1.3		0.5	36.7
C-2	2-12-6	1.6	2.4	1.0	130.8
C-3	4-12-4	1.1	4.4	1.0	188.1
C-4	Tenn. Br. Rock	31.7		0.5	893.9

To save time and for the sake of uniformity, it was directed that collaborators use the above total acidity corrections in the calculation of their results and the indicated weights of samples.

DIRECTIONS TO COLLABORATORS

The following directions were submitted to the collaborators:

(1) Determine the acid- or non-acid-forming quality as directed under 55,

[1] *Ind. Eng. Chem. Anal. Ed.*, **10**, 72 (1938).

pages 34 and 35, *Methods of Analysis, A.O.A.C.*, 1935. Use 0.5 *N* NaOH in place of 1.0 *N* NaOH in every case.

(2) Proceed as directed in (1) except as indicator use 10 drops of 1% bromophenol blue in 50% ethyl alcohol. Titrate to the color change (corresponding to *p*H 4.3–4.5) from yellow or orange magenta to grey or blue magenta when observed by transmitted light through a thin layer of solution as around the edges of a tipped Erlenmeyer flask. This color change occurs just before the final change to definite blue.

(3) Proceed as directed in (1) except as an indicator use the following: Weigh 0.1 gram of bromocresol green and 0.02 gram of methyl orange into an agate mortar, grind with small amounts of NaOH, using a total of 2 cc. of 0.25 *N* NaOH or its equivalent and make up to 100 cc. volume with water. Use 10 drops of solution per 150 cc. of solution titrated. Titrate to a light green color (*p*H 4.3), described as where the green definitely predominates over the yellow. For further details see *J. Ind. Eng. Chem. Anal. Ed.*, **10**, 75 (1938).

In one of the duplicate determinations under (1), (2), or (3) for each sample use a filter paper cone to minimize spattering as follows: After addition of the Na_2CO_3-sucrose solution to the sample in the beaker and before evaporation on the sand bath, insert a filter paper (low ash) cone folded so the base will just slip into the beaker, rest on the bottom, and touch the sides all the way around. Cut off the apex of the cone to form an open vent about 3 mm. in diameter.

Please mark (with asterisk or similar sign) on report blank those determinations where filter paper cone was used. If used in all determinations please note on report blank.

If convenient, a determination of the *p*H of the average end point obtained with each indicator will be of additional value.

In case all samples can not be completed it is recommended that one, two, or three samples be run through with each indicator.

Your observations, opinions, and preferences will be especially valuable.

RESULTS OF COLLABORATORS

In Table 2 the equivalent acidities or basicities for each sample, as determined by each collaborator by both methods, are given in pounds of calcium carbonate per ton of fertilizer. The results represent the average values of two or more separate determinations. A summary of the results in Table 3 shows a comparatively small difference in values obtained with each of the three indicators.

COLLABORATORS*

1. W. A. Morgan, Wilmington, Del.
2. R. L. Jones, Navassa, N. C.
3. H. C. Batton, Baltimore, Md.
4. Paul Caldwell, East St. Louis, Ill.
5. E. W. Cowan and L. D. Haigh, Columbia, Mo.
6. Henry A. Davis, Durham, N. H.
7. Oscar I. Struve, Buffalo, N. Y.
8. R. D. Caldwell, Atlanta, Ga.
9. L. V. Rohner, Syracuse, N. Y.
10. Frank O. Lundstrom, Washington, D. C.

* Of the 37 collaborators submitting reports, 20 are from the laboratories of fertilizer or allied companies, 16 are from Experiment Stations or similar institutions, and one is from a commercial laboratory.

TABLE 2.—*Collaborative results on determination of acidity and basicity of fertilizers*

SAMPLE C-1				SAMPLE C-2			
NUMBER OF COLLABORATOR	METHOD			NUMBER OF COLLABORATOR	METHOD		
	1	2	3		1	2	3
1	100B	100B	94B	1	21A	16A	17A
2	6B	5B	10B	2	62A	61A	66A
3	31A	1A	28A	3	92A	100A	105A
4	22A	28B	7A	4	73A	61A	101A
5	16A	22B	24A	5	103A	70A	77A
6	40A	7A	2A	6	91A	64A	68A
7	51A	22A	24A	7	107A	80A	80A
8	37A	34A	27A	8	83A	58A	78A
9	71A	29B	11A	9	139A	106A	94A
10	53A	27A	20A	10	98A	77A	64A
11	25B	25B	40B	11	54A	47A	59A
12	23A	1B	28A	12	73A	63A	71A
13	9A	9A	2A	13	77A	71A	82A
14	9A	3A	3A	14	76A	86A	76A
15	22A	2A	9A	15	81A	63A	51A
16	17A	27A	27A	16	86A	71A	81A
17	67A	87A	47A	17	116A	104A	94A
18	6B	17B	26B	18	75A	78A	75A
19	18B	38B	18B	19	58A	58A	53A
20	13A	1B	13B	20	75A	58A	70A
21	4A	3A	1A	21	78A	67A	75A
22	33B	86B	83B	22	48A	22A	21A
23	137A	112A	87A	23	146A	91A	141A
24	65B	12B	24B	24	86A	74A	68A
25	3B	63B	23B	25	71A	1A	36A
26	13A	8A	7A	26	89A	70A	78A
27	32A	2B	14B	27	98A	36A	62A
28	2A	58B	2A	28	51A	41A	31A
29	29B	34B	29B	29	55A	30A	78A
30	32B	48B	30B	30	54A	41A	61A
31	4A	13A	9A	31	77A	76A	68A
32	37A	22A	37A	32	76A	48A	81A
33	42A	17A	22A	33	96A	140A	128A
34	56A	26B	33B	34	83A	10A	32A
35	36A	35A	38A	35	68A	60A	53A
36	14A	27A	17A	36	68A	70A	74A
37	91B	103B	113B	37	17A	7A	1A
Av. Acidity or Basicity (lbs. $CaCO_3$ per ton)	12A	7B	2B	Av. Acidity (lbs. $CaCO_3$ per ton)	80A	63A	71A
Av. Deviation	30	32	28	Av. Deviation	18	20	18
Max. Deviation	125	119	111	Max. Deviation	66	77	70

TABLE 2.—*Collaborative results on determination of acidity and basicity of fertilizers—Continued*

SAMPLE C-3				SAMPLE C-4			
NUMBER OF COLLABORATOR	METHOD			NUMBER OF COLLABORATOR	METHOD		
	1	2	3		1	2	3
1	319A	303A	303A	1	254B	370B	347B
2	356A	353A	365A	2	246B	248B	217B
3	342A	347A	337A	3	151A	116A	156A
4	266A	341A	356A	4	114A	71B	11B
5	383A	351A	362A	5	67A	103B	87B
6	368A	348A	357A	6	38B	27B	56B
7	392A	375A	373A	7	53B	102B	94B
8	396A	331A	371A	8	36B	81B	26B
9	412A	381A	385A	9	126A	78B	74B
10	380A	366A	362A	10	73A	99B	29B
11	348A	341A	347A	11	53B	101B	97B
12	359A	347A	349A	12	59B	104B	88B
13	362A	366A	369A	13	54B	59B	59B
14	358A	366A	357A	14	266B	273B	261B
15	361A	351A	343A	15	71B	99B	104B
16	378A	378A	368A	16	127B	120B	115B
17	391A	388A	381A	17	6B	21B	1B
18	363A	348A	350A	18	64A	16B	3B
19	335A	338A	346A	19	61B	71B	51B
20	359A	351A	352A	20	80B	112B	78B
21	366A	354A	367A	21	56B	53B	61B
22	341A	307A	298A	22	126B	4B	186B
23	421A	368A	413A	23	14A	66B	6B
24	388A	357A	356A	24	124B	202B	215B
25	371A	318A	341A	25	31B	156B	61B
26	368A	345A	373A	26	43B	50B	36B
27	383A	342A	353A	27	66A	130B	59A
28	321A	316A	338A	28	217B	296B	226B
29	340A	310A	353A	29	142B	272B	117B
30	338A	328A	354A	30	142B	170B	163B
31	361A	362A	369A	31	—	—	—
32	371A	351A	358A	32	—	—	—
33	363A	366A	363A	33	111B	16B	131B
34	379A	332A	327A	34	62A	—	—
35	363A	373A	360A	35	110B	117B	118B
36	360A	357A	357A	36	—	—	184B
37	298A	295A	293A	37	194B	186B	179B
Av. Acidity (lbs. $CaCO_3$ per ton)	360A	350A	354A	Av. Basicity (lbs. $CaCO_3$ per ton)	58B	108B	90B
Av. Deviation	20	16	14	Av. Deviation	81	60	62
Max. Deviation	94	55	61	Max. Deviation	209	262	257

TABLE 3.—*Comparison of indicators as shown by average value of collaborators' results*

SAMPLE NUMBER	EQUIVALENT ACIDITY OR BASICITY VALUES (POUNDS $CaCO_3$/TON)			
	METHYL RED (1)	BROMOPHENOL BLUE (2)	BROMOCRESOL GREEN* M.O.	ELECTROMETRIC* *p*H 4.5
C-1	12A	7B	2B	18A
C-2	80A	63A	71A	60A
C-3	360A	350A	354A	367A
C-4	58B	108B	90B	184B

* Obtained by interpolation of values used in plotting curves of Fig. 1.

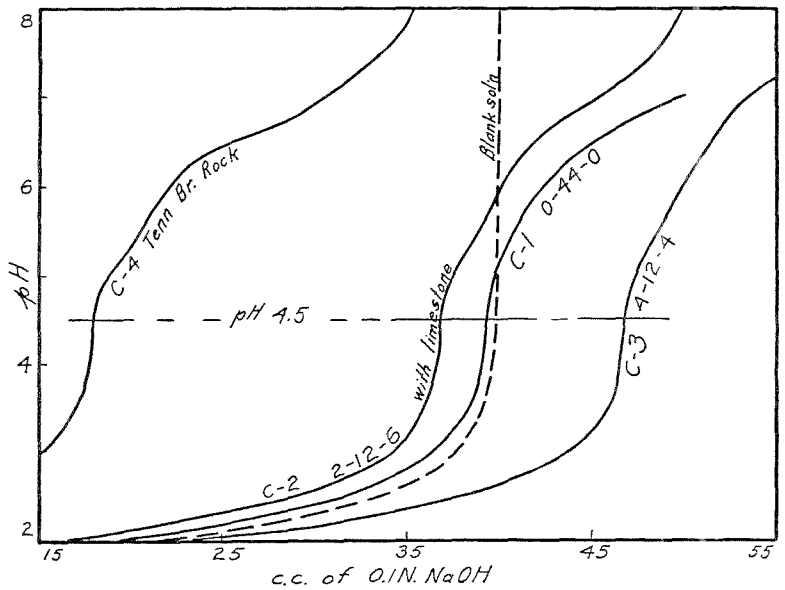

FIG. 1.—TITRATION CURVES OF FERTILIZER SAMPLES
(READINGS MADE WITH BECKMAN *p*H METER)

11. Mary C. Fox and C. Clifton Howes, Baltimore, Md.
12. R. C. Koch, Hammond, Ind.
13. G. S. McDaniel, Atlanta, Ga.
14. J. Preston Yarborough and Chas. Buchwald, Atlanta, Ga.
15. J. G. McCallister, Jr., Baltimore, Md.
16. Carl Neutzel, Baltimore, Md.
17. G. S. Fraps and T. L. Ogier, College Station, Tex.
18. H. R. Allen, Lexington, Ky.
19. C. L. Hare and T. H. Burton, Auburn, Ala.
20. P. J. Buchanan and R. C. Charlton, Baltimore, Md.
21. C. A. Butt and C. M. Cartledge, East Point, Ga.
22. Robt. P. Thornton, Tampa, Fla.

23. W. H. MacIntire and L. J. Hardin, Knoxville, Tenn.
24. Wm. C. Geagley and Mack M. Nasif, Lansing, Mich.
25. L. S. Walker and E. F. Boyce, Burlington, Vt.
26. Joe J. Scherer, Tallahassee, Fla.
27. Gordon Hart, Tallahassee, Fla.
28. C. R. Byers, Carteret, N. J.
29. Geo. E. Grattan and C. V. Marshal, Ottawa, Canada.
30. John B. Smith and D. R. Willard, Kingston, R. I.
31. W. R. Austin, Nashville, Tenn.
32. F. B. Carpenter and H. L. Moxon, Richmond, Va.
33. H. D. Haskins and A. F. Spelman, Amherst, Mass.
34. Geo. F. Moore and Thos. Beer, Tampa, Fla.
35. E. W. Magruder and W. A. Ryder, Norfolk, Va.
36. W. H. Pierre and R. W. Pearson, Ames, Iowa.
37. J. Morrisson, Chicago Heights, Ill.

The average deviation values summarized in Table 4 show that in case of three of the four samples the mixed indicator of bromocresol green and methyl orange gave more concordant results than did either methyl red or bromophenol blue.

TABLE 4.—*Comparison of indicators as shown by deviations from average values**

SAMPLE NUMBER	DEVIATION FROM AVERAGE VALUE FOR EACH INDICATOR (POUNDS $CaCO_3$/TON)					
	METHYL RED (1)		BROMOPHENOL BLUE (2)		BROMOCRESOL GREEN—M.O. (3)	
	AV.	MAX.	AV.	MAX.	AV.	MAX.
C-1	30	125	32	119	28	111
C-2	18	66	20	77	18	70
C-3	20	94	16	55	14	61
C-4	81	209	60	262	62	257
Av. Dev.	37		32		31	
Max. Dev.		124		128		125

* Average of all collaborative values for each sample.

COMMENTS OF COLLABORATORS

In response to the questionnaire sent to each collaborator, many valuable observations, opinions, and preferences in regard to various technical details of the method were obtained. In addition, several collaborators submitted experimental evidence of probable sources of error in the tentative method.

The recommendation of the Association that 0.5 *N* sodium hydroxide solution be used in the titration in place of 1.0 *N* sodium hydroxide solution, was endorsed by 30 out of 37 collaborators. Six preferred weaker alkali and only one favored stronger alkali. The preference for indicators was decidedly in favor of Pierre's mixture of bromocresol green and methyl orange.

The use of a filter paper cone for the prevention of spattering during drying and ignition of the sample was definitely favored by a significant

majority of the collaborators. Very few considered its use unnecessary and they usually avoided spattering by slow and carefully controlled or constantly attended evaporation. Many emphasized the greater accuracy and the increased speed of evaporation or drying possible when the cone is used. As a means for initial evaporation of the sample and sodium carbonate-sucrose solution, the sand bath and electric hot plate were about equal in preference among the collaborators. The temperatures favored were an initial one of 100°–120° C. up to 150°–200° C. final temperature before ignition in a furnace.

The final volume of the filtrate when titrated varied among the collaborators from 100 cc. to 250 cc., with 150 cc. the approximate volume titrated by a majority of the collaborators. This item is significant in some samples high in phosphate and lime in that the larger volume or more dilute solution decreases the possibility of interference by precipitation or cloudiness near the end point.

A matter of special importance in regard to its effect on results obtained by the present tentative method is the acid extraction of the ashed sample prior to filtration and titration. Although directions in the tentative method are explicit on this point, evidence was submitted in 1936 by C. L. Hare of Alabama showing the acid extraction of the ashed residue to be incomplete in the case of some samples. By means of a second extraction with additional acid, Hare found nine different fertilizers which yielded from 10 to 25 pounds calcium carbonate additional basicity. He suggested that this additional basicity might be due to larger particles of limestone (10–20-mesh fineness). However, with Sample A-5 used in the 1936 collaborative study, he obtained from 65 to 100 pounds of calcium carbonate additional basicity by a second acid extraction and this sample was ground to pass a 40-mesh sieve before being sent out by the Associate Referee. Hence it appears that during the ashing or ignition of the sample coarser basic aggregates are formed and that they are not completely dissolved during the acid extraction as prescribed in the tentative method. This year Collaborators Butt and Cartledge, using a 0.5 gram sample (C-4, Tennessee brown rock phosphate) as directed by the Associate Referee, submitted data showing approximately 200 pounds of calcium carbonate additional basicity obtained by extracting the ignited sample with 40 cc. of 1.0 *N* hydrochloric acid instead of 30 cc. of 1.0 *N* hydrochloric acid, as prescribed in the tentative method. According to the analysis of Sample C-4 and the present tentative method, its available phosphoric acid content of 3–4 per cent would call for the use of a 1 gram sample. However, in view of his past experience as well as that of Hare, the Associate Referee directed the use of a 0.5 gram sample in the hope of overcoming the above difficulty. In a recent communication this difficulty with Sample C-4 was confirmed by Pierre and Pearson. Pierre suggests sufficient excess acid

and sufficient time for digestion of samples that are very basic or where a large amount of coarse dolomitic limestone or ground bone is present.

ELIMINATION OF WATER-INSOLUBLE MATERIAL COARSER THAN 20-MESH

During the past year over 300 unground samples of commercial mixed fertilizers of all the more common analyses have been collected for an investigation by wet sieving to determine whether any fertilizers or class of fertilizers contain sufficient water-insoluble material coarser than 20-mesh of sufficient basicity to justify elimination of this portion before the tentative method is applied. Taylor and Pierre[2] found that these coarser portions of dolomitic limestone were not available during the year of application. This work by the Associate Referee has not been completed.

CALCULATION OF RESULTS

The directions for calculating results according to the present tentative method seem to be explicit, but the difficulty experienced by some collaborators both this year and in 1936 induced the Associate Referee to submit a formula to all collaborators who had been asked to recheck their calculations when submitted results were very much out of line with the average. In response to solicited opinions regarding the use of such a formula, many collaborators submitted formulas that had been used in their laboratories. Below is a composite of the various formulas proposed:

$$\left\{\frac{(\text{cc. } 0.5\ N \text{ NaOH blank} - \text{cc. } 0.5\ N \text{ NaOH sample}) \times 50 \text{ lbs. } CaCO_3}{\text{Wt. of sample used in grams}}\right\}$$

$$\text{minus } (\%\ \text{Total N} \times 35.7) \text{ minus } (\%\ \text{insol. } P_2O_5 \times 28.2)$$

When the formula is solved algebraically, a positive result indicates that the sample is basic or non-acid forming, while a negative result indicates sample is acid or acid-forming.

All collaborators who misinterpreted the directions for calculating results in the tentative method favored substitution of a formula of the above type.

RECOMMENDATIONS[3]

It is recommended—

(1) That in the present tentative method for the determination of acid- and base-forming quality of fertilizer, *Methods of Analysis, A.O.A.C.*, 1935, methyl red indicator, 34, 55(a), be replaced by the mixed indicator prepared by weighing 0.1 gram of bromocresol green and 0.02 gram of methyl orange into an agate mortar, and triturating while slowly adding

[2] *J. Am. Soc. Agron.*, 27, 764 (1935).
[3] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 53 (1939).

about 2 cc. of 0.1 *N* NaOH, and then diluting to 100 cc. with water (first action).

(2) That in the same method 0.4 cc. of the mixed indicator be used in titration in place of methyl red (first action).

(3) That the same method be modified by the substitution in the titration of 0.5 *N* sodium hydroxide in place of 1.0 *N* sodium hydroxide solution (final action).

(4) That the same method be modified by making optional the use of a filter paper cone for the prevention of spattering (first action). This cone should be of low ash paper and such size that when folded the base will just slip into the beaker, rest on the bottom, and touch the sides all around. The apex is cut off to provide a vent of about 3 mm. diameter, *This Journal*, **21**, 301 (1938).

(5) That in the same method the elimination of water-insoluble material coarser than 20 mesh before the method is applied be studied further.

(6) That the basicity of phosphate rock and other factors that affect the method be studied further.

(7) That in the same method, p. 34, in **55(b)**, the typographical error of "286 g of $Na_2CO_3 \cdot H_2O$" be corrected to read "286 g $Na_2CO_3 \cdot 10H_2O$."

The paper, entitled "A Comparison of the Official and MacIntire-Shaw-Hardin Methods for Determining Available P_2O_5," by J. Richard Adams, is published in this number of *This Journal*, p. 397.

The paper, entitled "Citrate Solubility of the Magnesium in Dolomite of Varying Particles Size," presented by Whittaker, Rader, and Zahn, was published in *This Journal*, **22**, 180 (1939).

REPORT ON CALCIUM, SULFUR, COPPER, AND ZINC

By GORDON HART (Chemical Division, Agricultural Department, Tallahassee, Florida), *Associate Referee*

The Bartlett-Tobey method for the determination of calcium was tried out in this laboratory by W. Y. Gary. The results appear to be very promising, and bromophenol blue seems a better indicator than methyl red. If the precipitation is made at *p*H 3.5–4.0, iron and alumina do not interfere, but manganese may interfere if there is much present.

It is recommended[1] that collaborative work on methods for the determination of calcium, sulfur, copper, and zinc be done.

The paper, entitled "Improved Molybdenum Blue Reagents for Determination of Phosphorus and Arsenic," by J. A. Schricker and P. R. Dawson, was published in *This Journal*, **22**, 167 (1939).

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 51 (1939).

MONDAY—AFTERNOON SESSION

REPORT ON EGGS

By H. A. LEPPER (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The investigations on eggs this year were curtailed by the press of other duties on the part of some of the associate referees.

It is recommended[1]—

(1) That the title of par. 16, page 301, *Methods of Analysis, A.O.A.C.*, 1935, be changed to read, "Chlorine," and that the directions be revised as recommended by the associate referee (see *This Journal*, 22, 77 (1939).

(2) That the method for the determination of dextrose and sucrose, page 301, 18, be amended as suggested by the Associate Referee, *ibid.*

(3) That the studies on methods for dried eggs be discontinued.

(4) That study of methods for glycerol be continued.

(5) That study of methods for decomposition be continued.

(6) That study of the method for the determination of cholesterol and fat be continued.

No report on unsaponifiable constituents and fat was given by the associate referee.

REPORT ON DETECTION OF DECOMPOSITION

By J. CALLAWAY, JR. (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The Associate Referee believes that there is a real need for more official chemical methods for the measurement of decomposition in eggs. In order to be of greatest use such methods should not require elaborate apparatus or excessive time. A rapid method for the determination of acidity of ether extract in liquid eggs has been adopted as tentative, and last year a rapid method for determination of ammonia nitrogen was submitted to collaborators. The latter method has the advantage of less complicated apparatus and also requires less attention than does the present tentative method. The final results, however, were not identical with those obtained by the tentative method, although there appeared to be a definite relationship between them.

Believing that more work was justified, the Associate Referee requested collaborators to study previous reports and to try and improve the absorption apparatus. After they had prepared and tested such apparatus they were requested to determine ammonia nitrogen by a new rapid method and by the present tentative aeration method on several samples

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 61 (1939).

of eggs, including both good and bad. At the suggestion of Shupe it was further directed that sodium or potassium fluoride be added to egg samples in the absorption apparatus to prevent possible increased decomposition during absorption of ammonia.

Reports were received from Manuel Tubis, Food and Drug Administration, Philadelphia, Pa., and Irwin S. Shupe, Food and Drug Administration, Kansas City, Mo., the same collaborators who reported last year. Tubis reports that he tried out a different type of absorption cell consisting of a Petri dish with a partition dividing the dish into two equal compartments. The dish was covered with a piece of ground-glass greased with vaseline. The dimensions of the dish were as follows: diameter 9.5 cm., height 2 cm., height of partition 1 cm. Each compartment had a capacity of about 29 cc. Recovery of ammonia from a standard solution of ammonium salt with this cell was practically 100 per cent.

The acid used was 0.005 *N* and it contained the mixed indicators recommended by Bandemer and Shaible.[1] The alkali was 0.0025 *N*. All the saturated potassium carbonate solution was also saturated with sodium fluoride, Tubis further reports.

The results presented (Table 1) were obtained on samples bought in

TABLE 1.—*Results obtained with A.O.A.C. and absorption methods*

SAMPLE	MG. AMMONIA NITROGEN PER 100 GRAMS EGG	
	A.O.A.C. METHOD	ABSORPTION METHOD
1[a]	1.98	3.08
1[b]	2.72	3.18†
2[a]	2.67	2.94
2[e]	2.88	4.01
3	3.04	4.15
3[e]		3.21
3[w]		3.35
4 (Yolk)	6.06	7.39
4 (White)	2.56	1.43[p]

[a] Kept refrigerated.
[b] Kept at room temperature.
† This difference only amounts to 0.02 mg./5 grams higher than by aeration.
[e] These determinations were made a day later on the refrigerated samples.
[w] Sample same as 3[e] but had 3 cc. of water added to the egg, showing dilution caused a slight change.
[p] This is lower than the corresponding result by aeration.

the open market, broken out in the laboratory, and mixed by shaking with glass beads. All were normal in appearance, and none had an abnormal odor.

Sample 1 was divided into two equal parts; one part, 1[a], was kept refrigerated, and the other, 1[b] was left in the laboratory at room temperature to induce a slight spoilage.

[1] *Ind. Eng. Chem. Anal. Ed.*, 8, 201 (1936).

In another sample, the use of 4 cc. of saturated carbonate instead of 3 cc. gave a result of 0.03 mg./100 grams higher.

The new dish was compared with the old cell (Table 2). The samples used had been diluted to about twice their original volume.

TABLE 2.—*Comparison of "new dish" and "old cell" methods*

SAMPLE	NEW DISH	OLD CELL
	mg./100 grams	
5	3.38	3.45
"Fresh egg"	2.86	2.77
"Second grade egg"	3.24	3.07
6	2.48	2.41
7	3.30	3.28
7*	3.76	3.96
Control (recoveries)	100%	100%

* Incubated for 6 hours at 37.5° instead of usual 5 hours.

The "new dish" method was compared with the aeration method on three subdivisions of a sample of commercial frozen eggs. The results are shown in Table 3.

TABLE 3.—*Comparison of "new dish" and aeration methods*

SUBDIVISION	MG. AMMONIA NITROGEN/100 GRAMS	
	NEW DISH	AERATION
1	2.62	2.12
2	2.84	2.25
3	2.32	2.14

Summarizing, the aeration method gives slightly lower results than the proposed method, and the "new dish" method tried gives comparable but slightly higher results than the proposed method.

Shupe also tried some modifications of the absorption apparatus. He found that a glass cylindrical cell divided in half was not so efficient as the one he had used the year previously, which was of the type recommended by Bandemer and Schaible.[1] He recommends that the inner cell have a clearance of at least 4 mm. from the glass plate used as cover.

Shupe reports as follows on work using the adsorption apparatus just described:

Two samples of whole eggs (A and B) were analyzed by the A.O.A.C. tentative aspiration method and by the absorption method. For the proposed rapid method 5 grams (+0.05) samples were weighed into the absorption cells and 3 cc. of water was mixed with the sample. A measured volume of standard 0.02 *N* acid, 5 cc. for B and 2 cc. for A, was added to the inner cell. The cover-glasses were put in place and 3 cc. of saturated potassium carbonate solution was added to the egg in the outer chamber. The cells were then allowed to stand 5 hours at room temperature

(27°–30° C.). Methyl red indicator and 0.02 *N* acid and alkali were used. The results obtained are given in Table 4.

TABLE 4.—*Results by proposed rapid absorption method*

SAMPLE	NH_3/100 GRAMS
A—without NaF	3.0
A—K_2CO_3 solution saturated with NaF	3.0
A—K_2CO_3 solution saturated with NaF	3.4
B—without NaF	23.2
B—without NaF	23.6
B—K_2CO_3 solution saturated with NaF	21.1
B—K_2CO_3 solution saturated with NaF	20.9

* STANDARD $(NH_4)_2SO_4$ EQUIVALENT TO MG. NH_3 AS FOLLOWS:	RECOVERY IN 5 HOURS AT ROOM TEMPERATURE
	per cent
0.34 (with NaF)	100
0.68 (with NaF)	95
1.02 (with NaF)	93
1.70 (with NaF)	96

SAMPLE	NH_3/100 GRAMS
	mg.
A—By A.O.A.C. Aspiration	2.9
B—By A.O.A.C. Aspiration	19.1

* Standards contained 1 cc. of 0.02 *N* acid in excess of amount required to neutralize the ammonia.

REMARKS: Slightly higher results were obtained by the absorption method than by the A.O.A.C. aspiration method. 5 hours at room temperature (27°–30° C.) seems to be an adequate time and high enough temperature for the absorption of the ammonia.

The use of sodium or potassium fluoride seems advisable.

CONCLUSION

The results of the collaborators show that the absorption method gives slightly higher results than does aeration. Since the aeration method requires a special set-up and close attention it becomes very time consuming when many samples are to be examined. The absorption method requires less apparatus and less attention.

The Associate Referee believes that more work on standardization of absorption apparatus should be done. He believes that on the whole the absorption method is preferable to the aeration method.

RECOMMENDATIONS[1]

It is recommended—

(1) That further work be done on the absorption method for the determination of ammonia nitrogen in liquid eggs.

(2) That additional chemical methods for measurement of decomposition in eggs be sought.

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 61 (1939).

REPORT ON ADDED GLYCEROL, SUGAR, AND SALT

By L. C. MITCHELL (U. S. Food and Drug Administration, Minneapolis, Minn.), *Associate Referee*

GLYCEROL

No collaborative work was done on the methods for the detection and determination of added glycerol in yolks. As the tentative method for the determination of glycerol is not applicable in the presence of sugars, the Associate Referee did some preliminary work on the possibility of separating glycerol from sugar by distillation with superheated steam (up to 235° C.), but found that the glycerol did not completely distil. Time did not permit further work.

SALT

The collaborators were asked to add 10 per cent previously dried salt to egg white and proceed as directed under XXIII, 16, *Methods of Analysis, A.O.A.C.*, 1935, correcting the results by subtracting the amount of salt found in the blank, which salt is due to the naturally occurring chlorine compounds found in eggs. The results are given in Table 1.

TABLE 1.—*Results on 10% added salt in egg white*

COLLABORATOR	SALT RECOVERED
	per cent
Donald A. Ballard, Food and Drug Administration, Seattle	10.02
	10.02
Edward O. Haenni, Food and Drug Administration, Washington	9.98
	9.96
O. S. Keener, Food and Drug Administration, Cincinnati	10.14
	10.00
	10.01
G. E. Keppel, Food and Drug Administration, New Orleans	9.95
	9.97
C. A. Kuehl, H. J. Heinz Company, Pittsburgh	9.93*
	9.99*
J. A. Mathews, Food and Drug Administration, Washington	10.02
	10.00
Average	10.00

* Gravimetric method. All other results volumetric.

SUGAR

The official method for the determination of dextrose and sucrose in eggs, *Methods of Analysis, A.O.A.C.*, 1935, XXIII, 18, has been found satisfactory, *This Journal*, 14, 397 (1931); 16, 74, 305 (1933), with the exception of the error due to the volume of the precipitate, which is appreciable in liquid yolks, dried yolks, or whole eggs. The errors may

be corrected by such well-known methods as (1) double dilution, (2) filtration, followed by washing the residue free from sugar and making filtrate up to a given volume, and (3) correction of the volume occupied by the precipitate.

Method (3) means that corrections must be made for the volume occupied by the added calcium carbonate (probably a constant), by the proteins, and by the fat, with the percentages of the proteins and fat varying with each sample. Fat is readily determined, so is the nitrogen, but further correction would be necessary on the nitrogen since some of it is found in the fatty substances (lecithin). Thus far the Associate Referee has made no headway in establishing a correction factor for the volume occupied by the precipitate. Method (2) was abandoned because prolonged washing caused some of the residue to pass through the filter. Method (1) was found to yield promising results.

The collaborators were accordingly asked to add 10 per cent previously dried sucrose to yolks and proceed as directed under XXIII, 18, *Methods of Analysis, A.O.A.C.*, 1935; and to run a second determination, using the same amount of sample and calcium carbonate, but twice the amount of salt solution and alcohol and making the volume to 500 cc. instead of 250 cc. as given in the method. To correct the error due to the volume occupied by the precipitate, they were also requested to subtract the percentage of sucrose found in the 250 cc. determination from twice that found in the 500 cc. The results are given in Table 2.

TABLE 2.—*Results on 10% added sucrose in egg yolks*

COLLABORATOR	SUCROSE RECOVERED
	per cent
Edward O. Haenni	10.05
	10.07
O. S. Keener	9.93
	9.94
G. E. Keppel	9.88
	9.99
C. A. Kuehl	9.83
J. A. Mathews	9.90
	9.98
Average	9.95

COMMENTS OF COLLABORATORS

Ballard.—Added salt. The method as outlined under XXIII, 16 (a), *Methods of Analysis, A.O.A.C.*, 1935, was used for the natural salt in the whites. However, for the sample the filtrate from the charred mass was diluted to 200 cc. and a 40 cc. aliquot used for the determination, since it was obvious that the entire filtrate would require an excessively large quantity of silver nitrate. The duplicate analyses reported are from separate 10-gram portions of whites, each treated according to the method with the above noted exception.

Haenni and Mathews.—In the method for the determination of sucrose we encountered difficulty in making the mixture to the mark in the flasks, due to the entrapment of considerable volumes of air in the precipitate. We found it advisable to remove this air by applying suction to the mixture before making it to volume, the addition of a few drops of alcohol and ether being used to facilitate this operation. The difficulty seems to be particularly great in the case of the 250 cc. dilution.

The method for the determination of chlorine in eggs and egg products, as given in *Methods of Analysis*, is even yet somewhat ambiguous since the reference to the final determination calls for filtering off and washing the excess silver chloride, whereas the solution is obviously prepared with the intention of back titrating an aliquot of the excess silver nitrate. In the present work the chlorine in the egg white itself was determined according to the procedure in XXIII, 16, 17, an aliquot of the solution containing the excess silver nitrate being back titrated. The egg white containing added salt was analyzed similarly, an aliquot of the prepared solution being used for the precipitation of the chlorine.

Would it not be possible to establish a correction factor based on this double dilution method, correlated, perhaps, with the total solids or other determination, so that the double determination involved in this procedure would not be necessary in routine work?

DISCUSSION

Collaborative work was reported in 1932, *This Journal*, 16, 298 (1933), for added salt and sugar in whole eggs. At that time no correction was made for any error due to the volume occupied by the precipitate in the sugar determination, as such error is not particularly large. This error, however, is appreciable in such products as yolks, dried yolks, dried whole eggs, or frozen yolks, the latter product being the one in which added sugar is most likely to be encountered.

The results for both added salt and sugar are well within the analytical error of the respective methods, and are remarkably close to the theoretical.

Collaborator Haenni called attention to the ambiguity of the method for preparation of solution and to the fact that the reference to XII, 35 (gravimetric) in the determination should be XII, 37 (volumetric). In order to clarify the procedure and to include samples containing added salt, the method has been modified for consideration of the Association. (The modified method was published in *This Journal*, 22, 77 (1939).)

In the presence of added salt, correction is made for the natural chlorine-bearing substances present in the type of egg product under examination. For eggs (hen) the corrections are approximately as follows, *This Journal*, 15, 310 (1932): 0.3 per cent on liquid eggs (whether yolks, whites, or whole eggs); 0.6 per cent on dried yolks, 1.1 per cent on dried whole eggs, or 2.4 per cent on dried whites (albumin) for the naturally occurring chlorine-bearing substances calculated as salt.

It is suggested for the consideration of the Association that the method proposed for correction of error due to the volume of the precipitate in samples containing added sugar be incorporated at the end of the para-

graph 18(a), These directions were published in *This Journal*, 22, 77 (1939).

The directions for inversion of sucrose in the method, XXIII, 19, are those used by the Association for many years, but they do not now conform to the general method for sugars. Accordingly, Recommendation (3) is offered for the consideration of the Association.

RECOMMENDATIONS[1]

It is recommended—

(1) That the official method for the determination of chlorine, *Methods of Analysis, A.O.A.C.*, 1935, XXIII, 16, be modified along the lines suggested by the Associate Referee.

(2) That the official method for the determination of dextrose and sucrose, 18, be modified as suggested by the Associate Referee and adopted as official (first action).

(3) That the words "add 5 cc. of HCl, and allow to stand overnight" under XXIII, 19, Reducing Sugars Invert, be deleted and that the following words, "and invert the sucrose as directed under XXXIV, 23(b) or (c)," be inserted.

(4) That the study of methods for glycerol be continued.

No report on dried eggs was given by the associate referee.

REPORT ON PRESERVATIVES

By WILLIAM F. REINDOLLAR (State of Maryland Department of Health, Baltimore, Md.), *Referee*

The special method for the determination of saccharin in non-alcoholic beverages was investigated, modified, and submitted to collaborative study, to learn whether or not it is applicable to semi-solid preparations. The results of this investigation indicate that this method is unsuitable for these products. It is recommended,[2] therefore, that further work on this method be discontinued.

REPORT ON BENZOATE OF SODA

By A. E. MIX (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

It was suggested that the Associate Referee study the determination of benzoate of soda in food products, for which a rapid chemical method giving accurate results is required. A number of methods were reviewed

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 61 (1939).
[2] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 66 (1939).

and it is believed that the requirements may be met by a modification of the Monier-Williams method.

The apparatus consists of a boiling flask fitted with reflux (bulb) condenser, a large test tube (constricted at the center) for holding 3 grams of magnesium turnings in the upper portion of the tube, and two pieces of glass tubing connected in such a manner as to fit into a rubber stopper, which closes the neck of the flask and at the same time receives the lower end of the condenser tube.

METHOD

The sample is mixed with water if necessary, and an excess of NaCl is added (40 grams salt per 100 cc. solution). The mixture is acidified with P_2O_5, and 2 cc. is added in excess. A boiling tube is added to the flask, the flask is connected with the condenser, and the sample solution is boiled. This does not require any attention until after 3 hours of boiling. The flask is disconnected, and the Mg benzoate formed in the test tube is extracted with the hot water used for rinsing the inside of the condenser tube and also for the glass tubes attached to the rubber stopper. Thorough extraction is required but the volume of this extraction should be kept as small as possible. (The magnesium turnings placed in a small beaker immersed in a small casserole of boiling water and allowed to heat for 10 minutes were more thoroughly extracted than if left in the tube.) The extracted solution is filtered through glass wool and made strongly alkaline with about 20 drops of a 40% solution of NaOH. It is cooled to 40°–50°C., and oxidized with a saturated solution of KMO_4 until the

Results on benzoate of soda by a modified Monier-Williams method

SAMPLE	NaC_6H_5COO WT. OF CHARGE	WEIGHT RECOVERED	YIELD	DURATION OF BOILING	SOLVENT MIXTURE USED, BEFORE TITRATION
	gram	*gram*	*per cent*	*hours*	
1	0.0594	0.05526	93.0	3	100 cc. H_2O
1A	0.0500	0.04850	97.0	3	25 cc. Et OH, 95%
2	0.0500	0.0513	101.9	3	30 cc. H_2O 10 cc. Et OH, 95%
3	0.0560	0.0616	110.5	3	100 cc. H_2O 25 cc. Et OH, 95%
4	0.0500	0.04818	96.4	3	10 cc. H_2O 30 cc. Et OH, 95%
5	0.0500	0.05784	115.7	3	10 cc. H_2O 30 cc. Et OH, 95%
6	0.0500	0.05123	102.4	3	10 cc. H_2O 30 cc. Et OH, 95%
7	0.0500	0.05061	101.2	3	10 cc. H_2O 30 cc. Et OH, 95%

Nos. 1, 2, 3, sodium benzoate weighed from stock bottle.

Nos. 4, 5, 6, 7, sodium benzoate made into a water solution (1 cc. = .0005 gram); 100 cc. was measured out for sample.

No. 1A, sample of tomato juice to which 0.05 gram of dried Na benzoate was added.

Min. recovery, 93.0% water solution; max. recovery, 115.7% water solution.

Av. recovery, 102.2+.

pink color persists for some minutes. The excess KMO_4 is destroyed by the addition of NA_2SO_3 crystals until an even brown color of MnO_2 is formed. A few drops of concentrated H_2SO_4 are added. The resulting solution is usually clear and colorless. This clear solution is saturated with NaCl and extracted with a 50–50 mixture of ethyl acetate and petroleum ether.

COMMENTS

It appears that 4 or 5 extractions with 25 cc. ethyl acetate and petroleum ether mixture are sufficient.

Violent shaking of separatory funnel should be avoided. The solution was evaporated at room temperature in a current of dry air and allowed to stand overnight (or until no acid odor could be detected).

The residue of benzoic acid was dissolved in various amounts of 95 per cent alcohol and water, 1–2 drops of phenolphthalein indicator were added, and the solution titrated with 0.02*N* NaOH.

Samples 3 and 5 appeared dry but had acid odor and gave high yield. Samples 2, 6, and 7, faintly acid, gave over 100 per cent yield; 1, 1A, and 4, dry, no acid odor, gave reasonable yield.

It appears that drying, retention of acid odor, and the water-alcohol mixture for dissolving the residue must be carefully controlled.

It is recommended[1] that the work on this method be continued and that the method be further studied collaboratively.

REPORT ON COLORING MATTERS IN FOODS

C. F. JABLONSKI (U. S. Food and Drug Administration, New York City), *Referee*

Last year's recommendations of the Committee requested the Referee to continue the collaborative investigation of the quantitative estimation of Ponceau SX in the presence of Ponceau 3R. With this purpose in view, the Referee sent out to the collaborators six sets of samples, consisting of five subdivisions each, and instructions to estimate the dye mixtures by a submitted method, which was a slight modification of the one tried last year.

The samples in question were of the following composition (based on titanium chloride titrations):

SAMPLE	PONCEAU SX	PONCEAU 3R	TOTAL COLORS
	per cent	*per cent*	*per cent*
1	none	85.38	85.38
2	19.22	67.26	86.48
3	69.46	19.85	89.31
4	90.45	none	90.45
5	48.99	39.19	88.18

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 66 (1939).

The reports of the collaborators as received are as follows:

O. L. Evenson, Food and Drug Administration, Washington, D. C.

SAMPLE	PONCEAU 5X	PONCEAU 3R	TOTAL COLORS
	per cent	*per cent*	*per cent*
1	none	83.70	83.70
2	20.70	64.80	85.50
3	66.60	21.30	87.90
4	90.00	none	90.00
5	46.80	39.55	86.35
S. S. Forrest, Food & Drug Administration, Washington, D. C.			
1	none	85.30	85.30
2	18.60	64.30	82.90
3	68.40	20.30	88.70
4	90.60	none	90.60
5	48.00	40.80	88.80
Mrs. A. P. Bradshaw, Bureau of Chemistry & Soils, Washington, D. C.			
1	none	84.60	84.60
2	21.00	64.60	85.60
3	70.40	18.40	88.80
4	91.80	none	91.80
5	49.70	37.30	87.00
L. Koch, H. Kohnstamm & Co., Brooklyn, N. Y.			
1	none	85.40	85.40
2	21.90	65.65	87.55
3	73.25	16.20	89.45
4 (direct)	90.35		90.35
(after treatment)	95.10	none	95.10
5	51.35	36.50	87.85
J. J. Morris, H. Kohnstamm & Co., Brooklyn, N. Y.			
1	none	85.67	85.67
2	22.81	65.51	88.32
3	73.24	16.69	89.93
4 (direct)	88.52		88.52
(after treatment)	90.35	none	90.35
5	51.63	36.15	87.78
J. L. Hogan, Food & Drug Administration, New York City.			
1	none	84.65	84.65
2	20.71	65.49	86.20
3	67.45	21.30	88.75
4 (before treatment)	88.72		88.72
(after treatment)	86.62	trace or none	86.62
5	47.42	39.31	86.73

The following comments and criticism were offered by the collaborators:

Evenson.—Fairly good check results were obtained in all cases.

Forrest.—The method seems satisfactory, check results being obtained in all cases. Some variation is noted, but in several titrations a majority check closely.

It may be noted that the total titration for Nos. 1 and 2 do not check with those of Evenson. We have checked this point carefully and believe that the difference may be due to moisture absorbed by one of the samples.

Bradshaw.—In Sample 4 the corrected volume after subtracting from the original titration left a value of −0.36 cc. of 0.1 *N* titanium trichloride; this was not considered in the calculations.

Koch and Morris.—As in previous collaboration, the method presented no analytical difficulties. However, the straight ponceau SX Sample 4 consistently resulted in a higher percentage after the peroxide treatment. It is suggested that the collaborative investigation be directed towards the determination of dye mixtures containing 0–20% ponceau SX.

Hogan.—I had no particular difficulties with the method except that the end point while distinct seemed to vary over a range of about 0.20 cc. 0.1 *N* $TiCl_3$. In Sample 4 the average difference between the unoxidized and oxidized material amounted to 2.10% of dye. This difference may be due to a slight error in the submitted chart.

DISCUSSION

To give a better understanding, the results are summarized as follows (per cent):

Sample							
1	Ponceau SX	none	none	none	none	none	none
	Ponceau 3R	83.70	85.30	84.60	84.65	85.67	85.40
2	Ponceau SX	20.70	18.60	21.00	20.71	22.81	21.90
	Ponceau 3R	64.80	64.30	64.60	65.49	65.51	65.65
3	Ponceau SX	66.60	68.40	70.40	67.45	73.24	73.25
	Ponceau 3R	21.30	20.30	18.40	21.30	16.69	16.20
4	Ponceau SX	90.00	90.60	91.80	88.72	88.52	90.35
	Ponceau 3R	none	none	none	none	none	none
5	Ponceau SX	46.80	48.00	49.70	47.42	51.63	51.35
	Ponceau 3R	39.55	40.80	37.30	39.31	36.15	36.50

At first glance the results may not appear to be satisfactory. However, it must be taken into consideration that the color value of Sample 1 and also of Sample 4 as reported by the collaborators showed considerable difference. The extreme reported for Sample 1, which consisted entirely of ponceau 3R, is 1.97 per cent. The extreme reported for Sample 4, which consisted of Ponceau SX, is 3.28 per cent. Whether these differences of the color value of the dyes can be attributed to absorbed moisture cannot be positively answered. It is quite conceivable that mixtures of those dyes would also deviate at least to that extent. Therefore, if due allowances are made for these variations, the submitted results can be considered acceptable, since each 0.1 cc. of 0.1 *N* titanium trichloride represents approximately 0.70 per cent of dye.

In Sample 4 a number of collaborators noted a perceptible difference between color percentage of the treated and untreated sample. The Referee proposes to investigate this phase in the near future.

RECOMMENDATIONS[1]

It is recommended—

(1) That collaborative work be continued on the quantitative estimation of ponceau SX in the presence of ponceau 3R.

(2) That investigational work be continued on the quantitative separation and estimation of tartrazine and sunset yellow FCF in mixtures.

(3) That investigational work be undertaken to separate and estimate quantitatively mixtures of light green SF yellowish, brilliant blue FCF, and fast green FCF.

REPORT ON METALS IN FOODS

By H. J. WICHMANN (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

ARSENIC AND ANTIMONY

Three important papers on the determination of arsenic appeared in 1938 and the early part of 1939. Alfred E. How[2] modified the Gutzeit method in three respects, but still left it an empirical procedure. He substituted a string in a capillary tube for the paper strip, a stick of alloy (zinc 99.5, tin 0.05, lead 0.01, and iron 0.0028 per cent, respectively) for the zinc activated with stannous chloride, and used a double water bath instead of the single bath of the Gutzeit method, providing a difference of 5° C. between the temperature of generator and absorption tube. In addition, he caused the electrolyte to circulate around the stick of alloy. How's results calculate to a standard deviation of 7.4–2.4 per cent for a range of 0.1–100 micrograms, respectively. This is a much better performance than any claimed for the present official Gutzeit method with respect to range, but the accuracy has not been increased proportionally.

Klein and Vorhes[3] recently described the formation and extraction of arsenic ethyl xanthate, insoluble in aqueous acid solutions but soluble in carbon tetrachloride. These authors determine the arsenic finally by the Zinzadze[4] molybdenum blue method with photometric measurement. They applied their method to spray residue quantities of arsenic, leaving to the future the problem of adapting it to smaller quantities. Their standard deviation varied from 3.5 to 1.1 per cent for 100–800 micrograms of As_2O_3. The unusual feature of this paper is the method of arsenic isolation presented. Antimony and tin compounds do not form molybdenum blues, nevertheless these compounds must be removed because of turbidities caused by hydrolysis of their bromides. The removal of the interfering metals is made possible by a difference in the behavior of the

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[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 66 (1939).
[2] *Ind. Eng. Chem. Anal. Ed.*, 10, 226 (1938).
[3] *This Journal*, 22, 121 (1939).
[4] *Ind. Eng. Chem. Anal. Ed.*, 7, 230 (1935).

xanthates in carbon tetrachloride solution, with concentrated hydrochloric acid. The arsenic xanthate is stable towards that acid and remains in the carbon tetrachloride, but the tin and antimony xanthates, as well as other xanthates that are soluble in carbon tetrachloride or collect at the interface, decompose to the chlorides and dissolve in the acid phase. Here, then, is another possible method for the separation of micro amounts of antimony and arsenic, which should allow their subsequent individual determination.

The third paper, by Cassil and Wichmann,[5] describes a rapid arsine evolution followed by an iodine titration of the arsine. The striking features of this method are the speed of the evolution, the extraordinary efficiency of the arsine absorption in 1 cc. of mercuric chloride solution, and the accuracy of the final micro titration in a small volume of solution. An arsenic determination, exclusive of sample preparation, can be made in 10 minutes, and a standard deviation, constant over a range of 5–500 micrograms of As_2O_3, should not exceed 0.85 per cent. No doubt modifications based on delicate colorimetric determinations of excess iodine can be developed suitable for the 1–10 microgram range, and an antimony method based on the same principles is anticipated this year.

In view of these new developments, the possible avenues for further work that they open, and the limited time available, it is difficult for the Referee to make proper recommendations for future work. Perhaps a comparative estimation of possibilities will give a clue to the prospects for the greatest ultimate progress.

How appears to have extended the Gutzeit range materially, increased the accuracy to a certain extent, but made the method more complex. No shortening of the time required for a determination can be expected. Its usefulness would probably be greatest in the 0.1–10.0 microgram range, where the other two methods, unless successfully modified, are the weakest. The Klein-Vorhes[3] xanthate extraction process may become established as another method of arsenic isolation. Suggestions have been made that it might be especially useful in the analysis of organic arsenic compounds. The Referee believes that the colorimetric molybdenum blue arsenic determination, after any system of isolation, should be further developed, preferably by photometric methods, to the lower ranges of arsenic, possibly 1–50 micrograms. If it can be applied on the same solution after an iodine titration, it would become particularly useful as a check determination. This would require a change in the phosphate buffer used in the titration method, but such a change, provided it did not introduce an interference, should not be difficult. The iodine titration method as at present developed is remarkable for its speed and accuracy for 5–500 micrograms of arsenic, and if some modification will take care of quantities less than 5 micrograms, this method

[5] *This Journal*, 22, 436 (1939).

should be generally applicable. In view of these considerations, the Referee believes it best to determine first whether 0.1–10.0 microgram quantities of arsenic can be analyzed by cerulean-molybdate or iodine methods, and to keep How's modified Gutzeit in reserve until this question is settled. In the meantime, collaborative work on larger quantities of arsenic by the iodine titration method may be started this year. Collaborative studies as reported by Klein and Vorhes on 100–800 microgram quantities by the molybdate method need not be repeated. It is necessary first to ascertain whether the molybdate method will work for small quantities under collaborative conditions.

Since the collaborative work designed to finish the sample preparation phase of the arsenic methods was not conclusive, it must be repeated. The newer methods of final determination should facilitate this work.

The Referee hopes that an iodine titration method for antimony, in the absence of arsenic, will be ready before the next meeting. If this expectation is fulfilled, the next problem is the separation of micro quantities of arsenic and antimony. The Referee has in mind three or four possibilities, one or more of which may materialize during the year.

The action of arsine or stibine on gold or silver solutions was not studied during the year. The Referee believes that this problem is still worth some investigation, particularly for small quantities. The apparatus described by Cassil and Wichmann[5] should allow the development of these sols in small volumes, followed immediately by photometric measurement. The general and associate referees would welcome the assistance of interested analysts in this development work.

COPPER

The Referee's previous recommendations were to the effect that the colorimetric carbamate method for the determination of copper be placed on a photometric basis. The elements that may interfere in a copper determination are bismuth, cobalt, and nickel, which can produce carbamates of yellowish shades. The associate referee has obtained the absorption curves of the copper complex as well as those of the interfering metals. The striking feature of these curves is the fact that the absorption of the copper-carbamate complex at all wave lengths is so much greater than that of the others that small contaminations of bismuth, cobalt, or nickel are practically negligible. In biological samples these metals are probably seldom encountered in amounts in excess of 0.1 milligram and, therefore, do not cause serious errors. The Referee desires to point out, however, that there is at present considerable interest in the determination of copper in tomato products, where nickel interference may be more important because of contamination from nickel-containing equipment. It is unknown at present whether manufactured tomato products ever will contain sufficient nickel to cause an appreciable error in the

copper determination without previous separation of the two metals. The associate referee indicates that this can be done, if necessary, by precipitation of copper in acid solution with hydrogen sulfide. This is true, but former Associate Referee Coulson recommended with good reason, that precipitation methods be avoided in the determination of micro quantities of copper. The Referee, therefore, believes that an examination of the principle of competitive complexes with reference to cobalt and nickel might be profitable and help to avoid sulfide precipitations. The associate referee utilized this principle when he found that potassium cyanide inhibited the copper carbamate reaction but not that of bismuth, enabling him to correct for the small color effect of bismuth, and determined copper by difference.

The associate referee recommends iso-amyl acetate as the solvent for the copper-carbamate complex rather than amyl alcohol or the heavier carbon tetrachloride. He finds that the acetate solvent provides better agreement with Beer's law than either of the other two solvents. In addition he desires to avoid the use of separatory funnels.

The associate referee believes that the copper carbamate method with photometric measurement is now ready for use. The Referee is inclined to agree with this view, but natural caution inclines him to put the method to further collaborative test. He, therefore, recommends that the associate referee formulate a precise copper method based on the experience of former Associate Referee Coulson and his own, including simple directions for making color measurements with several types of instruments, and submit it with some samples to collaborators. In this connection the practice of Coulson in sending ashed samples containing known amounts of copper and interfering elements might well be followed. In such samples the interfering elements of special interest would be bismuth, cobalt, and especially nickel. If a satisfactory micro method for copper can be advanced to tentative adoption at the next meeting of this Association, it can be included in the next edition of *Official and Tentative Methods of Analysis, A.O.A.C.*

The associate referee recommends this photometric copper method for 20–50 micrograms. It could undoubtedly be extended to 100 micrograms by merely increasing the volume of the solvent, but how about the lower limit? The experience this Association has had with the determination of other metals indicates that sooner or later a demand will arise for methods that will satisfactorily determine smaller quantities. The next associate referee might, therefore, think about methods capable of determining 5 micrograms of copper or less.

ZINC

The associate referee and his associate tested 28 metals with dithizone and carbamate reagents in 0.02 *N* hydrochloric acid and 0.02 *N* am-

monium hydroxide buffered with ammonium citrate. The Referee would have preferred to work at definite hydrogen-ion concentration rather than at definite normalities, because *p*H is more in line with his conception of the principles governing the dithizone system of separations and analysis. Experience with lead determinations indicates that hydrogen-ion concentration has much to do with optimum extraction of a metal, and that losses may occur if metals are extracted from ammoniacal solutions, by dithizone, at too high a *p*H, or if dithizonates in carbon tetrachloride are washed with insufficiently buffered ammonia solutions.

The associate referees propose a method for the determination of zinc based on dithizone separations and carbamate inhibition of certain dithizone reactions and suggest its collaborative trial. Since the copper method the Association is investigating also depends on the formation of a carbamate complex the two associate referees are approaching a common ground. The Referee concurs in the recommendation for collaborative testing of the proposed zinc method. In addition he suggests that the Associate Referee on Zinc devote some time to a study of the photometric determination of zinc. Other referees are finding photometric determinations to be far superior to ordinary colorimetric methods. Fisher and Weyl[6] state that the maximum absorption for zinc dithizonate in carbon tetrachloride is at 538 mμ. The proper color filter or wave length for use in a dithizone zinc determination is therefore already known.

In addition the Referee hopes that the associate referee will be able to present at the next meeting quantitative data concerning the efficiency of the separations and the inhibitions utilized in the proposed method. The Association should know if small quantities of copper, cobalt, nickel, or other metals are ever transferred with lead and zinc back to the aqueous phase in the separation in the method and if so whether interfering metals are quantitatively inhibited by the mixed reagent. These reactions are equilibrium phenomena that are influenced by many factors, and the success of a separation depends upon choosing optimum conditions. The Referee is particularly interested in learning whether the colored carbamates of cobalt, nickel, bismuth, and especially of copper can ever offer an interference in the determination of zinc in instances where they have not previously been completely separated from zinc. The Associate Referee on Copper finds that the copper carbamate complex has a decided absorption at 540 mμ, in iso-amyl acetate, but that the absorption of cobalt, nickel, and bismuth carbamates is very much smaller. Since 538 mμ is the optimum wave length for the absorption of zinc dithizonate in carbon tetrachloride it can be readily seen that efficient separation of these possibly interfering metals from zinc, or the

[6] *Wiss. Veröffent. Siemens-Werken*, 14, No. 2, 41 (1935).

complete suppression of both their carbamate and dithizone complexes is necessary.

FLUORINE

The Associate Referee on Fluorine assumed new duties during the year, therefore his report is a summary of the present fluorine situation and of what remains to be done. The Referee has nothing to add and recommends that the Association's work on fluorine be continued along the lines indicated by the associate referee.

LEAD

The associate referee has revised paragraphs 30 and 33 of the rapid spray residue lead methods restricted to the determination of lead on apples and pears, adopted as official, first action, last year. The revision was necessitated because it was found last year that sporadic addition of lime sulfur to washing solutions caused abnormally low lead results if hydrochloric acid was used in the acidification of the apple strip solutions. The remedy is the deletion of the choice of hydrochloric or nitric acid and making the use of nitric acid mandatory. The other revisions concern the deletion of the direct electrolytic lead determination on strip solutions and the substitution of an intermediate dithizone extraction. This safety measure is intended to stop the occasional incomplete recovery of lead from solutions containing abnormal amounts of sugars. These changes do not affect the principles of the methods nor the results. Therefore, the collaborative results reported last year are still valid and the Referee sees no reason why this revision of the rapid spray residue methods should not be adopted. No reports of other difficulties with the spray residue methods on apples and pears have been received during the yea r and adoption of the methods (final action) may be recommended.

The associate referee also makes some observations on the determination of lead in maple products and baking powders where some difficulties have arisen with respect to sample preparation. It is hoped that information may be obtained before the next meeting on the reported loss of lead on ashing maple sirups. The Referee recommends that some collaborative work on methods for lead determination in these products be started this coming year. The determination of lead in oils or fatty foods has not been investigated. The simplification of methods for removing interferences (bismuth, tin, and thallium) in the lead methods needs attention before the preparation of a new edition of *Official and Tentative Methods of Analysis, A.O.A.C.* The Referee hopes that most of the defects in the lead methods may be remedied before the time for revision, but doubts whether there is time available for completing the project.

MERCURY

The associate referee continued his efforts to shorten the sample preparation and isolation procedures of his dithizone mercury method, now

tentative. Partial oxidation of organic matter followed by concentration of mercury by precipitation on a small filter bed of finely divided metallic zinc or coprecipitation with ferric hydroxide promises to shorten appreciably the amount of oxidation and therefore the time required for sample preparation.

The isolation of mercury from other elements, particularly copper, is making progress. The Referee believes that the possible interference of silver and bismuth should be given some attention. These metals are also neighbors of mercury in the dithizone system and may, under some circumstances, be co-extracted and cause trouble in a mercury determination. Silver and bismuth should not be entirely neglected in mercury determinations in foods or biological samples in general, merely because they are rarely found in such materials.

Since the associate referee determines mercury photometrically by measuring the absorption of residual dithizone rather than of mercury dithizonate, he must by all means avoid oxidation of dithizone in his final extraction. The results seem to show that he is succeeding in doing this by the use of hydroxylamine and hydrazine salts. It seems that the photometric mercury method promises greater accuracy than that possessed by the present tentative titrametric method. The associate referee's future program, especially that of collaborative work, is approved, and it is hoped that at the next meeting decided improvements in all parts of the mercury method may be evident.

SELENIUM

The collaborative results for selenium obtained this year by the volumetric thiosulfate method, together with those obtained in former years by the associate referee and his associates, warranted a recommendation for tentative adoption of this method. A slight, though perhaps significant, loss of selenium was noted if samples were hastily digested in an open system, even in the presence of mercury. It seems, therefore, that analysts should be particularly careful not to hurry the sample preparation in the beginning. If later associate referees consider a closed system advisable, it can be included when the status of the method is changed to official. The Referee also approves of the recommendation for tentative adoption of the thiosulfate method for micro quantities of selenium as applied to foods and biological products, in amounts from 5 micrograms upwards. He believes that future work on selenium should be directed towards the lower ranges (1–10 or 20 micrograms) and that this may be done by (1) refinement of the volumetric thiosulfate procedure, (2) utilization of the very sensitive iodine methods for indirect selenium determinations, and (3) colorimetric methods. The Referee suggests that more sensitive titration methods than are available at present might be developed if the iodine produced by the action of selenium dioxide on po-

tassium iodide in acid solution could be separated from selenium by suitable solvents or other means and oxidized to iodate, and the iodine then liberated with potassium iodide and acid and titrated with thiosulfate to a starch-iodine or electrometric end point. An alternative method for the indirect determination of selenium might be developed by photometric determination of the iodine liberated by selenium dioxide from iodides and then dissolved in a suitable solvent, provided these highly colored solutions follow Beer's law. Selenium dissolves in carbon bisulfide, but its solubility in chloroform or carbon tetrachloride is said to be very low or negligible, although it tends to collect at the interface. The color interference of colloidal selenium to iodine dissolved in these solvents should therefore be slight, although a filtration might be necessary.

As a possible basis for a direct colorimetric determination of selenium suitable for photometric determination of small amounts, the Referee suggests (1) the yellow color produced by selenium dissolved in carbon bisulfide; (2) the red color of colloidal selenium, if it can be properly dispersed by some colloid and the particle size maintained constant long enough for a measurement; and (3) the blue or blue-green color of the selenium-codeine complex in sulfuric acid. It is not expected that all of these color suggestions will be suitable, because of a probable lack of the desired accuracy or sensitivity. The criterion to be used in evaluating them should, in the Referee's opinion, be their value in determining small quantities of selenium of the order of 1–10 or possibly 20 micrograms.

FUMIGATION RESIDUES

This is the first year that a report on the determination of fumigation residues in foods has been given in this section. Since the study of the determination of spray residues was perhaps the principal reason for the existence of the section, the inclusion of the determination of fumigation residues might follow logically. The work of the first associate referee was limited to the study of the determination of cyanides.

It was generally thought heretofore that hydrocyanic acid absorbed by foods did not remain to any dangerous extent or for any great length of time, being either volatilized or changed into the stable cyanohydrin-aldehyde complex, which is believed to be of low toxicity. Last year, however, raisins containing dangerous amounts of cyanides as the result of fumigation with liquid hydrocyanic acid were distributed in interstate commerce. The methods used for the determination of the cyanide residues were neither very accurate nor satisfactory and the problem was given to a new associate referee. The associate referee's first report indicates that a change from distillation to aeration from heated solutions, with alkaline silver titration of the isolated hydrocyanic acid, will accom-

plish much towards sharpening the accuracy of the determination. Since samples containing hydrocyanic acid or cyanides are unstable, collaborative samples can not be distributed, but collaborators can determine their own recoveries and in this manner check the associate referee's work. The Referee hopes that a sufficient number of recovery experiments will be made to form the basis for a recommendation for tentative adoption next year. A method for cyanides accurate to .5 p.p.m. should be satisfactory for all ordinary purposes. If greater sensitivity is demanded, the associate referee has two colorimetric methods of considerable promise in reserve for development. Therefore the Referee recommends that the project be continued.

RECOMMENDATIONS OF THE REFEREE[1]

It is recommended—

(1) That studies be continued on methods of sample preparation of products containing organic arsenic or substances that inhibit the evolution of arsine.

(2) That the arsine evolution-iodine titration method for the determination of 5–500 micrograms of arsenic be studied collaboratively, and that a similar method for antimony and a colorimetric-iodine method for 1–20 micrograms of arsenic be investigated.

(3) That colorimetric methods for arsenic, after adequate systems of isolation, be studied photometrically. The molybdenum blue method for arsenic and gold or silver sol formation by arsine or stibine are especially recommended for attention.

(4) That separation of micro quantities of arsenic and antimony where they occur simultaneously be studied.

(5) That studies on micro methods for the determination of copper be continued.

(6) That the study of methods for the determination of micro amounts of fluorine in foods be continued and that special attention be given to sample preparation of organic materials.

(7) That the revision of paragraphs 30 and 33 of the rapid lead methods restricted to the determination of lead on apples and pears recommended by the associate referee be adopted and that the revised method be made official (final action).

(8) That studies concerned with the determination of lead in oils, baking powders, and maple sirup and with simplification of methods for separation of the interfering metals, bismuth, tin, and thallium, be continued.

(9) That studies on mercury methods be continued.

(10) That the thiosulfate selenium method described by the associate referee last year and subjected to collaboration this year be adopted as

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).

tentative for the determination of selenium in foods in amounts from 5 micrograms upward and that studies be continued on methods especially designed for 1–20 micrograms of selenium.

(11) That the study of micro methods for the determination of zinc be continued.

(12) That checks on the recovery of cyanides added to foods by the aeration-titration method be made and that development of the two colorimetric methods for the determination of cyanides described by the associate referee be continued.

REPORT ON ARSENIC

By C. C. Cassil (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*

The collaborative studies made during 1938 on sample preparation, as proposed by the Associate Referee in 1937, *This Journal*, 20, 171 (1937), were not satisfactory. Composite samples of shrimp and tobacco were sent to six collaborators. Each analyst was given instructions to prepare the samples by a wet sulfuric-nitric-perchloric acid digestion and also by a dry ashing procedure. It was further specified that the official Gutzeit method be used for the final determination.

The collaborators obtained results varying from 4 to 20 micrograms of arsenious oxide per gram of shrimp and from 10 to 51 micrograms of arsenious oxide per gram of tobacco, whereas the Associate Referee and two collaborators working on the same samples the previous year found 20–21.5 and 30–31.5 micrograms for the shrimp and tobacco, respectively. The Associate Referee believes that such erratic results are due to variations in the Gutzeit procedure and not to the sample preparation.

It is suggested that collaborative work on sample preparation for arsenic be dropped until there is available an arsenic method that has a higher degree of accuracy and precision than the present official method. Collaborative work on the new method mentioned below will be done during 1939, and if it proves satisfactory it is hoped that the sample preparation studies can be completed in 1940.

A method for determining from 5 to 500 micrograms of arsenious oxide that requires approximately only 10 minutes has been developed. An average recovery of 99.5 per cent with a standard deviation of 0.85 per cent was obtained from 34 determinations. This procedure is described in detail in a paper, entitled "A Rapid Volumetric Micro Method for Arsenic," by C. C. Cassil and H. J. Wichmann in *This Journal* (see p. 436). Some preliminary experiments show that this procedure may also be adapted to the determination of antimony, and that it may be possible to publish a paper during the coming year on antimony and the separation of antimony and arsenic.

RECOMMENDATIONS[1]

It is recommended—

(1) That the rapid volumetric method for quantities of arsenious oxide between 5 and 500 micrograms be studied by collaborators.

(2) That further work be done by the Associate Referee toward adapting this rapid isolation method to quantities of arsenic less than the equivalent of 5 micrograms of arsenious oxide.

(3) That some attention be given to the molybdenum blue method for determining quantities of arsenic less than the equivalent of 50 micrograms of arsenious oxide.

REPORT ON COPPER

By DAVID L. DRABKIN (Department of Physiological Chemistry, Medical School, University of Pennsylvania, Philadelphia, Pa.), *Associate Referee*

In this report an improved method for the determination of small amounts of copper by means of the sodium diethyl dithiocarbamate reagent is presented.

Improvement in technic and increased reliability have been accomplished by appropriate changes in (a) the ashing of the sample and treatment of the ash, (b) the use of a new solvent, iso-amyl acetate, for extraction of the colored copper carbamate complex, and (c) the use of several monochromatic light filters for more precise photometry of the colored solution. The interference of nickel, cobalt, and bismuth, when present in sufficient quantities to be significant, has also been studied.

The literature on the carbamate methods does not include precise data on the light transmission properties of the metallic complexes of diethyl dithiocarbamate, nor on suitable light filters. Such data, obtained both by visual spectrophotometry and by a photoelectric filter photometer, are presented. The choice of solvent and of light filters, and the decision as to the optimal concentrations of copper suitable for photometric measurement are based upon the spectrophotometric findings.

ASHING OF THE SAMPLE

Proper care must be exercised in the ashing of samples, both from the standpoint of possible adventitious contamination with copper as well as losses of the metal (in the form of volatile salts), when the ashing is at too high a temperature. The size of the sample should be such as to provide 20–50 micrograms of copper for analysis. A simultaneous determination should be run on a sample that has been enriched by at least 50 per cent by the addition of standard copper solution, providing a recovery check. Different materials require appropriate modifications in the ashing procedure. Several examples follow:

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).

Milk.—A 200 cc. sample is placed into a large evaporating dish (300 cc. capacity), of silica or platinum, preferably the latter. The sample is heated to a temperature of about 40° C., and while being gently stirred, dilute HCl is added dropwise until isoelectric precipitation of the casein is effected. This procedure is necessary to prevent frothing in the subsequent evaporation of the whey and curd. Evaporation to a caramelized dry mass is carried out upon an electric hot plate, covered with a layer of sand (to insure greater constancy of temperature). During this procedure the evaporating dish is kept covered with an inverted wide-stemmed glass powder funnel of suitable size (approximately 150 mm. diameter). The evaporating dish containing the relatively dry sample is now transferred uncovered to a muffle furnace preheated to scarcely perceptible dull red heat (500°–550° C.). The door of the furnace is kept open until incineration is over. The door is then closed and ashing accomplished.

Elixirs.—Sirupy material, containing a relatively large amount of carbohydrate and aromatic substances, is best ashed by a combination of wet and dry ashing. Partial wet ashing is carried out by successive additions to the sample of small amounts of concentrated nitric acid, with evaporation after each addition upon an electric hot plate covered with a layer of sand. The best commercial grades of C.P. nitric acid, sp. gr. 1.42, should be redistilled in glass at a temperature of 120° C., thereby rendering the acid practically copper free. After partial wet ashing, the sample is completely dry ashed in a muffle furnace at 500° C.

Animal tissues.—In metabolism experiments, if possible the tissues should be relatively free of blood. To obtain such tissue samples the animal may be slowly exanguinated, while the circulatory system is perfused with 0.9 per cent sodium chloride. Appropriately sized tissue samples are then withdrawn, washed quickly with copper-free distilled water, blotted upon filter paper, and quickly weighed in silica dishes of approximately 50 cc. capacity. The samples are then dried by heating in an oven at 110° C. for 24 hours, and re-weighed to furnish the dry weight. In the case of tissues relatively rich in copper, 1–5 grams of wet tissue suffices; in the case of other tissues as much as 20 grams is needed. One to 2 grams of liver, 5 grams of kidney, 20 grams of spleen, and 20 cc. of blood are examples of the size of sample usually taken for analysis. The dried tissue samples may be ashed by a combination of wet and dry ashing as described previously. A more rapid procedure is the following: The sample is partially wet ashed with nitric acid and heated to dryness upon an electric hot plate. The sample is cooled, and 5 cc. of an oxidation mixture, composed of 20 per cent magnesium nitrate saturated with magnesium carbonate is added. The material is ignited and ashed. The ash is taken up with dilute hydrochloric acid and filtered. This is a slight modification of the procedure recently recommended by van Niekerk

(1). Copper checks should be run upon the oxidation mixture, or the standard solutions should also be exposed to the oxidation procedure.

ISO-AMYL ACETATE AS SOLVENT FOR THE COPPER DIETHYL DITHIOCARBAMATE COMPLEX

Since metallic complexes with such reagents as ethyl xanthate, diphenylthiocarbazone, and diethyl dithiocarbamate are but sparingly soluble in water, various non-polar organic solvents have been suggested for their extraction. In the case of copper diethyl dithiocarbamate, McFarlane (2) has suggested the use of iso-amyl alcohol, while Haddock and Evers (3) used carbon tetrachloride. The latter solvent has the advantage of far lower solubility in water than the former. Carbon tetrachloride is, however, more volatile than iso-amyl alcohol, and, being heavier than water, extraction must be carried out in separatory funnels. To avoid contamination with stopcock grease, the separatory funnels must be used with ungreased stopcocks, which is relatively troublesome.

The writer has found iso-amyl acetate to possess decided advantages over both above solvents for the extraction of copper diethyl dithiocarbamate from weakly ammoniacal solutions (*p*H 8.5–9.0). In comparison with iso-amyl alcohol, iso-amyl acetate has a slightly higher boiling point (139° against 130.5° C.) and about one-twentieth the solubility in water at room temperature (0.16 per cent at 25° C. against 3.3 per cent at 22° C.). The latter property no doubt accounts for a far more efficient and rapid stratification of the aqueous (below) and organic solvent (above) layers after thorough mixing. The recovery of iso-amyl acetate is appreciably more quantitative than that of iso-amyl alcohol under the conditions used, which may be partly responsible for the superior photometric results (in respect to conformity with Beer's law) which have been obtained.

It is well recognized that commercial, C.P. grades of solvents which go under the name of "amyl acetate" are largely iso-amyl acetate. The C.P. grade of amyl acetate should be fractionated by distillation. The distillate obtained below 136° C. (sometimes as much as 60 per cent of the material) is discarded. The distillate obtained between 136° and 140° C. is used. This is the solvent referred to as iso-amyl acetate.

Before the data presented in the figures are described, a brief explanation of Beer's law (4) and the term extinction coefficient, ϵ, fundamental in photometry, seems appropriate. Beer's law may be formulated as follows: If by transmission through a layer of absorbing solution of depth 1 (as 1 cm.), light of intensity I is reduced to intensity $I \cdot 1/n$, then by passing through two such layers it will be reduced to intensity $I \cdot 1/n \cdot 1/n$ or $I \cdot 1/n^2$ and, by passing through d such layers, the resulting intensity will be $I \cdot 1/n^d$. Thus as the depth of absorbing stratum increases in arithmetic ratio, the intensity of the light passing through decreases

exponentially or logarithmically. A general equation, expressing Beer's law, may be written—

$$I' = I \cdot \frac{1}{n^d}, \tag{1}$$

where I equals the intensity of incident light, and I' equals the intensity of the transmitted light, after it has traversed an absorbing solution of some finite depth, d. This equation may be written in logarithmic form

$$\log n = -\frac{\log \frac{I'}{I}}{d}. \tag{2}$$

It is not necessary to develop the equation further here, except to say that when a spectrophotometer is employed the measurement which is obtained is that of fraction of light transmitted, T, which is equal to I'/I. Since the chemist is interested in the absorption of light and not primarily the transmission, the extinction coefficient, ϵ, is calculated from the transmission measurement. By Bunsen and Roscoe's definition (5), ϵ is equal to log n in equation 2, and the equation may be written—

$$\epsilon = -\frac{\log T}{d}. \tag{3}$$

Since the depth, d, is referred commonly to unity, as 1 cm., the equation becomes

$$\epsilon = -\log T. \tag{4}$$

In the Bausch and Lomb spectrophotometer, T is equal to $\tan^2\theta$, the angle at which solvent and solution match (6). Thus, Beer's law is implicit in the equation for the extinction coefficient, and ϵ is directly proportional to the concentration when Beer's law applies. It must be remembered, however, that Beer's law holds strictly true only for monochromatic light. It is this consideration that calls for the use of monochromatic light filters when photometric measurements are carried out with comparators of the Duboscq "colorimeter" type, or with the usual type of photoelectric comparator.

Figure 1 includes data obtained by means of precise, visual spectrophotometry in which a Bausch and Lomb spectrophotometer was used, and similar data obtained by means of a photoelectric filter photometer, the Evelyn apparatus (7). The amounts of copper exposed to extraction by 10 cc. of solvent are given in the abscissa. The values of ϵ appear in the ordinate. The subscript and superscript attached to ϵ denote that the value ϵ was in all cases calculated for the same concentration, $c = 0.1$ mg. per 10 cc. and for the same depth, 1 cm. The depth of cuvette employed in the spectrophotometer was exactly 1 cm., the optical ends

of the cuvette being polished, parallel glass end-plates. In the Evelyn apparatus matched test tubes are employed as cuvettes. The internal diameter of the test tubes was 1.98 cm. The ϵ values in this case were divided by 1.98 (see Equation 3) to obtain ϵ for a depth of 1 cm. This correction is, of course, a very rough one, since the effective optical depth of an imperfect cylinder, only part of which is exposed to the passage of light (as in the Evelyn apparatus) is very difficult to determine

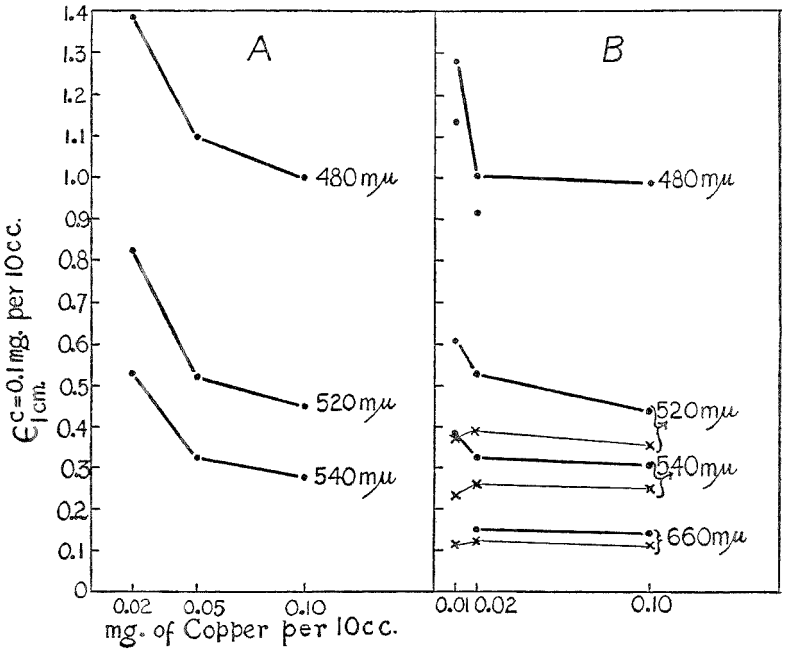

FIG. 1.—COMPARISON OF EXTINCTION COEFFICIENT VALUES, ϵ, YIELDED AT DIFFERENT WAVE-LENGTHS BY THE COMPLEX OF COPPER DIETHYL DITHIOCARBAMATE IN A, ISO-AMYL ALCOHOL, AND IN B, ISO-AMYL ACETATE.

Heavy lines show data obtained by precise, visual spectrophotometry (Bausch and Lomb spectrophotometer), while light lines give data obtained by means of photoelectric filter photometry (Evelyn apparatus).

The amount of copper contained in 10 cc. of the solvent in a particular determination is given in the abscissa. The ϵ values, however, have all been calculated for a concentration of 0.1 mg. per 10 cc. and for a cuvette depth of 1 cm. When Beer's law is obeyed strictly, this manner of plotting data should produce lines parallel with the horizontal ordinate.

precisely. For this as well as other reasons (use of filters with broad spectral intervals instead of true monochromacy attainable in the spectrophotometer), ϵ values obtained with filter photometers are not expected to duplicate the ϵ values obtained by spectrophotometry, which may be considered to be the ultimate method in determinations of this type. With solutions of the type under discussion, however, there is a good parallelism of results obtained by the two methods of measurement. When

results are plotted as in Figure 1, perfect agreement with Beer's law should produce straight lines parallel with the abscissa. It is evident that with iso-amyl alcohol satisfactory agreement with the law (proportionality of ϵ values and concentration) was not obtained, whereas fair agreement was found when iso-amyl acetate was used, particularly in concentrations between 0.02 mg. and 0.1 mg. per 10 cc. solvent used for extraction.

In Figure 2 are shown the absorption spectra of $CuSO_4 \cdot 4NH_3 \cdot H_2O$, Cu diethyl dithiocarbamate, Ni diethyl dithiocarbamate, Co diethyl dithiocarbamate, and Bi diethyl dithiocarbamate. The original copper salt used was prepared from Bureau of Standards Cu, as described by Coulson (8). C.P. nickel chloride and C.P. cobalt chloride were dissolved in water, while bismuth nitrate was dissolved in dilute nitric acid. The carbamate complexes of these various metals were obtained as in the procedure for copper described by Coulson (8). The carbamate metallic complexes were all extracted by means of iso-amyl acetate from the weakly ammoniacal solutions at *p*H 8.5–9.0. The curves are based upon measurements with the Bausch and Lomb spectrophotometer. The extinction coefficient values are calculated for a concentration of 1 gram molecular weight of metal per liter, at a depth of 1 cm. ϵ values, under such conditions, are often referred to as molecular extinction coefficients. The legend of the figure gives the concentrations employed in the actual measurements. The curve for cupric ammonium sulfate is based on the results of Drabkin and Austin (6) and is given for the interesting comparison it affords as to the relative amount of absorption produced by this copper complex, and that produced by copper diethyl dithiocarbamate. As the figure shows, the relative optical densities are such that the curves had to be drawn against different ordinates. Copper diethyl dithiocarbamate is a far more intense pigment, having approximately 30 times greater absorption in the red-yellow, 100 times greater absorption in the green, and 13,000 times greater absorption in the blue-violet spectral regions than cupric ammonium sulfate.

The two absorption curves for the copper diethyl dithiocarbamate complex were obtained with the respective concentrations of 0.1 mg. and 0.02 mg. copper per 10 cc. of solvent used for extraction. Since the ϵ values are calculated to a common concentration, the two curves should be practically identical within the limits of the spectrophotometric method, provided Beer's law is obeyed. It is evident that fair agreement was attained at all wave-lengths except in the blue-violet. In this region (470–420 mμ) of the spectrum agreement would have been accidental, since the sensitivity of the eye is very poor for light of these wave-lengths. It is to be noted that there is an absorption maximum at wave-length 440 mμ. Ordinarily, a region of maximum of absorption is an ideal place in the spectrum to choose for establishing absorption constants. It is

usually also the best region to isolate by means of monochromatic light filters when measurements are to be carried out by means of a filter photometer. In the present instance, however, the absorption maximum lies beyond the region of ordinary visual sensitivity and is not suitable for accurate visual measurements. Under such circumstances, the next

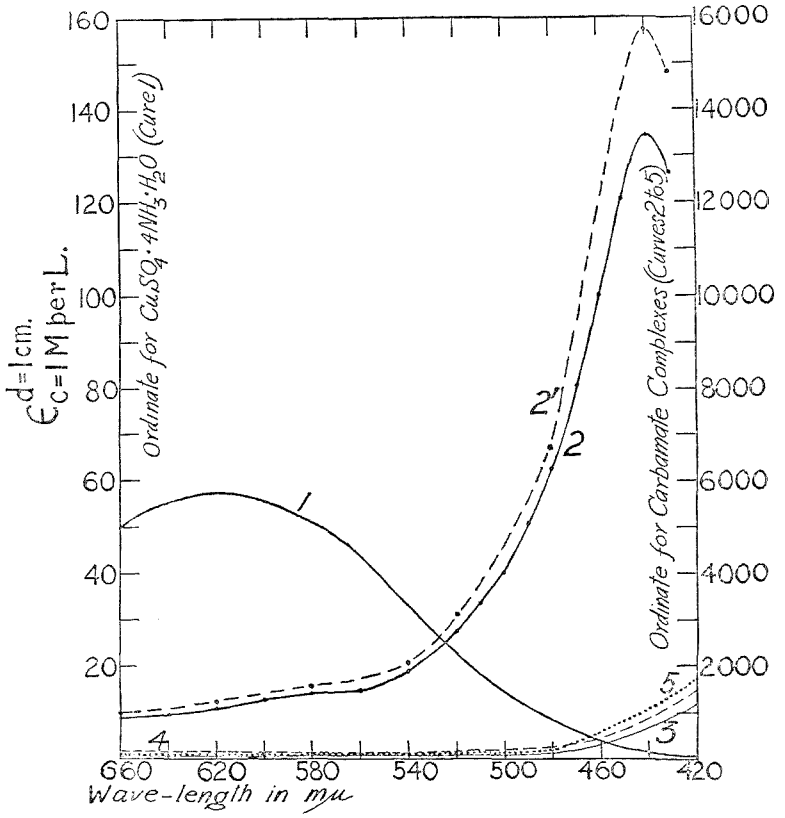

FIG. 2.—ABSORPTION SPECTRA OF METALLIC COMPLEXES OF COPPER, NICKEL, COBALT AND BISMUTH, IN ISO-AMYL ACETATE, OBTAINED BY PRECISE, VISUAL SPECTROPHOTOMETRY (BAUSCH AND LOMB SPECTROPHOTOMETER). THE MOLECULAR EXTINCTION COEFFICIENT (I.E. ϵ FOR A CONCENTRATION OF 1 GRAM MOLE OF METAL PER LITER AND FOR A CUVETTE DEPTH OF 1 CM.) IS PLOTTED AGAINST WAVE-LENGTH. THE ORDINATE ON THE LEFT GIVES VALUES WHICH APPLY ONLY TO CURVE 1, WHILE THE ORDINATE ON THE RIGHT IS APPLICABLE TO CURVES 2 TO 5.

Curve 1. The molecular extinction coefficient curve of $CuSO_4.4NH_3.H_2O$.
Curve 2. The molecular extinction coefficient curve of copper diethyl dithiocarbamate in iso-amyl acetate. The concentration used in the determination was 0.1 mg. of Cu extracted by 10 cc. of solvent.
Curve 2'. Same as curve 2, but determinations were upon a solution containing 0.02 mg. of Cu extracted by 10 cc. of solvent.
Curve 3. The molecular extinction coefficient curve of nickel diethyl dithiocarbamate in iso-amyl acetate. The concentration used in the determination was 0.1 mg. of Ni extracted by 10 cc. of solvent.
Curve 4. The molecular extinction coefficient curve of cobalt diethyl dithiocarbamate in iso-amyl acetate. The concentration used in the determination was 0.1 mg. of Co extracted by 10 cc. of solvent.
Curve 5. The molecular extinction coefficient curve of bismuth diethyl dithiocarbamate in iso-amyl acetate. The concentration used in the determination was 0.1 mg. of Bi extracted by 10 cc. of solvent.

best spectral region for monochromatic isolation would be closer to the region of maximum visual acuity and one in which the absorption of the pigment being measured was not changing with respect to wave-length too steeply. Such a region, for copper diethyl dithiocarbamate is, for example, at wave-length 540 mμ. The best practice, however, for filter photometry of pigments of this type is the use of several monochromats, permitting measurements in at least three broad spectral regions, for example, red, green and blue. Filters which may be employed for this purpose will be described below.

The absorption spectra of nickel, cobalt, and bismuth diethyl dithiocarbamates were determined because, under the present conditions of ashing, solution of the ash, and conversion into carbamates, these metals may possibly interfere with the accurate determination of copper. The color of the nickel and bismuth complexes may be described as approximately similar to that of the copper carbamate complex, though perhaps slightly yellower. The color of the cobalt complex is greenish in comparison with the others. The difference in tint is probably accounted for by a minimum in absorption in the case of cobalt diethyl dithiocarbamate in the green spectral region at wave-length 540 mμ. This is difficult to see in the plotted curve, due to the coordinates employed. Under the conditions used it is probable that only a fraction of the bismuth finds its way into the iso-amyl acetate, since extraction is at *p*H 8.5–9.0. The absorption of the copper complex as Figure 2 indicates, is approximately 20–30 times greater than that of the other metals. This means, for example, that a sample of 0.05 mg. copper, contaminated with 0.025 mg. of nickel, cobalt, or bismuth, would give an increase in absorption (in comparison with pure copper) amounting to 2.5 per cent. Since ϵ is proportional to the concentration, the determination would yield results 2.5 per cent too high, or 0.0513 mg. copper. In these circumstances any correction for contamination by nickel, cobalt, or bismuth may well be neglected. This has been confirmed by subjecting mixtures of copper and the other metals to analysis. When contamination of copper with appreciably more significant amounts of nickel, cobalt or bismuth is suspected, appropriate separation of the metals or corrections must be employed.

In Figure 3 are presented absorption curves of copper diethyl dithiocarbamate obtained by means of the Evelyn photoelectric filter photometer. The concentrations of copper exposed to extraction by 10 cc. of iso-amyl acetate varied from 0.01 mg. to 0.40 mg. It is to be noted that fair agreement with Beer's law was obtained in the red and green spectral regions (wave-length 660 mμ–520 mμ), but not in the blue-violet region. In the Evelyn apparatus, the photoelectric current is measured by means of a galvanometer, with a scale giving transmission, T, directly in units from 0 to 100 per cent. The instrument employs a so-called barrier-layer photocell, which generates a current as light strikes the sensitive surface,

without need for an external e.m.f. Instruments of this type work best under conditions of relatively high luminous flux, and therefore call for the use of relatively dilute solutions, if a battery operated light source

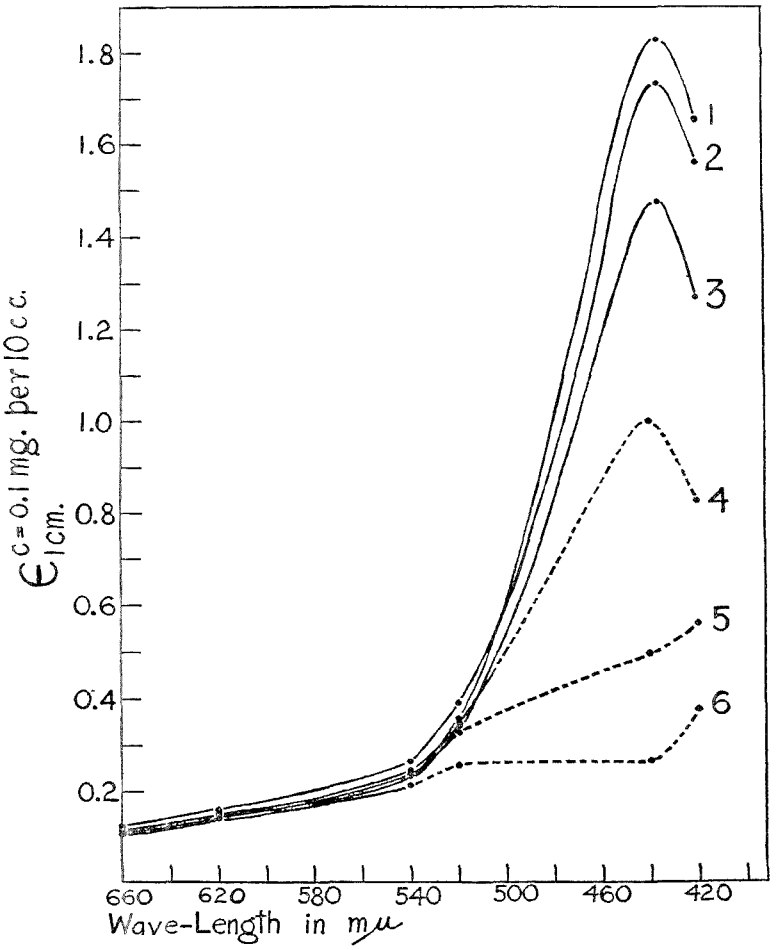

FIG. 3.—ABSORPTION CURVES YIELDED BY THE COMPLEX OF COPPER DIETHYL DITHIOCARBAMATE IN ISO-AMYL ACETATE.

The determinations of transmission were made by means of an Evelyn photoelectric filter photometer in six relatively broad spectral regions, isolated by means of monochromatic filters. The maximum transmissions of the respective monochromats were at wave-lengths 660 mμ, 620 mμ, 545 mμ, 520 mμ, 440 mμ, and 420 mμ. The solid lines were drawn as smooth curves joining dots representing the individual determinations. The broken lines are used to denote uncertainty, due to reading at very low transmission (see text). The concentration of copper in 10 cc. of solvent was

Curve 1, 0.01 mg.
Curve 2, 0.02 mg.
Curve 3, 0.04 mg.
Curve 4, 0.10 mg.
Curve 5, 0.20 mg.
Curve 6, 0.40 mg.

All results, however, have been plotted in terms of ϵ for a concentration of 0.1 mg. of copper per 10 cc. and for a cuvette depth of 1 cm.

of relatively low intensity (6v. flashlight Mazda) is employed. With concentrations of 0.1 mg.–0.4 mg. of copper complex in 10 cc. of solvent, and a test tube cuvette with internal diameter of 1.98 cm., the transmission was too low in the blue spectral region to be determined accurately in an apparatus of this type. The data obtained under these conditions are shown by broken lines. This is an instrumental error, and is not to be taken as indicating necessarily serious deviation from Beer's law in the blue spectral region with higher concentrations of copper diethyl dithiocarbamate. It is possible, however, that such deviations may exist. With concentrations of copper of the order of 0.02–0.05 mg. per 10 cc., excellent reproducibility of determinations was obtained by means of the Evelyn instrument, as shown in Table 1. These results

TABLE 1.—*ϵ values obtained by means of a photoelectric filter photometer upon copper diethyl dithiocarbamate solutions*

	SOLUTIONS				
Wave-length	A	B	C	D	Average
$m\mu$	ϵ^*	ϵ^*	ϵ^*	ϵ^*	ϵ^*
660	0.122	0.125	0.110	0.120	0.119 ± 0.005
545	0.263	0.267	0.260	0.265	0.264 ± 0.002
520	0.394	0.399	0.405	0.395	0.398 ± 0.004
420	1.543	1.560	1.650	1.545	1.575 ± 0.038

* ϵ calculated for concentration of 0.1 mg. copper per 10 cc. of iso-amyl acetate used for extraction. Original amount of copper in each sample was 0.02 mg.

may be compared by assuming solution A to be the standard, containing 0.02 mg. copper per 10 cc. The concentration of the other solutions may then be obtained by a ratio of the ϵ values of standard and unknown at each wave-length. For example, at wave-length 660 mμ, 0.02 mg. $\times 0.125 / 0.122 = 0.0205$ mg., the concentration of solution B. This calculation is carried out at each of the 4 wave-lengths, and the values obtained are averaged. When this is done, in comparison with standard $A = 0.02$ mg. copper, solution $B = 0.0202$ mg., solution $C = 0.0199$ mg., and solution $D = 0.0200$ mg. This is a far greater order of accuracy than has been attained heretofore (8) by non-photometric determinations of copper diethyl dithiocarbamate. Of course, this order of accuracy need not be expected on samples of material which have to pass through an ashing procedure and which may be contaminated with interfering impurities.

Figure 4 presents the transmission curves of various light filters or combinations of filters, which may be used to attain relative monochromacy. The data were obtained with the Bausch and Lomb spectrophotometer. The use of such filters by the writer in the copper diethyl dithiocarbamate method was mentioned in preliminary reports (8 and 9). For visual photometry an ideal monochromat should transmit light effi-

ciently in only one spectral region as narrow as possible. The transmission curve should indicate a sharp, relatively symmetrical falling off in transmission (i.e. increase in light absorption) on the two sides of maximal transmission. Many simple light filters, for example Wratten filter No. 62 (mercury green) and Wratten filter No. 75η (blue green), have good monochromacy in the green and blue spectral regions, respectively, but also transmit light very efficiently in the deep red (Curve 2′, Figure 4).

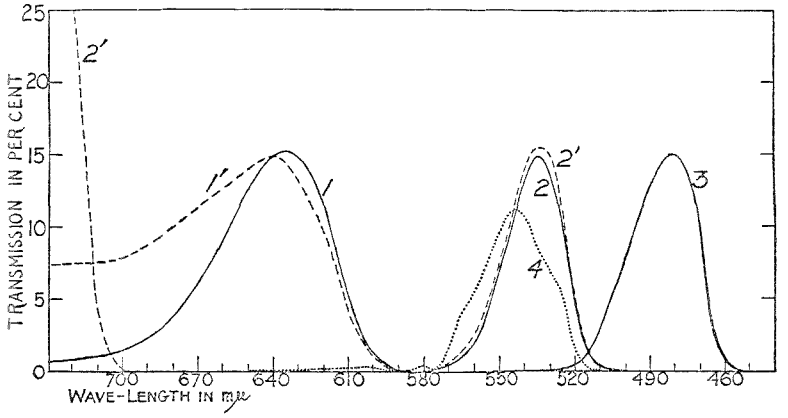

FIG. 4.—THE TRANSMISSION CURVES OF SEVERAL MONOCHROMATIC FILTERS, OBTAINED BY PRECISE, VISUAL SPECTROPHOTOMETRY (BAUSCH AND LOMB SPECTROPHOTOMETER).

Curve 1. The transmission curve of Wratten filter No. 29 F (red for additive synthesis) plus a 1 cm. layer of 0.2805 *M* $CuSO_4$ (7.0 grams of crystalline $CuSO_4 \cdot 5H_2O$ made up to 100 cc.).
Curve 1′. The transmission curve of Wratten filter No. 29 F plus Corning glass filter No. 430 (dark blue green), 0.95 mm. in thickness.
Curve 2. The transmission curve of Wratten filter No. 62 (mercury green) plus a 1 cm. layer of 0.2805 *M* $CuSO_4$.
Curve 2′. The transmission curve of Wratten filter No. 62, same as above, but without the liquid $CuSO_4$ filter. The high transmission in the deep red, which the $CuSO_4$ eliminates, is evident
Curve 3. The transmission curve of Wratten filter No. 75η (blue green) plus a 1 cm. layer of 0.2805 *M* $CuSO_4$.
Curve 4. The transmission curve of a composite Corning glass monochromat of three elements, No. 430 (dark shade blue green), 5.5 mm. thick, No. 351, 2 mm. thick, and No. 512 (didymium glass), 2 mm. thick. This is an example of one of the composite monochromats used in the Evelyn photoelectric photometer.

While such filters may be used unmodified, true monochromacy will not be attained unless means are provided to cut out the red and infra-red transmission. This is particularly desirable if the monochromats are to be employed in photoelectric photometers, since most photocells are very sensitive to the infra-red. The removal of red and infra-red transmission may be accomplished by combining such filters with a liquid filter—a 1 cm. layer of 0.2805 *M* copper sulfate (7.0 grams of crystalline copper sulfate made up to 100 cc., including a few drops of H_2SO_4 to insure greater stability of the solution). Curve 2, Figure 4, shows the transmission of Wratten filter No. 62, plus a 1 cm. layer of 0.2805 *M* copper sulfate. A colored glass that exactly duplicates the light transmission

properties of copper sulfate solution is not available. The Corning glass filter No. 430 (dark blue green) very roughly approximates the transmission properties desired. A thickness of 0.95 mm. of the glass may be substituted for a 1 cm. layer of 0.2805 *M* copper sulfate (Curves 1 and 1′, Figure 4). While this is not ideal, it is simpler practice than the use of liquid filters in appropriate special containers.

Three monochromats, each with approximately 15 per cent relative transmission at their respective minima of absorption, are recommended for use in the photometry of copper diethyl dithiocarbamate. They are:

1. Wratten filter No. 29F (red for additive synthesis), plus Corning glass filter No. 430, 0.95 mm. in thickness. The maximum transmission of this combination is in the red region at wave-length 640 mμ.
2. Wratten filter No. 62 plus the above Corning glass filter. The maximum transmission of the combination is in the green region at wave-length 535 mμ.
3. Wratten filter No. 75η plus the above Corning glass filter. The maximum transmission is in the blue region at wave-length 480 mμ.

By the use of such filters a comparator of the Duboscq type is converted into a filter photometer, and a photoelectric comparator into an abridged spectrophotometer (10). This set of three inexpensive monochromats, which requires only 4 filter elements, will be found useful in a great many other determinations besides that of copper diethyl dithiocarbamate. The Wratten filters should be ordered mounted in B glass. A suitable size, which may be fitted into the eye-piece of a Duboscq instrument, is a 3/4 inch circle. To facilitate determinations upon a solution at each of the three wave-lengths, it may be found convenient to place the Corning glass filter within the eye-piece of the comparator and to mount the three Wratten filters in a simple holder, above the eye-piece, sliding each filter into place as needed. The particular filter combinations that have been discussed are superior in monochromacy and considerably less expensive than corresponding filter combinations of colored glass elements, such as those used in the Evelyn apparatus (Curve 4, Figure 4).

SEPARATION OF COPPER FROM NICKEL AND COBALT AND CORRECTION FOR BISMUTH CONTAMINATION

The most common contaminant of copper in biological materials is iron. Iron forms colored complexes with diethyl dithiocarbamate. Even large quantities of iron (greater than 10 mg.), however, do not interfere with the determination of as little as 0.02 mg. of copper when the copper carbamate complex is extracted in the presence of ammonium citrate at *p*H 8.5–9.0 (3) and (8). Another effective method for preventing color development due to iron is the use of 4 per cent sodium pyrophosphate, which forms insoluble ferric pyrophosphate, while the soluble copper pyrophosphate is left free to react with diethyl dithiocarbamate. This method for preventing interference by iron in copper determinations,

introduced by Drabkin and Waggoner (11), has been found effective by various workers (1), (2), and (12). The use of pyrophosphate is effective at a *p*H greater than 7.5 (12), and may possess advantages over the citrate method if it is desirable to work at a *p*H below 9.0 (as in the presence of excessive phosphates in the material). The advantage of either method is, of course, the avoidance of the necessity for separating copper from iron by means of hydrogen sulfide treatment.

In most biological materials of animal origin, the contamination of copper by nickel, cobalt, or bismuth will be of an order of magnitude that will not produce serious error in the determination of copper as copper diethyl dithiocarbamate, since the development of color due to the other metals will be very small. When considerable contamination by nickel, cobalt, or bismuth is present, neither citrate nor pyrophosphate prevents the formation of the respective carbamate complexes. The only effective means for separating copper from nickel or cobalt appears to be a hydrogen sulfide treatment in acid solution. Under these conditions only the copper forms an insoluble sulfide. An effective method for the formation of cuprous sulfide and its separation is that described by Coulson (13) as Method 1, involving filtration on alundum filtering crucibles and solution in hot nitric acid.

A very effective method has been found by the writer for correcting for contamination of copper by bismuth. One determination is carried out by the usual technic. This yields a value for total pigment as Cu+Bi diethyl dithiocarbamate. In a second determination, after adjustment of the *p*H but before the addition of diethyl dithiocarbamate, 5 cc. of a 0.08 *M* solution of potassium cyanide is added (approximately 33 mg. per 5 cc.). The color development obtained in this case is due solely to the bismuth present. The difference between the two analyses represents the color due to copper. It is interesting that nickel and cobalt, besides copper, form very firm complexes with cyanide, probably respectively represented by $Ni(CN)_4^-$, $Co(CN)_6^-$, and CuCN, under the conditions employed. Bismuth forms no compound with cyanide.

The Associate Referee believes that with the incorporation of the various improvements in the analytical procedure for copper diethyl dithiocarbamate that have been presented the method may be ready for consideration for acceptance as an official method.

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REPORT ON ZINC*

By E. B. HOLLAND and W. S. RITCHIE (Massachusetts Agricultural Experimental Station, Amherst, Massachusetts), *Associate Referee*

Work on the colorimetric method for the determination of zinc in foodstuffs was continued along essentially the same lines as previously reported. The process has been modified in numerous details and expanded to permit the determination of both copper and lead when desired.

REAGENTS

The purification and preparation of the reagents required more attention than would be expected. The presence of reacting metals in the distilled water, hydrochloric acid, and ammonium hydroxide necessitated careful distillation. Some lots of citric acid or of ammonium citrate required repeated extractions with dithizone and carbon tetrachloride to eliminate the lead, zinc, and copper. After standing in glass containers these same solutions would again react, probably due to the assimilation of zinc or lead from the glass. Therefore frequent checks are obligatory in the analytical work, although there seemed to be some tendency towards stability with continued use.

Care should be exercised in the daily preparation of the dithizone reagent to insure a uniform product and to protect it from unnecessary exposure, especially to light. Most of the carbon tetrachloride may be recovered by treatment with anhydrous sodium sulfate and distillation.

REACTING METALS

To determine the relative response of various metals to dithizone and carbamate reagents chlorides, mostly of high grade, were secured, and solutions were prepared from amounts calculated to contain 1 mg. of the metal to 500 cc. of solution (0.000002 gram per cc. or 2 p.p.m.). Ten cc. (0.02 mg.) was used for each test in 0.02 *N* hydrochloric acid and ammonium hydroxide solutions. Five cc. of 0.20 *N* in 50 cc. gave the desired concentration.

As the reagents were known to react after standing the various portions were shaken out with dithizone and carbon tetrachloride to a green color,

* Contribution No. 338, Massachusetts Agricultural Experiment Station.

and the solvent was discarded immediately before the addition of the metal so that there could be no question as to the source of any colored complex produced. The insolubility of some of the chlorides, such as bismuth, mercurous, palladous, silver, and thallous, may retard or even prevent a reaction that has been reported by others working under a different *p*H.

The scheme followed and the results obtained are summarized in the following tables.

TABLE 1.—*Method of treatment*

ACID SOLUTION	DITHIZONE 0.01%	CARBAMATE 0.25%	DITHIZONE AND CARBAMATE
	cc.	*cc.*	*cc.*
Water	34	30	29
Metal	10	10	10
Hydrochloric acid, 0.2 *N*	5	5	5
Dithizone and carbamate	<1	5	<1 & 5
Carbon tetrachloride	10	10	10
AMMONIACAL SOLUTION			
Water	24	20	19
Metal	10	10	10
Ammonium hydroxide, 0.2 *N*	5	5	5
Ammonium citrate, 10%	10	10	10
Dithizone and carbamate	<1	5	<1 & 5
Carbon tetrachloride	10	10	10

The results (Table 2) were derived largely from single samples of the various salts, but they appear to be consistent. The colored complexes are yellow or pink. All of the reacting metals so far determined belong to one of three small groups of closely related members in the periodic table as follows:

A	B	C
27 Cobalt	46 Palladium, ous	79 Gold
28 Nickel	47 Silver	80 Mercury, ic
29 Copper, ic & ous	48 Cadmium	81 Thallium, ous
30 Zinc		82 Lead
		83 Bismuth

Under the conditions enumerated only copper and mercury (ic) reacted in an acid solution with dithizone reagent. With palladium (ous) and silver the reaction was slight and may be disregarded.

In an ammoniacal solution cobalt, copper (ic and ous), zinc, silver, cadmium, mercury (ic), lead, and bismuth reacted with dithizone although bismuth was very slow. Gold and thallium (ous) may be disregarded.

TABLE 2.—*Color reaction of the metals*

METALS	DITHIZONE		CARBAMATE		DITHIZONE & CARBAMATE	
	0.02 *N* HCl	0.02 *N* NH_4OH	0.02 *N* HCl	0.02 *N* NH_4OH	0.02 *N* HCl	0.02 *N* NH_4OH
Aluminum	green	green	none	none	green	green
Antimony, ous	green	green	none	none	green	green
Beryllium	green	green	none	none	green	green
Bismuth	green	green[1]	none	none	green	green
Cadmium	green	pink	none	none	green	green
Cerium, ous	green	green	none	none	green	green
Chromium	green	green	none	none	green	green
Cobalt	green	pink	light yellow	light yellow	green	green
Copper, ic	pink	yellow	bright yellow	bright yellow	green	green
Copper, ous	pink	yellow	bright yellow	bright yellow	green	green
Gold	green	purplish[5]	none	none	green	green
Iron, ic	green	green	none	none	green	green
Lead	green	pink	none	none	green	green
Manganese	green	green	none	none	green	green
Mercury, ic	yellow[2]	yellow	none	none	green	green
Mercury, ous	green	green	none	none	green	green
Nickel	green	green	light yellow	light yellow	green	green
Palladium, ous	green[3]	green	none	none	green	green
Platinum, ic	green	green	none	none	green	green
Silver	green[4]	yellow	none	none	green	green
Thallium, ous	green	green	none	none	green	green
Thorium	green	green	none	none	green	green
Tin, ic	green	green	none	none	green	green
Tin, ous	green	green	none	none	green	green
Uranium	green	green	none	none	green	green
Vanadium	green	green	none	none	green	green
Zinc	green	pink	none	none	green	pink
Zirconium	green	green	none	none	green	green

[1] Bismuth reacted yellow on long standing.
[2] Mercury tended to fade.
[3] Palladium, no positive reaction.
[4] Silver, a slight yellow reaction, destroyed by additional reagent.
[5] Gold, no positive reaction.

Cobalt, nickel, and copper (ic and ous), reacted with carbamate reagent in both acid and ammoniacal solutions. Copper manifested a strong affinity for carbamate and produced a deeper color than did cobalt and nickel. Carbamate with dithizone inhibited the reaction of all these metals except zinc.

PROCESS OF SEPARATION

Eight metals (excluding bismuth) are shown to readily form colored complexes with dithizone or carbamate reagents in 0.02 *N* acid or ammo-

niacal solutions. These complexes are used for the separation and for the colorimetric determination of specific metals such as lead and zinc.

As the amount of non-reacting bases and acids in the ash solution of most samples materially exceeds that of the reacting metals a preliminary extraction with dithizone and carbon tetrachloride from a 0.02 *N* ammonium hydroxide solution, buffered with ammonium citrate, is deemed advisable to prevent possible interference. The ammoniacal aqueous layer is of no value and may be discarded.

The solvent layer containing the reacting metals is drawn off and extracted with 0.02 *N* hydrochloric acid for the removal of lead and zinc (also cobalt, silver, and cadmium) in solution in the acid aqueous layer as chlorides, leaving the copper (also mercury) in the original solvent. The copper in the solvent layer can be determined if desired.

To the acid aqueous layer is added sufficient ammonium hydroxide to produce a 0.02 *N* solution. The solution is buffered with ammonium citrate, dithizone is added, and it is then extracted with carbon tetrachloride in the presence of carbamate reagent,* which inhibits the lead and leaves the zinc complex in the solvent layer for determination against a standard solution in a Duboscq colorimeter or other instrument.

If the carbamate reagent is omitted and potassium cyanide is added, zinc can be inhibited in the ammoniacal solution and the lead complex determined in the solvent layer in a colorimeter against a standard solution.

With this preamble the various steps of the detailed method should be easily comprehended. The method follows:

ZINC METHOD IN DETAIL

(All water must be redistilled from glass.)

REAGENTS

(a) *Standard zinc solution.*—Dry reagent Zn (30-mesh or finer), transfer 2 grams to a volumetric flask, and add about 200 cc. of water and gradually a slight excess of HCl. Boil until solution is complete and make to 2000 cc. at 25° C. 1 cc. contains 0.001 gram of Zn.

(b) *Dilute zinc solution.*—To 4 cc. of the standard zinc solution add sufficient water at 25° C. to make 2000 cc. 1 cc. contains 0.000002 gram of Zn.

(c) *Hydrochloric acid.*—0.20 *N*. Distil the HCl into cold "metal-free" water by allowing the concentrated acid to drip from a separatory funnel into hot H_2SO_4 below the surface and dilute to the required strength.

(d) *Ammonium hydroxide.*—0.20 *N*. Distil the NH_4OH to about 70° C. into cold "metal-free" water and dilute to the required strength.

(e) *Sodium diethyl dithiocarbamate.*—2.5 grams per 1000 cc.

(f) *Diphenylthiocarbazone (dithizone).*†—Dissolve 0.015 gram of the dithizone in 10 cc. of the NH_4OH (d), crushing the aggregates to facilitate solution, and transfer to a 250 cc. pear-shaped separatory funnel with 90 cc. of water. Shake out with 10 cc. portions of CCl_4 to a green color, discard the solvent layers, and filter the aqueous portion through washed ashless paper. Prepare a fresh solution daily.

* So far as noted R. H. Caughey was the first to observe the action of carbamate in this connection.
† One cc. of reagent is generally sufficient for 0.01 mg. of reacting metals.

(g) *Ammonium citrate solution.*—225 grams per 2000 cc.* Dissolve the NH_4 citrate in water, add distilled NH_4OH until sharply alkaline to litmus, and make to volume. Transfer 250 cc. to a 750 cc. pear-shaped separatory funnel, add an excess of dithizone (usually 3, 2, and 1 cc., respectively), and shake out with three 25 cc. portions of CCl_4 to a green color. Discard the solvent layers and filter the aqueous portions through washed ashless paper.

(h) *Potassium cyanide solution.*—10% (50 grams per 500 cc.).

(i) *Carbon tetrachloride.*

NOTE: The reagents and the ash solutions are vitiated by standing in contact with glass for any length of time, probably due to the absorption of zinc or possibly lead. This necessitates frequent preparation of reagents or their purification and the prompt handling of analytical solutions. Purified or synthetic ceresin, paraffin, and other wax mixtures have been suggested as protective coatings for the inside of the reagent bottles, and if found to adhere at laboratory temperature may prevent contamination of the solutions.

PROCEDURE

Transfer 4 grams of finely ground (1 mm.) air-dry material to a flat-bottomed platinum dish and calcine to a white or gray ash in an electric muffle at a temperature below visible redness. Pulverize with an agate pestle and reheat if necessary to destroy carbon particles. Transfer the powdered ash to a 100 cc. volumetric flask with small portions of water and a policeman if needed. Add distilled HCl dropwise until the mixture is faintly acid to litmus and then 20 cc. of the 0.20 *N* HCl in excess. Heat on a steam bath to insure complete solution of the metals, cool, make to volume, and filter through dry ashless paper.

Pipet 10 cc.† (0.40 gram) of the ash solution into a 250 cc. glass-stoppered separatory funnel (pear-shaped with a short delivery tube), add 20 cc. of water, 7 cc.‡ of the 0.20 *N* NH_4OH solution, 10 cc. of the NH_4 citrate solution, and sufficient dithizone reagent to impart a yellow color to the solution. Add 10 cc. of CCl_4 and shake out vigorously for at least 2 minutes to extract copper, lead, and zinc (also cobalt, cadmium, and mercury when present). Allow the mixture to separate and draw off the solvent layer into a second separatory funnel. Discard the ammoniacal aqueous portion, which should be slightly yellow and contain the non-reacting bases and acids.

To the carbon tetrachloride layer add 45 cc. of water and 5 cc. of the 0.20 *N* HCl solution and shake out to isolate the copper, removing the lead and zinc as chlorides in the acid aqueous solution, which should be colorless. Draw off the solvent layer and use for the determination of copper if desired, although a 25 cc. aliquot§ of the original ash solution is preferable for copper and lead.

To the acid aqueous layer, add 19 cc. of water, 15 cc. of the 0.20 *N* NH_4OH solution, 10 cc. of the NH_4 citrate solution, 5 cc. of the carbamate reagent, and sufficient dithizone in small portions to impart a yellow tint to the solution. Add 10 cc. of CCl_4 and shake out to extract the colored zinc salt. Allow to separate, rinse the delivery tube with a few drops of the solvent layer, and draw off the remainder through a dry ashless filter (to remove traces of moisture) into a weighing bottle and stopper to prevent evaporation.

Compare the color in a Duboscq colorimeter, using micro cups and a green color filter against 5 cc. of the dilute zinc solution treated in exactly the same manner.

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* Or 210 grams of citric acid substantially free from lead.

† The size of the aliquot may be varied by the analyst. Some prefer to match a light color from a small aliquot and others a darker shade from larger amounts.

‡ 2 cc. to neutralize and 5 cc. to produce a 0.20 *N* solution.

§ The quantity of lead in foodstuffs is relatively small in most instances; copper ranges from 4 to 70 p.p.m. in dry matter, while zinc averages higher and ranges from 10 to over 80 p.p.m. (unpublished data).

$$\% = \frac{sR}{R_1} \times 250 \text{ for 0.40 gram (F);}$$

$$= \frac{0.0025R}{R_1}$$

$$\text{p.p.m.} = \frac{25R}{R_1}, \text{ in which}$$

s = grams of the standard used.
R = scale reading at which the standard was set.
R_1 = scale reading of the unknown.
F = factor for converting the aliquot to percentage or p.p.m.

Since a small quantity of zinc (about 2 p.p.m.) will usually be found in the blank after careful purification of the reagents, $\frac{(s+B)R}{R_1}$ should be substituted in the calculation and the blank deducted.

Lead may be determined in a similar manner by adding 10 cc. of potassium cyanide instead of carbamate in the third extraction to inhibit the zinc.

The method presented has given promising results and warrants collaborative work on the part of the Association.[1]

REPORT ON FLUORINE IN FOODS

By DAN DAHLE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The titration of fluorine with thorium nitrate was studied. Such factors as (1) the amount of indicator used, (2) variations in *p*H, and (3) the presence of neutral salts like sodium chloride and sodium perchlorate and their effects on the accuracy of the titration were given consideration. The details of this work were published in *This Journal*, 21, 459 and 468. In general, it seems that the thorium nitrate titration can be depended on for great accuracy in the determination of small quantities of fluorine when applied to distillates free from weak acids, sulfates, and phosphates, and containing only moderate amounts of chlorides and perchlorates.

There are indications that during the distillation the glassware contributes minute quantities of fluorine. This in turn seems to be compensated by a slight deficiency in the recovery of the distilled fluorine. For practical purposes these errors do not appreciably influence the accuracy of the determination of fluorine in foods, but they may require study and consideration where very minute quantities of fluorine are concerned.

Titrations of distillates from samples distilled without previous destruction of organic matters were only partly successful, which indicates that a complete absence of organic acids in the distillate is desirable for accurate results on fluorine. Ashing of all food materials prior to the

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).

distillation, therefore, seems to be a necessity if the determination is to be made by titration with thorium nitrate.

The problem that remains to be solved is that of separating small quantities of fluorine from large amounts of organic matter without loss or gain of fluorine. So far, no entirely satisfactory ashing procedure is available. The fluorine fixatives suggested by different experimenters have turned out to be unsatisfactory in one way or another. The main troubles seem to have been: (1) a fluorine content in the fixative itself; (2) poor solubility of the fixative, resulting in lack of proper contact between sample and fixative; and (3) various difficulties in getting proper ashing or in the subsequent distillation of the fluorine.

RECOMMENDATIONS[1]

It is recommended—

(1) That the work on fluorine in foods be continued.

(2) That the procedure for titrating small quantities of fluorine be studied collaboratively.

(3) That the question of preparation prior to the distillation be given further study.

REPORT ON LEAD

By P. A. CLIFFORD (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

SPRAY RESIDUE

For the reasons outlined in the Associate Referee's report of last year, *This Journal*, **21**, 218 (1938), it is recommended that nitric acid, exclusively, be used for acidification of the wash solution in the rapid solvent method for the determination of spray residue lead on apples and pears. Hydrochloric acid may be retained in the rinse, if desired, to provide for a rapid Gutzeit arsenic determination upon a further aliquot of the same alkaline wash solution, after acidification with hydrochloric acid. It was likewise found desirable to eliminate the *direct* electrolytic determination of lead upon the acidified wash filtrate. Therefore it is recommended that pars. **30** and **33**, *Methods of Analysis, A.O.A.C.*, 1935, 391, 393, be amended. The changes were published in *This Journal*, **22**, 85 (1939). They do not affect the collaborative work reported last year.

MAPLE SIRUP

The analysis of maple sirup for lead has developed into a major project for regulatory chemists within the past two or three years. Accordingly, a rapid colorimetric dithizone method presented by Perlman[2] is of in-

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).
[2] *Ind. Eng. Chem. Anal. Ed.*, 10, 134 (1938).

terest. This author appears to have surmounted the interferences of zinc and tin in this colorimetric procedure, and his own figures and the results obtained by the Associate Referee on a number of samples indicate that the method yields results comparable in accuracy to those obtained by the ash-electrolytic or mush-electrolytic methods. A rapid method, similar in scope to the colorimetric dithizone method for lead on apples and pears, seems highly desirable for maple sirups, and it is the Associate Referee's plan to study Perlman's procedure collaboratively, perhaps to revise it slightly so as to employ a larger sample and less awkward aliquoting, and to contrast results with those obtained by the ash-electrolytic and mush-electrolytic methods.

BAKING POWDERS

A procedure for the determination of lead in baking powder was proposed last year by the Associate Referee, *This Journal*, **21**, 437 (1938). Further work, however, showed that certain combination powders yielded a highly refractory ash, which came into solution only after prolonged digestion with hydrochloric acid. As this rendered the analysis of these powders very troublesome, it was thought best to postpone collaborative study until these difficulties were adjusted. It was found later that treatment of the baking powder ash with hot 30 per cent sodium hydroxide solution, followed by an excess of hydrochloric acid, is very effective in securing a clear ash solution. If any appreciable amount of insoluble material remains after this treatment, the writer's practice has been to transfer the ash solution and residue from the casserole to a centrifuge bottle, clarify, pour off the clear solution, and rinse the residue (chiefly silicates and sulfates) into a platinum dish. The dry residue is then treated with hydrofluoric acid plus a few cc. of sulfuric acid, and usually no trace of insoluble material remains. The dish is then rinsed into the main body of the solution and the analysis continued as usual. This procedure will be studied collaboratively next year.

It might be well at this point to stress the necessity of bringing *all* ash material into solution by suitable means whenever a dry ashing procedure is used in the determination of lead. Appreciable amounts are apt to be occluded in refractory silicate or phosphate residues.[1] The same precaution applies to a calcium sulfate residue after a wet digestion, and here, even small amounts of barium can cause large losses of lead due to the isomorphous nature of lead and barium sulfate. Hydrochloric acid is usually the best solvent for a dry ash, but in some cases perchloric acid has been found very effective.

PERMANENCY OF DITHIZONE SOLUTIONS

The results of a study of the permanency of dithizone solutions were published by the writer in *This Journal*, **21**, 695 (1938). Carbon tetra-

[1] Fairhall, *Am. J. Pub. Health*, **28**, 825 (1938).

chloride solutions of dithizone can be preserved almost indefinitely if the solvent is first purified and the solution stored in the cold under dilute sulfur dioxide water. Stabilization of chloroform solutions of dithizone has not been as successful. Recently, W. O. Winkler[1] advises that dilute sulfuric acid has a stabilizing effect upon chloroform solutions of dithizone. To test this, two portions of the same dithizone solution were stored under ordinary laboratory conditions in well-stoppered white glass flasks with a layer of sulfuric acid (1+100) overlaying one portion. Decomposition rates were followed photometrically over a period of 55 days. At the end of that time, the untreated portion had fallen to 83 per cent, and the acid-treated portion had fallen to 87 per cent, of the original strength. Thus an appreciable stabilizing effect was noted. In a subsequent experiment, however, this stabilizing effect could not be reproduced, and while an untreated solution of dithizone in chloroform dropped to only 91 per cent strength over a period of 33 days, a portion of the same solution overlaid with dilute sulfuric acid fell to 73 per cent of the original strength. In fact, dilute acetic acid seemed to stabilize better than the dilute sulfuric acid in this case as photometric readings on a portion of the same solution overlaid with acetic acid (1+100) indicated a strength of 89 per cent at the end of 33 days.

Thus, the value of dilute acids as stabilizers for chloroform solutions of dithizone is problematical, and until better stabilizers are found, the Associate Referee believes stress should be placed on the absolute purity of the solvent and the dithizone with which dithizone solutions are prepared.

DETECTION OF INTERFERENCES IN THE "MIXED-COLOR" DITHIZONE METHOD FOR LEAD

The remarks of the Associate Referee on the photometric detection of the interferences of bismuth, tin, and thallium in the lead method and on the co-determination of lead and bismuth have been expanded into a contributed paper, which will appear in an early issue of *This Journal.*

It is recommended[2] that paragraphs 30 and 33 be revised as suggested and that collaborative work on lead be continued.

REPORT ON MERCURY

By W. O. WINKLER (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The work on mercury this year has dealt with concentration and isolation procedures and with the photometric method of determination. In accordance with last year's report, *This Journal,* 21, 220 (1938), work

[1] Private communication.
[2] For report of Subcommittee C and action by the Association, see *This Journal,* 22, 62 (1939).

was also continued on methods of concentration to avoid oxidation of the sample.

CONCENTRATION PROCEDURES

(*A*) *Zinc.*—The method of concentration by precipitation with powdered zinc, reported last year, was not further investigated to any extent this year. This is largely due to the fact that the Associate Referee was unable to secure powdered zinc of the desired purity. A few experiments with zinc of 40–80 mesh showed that too large a quantity of this material is necessary to remove all the mercury from solution.

If the accuracy desired does not exceed 5–10 micrograms (.005–.01 mg.) powdered zinc may be used. It is probable that the quantity of zinc necessary for a determination can be reduced by a system of filtrations of the mercury solution through the powdered zinc metal. The Associate Referee shall endeavor to do this and also to secure a metal more nearly mercury-free.

(*B*) *Ferric Hydroxide.*—The Associate Referee last year expressed the intention of using ferric hydroxide as a gatherer or concentrating agent for mercury, and the work this year has shown the value of this reagent for this purpose, which is due to its great powers of adsorption. By a very simple procedure it is possible to completely remove mercury from its solution. The procedure follows:

REMOVAL OF MERCURY FROM SOLUTION

Add to the acid solution of mercury (such as the HNO_3 extract of a food sample) sufficient strong NaOH (35–50%) to neutralize most of the acid. Transfer the solution to one or more centrifuge bottles with the aid of a wash bottle. Add to each bottle (150–180 cc. volume) 2 cc. of a nearly saturated solution of Fe $NH_4(SO_4)_2$ and continue the addition of the NaOH slowly with stirring until the solution is basic to litmus. Add 1 cc. of NaOH in excess and continue stirring the liquid for about 1 minute. Place the bottles in a centrifuge and whirl 8–10 minutes at high speed. After decanting the supernatant liquid, immediately add 15 cc. of HNO_3 (1+5) and allow the bottle to stand with occasional rotary shaking until the $Fe(OH)_3$ is dissolved. Place the covered bottle on the steam bath for 5 minutes. Remove, and add sufficient $KMnO_4$ solution (saturated) to oxidize any organic matter and bring any reduced mercury to the mercuric state. Replace the bottle (covered) on the steam bath and again heat several minutes. (If a considerable quantity of organic matter has been precipitated with the $Fe(OH)_3$ the HNO_3 mixture should be placed in the reflux apparatus used in preparation of the original sample extract and boiled with sufficient $KMnO_4$ to maintain a purple color.)

Remove the bottle from the bath, dilute with 25–30 cc. of water, and reduce the $KMnO_4$ etc., with a 10% solution of oxalic acid. Partially neutralize the excess acid with 3 cc. of NH_4OH (1+1) and dilute to about 200 cc. with water. Extract the mercury from the solution with dithizone as described in a previous report, *This Journal*, 18, 641 (1935). Use carbon tetrachloride as the solvent for the extractant if the extracts are to be treated according to procedure No. 2.

TREATMENT OF DITHIZONE EXTRACTS

Treat the extracts by one of the following procedures:

(1) Oxidize the extracts directly in the $CHCl_3$ or CCl_4 solution by the modification of the original methods, *This Journal*, **19**, 236 (1936), given below.

(2) Transfer the mercury to an aqueous solution by shaking the dithizone extracts with an acid aqueous solution containing thiosulfate and oxidize the thiosulfate by Method 2. See also *Ibid.*, **21**, 220 (1938).

If copper is present, remove by the acid iodide treatment given in a previous report, *Ibid.*, **18**, 642 (1935), reextract the mercury from the solution after making ammoniacal and follow by procedure No. 2.

(1) DIRECT OXIDATION OF EXTRACTS

Direct oxidation of the extracts is accomplished by shaking them with an acid solution of permanganate that has been warmed to 50°–55° C. The use of nitric acid in the oxidation solution has been substituted for sulfuric acid because of the greater solvent power of nitric acid for mercury. A volume of 1.5 cc. of nitric acid (1+1) is used. Some results by this modified treatment are given in Table 1. The determinations were made photometrically.

TABLE 1.—*Recovery of mercury by direct oxidation of extracts*

MERCURY ADDED	MERCURY FOUND	DIFFERENCE
mmg.	*mmg.*	*mmg.*
10.0	10.0	0
15.0	14.9	0.1
1.0	1.6	0.6
2.0	2.6	0.6
15.0	16.1	1.1
10.0	11.1	1.1

The seemingly high results are thought to be due to the attack of the chloroform solvent by the oxidizing mixture, and further experiment with other procedures indicates that the reducing agent used was not entirely satisfactory. The use of hydroxylamine hydrochloride as the reducing agent, reported last year, has not proved quite so effective as was anticipated. Reduction with hydroxylamine sulfate followed by addition of hydrazine sulfate has been found to maintain the stability of the dithizone very effectively. The fact that the excess mercury recovered is not uniform does not permit the conclusion that it was contributed by the reagents, but rather shows that the dithizone reagent was affected. The results indicate that the procedure is promising.

(2) TRANSFER TO AQUEOUS SOLUTION WITH THIOSULFATE AND OXIDATION OF THE AQUEOUS SOLUTION

The transfer of the mercury from the dithizone extracts to the acid thiosulfate solution appears to be complete. In the procedure given last year, the oxidation following the transfer was accomplished by adding potassium permanganate in the cold. As occasional losses have been encountered by this method of oxidation, the oxidation is now done in the presence of nitric acid and sulfuric acid, accompanied by heating on the steam bath for 10 minutes. Heating the solution to the temperature

of the steam bath makes necessary removal by filtration of any dithizone solvent that may cling to the aqueous solution. The procedure is as follows:

Add to the combined dithizone extracts in a 150 cc. separatory funnel, 45 cc. of water, 2 cc. of H_2SO_4 (1+50) and 3–4 cc. of 2% $Na_2S_2O_3 \cdot 5H_2O$ in the order given. Shake the funnel vigorously for 30–45 seconds. Allow the layers to separate and immediately draw off the lower CCl_4 layer. Filter the aqueous layer through a small wet pledget of cotton in a short-stemmed funnel and collect the filtrate in a 150 cc. beaker. Wash the separatory funnel and filter with 2 or 3 small portions of water from a wash bottle. Add to the aqueous solution 4 cc. of a 1 to 1 mixture of H_2SO_4 and HNO_3 and then add 5 cc. of saturated $KMnO_4$ solution with stirring. Place the covered beaker on the steam bath for 10 minutes, stirring occasionally. Remove, and cool the solution to room temperature, then neutralize most of the acid with 13 cc. of NH_4OH (1+1). Reduce the $KMnO_4$ by adding dropwise a 10% $NH_2OH \cdot H_2SO_4$ solution with stirring until a clear solution is obtained. When the solution is clear, add 2 cc. of a saturated solution of $(NH_2)_2 \cdot H_2SO_4$. Make the solution to the proper volume and make the determination.

Results obtained on four samples with this procedure are given in Table 2.

TABLE 2.—*Recovery of mercury by transfer to dilute acid solution with thiosulfate and subsequent oxidation of the thiosulfate*

MERCURY ADDED	MERCURY FOUND	DIFFERENCE
mmg.	*mmg.*	*mmg.*
10.0	10.9	+0.9
0	0.9	+0.9
12.0	12.7	+0.7
14.0	14.6	+0.6

The results (Table 2) are consistently high, which fact indicates that some mercury was contributed by one or more of the reagents. The fact that the readings were constant lends support to the conclusion that the excess mercury found was not due to any instability of the reagent. If the amount of mercury contributed was equal to the average of increases found, then the results obtained are within 0.1–0.2 microgram of the mercury actually present. Further work should demonstrate the possibilities of this procedure.

OXIDATION AND REDUCTION

Before the above method of reducing the oxidant with hydroxylamine sulfate followed by hydrazine sulfate was tried, reduction was made with 30 per cent hydrogen peroxide or hydroxylamine hydrochloride. Provided the oxygen liberated in the reduction is entirely removed the hydrogen peroxide produces a solution with which the dithizone "stays put" very well in the determinative extraction. This is usually accomplished by heating on the hot plate or the steam bath. The main precautions to

avoid loss of mercury in this case are to insure the absence of chlorides, and to heat under a reflux in the presence of excess sulfuric acid. Although the mercuric sulfate is reputedly not volatile in the presence of sulfuric acid occasional losses of mercury have occurred on boiling the solution obtained above in a covered beaker.

Several samples were submitted to one collaborator and the Associate Referee, and the mercury was determined by precipitation with ferric hydroxide followed by the solution in nitric acid, extraction with dithizone, and transfer to the aqueous phase with acid thiosulfate. The sodium thiosulfate was oxidized as previously indicated and excess permanganate reduced with hydrogen peroxide followed by boiling to remove free oxygen and neutralization of most of the acid with ammonium hydroxide (1+1). Results are given in Table 3.

TABLE 3.—*Recovery of mercury by acid thiosulfate transfer followed by oxidation and reduction with hydrogen peroxide*

Hg ADDED	Hg FOUND	DIFFERENCE
mmg.	*mmg.*	*mmg.*
10.0	10.3	+0.3
12.0	12.8	+0.8
14.0	14.0	+0.0
12.0	11.6	−0.4
10.0*	10.2	0.2
12.0*	12.0	0.0
10.0*	9.8	−0.2

* Determinations made by the Associate Referee.

The results (Table 3) obtained on quantities of 10–14 micrograms are within 3–4 per cent of the correct amount in all cases but one. The Associate Referee believes this to be a good average, and the accuracy is sufficient for most purposes where minute quantities of mercury are to be determined.

MANIPULATION OF THE PHOTOMETRIC DETERMINATION

The results shown in Tables 1–3 were obtained by the photometric method, *This Journal*, **21**, 226 (1938), with chloroform solutions of dithizone of 5 and 10 mg. per liter. The Associate Referee prefers a solution of 5 mg. per liter, of which 10 cc. is taken for quantities between 0 and 17.0 micrograms. The appreciable solubility of chloroform in aqueous solutions makes it necessary that the determinative extraction be made from a definite volume and the standards made by extraction from the same volume. The volume of 120 cc. was chosen as convenient for the "thio" transfer potassium permanganate oxidation procedure. The practice was to mark the separatory funnel from which the extraction was to be made at this volume with a glass marking pencil and make the solution

to the mark. Readings were made with a one-inch cell for the solution containing 5 mg. per liter.

Solutions containing 10 mg. per liter were read in a half-inch cell, only 5 cc. of solution being used in most cases. The practice in this case was to saturate the solution to be determined with chloroform before making the determinative extraction, remove the excess chloroform by filtering through a pledget of cotton, and wash with dilute acid solution saturated with chloroform. This last procedure is essential in making the determination following direct oxidation of dithizone in the chloroform solution, in which case the sample solution is already saturated with chloroform.

It has been observed that the mercury dithizone solution does not come to equilibrium in the photometer immediately, and in most cases the reading will be observed to rise. The reading recorded should be at the maximum when equilibrium has been obtained. If the reading starts dropping again while in the photometer, the maximum reading should be used.

RECOMMENDATIONS[1]

It is recommended—

(1) That collaborative work be done on the ferric hydroxide precipitation method of concentration, and that the acid sodium thiosulfate method of isolation given in this report be followed.

(2) That further work be done on the zinc precipitation method.

(3) That work on the photometric method for mercury be continued.

REPORT ON SELENIUM

By R. A. OSBORN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The simplified procedure for the determination of selenium as published in *This Journal*, **21**, 228 (1938) was studied collaboratively. The collaborators were given three samples of dry vegetation that had previously been thoroughly ground and mixed, and a standard selenium solution containing 20 micrograms of selenium per milliliter for use as the ultimate standard. The selenium content of each sample was not large. The analysts were directed to digest duplicate 10 gram portions and to titrate with approximately 0.001 *N* iodine and thiosulfate solutions. Table 1 summarizes the results of the collaborators.

COLLABORATORS

(A) V. H. Morris and Wesley H. Stoneburner, Ohio Agr. Expt. Sta., Wooster, Ohio.

(B) Percy O'Meara, State of Michigan Department of Agriculture, Lansing, Mich., with cooperation of L. H. Greathouse, Michigan State College Experiment Station, Lansing.

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).

(C) K. T. Williams, U. S. Bureau of Chemistry and Soils, Washington, D. C.
(D) H. W. Lakin, U. S. Bureau of Chemistry and Soils, Washington, D. C.
(E) L. H. McRoberts and A. K. Klein, U. S. Food and Drug Administration, San Francisco, Calif.
(F) A. L. Curl, U. S. Food and Drug Administration, Washington, D. C.
(G) N. J. Menard, U. S. Food and Drug Administration, Washington, D. C.
(H) R. A. Osborn, U. S. Food and Drug Administration, Washington, D. C.

TABLE 1.—*Collaborative results*

COLLABORATOR	SAMPLE 1		SAMPLE 2		SAMPLE 3	
	p.p.m.	*Av.*	*p.p.m.*	*Av.*	*p.p.m.*	*Av.*
A	0.36*		12.3*		4.5*	
	0.97		11.2		4.4	
	0.76	0.70	11.7	11.7	4.5	4.45
B	0.14		8.9		3.6	
	0.3		12.4		2.5	
	0.5	0.3	11.4	10.9	2.8	3.0
C			9.6		3.3	
			9.5		3.0	
			9.8	9.6	2.7	3.0
D			5.3		2.7	
			9.6		2.6	
			7.3	7.1	2.4	2.6
E	0.4		9.1		4.5	
	0.4		12.1		3.8	
	0.2	0.3	10.3	10.5	4.9	4.4
F	0.2		12.8		4.0	
	0.2	0.2	13.0	12.9	3.9	3.95
G	0.16		12.7		3.4	
	0.18	0.17	13.0	12.8	3.3	3.35
H	0.20		13.2		3.9	
	0.21		12.7		3.2	
	0.22	0.21	13.3	13.1	4.2	3.8
Grand average		0.35		11.1		3.57

* Regular Robinson still used for the distillation instead of the special still equipped with a thermometer, *Ind. Eng. Chem. Anal. Ed.*, 6, 274–276 (1934).

DISCUSSION

In general the collaborative results are in excellent agreement. Lack of experience in the titrations with 0.001 *N* solutions may account for variations as large as 1 microgram. It is the experience of the Associate Referee that artificial light is not so desirable as natural daylight for viewing the end point of the titration. It is necessary also to complete the titrations rapidly and to use as nearly as possible the same conditions and amounts of reagents in the titration of the unknowns as are used in

the standardization of the iodine and thiosulfate with the standard selenium solution. Collaborators C and D did not use mercury in the digestion. As it is also the Associate Referee's experience that the mercury functions to reduce volatility of selenium, their lower results may be attributable to its omission.

Additional studies were made of the method of digestion in order to determine whether a closed or trapped system of digestion is better than the open digestion. Eight additional samples of dry vegetation were analyzed. Duplicate 10 gram portions of the samples were digested by (1) complete digestion with the modified Soxhlet apparatus, *This Journal*, 20, 201 (1937), and a very low flame for approximately 3 hours, and the trapped liquid was redistilled; (2) a very slow digestion (about 2 hours) in a Kjeldahl flask until a dark brown color was obtained, *This Journal*, 21, 234 (1938); (3) as in (2) except a rapid digestion which was discontinued when the mixture became a dark brown color (20–50 minutes). In all instances mercury was used in the digestion and after distillation and precipitation the selenium was measured volumetrically, starch being used as indicator. Samples 5–9 inclusive were titrated with 0.01 *N* solution, and with Samples 10–12 inclusive 0.001 *N* solutions were used.

TABLE 2.—*Further study of digestion procedures*

SAMPLE	DESCRIPTION	PARTS PER MILLION OF SELENIUM		
		PROCEDURE (1)	PROCEDURE (2)	PROCEDURE (3)
5	Broccoli No. 231	285 284	283 282	282 280
6	Alfalfa No. 234	93 93	94 92	92 91
7	Vegetation No. 264	105 104	103 103	101 97
8	Vegetation B 14447	1208 1205	1211 1208	1199 1198
9	Vegetation No. 122	115 (5. g)	119 (5. g)	115 (5. g)
10	Gluten Flour	1.5 1.3 (5. g)	1.6 1.4 (5. g)	—
11	Flour—Plot 6 (1935)	1.4 1.4	1.3 1.3	—
12	Wheat Plot 2 (1935)	1.5 1.5	1.5 1.4	—

The results in Table 2 were obtained by A.L. Curl.

There is excellent agreement between the results with the modified Soxhlet and slow digestion in a Kjeldahl flask. It appears that small

losses of selenium may occur even when mercury is present if the digestion is carried out too rapidly. Incidentally, Sample 5 was also digested by attaching a vertical condenser to the reaction flask and refluxing gently for several hours, the condenser then removed and the digestion continued until SO_3 fumes appeared. The selenium found for 5 gram duplicate portions was 284 and 283 p.p.m. Further study of this procedure may be warranted since it eliminates the trap and appears to give good results

Those carrying out routine selenium determinations may wish to recover the used hydrobromic acid. It is practical to discard all material distilling below 120° C. and to collect and redistil the 120–124° C. fraction. The fraction distilling from 124 to 128° C. need not be redistilled. The Associate Referee uses the recovered acid for the distillation of selenium but the reagent grade acid for the titration, especially in the lower ranges.

Standard selenium solutions are usually stable. Some precipitation of red selenium around the necks of bottles containing standard solutions have been observed, but no material change was apparent in the concentration of the solutions even when they were one or two years old.

It is recommended[1] that the procedure presented by the Associate Referee for the determination of selenium in foods, which has been studied collaboratively, be adopted by the Association as tentative. It is further recommended that studies on the determination of selenium be continued with the view to further simplification or greater precision.

REPORT ON FUMIGATION RESIDUES IN FOODS

By W. O. WINKLER (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The Associate Refereeship on Fumigation Residues in Foods was created during the year following the epidemic of cyanided fruits, mainly raisins. As a result it was the interpretation of the Associate Referee that although considerable work might well be directed to the detection of residues from a number of other fumigation materials, for the present all efforts should be directed toward the detection of residual hydrocyanic acid in food products.

In cooperation with the Referee on Metals in Foods an attempt was made to increase the sensitivity and accuracy of the present A.O.A.C. methods, *Methods of Analysis, A.O.A.C.*, 1935, 348, as well as to introduce new methods for qualitative and quantitative determinations. The work has progressed satisfactorily.

In order to increase the sensitivity of the present methods for the determination of hydrocyanic acid in stock feeds, it seemed to be necessary to reduce materially the volume of liquid in which the determination

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 63 (1939).

is made. The isolation of the hydrocyanic acid by direct or steam distillation gave a volume of liquid too large to permit of real sensitivity, being only about 15–20 p.p.m. It was also frequently found in the case of fruits such as raisins that the distillate possessed an opalescence that further obscured the end point. The Associate Referee prefers the basic titration method because it is simpler and more specific for hydrocyanic acid than the acid precipitation titration method.

Aeration appeared to be the most logical method of isolating and concentrating the hydrocyanic acid, and since the Associate Referee had previously used the Labatti[1] aeration method, a modification of this procedure was tried. The following very satisfactory procedure, combining the isolation by aeration and determination by the basic titration method, resulted. The apparatus set-up is shown in Figure 1.

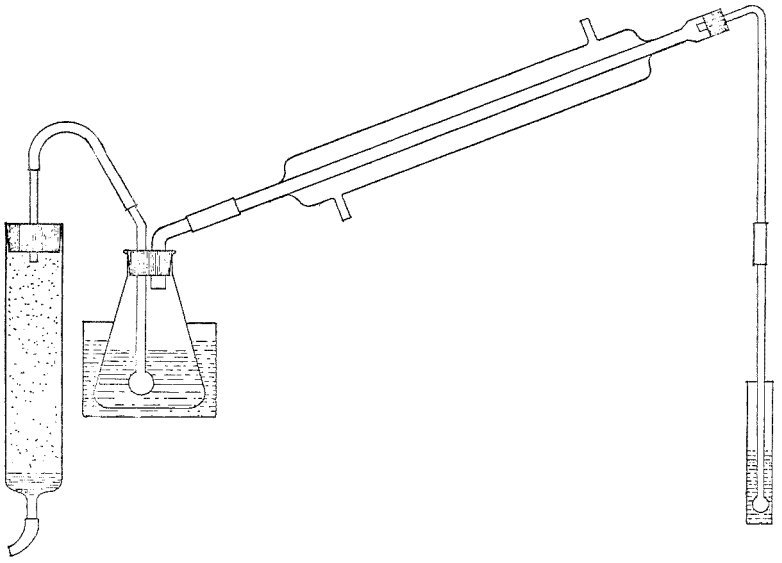

FIG. 1.—APPARATUS FOR DETERMINATION OF HCN BY AERATION METHOD.

DETERMINATION OF HYDROCYANIC ACID IN FOODS

Fit the aeration flask (300 cc. Erlenmeyer) with a two-holed No. 6 rubber stopper. Through one of the perforations pass a spray tube having a bulb at the lower end containing a number of small holes, and extend it nearly to the bottom of the flask. In the other perforation place a 9 mm. (internal diameter) glass tube, terminating just below the stopper and extending about an inch above it. Connect this latter tube, by means of a piece of 9 mm. rubber tubing, to a Liebig condenser inclined at about 45°. Connect the spray tube to the soda lime tower and this in turn to the compressed air outlet by means of rubber tubing. Fit the upper end of the condenser with a one-holed rubber stopper carrying a glass tube bent to extend vertically downward. Connect the latter to a spray tube, which is immersed nearly

[1] *J. Soc. Chem. Ind.*, 54, 275 T (1935).

to the bottom of the absorbing liquid contained in a 15–20 mm. by 6 inch glass receiving tube. (The receiving tube should have a flat bottom if the greatest sensitivity is desired. For ordinary determination a $6 \times \frac{5}{8}$ inch (15 mm.) test tube may be used.)

Place 11 cc. of water and 2.0 cc. of 10% NaOH in the tube to absorb the HCN before placing the sample in the aeration flask. (For very small amounts use only 1 cc. of 10% NaOH and 8 cc. of water in a 15 mm. flat-bottomed tube.) Before weighing out the sample adjust the compressed air outlet so that there is a flow of 800–1000 cc. per minute, tested by collecting the air over water in a liter flask and using a stop-watch.

Thoroughly grind the sample in a mortar or food chopper and weigh 25 grams directly into the flask, or on a small filter paper in the case of dried fruits. Add 180 cc. of H_2SO_4 (1+50), stopper the flash with a solid rubber stopper, and shake a few minutes to partially break up the sample. Rinse off the stopper with a little water and connect the flask with the apparatus by means of the stopper carrying the spray and distillation tubes, connect to the condenser, and raise the flask into place. Place a small bath so that the lower part of the aeration flask is immersed to the depth of 1–1.5 inches in a 35% glycerol solution. Immediately connect the air flow, heat the bath rapidly to about 104° C., and then lower the flame so that the temperature remains at 105°–110° C. After the temperature of the bath reaches 100° C., continue the flow of air for 15 minutes. then remove the flame and disconnect the air from the distillation flask. Disconnect the spray tube but allow it to remain in the absorption solution to be used as a stirrer. Add 2 cc. of NH_4OH (1+2) and 1 cc. of 5% KI and titrate with 0.02 *N* $AgNO_3$ to the appearance of a turbidity, using a black background. 1 cc. of the 0.02 *N* $AgNO_3$ is equal to 1.08 mg. of HCN.

RECOVERIES BY THE METHOD

To test the recovery of the procedure, known quantities of potassium cyanide were added to food samples. Although this practice may not be entirely in accord with food samples containing hydrocyanic acid obtained on the market, there appeared to be no other practical way of adding a definitely known quantity of the hydrocyanic acid.

The quantities of potassium cyanide or hydrocyanic acid taken and the recoveries on samples of raisins are given in Table 1.

The results given in the table indicate that the method gives excellent recoveries when the directions are followed closely. Previous experiments

TABLE 1.—*Recovery of HCN from raisins*

SAMPLE	KCN ADDED	HCN EQUIVALENT	HCN FOUND	RECOVERY
	mg.	*mg.*	*mg.*	*per cent*
1	10.00	4.15	4.18	100.7
2	1.00	0.415	0.438	105.6
3	40.00	16.60	16.3	97.3
4	40.00	16.60	16.6	100.0
5	40.00	16.60	16.65	100.3
6	20.00	8.30	8.15	98.2
7	4.00	1.66	1.66	100.0
8	20.00	8.30	8.28	99.7
9	40.00	16.60	16.35	98.5

on samples of raisins showed that a change in the order of adding the potassium cyanide and the acid made a difference in the recovery obtained. If water, then potassium cyanide, then acid were added, recoveries of only 75–85 per cent were obtained. If the acid were added before the potassium cyanide, the recoveries were the same as those given in the table. This difference is attributed to the cyanohydrin addition of some of the potassium cyanide to the aldehyde group of the dextrose in the raisins. This reaction proceeds much more rapidly at a high *p*H than at a low *p*H. The potassium cyanide or hydrocyanic acid thus changed is not recovered in the determination. However, in the proposed procedure sufficient acid is present to prevent the cyanohydrin reaction when the aqueous solution is added, and all hydrocyanic acid present should be recovered.

LIMIT OF ACCURACY OF THE BASIC TITRATION DETERMINATION

A minimum of about 8 cc. of absorbing solution containing 1 cc. of 10 per cent sodium hydroxide appears to be necessary to effect complete retention of all the hydrocyanic acid with the flow of air used in the method. A tube of about 10/16 inch in diameter, with a flat bottom, appears to be the best suited to the purpose in the Associate Referee's opinion. Using a tube of this kind containing 8 cc. of the absorption solution to which was added 2 cc. of ammonium hydroxide (1+2) and 0.5 cc. of 5 per cent potassium iodide he was able to distinguish the turbidity indicating the end point against a dark background with 0.02–0.03 cc. of 0.01 *N* silver nitrate, which quantity represents 0.011 mg. of hydrocyanic acid or is equivalent to 0.5 p.p.m. on a 25 gram sample. This should be satisfactory for most purposes.

NEW METHODS

Phenolphthalin test.—Among the more promising methods or tests is the phenolphthalin test.[2,3] While apparently not used to any extent in this country, this method has found considerable use in Great Britin and is claimed by Moffitt and Williams[2] to be the most sensitive test for hydrocyanic acid. The method is based on the oxidation of the colorless phenolphthalin to the indicator phenolphthalein by hydrocyanic acid, which gives the characteristic pink color in alkaline solution. The reagent is prepared according to Childs and Ball[3] as follows:

Ten cc. of a 1% solution of phenolphthalin containing 0.2% NaOH is diluted to 50 cc. with a 2.5% solution of glycerol. To this solution is added 50 cc. of a 0.3% solution of Cu acetate, and the whole is well mixed, and filtered if cloudy, and kept in a stoppered glass bottle. The reagent must be freshly prepared. One cc. of the reagent is added to the solution to be tested and this made basic with NaOH.

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[2] *Analyst*, 62, 101–6 (1937).
[3] *Ibid.*, 60, 294 (1935).

Phenolphthalin qualitative test.—This reagent is well adapted to the qualitative detection of hydrocyanic acid as well as to its quantitative determination. In the opinion of the Associate Referee the reagent is much superior to the gum guaiac qualitative test for hydrocyanic acid. The following simple qualitative test may be made on such foods as dried fruits in a manner similar to the guaiac test.

Place 30–50 grams of sample in a 200 cc. Erlenmeyer flask and close with a rubber stopper for 20–30 minutes. Then moisten a strip of filter paper with a dilute NaOH solution (5%), raise the stopper, and quickly insert the strip and suspend it by the end that is held between the stopper and the flask. Remove the strip after 45 seconds, and place a drop or two of the reagent on it. Immediately there is produced the pink of phenolphthalein in alkali if cyanide is present. If the paper remains white, no cyanide is present. The test is not specific for cyanide, however, as free halogens will also give the test and possibly other volatile oxidizing agents.

Phenolphthalin quantitative determination of cyanide.—The phenolphthalin reagent can be added directly to the solution in which the hydrocyanic acid has been absorbed in the aeration method after making to definite volume. The color developed is compared with standards containing the same amount of alkali and reagent in an equal volume of solution. As stated above, the test is not specific for cyanide but ferricyanides and aqueous solutions of the halogens give a similar coloration. Sulfides interfere and if present should be removed by addition of lead nitrate or acetate before distillation or aeration. Ferrocyanide, chromates, nitric acid, ferric chloride, and halogen salts give no reaction with the reagent.[3]

Photometric method.—Because of the nature of the color produced, it was thought that the phenolphthalin method would lend itself readily to photometric determination, but several difficulties were encountered in attempting to do this. The pink color in aqueous solution was found to fade rapidly so as to make a definite reading almost impossible. In alcoholic solution the color was found to be much less intense than in aqueous solution and gradually increased in intensity upon standing.

Experimentation showed that in 30 per cent alcohol the color was nearly as intense as in water, and the color remained quite constant for an hour or more. Standards developed in 50 cc. of 30 per cent alcohol containing progressive amounts of hydrocyanic acid showed that the color produced was not a linear function of the hydrocyanic acid present. The curve produced is given in Figure 2 and appears to be parabolic. The readings were made in a Clifford and Brice type photometer, *This Journal*, 19, 132 (1936), with a one-inch cell. The photometric method offers considerable promise, in the opinion of the Associate Referee, and may afford a way of reaching the near gamma range by decreasing the volume in which the color is developed and increasing the length of the cell. A No. 56 light filter should be used.

Thiocyanate method.—An effort was made to devise a thiocyanate

method since this would be delicate and sensitive and practically specific for cyanide. Criticisms of the ferric thiocyanate color in the past have been its rapid fading and variation in different acid strengths. A recent method for iron[4] based on the thiocyanate reaction appeared to offer a solution to the instability of the color. Instead of developing the color in aqueous solution it was done in 2 methoxy ethanol. In the latter solution the color was claimed to be more intense and also permanent.

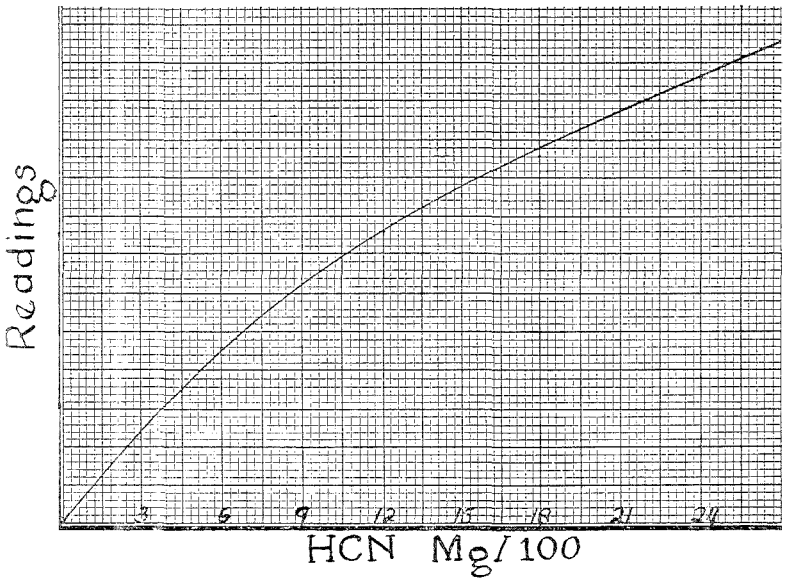

FIG. 2.

An attempt was made to reverse the reagents and use a similar determination for cyanide. To convert the hydrocyanic acid to thiocyanate it is necessary, of course, to evaporate the solution to dryness with ammonium polysulfide or sodium sulfide. Some determinations in which the residue was taken up in a few cc. of 1 to 1 methyl cellosolve and water gave good results in some cases, but the presence of dissolved sulfur offers some objection and causes a fading of the color. The present problem is to find a way of removing all sulfur without too much manipulation. The method appears to give promise for micro quantities if the sulfur can be removed without too much difficulty.

RECOMMENDATIONS[5]

It is recommended—

(1) That the recoveries of hydrocyanic acid by aeration and basic titration given in this report be checked by other analysts.

[4] Ind. Eng. Chem. Anal. Ed., 9, 453 (1937).
[5] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).

(2) That the phenolphthalin method given in this report be further studied.

(3) That the work on a thiocyanate method be continued.

REPORT ON FRUITS AND FRUIT PRODUCTS

By B. G. HARTMANN (U. S. Food and Drug Administration, Washington, D. C.), *General Referee*

This year's schedule on the subject of Fruits and Fruit Products lists five recommendations. Time did not permit of work on all the assignments, but progress has been reported on most of them. Much of the work is preliminary in character.

SOLUBLE SOLIDS AND EFFECTS OF ACIDS ON SUGARS ON DRYING

This assignment has been carried by the Association for several years without report.

The determination of soluble solids in fruit and fruit products has been the subject of extensive investigations by H. J. Wichmann, Kathryn Breen, P. L. Gowan, T. Macara, L. H. McRoberts, and P. A. Clifford. McRoberts, *This Journal*, **16**, 374 (1932), briefly summarizes the work of these investigators, and the report by Clifford appears in **17**, 215 (1934).

From supporting evidence obtained in these investigations the general conclusion is reached that of the methods studied the refractometric procedure is best suited for the determination of soluble solids in fruits and fruit products.

T. Macara[1] pointed out that in the examination of solutions containing invert sugar, glucose solids, and citric acid, corrections are necessary when the Schönrock tables for sucrose are used. McRoberts corroborated Macara's findings, and in consequence of his work he was appointed by the Association to study this phase of the project. In 1934 McRoberts reported sufficient experimental work to show conclusively that for the determination of the soluble solids of fruit products by means of the refractometric index, the Schönrock table for sucrose is not applicable without correction in the case of products high in invert sugar, such as jellies. McRoberts was not able to follow up the work, and for the past few years no report has been received on the subject.

The Beverage Section of the Food and Drug Administration, Washington, D. C., recommends the use of the refractometric procedure for the determination of soluble solids in preserve and jelly work. The method, it is believed, is at least as accurate as methods based on evaporation of an aliquot of the sample solution and drying the residue in vacuo at 70° C. It has the further advantage of requiring less time than do drying methods.

[1] *Analyst*, 56, 391 (1931).

Apparently the refractometric method is suitable for regulatory needs, despite the fact that in the evaluation of solids, the Schönrock sucrose tables are used. In view of the fact that the refractometric method has been used in official work for several years with entire satisfaction, there seems to be no immediate necessity of pursuing the rather involved study necessary to correct the hitherto serviceable Schönrock table, and it is recommended that the work on soluble solids be dropped for the present.

PROPOSED CHANGES IN THE ANALYSES OF PRESERVES AND JAMS

In the paper, entitled "Chemical Analysis of Preserves and Jams," by J. W. Sale, *This Journal*, **21**, 503 (1938), modifications of the methods used for analyzing preserves and jams are discussed. They are the result of several years of experience in the examination of these products and are offered for the purpose of correcting the apparent uncertainties existing in the mind of the analyst regarding the provisions of some of the official procedures. As these modifications do not affect the principles of the official procedures, but apply only to changes and additions in their text, it seems unnecessary to submit them to collaborative study. Accordingly, they are recommended for adoption as official (first action). The changes were published in *This Journal*, **22**, 78 (1939).

Much thought has been given to the improvement of the official methods for the determination of the insoluble solids in products containing pits and large seeds, such as damson plums and grapes. It is evident that the 25 gram sample (official method) of a damson plum preserve, with its large pits, is not conducive to an accurate determination. In 1936 Samuel L. Alfend of the St. Louis Station, *This Journal*, 20 (1937) proposed the following procedure for fruits of this type:

> Pulp the entire material as thoroughly as possible in a mortar without grinding the seeds or pits, or attempting to break up the skins. Weigh out 200 grams of actual fruit, or, in the case of preserves, jams, etc., weigh out 300 grams of sample, boil with water for the usual time, filter at once through a weighed cotton pad, wash until the wash water is free of solids, and make to 2 liters. After obtaining the weight of the dried material, pick out seeds or pits and soak them thoroughly in water to remove adhering insoluble matter. Rinse, dry, and subtract their weight from the entire weight of insoluble solids.

Obviously this procedure should give a more accurate result than that which is obtained by the official 25 gram sample. No collaborative work has been done on this important subject, and it is therefore suggested that in the coming year the above method be made the subject of collaborative study.

INACTIVE MALIC, ISOCITRIC, AND LACTIC ACIDS

No collaborative study on the determination of inactive malic acid and lactic acid was made.

The hope expressed in the Referee's last report regarding a possible method for isocitric acid did not materialize. It developed that before any progress could be made in the devising of a method, it would be necessary to find some means of removing tannin and coloring matter from the sample. As it was believed that charcoal would answer the purpose, an investigation was made with "Nuchar W," one of the charcoals used in analytical procedures. The results are shown in Table 1.

TABLE 1.—*Adsorption of organic acids on charcoal*
Nuchar (W), 0.200 gram. Volume of Solution, 110 cc. at 20°. Time, 2 Minutes

ACID	ACID ADSORBED (PER CENT)				
	SUCCINIC	CITRIC	MALIC (LEVO)	TARTARIC	ACETIC
grams in 110 cc. soln					
.0125	57.6	52.0	45.6	40.8	39.2
.0376	34.3	33.5	26.9	23.1	15.7
.0626	24.8	23.0	18.7	15.8	11.5
.0875	19.2	17.9	15.0	13.7	9.4
.1125	16.4	14.9	12.9	12.3	7.6
.1375	14.1	12.5	11.1	9.5	6.6
.1625	12.6	11.0	9.8	8.4	6.2
.1875	11.0	9.9	8.8	7.6	5.5
.2125	10.1	9.1	8.1	6.8	5.0
.2377	9.2	8.0	7.4	6.3	4.6

The data (Table 1) require no comment. They show conclusively that charcoal is not suitable for the quantitative determination of acids, particularly when the acid concentration is low.

TOTAL ACIDS (FREE AND COMBINED)

In the course of the investigation of methods for the determination of malic, inactive malic acid, and isocitric acids, it became necessary to determine accurately the sum of these acids (free and combined).

After considerable experimentation it was found that the lead salts of the polybasic acids, ordinarily found in fruits after removal of lead with hydrogen sulfide, yielded solutions that could be accurately titrated for their total acid content.

Fifty cc. of the solution containing the quantity of acid indicated (Table 2) was pipetted into a centrifuge bottle. Lead acetate solution and about 200 cc. of 95 per cent alcohol were added, and the mixture was shaken vigorously for about 2 minutes and centrifuged. The supernatant liquor was decanted, and the lead salts were washed with 80 per cent alcohol decomposed with hydrogen sulfide, transferred to a 110 cc. volumetric flask, made to mark with water, and filtered; 100 cc. of the clear filtrate was transferred to a 400 cc. beaker, evaporated to about 50 cc. and titrated with 0.1 *N* alkali.

MALIC ACID

A question often arises as to the necessity in the official method for determination of malic acid in fruit products, *Methods of Analysis A.O.A.C.*, 1935, 328, for subjecting the sample solution to the rather tedious "tribasic lead acetate" step. The answer to the question depends entirely on the nature of the acids contained in the material under examination.

Since the final determination of the acid involves a polarimetric reading, it is necessary to remove all interfering, optically-active compounds, such as sugars, tartaric and isocitric acids, pectin, and tannins, before the polarization is undertaken. The removal of sugars, pectin, and tartaric acid is easily accomplished, but iso-citric acid, tannin, and coloring matter require the treatment with tribasic lead acetate. Tannin and coloring

TABLE 2.—*Recovery of acids from lead precipitate*

ACID	ACID TAKEN	ACID RETURNED		ACID RETURNED
	mg.	*mg.*		*per cent*
Citric	99.3	99.1 98.9	99.0	99.7
Tartaric	99.4	99.0 99.2	99.1	99.7
Malic	98.2	96.8 97.2	97.0	98.8
Succinic	99.4	98.6 98.9	98.8	99.4
Citric and Tartaric	49.7 49.7 (99.4)	99.6 99.3	99.5	100.1
Potassium Acid Tartrate	124.5	124.0 124.0	124.0	99.6

matter are present in all fruit products; isocitric acid has been found only in the blackberry. Obviously, tannin and coloring matter are the chief offenders in the determination of malic acid. Tannin, besides being optically active, also exhibits a distressing tendency to produce brownish solutions when treated with uranium acetate, thus still further vitiating polarimetric readings.

There is at present no method available for the removal of tannin. Treatment with charcoal is not suitable for the purpose, as was shown elsewhere in this report. Experiments with zinc salts for the precipitation of tannin were not successful.

Until a procedure for removing tannin has been devised it will be necessary to subject the sample to the tribasic lead acetate treatment. In the analysis of samples known to be free of isocitric acid and low in tannin it should be permissible to omit the step in cases where an accurate determination is not desired. Recent work has shown that it may be possible to shorten the malic acid method.

It has been found that the zinc salts of citric and isocitric acids are precipitable in 75 per cent alcohol, while those of malic acid are readily soluble in this concentration of alcohol. In case the separation is found to be quantitative the further advantage of an increase in the quantity of sample, which is now restricted through the empiricism of the present method, will have been gained. This is important in cases where the total acid is relatively high but the content of malic acid is low, as in currants, grapefruit, pineapples, and raspberries.

CITRIC ACID BY THE PENTABROMACETONE METHOD

The official method for the determination of citric acid in foods by pentabromacetone has been criticized in the literature, particularly that part of it which deals with the oxidation of the brominated solution with permanganate.

In a recent article R. S. Paul[1] has the following to say regarding the determination of the acid:

> The pentabromacetone method is now coming to be generally recognized as the most satisfactory available. None of the descriptions of the method given in the literature was entirely satisfactory, the best at the time being that of the A.O.A.C., which allows for the slight solubility of pentabromacetone in water. It is further stated that the purity of the pentabromacetone depends on the care with which the oxidation is carried out. Rapid oxidation tends to the formation of products which are largely of such a degree of volatility as to interfere with the drying to a constant weight in a vacuum desiccator.

In an investigation by Lampitt and Rooke,[2] the following conclusions are reached regarding the oxidation of citric acid and the collection of the pentabromacetone:

1. There is no important difference between the results whether 50 ml. or 100 ml. of original citric acid solution are taken for analysis, but owing to the solubility of pentabromacetone it is advisable to keep the volume of the reaction solution as low as possible.
2. The temperature of the oxidation is immaterial (below 50° C.) but at lower temperature a longer reaction time is necessary.
3. Too rapid addition of permanganate is harmful, but excess does not matter; more ferrous sulfate solution, however, is then necessary.
4. It is advisable to cool the reaction mixture in the ice-chest overnight before filtration.
5. The volume of wash water should be kept as low as possible—not more than 25 ml.

Apparently the official method, with the exception of the manner of oxidizing the brominated citric acid solution, fulfils the requirements cited in the above reference.

Data obtained by rapid and slow oxidation of solutions containing known quantities of pure citric acid are presented in Table 3.

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[1] *Analyst*, 60, 635 (1935).
[2] *Ibid.*, 61, 654 (1936).

The results recorded under "Rapid Oxidation" (Table 3) were obtained by following the requirements for the oxidation of citric acid and the collection of the pentabromacetone laid down in the official method. For those under "Slow Oxidation" the same procedure was followed except that the permanganate was added slowly instead of all at once. Following bromination the permanganate was added from a buret in 2 cc. portions, and the mixture was shaken a few seconds after each addition. After the introduction of the 25 cc. permanganate solution, which required about 1.5 minutes, the mixture was shaken about 1 minute and allowed to stand for 3 minutes. The ferrous sulfate solution was then added and the pentabromacetone determined as directed in the official method.

TABLE 3.—*Rapid and slow oxidation of citric acid*

CITRIC ACID IN SOLUTION	CITRIC ACID DETERMINED*	
	RAPID OXIDATION .424 P (MG.)	SLOW OXIDATION .424 P (MG.)
mg. 1.53	No precipitate	Slight precipitate
3.89	1.31, 1.53 Av. 1.42	1.82, 1.82 Av. 1.82
7.78	4.79, 4.75 Av. 4.77	5.43, 5.55 Av. 5.49
38.29	32.61, 32.99 Av. 32.80	34.48, 34.68 Av. 34.58
114.87	103.62, 103.96 Av. 103.79	108.33, 108.54 Av. 108.44

* The volume of the reaction mixture was about 150 cc. in all cases; 25 cc. each of $KMnO_4$ and $FeSO_4$ solution was used.

The results show that slow oxidation yields materially more pentabromacetone than does rapid oxidation. This circumstance would indicate that the loss of pentabromacetone in the official method is not due entirely to solubility but in part either to incomplete oxidation of citric acid or to the formation of volatile bromine compounds. It was noticed that the pentabromacetone obtained in slow oxidation was more crystalline than that produced in rapid oxidation, a circumstance that makes for better filtration and drying.

There can be no question that slow oxidation is preferable to rapid oxidation. However, more work is needed before a revised procedure for this all important step can be written into the official method; apparently the solubility factor is materially smaller with slow oxidation.

It is recommended that the slow versus rapid oxidation be investigated as to preference in the conversion of citric acid into pentabromacetone.

POLARISCOPIC METHODS FOR JAMS, JELLIES, AND PRESERVES

In 1935 the Referee on Sugar and Sugar Products pointed out that certain constituents of food products, notably pectins, interfere with the polarimetric determination of sugars and recommended a study of the effect of clarifying agents for the purpose of their elimination.

The necessity for such a study is evident when it is considered that food products very frequently contain large quantities of interfering optically active compounds other than pectins, which if not removed will cause serious errors in sugar determinations.

Fortunately the lead reagents generally used for clarifying sugar solutions precipitate the majority of these interfering substances, but owing to the solubility of the lead salts in aqueous solution their removal is far from quantitative. It would seem, therefore, that in order to suppress solubility other vehicles than water should be tried. It has been found that the lead salts of pectin and of the acids are almost completely removed in 70 per cent alcohol.

Although it is realized that the possible occlusion of sugars in such a system may vitiate the determination, it is believed that the idea is deserving of a trial.

It is recommended that this study be continued.

ELECTROMETRIC TITRATION OF ACIDS

In continuation of last year's work, the associate referee reports progress in the development of fundamental data for the interpretation of titration curves obtained on mixtures of fruit acids.

It is recommended that this work be continued next year.

P_2O_5 IN JAMS, JELLIES, AND OTHER FRUIT PRODUCTS

No report was received from the associate referee on this assignment. It is recommended that this study be continued next year.

RECOMMENDATIONS[1]

It is recommended—

(1) That study of soluble solids be discontinued for the present.

(2) That study of electrometric titration be continued.

(3) That the changes in the official methods for the analysis of preserves and jams indicated in this report be considered for adoption.

(4) That study of methods for inactive, malic, isocitric, and lactic acid be continued.

(5) That the effect of slow oxidation on the yield of pentabromacetone in the determination of citric acid be studied.

[1] For report of Subcommittee D and action by the Association, see *This Journal* 22, 66 (1939).

(6) That study of polarimetric methods for jams, jellies, and preserves be continued.

(7) That the study of P_2O_5 in jams, jellies, and other fruit products be continued.

No report on soluble solids and effects of acids on sugar on drying was given by the associate referee.

REPORT ON ELECTROMETRIC TITRATION OF ACIDITY

By ROBERT U. BONNAR (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

During the past two years the Associate Referee has considered the problem of measuring the components of a mixture of weak acids from the titration curve of the mixture. As the introductory step, the mathematical basis of such measurements has been developed from the buffer properties of weak acids.

At constant temperature and constant ionic strength, the pH of a partially neutralized solution of a weak monobasic acid of concentration C and dissociation constant K is given by

$$1. \qquad pH = pK + \log \frac{X}{C - X},^{1}$$

where pK=log 1/K and X is the concentration of the salt of the weak acid arising from neutralization. If several buffer solutions be formed of one initial acid concentration, pH of the j-th buffer solution is

$$2. \qquad pH_j = pK + \log \frac{X_j}{C - X_j},$$

X_j being the concentration of neutralized acid in the j-th solution. Now, if i monobasic acids be present, the equation becomes

$$3. \qquad pH_j = pK_i + \log \frac{X_{ij}}{C_i - X_{ij}},$$

X_{ij} being the concentration of the i-th acid taken up by neutralizer in the j-th buffer solution. This equation may now be solved for X_{ij}. Letting $W_{ij}=pH_j-pK_i$

$$\frac{X_{ij}}{C_i - X_{ij}} = 10^{W_{ij}}$$

$$X_{ij} = 10^{W_{ij}}C_i - 10^{W_{ij}}X_{ij},$$

$$4. \qquad = \frac{10^{W_{ij}}}{1 + 10^{W_{ij}}} C_i.$$

[1] W. M. Clark, The Determination of Hydrogen Ions, 3rd Ed., 1928, Chapter I, p. 22, Eq. 23.

5. $$\text{Let} \quad r_{ij} = \frac{10^{W_{ij}}}{1 + 10^{W_{ij}}}, \quad \text{then}$$

6. $$X_j = r_{ij}C_i.^{2}$$

Here X_j is the total concentration of neutralized acid, corresponding to the number of equivalents of alkali added in titration, at $p\mathrm{H}_j$. The titration curve is thus represented by the formation of successive buffer solutions of i acids, each having its own dissociation constant and present in its own concentration. From j points along the titration curve, a set of j simultaneous equations in i unknowns can be constructed, which will allow the determination of each of the i acids, with an estimate of error based on $j-i$ degrees of freedom.

The chemical measurements available are X, the number of equivalents of alkali added, and the $p\mathrm{H}$ of the solution. In order to obtain r for an unknown sample, the dissociation constants of the acids present must be known. Then $W_{ij}=p\mathrm{H}_j-p\mathrm{K}_i$ must be calculated for each particular acid. Table 1 has been constructed to give r for positive values of W from the relation

5. $$r = \frac{10^{W}}{1 + 10^{W}} \cdot$$

This table is applicable to any weak acid. Since r is tabulated only for the positive values[3] of W, and since the table is symmetrical about $W=0$, $r=\frac{1}{2}$,

7. $$r(-W) = 1 - r(|W|).$$

Experimentally, the problem is to determine W_{ij}. If the titration curve of known amounts of a pure acid be measured over the concentration range of interest, $p\mathrm{K}_i$ can be calculated, since the $p\mathrm{H}$ is known for each point of the curve.

This solution for $p\mathrm{K}_i$ is here called the "backward solution," to distinguish it from the solution for C_i, known as the "forward solution," which is the ultimate aim of the investigation. For monobasic acids, the backward solution is simple,

$$X_j = r_jC \quad \text{and}$$

8. $$\frac{X_j}{C} = r_j.$$

W_{ij} can be found by inverse interpolation in Table 1, and $p\mathrm{K}_i=p\mathrm{H}_j-W_{ij}$. Thus an average value of $p\mathrm{K}_i$ and a standard deviation with $j-1$ degrees of freedom can be directly calculated from the j measurements.

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[2] In this equation the tensor notation indicates summation according to the repeated subscripts. Thus, if $i=1, 2, 3, 4, 5$,

$$X_j = r_{1j}C_1 + r_{2j}C_2 + \cdots + r_{5j}C_5$$

[3] This artifice reduces by half the tabulation necessary. r for negative values of W is the difference between unity and r for the tabular absolute value of W.

TABLE 1. $r = \frac{10^W}{1+10^W}$

w	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
3.0	.99900	.99902	.99905	.99907	.99909	.99911	.99913	.99915	.99917	.99919
2.9	.99874	.99877	.99880	.99883	.99885	.99888	.99890	.99893	.99895	.99898
.8	.99842	.99845	.99849	.99852	.99856	.99859	.99862	.99685	.99868	.99871
.7	.99801	.99805	.99810	.99814	.99818	.99822	.99827	.99830	.99834	.99838
.6	.99750	.99755	.99761	.99766	.99771	.99777	.99782	.99787	.99792	.99796
.5	.99685	.99692	.99699	.99706	.99712	.99719	.99725	.99732	.99738	.99744
.4	.99603	.99612	.99621	.99630	.99638	.99646	.99654	.99662	.99670	.99677
.3	.99501	.99513	.99524	.99534	.99545	.99555	.99565	.99575	.99585	.99594
.2	.99373	.99387	.99401	.99415	.99428	.99441	.99453	.99466	.99478	.99490
.1	.99212	.99230	.99247	.99264	.99281	.99297	.99313	.99328	.99344	.99359
2.0	.99010	.99032	.99054	.99075	.99096	.99117	.99137	.99156	.99175	.99194
1.9	.98757	.98785	.98812	.98839	.98865	.98890	.98915	.98940	.98964	.98987
.8	.98440	.98475	.98509	.98542	.98575	.98607	.98638	.98669	.98699	.98728
.7	.98044	.98087	.98130	.98172	.98213	.98253	.98292	.98330	.98368	.98404
.6	.97550	.97604	.97657	.97709	.97760	.97810	.97859	.97907	.97953	.97999
.5	.96935	.97002	.97069	.97133	.97197	.97259	.97320	.97379	.97437	.97494
.4	.96171	.96255	.96337	.96418	.96497	.96573	.96649	.96723	.96795	.96865
.3	.95227	.95331	.95432	.95532	.95629	.95724	.95817	.95909	.95998	.96086
.2	.94065	.94192	.94317	.94439	.94559	.94676	.94791	.94903	.95014	.95122
.1	.92641	.92796	.92949	.93099	.93245	.93388	.93529	.93667	.93803	.93935
1.0	.90909	.91098	.91282	.91464	.91642	.91817	.91988	.92156	.92321	.92482
0.9	.88818	.89045	.89268	.89486	.89701	.89912	.90119	.90322	.90521	.90717
.8	.86319	.86589	.86854	.87115	.87371	.87623	.87870	.88114	.88353	.88588
.7	.83366	.83683	.83995	.84302	.84604	.84902	.85195	.85483	.85766	.86045
.6	.79924	.80291	.80653	.81010	.81361	.81708	.82050	.82386	.82718	.83045
.5	.75975	.76392	.76805	.77213	.77616	.78013	.78405	.78792	.79175	.79552
.4	.71525	.71992	.72454	.72911	.73364	.73811	.74253	.74691	.75124	.75552
.3	.66614	.67124	.67630	.68133	.68629	.69123	.69613	.70097	.70578	.71054
.2	.61314	.61858	.62400	.62938	.63474	.64007	.64535	.65061	.65583	.66101
.1	.55731	.56298	.56863	.57429	.57990	.58549	.59107	.59663	.60216	.60767
0.0	.50000	.50576	.51150	.51726	.52301	.52875	.53449	.54021	.54593	.55161

For dibasic and polybasic acids, the titration is complicated by the different values of pK_1 and pK_2 for the different acids. Here, one form of the backward solution has been derived as follows—

6. $X_j = r_{ij}C_i$. Let $i = 1$ and 2

9a. $X_j = r_{1j}C_1 + r_{2j}C_2$

for the same dibasic acid, $C_1 = C_2 = \frac{1}{2}C$, so

b. $X_j = \frac{1}{2}C(r_{1j} + r_{2i})$

c. $$\frac{2X_j}{C} = r_{1j} + r_{2j}.$$

Substituting the equivalents for r_{1j} and r_{2j} from Equation 5,

d. $$\frac{2X_j}{C} = \frac{10^{W_{1j}}}{1 + 10^{W_{ij}}} + \frac{10^{W_{2j}}}{1 + 10^{W_{2j}}}.$$

$$\text{Let} \quad 10^{W_{ij}} = \frac{1}{S}, \qquad 10^{W_{2j}} = \frac{1}{N}, \qquad \frac{2X_j}{C} = R.$$

Then substituting and clearing,

10. $$R = \frac{1}{S+1} + \frac{1}{N+1}. \qquad = \frac{N + S + 2}{NS+S+N+1}.$$

The next step is to proceed to curve-fitting.[4]

In the process of curve-fitting, there is a function F_0 such that, for this case,

11a. $$F_0 = N + S + 2 - RNS - RS - RN - R.$$

b. $$= (1 - R)N + (1 - R)S + (2 - R) - RNS$$

Now, for N, substitute its equivalent $10^{-W_{2j}} = 10^{(pK_2 - pH_j)}$

$$= \frac{10^{pK_2}}{10^{pH_j}}, \quad \text{and for } S, \quad \frac{10^{pK_1}}{10^{pH_j}}$$

12. $$F_0 = \frac{1 - R}{10^{pH_j}} \cdot 10^{pK_2} + \frac{1 - R}{10^{pH_j}} \cdot 10^{pK_1} + (2 - R) - \frac{R \cdot 10^{pK_1} \cdot 10^{pK_2}}{10^{2pH_j}}$$

$$\text{Let} \quad \frac{1 - R}{10^{pH}} = Y_1, \qquad \frac{R}{10^{2pH}} = Y_2, \qquad 2 - R = Y_3$$

$$\text{and} \quad 10^{pK_1} = A, \qquad 10^{pK_2} = B. \qquad \text{Then}$$

13a. $$F_0 = Y_1B + Y_1A + Y_3 - Y_2AB$$

b. $$= (A + B)Y_1 - ABY_2 + Y_3.$$

Observe now that the measurements $R = 2X_j/C$ and the pH are combined to form the variables, while the pK values are involved in the parameters to be determined. In this solution, the exact value of the Lagrangian function must be used, instead of an assumed unity. The partial derivatives for curve-fitting are:

14. $$F'_A = Y_1 - BY_2 \qquad F'_{Y_1} = A + B$$
$$F'_B = Y_1 - AY_2 \qquad F'_{Y_2} = -AB$$
$$F'_{Y_3} = 1.$$

[4] The process of curve-fitting here used is that of W. E. Deming, Some Notes on Least Squares, U. S. D. A. Graduate School, 1938, 181 pp. Other published works by Deming on the subject are *Phil. Mag.* 11, 146–158 (1931); 17, 804–928 (1934); 19, 389–402 (1935).

The Lagrangian function then becomes

15. $$L = \frac{(A + B)^2}{Wt.Y_1} + \frac{(AB)^2}{Wt.Y_2} + \frac{1}{Wt.Y_3} \cdot$$

The prior estimates A_0 and B_0 of A and B can be taken from existing tables, from publications, or from approximations from the titration curve. The weights are computed as follows;[5]

16a. $$\frac{1}{Wt.Y_1} = \frac{1}{Wt.R}\left(\frac{dY_1}{dR}\right)^2 + \frac{1}{Wt.p\mathrm{H}}\left(\frac{dY_1}{dp\mathrm{H}}\right)^2$$

since $$Y_1 = \frac{1 - R}{10^{p\mathrm{H}}} \qquad \frac{dY_1}{dR} = -10^{-p\mathrm{H}}$$

$$\frac{dY_1}{dp\mathrm{H}} = (1 - R)10^{-p\mathrm{H}} \log_e 10 = (-\log_e 10)Y_1$$

b. $$\frac{1}{Wt.Y_1} = \frac{1}{Wt.R} \cdot 10^{-2p\mathrm{H}} + \frac{Y_1^2(\log_e 10)^2}{Wt.p\mathrm{H}},$$

where $Wt.R = n/S^2_R$, the reciprocal of the variance of the mean of R due to experimental error,

$Wt.p\mathrm{H} = n/S^2_{p\mathrm{H}}$, $S^2_{p\mathrm{H}}$ being the variance of the errors of pH measurement.

In like manner

17a. $$\frac{1}{Wt.Y_2} = \frac{1}{Wt.R}\left(\frac{dY_2}{dR}\right)^2 + \frac{1}{Wt.p\mathrm{H}}\left(\frac{dY_2}{dp\mathrm{H}}\right)^2$$

b. $$\frac{1}{Wt.Y_2} = \frac{1}{Wt.R} \cdot 10^{-4p\mathrm{H}} + \frac{1}{Wt.p\mathrm{H}} (2 \log_e 10)^2 Y_2^2$$

18. $$\frac{1}{Wt.Y_3} = \frac{1}{Wt.R} \cdot$$

The applicability of the "backward solution" for dibasic acids was tested with malic acid and succinic acids. Series of eighteen buffer solutions of approximately 0.01 M concentration in the acid were made by titration with sodium hydroxide and dilution to a uniform 50 cc. volume. These buffers were measured with the glass electrode. Sealed electrodes and the Beckman model F hydrogen ion meter standardized with 0.0500 M potassium biphthalate at pH 4.00 at 25° C. were used. S^2_R was experimentally determined as 0.0119 for nine degrees of freedom. $S^2_{p\mathrm{H}}$ was assumed to be 0.0001 when $S = 0.01$ pH was used as the least count of the instrument. A_0, the prior estimate of A, was taken as $10^{4.18}$ for suc-

[5] Deming, Least Squares, p. 30, Ex. 11.

cinic acid and $10^{3.48}$ for malic acid. B_0, the prior estimate of B, was taken as $10^{5.57}$ for succinic acid and $10^{5.11}$ for malic acid.[6] $p\mathrm{K}_1$ was then found to be 4.20 for succinic acid and 3.48 for malic acid, with standard devia-

TABLE 2. $\left(\frac{dr}{dW}\right)^2 = 5.30190\, r^2(1-r)^2$ *for values of r from Table 1*

w	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
3.0	.00001	.00001	.00000	.00000	.00000	.00000	.00000	.00000	.00000	.00000
2.9	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001
.8	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001
.7	.00002	.00002	.00002	.00002	.00002	.00002	.00002	.00002	.00001	.00001
.6	.00003	.00003	.00003	.00003	.00003	.00003	.00003	.00002	.00002	.00002
.5	.00005	.00005	.00005	.00005	.00004	.00004	.00004	.00004	.00004	.00003
.4	.00008	.00008	.00008	.00007	.00007	.00007	.00006	.00006	.00006	.00005
.3	.00013	.00012	.00012	.00011	.00011	.00010	.00010	.00009	.00009	.00009
.2	.00021	.00020	.00019	.00018	.00017	.00016	.00016	.00015	.00014	.00014
.1	.00032	.00031	.00030	.00028	.00027	.00026	.00025	.00024	.00023	.00022
2.0	.00051	.00049	.00047	.00045	.00043	.00041	.00039	.00037	.00035	.00034
1.9	.00080	.00076	.00073	.00070	.00067	.00064	.00061	.00058	.00056	.00053
.8	.00125	.00120	.00114	.00109	.00105	.00100	.00096	.00091	.00087	.00084
.7	.00195	.00187	.00179	.00171	.00163	.00156	.00149	.00143	.00137	.00131
.6	.00303	.00290	.00278	.00266	.00254	.00243	.00233	.00223	.00213	.00204
.5	.00468	.00448	.00429	.00411	.00394	.00377	.00361	.00345	.00331	.00316
.4	.00719	.00689	.00660	.00632	.00606	.00581	.00556	.00532	.00510	.00489
.3	.01095	.01050	.01008	.00966	.00926	.00888	.00851	.00816	.00783	.00750
.2	.01652	.01587	.01523	.01462	.01403	.01347	.01293	.01241	.01190	.01142
.1	.02464	.02369	.02277	.02188	.02103	.02022	.01942	.01865	.01791	.01721
1.0	.03621	.03487	.03358	.03232	.03110	.02993	.02880	.02770	.02665	.02563
0.9	.05230	.05045	.04866	.04693	.04525	.04362	.04204	.04051	.03904	.03760
.8	.07394	.07150	.06912	.06680	.06455	.06236	.06023	.05816	.05614	.05419
.7	.10195	.09885	.09582	.09285	.08996	.08712	.08435	.08165	.07902	.07644
.6	.13650	.13277	.12909	.12548	.12193	.11844	.11501	.11165	.10835	.10511
.5	.17664	.17244	.16827	.16413	.16003	.15599	.15199	.14805	.14414	.14029
.4	.21993	.21556	.21119	.20683	.20246	.19811	.19378	.18946	.18516	.18089
.3	.26224	.25819	.25409	.24994	.24576	.24152	.23724	.23295	.22862	.22428
.2	.29830	.29514	.29186	.28848	.28499	.28140	.27773	.27396	.27012	.26621
.1	.32272	.32094	.31900	.31690	.31466	.31228	.30975	.30708	.30428	.30135
0.0	.33137	.33128	.33102	.33058	.32997	.32918	.32822	.32710	.32580	.32435

tions of 0.008 and 0.014 based on sixteen degrees of freedom, respectively; $p\mathrm{K}_2$ was found to be 5.41 for succinic acid and 4.93 for malic acid with standard deviation (sixteen degrees of freedom) of 0.01 and 0.013, respectively. This shows that $p\mathrm{K}$ values can be found from glass electrode

[6] Clark, W. M. Determination of Hydrogen Ions, p. 678.

measurements of a titration curve to within the least count of present-day instruments.

These values were then used with nine-point titration curves in two attempts to resolve mixtures of approximately equal parts of malic and succinic acids.

Reverting to Equation 6, for two dibasic acids this becomes:

19. $$X_j = r_{1j}C_1 + r_{2j}C_1 + r_{3j}C_2 + r_{4j}C_2.$$

The curve-fitting equation is:

20. $$F_0 = (r_1 + r_2)C_1 + (r_3 + r_4)C_2 - X.$$

The partial derivatives of F_0 are:

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a. $F'_{C_1} = r_1 + r_2$ e. $F'_X = -1$

b. $F'_{C_2} = r_3 + r_4$ f. $F'_{r_3} = C_2$

c. $F'_{r_1} = C_1$ g. $F'_{r_4} = C_2$.

d. $F'_{r_2} = C_1$

The Lagrangian function is:

22. $$L = \frac{C_1^2}{Wt.r_1} + \frac{C_1^2}{Wt.r_2} + \frac{C_2^2}{Wt.r_3} + \frac{C_2^2}{Wt.r_4} + \frac{1}{Wt.X}$$

where

$$\frac{1}{Wt.r} = \frac{1}{Wt.W}\left(\frac{dr}{dW}\right)^2$$

24a. since $$r = \frac{10^W}{1 + 10^W}, \qquad Ln\, r = Ln\, 10^W - Ln(1 + 10^W)$$

b. $$\frac{dr}{r} = \frac{2.303\ 10^W}{10^W}\,dW - \frac{2.303\ 10^W}{1 + 10^W}\,dW$$

c. $$= 2.303(1 - r)dW$$

d. $$\frac{dr}{dW} = (2.303)r(1 - r)$$

25. $$\frac{1}{Wt.r} = \frac{1}{Wt.W}\,(2.303r)^2(1 - r)^2.$$

Table 2 gives $(dr/dW)^2$ for the values of r given in Table 1. Since dr/dW is symmetrical with respect to $r=\frac{1}{2}$, the entry in Table 2 for r greater than .5 is also the entry for $1-r$ greater than .5, and so, as tabulated, for the absolute value of W.

Recalling Equation 19, it is noted that, since dibasic acids are involved, C_1, the number of equivalents of the first hydrogen of malic acid, is equal to the number of moles of the acid, and likewise C_2 is both the number of equivalents of the first hydrogen and the number of moles of succinic acid. Therefore molal concentrations may be used. Table 3 shows the

TABLE 3

TRIAL	ACID	PRIOR ESTIMATE	PRESENT	FOUND
		moles/liter	*moles/liter*	*moles/liter*
I	Malic	.00505	.00481	.00473
	Succinic	.00505	.00497	.00508
	Total	.0101	.00978	.00981
II	Malic	.00606	.00577	.00555
	Succinic	.00404	.00398	.00419
	Total	.0101	.00975	.00974

prior estimates, the concentrations present, and the concentrations found in these two experiments. Since the full least squares solution, involving the Lagrangian function, was necessary, the statistical weights are given by

$1/Wt.X = 1.349066 \times 10^{-10}$ by conversion of $Wt.R$, to a concentration basis

$1/Wt.W = S^2_{pH} + S^2_{pK}/K$ where S^2_{pH} and S^2_{pK} are taken from the "backward solution" for $K = 16$ degrees of freedom,

$1/Wt.W = 1/53334$ for malic acid and

$1/Wt.W = 1/80000$ for succinic acid.

It is believed that these experiments, although under conditions too idealized to be regarded as constituting a method of analysis, confirm the usefulness of this approach to the determination of organic acids in mixtures. It is therefore recommended that the studies of the electrometric titration curve be continued.

For report on malic, isocitric, and lactic acids, see Report of the Referee on Fruits and Fruit Products.

No report on polariscopic methods for jams, jellies, and preserves was given by the associate referee.

No report on P_2O_5 in jams, jellies, and other fruit products was given by the associate referee.

REPORT ON CANNED FOODS

By V. B. BONNEY (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

During the past packing season the Canned Food Section of Food Division of the Food and Drug Administration collaborated with the National Canners Association laboratory in San Francisco, in experimental packs of canned Royal Anne cherries, whole unpeeled apricots, clingstone peaches—both halves and slices—and Bartlett pears in halves. In these studies a large number of packs of 36 cans each were prepared, and the exact put-in weight of fruit in each can was recorded. The packs covered variations in packing medium (water, or sugar sirups of various strengths), degrees of ripeness of fruit, fruit from different growing conditions, different methods of cooking, and the different sizes of cans commonly used.

The plans for these studies include an arrangement whereby 3 cans from each pack are examined the 60th, 90th, and 180th days after packing in the National Canners Association laboratory in San Francisco and 3 other cans are examined the same days in the Canned Food Section laboratory in Washington. In order to insure uniform procedure the following method is being used in each of the laboratories for the determination of drained weight, one of the most important factors in the studies:

> Pour the entire contents of the container on a round sieve with a No. 8 standard screen (diameter of wire 0.84 mm. and size of opening 2.38 mm.). Use a sieve 8 inches in diameter for cans under 3 pounds net weight, and a sieve 12 inches in diameter for larger containers. If the units of the product are "cupped" (for example, peaches in halves), turn over as quickly and gently as possible any units which may have fallen with cups up, then tilt the screen as much as possible without shifting of the units. After draining for two minutes from the time the product is poured on the screen, transfer contents to a tared dish and determine drained weight.

Using this method the two laboratories have determined drained weight in the cans of fruit indicated (Table 1).

No analysis of the data to determine differences in individual cans from the same lot has been made, except the general observation that variations in any three cans cut at either laboratory are usually as great as the variations noted in the entire six cans, cut three at each of the laboratories. There are too many factors affecting the drained weight of canned fruit to permit the preparation of duplicate cans, with any assurance that the drained weight of each can will be identical. Therefore the only significant check between two analysts is the average by each analyst of the analyses of a large number of cans with known put-in weights of fruit.

In view of the close agreement in relationship between put-in weight of fruit and drained weight, as shown by the above results on over 900 cans of fruit examined by two laboratories using the above method, it is recommended that this method be substituted in the present official method for the last clause in the second sentence of the first paragraph, all the last sentence of that paragraph, and the first two sentences in the second paragraph. While the collaborative work mentioned was done entirely on canned fruits, work in the Canned Food Section shows the method to be equally applicable to canned vegetables, with the exception of canned tomatoes. The 8-mesh screen is too fine for satisfactory results in draining the latter article, therefore it is further recommended that a statement be added to the official method showing that it is not applicable to canned tomatoes.

TABLE 1.—*Drained weight*

	CANNED FOOD SECTION		NAT. CANNERS ASS'N	
	NO. OF CANS	DRAINED WEIGHT—% OF PUT-IN WEIGHT	NO. OF CANS	DRAINED WEIGHT—% OF PUT-IN WEIGHT
Apricots, whole unpeeled	377	90.0	378	90.7
Cherries, Royal Anne	264	94.1	264	94.3
Peaches, Yellow Cling, halves	195	94.0	195	94.8
Peaches, Yellow Cling, sliced	47	93.0	48	94.2
Pears, Bartlett, halves	32	95.9	32	95.3

The Referee's Laboratory has undertaken further studies on the relationship between the alcohol-insoluble solids of canned peas and the observed maturity, in the field, of those peas before being canned. S. C. Rowe and L. M. Beacham of the Laboratory studied field conditions and made detailed observations of the maturity of Alaska variety peas being harvested for canning and obtained samples of the observed peas after canning, at thirteen plants located in Virginia, Maryland, Delaware, and Wisconsin.

After making arrangements with the canner for conducting the experiment, Rowe and Beacham observed the field from which peas were being harvested and noted the general appearance of vines and pods. A load of peas was then followed to the viner and a sample, as nearly representative as possible, was taken from the load. The pods in this sample were removed from the vines and were classified according to their apparent maturity, as follows:

F—Pods in which the peas had not developed sufficiently to give any peas large enough for canning.

M_1—Pods in which the peas had developed, but in which they were still too small to completely fill the pods.

TABLE 2.—*Relationship of pod maturity to alcohol-insoluble solids in canned Alaska peas*

CODE	F PODS	M_1 PODS	M_2 PODS	M_3 PODS	M_4 PODS	SIEVE SIZE	TOTAL	A.I.S.
			per cent				*per cent*	
WMPA	17	25.6	56.8	0.7	0	1	5.0	—
2						2	20.0	10.4
3						3	42.5	14.7
4						4	32.5	18.8
TCB 1–2	2.8	38.8	57.5	0.8	0	1–2	36.4	—
3						3	54.5	18.3
4						4–5	9.1	19.6
TCA 1–2	3.1	31.8	57.8	2.3	0	1–2	34.7	13.11
3						3	52.1	18.62
4						4–5	13.0	20.08
WVA 1	10.7	14.0	71.8	3.5	0	1	13.0	9.5
2						2	27.0	12.1
3						3	35.0	15.8
4						4	25.0	19.3
TCD 1–2	3.2	33.3	58.9	4.6	0	1–2	19.0	10.8
3						3	36.0	15.1
4						4–5	45.0	18.6
TCC 1–2	3.9	18.3	72.8	5.1	0	1–2	33.3	16.7
3						3	55.5	21.3
4						4–5	11.2	22.2
SAPR	20.7	20.0	51.3	8.0	0	Pod Run	100.0	13.6
WMPB 1	0	0	79.3	20.7	0	1	1.5	9.7
2						2	12.5	12.1
3						3	48.0	16.5
4						4	36.5	19.6
5						5	1.5	21.3
TCE 1–2	0	29.5	47.7	22.8	0	1–2	16.7	—
3						3	33.3	16.8
4						4–5	50.0	20.4
WBB	2.5	4.1	84.3	8.7	0.4	1	3.0	—
2						2	20.0	12.3
3						3	60.0	18.4
4						4	15.0	20.1
5						5	2.0	22.9
WMPC 1	1.3	6.6	66.9	24.7	0.5	1	1.0	10.0
2						2	12.5	14.5
3						3	46.5	19.4
4						4	39.0	21.3
5						5	1.0	23.3
LAPR	12.7	23.0	52.5	10.6	1.2	Pod Run	100.0	15.6
WWA	10.0	1.8	78.0	8.8	1.4	1	1.0	—
2						2	15.0	12.5
3						3	55.0	16.7
4						4	25.0	22.1
5						5	4.0	21.8
DAPR	4.5	4.2	59.6	30.1	1.6	Pod Run	100.0	19.8

TABLE 2.—*Relationship of pod maturity to alcohol-insoluble solids in canned Alaska peas—Continued*

CODE	F PODS	M_1 PODS	M_2 PODS	M_3 PODS	M_4 PODS	SIEVE SIZE	TOTAL	A.I.S.
			per cent				*per cent*	
TCF 1–2	1.3	3.2	47.9	45.0	2.6	1–2	14.4	11.8
3						3	42.8	16.9
4						4–5	42.8	21.0
KC 1–2	3.0	9.1	37.2	47.3	3.4	1–2	13.1	17.7
3						3	34.8	21.5
4						4	52.1	23.6
WSCA	0	3.3	62.3	30.8	3.7	1	1.0	—
2						2	12.0	13.1
3						3	50.0	17.0
4						4	35.0	21.5
5						5	2.0	23.5
KEPR	0.4	3.7	35.1	56.7	4.3	Pod Run	100.0	23.8
KFPR	0.6	4.1	38.6	52.2	4.5	Pod Run	100.0	23.7
FB 1–2	2.5	6.7	46.5	39.4	4.6	1–2	20.0	17.2
3						3	50.0	22.4
4						4–5	30.0	23.0
FD 1–2	3.5	8.8	29.4	52.7	5.7	1–2	20.4	18.2
3						3	48.4	21.4
4						4–5	31.2	22.9
FE 1–2	6.5	8.7	36.0	42.6	6.3	1–2	20.0	16.7
3						3	50.0	21.0
4						4–5	30.0	22.5
KB 1–2	1.6	7.3	38.9	45.5	6.7	1–2	12.0	17.6
3						3	40.0	20.6
4						4–5	48.0	22.6
LBPR	4.9	6.6	38.0	43.8	6.7	Pod Run	100.0	22.0
FC 1–2	2.2	8.7	39.9	41.8	7.4	1–2	22.8	14.4
3						3	50.0	19.7
4						4–5	27.2	22.6
KDPR	2.5	7.6	38.8	43.5	7.7	Pod Run	100.0	23.1
KA 1–2	2.4	7.0	47.8	34.8	8.0	1–2	6.8	14.1
3						3	26.6	17.3
4						4–5	66.6	22.0
WSCB	0.7	4.8	53.4	33.0	8.0	1	2.0	—
2						2	12.0	13.5
3						3	45.0	17.9
4						4	40.0	21.5
5						5	1.0	21.7
DCPR	6.0	8.0	30.0	47.1	8.9	Pod Run	100.0	20.6
WBA	0.6	2.3	30.2	54.8	12.1	1	2.0	—
2						2	11.0	20.0
3						3	55.0	22.2
4						4	28.0	25.0
5						5	4.0	26.4
LCPR	2.8	7.2	33.7	37.9	18.5	Pod Run	100.0	23.1
SBPR	1.5	3.2	30.7	43.7	20.8	Pod Run	100.0	22.4

TABLE 2.—*Relationship of pod maturity to alcohol-insoluble solids in canned Alaska peas—Continued*

CODE	F PODS	M_1 PODS	M_2 PODS	M_3 PODS	M_4 PODS	SIEVE SIZE	TOTAL	A.I.S.
			per cent				*per cent*	
DBPR	8.4	3.9	25.8	41.1	20.8	Pod Run	100.0	22.4
LDPR	2.1	4.9	30.1	36.1	26.8	Pod Run	100.0	23.8
WELA	0	1.0	8.7	57.8	32.5	1	2.8	—
						2	1.4	—
3						3	27.8	23.7
						4	1.4	—
5						5	66.6	24.3
WKA	0	0.4	11.1	53.8	34.8	1	2.0	—
						2	10.0	—
3						3	35.0	21.5
4						4	35.0	21.4
5						5	10.0	23.6
6						6	8.0	24.5
FA 1–2	0.6	2.4	27.6	29.5	39.8	1–2	23.5	17.8
3						3	53.0	21.7
4						4–5	23.5	23.4

M_2—Pods in which the peas had developed sufficiently so that they were tight in the pod, but with shells still full of juice and brittle.

M_3—Pods in which the peas were tight but the shells thin (papery) and tough. Pods that had whitened and started to wrinkle slightly on the lower edge were included in this group.

M_4—Pods in which the peas were beginning to loosen because of shrinkage, with the shells thin (papery) and tough. Pods with yellow shells were also included in this group.

After the sample had been analyzed in this way, and knowledge obtained of the maturity of the peas in the load, the load was vined. These vined peas were kept separate in marked boxes, and a record was kept of the weight of the peas so obtained. In those plants where the peas were graded for size, the peas under observation were graded separately and a record kept of the weight of each sieve size resulting. The peas were then sent through the regular factory canning process, care being taken to keep them identified until they had passed through the closing machine. At the closing machine a representative sample of 12 cans of each sieve size, or 24 cans if the peas were ungraded for size, was taken and identified with code marks. These cans were then given the regular factory cook and cooling process.

This procedure was usually repeated at each plant several times, and thus samples from fields at several different stages of maturity were secured. A record was kept of other variable factors such as the time elapsing between cutting and vining, and between vining and canning,

but these did not show a significant effect upon the alcohol-insoluble solids content.

All the cans obtained in this way have not been analyzed for alcohol-insoluble solids, but all cans in some of the lots have been examined, and a preliminary test of at least 1 can from each lot has been made. As shown in Table 2, 10 of the 95 lots show an alcohol-insoluble solids content of more than 23.5 per cent. The lowest percentage of M_4 pods found in a sample having an alcohol-insoluble solids content of more than 23.5 per cent was in the case of KC-4, where 3.4 per cent M_4 pods were found in the unvined peas and the alcohol-insoluble solids content of the Sieve 4 peas was 23.6 per cent. Twenty-two of the lots had 3.4 per cent or more M_4 pods, while only 10 of the analyses on the corresponding canned peas showed an alcohol-insoluble solids content of 23.5 per cent or more.

The results from these analyses show that a definite relationship exists between the maturity of the peas used for canning and the alcohol-insoluble solids of the canned peas. While the alcohol-insoluble solids of the larger sieve sizes consistently ran higher than those of smaller sizes of the same lot, even in the large sieve sizes 23.5 per cent was exceeded only when a considerable number of mature pods were present. In lots that showed no pods of M_4 maturity, the alcohol-insoluble solids remained below 23.5 per cent, approaching this figure as the proportion of the M_1 and M_2 pods decreased and that of the M_3 pods increased. When a large per cent of M_3 pods was found with several per cent of M_4 pods, the alcohol-insoluble solids usually increased slightly beyond 23.5 per cent for the larger sieve sizes, and continued to increase slightly as more M_4 pods were noted. In many cases large quantities of M_4 pods were encountered where the alcohol-insoluble solids ran less than 23.5 per cent. In every case, however, where alcohol-insoluble solids of 23.5 per cent or more were observed, peas containing a preponderance of M_3 pods and considerable amounts of M_4 pods had been used for canning.

Table 3 shows the relatively close agreement in the alcohol-insoluble solids content of different cans from the same lot. These cans were taken consecutively, and at regular intervals, so that Can No. 1 represents the first, and Can No. 24, the last peas canned from the lot.

RECOMMENDATIONS[1]

It is recommended that the method for determining alcohol-insoluble material in canned peas, *This Journal*, **21**, 89 (1938), be clarified as follows:

(a) Change the title to read, "Alcohol-Insoluble Solids in Canned Peas and Canned Dried Peas."

(b) Change the 3rd sentence on p. 90 to read as follows: "Grind the drained peas in a food chopper until the cotyledons are reduced to a smooth homogeneous paste, stir, and weigh 20 g. of the ground material into a 600 cc. beaker."

[1] For report of Subcommittee C and action by the Association, see *This Journal*, **22**, 60 (1939).

TABLE 3.—*Alcohol-insoluble solids content of different cans from the same lot*

CAN NO.	SIEVE SIZE	ALCOHOL-INSOLUBLE SOLIDS
		per cent
1	1 & 2	12.86
2	1 & 2	13.27
3	1 & 2	12.98
4	1 & 2	12.82
5	1 & 2	12.92
6	1 & 2	12.88
7	1 & 2	13.46
8	1 & 2	13.13
9	1 & 2	13.28
10	1 & 2	13.11
11	1 & 2	13.33
12	1 & 2	13.31
13	1 & 2	12.95
14	1 & 2	12.91
15	1 & 2	12.77
16	1 & 2	13.07
17	1 & 2	13.30
18	1 & 2	13.24
19	1 & 2	13.26
20	1 & 2	13.00
21	1 & 2	13.05
22	1 & 2	13.42
23	1 & 2	13.17
24	1 & 2	13.07
1	3	18.54
2	3	18.35
3	3	18.60
4	3	18.56
5	3	18.67
6	3	18.42
7	3	18.64
8	3	18.81
9	3	18.41
10	3	18.63
11	3	18.44
12	3	18.90
13	3	18.74
14	3	18.60
15	3	18.60
16	3	18.90
17	3	18.76
18	3	18.84
19	3	18.47
20	3	18.55
21	3	18.54
22	3	18.78
23	3	18.39
24	3	18.80

TABLE 3.—*Alcohol-insoluble solids content of different cans from the same lot—Continued*

CAN NO.	SIEVE SIZE	ALCOHOL-INSOLUBLE SOLIDS
		per cent
1	4	20.05
2	4	20.14
3	4	19.89
4	4	19.96
5	4	19.92
6	4	19.85
7	4	20.02
8	4	20.07
9	4	20.20
10	4	20.03
11	4	19.97
12	4	20.26
13	4	20.00
14	4	19.85
15	4	20.53
16	4	20.54
17	4	20.23
18	4	20.14
19	4	20.10
20	4	19.80
21	4	20.17
22	4	19.92
23	4	20.15
24	4	20.03

(c) Combine the first and second complete paragraphs on p. 90 to read as follows: "Fit into a Büchner funnel a filter paper of appropriate size, previously prepared by drying in a flat-bottomed dish for 2 hours at the temp. of boiling H_2O, covering with a tight-fitting cover, cooling in a desiccator, and weighing at once. Apply suction and transfer the contents of the beaker to the Büchner funnel in such a manner as not to run over the edge of the paper; suck dry and wash the material on the filter with 80% alcohol until the washings are clear and colorless."

CORRECTIONS

Vol. 22, No. 1, *This Journal:*

Page 72, end of line 23, "10.2" should read "±0.1." Line 24, "1.0" should read 1.0%."

Page 125, line 5 above heading "Discussion," "4X" should read "for X."

Page 195, line 10, "(8″×10″)" should read "(8″×1″)."

CONTRIBUTED PAPERS

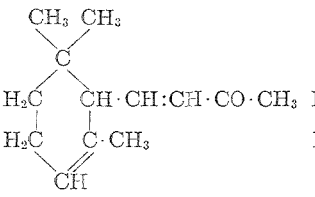
IDENTIFICATION OF FLAVORING CONSTITUENTS IN COMMERCIAL FLAVORS

V. QUANTITATIVE DETERMINATION OF β-IONONE

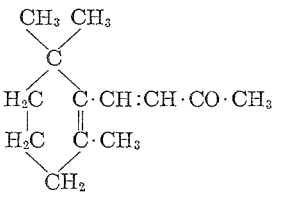
By JOHN B. WILSON*

The preparation of ionone by Tiemann and Krueger is considered one of the outstanding triumphs of modern chemical research. It is discussed in detail in works on perfumery and essential oils (1) (2) since ionone was the original artificial violet perfume. Later, ionone was found to be a mixture of two isomeric ketones, differing in the position of the double bond. Tiemann and Krueger were really aiming to produce irone, a third isomer, which they had found to be the characteristic odorous constituent of orris root (3). Irone differs from both α- and β-ionone by the position of the double bond. The constitutional formulas attributed to the three compounds are given below.

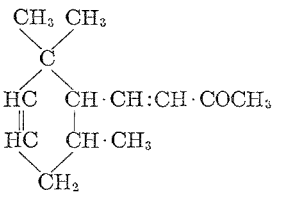
α-ionone



β-ionone



β-irone



Besides the three compounds under discussion, fifteen other isomers having the formula $C_{13}H_{20}O$, including α-irone, iso-irone, and tuberone, are described in the literature (4), but none has a commercial importance approaching that of α- and β-ionone, which have been used extensively in the perfume industry for almost a half century.

Some years ago manufacturers began to add β-ionone to flavors because when diluted this chemical perfume gives an odor resembling that of raspberries. Raspberries are among the most easily manipulated fruits for use in the manufacture of flavors. The color of raspberries, especially the black varieties, is sufficiently intense to permit considerable dilution before it fades into insignificance. The flavor of raspberries, in spite of its delicacy, is quite characteristic, and it usually survives manipulations better than do the flavors of most other fruits. However, the addition of 1 or 2 mg. of β-ionone to 1 ounce of a so-called true fruit raspberry flavor will permit dilution to one gallon (128 fluid ounces) of bottler's or fountain sirup whereas only 30 fluid ounces would be possible without such addition. In the preparation of dry products, such as gelatin desserts, even

* Contribution from the Beverage Section of the Food Division, Food and Drug Administration, U. S. Department of Agriculture. Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November 14–16, 1938.

greater dilutions are possible because the evanescent fruit flavor may be lost before the product reaches the ultimate consumer, while the less volatile β-ionone remains to give an artificial raspberry flavor to the final product.

REVIEW OF LITERATURE

A review of the available literature brought to light a number of methods designed for use in the examination of commercial ionones for purity. Five derivatives of β-ionone are described in Beilstein (4). The oxime is an oil, while the oxime acetic acid and ketazine are described as crystalline substances melting at 103° and 105° C., respectively. The semicarbazone and thiosemicarbazone melt at 148°–149° C. and 153° C., respectively.

The procedures given by Tiemann (5) for preparation of the first four of the derivatives mentioned above are recommended for use in the commercial purification of ionone and are designed for this purpose. Tiemann (5) also gives directions for the preparation of the *p*-bromophenylhydrazone, oxyionolactone, and other compounds of value in separating the isomers commercially.

A number of other procedures recommended especially for ionone by various authors were tried out, as well as some reagents frequently used for the detection and determination of aldehydes and ketones. The results are given below.

EXPERIMENTAL

The purity of the β-ionone used in the early experiments was determined by the method of Hendrikse and Reclaire (6) and found to be 93.2 per cent and 92.96 per cent in two duplicate determinations. Two duplicate determinations by the method of Radcliffe and Swan (7) for total ketones gave 91.88 per cent and 91.66 per cent, respectively. These procedures are applicable to comparatively pure commercial ionones, but not to quantities of 100 mg. or less in complex mixtures.

The most promising method found in the literature, from the point of view of determining moderate quantities of ionone, was that of S. Ito (8). This method, which is recommended for use on about 200 mg. of sample, precipitates the ionone as semicarbazone. It was applied by the writer to solutions containing various quantities of β-ionone, with the results given in Table 1. As Ito's method is not readily available in English, it is recorded here. The free translation given was prepared by the writer from a literal translation made by Saburo Katsura from the original article in Japanese.

Dissolve a weighed portion of the ionone in a small quantity of alcohol, add a saturated water solution of semicarbazide HCl containing three times the quantity necessary for combination with the ionone believed to be present. Add more alcohol if necessary to obtain a clear solution. Permit the mixture to stand for 24 hours.

Add 200 cc. of cold water, place in a refrigerator, and after 24 hours filter, dry, and weigh. (Ito used .15–.40 gram of sample in testing the procedure.)

The semicarbazone formed is a white sticky precipitate that filters readily but melts even on drying at 80° C. in vacuo in spite of a recorded melting point of 148°–149° C. On cooling the melted substance gives the appearance of rosin. The data (Table 1) show a good recovery for quantities approaching 200 mg., but recovery falls off rapidly as the quantity of ionone is decreased.

TABLE 1.—*Determination of β-ionone by Ito's method*

EXPERIMENT NO.	β-IONONE PRESENT	SEMICARBAZONE	β-IONONE FOUND	RECOVERY
	gram	*gram*	*gram*	*per cent*
111	0.2307	0.2814	0.2170	94.06
112	0.1355	0.1596	0.1231	90.85
113	0.0757	0.0850	0.0655	86.53
114	0.0372	0.0382	0.0295	79.30
115	0.0100	0.0043	0.0033	33.00

The writer also attempted to prepare crystalline β-ionone semicarbazone as directed by Veibel (9), Tiemann and Krueger (10), and Chuit (11), but was not successful.

The thiosemicarbazone formed when the determinations of purity were made by the Radcliffe and Swan method (7) was recovered by evaporation of the carbon bisulfide and found to be a thick oily liquid that did not crystallize despite all efforts to cause it to do so. A portion of the compound was dissolved in alcohol, but it did not crystallize even when kept in the refrigerator for two weeks after dilution with water. No crystalline β-ionone thiosemicarbazone was obtained when the procedure recommended by Chuit (11) was used, and when silver nitrate was added to the alcohol solution of the salt as recommended by Neuberg and Neimann (12) for precipitation of metallic salts of thiosemicarbazones of aldehydes and ketones, the white precipitate that formed at once turned dark brown in a few moments, probably due to the reducing effect of the double bond in the ionone.

Tiemann (5) states that when concentrated solutions of *p*-bromophenylhydrazine and β-ionone in acetic acid are mixed, crystals of β-ionone-*p*-bromophenylhydrazone separate out. The writer added a solution of 0.2 gram of *p*-bromophenylhydrazine hydrochloride and 0.2 gram of sodium acetate in 10 cc. of acetic acid to 100 mg. of β-ionone in 5 cc. of acetic acid. As no precipitate had formed after 48 hours, 15 cc. of water was added to the solution, which then became cloudy and began to deposit crystals within 15 minutes. Gradually, 40 cc. of water was added with perceptible increases in the quantity of crystals formed. After the solution had stood 48 hours the crystals were filtered off, washed with

water, dried at 100° C., and weighed. The weight of the crystals corresponded to 58.4 mg. of β-ionone, showing a recovery of 62.8 per cent based on the purity of 93 per cent β-ionone in the sample taken. The crystals were dark in color.

As these reagents did not give acceptable results, the writer tried a large number of reagents known to have produced crystalline precipitates with one or more aldehydes or ketones, and at the same time made a blank determination to obviate errors due to insolubility of the reagent under the conditions obtaining.

Para-nitrophenylhydrazine, methylphenylhydrazine, diphenylhydrazine, benzylphenylhydrazine, betanaphthyl-hydrazine, hydroxylamine, thiosemicarbazide, dimethylhydroresorcinol, and 4-4-diphenylsemicarbazide, when used as directed in "Analyse Konstitutionsermittlung Organisches Verbindungen," by Hans Meyer, yielded with β-ionone oily or resinous precipitates ill-suited to quantitative work.

β-ionone gave a small amount of highly colored crystals with 2-4 dinitrophenylhydrazine (13) and with semioxamizid white crystals similar to those obtained in the blank.

META-NITROBENZHYDRAZIDE AS A PRECIPITANT FOR β-IONONE

Finally the writer turned to *m*-nitrobenzhydrazide, which was recommended as a precipitant for aldehydes and ketones by Curtius and his coworkers (14). A quantity (0.3 gram) of *m*-nitrobenzhydrazide was dissolved in 20 cc. of alcohol (1+1), and 0.23 gram of β-ionone dissolved in 10 cc. of alcohol was added. After two days it was found that crystals had formed in the liquid. About 30 cc. of water was added a little at a time until the solution became cloudy. The flask was stoppered and again set aside for two days, at the end of which time a perceptible increase in the quantity of precipitate was noted. Some of the precipitate was crystalline and some oily in appearance.

Encouraged by the results of this experiment the writer added 0.3 gram of *m*-nitrobenzhydrazide dissolved in 20 cc. of alcohol (1+1) to 10 cc. of alcohol containing 200 mg. of β-ionone. The flask was stoppered, and after three days the well-formed crystals were filtered off, washed with 18 cc. of dilute alcohol (3+4), then with 100 cc. of water, dried at 100° C., and weighed. The precipitate amounted to 0.1366 gram, equivalent to 73.9 mg. of β-ionone, or about 37 per cent of the quantity taken. Several more precipitations were made, and it was found that by diluting the solution with water the yield could be increased to 87 per cent, but in every case after dilution some oily matter was present in the precipitate.

As directed by Curtius and Reinke (15) for precipitation of aldehydes and ketones with *m*-nitrobenzhydrazide, 10 cc. of alcohol containing 100 mg. of β-ionone was diluted to 350 cc. with water, and 0.3 gram of the reagent dissolved in 30 cc. of alcohol (2+1) was added. The mixture was

refluxed on the steam bath for 30 minutes, cooled, and after being allowed to stand 3 days it was filtered. The precipitate was washed, dried, and found to weigh 89 mg., equivalent to 48 mg. of β-ionone. The filtration was very slow, and as the precipitate adhered to the flask in an alarming manner, it was washed out with ether, dried, and found to weigh 22 mg., corresponding to 12 mg. of β-ionone. Other quantities of ionone yielded similar recoveries.

A variation of the above procedure suggested by S. Reznek of this Administration was also tried. The *m*-nitrobenzhydrazide was dissolved in acetic acid (1+3) and added to 100 cc. of 5 per cent alcohol containing 50.3 mg. of β-ionone. After standing overnight the mixture was quite cloudy. After being shaken violently for 5 minutes, it was found that the precipitate was no longer attached to the sides and bottom of the flask but floated about in the otherwise clear solution. This precipitate, as well as two others obtained from other quantities of β-ionone, was filtered, dried, and weighed. The results are given in Table 2.

TABLE 2.—*Determination of β-ionone as m-nitrobenzhydrazide as directed by S. Reznek*

EXPERIMENT NO.	β-IONONE PRESENT	WT. OF PRECIPITATE	β-IONONE FOUND	RECOVERY
	mg.	*mg.*	*mg.*	*per cent*
186	50.3	81	43.8	87
247	50.0	77	41.7	83
248	20.0	30	16.2	81

It was found that precipitation occurred in the reagent after a few days, which caused doubt as to the composition of the precipitates obtained above. This factor, in conjunction with the low yields, caused the writer to abandon work on this procedure.

As a preliminary step to increasing the yield of β-ionone-*m*-nitrobenzhydrazide obtained by the first procedure tried, experiments were conducted to ascertain the solubility of this salt in various strengths of alcohol. A weighed quantity of substance was placed in a small Erlenmeyer flask, dissolved in a mixture of alcohol and water by warming on the steam bath, stoppered, and set aside for about 1 hour to cool. Some of the mixture was left in the room, and some was placed in an electric refrigerator kept at about 10° C. After 24 hours the precipitate was filtered, washed twice with 5 cc. portions of dilute alcohol (30 cc. of 95 per cent alcohol made up to 100 cc. with water), dried at 100° C., and weighed. The results are given in Table 3.

The data in Table 3 show that cold 30 per cent alcohol has little solvent action upon β-ionone-*m*-nitrobenzhydrazide, especially in the presence of excess reagent and acetic acid, and is therefore a suitable medium for precipitating and washing this compound.

After a considerable amount of experimentation with a view to obtaining as large a yield as possible and at the same time obtaining a crystalline precipitate containing as little oily material as possible, a procedure for precipitating β-ionone as *m*-nitrobenzhydrazide was worked out as given below.

TABLE 3.—*Solubility of β-ionone-m-nitrobenzhydrazide in alcohol of various strengths*

EXPERIMENT NO.	ALCOHOL USED		TEMPERATURE	β-IONONE-M-NITROBENZHYDRAZIDE			SOLUBILITY
	VOLUME	STRENGTH		TAKEN	RECOVERED	DISSOLVED	
	cc.	*per cent*		*mg.*	*mg.*	*mg.*	*mg. per 100 cc.*
295	12.0	55.4	Room	102.4	81.4	21.0	175.0
299	19.0	45.0	Room	101.8	94.7	7.1	37.4
298	17.0	39.1	Room	100.0	95.0	5.0	29.4
301	14.0	47.5	Room	102.9	92.3	10.6	75.7
303	20.0	47.5	Room	106.2	98.5	7.7	38.5
296	12.0	55.0	Refriger.	113.0	102.0	11.0	91.7
302	14.0	47.5	Refriger.	100.9	95.8	5.1	36.4
304	20.0	47.5	Refriger.	99.6	93.6	6.0	30.0
300	19.0	45.0	Refriger.	114.5	108.8	5.7	30.0
318*	16.0	32.7	Refriger.	51.5	49.8	1.7	10.6
323*	15.5	30.6	Refriger.	50.0	50.0	0.0	0.0
311*	15.0	30.0	Refirger.	115.8	113.8	2.0	13.3
310*	15.0	30.0	Refriger.	113.5	112.9	0.6	4.0
309*	15.0	30.0	Refriger.	102.5	101.3	1.2	8.0

* 0.05 of *m*-nitrobenzhydrazide and 0.2 cc. acetic acid added.

QUANTITATIVE PRECIPITATION OF β-IONONE AS *M*-NITROBENZHYDRAZIDE

Place 5 cc. of alcohol containing 10–100 mg. of β-ionone in a 125 cc. conical flask. Add 95–100 mg. of solid *m*-nitrobenzhydrazide and dissolve by warming the solution on the steam bath, taking precautions to prevent loss of alcohol through evaporation. Add 5 cc. of water, and if the solution becomes cloudy, warm until clear. Remove the solution from the steam bath, add 0.2 cc. of glacial acetic acid, stopper the flask lightly, and place upon a wooden surface to prevent too rapid cooling. If about 20 mg. or more of β-ionone is present, crystals will begin to form within 30 minutes after the contents of the flask have reached room temperature. Let stand in the room for at least 2 hours (overnight does no harm) and add 5 cc. of water dropwise, mixing the solution continuously during the addition by rotating the flask. Stopper, let stand in the room for at least 1 hour, and place in the refrigerator overnight or up to 48 hours. Filter through a No. 4 sintered glass crucible, wash with 30 cc. of cold 30% alcohol, using a wet policeman to remove precipitate adhering to the flask, and dry at 100° C. Weight of precipitate multiplied by 0.541 gives the corresponding weight of β-ionone.

A number of commercial β-ionones were purchased on the open market, and duplicate ionone determinations were made by the above procedure, 100 mg. of sample and 100 mg. of the reagent being used. In addition, determinations of total ketones as ionone were made by the Radcliffe and Swan method (7). The results are given in Table 4.

TABLE 4.—*Ionone and total ketones, as ionone, in commercial β-ionones*

SAMPLE NO.	IONONE	TOTAL KETONES AS IONONE
	per cent	*per cent*
1	94.68	93.20
	93.80	92.96
2	96.14	100.00
	95.71	
3	95.19	99.19
	94.88	
4	85.04	86.83
	84.08	

To establish the composition of the precipitate, four 5 cc. portions of alcoholic solution containing 50 mg. of a commercial β-ionone were precipitated in the usual manner and filtered upon Gooch crucibles. After they had been dried and weighed, the precipitate and asbestos were mixed with prepared copper oxide and transferred quantitatively to the combustion boat of an apparatus for the determination of nitrogen by the Dumas method. The results are given in Table 5.

TABLE 5.—*Determination of nitrogen on β-ionone-m-nitrobenzhydrazide precipitates by the Dumas method*

NO.	β-IONONE	PRECIPITATE	β-IONONE		NITROGEN	
	mg.	*mg.*	*mg.*	*per cent*	*mg.*	*per cent*
452	50	82.4	44.9	89.8	9.89	11.92
454	50	83.6	45.2	90.4	10.06	12.03
456	50	86.7	46.9	93.8	10.79	12.44
458	50	92.5	50.0	100.0	10.81	11.69
Theory	50	92.5	50.0	100.0	10.94	11.83

The data in Table 5 show that the precipitate has the composition expected for β-ionone-*m*-nitrobenzhydrazide.

To test the applicability of the method to other quantities of β-ionone, a series of solutions was prepared to contain varying quantities of substance. For the stock solution 6.0035 grams of β-ionone was dissolved in 95 per cent alcohol and diluted to 300 cc. with the same solvent. Various quantities of the stock solution were diluted with alcohol to 100 cc., and ionone was determined by the proposed method. The data are given in Table 6.

The data in Table 6 indicate that the accuracy of the proposed method for β-ionone is substantially the same for quantities ranging from 10 to 100 mg. in 5 cc. of alcohol solution.

The low solubility of β-ionone-*m*-nitrobenzhydrazide in 30 per cent alcohol, in which solvent the precipitation was made, and the uniformity of the ionone content found when such widely different quantities of

ionone were used for the precipitation lead to the conclusion that the commercial ionone used for these experiments actually contains 96 per cent of ionone and 4 per cent of impurities. The material used here was from Sample 2, reported in Table 4 as showing 96.14 per cent and 95.71 per cent of β-ionone by the recommended procedure and 100 per cent total ketones as ionone by the Radcliffe and Swan method. No evidence has been obtained as to the composition of the impurities present in this product.

TABLE 6.—*β-ionone in stock solution and its varying dilutions*

SOLUTION	VOLUME OF STOCK DILUTED TO 100 CC.	β-IONONE IN 5 CC. ALIQUOT	PRECIPITATE	β-IONONE	RECOVERY
	cc.	*mg.*	*mg.*	*mg.*	*per cent*
A	100	100	175.4	94.9	94.9
B	50	50	89.2	48.2	96.4
C	40	40	70.4	38.1	95.2
D	30	30	53.0	28.7	95.7
E	20	20	35.6	19.3	96.5
F	10	10	18.0	9.7	97.0

When applied to a number of commercial products for use in violet perfumes, about 1 gram was dissolved in alcohol and diluted to 100 cc. with the same solvent, and 5 cc. was treated as described above. Total ketones were also determined by the method of Radcliffe and Swan, a 1 gram sample and about 1 gram of thiosemicarbazide being used. The β-ionone found in these products, which from the standpoint of their use may well be grouped under "imitation violet oil," is given in Table 7.

TABLE 7.—*β-ionone and total ketone content of imitation violet oils*

MANUFACTURER	β-IONONE	KETONES AS IONONE
	per cent	*per cent*
A	81.51	85.19
	80.10	
B	73.68	71.58
	73.56	
C	64.40	63.16
	63.96	
D	52.06	

As many raspberry flavors, especially those of the so-called "True Fruit Type," contain a base of concentrated raspberry juice or alcoholic extract of fresh or dried raspberries to which may be added distillates from the berries and possibly small quantities of synthetic ingredients, the procedure of steam distillation was applied as a means of separating any β-ionone from the commercial flavor. The procedure of steam dis-

tillation was applied successfully in Part II of this series (16) and in studies of the volatile constituents of fruits (17) (18) (19).

Since the publication of the writer's paper in 1932, the advent of the standardized ground-glass joint has made it possible to improve the apparatus used for steam distillation by substituting all glass equipment of standard dimensions, which is listed below.

As previous experiments had shown that the procedure for aldehydes and ketones described in Part II of this series was not applicable to β-ionone, it was thought possible that after steam distillation the ionone could be extracted with a volatile solvent, held back by the reagent during evaporation of the solvent, and afterwards recrystallized or reprecipitated in the same manner as described above for quantitative precipitation of β-ionone. After considerable experimentation the procedure given below was devised. Both petroleum ether and sulfuric ether were tried for the extraction; the number of extractions needed were subjected to test; and the time of standing varied within certain limits.

β-IONONE IN RASPBERRY FLAVORS

APPARATUS

(a) *Steam generator filled with water.*—An oil can holding 1 gallon will serve the purpose.

(b) *Distillation flask.*—Round-bottomed boiling flask having interchangeable ground-glass connection 24/40, capacity about twice the volume of sample to be used.

(c) *Still head.*—Adapter, 75° angle, with interchangeable male connections 24/40 at bottom and side and female connection 14/35 at top, with side arm lengthened and bent to fit vertical condenser (like Ace Glass Co. No. 1180).

(d) *Spray tube.*—Adapter, for use with Woulff bottles equipped with interchangeable ground-glass connection, aeration tube with connection 14/35, holes in bulb approximately 2 mm. in a diameter, length of tubing such that when the apparatus is set up, the bulb is situated not more than 20 mm. above the bottom of the distilling flask (like Ace Glass Co. 1300-C).

(e) *Condenser.*—Coil type with interchangeable female connection 24/40 at top with 250 to 300 mm. jacket and outlet tube lengthened to about 200 mm. to reach bottom of receiving flask.

(f) *Receiving flask.*—Conical flask of 500 cc. capacity.

REAGENTS

(a) *Ethyl ether.*—Containing practically no alcohol.

(b) *M-nitrobenzhydrazide.*—Obtainable from Eastman Kodak Co.

(c) *Glacial acetic acid.*—Reagent grade.

(d) *Alcohol.*—95% by volume.

(e) *Dilute alcohol.*—Place 30 cc. of alcohol in a 100 cc. volumetric flask and dilute to the mark with water. Keep in a refrigerator.

PROCEDURE

Place 250–1000 cc. of sample (which should contain not more than 100 mg. of β-ionone) in the distilling flask and connect with the apparatus. Add enough water to the receiving flask to just cover the outlet of the condenser. Heat the sample

nearly to boiling on an asbestos mat with a flame or by immersing it in a boiling water bath. As soon as the sample has reached the temperature of the bath or has just begun to boil, connect with the steam generator and pass a rapid current of steam through the sample until 300–500 cc. of distillate has been collected.

Add sufficient water to the distillate to reduce the alcohol content to about 10 per cent or less and transfer to a large separatory funnel. Add 150–200 cc. of ether, depending upon the volume of solution, so that about 100 cc. will be obtained upon separation. Shake thoroughly and separate. Transfer the ether solution to a 125 cc. conical flask containing 95–100 mg. of *m*-nitrobenzhydrazide. Add 0.2 cc. of acetic acid and dissolve the solid reagent by stirring and breaking up lumps with a glass rod, warming if necessary to complete the solution. Permit the mixture to stand for about 1 hour and evaporate on the steam bath to about 10 cc., passing a current of air into the flask to hasten the evaporation and keep down the temperature. In the meantime make a second extraction of the distillate, using 100 cc. of ether. Add the separated ether solution to the flask containing the residue from the first ether extract and after allowing to stand about 15 minutes evaporate to 10 cc. as before. In a similar manner make a third extract, using 100 cc. of ether, add to the flask, and evaporate as before until only 1–3 cc. of watery liquid and perhaps some oily residue remain.

While the flask is still warm, add 5 cc. of alcohol from a pipet, allowing the liquid to wash down the sides of the flask, and dissolve the residue completely by warming on the steam bath, protecting the liquid against loss by evaporation. Add 5 cc. of water and warm if necessary to obtain a clear solution. Add 0.2 cc. of acetic acid, close with a cork stopper, and place the flask on a wooden surface to prevent too rapid cooling.

After 2 hours add 5 cc. of water dropwise, mixing the liquid by continuously rotating the flask, stopper, and keep at room temperature for at least 1 hour (overnight does no harm), then place in the refrigerator overnight or up to 48 hours.

Filter on a fritted glass crucible of porosity 4 and wash with about 30 cc. of dilute alcohol. Dry in a vacuum over at 70° C. and weigh. Wt. of ppt. $\times$ 0.541 = β-ionone.

This procedure was applied to the same diluted solutions that were used for testing the precipitation method and reported in Table 6. In each determination 5 cc. of the solution of β-ionone was added to 20 cc. of alcohol and 225 cc. of water in the steam distillation apparatus, and the procedure was carried out as described above. The results are given in Table 8.

TABLE 8.—*Recoveries of β-ionone by the proposed method*

SOLUTION	PRESENT	FOUND BY DETERMINATION				
		1	2	3	AV.	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*
A	100	94.7	96.0	96.8	95.8	95.8
B	50	47.0	47.5	47.8	47.4	94.8
C	40	37.9	38.1	38.4	38.1	95.2
D	30	28.5	28.7	28.8	28.7	95.7
E	20	18.8	18.9	19.0	18.9	94.5
F	10	9.4	9.0	9.5	9.3	93.0

The method was also applied to a number of commercial samples of products, labeled "True Fruit Raspberry," and while several products were found to contain no ionone, in a number of other cases the following quantities were determined: 50, 51, 72, 75, 77, 120, 132, and 160 mg. of β-ionone per liter of flavor. For several samples of both red and black raspberries negative results were obtained in every case. A detailed description of the procedure used is given in Part VI of this series, which follows this paper. As *m*-nitrobenzhydrazide combines with a number of aldehydes and ketones, precipitates obtained by the methods given here should be identified by the procedure given in Part VI.

SUMMARY

Several procedures recommended by various authors for the determination of ionone were tried out and found to give unsatisfactory results. A number of procedures involving the use of 14 different reagents recommended for the precipitation of aldehydes or ketones were then tried, and it was found that one reagent, *m*-nitrobenzhydrazide, gave a precipitate with β-ionone that appeared to be suited to its quantitative determination. Several procedures recommended for the application of this reagent to aldehydes and ketones were subjected to test and the most suitable one selected. A method was then developed for the quantitative determination of β-ionone as *m*-nitrobenzhydrazide and found to be accurate for quantities ranging from 10 to 100 mg. of β-ionone. The writer expects to apply this procedure to other similar ketones as a possible means of their identification.

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IDENTIFICATION OF FLAVORING CONSTITUENTS OF COMMERCIAL FLAVORS

VI. IDENTIFICATION OF β-IONONE AS *M*-NITROBENZHYDRAZIDE

By JOHN B. WILSON and GEORGE L. KEENAN*

Part V (1) of this series of papers deals with the quantitative determination of β-ionone. The reagent used is *m*-nitrobenzhydrazide, which was recommended as a precipitant for aldehydes and ketones by Curtius (2) and his co-workers, and was used for the quantitative determination of vanillin by Hanus (3). However, the writers did not consider the formation of a precipitate as sufficient evidence of the presence of β-ionone in commercial flavors, and wished to obtain a positive means of identifying β-ionone in products of unknown composition. To accomplish this end they had recourse to the immersion method used for the identification of certain aldehydes and ketones and reported in Part I of this series (4).

The procedure employed in Part I could not be used for preparation of a derivative since semicarbazide had been shown unsuitable for this purpose, as reported in Part V. However, the precipitate of β-ionone *m*-nitrobenzhydrazide that was found (Part V) to serve so well as a means of determining β-ionone, appeared to be crystalline to the naked eye. Accordingly, a quantity of the substance was prepared for microscopic study.

PREPARATION OF β-IONONE-*M*-NITROBENZHYDRAZIDE

After several experiments it was found that well crystallized β-ionone-*m*-nitrobenzhydrazide can be prepared in the following manner:

> Place about 500 mg. of β-ionone and 600 mg. of *m*-nitrobenzhydrazide in a 200 cc. conical flask, add 50 cc. of alcohol, and dissolve by warming on the steam bath. When all the hydrazide is in solution, add 50 cc. of hot water, and continue the heating on the steam bath for about 5 minutes. Stopper the flask and set aside for about 20 minutes, then add 2 cc. of acetic acid and let stand overnight at room temperature and then in the refrigerator for 5–6 hours. Filter through a fritted glass crucible of No. 4 porosity, wash with several portions of 30 per cent alcohol totaling about 50 cc., and dry in vacuo at 70° C.

β-ionone-*m*-nitrobenzhydrazide made in this manner was found to be well suited to microscopic identification. When nitrogen was determined by the Dumas method the results obtained were 13.20, 13.27, and 13.24 per cent, which is somewhat higher than the theoretical result, 11.83 per cent.

This material was examined microscopically by the method described in Part I (4) and its properties are described below.

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* Joint contribution from the Beverage Section of the Food Division and the Microanalytical Division, Food and Drug Administration, U. S. Department of Agriculture. Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November 14–16, 1938.

OPTICAL CRYSTALLOGRAPHIC PROPERTIES OF β-IONONE-*m*-NITROBENZHYDRAZIDE

To the naked eye, this substance in mass has a yellowish color. When examined in ordinary light under the microscope, the material is essentially colorless and crystallizes in thin, rod-like plates, many of them having lath-like or frayed ends, some of them having a six-sided outline. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation negative. The refractive indices as determined by the immerson method were the minimum and maximum values, these being $n_\alpha = 1.548$, invariably shown on the elongated fragments when their long dimension is parallel to the vibration plane of the lower nicol (lengthwise), and $n_\gamma = 1.648$ usually shown on the elongated fragments when their long dimension is at right angles to the vibration plane of the lower nicol (crosswise).

To identify β-ionone in a food product, steam distil a suitable sample of the food. Extract the distillate with ether and follow the procedure for the determination of β-ionone as given in Part V of this series. If a precipitate is obtained, examine it microscopically by the immersion method. If the precipitated material consists of oily matter mixed with crystalline matter, place the fritted glass crucible in a Gooch holder attached to a suction flask. By means of a wire support a test tube within the suction flask in such a manner as to catch any liquid that may pass through the crucible. Add about 5 cc. of petroleum ether, cover the crucible, and let stand for about 5 minutes. Turn on the suction just long enough to carry through any of the solvent that may remain in the crucible. Transfer the petroleum ether solution to a small beaker and allow it to evaporate spontaneously. Repeat several times until no more soluble matter is obtained by the extraction. Examine the remaining contents of the crucible and the several residues microscopically for crystals of β-ionone-*m*-nitrobenzhydrazide.

This procedure was applied to a number of commercial raspberry flavors and β-ionone was identified as a constituent. Subsequently, two of the manufacturers admitted that this substance had been added to their products. Several other commercial raspberry flavors were examined and no β-ionone-*m*-nitrobenzhydrazide was found in the insoluble matter.

In order to establish the presence or absence of β-ionone as a constituent of raspberries, a number of samples were examined. Two one quart samples of red raspberries weighing 668 grams and 632 grams, respectively, and two one quart samples of black raspberries weighing 615 grams and 654 grams, respectively, were examined by the recommended procedure, and no crystalline material whatsoever was found when the insoluble matter obtained was examined microscopically.

Larger samples were used for the next experiments. Three 3 kg. portions of frozen pack red raspberries (3+1) totaling 15 pounds of the berries were steam distilled. The steam distillates were extracted with ether in rotation, and the ether extracts were evaporated in a small flask containing 100 mg. of *m*-nitrobenzhydrazide, which was dissolved in the first portion of ether extract by warming slightly, and 0.2 cc. of acetic acid was added. The evaporation was carried out on a steam bath while a current of air played upon the surface of the liquid, the surface of the flask being cold to the touch at all times. When the solution had been evaporated to about 10 cc. the second portion of ether was added, allowed

to stand in contact with the reagent 15–20 minutes, then evaporated as before. This process was repeated until all the ether extracts had been evaporated in the same flask after being left in contact with the reagent long enough for the formation of the *m*-nitrobenzhydrazide of any β-ionone that might have been present in the distillate. The final evaporation was continued until only 2–3 cc. of watery liquid and some oily drops remained in the flask. The residue had the odor of raspberries, but no odor of β-ionone could be detected. The residue was then dissolved in 5 cc. of 95 per cent alcohol, and 5 cc. of water and 0.2 cc. of acetic acid were added. The solution stood overnight at room temperature to permit β-ionone-*m*-nitrobenzhydrazide (if present) to crystallize. The next day 5 cc. more water was added, and the solution was allowed to remain in the refrigerator for about 48 hours. It was then removed, filtered, washed with 30 per cent alcohol, dried, and weighed.

When submitted to microscopic examination, no crystals of β-ionone-*m*-nitrobenzhydrazide were present, nor could any other crystalline material of any kind be found in the crucible. The crucible contained only a minute quantity of oily or gummy material, which had partially soaked into the fritted glass of the crucible and still retained the odor of the berries, which odor was also apparent in the filtrate. When washed with petroleum ether, none of the matter in the crucible dissolved or came through the crucible.

The test was repeated on another sample of red raspberries consisting of four portions aggregating 13 kg. of frozen pack red raspberries with sugar, containing not less than 24 pounds of red raspberries. Here again no crystals of β-ionone-*m*-nitrobenzhydrazide occurred, nor was any other crystalline material found.

A 12.2 kg. sample of frozen pack black raspberries with sugar, containing not less than 22 pounds of black raspberries, and a 10 kg. sample of frozen pack black raspberries (3+1) seedless, containing not less than 16 pounds of fruit, were examined in the same manner. The microscope showed that the insoluble matter contained no crystals of β-ionone-*m*-nitrobenzhydrazide nor any other crystalline material whatsoever.

In the opinion of the writers, these data constitute a negative result for the test for β-ionone in either red or black raspberries.

SUMMARY

A quantity of β-ionone-*m*-nitrobenzhydrazide was prepared, and its optical crystallographic properties were determined. Three samples each of red and black raspberries, consisting of as much as 24 pounds and 22 pounds, respectively, were examined, and no β-ionone was found to be present.

In certain cases samples of commercial so-called true fruit raspberry flavors were found to contain β-ionone, and in other cases none was found.

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IDENTIFICATION OF FLAVORING CONSTITUENTS IN COMMERCIAL FLAVORS

VII. QUANTITATIVE DETERMINATION OF COUMARIN

By JOHN B. WILSON*

During the past few years the physiological effects of coumarin and its degradation products in cured hay gave rise to the need for a precise method for the determination of coumarin for the use of the plant physiologists who have been conducting breeding experiments on grasses with a view to eliminating this constituent from this type of forage. This need has now been met by the work of several investigators. Clayton and Larmour (1) appear to have been the first to suggest the use of the color produced by coupling coumarin with diazo-*p*-nitroaniline in alkaline solution for this purpose, although the original work on this condensation was reported by Mitchell (2), and the same color used by Chakravati (3).

After a thorough investigation Roberts and Link (4) published a method for the determination of coumarin, melilotic acid, and coumaric acid in plant tissue. Duncan and Dustman, whose steam distillation method for coumarin (5) was published several years ago, have revised their procedure and are now (6) substituting a modified form of the Roberts and Link procedure for the actual colorimetric measurement of coumarin for their previous color test and recommend the distillation method for the determination of coumarin in vanilla.

The writer desired to find a quick method for the determination of coumarin in imitation vanilla, which according to trade practice may contain from 0.05 to 0.20 per cent of coumarin, with vanillin in proportions ranging from 0.10 to 0.70 per cent, or even more on occasion. As the official method for coumarin (7) is somewhat tedious, and as Duncan and Dustman (6) have shown that at least two and sometimes three steam distillations under reduced pressure are needed to completely recover added coumarin from vanilla products, the writer sought to use the colorimetric procedure without distillation.

EXPERIMENTAL

Solutions of vanillin and coumarin were prepared of such strength that the colorimetric test could be applied to different quantities of each,

* Contribution from the Beverage Section of the Food Division, Food and Drug Administration, U. S Department of Agriculture. Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November 14–16, 1938.

either alone or mixed in varying proportions. The test of Duncan and Dustman (6) was applied, and it was found that both substances give a color to the naked eye. However, with the assistance of P. A. Clifford of the U. S. Food and Drug Administration the writer found that when the several solutions were examined in a spectrophotometer designed by Clifford and Brice and the proper filter used, coumarin could be determined even in the presence of vanillin. It was also found that filter No. 49 gave the best spread over a workable range with coumarin and at the same time registered the least interference from vanillin.

As it had been recorded by Duncan and Dustman that vanillin interferes with this colorimetric determination, the first experiments were made with increasing quantities of coumarin and with a quantity of vanillin five times as great as the maximum quantity of coumarin. This is a ratio frequently found between these two flavoring ingredients in imitation vanilla. The results of this experiment are given in Table 1.

TABLE 1.—*Measurement of color intensity of coumarindiazo-p-nitraniline*

VANILLIN IN 50 CC.	COUMARIN IN 50 CC.	READING FILTER NO. 49 AFTER—	
		2 HRS.	24 HRS.
mg.	*mg.*	*mm.*	*mm.*
3	0.0	3.5	9.8
3	0.1	25.5	31.5
3	0.2	48.5	54.8
3	0.3	72.2	78.3
3	0.4	94.4	99.4
3	0.5	125.0	128.6
3	0.6	off scale	

The results given are in all cases the average of 5–10 individual readings.

The data in Table 1 indicate that quantities of coumarin up to 0.5 mg. in the aliquot used may be determined with the spectrophotometer even in the presence of vanillin and that the color deepens somewhat on standing for 24 hours.

The next experiment was to ascertain the effect upon solutions of the same coumarin content of varying the proportion of vanillin. As 0.3 mg. of coumarin appears to be the mean of the workable range, this quantity was used, and the vanillin was varied from 0.0 to 10.0 mg., which last quantity is 33 times the quantity of coumarin present. The data are given in Table 2.

As heliotropin (piperonal) is sometimes used in imitation vanilla, the effect of this substance was tried in a similar manner (Table 3).

These two experiments show that neither vanillin nor heliotropin has a deleterious effect upon the determination of coumarin by the procedure recommended.

TABLE 2.—*Effect of varying proportions of vanillin on coumarin determination*

COUMARIN IN 50 CC.	VANILLIN IN 50 CC.		READING FILTER NO. 49 AFTER—	
			2 HRS.	24 HRS.
mg.	*mg.*	*ratio, 1 to—*		
0.3	0.0		72.9	80.4
0.3	0.5	1.7	71.5	80.2
0.3	1.0	3.3	71.1	80.9
0.3	1.5	5.0	70.3	81.7
0.3	2.5	8.3	70.1	79.8
0.3	4.0	13.3	68.8	80.8
0.3	5.0	16.6	71.7	82.1
0.3	7.5	25.0	71.1	83.7
0.3	10.0	33.3	71.5	88.0*
			Av. 71.0	81.2

* Omitted from the average.

TABLE 3.—*Effect of varying proportions of heliotropin on coumarin determination*

COUMARIN IN 50 CC.	HELIOTROPIN IN 50 CC.		READING FILTER NO. 49 AFTER—	
			2 HRS.	24 HRS.
mg.	*mg.*	*ratio, 1 to—*		
0.3	0.0		75.9	80.2
0.3	0.0		76.2	80.7
0.3	5.0	17	76.0	80.2
0.3	10.0	34	76.4	80.4
0.3	15.0	50	76.6	80.6
0.3	20.0	67	77.5	80.3

Experiments were then conducted to determine the applicability of a clarification procedure such as is used in the Folin-Denis colorimetric method for vanillin. Since it was evident that the addition of sodium carbonate as a reagent in the colorimetric method for coumarin would cause a precipitate with any residual lead acetate, it was decided to remove the lead with sodium oxalate before applying the color reaction. In order to increase the range of reading in the spectrophotometer it was decided also to increase the quantities of reagent. The method given below was tried out.

COUMARIN IN IMITATION VANILLA

REAGENTS

Standard coumarin solution.—Dissolve 0.1000 gram of pure coumarin in 10 cc. of alcohol and dilute to 100 cc. with water. To prepare a solution of coumarin containing 0.1 mg. per 1 cc., dilute 10 cc. of the standard solution to 100 cc. with water.

Lead acetate solution.—Dissolve 50 grams of neutral Pb acetate and 50 grams of basic Pb acetate in hot water, dilute to 1 liter, cool, and filter.

Sodium oxalate.—Anhydrous.

Sodium carbonate solution.—Dissolve 5 grams of anhydrous Na_2CO_3 in water and dilute to 500 cc.

Solution A: Dissolve 0.7 gram of *p*-nitraniline in 9 cc. of HCl and dilute to 100 cc. with water.

Solution B: Dissolve 5 grams $NaNO_2$ in water and dilute to 100 cc.

Diazonium solution.—Chill a 100 cc. flask and Solutions A and B to about 3° C. in a refrigerator or cold room or in chopped ice. Pipet 5 cc. of each solution into the flask, mix, and let stand in refrigerator 5 minutes. Add 10 cc. more of Solution B, return to the refrigerator for 5 minutes, then fill the flask to the mark with ice-cold water. The solution is ready for use in 15 minutes but must be discarded after 24 hours.

PREPARATION OF GRAPH

Place 1, 3, and 5 cc. portions of coumarin solution (1 cc. = 0.1 mg.) in as many 100 cc. volumetric flasks and add enough water to bring the volume to 20 cc.; add 10 cc. of Na_2CO_3 solution and heat on a water bath at 85° for 15 minutes or in a boiling water bath for 5 minutes. Allow the solutions to cool gradually; when they have reached room temperature, add 10 cc. of diazonium solution to each, fill to the mark with water, and mix. Let stand 1.5–2.0 hours and read in a spectrophotometer, using a No. 49 filter and a ½ inch cell. Plot the results on coordinate paper so that the quantity of coumarin can be read in terms of grams per 100 cc. of the original sample.

DETERMINATION

Pipet 5 cc. of imitation vanilla into a 100 cc. volumetric flask, and add 75 cc. of tap water and 5 cc. of Pb acetate solution. Fill to the mark with tap water. Mix, and filter through a folded filter. To the filtrate add 0.2 gram of anhydrous Na oxalate and dissolve in the filtrate by rotating the container. After the reagent has dissolved completely, rotate again for a few seconds, let stand at least 5 minutes, and filter through a 11 cm. S & S filter 589.

Transfer 5 cc. of the filtrate to a 100 cc. volumetric flask and treat in the same manner as were the standards used in preparing the graph. The final solution has been subjected to a dilution of 400 times if the quantities recommended were used.

The above procedure was applied to a set of imitation vanillas of known composition. The results given in Table 4 were obtained.

The data in Table 4 show that results of reasonable accuracy can be obtained when the colorimetric method for coumarin is applied to imitation vanilla clarified with lead acetate and that the distillation with steam under reduced pressure, as recommended by Duncan and Dustman, may be dispensed with when quick determination of coumarin is desired.

When the method was applied to several vanilla extracts purchased on the open market, the readings obtained showed substantial amounts of coumarin when there was no indication of its presence from the taste or odor of the extract. Further work should be done to establish the composition of the substance in the vanilla that gives a reaction similar to that of coumarin under these circumstances. The data given in this paper clearly show that the color obtained on vanilla extract is not due to vanillin as was reported by Duncan and Dustman. The proportions of

TABLE 4.—*Coumarin in imitation vanilla*

VANILLIN	COUMARIN	VANILLA EXTRACT	CARAMEL TO COLOR	COUMARIN FOUND
g./100 cc.	*g./100 cc.*	*cc./1000 cc.*		*g./100 cc.*
0.60	0.151	50	+	0.147 0.152 0.144
0.65	0.050	40	+	0.058 0.064 0.06
0.70	0.175	60	+	0.174 0.164 0.180
0.72	0.200	30	+	0.196 0.204
0.50	0.100	90	+	0.104 0.088 0.110

coumarin indicated by this method as being present in the true vanilla samples were 0.057, 0.035, 0.09, 0.03, 0.038, and 0.04 gram per 100 cc. It will be remembered, of course, that the official gravimetric method for coumarin frequently yields as much as 0.04 gram per 100 cc. of extract (8) that does not respond to qualitative tests for coumarin nor does the residue have an odor in any way similar to coumarin.

SUMMARY

The condensation of coumarin with diazo-*p*-nitraniline has been shown to be applicable to imitation vanilla when clarified with lead acetate solution, thus forming the basis of a quick method for the determination of coumarin in imitation vanilla.

True vanilla extracts have been shown to contain a substance, not vanillin, that yields color with the reagent, so that in the case of true vanilla the test should be applied to a distillate, as recommended by Duncan and Dustman, before it may be concluded that this ingredient has been added to the vanilla.

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(3) CHAKRAVATI, *J. Indian Chem. Soc.*, **8**, 503 (1931).
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(6) ———, *Ibid.*, **9**, 416 (1937).
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A COMPARISON OF THE OFFICIAL AND MACINTIRE-SHAW-HARDIN METHODS FOR DETERMINING AVAILABLE PHOSPHORIC ACID

By J. Richard Adams (Bureau of Chemistry and Soils, Washington, D. C.)

At the 1937 meeting of the Association of Official Agricultural Chemists a paper was presented by MacIntire, Shaw, and Hardin[1] on a direct method for the determination of available P_2O_5. The method is claimed by the authors[2] to be a simple, rapid, and economical direct analytical procedure applicable to all types of phosphatic fertilizers. It consists in leaching a weighed sample of the fertilizer with 100 cc. of an ammonium nitrate-ammonium citrate solution of *p*H 4.2. The leached residue is then steam digested in another 100 cc. portion of this special citrated ammonium nitrate solvent for 30 minutes. The leachate and digestate are combined, made up to volume, and filtered. The P_2O_5 content of the filtrate is determined by the official method. This value is taken as a measure of the available P_2O_5 of the sample. The MacIntire-Shaw-Hardin method is thus a direct one in that the available P_2O_5 is determined as such rather than by the difference between the total and citrate-insoluble P_2O_5 as in the official method.

In fertilizer laboratories records are kept of both the total and citrate-insoluble forms of P_2O_5, and the available P_2O_5 obtained by the official method entails no additional determination. In over 80 per cent of the control laboratories, however, only the available P_2O_5 in a sample is required and in these laboratories the MacIntire-Shaw-Hardin method would offer an advantage over the official method in that it involves only one determination.

Because of the possibilities of this proposed method, it was suggested that a comparative study should be made of the official and MacIntire-Shaw-Hardin methods for determining available P_2O_5. In compliance with this suggestion and under the direction of the Associate Referee on Phosphoric Acid, the work presented here was carried out in order to determine how close an agreement exists between these two methods when used to determine available P_2O_5 in various phosphatic materials.

The steam digestion apparatus used is somewhat different from the one described by MacIntire and coworkers. The modified apparatus is shown in Figure 1. Steam, generated in a one-half horse power boiler, is regulated by means of needle valves in the manifold and is injected into the boiling solution through a tube having a spiral outlet. This ensures a swirling motion of the steam in the solution and causes complete agitation. A pressure release tube opening into a trap on the back of the

[1] *This Journal*, **21**, 113 (1938).
[2] *Ind. Eng. Chem. Anal. Ed.*, **10**, 143 (1938).

apparatus prevents any mechanical loss of the solution. The solution is maintained at a constant volume of 100 cc. by proper regulation of the steam and the burner temperature.

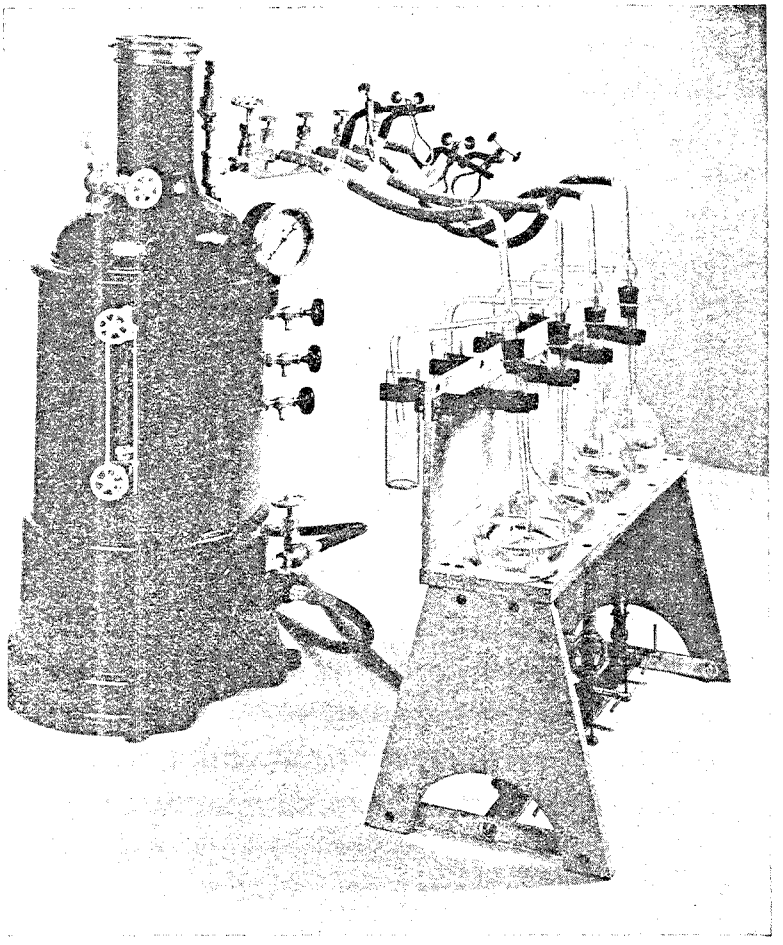

FIG. 1.—APPARATUS FOR DETERMINING AVAILABLE P_2O_5 BY THE MACINTIRE-SHAW-HARDIN METHOD.

It was found necessary to exercise some care in the use of the citrated ammonium nitrate solution because of its tendency to mold. This mold formation increases the acidity of the solution and consequently gives high availability values. The mold forms only when the solution is allowed to stand for several weeks. Any possibility of trouble from this source can be avoided by preparing only enough solution for the work at hand.

The samples analyzed cover a wide range of availability and include

monopotassium phosphate, di- and tricalcium phosphates, calcium hydroxyphosphate, calcined phosphate, ammoniated superphosphate, steamed bone meal, basic slag, raw rock phosphates, and mixtures containing one or more of these phosphatic components in different combinations. The samples, in all cases, were ground to pass an 80-mesh sieve, and the analyses were made in accordance with the published procedures. The results of both methods of analysis are given in the table.

The tabulated figures are the average of closely agreeing duplicates obtained on each of two samples, or four determinations in all. Fully as good agreement was obtained between duplicates by the MacIntire-Shaw-

Available P_2O_5 in various phosphatic materials as determined by the A.O.A.C. and MacIntire-Shaw-Hardin methods

SAMPLE	A.O.A.C. METHOD				MACINTIRE-SHAW-HARDIN METHOD		
	TOTAL P_2O_5	CITRATE-INSOLUBLE P_2O_5	AVAILABLE P_2O_5	AVAILABLE P_2O_5, % OF TOTAL	WT. OF SAMPLE	AVAILABLE P_2O_5	AVAILABLE P_2O_5, % OF TOTAL
	per cent	*per cent*	*per cent*		*grams*	*per cent*	
KH_2PO_4	52.13	0.00	52.13	100.00	0.5	52.43	100.58
$Ca_3(PO_4)_2$	35.69	21.95	13.74	38.50	0.5	27.06	75.82
$CaHPO_4$	48.53	3.66	44.87	92.46	0.5	46.26	95.32
$CaHPO_4+CaCO_3$(1:1)	26.31	5.36	20.95	79.63	1.0	14.74	56.02
$CaHPO_4+CaSO_4$(1:1)	26.69	0.18	26.51	99.33	1.0	23.58	88.34
Calcium hydroxyphosphate[a]	41.83	31.46	10.37	24.79	0.5	41.04	98.11
Calcium hydroxyphosphate[a]					1.0	36.05	86.18
Calcium hydroxyphosphate[a,b]	43.57	26.80	16.77	38.49	0.5	30.12	69.13
Calcined phosphate	37.05	21.32	15.73	42.46	1.0	21.99	59.35
Steamed bone meal	34.42	18.52	15.90	46.19	1.0	32.59	94.68
Basic slag	11.28	9.86	1.42	12.59	1.0	3.15	27.93
Florida pebble phosphate[c]	35.25	32.72	2.53	7.17	1.0	3.76	10.58
Florida pebble phosphate	30.88	27.45	3.43	11.09	1.0	4.26	13.61
Tenn. brown rock phosphate[d]	32.82	30.32	2.50	7.71	1.0	3.10	9.38
Tenn. brown rock phosphate	33.77	31.19	2.58	7.05	1.0	3.12	9.24
Ammoniated superphosphate[e]	19.76	1.33	18.43	93.27	1.0	19.74	99.90
Ammoniated superphosphate[f]	18.40	4.13	14.27	77.55	1.0	18.20	98.91
6-8-4 mixture contg. raw rock	21.88	13.77	8.11	37.07	1.0	10.88	49.73
5-10-5 mixture contg. C.S.M.	11.29	0.22	11.07	98.05	1.0	11.20	99.20
4-8-4 mixture	8.16	0.13	8.03	98.41	1.0	8.01	98.16
4-16-4 mixture	15.43	1.01	14.42	93.45	1.0	15.56	100.84
1938 A.O.A.C. sample No. 1	17.57	3.22	14.35	81.67	1.0	17.17	97.72
1938 A.O.A.C. sample No. 2	15.69	4.69	11.00	70.11	1.0	14.38	91.65
1938 A.O.A.C. sample No. 3	40.19	2.45	37.74	93.90	1.0	40.50	100.77
1938 A.O.A.C. sample No. 4	40.17	3.05	37.12	92.41	1.0	39.77	99.00

[a] P_2O_5/CaO = 0.788; theoretical = 0.760.
[b] Heated in an atmosphere of steam for 30 minutes at 1400° C.
[c] Bureau of Standards Standard Sample No. 120.
[d] Bureau of Standards Standard Sample No. 56a.
[e] NH_3 = 4.3 %.
[f] NH_3 = 6.8 %.

Hardin method as by the official method. In 50 per cent of the samples analyzed very good agreement was found to exist between the results obtained by the two methods. The MacIntire-Shaw-Hardin method gave high results for 42 per cent of the samples. These high values were particularly marked in the case of tricalcium phosphate and calcium hydroxyphosphate. The trend towards the higher availability values tends to fall in line with the published results of vegetative tests[3] and adds greatly to the promise of the MacIntire-Shaw-Hardin method. However, as both of the methods under discussion are of an arbitrary nature, further vegetative tests would seem to be advisable before their relative accuracy can be finally estimated.

The Associate Referee on Phosphoric Acid has made a recommendation[4] that further consideration be given to the MacIntire-Shaw-Hardin method for the determination of available P_2O_5.

EFFECT OF FLUORINE IN THE DETERMINATION OF CITRATE-INSOLUBLE PHOSPHORIC ACID BY THE OFFICIAL METHOD

By L. F. RADER, JR., and WILLIAM H. ROSS (Fertilizer Research Division, Bureau of Chemistry and Soils, Washington, D. C.)

At the last meeting of this Association a report was presented by Ross, Rader and Beeson[1] on the "Citrate-insoluble Phosphoric Acid in Ammoniated Mixtures Containing Dolomite." In the study that was made of this subject it was found that:

(1) The citrate-insoluble P_2O_5 in a fluorine-free ammoniated superphosphate remained unchanged when stored with or without dolomite at 30° C. for 180 days.

(2) Storage of a fluorine-free ammoniated superphosphate at 75° C. for the same length of time caused a slight increase in citrate-insoluble P_2O_5 in the absence of dolomite and a marked increase when dolomite was present.

(3) The presence of fluorine as calcium fluoride in an ammoniated superphosphate caused a slight increase in citrate-insoluble P_2O_5 when stored at 30° C. for 180 days in the absence of dolomite and a marked increase in the presence of dolomite.

(4) An increase in the storage temperature of a fluorine-containing ammoniated superphosphate or ammoniated superphosphate-dolomite mixture from 30 to 75° C. reduced still further the availability of the P_2O_5 in the mixture.

These results may be explained if it is assumed that the dicalcium phosphate initially formed in the ammoniation of superphosphate (Equation 1) undergoes hydrolysis in storage at temperatures above normal to form calcium hydroxyphosphate as represented in Equation 2, and that the presence of dolomite increases the extent to which calcium hydroxy-

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[3] Ross, Jacob, and Beeson, *This Journal*, 15, 227 (1932).
[4] Ross and Adams, *This Journal*, 22, 254 (1939).
[1] *This Journal*, 21, 258 (1938).

phosphate is formed by reacting at temperatures above normal with the monoammonium phosphate formed by ammoniation (Equation 1) as indicated in Equations 3 and 4. In the presence of fluorine, the reactions apparently proceed to the formation of fluorapatite as suggested by MacIntire and his coworkers.[2] If this reaction proceeds as indicated in Equation 5, any increase in citrate-insoluble P_2O_5 should be accompanied by a corresponding increase in water-soluble P_2O_5.

$$Ca(H_2PO_4)_2+NH_3=CaHPO_4+NH_4H_2PO_4 \quad (1)$$

$$10CaHPO_4+2H_2O=[3Ca_3(PO_4)_2]\cdot Ca(OH)_2+4H_3PO_4 \quad (2)$$

$$5NH_4H_2PO_4+3CaCO_3\cdot MgCO_3=Ca_3(PO_4)_2+3MgNH_4PO_4+2NH_3+6H_2O+6CO_2 \quad (3)$$

$$10Ca_3(PO_4)_2+2NH_3+6H_2O=3[3Ca_3(PO_4)_2]\cdot Ca(OH)_2+2NH_4H_2PO_4 \quad (4)$$

$$9CaHPO_4+CaF_2=[3Ca_3(PO_4)_2]\cdot CaF_2+3H_3PO_4 \quad (5)$$

That an increase in water-soluble P_2O_5 does take place in mixtures of an ammoniated superphosphate and calcium fluoride was shown by the results given in last year's report.[1] If the reactions taking place when dolomite is added to a mixture of this kind proceed as represented in Equations 3, 4 and 5, then there should be at first a decrease and later an increase in water-soluble P_2O_5. This was found to be true by chemical analysis.

EXPERIMENTAL

In continuing the work as recommended at the last meeting of the Association,[1] the writers made further tests on the effect of storage at temperatures above normal on the citrate-insoluble P_2O_5 in commercial ammoniated superphosphates. All tests were made with samples prepared by ammoniation of the same Tennessee superphosphate. The results obtained (Table 1) agree with those previously reported[1] in showing that the citrate-insoluble P_2O_5 in a commercial ammoniated superphosphate increases slowly at 30° C. and more rapidly at higher temperatures. Tests made with an ammoniated superphosphate of varying particle size (Samples 181–211) indicate that the size of the particle has little or no effect on the reversion of the P_2O_5 in storage.

In a second series of tests, samples of ammoniated superphosphates were stored at a temperature below normal (5° C.) as well as at the higher temperatures. Two of the samples used in these experiments were commercial ammoniated superphosphates and the third was a synthetic product prepared by ammoniating a mixture of C.P. monocalcium phosphate and calcium fluoride.* The results given in Table 2 show that ammoniated superphosphate mixtures of the ordinary type can be stored at 5° for 180 days with no significant loss of available P_2O_5, but that

[2] *Ind. Eng. Chem.*, **10**, 143 (1938); *This Journal*, **21**, 113 (1938).

* The "C.P." calcium fluoride used in this test was found to contain a small amount of a soluble fluoride. It was replaced in subsequent tests with a higher grade calcium fluoride.

TABLE 1.—*Effect of storage on the citrate-insoluble P_2O_5 in commercial ammoniated superphosphates*

SAMPLE NO.	PARTICLE SIZE	NH_3	PHOSPHORIC ACID (P_2O_5)		INCREASE IN CITRATE-INSOLUBLE P_2O_5 AFTER STORAGE FOR 6 MONTHS AT		
			TOTAL	CITRATE-INSOLUBLE[a]	30°	45°	75°
	mesh			*per cent*			
223	< 8	2.78	21.04	0.53	0.92	1.44	2.85
181[b]	>10	1.98	21.18	0.50	0.59	0.88	2.22
191[b]	10–20	2.20	21.40	0.46	0.46	0.79	1.34
201[b]	20–40	2.52	22.07	0.59	0.56	1.04	2.14
211[b]	<40	2.46	20.43	0.60	0.75	0.65	1.65
221	< 8	0.93	21.32	0.43	0.14	—	1.37
222	< 8	1.49	21.54	0.45	0.30	—	1.65
241	<20	2.24	20.08	0.43	0.12	0.44	1.47
231	<40	2.25	21.32	0.51	0.59	0.49	1.59

[a] All samples ground to pass a 40-mesh screen before analysis.
[b] Prepared by screening Sample 223.

TABLE 2.—*Per cent citrate-insoluble P_2O_5 in ammoniated superphosphates stored at temperatures above and below normal*
(Ammonia content = 3%[a])

SAMPLE NO.	SOURCE OF PHOSPHATE	TOTAL P_2O_5	INITIAL CITRATE-INSOLUBLE P_2O_5	INCREASE IN CITRATE-INSOLUBLE P_2O_5 AFTER STORAGE AT					
				5° FOR			30° FOR		45° FOR 180 DAYS
				30 DAYS	60 DAYS	180 DAYS	15 DAYS	180 DAYS	
161	Florida pebble rock	20.66	0.64	0.00	0.00	0.00	—	2.16	3.91
171	Tennessee brown rock	20.16	0.54	0.00	0.00	0.04	—	1.71	2.51
471	$Ca(H_2PO_4)_2 + CaF_2$[b]	52.25	0.20	0.18	0.28	1.18	3.50	—	—

[a] On basis of superphosphate containing 20% P_2O_5
[b] Fluorine in ammoniated product = 1.5%.

some increase in citrate-insoluble P_2O_5 occurs at this temperature in the synthetic ammoniated product. This is in agreement with the previous observation[1] that in ammoniated mixtures of pure materials containing fluorine the rate of the reversion of P_2O_5 is much greater than it is in the corresponding commercial products.

REVERSION OF P_2O_5 IN THE PROCESS OF ANALYSIS

If the loss of available P_2O_5 that occurs in ammoniated superphosphate mixtures during storage is due to the reaction represented in Equation 5, then the rate of the reaction should be increased by any treatment, such as washing, that would remove the free phosphoric acid formed. It has

also been observed by Ross and Jacob[3] that tricalcium phosphate is changed into calcium hydroxyphosphate by digestion in neutral ammonium citrate solution. It seemed possible, therefore, that a portion of the citrate-insoluble P_2O_5 found in ammoniated mixtures might be due to a reversion of the phosphoric acid in the process of analysis.

In this study of the problem, samples were prepared that contained varying combinations of the phosphatic compounds commonly occurring in ammoniated fertilizer mixtures. The materials and the proportion of each used in the preparation of the samples were as follows:

MATERIAL	PERCENTAGE COMPONENTS OF SAMPLE				
	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5
Ammonia	7	—	7	—	—
Monoammonium phosphate	—	—	—	5	—
Monocalcium phosphate monohydrate	88	—	57	—	—
Dicalcium phosphate, dihydrate	—	95	31	55	—
Tricalcium phosphate, anhydrous	—	—	—	—	95
Tricalcium phosphate, precipitated	—	—	—	25	—
Ammonium sulfate	—	—	—	10	—
Quartz flour	5	5	5	5	5
Total	100	100	100	100	100

All materials were ground to pass a 100-mesh sieve before being mixed. The composition of the samples is given in Table 3.

TABLE 3.—*Analysis of standard phosphate samples*

SAMPLE NO.	NH_3	CaO	TOTAL P_2O_5
		per cent	
1	7.31	18.59	51.33
2	—	31.00	39.13
3	6.90	25.30	47.27
4	3.31	35.46	40.17
5	—	51.33	43.02

FRESHLY-PREPARED MIXTURES

Samples 1 to 5 were analyzed for citrate-insoluble P_2O_5 as prepared and again when the quartz flour was replaced in part with a sufficient quantity of a fluorine compound to give a dry mixture containing 1.5 per cent of fluorine. The fluorine compound was mixed with each of the samples and the resulting mixture was then analyzed immediately to insure against any reversion of the P_2O_5 other than that which occurred during the process of analysis.

[3] *This Journal*, 14, 182 (1931).

The results obtained, given in the first column of figures in Table 4, show (1) that the presence of calcium fluoride causes little or no increase in citrate-insoluble P_2O_5 during the process of analysis of freshly-prepared mixtures over that found when fluorine is absent; and (2) that a marked reversion of P_2O_5 occurs during the process of analysis of freshly-prepared mixtures containing sodium fluoride or other water-soluble fluorine compound.

TABLE 4.—*Variation in citrate-insoluble P_2O_5 during washing and digestion of freshly-prepared phosphatic mixtures containing water-soluble or water-insoluble fluorides*

MIXTURE	PER CENT CITRATE-INSOLUBLE P_2O_5					
	TIME OF WASHING[a]			TIME OF DIGESTION[b]		
	1 HR.	4 HRS.	24 HRS.	1 HR.	4 HRS.	24 HRS.
Sample 1 with quartz	0.00	0.00	0.00	0.00	0.00	0.00
Sample 1 with CaF_2	0.00	0.00	0.00	0.00	0.00	0.00
Sample 1 with NaF	1.03	2.63	3.95	1.03	1.10	3.27
Sample 2 with quartz	0.00	0.00	0.00	0.00	0.00	0.00
Sample 2 with CaF_2	0.00	0.00	0.00	0.00	0.00	0.00
Sample 2 with NaF	0.30	0.95	5.15	0.30	0.40	5.90
Sample 3 with quartz	0.08	0.06	0.07	0.08	0.07	0.11
Sample 3 with CaF_2	0.08	0.08	0.15	0.08	0.08	1.43
Sample 3 with NaF	4.57	5.05	7.05	4.57	4.46	7.09
Sample 3 with NH_4F	5.20	4.95	7.12	5.20	4.20	8.21
Sample 3 with Na_2SiF_6	4.05	4.08	5.60	4.05	4.95	6.65
Sample 3 with $CaSiF_6 \cdot 2H_2O$	6.43	6.95	9.03	6.43	5.68	7.73
Sample 3 with $CaCl_2$	0.00	0.00	0.00	0.00	0.00	0.40
Sample 4 with quartz	6.30	6.26	6.59	6.30	6.13	7.73
Sample 4 with CaF_2	5.95	6.20	6.68	5.95	6.18	9.15
Sample 4 with NaF	9.18	11.60	15.50	9.18	9.76	15.18
Sample 4 with $CaSO_4 \cdot 2H_2O$	5.99	6.40	6.47	5.99	6.24	7.48
Sample 5 with quartz	24.75	25.00	24.70	24.75	21.25	16.25
Sample 5 with CaF_2	25.26	25.44	25.77	25.26	21.51	17.00
Sample 5 with NaF	26.57	26.19	26.72	26.57	23.28	17.31

[a] Time of digestion in citrate solution, 1 hour.
[b] Time of washing, 1 hour.

The effect of varying the time of washing and of the citrate digestion of the washed residues on the reversion of the P_2O_5 in the samples was determined by varying the period of either treatment from 1 to 24 hours while the other treatment remained constant. The quantity of wash water used in each determination was limited to 250 cc. The results obtained (Table 4) indicate that fluorine-free mixtures undergo little or no increase in citrate-insoluble P_2O_5, either with prolonged washing of the sample or when the washed residues are digested in citrate solution for a period of 4 hours or less. Sample 4, which contained precipitated

tricalcium phosphate, showed some increase in citrate-insoluble P_2O_5 when the washed residue was digested for 24 hours, whereas the citrate-insoluble P_2O_5 in Sample 5, which contained anhydrous tricalcium phosphate as the only phosphatic component, decreased on prolonged digestion of the washed residue. Ross, Jacob, and Beeson[4] found that precipitated or hydrated tricalcium phosphate is more soluble in citrate solution than the anhydrous or ignited product. The opposite results obtained with Samples 4 and 5 indicate that the digestion of Sample 5 in citrate solution results not only in a partial hydrolysis of the anhydrous tricalcium phosphate but also in its partial hydration to the more soluble hydrated form.

Table 4 further shows that prolonged washing of the sample, or a 1–4 hour digestion in citrate solution subsequent to washing, causes little or no increase in citrate-insoluble P_2O_5 when calcium fluoride is included in the mixture, and that a marked increase in citrate-insoluble P_2O_5 takes place under the same conditions when the calcium fluoride is replaced by a more soluble fluoride or fluosilicate. No appreciable increase in citrate-insoluble P_2O_5 takes place, however, when the fluorine compound is replaced by a calcium salt such as the sulfate or chloride.

The results obtained when Samples 1 and 3 alone and in mixture with calcium fluoride were digested in citrate solution without previous washing are given in Table 5. A comparison of these results with those in Table 4 shows that failure to remove soluble phosphates by washing increases the rate at which samples containing fluorine undergo reversion during digestion in citrate solution.

TABLE 5.—*Variation in citrate-insoluble P_2O_5 with time of digestion without prior washing with water*

MIXTURE	PER CENT CITRATE-INSOLUBLE P_2O_5		
	TIME OF DIGESTION		
	1 HR.	4 HRS.	7 DAYS
Sample 1 with quartz	0.00	0.00	0.00
Sample 1 with CaF_2	0.25	0.17	10.02
Sample 3 with quartz	0.08	0.17	0.44
Sample 3 with CaF_2	0.85	0.89	14.90

STORED MIXTURES

It was shown by Ross, Rader, and Beeson[1] that ammoniated mixtures containing calcium fluoride undergo slight reversion in storage at ordinary temperatures and a more rapid reversion at higher temperatures. Tests were accordingly undertaken to determine whether the reversion that takes place during storage is accompanied by a further reversion

[4] *This Journal*, 15, 227 (1932).

during the process of analysis by the present official method. In this series of experiments Sample 3, in mixture with calcium fluoride and again with sodium fluoride, was stored with a moisture content of 7 per cent for 7 and for 28 days at 20°–25° C. and for 7 and 21 days at 100° C. Mixtures of Sample 2 with calcium fluoride and with sodium fluoride were also stored for 7 and for 21 days at 100° C.

TABLE 6.—*Effect of storage on citrate-insoluble P_2O_5 in phosphatic mixtures containing water-soluble or water-insoluble fluorides*

MIXTURE	WATER-SOLUBLE		CITRATE-INSOLUBLE	
	P_2O_5	F	P_2O_5	F
		per cent		
Fresh mix, analyzed at once				
Sample 2 with CaF_2	1.45	0.01	0.00	1.18
Sample 2 with NaF	9.98	0.43	0.30	0.62
Sample 3 with CaF_2	19.33	0.01	0.08	1.22
Sample 3 with NaF	27.33	0.48	4.57	0.33
Stored 7 days at 20°–25° C.				
Sample 3 with CaF_2	19.30	0.01	0.00	1.15
Sample 3 with NaF	27.34	0.02	13.05	0.68
Stored 28 days at 20°–25° C.				
Sample 3 with CaF_2	19.56	0.01	0.10	1.16
Sample 3 with NaF	27.61	0.01	14.02	0.74
Stored 7 days at 100° C.				
Sample 2 with CaF_2	7.74	0.02	1.19	1.40
Sample 2 with NaF	15.61	0.02	17.20	1.48
Sample 3 with CaF_2	25.65	0.01	18.60	1.22
Sample 3 with NaF	27.30	0.02	17.14	1.00
Stored 21 days at 100° C.				
Sample 2 with CaF_2	1.52	—	5.27	0.87
Sample 2 with NaF	12.46	—	17.67	1.27
Sample 3 with CaF_2	25.63	—	19.40	1.15
Sample 3 with NaF	28.05	—	16.83	0.83

The results obtained (Table 6) show that the citrate-insoluble P_2O_5 found in the calcium fluoride mixtures stored at normal temperatures was little, if any, greater than in the freshly-prepared mixtures. When stored at 100° C. the calcium fluoride as well as the sodium fluoride mixtures undergo a marked increase in citrate-insoluble P_2O_5 over the corresponding freshly-prepared mixtures. The data in Table 6 further show that in the sodium fluoride mixtures the water-soluble fluorine decreases while the citrate-insoluble fluorine increases with storage. This affords further evidence of fluorapatite formation in ammoniated mixtures or mixtures containing di- or tricalcium phosphates when fluorine is present.

The citrate-insoluble P_2O_5 in the stored mixtures listed in Table 7 was determined by washing and by digesting the washed residues for 1 hour each as directed in the present official method. The results obtained when each treatment was increased to 6 hours and to 24 hours while the other treatment remained at 1 hour are also shown in Table 7. The data given in the table are in general agreement with the results obtained in the

TABLE 7.—*Variation in citrate-insoluble P_2O_5 during washing and digestion of stored phosphatic mixtures containing water-soluble or water-insoluble fluorides*

MIXTURE	PER CENT CITRATE-INSOLUBLE P_2O_5					
	TIME OF WASHING[a]			TIME OF DIGESTION[b]		
	1 HR.	6 HRS.	24 HRS.	1 HR.	6 HRS.	24 HRS.
	Stored 7 days at 20°–25° C.					
Sample 1 with NaF	10.76	10.87	11.53	10.76	10.62	10.29
Sample 2 with NaF	12.90	15.57	16.28	12.90	12.67	12.82
	Stored 28 days at 20°–25° C.					
Sample 3 with CaF_2	0.10	0.10	0.20	0.10	0.10	1.50
Sample 3 with NaF	14.02	14.16	14.54	14.02	13.56	14.30
	Stored 7 days at 100° C.					
Sample 1 with CaF_2	13.42	13.62	14.00	13.42	12.96	11.91
Sample 1 with NaF	13.01	14.64	15.79	13.01	12.00	12.35
Sample 2 with CaF_2	1.19	1.24	1.38	1.19	1.48	1.46
Sample 2 with NaF	17.20	17.22	17.69	16.72	16.83	16.97
	Stored 21 days at 100° C.					
Sample 3 with CaF_2	19.40	19.52	19.48	19.40	18.83	18.23
Sample 3 with NaF	16.83	16.67	16.57	16.83	16.27	15.63

[a] Time of digestion in citate solution, 1 hour.
[b] Time of washing, 1 hour.

collaborative study that was made of the extent to which the citrate-insoluble P_2O_5 in phosphatic mixtures varied with the time interval between the water extraction and citrate digestion.[5] This study showed that in ammoniated mixtures containing fluorine there was a slight but more or less constant increase in citrate-insoluble P_2O_5 with the time interval between washing and citrate digestion. The results given in Table 7 indicate that the citrate-insoluble P_2O_5 in stored ammoniated mixtures containing fluorine also undergo a more or less constant increase with the time of washing and with the time of digestion in citrate solution. This rate of increase in the case of the stored samples is such as to indicate a small but insignificant increase during the time (1 hour) specified in the official method for washing the sample and for making the citrate digestion.

[5] Ross and Adams, *This Journal*, 22, 254 (1939).

SUMMARY AND CONCLUSIONS

1. Neither freshly-prepared nor stored fluorine-free phosphate mixtures undergo any significant increase in citrate-insoluble P_2O_5 during the process of analysis when each of the different steps in the procedure is completed within one hour.

2. The presence of calcium fluoride causes little or no increase in citrate-insoluble P_2O_5 during the process of analysis of freshly-prepared ammoniated mixtures or mixtures containing di- or tricalcium phosphate over that found when fluorine is absent.

3. A marked reversion of P_2O_5 occurs during the process of analysis of freshly-prepared ammoniated mixtures or mixtures containing di- or tricalcium phosphate in the presence of sodium fluoride or other water-soluble fluorine compound.

4. In the presence of calcium fluoride, ammoniated mixtures or mixtures containing di- or tricalcium phosphate undergo a very slow reversion in storage at ordinary temperatures. Increasing the temperature of storage or replacing the calcium fluoride with sodium fluoride increases the rate of reversion.

5. In the presence of a fluorine compound, stored ammoniated mixtures may undergo a small increase in citrate-insoluble P_2O_5 during the process of analysis, but the increase is insignificant if each of the different steps in the procedure is completed within a period of one hour.

DETERMINATION OF ROTENONE IN DERRIS AND CUBE POWDERS

USE OF DECOLORIZING CARBON IN THE CHLOROFORM EXTRACTION METHOD

By J. J. T. GRAHAM (Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

In the determination of rotenone in derris and cube powders, several difference solvents and methods of extraction have been proposed, but in all the recent methods carbon tetrachloride has been used exclusively as a crystallizing medium.

It was observed by Jones and Graham[1] that in general it is easier to get complete extraction of the rotenone from cube than from derris, but that the rotenone crystallizes more slowly from the cube extracts than from derris extracts of comparable rotenone content; furthermore, the crystalline precipitates from the cube extracts are usually more highly colored and retain the resin contaminations more tenaciously.

It has also been recognized by a number of workers[2,3,4,5] that the derris

[1] *Ind. Eng. Chem. Anal. Ed.*, **10**, 19 (1938).
[2] Cahn and Boam, *J. Soc. Chem. Ind.*, **54**, 37T (1935).
[3] Jones, H. A., *Ind. Eng. Chem. Anal. Ed.*, **9**, 206 (1937).
[4] Seaber, W. M., *J. Soc. Chem. Ind.*, **56**, 168T (1937).
[5] Worsley, R. R. Le G., *Ibid.*, **55**, 349T (1936).

and cube resins interfere with the crystallization of the rotenone from the carbon tetrachloride solution. However, the work of Jones[3] indicates that this can be overcome by the addition of sufficient pure rotenone in the crystallization medium to bring the concentration up to a certain minimum quantity, provided the crystallization is allowed to proceed for a sufficient period of time.

After a study of the more promising methods, Jones and Graham[1,6] recommended a procedure that is essentially a combination of the chloroform extraction method of Beach[7] and the crystallization method of Jones.[3]

Recently the suggestion was made to the writer* that higher results for rotenone on cube samples could be obtained by the Jones-Graham method by the addition of decolorizing carbon in the extraction flasks. Accordingly a series of cube and derris samples was analyzed for rotenone by the method as published[6] and also with the addition of decolorizing carbon (Norit-A was used in this work). The sample and the carbon were mixed in the flask before the chloroform was added for extraction. The results of these analyses are given in Table 1.

TABLE 1.—*Results for rotenone and total chloroform extract on cube and derris powders* (30 grams of sample and 300 cc. of chloroform used)

SAMPLE		ROTENONE		PURITY OF THE SOLVATE		CHLOROFORM EXTRACT	
		WITHOUT CARBON	10 G.* CARBON	WITHOUT CARBON	10 G. CARBON	WITHOUT CARBON	10 G. CARBON
		per cent		*per cent*		*per cent*	
3230	Cube	3.8	4.3	77.1	90.9	18.8	15.0
3004	Cube	2.8	3.6	79.2	93.1	16.2	12.9
1938-2	Cube	4.3	5.1	79.6	90.7	22.4	17.7
3596	Cube	3.9	4.5	75.2	88.9	18.7	14.8
NCA	Cube	4.0	4.6	79.5	89.3	—	—
2119	Cube	5.1	5.8	74.7	85.8	19.9	17.0
25095-D	Cube	3.1	3.8	80.3	93.1	15.3	12.0
2711	Cube	1.7	2.2	84.3	97.0	9.1	6.2
3126	Derris	5.6	5.5	86.5	89.8	16.1	14.1
3002	Derris	2.0	2.1	82.7	88.0	12.5	10.2
3006	Derris	3.7	3.8	79.7	84.1	16.2	13.6
1937-1	Derris	4.5	4.6	83.0	92.7	—	—
1938-1	Derris	4.5	4.8	83.5	92.4	16.1	13.7

* Sample 3596 when treated with 15 grams of carbon gave results no different from those obtained by the use of 10 grams of carbon.

In every case the samples of cube gave higher values for rotenone when the carbon was mixed with the powder in the extraction flask, the increase ranging from 0.5 to 0.8 per cent. The differences in the values for rotenone in the derris samples are within the limits of reproducibility of the method.

[6] *This Journal*, 21, 148 (1938).
[7] *Soap*, 12, 109, 111 (1936).
* Credit for this suggestion is due C. A. Greenleaf of the National Canners Association, Washington, D. C.

The quantity of chloroform extract was determined by evaporating 10 cc. of the filtered chloroform solution to dryness and heating at 105° C. to constant weight. The extract in which carbon was used showed a reduction of the dried residue varying from 2.9 to 4.7 per cent in the case of the cube samples and from 2.0 to 2.6 per cent in the derris samples.

Cahn, Phipers, and Boam[8] found that passage of a rotenone-rich extract of derris in benzene twice through a short column of charcoal gave, after removal of the solvent from the percolate, a completely colorless, transparent resin from which rotenone crystallized spontaneously in clusters of radiating needles, but that only a part of the rotenone originally present could be separated, as some was adsorbed on the charcoal.

While determining rotenone in *Mundulea* bark Worsley[5] experienced much trouble with chlorophyl and other coloring compounds that were extracted along with the resins by the ethyl acetate used as a solvent. He found that if the resins were dissolved in alcohol and shaken with charcoal the colors were removed and also a little rotenone, but that no loss of rotenone occurred when the charcoal was first mixed with the powdered bark and then extracted in his special apparatus.

The possibility of loss of rotenone when using decolorizing carbon was recognized by the writer; therefore investigations were conducted to settle this point. It was found that when 1.75 grams of pure rotenone was mixed with 10 grams of carbon and then carried through the extraction, crystallization, and purification procedures about 30 per cent of the rotenone was lost. However, because derris samples analyzed both with and without the use of carbon gave no significant difference in the rotenone values, the indications were that the carbon had no adsorptive effect on the rotenone under the conditions of this analysis, where the other chloroform-soluble constituents were present. To determine this point, one sample of cube and two samples of derris of low rotenone content were selected, and analyses were made with and without the addition of carbon, and with and without the addition of rotenone in the extraction flasks. The rotenone added to the sample in the flask varied from 0.8 to 1.5 grams, and it was mixed with the carbon before the chloroform was added. The results of these analyses are given in Table 2.

In the case of the derris samples the percentages of rotenone recovered in the determinations in which carbon was used agreed with those recovered in the absence of carbon, after corrections for the quantities of rotenone added to the extraction flasks. In the case of the cube sample the recovered rotenone, after correction for that added, checked that obtained when carbon was used and no rotenone was added to the extraction flask, but both results were slightly higher than the result obtained without the use of carbon, which was to be expected. These results indicate that no rotenone was adsorbed by the carbon, otherwise lower results would have been obtained.

[8] *J. Soc. Chem. Ind.*, 57, 200 (1938).

TABLE 2.—*Recovery of rotenone in presence of carbon and added rotenone in the extraction flasks*

(30 grams of sample and 300 cc. of chloroform used)

SAMPLE		WITHOUT CARBON	10 GRAMS OF CARBON IN EXTRACTION FLASK. ROTENONE ADDED IN EXTRACTION FLASK—			
			NONE	0.8 g.	1.2 g.	1.5 g.
		per cent	*per cent*	*per cent**	*per cent**	*per cent**
3354	Derris	0.6	0.8	—	—	0.8
3002	Derris	2.0	2.1	2.1	2.1	—
2711	Cube	1.7	2.2	—	2.2	—

* Percentages of rotenone corrected for the pure rotenone added.

In the extracts obtained with carbon, the rotenone crystallized rapidly and the precipitates were white in contrast to the brown color frequently encountered in the analysis of cube powders. Evidently the carbon removes the portion of the resin that has the greatest retarding effect on the crystallization of the rotenone.

SUMMARY

In the analysis of cube powders by the Jones-Graham method higher percentages of rotenone were obtained, and the rotenone-carbon tetrachloride solvate crystallized more readily and had a purer composition when carbon was used in the extraction flask.

The use of carbon in the extraction flask, in the case of the derris powders tested, caused no significant difference in the results for rotenone.

THE DETERMINATION OF THALLOUS SULFATE IN ANT POISONS

By C. G. DONOVAN (Insecticide Division, U. S. Food and Drug Administration, Washington, D. C.)

The use of thallium as a rodent poison apparently originated about 1920,[1] when a company in Germany introduced a proprietary thallium rat poison. In recent years it has come into commercial use in the United States. It now appears in the form of thallous sulfate in a number of ant poisons, either in solutions with sugar or in paste form mixed with sugars, starches, greases, and absorbent material. The appearance and increasing number of these poisons have necessitated the development of an accurate method for the determination of thallium in products of this nature.

Hillebrand and Lundell[2] have published methods for the determination of thallium in inorganic compounds by weighing it as the chromate, thallic oxide, or thallous iodide. Among other proposed gravimetric

[1] U. S. Dept. Agr. Bur. Biol. Survey Bull. 238, p. 1.
[2] Applied Inorganic Analysis, pp. 376–78.

methods are those in which thallium is weighed as thallous sulfostannate, Tl_4SnS_4; as thallous acid sulfate, $TlHSO_4$; or as the neutral sulfate, Tl_2SO_4; and as the chloroplatinate, Tl_2PtCl_6. Sikazo Nisihuko[3] has determined thallium gravimetrically as the cobaltinitrite, $Tl_3(Co(NO_2)_6)$.

Among the volumetric methods are those based on the oxidation of thallous to thallic sulfate in hydrochloric acid solution by potassium permanganate,[4] potassium bromate,[5] potassium iodate,[6] and ceric sulfate.[7] For small amounts of thallium, a colorimetric method has also been used.[8]

Of the above listed methods, the thallous iodide procedure appeared to the writer to be most promising provided conditions could be established to minimize the solubility of the precipitated thallous iodide, which is soluble to some extent in water. According to F. Kohlrausch,[9] 1 liter of water dissolves 0.0847 gram of thallous iodide at 26° C., but it is less soluble in solutions containing potassium iodide, alcohol, or a little acetic acid; and is nearly insoluble in solutions containing ammonium hydroxide or sodium thiosulfate.

A number of trials and modifications involving the oxidation of the organic matter, reduction of the thallium from the thallic to thallous condition, and precipitation of the thallium with varying amounts of potassium iodide, finally led to the development of the thallous iodide method, which is described as follows:

PROCEDURE

Weigh a quantity of the sample containing 0.1–0.15 gram of thallous sulfate (usually about 10 grams), transfer to an 800 cc. Kjeldahl flask, and add 25 cc. of concentrated H_2SO_4 followed by 5–10 cc. of concentrated HNO_3. After the first violent reaction has ceased, heat on a Kjeldahl digestion apparatus until white fumes of H_2SO_4 are evolved. Add a few drops of HNO_3 and continue the heating and addition of HNO_3 until the organic matter has been destroyed, as evidenced by a colorless or light yellow solution. Cool, add 10–15 cc. of water, again cool, and wash the contents of the flask into a 400 cc. beaker, continuing the washing until the volume is 60–70 cc. Boil several minutes to remove all the HNO_3, cool, and filter into a 400 cc. beaker. Wash with hot water until the volume in the beaker is 175 cc., neutralize with NH_4OH, and then slightly acidify with H_2SO_4 (1+4). Add 1 gram of $NaHSO_3$ to insure reduction of the thallium from the thallic to the thallous form. Heat to boiling, add 50 cc. of 10% KI solution, stir, and let stand overnight. Filter through a tight Gooch crucible containing two disks of S & S 589 white ribbon filter paper covered by a medium pad of asbestos. Wash 4 or 5 times with 10 cc. portions of 1% KI solution, and finally with absolute alcohol. Dry to constant weight at 105° C. (1-1½ hours in oven), and weigh as thallous iodide, TlI. From this weight calculate the percentage of thallium as thallous sulfate, Tl_2SO_4, using the factor 0.7616.

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[3] *J. Soc. Chem. Ind.*, Japan 37, Suppl. binding 180 (1934).
[4] Hawley, *J. Am. Chem. Soc.*, 29, 300 (1907).
[5] Zintl and Reinäcker, *Z. Anorg. Elem.*, **153**, 276 (1926).
[6] Berry, *Analyst*, 51, 137 (1926).
[7] *Ibid.*, 54, 461 (1929).
[8] Shaw, *Ind. Eng. Chem. Anal. Ed.*, 5, 93 (1933).
[9] *Z. Phys. Chem.*, 64, 168 (1908).

DISCUSSION

Analyses were made on some thallous sulfate-sugar solutions without destroying the organic matter. Accurate results were obtained with some mixtures, but as would be expected the precipitated thallium in most cases appeared colloidal and had a tendency to pass through the Gooch crucible during filtering and washing.

The specified amount of potassium iodide added in the precipitation of the thallous iodide is necessary, as it appears that the slight acidity of the solution before precipitation, together with the excess potassium iodide and presence of ammonium salts, renders the precipitate practically insoluble.

The presence of substances, such as silver compounds, that are soluble in sulfuric acid and precipitated by potassium iodide would interfere with the results obtained by this method. However, no samples have been encountered that contained interfering substances.

ANALYSES OF PREPARED SOLUTIONS AND SAMPLES

A 1 per cent solution of C. P. thallous sulfate was prepared at 25° C. The thallous sulfate crystals had previously been dried in an oven at 105° C., and sulfate determinations indicated a high degree of purity. At the same temperature, aliquots containing 0.075, 0.1, 0.125, and 0.15 gram, respectively, of thallous sulfate were measured from a buret; and with the exception of oxidation of the organic matter, the thallous sulfate was determined by the method described. The amounts of thallous sulfate in the different aliquots correspond to the varying amounts of thallous sulfate present in 10–15 grams of commercial ant baits. Results illustrating the recovery of the thallous sulfate are shown in Table 1.

TABLE 1.—*Recovery of thallous sulfate from water solutions*

ALIQUOT OF 1% Tl_2SO_4	EQUIVALENT Tl_2SO_4 TAKEN	TlI OBTAINED	Tl_2SO_4 RECOVERED	ERROR
cc.	*gram*	*gram*	*gram*	*per cent*
7.5	0.075	0.0984	0.0749	−0.13
10.0	0.100	0.1316	0.1002	+0.20
12.5	0.125	0.1639	0.1248	−0.16
15.0	0.150	0.1970	0.1500	0.00

With similar aliquots of the thallous sulfate solution, liquid and paste mixtures analogous in composition to certain proprietary products were prepared, and their thallous sulfate content was determined. The liquid mixtures consisted of thallous sulfate solution, two grams of honey, and three grams of granulated sugar. Two grams of honey, three grams of granulated sugar, one gram of ground walnut meat, and the varying aliquots of thallous sulfate solution were used in preparing the pastes. The results of the analysis are shown in Tables 2 and 3.

TABLE 2.—*Recovery of thallous sulfate from prepared solutions containing honey, granulated sugar, and thallous sulfate*

ALIQUOT OF 1% Tl_2SO_4	EQUIVALENT Tl_2SO_4 TAKEN	TlI OBTAINED	EQUIVALENT Tl_2SO_4 RECOVERED	ERROR
cc.	*gram*	*gram*	*gram*	*per cent*
7.5	0.075	0.0982	0.0748	−0.27
10.0	0.100	0.1312	0.0999	−0.10
12.5	0.125	0.1632	0.1243	−0.56
15.0	0.150	0.1966	0.1497	−0.20

TABLE 3.—*Recovery of thallous sulfate from prepared pastes containing honey, granulated sugar, walnut meat, peat moss, and thallous sulfate*

ALIQUOT OF 1% Tl_2SO_4	EQUIVALENT Tl_2SO_4 TAKEN	TlI OBTAINED	EQUIVALENT Tl_2SO_4 RECOVERED	ERROR
cc.	*gram*	*gram*	*gram*	*per cent*
7.5	0.075	0.0984	0.0749	−0.13
10.0	0.100	0.1310	0.0998	−0.20
12.5	0.125	0.1646	0.1254	+0.32
15.0	0.150	0.1961	0.1493	−0.47

J. J. T. Graham of this laboratory, using this method on two samples, each containing 0.1 gram of thallous sulfate and similar in nature to those mentioned in Table 3, obtained a recovery of 0.1002 and 0.0995 gram, or 99.85 per cent.

DETERMINATION OF VOLATILE FATTY ACIDS AS AN APPROACH TO THE EVALUATION OF SPOILAGE IN CANNED SARDINES

By FRED HILLIG (Food Division,* Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

In previous communications[1,2,3] report was made on the determination of volatile fatty acids in canned salmon, tuna fish and herring roe, as an approach to the problem of evaluating spoilage.

The work has now been extended to cover the determination of volatile acids in sardines (California) and the purpose of this paper is to present the facts developed.

It was found that canned sardines prepared from the freshest possible raw material contain small quantities of volatile fatty acids. As decomposition becomes more and more extensive there is a progressive increase in the amount of these acids present.

* W. B. White, Chief.
[1] *This Journal*, 21, 684 (1938).
[2] *Ibid.*, 688.
[3] *Ibid.*, 22, 116 (1939).

Volatile and formic acid numbers were then determined on an authentic pack of sardines. Results are given in Table 1. A description of the packs follows.

Code 2.—Packed 3-3-36, 6:00 p.m. Fish out of water 12 hours. They were in good condition, with no detectable decomposition.

Code 5.—Packed 3-4-36, 7:00 p.m. Fish out of water 37 hours. In general the eyes were fairly bright. In many fish the gills were bright on one side, but faded and darkened on the other side. The flesh was somewhat soft and had lost its translucent appearance, becoming slightly opaque.

Code 8.—Packed 3-5-36, 8:00 a.m. Fish out of water 50 hours. The eyes of the fish were reddened, the gills foul, and the flesh soft. The entire lot had a distinct odor of decomposition.

Code 14.—Packed 3-7-36. Fish badly decomposed, soft, spongy, and sour. Flesh reddened and gassy.

All packs were made at Los Angeles from the same lot of fish.

TABLE 1.—*Analysis of canned sardines*

CAN NO.	CODE 2		CODE 5		CODE 8		CODE 14	
	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER
	mg./ 100 g.	*cc. 0.01 N per 100 g.*	*mg./100 g.*	*cc. 0.01 N per 100 g.*	*mg./100 g.*	*cc. 0.01 N per 100 g.*	*mg./100 g.*	*cc. 0.01 N per 100 g.*
1	1.3	15.8	1.6	21.6	3.9	57.1	15.5	156.1
2	1.2	15.8	1.7	22.1	3.3	58.1	17.5	168.2
3	1.3	15.9	1.6	22.2	5.8	60.2	18.1	170.8
4	1.4	16.4	1.5	22.5	3.9	61.7	18.3	174.2
5	1.2	16.5	1.5	22.8	4.0	65.3	19.5	181.1
6	1.4	16.7	1.8	22.8	3.9	65.3	18.4	181.8
7	1.5	16.9	1.7	23.5	5.0	66.4	18.4	183.5
8	1.3	17.1	1.7	23.6	4.6	67.8	18.9	183.5
9	1.5	17.4	1.5	23.9	5.9	71.1	18.6	189.5
10	1.8	18.6	1.7	24.5	6.0	80.8	21.1	201.5
Av.	1.4	16.7	1.6	23.0	4.6	65.4	18.4	179.0
Max.	1.8	18.6	1.8	24.5	6.0	80.8	21.1	201.5
Min.	1.2	15.8	1.5	21.6	3.3	57.1	15.5	156.1

The presence of acetic acid in sauce-packed sardines, especially mustard packs, may limit the value of a determination of volatile acidity. However, if formic acid is determined on the distillate the method becomes valuable as an index of the condition of the sample under examination. Formic acid was determined on sardines packed in mustard and tomato sauces, with the results given in Table 2.

Codes 5M and 5T were packed from the same lot of sardines as was Code 5, Table 1; likewise Codes 8M and 8T were packed from the same lot as was Code 8. It will be noticed that the formic acid obtained in Codes 5M and 8M check closely with that found in Codes 5 and 8, while

TABLE 2.—*Analysis of sardines packed in mustard and tomato sauces*

CAN NUMBER	MUSTARD SAUCE PACK		TOMATO SAUCE PACK	
	CODE 5M	CODE 8M	CODE 5T	CODE 8T
	FORMIC ACID NUMBER	FORMIC ACID NUMBER	FORMIC ACID NUMBER	FORMIC ACID NUMBER
	mg./100 g.	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*
1	1.7	3.5	2.1	4.7
2	1.8	3.6	2.8	4.8
3	1.8	4.0	2.9	5.1
4	1.8	4.1	2.9	5.7
5	1.8	4.1	3.2	6.9
6	1.9	4.1	—	—
7	1.9	4.3	—	—
8	1.9	4.3	—	—
9	2.0	4.6	—	—
10	—	4.6	—	—
Av.	1.8	4.1	2.8	5.4
Max.	2.0	4.6	3.2	6.9
Min.	1.7	3.5	2.1	4.7

the quantities in Codes 5T and 8T show a tendency to be slightly higher. A sample of the sauce was not available for analysis, and no definite statement can be made as to its formic acid content.

The methods of analysis used were those given in a previous report.[2]

An attempt was then made to identify the individual acids comprising the acid mixture following the procedure of fractionation previously used.[2]

Since Curve 1 falls between the acetic and formic acid lines, the presence of formic acid and one or more acids higher in the series is indicated. Fractionation Curve 6 starts just below the line for propionic acid. The angle at which it crosses the propionic acid line indicates that the highest detectable member of the series of acids present is propionic acid, with possible traces of one or more of the higher members of the series.

A study of the table of rates of distillation of this series of acids up to iso-butyric acid[1] shows that 500 cc. of distillate collected under the conditions laid down will contain practically all of the N-butyric and iso-butyric acids, and that 1.5 per cent of the propionic acid and 12.5 per cent of the acetic acid will be left in the distillation flask. With these facts in mind the writer attempted to verify the above conclusion as to the presence of propionic acid. The neutralized distillates obtained in preparing the curves shown in Figure 1 were evaporated to dryness, the formic acid was destroyed, and the remaining acids were recovered as previously described.[2] A new distillation curve was then prepared (Curve 1, Figure 2). Since the curve passes between the lines for acetic and pro-

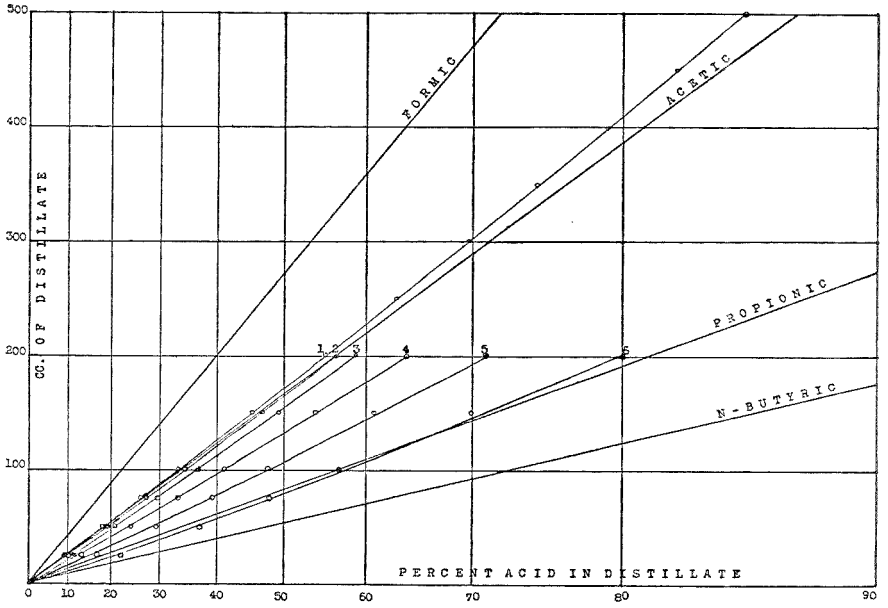

FIG. 1.

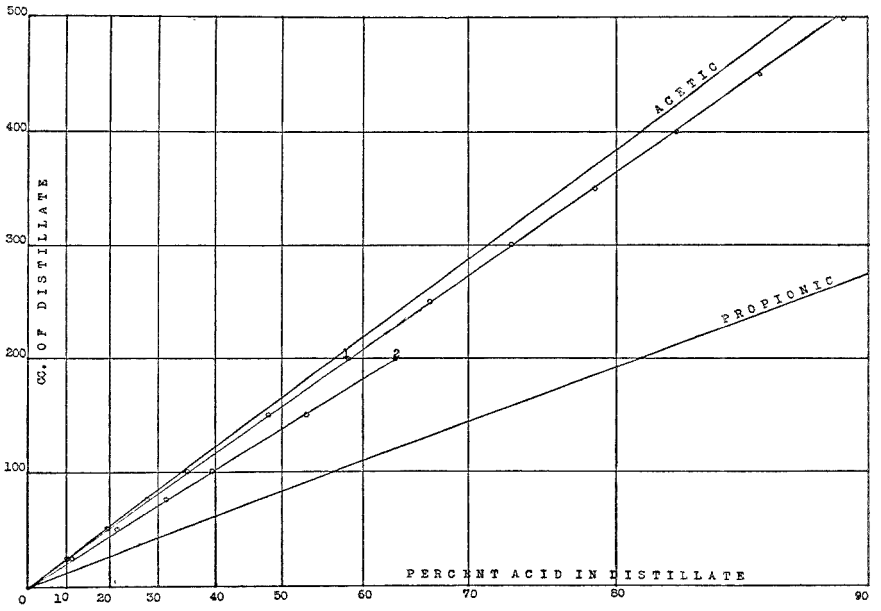

FIG. 2.

FRACTIONATION OF VOLATILE FATTY ACIDS FROM SARDINES, CODE 8

pionic acids, the presence of acetic acid and one or more acids higher in the series is indicated. Curve 2 was prepared from the distillation of the acids remaining in the flask after the 500 cc. of distillate used to prepare Curve 1 had been collected. Since the butyric acids, if present, have been practically eliminated in the first 500 cc. of distillate, and formic acid has been destroyed, Curve 2 should coincide with the acetic acid line if propionic acid is absent. The fact that Curve 2 falls materially below the acetic acid line is a further indication of the presence of propionic acid in the sample under investigation.

SUMMARY

A method for the evaluation of spoilage in canned sardines is proposed. The procedure is simple and yields diagnostic and consistent results.

THE ALCOHOLS AS A MEASURE OF SPOILAGE IN CANNED FISH

By DUNCAN A. HOLADAY (U. S. Food and Drug Administration, Washington, D. C.)

During a study of spoilage in canned sea foods it appeared that a determination of the amounts of alcohols present would be useful as an index of decomposition.

Experimental packs of fish were used for the investigation. The material ranged from fresh fish to putrid fish, and it was classified organoleptically before processing was begun. The method used for the determination of alcohols was essentially that of Friedemann and Klaas,[1] and their original paper should be studied. This method, which involves a controlled alkaline permanganate oxidation following the removal of interfering substances, is quite specific for alcohols as a group.

Table 1 shows the results that were obtained on packs of mackerel, salmon, and brine-packed sardines. Since these results may involve more than one alcohol, they are expressed as cubic centimeters of 0.02 *N* permanganate per 100 grams of sample. It appears that good fish contain very small amounts of alcohol, and that as decomposition proceeds the quantities present become progressively larger.

An attempt was made to identify the alcohols present. For this study, 2.6 liters of filtrate, representing 360 grams of putrid fish, were purified exactly as directed in the regular method. The purified distillate was concentrated to a final volume of 8 cc. by successive fractional distillations, and 50 per cent of the volume was collected each time. From 2 cc. of this concentrate fractionated in a micro-fractionating column about 0.05 cc. of distillate with a strong odor of ethyl alcohol was obtained. This distillate was treated with 3-5 dinitrobenzoyl chloride. After three re-

[1] *J. Biol. Chem.*, 115, 47 (1936).

TABLE 1.—*Alcohols in canned fish*
(cc. 0.02 *N* $KMnO_4$ per 100 grams)

CAN NUMBER	CODE 1	CODE 2	CODE 3	CODE 1	CODE 2	CODE 3	CODE 1	CODE 2	CODE 3
	MACKEREL			SALMON			SARDINES		
1	4.2	10.0	52.3	5.0	16.4	35.4	10.2	18.9	106.8
2	3.2	17.2	57.4	3.7	7.5	62.2	4.1	18.5	75.7
3	3.4	13.3	27.6	7.1	9.8	40.5	6.5	27.3	68.9
4	3.9	8.6	50.3	5.2	14.9	60.4	3.3	12.1	91.0
5	3.6	10.9	39.5		16.4	61.7	3.8	13.6	70.6
6		8.8	39.8		6.5	46.2	6.8	16.7	65.8
7		10.6	32.3		17.2	33.4	9.0	11.6	77.0
8		8.8	70.0		10.1	25.2	6.0	12.5	73.7
9		16.8	28.5		16.7	58.7	3.8	16.1	92.3
10		18.0	30.9		10.1	52.5	5.6	22.7	117.6
Average	3.6	12.3	42.9	5.3	12.6	47.7	5.9	17.0	83.9
Maximum	4.2	18.0	70.0	7.1	17.2	62.2	10.2	27.3	117.6
Minimum	3.2	8.6	27.6	3.7	6.5	25.2	3.3	11.6	65.8

Mackerel: Packed at Terminal Island, Calif.

* Code 1 packed 10-3-34, 7 p.m. Fish selected for freshness. Out of water 11 hours.

* Code 2 packed 10-4-34, 9 a.m. Fish from the same lot as Code 1, but out of water 14 hours longer. Fish were somewhat wrinkled, with a dull luster; some had begun to turn red around the eyes and were a trifle soft. The odor of the fish after washing was slightly sour.

* Code 3 packed 10-4-34, 6 p.m. Fish from the same lot, but out of water 9 hours longer than Code 2. Fish were all bloody around the eyes, and were somewhat soft after cleaning. Fish had a persistent sweetish odor of decomposition which could not be removed by washing.

Salmon: Code 1 packed 7-12-35, Cordova, Alaska. Fish out of water 24 hours. Eyes bright, gills odorless, flesh firm.

* Code 2 packed 6-27-36, Bristol Bay, Alaska. Fish out of water 45–57 hours. Gills of some fish were quite white, others pink to red. Eyes on the upper side slightly dull, on the under side more or less red. Odor of some was sour, while others were practically odorless.

* Code 3 packed 6-28-36 from the same lot as Code 2. Out of water 62–74 hours. Eyes somewhat sunken, very dull. Slime quite sour in odor. Flesh had lost its resilience but was firm. The flesh had a slightly putrid odor.

Sardines: Packed at Terminal Island, Calif.

Code 1 packed 3-3-36, 6 p.m. Fish out of water 12 hours. Fish were in good condition; with no detectable decomposition.

Code 2 packed 3-4-36, 7 p.m. Fish out of water 37 hours. In general the eyes were fairly bright. In many fish the gills were bright on one side, but faded and darkened on the other side. The flesh was somewhat soft and had lost its translucent appearance, becoming slightly opaque.

Code 3 packed 3-5-36, 8 a.m. Fish out of water 50 hours. The eyes of the fish were reddened, the gills foul, and the flesh soft. The entire lot had a distinct odor of decomposition.

All packs from the same lot of fish.

* These codes are identical to the same numbered codes used by Hillig and Clark, *This Journal*, 21, 688 (1938).

crystallizations the dinitrobenzoates obtained melted at from 86° to 89° C. The melting points of some pure dinitrobenzoates are: Ethyl, 92.7°; propyl, 73°; and butyl, 62.5° C. From these data it appears that the major portion of the alcohol present is ethyl.

The method follows:

METHOD FOR DETERMINATION OF ALCOHOLS IN CANNED FISH

REAGENTS

1. *Calcium hydroxide suspension.*—Slake 100 grams of CaO (reagent quality) and make up the suspension to 1 liter.
2. *Sodium hydroxide.*—5 *N*. Keep in a glass-stoppered bottle *not* lined with paraffin.
3. *Potassium permanganate.*—Make up 0.1 *N* stock solution and dilute as needed.
4. *Sodium thiosulfate.*—Make up 0.1 *N* solution and dilute as needed.

APPARATUS

Use all-glass apparatus. Two 500 cc. round-bottomed boiling flasks, a 75° angle adapter, and a Friederichs condenser, standard taper joints No. 29/42, are recommended.

PROCEDURE

Pass the entire contents of a can of fish through a meat chopper three times and thoroughly mix the material after each grinding. Weigh 50 grams of this material into a 250 cc. beaker, stir to a uniform suspension with 100 cc. of water, and transfer quantitatively to a 250 cc. volumetric flask. Add 15 cc. of 2 *N* H_2SO_4, mix well, and add 15 cc. of 20% phosphotungstic acid solution. Dilute the mixture to 250 cc., *shake vigorously*, allow to stand 5 minutes, and filter through a folded filter paper. Place 150 cc. of the filtrate in a 500 cc. boiling flask and add 10 cc. of Deniges' reagent (U.S.P. XI) and a few glass beads. Make to 200 cc. and distil off 100 cc. into another 500 cc. boiling flask. Add 5 cc. of Deniges' reagent to the distillate and add lime suspension until the mixture becomes *orange in color*. Shake vigorously, make to 150 cc., and distil off 100 cc., collecting the distillate in a 100 cc. volumetric flask. Pipet an aliquot into a 200 cc. Erlenmeyer flask, add 10 cc. of 5 *N* NaOH and 25 cc. of 0.02 *N* permanganate with constant rotation. Cover with a small beaker, place in a boiling water bath, and heat for at least 20 minutes. Cool, add 10 cc. of 10 *N* H_2SO_4, enough KI to react with the remaining permanganate, and titrate the liberated I with 0.02 *N* thiosulfate. Calculate the quantity (cc.) of permanganate oxidized per 100 grams of sample, neglecting the volume of solids.

Choose the aliquots so that not more than 6 cc. of permangante is consumed in the reduction. For most samples, a 25 cc. aliquot will be correct, but if a smaller aliquot is used, add distilled water to make the volume in the reduction flask up to 60–70 cc. (This volume is important. The oxidation of alcohol is not quantitative, but if the alkalinity, volume, and time are controlled, it is consistent.)

Take special care to have clean glassware. Wash all the apparatus frequently with cleaning solution and rinse with distilled water *poured from an all-glass wash bottle*. Do not allow the lower parts of the pipet to touch the hands or the desk, as any dust or organic matter will give variable results. It is advisable to plug the stems of the pipets with cotton.

Use the following factors for converting from permanganate to ethyl alcohol:

cc. $\times 0.0855$ = mg. ethyl alcohol (for 0.02 *N*);
cc. $\times 0.0427$ = mg. ethyl alcohol (for 0.01 *N*);
cc. $\times 0.0215$ = mg. ethyl alcohol (for 0.005 *N*).

SUMMARY

A determination of the amount of alcohol present is proposed as a measure of the extent of decomposition of canned fish.

CHEMICAL COMPOSITION OF COTTONSEED HULL BRAN*

By D. M. Musser†

This work was undertaken as one phase of a broad investigation‡ of the chemical and physical properties of all constituents of the cotton

* Contribution from the Cotton Research Foundation Fellowship, Mellon Institute.
† Industrial Fellow, Mellon Institute.
‡ Supported by the Cotton Research Foundation, a philanthropic organization with headquarters at Memphis, Tenn.

plant, with the objective of improving the economic status of the cotton industry through the development of new uses.

Cottonseed hull bran was subjected to a detailed analysis, including a spectrographic examination of the ash. An integrated chemical study of this material has never been reported in the literature, although various constituents have been determined by several investigators. Hudson and Harding[1] determined the yield of xylose obtainable from cottonseed hull bran. Markley[2] studied the non-nitrogeneous components and reported analyses for furfural, xylose, cellulose, and lignin. Anderson and his associates[3,4] described the isolation of a hemicellulose and its hydrolytic products. McBryde,[5] McHargue,[6] and Sheets and Thompson[7] investigated the mineral matter of cottonseed hull ash.

EXPERIMENTAL

Analyses for the following constituents were made on a representative sample of hull bran according to the methods cited: cellulose by the chlorination procedure of Cross and Bevan;[8] lignin by the method of Ritter, Seborg, and Mitchell;[9] furfural and pentosans by the A.O.A.C. procedures;[10] hydrolysis number by the method of Hawley and Fleck;[11] and methoxyl, acetic acid, ash, moisture, ether extract, one per cent sodium hydroxide extract, cold water extract, and hot water extract, as suggested by Schorger.[12]

The cottonseed hull bran was first freed as completely as possible from hull fibers, then ground to pass a 40-mesh sieve, and finally oven-dried (105° C.). The results, which in all cases represent the average of two or more determinations, are recorded in Table 1.

Markley reported 51.7 per cent cellulose, 23.83 per cent lignin, and 25.33 per cent furfural.

In preparing samples of cottonseed hull ash for spectrographic examination, the hull material was obtained from hand-ginned seeds, and special precautions were taken to avoid contaminants. When the ashing was carried out in porcelain crucibles at a temperature of about 600° C., some variations in relative amounts of the elements were observed. Satisfactory results were obtained, however, when the hulls were ashed in platinum crucibles at a temperature of approximately 850° C.

The spectrographic analyses were made with a Hilger E-1 quartz spectrograph in the region 2450–6700 Å. The ashed samples were vaporized in a graphite arc operated from a 220-volt d.c. source at 6–12 amperes

[1] *J. Am. Chem. Soc.*, 39, 1038 (1917).
[2] *J. Am. Soc. Agron.*, 20, 1102 (1928).
[3] Anderson and Kinsman, *J. Biol. Chem.*, 94, 39–47 (1931).
[4] Anderson, Hechtman, and Seeley, *Ibid.*, 126, 175 (1938).
[5] Tenn. Agr. Expt. Sta. Bull. 4, No. 5 (1891).
[6] *J. Am. Soc. Agron.*, 18, 1076 (1926).
[7] U. S. Dept. Agr. Farmers Bull. 1179 (1920).
[8] Doree, The Methods of Cellulose Chemistry, D. Van Nostrand Co., New York, 1933, p. 331.
[9] *Ind. Eng. Chem. Anal. Ed.*, 4, 202 (1932).
[10] *Methods of Analysis, A.O.A.C.*, 1935, p. 344.
[11] *Ind. Eng. Chem.*, 19, 850 (1927).
[12] *Ibid.*, 9, 556 (1917).

TABLE 1.—*Composition of oven-dried cottonseed hull bran*[a]

	per cent
Cross and Bevan cellulose	53.40
Pentosans in Cross and Bevan cellulose	19.05
Hydrolysis number (loss in cellulose due to 15% H_2SO_4 hydrolysis)	33.40
Furfural	22.50
Total pentosans	38.40
Lignin	23.40
Nitrogen (Kjeldahl) in lignin	0.52
Total nitrogen (Kjeldahl)	0.54
Methoxyl	2.16
Acetic acid by hydrolysis	4.98
Ash	2.28
Ether-soluble	0.27
1% Alkali-soluble	20.22
Cold-water soluble	1.87
Hot-water soluble	7.52

[a] Original hull bran contained 8.11% moisture.

with electrodes of the "special, highest purity" spectrographic grade of graphite.

The spectrograms§ of the ashed hulls showed the presence of large quantities of calcium, magnesium, sodium, and potassium. In addition, iron, manganese, copper, boron, phosphorus, silicon, barium, aluminum, and traces of zinc and nickel were present.

SUMMARY

Cottonseed hull bran has been analyzed for the following constituents: cellulose, lignin, methoxyl, acetic acid, ash, furfural, pentosans, and extractives by different solvents. Spectrographic analysis of the ash is also reported.

STUDIES ON THE QUANTITATIVE ESTIMATION OF LIGNIN

IV. EFFECT OF CERTAIN PROTEINS ON THE DETERMINATION OF LIGNIN BY THE FUMING HYDROCHLORIC ACID METHOD

By MAX PHILLIPS (Industrial-Farm Products Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture)

In a previous communication from this laboratory,[1] results were presented on the action of 42–43 per cent and 5 per cent hydrochloric acid on various carbohydrates in relation to the determination of lignin by the method of Goss and Phillips.[2] In this paper results of a similar study

§ The cooperation of Dr. Mary E. Warga of the University of Pittsburgh, who made the spectrographic analyses, is gratefully acknowledged.

[1] Phillips and Goss, *This Journal*, **21**, 140 (1938).

[2] *This Journal* 19, 341 (1936).

are given to show the effect on a number of proteins of the reagents used in this determination.

Several investigators have pointed out that whereas lignin isolated from wood by hydrolysis with either 42–43 per cent hydrochloric acid or 72 per cent sulfuric acid contains practically negligible quantities of nitrogen, this is not the case with lignin isolated from plants containing greater quantitites of nitrogenous constituents.

Paloheimo[3] has called attention to the fact that proteins are not completely hydrolyzed by the strong mineral acids used in the determination of lignin and he has, accordingly, suggested that from the weight of lignin obtained, be deducted the weight of the crude protein in the lignin ($N \times 6.25$). Although similar procedure has been followed in this laboratory, it has always been realized[4] that this involves the assumption that the nitrogenous complexes associated with the lignin are of protein character—a fact by no means established. It has also been fully recognized that the lignin figures thus obtained are approximations only.

Norman and Jenkins[5] found that no precipitate is obtained when egg-albumin or caseinogen is allowed to stand for 16 hours with 72 per cent sulfuric acid, and then diluted to 3 per cent and boiled. However, it was noted that when these proteins were added to straw and the mixture was treated as above described, there was an increase in the apparent lignin content of the straw. This, the authors believe, is probably due to an interaction of certain degradation products of protein with the lignin. In a later publication Norman[6] concludes that the increase in the weight of the crude lignin is caused by the condensation of the lignin with large protein fragments, partially deaminated, and that amino acids do not appreciably increase the apparent lignin content of straw.

EXPERIMENTAL

For this investigation there were used six proteins of vegetable origin: namely, zein, α-globulin of sesame seed, α-globulin of tomato seed, coagulated adsuki bean proteins, coagulated Georgia velvet bean proteins and coconut globulin, and one protein of animal origin, namely, lactalbumin. A series of experiments was first conducted for the purpose of ascertaining the effect on the several proteins studied of successive treatments with cold fuming hydrochloric acid and boiling dilute hydrochloric acid. All conditions were those prescribed by Goss and Phillips[2] for the quantitative estimation of lignin by the fuming hydrochloric acid method. The results obtained are recorded in Table 1.

It will be observed that the quantity of residual material obtained when the several proteins under examination were subjected to the

[3] *Biochem. Z.*, 165, 463 (1925); Transactions of the Agricultural Society of Finland, Part 13, Helsiniki (1926); *Biochem. Z.*, 214, 161 (1929).
[4] Phillips, *This Journal*, 15, 126 (1932); 18, 390 (1935).
[5] *Biochem. J.*, 28, 2160 (1934).
[6] *Ibid.*, 31, 1567 (1937).

TABLE 1.—*Results of treatment of proteins with cold fuming and boiling dilute hydrochloric acid*

PROTEIN	WEIGHT OF PROTEIN	N IN PROTEIN*	INSOLUBLE RESIDUE		N IN INSOLUBLE RESIDUE		
			WEIGHT	CALCULATED ON BASIS WEIGHT OF ORIGINAL PROTEIN	PER CENT	WEIGHT	CALCULATED ON BASIS OF ORIGINAL N PRESENT
	grams	*per cent*	*grams*	*per cent*		*grams*	*per cent*
Lactalbumin	0.5000	13.78	0.0038	0.76	5.13	0.00019	0.28
	1.0000		0.0083	0.83	4.16	0.00034	0.25
Zein	0.5000	15.15	0.0287	5.74	13.52	0.00388	5.12
α-Globulin of Sesame Seed	1.0000	16.89	0.0086	0.86	13.51	0.00116	0.69
α-Globulin of Tomato Seed	1.0000	16.36	0.0042	0.42	14.70	0.00062	0.38
Coagulated Adsuki Bean Proteins	1.0000	13.84	0.0344	3.44	12.29	0.00423	3.06
Coagulated Georgia Velvet Bean Proteins	1.0000	13.89	0.0138	1.38	12.23	0.00169	1.22
Coconut Globulin	0.5000	15.84	0	—	—	—	—
	1.0000		0.0026	0.26	17.06	0.00044	0.28

* Not corrected for moisture.

successive action of cold fuming hydrochloric acid and boiling dilute hydrochloric acid varied considerably. In the case of zein it amounted to 5.74 per cent, whereas the percentage of unhydrolyzed material was practically negligible in the case of the coconut globulin. The percentage of nitrogen in the residual material was generally less (and in certain cases considerably so) than in the original material.

The data presented in Table 2 show the effect of varying quantities of added protein materials on the yield of lignin obtained from rye straw. In all experiments there was used one gram of rye straw that had been successively extracted with a 1:2 alcohol-benzene solution, hot water, and boiling one per cent hydrochloric acid, as directed by Phillips.[7] The amounts of protein added to the one gram of rye straw were 0.1, 0.2, and 0.3 gram, respectively. In this series of experiments, as well, the conditions were exactly those prescribed by Goss and Phillips[2] for the quantitative estimation of lignin in plant materials.

It will be observed from Table 2 that, with only two exceptions, the yield of ash-free crude lignin increased with the increase in the quantity of protein added. The nitrogen in the crude lignin, calculated as per cent

[7] *This Journal*, 18, 386 (1935).

TABLE 2.—*Effect on yield of lignin from rye straw of adding varying amounts of protein*

1,000 grams of rye straw used for each experiment.[1] Per cent nitrogen in the extracted straw, 0.63.

PROTEIN ADDED	WEIGHT OF ADDED PROTEIN	WEIGHT OF N IN ADDED PROTEIN	TOTAL N ORIG. ADDED	WEIGHT OF CRUDE LIGNIN (ASH-FREE)	N IN CRUDE LIGNIN			INCREASE IN WEIGHT OF CRUDE LIGNIN DUE TO ADD. OF PROTEIN	FACTOR FOR CORRECTING FOR INCREASE IN WEIGHT OF N
					CALCULATED ON ASH-FREE BASIS	INCREASE IN WEIGHT OF N IN CRUDE LIGNIN	CALCULATED ON BASIS OF TOTAL N ORIG. PRESENT		
	gram	*gram*	*gram*	*gram*	*per cent*	*gram*	*gram*	*per cent*	*gram*	
None	0	0	0.0063	0.1970	1.81	0.00356	—	56.50	—	—
Lactalbumin	0.1000	0.01378	0.02008	0.2003	2.42	0.00484	0.00128	24.10	0.0033	2.58
"	0.2000	0.02756	0.03386	0.2033	2.68	0.00545	0.00189	16.09	0.0063	3.33
"	0.3000	0.04134	0.04764	0.2071	2.79	0.00578	0.00222	12.13	0.0101	4.55
Zein	0.1000	0.01515	0.02145	0.1995	2.41	0.00481	0.00125	22.42	0.0025	2.00
"	0.2000	0.03030	0.03660	0.2067	2.78	0.00574	0.00218	15.68	0.0097	4.45
"	0.3000	0.04545	0.05175	0.2164	3.03	0.00655	0.00299	12.65	0.0194	6.49
α-Globulin of Sesame Seed	0.1000	0.01689	0.02319	0.2102	2.42	0.00509	0.00153	21.95	0.0132	8.63
"	0.2000	0.03378	0.04008	0.2170	3.06	0.00664	0.00308	16.57	0.0200	6.49
"	0.3000	0.05067	0.05697	0.2094	2.97	0.00622	0.00266	10.92	0.0124	4.66
α-Globulin of Tomato Seed	0.1000	0.01636	0.02266	0.2020	2.49	0.00503	0.00147	22.20	0.0050	3.40
"	0.2000	0.03272	0.03902	0.2105	2.87	0.00604	0.00248	15.48	0.0135	5.44
"	0.3000	0.04908	0.05538	0.2157	3.19	0.00688	0.00332	12.42	0.0187	5.63
Coagulated Adsuki Bean Proteins	0.1000	0.01384	0.02014	0.2048	2.26	0.00463	0.00107	22.99	0.0078	7.29
"	0.2000	0.02768	0.03398	0.2054	2.56	0.00526	0.00170	15.48	0.0084	4.94
"	0.3000	0.04152	0.04782	0.2167	2.67	0.00578	0.00222	12.09	0.0197	8.87
Coagulated Georgia Velvet Bean Proteins	0.1000	0.01389	0.02019	0.2032	2.00	0.00406	0.00050	20.11	0.0062	12.40
"	0.2000	0.02778	0.03408	0.2052	2.23	0.00457	0.00101	13.41	0.0082	8.12
"	0.3000	0.04167	0.04797	0.2062	2.42	0.00499	0.00143	10.40	0.0092	6.43
Coconut Globulin	0.1000	0.01584	0.02214	0.2029	2.18	0.00412	0.00086	19.96	0.0059	6.86
"	0.2000	0.03168	0.03798	0.2068	2.35	0.00486	0.00130	12.80	0.0098	7.54
"	0.3000	0.04752	0.05382	0.2001	2.45	0.00490	0.00134	9.10	0.0031	2.31

[1] Rye straw extracted successively with 1:2 alcohol-benzene solution, hot water, and boiling 1% HCl solution.

of the total nitrogen originally present in the straw and in the added protein, decreased in every case with the increase in the quantity of nitrogen in the sample. This is due, no doubt, to the fact that the proteins studied are much more susceptible to the hydrolytic action of the strong mineral acid used than are the nitrogenous complexes present in the hydrolyzed straw. It will be observed that 56.50 per cent of the nitrogen originally present in the hydrolyzed straw was found in the lignin residue, and that when proteins had been added, the results ranged from 9.10 per cent, in the case of coconut globulin, to 24.10 per cent in the case of lactalbumin. Judged by their relative resistance to hydrolysis, the nitrogenous complexes of rye straw must be considered as being quite unlike those of protein in character.

In the last column of Table 2 are recorded the ratios between the increase in the weight of crude lignin due to the addition of proteins and the increment of nitrogen in the crude lignin. It will be observed that this ratio varies both with the quantity and type of protein added. In a certain limited number of cases the ratios approach the figure 6.25, the conventional factor used for calculating the percentage of nitrogen found into percentage of crude protein, but in most instances this is not true. Because of this wide variability, it is not possible to compute the ratio between the increase in the weight of the crude lignin and the increment of nitrogen in the lignin that would be applicable in all cases. Moreover, of the total nitrogen in such materials as straws, stalks, hulls and cobs, only a portion can be considered as arising from proteins. The nature of the remaining nitrogen is, in the main, unknown. To assume, therefore, that the nitrogen in the crude lignin obtainable from such materials when analyzed by the method of Goss and Phillips is protein in character is, of course, not justified in a strict sense. When the nitrogen content of the lignin is small the error thus introduced is not appreciable. However, in determining the lignin content of materials rich in protein, it is not possible to apply a suitable correction for the nitrogenous complexes in the lignin because of the variability in the ratio between the increase in the weight of the crude lignin and the increment of nitrogen in this material. In such instances all that can be done is to report merely the weight of the ash-free crude lignin and the percentage of nitrogen in the lignin.

SUMMARY

The effect of proteins on the determination of lignin by the method of Goss and Phillips has been investigated. The results indicate that the resistance to hydrolysis of the several proteins studied (either alone or in the presence of different proportions of lignified plant material) by cold 42–43 per cent and boiling 5 per cent hydrochloric acid is quite different. Because of this variability, it is not possible to compute a fixed ratio

between the increase in the weight of the crude lignin and the increment of nitrogen in this material.

The writer wishes to express his thanks to Dr. D. B. Jones of the Protein Research Division of this bureau for supplying the proteins used in this investigation.

THE PHOTOMETRIC DETERMINATION OF NICOTINE ON APPLES, WITHOUT DISTILLATION

By L. N. MARKWOOD (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.)

In the method for determining nicotine on apples sprayed with nicotine bentonite previously described by the writer[1] the nicotine, after removal from the fruit, is distilled and converted to the silicotungstate according to conventional procedure.[2] The silicotungstic acid precipitation has been generally regarded as the most sensitive as well as the most accurate means for determining nicotine. Several micro-procedures are based on it.[3] Recently, however, a color test was described by Barta and Marschek[4] for the determination of nicotine, and was applied by them to the examination of tobacco. It shows promise of displacing the silicotungstate method for micro amounts of nicotine. When this test was described earlier by Barta[5] as a means of determining pyridine it was pointed out that nicotine also responds to the test, with the development of a red color the same as pyridine, but with a sensitivity not equal to that with pyridine. The reagents used were an aqueous solution of cyanogen bromide and an alcoholic solution of β-naphthylamine.

In view of the claim that nicotine can be determined in tobacco directly, without distillation, simply by extracting the tobacco with these two reagents, filtering, and measuring the color intensity or the absorption coefficient,[4] it seemed appropriate to investigate the possible application of this reaction to a direct determination of nicotine deposits on sprayed apples.

Barta[5] states that the solution to be tested should ideally contain only pyridine, as acids weaken the color and ammonia causes orange-yellow instead of red to appear. Obviously it is necessary to use some reagent to remove a water-insoluble nicotine insecticide from the surface of an apple, and if distillation is to be dispensed with this reagent must be considered in the color formation.

Experiments were conducted on apples sprayed with a water-insoluble nicotine insecticide (nicotine bentonite). The results show that the nico-

[1] *This Journal*, **21**, 151 (1938).
[2] *Methods of Analysis, A.O.A.C.*, 1935, 60.
[3] T. Kozu, *J. Agr. Chem. Soc. Japan*, **7**, 977 (1931); L. Nagy, *Biochem. Z.*, 249, 404 (1932); J. R. Spies, *Ind. Eng. Chem., Anal. Ed.*, **9**, 46 (1937); L. D. Goodhue, *Ind. Eng. Chem., Anal. Ed.*, **10**, 52 (1938). *Mezögazdasági Kutatások*, **10**, 29 (1937).
[5] *Biochem. Z.*, **277**, 412 (1935).

tine deposit can be determined directly and accurately through the color reaction mentioned and that the method is suitable for control operations where routine analyses are to be made.

Nicotine bentonite was chosen as the insecticide for experimental work, because it is the most important of the fixed nicotines and is now coming into commercial use. The nicotine in this product is only partially soluble in water and in acid solutions, but it is brought completely into solution by a weak concentration of fixed alkali. When apples coated with nicotine bentonite are shaken in a closed container with this alkaline solution, the nicotine is completely removed. This constitutes the first step in the determination of nicotine on apples. The procedure necessary to bring the alkaline extract to the condition under which the color test can be applied is presented in this paper.

PREPARATION OF STANDARD NICOTINE SOLUTIONS

In the preparation of known nicotine solutions, which are needed for the evaluation of unknowns, it is convenient to employ nicotine bentonite as the reference material. With ordinary care this product is stable over long periods. It can readily be made by the method described by C. R. Smith.[6] The nicotine content is determined by the official method[2] and ordinarily it is 5 to 8 per cent.

Although nicotine bentonite can be dispersed in sodium hydroxide solution to bring the nicotine into true solution as the free base, the bentonite forms a colloidal suspension that is not readily filterable. Acidification or neutralization renders the suspended matter flocculent and filterable, but causes re-formation of insoluble nicotine bentonite. For example, if the alkaline suspension is neutralized with acetic acid to the phenolphthalein end point, the filtrate contains only about 70 per cent of the total nicotine. The suspension must therefore be kept alkaline to prevent any loss of nicotine on filtration. Flocculation and filterability, without reprecipitation of nicotine, can be accomplished by treatment with a suitable salt, such as calcium acetate, which in this case precipitates "calcium bentonite." The latter soon flocculates and settles out, leaving a practically clear supernatant liquid, which is readily filterable to a crystal-clear filtrate containing all the nicotine.

The flocculation of the calcium bentonite serves the further important purpose of removing the coloring matter that is brought out of apple skin by alkali, which removal is desirable in a solution that is later to be tested for light absorption.

To prepare solutions for the standard curve, a single quantity of nicotine bentonite powder is treated as outlined and the alkaline mixture is made to a fixed volume. Various aliquots of the alkaline filtrate are taken and adjusted to the same sodium hydroxide content as that of the

[6] U. S. Patent 2,096,566, Oct. 19, 1937. Also *J. Am. Chem. Soc.*, 56, 1561 (1934).

largest aliquot, also the same as that of the unknown. The purpose of this adjustment with alkali is to provide a uniform concentration of sodium salt in each standard solution. In other work to be published the writer has found that the concentration of sodium acetate formed on neutralization with acetic acid influences to some extent the degree of color development.

There is still variation in the small quantity of calcium found in the standards, due to the calcium acetate, all the calcium of which is not precipitated by the bentonite. However, the intensity of color does not appear to be affected by such variations at these low concentrations, and therefore no adjustment for calcium need be made.

Color development is markedly influenced by the hydrogen-ion concentration of the solution. Strong alkalinity prevents it altogether. As alkalinity decreases, intensity of color in a given time increases until a maximum is reached at the phenolphthalein end point. Further additions of acid cause a progressive reduction in intensity. Therefore, to achieve maximum sensitivity the solution is neutralized (decolorized) to phenolphthalein with acetic acid and made to a fixed volume. A small, fixed portion of each of these standard solutions is then treated with the color-forming reagents, as described later. The coincidence of maximum sensitivity with the phenolphthalein end point is fortunate, since it is a simple matter to make this adjustment and the solution is left in a colorless condition. In practice, first a strong and then a weaker solution of acetic acid is used. To prevent the reappearance of the pink color, one drop of acetic acid beyond the disappearance of the color is added.

The color-forming reagents are an aqueous solution of cyanogen bromide and an alcoholic solution of β-naphthylamine. A definite proportion of volume of reagents to volume of test solution must be used to get the proper color (red after yellow) and the maximum intensity in a given time, that is the same conditions must prevail throughout. The reagents, 1 cc. of cyanogen bromide and 5 cc. of β-naphthylamine, should be added to 5 cc. of solution in the order stated. The color reaches a maximum intensity in 50 to 70 minutes, or about 1 hour, at which time readings are made in a suitable photometer. In the work described here there was used a neutral-wedge (visual) photometer equipped with three absorption tubes (1, 3, and 10 cm. in length) and with an approximately monochromatic blue filter (optical center at 0.49 micron).[7]

The photometer readings, when plotted against concentrations, give a straight line, in conformity with Beer's law. The mechanism of the color reaction is not fully understood.[8] Addition of the cyanogen bromide alone produces no color if the nicotine concentration is quite low, but if it is sufficiently high a yellow color soon appears. On addition of the

[7] *This Journal*, **19**, 130 (1936).
[8] Kulikow and Krestowosdwigenskaja, *Z. Anal. Chem.*, **79**, 452 (1930).

naphthylamine the color changes slowly through yellow and orange to red or pink.

The method follows:

REAGENTS

(a) *Sodium hydroxide solution.*—0.5%.

(b) *Calcium acetate solution.*—Containing 4 grams of Ca per liter. This may be made by warming 10 grams of $CaCO_3$ with a dilute solution of 12–13 grams of glacial acetic acid, and diluting to 1 liter.

(c) *Phenolphthalein.*—Usual indicator strength.

(d) *Acetic acid solutions.*—A strong solution (about 30%) and a weaker solution (about 2%).

(e) *Cyanogen bromide.*—Prepared fresh before using. A fresh 10% KCN solution was added dropwise to saturated bromine water until the latter was just decolorized. The solution was then diluted to five times its volume.

(f) *β-naphthylamine.*—0.2 gram of the pure product was dissolved in 100 cc. of 95% alcohol. (This solution should be prepared fresh before use and kept away from sunlight to avoid coloration. A moderate fluorescence in direct daylight is normal.)

(g) *Bentonite.*—The powdered commercial mineral.

PROCEDURE

Four-tenths gram of nicotine bentonite was treated with 400 cc. of the NaOH solution in a 1 liter volumetric flask and allowed to stand a few minutes with occasional shaking. To this was added 400 cc. of distilled water, and then 20 cc. of the Ca acetate solution, with swirling. The solution was made to volume and mixed well. After 15 minutes, by which time the flocculent precipitate had settled, the mixture was filtered rapidly through a fluted paper. To avoid disturbing the settled precipitate on decanting, the mixture was usually first transferred to a conical flask.

Aliquots of the clear filtrate were pipetted into 250 cc. volumetric flasks. These aliquots normally were 50, 100, 150, and 200 cc., and they contained 20, 40, 60, and 80 cc., respectively, of the NaOH solution. To bring them to the same alkali content there was added 60, 40, 20, and 0 cc., respectively, of the NaOH solution. After the addition of 2 or 3 drops of phenolphthalein indicator, each solution was treated with strong acetic acid almost to decolorization and then just decolorized, plus one drop over, with the weaker acid. The solution was then made to volume. A blank was also prepared but with ordinary bentonite replacing the nicotine bentonite. In this case a single aliquot of 200 cc. was taken.

A 5 cc. portion of each standard solution was pipetted into a test tube, exactly 1 cc. of the CNBr solution was added, and the solution was mixed by swirling. Then exactly 5 cc. of the naphthylamine solution was added, and the solutions were mixed well by further swirling. The tubes were stoppered and set aside in a dark cabinet for 1 hour, when photometric readings were made.

A graph was constructed showing the relationship between concentration and photometric reading. A straight line was obtained for the range investigated, viz., 0–16 micrograms of nicotine per cc. This line passes through the blank as well as through the other points. A typical graph is shown in Figure 1.

TREATMENT OF APPLES

The sample of fruit was placed in a suitable closed container and shaken with the sodium hydroxide solution. The container may be a tin can, a glass bottle, or a bell jar having a glass plate clamped over the open end,

and should be provided with a draw-off valve. When the sample is large a tin can is the most suitable container. Since the number and size of the fruit vary, a representative procedure is outlined.

The sample of 10 mature apples was placed in the container, 200 cc. of sodium hydroxide solution was added, and the container was shaken about 3 minutes. The liquid was drawn off into a 500 cc. volumetric

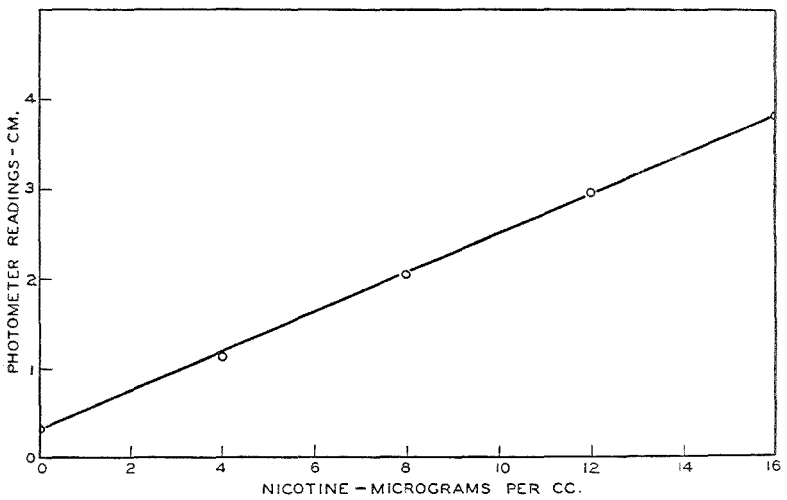

FIG. 1.—TYPICAL STANDARD CURVE FOR PHOTOMETRIC DETERMINATION OF NICOTINE.

flask. (When the apple sample is obviously too large for this amount of liquid, it will be necessary to increase the volume of sodium hydroxide solution as well as the quantities of wash water, and to adjust the final volume to the ratio shown later, viz., 500 cc. to each 200 cc. of alkali.) The apples were next shaken a minute or two, first with 180 cc. and then with 80 cc. of water, and each washing was drawn into the flask.* About 0.2 gram of powdered bentonite was then dispersed in the solution. (The object in adding this reagent here was to ensure the presence of enough bentonite for the calcium bentonite precipitate which follows.) Ten cubic centimeters of calcium acetate solution was added, then water to the mark, and the solution was mixed well. The mixture was filtered, and a 200 cc. aliquot was treated as described under "Procedure," beginning "After the addition of 2 or 3 drops." (In some cases the bentonite fails to decolorize the solution completely, leaving a slight yellowish tint, but this is of no consequence as the residual color normally disappears on neutralization. If on standing any turbidity develops in the neutralized

* The writer is indebted to R. D. Chisholm for suggesting the following shortened manipulation in the treatment of the apples: Shake the apples with exactly 200 cc. of the NaOH solution as described originally. Draw an 80 cc. aliquot into a 250 cc. volumetric flask and add about 100 cc. of water, then the bentonite, etc. By this procedure washing of the apples is eliminated. It is assumed that the apple surface is dry.

solution, it may be removed by a second filtration before the color reagents are applied.)

The color test was made as described above. The concentration of nicotine in the unknown was read directly from the standard curve. Calculations were made back to the total sample with due regard for the volumes used.

When the neutral-wedge photometer[7] is used, it appears to be unnecessary to run a new set of standards for each unknown, except as an occasional check, but if a given calibration line is to be used safely with each unknown, the conditions must be uniform, especially the freshness of the color reagents and the interval allowed for color development.

Since a blank run on apples carrying no nicotine gave a reading agreeing closely with the blank of the standards an apple blank can ordinarily be dispensed with.

The sodium hydroxide solution does not give complete wetting of the waxy apple surface. When the apples are shaken with this solution, however, all the white spots of nicotine bentonite disappear, indicating that the insecticide has been effectively removed notwithstanding the apparently inadequate wetting. In comparative tests to ascertain the effectiveness of soap, which was found to give complete wetting, the recovery of nicotine was no higher when soap was used.

It is well known that apples build up a heavy coating of wax during storage, and as a result the removal of underlying spray residues, such as arsenicals, is made difficult. No tests have been made on nicotine-sprayed apples that have been kept in storage, and hence no claim is made that the method described here is applicable in such cases. This method was developed chiefly for use on fruit to be examined soon after picking.

Mature Winesap apples were used in the tests reported here. The skin was dark red and waxy.

The over-all recovery by this method was investigated by adding known quantities of nicotine bentonite to unsprayed apples. The results (Table 1) show that recovery is complete. The slight excess of nicotine recovered is without significance; it may be due to a slight concentrating effect during filtration, since the standards were filtered only once for the removal of the calcium bentonite, whereas the apple solutions were filtered again after neutralization to remove a slight turbidity.

Typical data are given in Table 2 on lots of apples sprayed at two different times (Series 1 and 2). The apples were especially sprayed for this work, and analyses were made about 4 days after spraying. A different photometer was employed in this and subsequent work, therefore no comparison of scale readings can be made with previous data.

The color reaction used is probably the most sensitive means known of determining nicotine. The sensitivity in actual use depends, of course, on the thickness of the layer of liquid being measured. By using the

TABLE 1.—*Over-all recovery of nicotine by proposed method*
(Ten apples per lot, total weight 2 pounds 5 ounces (1020 grams))

MATERIAL	PHOTOMETER READING	NICOTINE			RECOVERY
		PER CC.*	IN ENTIRE SAMPLE		
			FOUND	ADDED†	
	cm.	*mmg.*	*mg.*	*mg.*	*per cent*
Standards:					
Blank	2.11	0.00			
50-cc. aliquot	2.78	4.00			
100-cc. aliquot	3.53	8.00			
150-cc. aliquot	4.23	12.00			
200-cc. aliquot	4.92	16.00			
Apples:					
Lot 13	2.79	4.02	2.51	2.50	100.4
Lot 14	3.54	8.02	5.01	5.00	100.2
Lot 15	4.26	12.09	7.56	7.50	100.8
Lot 16	4.95	16.10	10.06	10.00	100.6

* Neutralized solution.
† As nicotine bentonite.

TABLE 2.—*Photometric determination of nicotine on lots of 10 apples*

SERIES	MATERIAL	PHOTOMETER READING	NICOTINE	
			PER CC.*	IN ENTIRE SAMPLE
		cm.	*mmg.*	*mg.*
1	*Standards:*			
	Blank	0.34	0.0	
	50-cc. aliquot	1.13	4.0	
	100-cc. aliquot	2.03	8.0	
	150-cc. aliquot	2.96	12.0	
	200-cc. aliquot	3.82	16.0	
	Apples: Wt. per lot, 2 lbs., 8 oz. (1133 grams)			
	Lot 2 (sprayed)	2.85	11.6	7.25
	Lot 9 (unsprayed)	0.36	—	—
2	*Standards:*			
	Blank	0.29	0.0	
	50-cc. aliquot	1.12	4.0	
	100-cc. aliquot	2.03	8.0	
	150-cc. aliquot	2.96	12.0	
	200-cc. aliquot	3.79	16.0	
	Apples: Wt. per lot, 2 lbs., 6 oz. (1077 grams)			
	Lot 11	2.85	11.7	7.31
	Lot 12	2.84	11.7	7.31

* Neutralized solution.

largest (10 cm.) tube with which the writer's photometer was equipped, a nicotine concentration of 1 part in 4 million, or 0.25 microgram per cc., could be determined. With a sensitivity of this order it was considered possible to determine the nicotine coverage on a single apple and this has been done.

Data for individual apples are given in Table 3. Each apple was treated with one-tenth the quantities of reagents specified for the group of 10 apples, or for the standards. The final volume for each apple was therefore 25 cc. as compared with 250 cc. for each of the standards, and this difference is taken into account in calculating the total nicotine. The results also show the uniformity of coverage that can be attained by careful spraying.

TABLE 3.—*Photometric determination of nicotine on single apples*
(Weight per apple, 4.5 ounces (127 grams))

MATERIAL	PHOTOMETER READING	NICOTINE	
		PER CC.*	IN ENTIRE SAMPLE
	cm.	*mmg.*	*mg.*
Standards:			
Blank	0.30	0.0	
50-cc. aliquot	1.18	4.0	
100-cc. aliquot	2.08	8.0	
150-cc. aliquot	2.93	12.0	
200-cc. aliquot	3.79	16.0	
Apples:			
A	3.20	13.2	0.82
B	3.18	13.2	0.82
C	3.11	12.8	0.80
D	3.13	12.9	0.81
E	3.13	12.9	0.81

* Neutralized solution.

INTERFERING SUBSTANCES

Since nicotine-sprayed fruit may also be coated with other insecticides, as well as fungicides, it becomes necessary to broaden the investigation to a study of the interference, if any, caused by these extraneous substances and means for their elimination. Accordingly, the following materials were investigated: Lead arsenate, calcium arsenate, cryolite, lime-sulfur, Bordeaux mixture, copper ammonium silicate, and a wettable sulfur containing bentonite.

In making these tests equal quantities of the supplementary material and the nicotine bentonite were used. Specifically, 0.4 gram of each was treated as described under "Procedure," that is, with 400 cc. of sodium hydroxide solution, 400 cc. of water, and 20 cc. of calcium acetate solu-

tion, after which the mixture was made to 1 liter and filtered. For convenience an aliquot of 80 cc. was neutralized and made to 100 cc. This neutralized solution was compared with a similar solution prepared from nicotine bentonite only. The results of these tests are summarized as follows:

Lead arsenate, calcium arsenate, Bordeaux mixture, copper ammonium silicate, and wettable sulfur do not interfere.

Cryolite permits a recovery of only about 84 per cent. Some cryolite is dissolved by the alkali, as shown by the appearance of a precipitate on neutralizing; moreover, fluoride ion can be detected in the solution. When this ion is removed by treating the alkaline solution with calcium oxide and filtering off the insoluble residue, the resulting solution on neutralization gives the correct reading.

Lime-sulfur (dry) causes the most interference of any added material, the recovery amounting to only about 76 per cent. The following ions can be detected in the alkaline solution: Sulfide and polysulfide (strong test), sulfate (weak), sulfite (appreciable), and thiosulfate (stronger than sulfite). Calcium is also present, but in about the same quantities as in the standards. Removal of sulfide and polysulfide sulfur (via copper carbonate) raises the recovery to 82 per cent; removal of all the forms of sulfur mentioned brings it to only 86 per cent. This is the highest recovery attained and it is still inadequate. It appears, therefore, that some factor connected with the complex nature of lime-sulfur, a full understanding of which is still lacking, is responsible for the interference. Since no remedial procedure was found, it will be necessary to separate the nicotine by distillation, submit the distillate to the color test, and compare with standards containing only nicotine in solution.

Among possible organic compounds, pyridine and its derivatives (of which nicotine is one) also respond to the color test described here. In some cases the color formed is not red; e.g., nicotinic acid produces a yellow color. Pyridine itself gives a more intense reaction than any of its derivatives, including nicotine; accordingly, if pyridine is present in a nicotine determination the results will be high. Since the simple homologues of pyridine, such as the picolines, give relatively weak colors, their presence will not seriously affect the results.[9]

The presence of pyridine or simple homologues in a spray residue is unlikely. Pyridine has been found in commercial nicotine, but it does not form so insoluble a bentonite as nicotine. Furthermore, any pyridine in water-soluble form deposited on fruit would probably soon disappear because of the volatile nature of that base. If nicotinic acid, an oxidation product of nicotine, is present, it will be removed as the insoluble calcium compound. In general, therefore, no interfering organic substances will be found in the final solution.

[9] L. Barta and Z. Marschek, *Biochem. Z.*, 293, 118 (1937).

SUMMARY

A direct photometric method is presented for determining the amount of nicotine spray-deposit on apples. The method involves stripping of the fruit with dilute sodium hydroxide solution, purification of the extract by means of a calcium bentonite coagulate, formation of a colored compound from nicotine by means of cyanogen bromide and β-naphthylamine, and measurement of the color with a photometer. Since no distillation is required, the method is suitable for rapid mass operation.

ACKNOWLEDGMENT

Appreciation is expressed to E. H. Siegler of the Division of Fruit Insect Investigations of this Bureau for spraying the apples.

A RAPID VOLUMETRIC MICRO METHOD FOR DETERMINING ARSENIC

By C. C. Cassil (Bureau of Entomology and Plant Quarantine) and H. J. Wichmann (Food and Drug Administration, U. S. Department of Agriculture)

The inherent errors and limitations of the Gutzeit and other colorimetric methods for determining arsenic are generally recognized. In an effort to find a substitute, the writers have developed a rapid titration method that will determine 5–500 micrograms of arsenious oxide. It involves an arsine evolution, absorption of the arsine in mercuric chloride, and the stoichimetric oxidation of the arsine to arsenic oxide.

The principle of this method was described by Smith,[1] who showed that quantities of arsenious oxide ranging from 2 to 10 mg. could be evolved as arsine and determined with a fair degree of accuracy. Tabor[2] tried to adapt this procedure to smaller quantities but failed to obtain recoveries better than 95 per cent, principally because he worked with large volumes of solution. Winterfelt et al.[3] developed a similar procedure for quantities ranging from 50 to 500 micrograms of arsenious oxide, but their method is extremely slow, requiring a 45-minute evolution and from ½ to 2 hours for the iodine oxidation. In some preliminary work Cassil[4] succeeded in materially shortening the time required for a determination. The method finally developed and presented in detail in this paper can be used for apple strip solutions, for material that has been submitted to a wet digestion, and perhaps for other methods of preparation.

EXPERIMENTAL PROCEDURE

ISOLATION REAGENTS

(a) *Hydrochloric acid.*—Use a C.P. concentrated acid that is arsenic-free.

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[1] U. S. Dept. Agr. Bur. Chem. Circ. 102 (1912).
[2] *This Journal*, 13, 417 (1930); 14, 436 (1931).
[3] *Arch. Pharm.*, 273, 457 (1935).
[4] *This Journal*, 21, 200 (1938).

(b) *Zinc.*—Use a good grade of 20- or 30-mesh granulated zinc. It should be as free from arsenic as possible because the arsenic in the zinc predominantly determines the size of the blank.

(c) *Potassium iodide solution.*—Dissolve 15 grams of KI in water and dilute to 100 cc.

(d) *Stannous chloride solution.*—Dissolve 40 grams of As-free $SnCl_2 \cdot 2H_2O$ in 100 cc. of concentrated HCl.

(e) *Absorbing solution.*—Dissolve 1.6 grams of $HgCl_2$ (recrystallized if necessary to eliminate titration reagent blank) and 0.05 gram of U.S.P. gum arabic in water and dilute to 100 cc.

(f) *Lead acetate.*—Dissolve 10 grams $Pb(C_2H_3O_2)_2$ in 80 cc. of water, add sufficient acetic acid to have the solution just acid to litmus paper, and make to 100 cc. with water.

TITRATING REAGENTS

(g) *Potassium iodide solution.*—Dissolve 20 grams of KI, previously recrystallized in the presence of excess iodine, in water and dilute to 100 cc. This reagent must be recrystallized in the presence of excess I_2 because most KI samples purchased on the open market have impurities which reduce iodine, thereby causing an undesirably large blank. There is no waste of KI by recrystallization, because the mother liquor can be evaporated to dryness and the recovered KI used for reagent (c).

(h) *Buffer solution.*—Dissolve 10 grams of $Na_2HPO_4 \cdot 12H_2O$ in water and dilute to 100 cc.

(i) *Standard iodine solutions.*—Prepare an approximately 0.05 *N* stock solution of I_2 by dissolving 6.35 grams of pure I_2 and 12.7 grams of KI in a small quantity of water; filter, and dilute the filtrate to 1 liter. Proper dilutions of this stock solution are used to prepare approximately 0.001 *N*, 0.005 *N*, and 0.01 *N* I_2 solutions. An additional amount (25 grams/liter) of KI is added to each of the dilute I_2 solutions.

(j) *Standard arsenic solutions.*—Prepare a stock solution by dissolving 1 gram of standard As_2O_3 in 25 cc. of a 20% NaOH solution. Saturate the solution with CO_2 and dilute to 1 liter with recently boiled water. 1 cc. of this solution contains 1 mg. of As_2O_3. Make three standard solutions containing 50, 250, and 500 micrograms of As_2O_3 per cc., respectively, by proper dilution of the stock solution.

(k) *Starch indicator.*—Mix about 2 grams of finely powdered potato starch with cold water to a thin paste. Add about 200 cc. of boiling water, stirring constantly, and immediately discontinue heating. The solution is preserved indefinitely by the addition to the reagent bottle of approximately 1 cc. of metallic Hg.

APPARATUS

Use a 125 cc. Erlenmeyer flask fitted with a 24/40 standard taper ground-glass joint for the generator, and attach this to an 18 cm. water-cooled condenser that has a 18/38 ground-glass joint on the upper end. Fit an adapter to the upper end of the condenser and connect the other end of the adapter to the delivery tube by means of a 10/30 ground-glass joint. The end of the delivery tube is made of methyl methacrylate resin to prevent sticking of the mercury arsenide on the inside. (Bakelite tubing has also been used satisfactorily.) The baffle on the resin tube is an aid in stirring the solution. Fill the adapter with two wads of dry Pyrex glass wool (As-free) that has been previously saturated with reagent (f). (The glass wool acts as a scrubber to remove any H_2S that may be generated during the evolution. The first wad of glass wool is efficient in removing the H_2S, but it becomes saturated in about 15 determinations. The other wad acts as an indicator to show when the first wad is so spent as to need replacement with a fresh piece of impregnated glass

wool.) Construct the receiver so that the constricted end will hold approximately 1 cc. of solution in a 4 cm. length and also allow the delivery tube to extend to the bottom. The upper end of the receiving tube is approximately 25 mm. in outside diameter and about 12 cm. long. The opening in the lower end of the delivery tube should not be over 2 mm. in diameter. See Figure 2.

NOTE: A 1 mm. glass capillary tube has been used successfully in place of the methyl methacrylate delivery tube for quantities of arsenic not exceeding the equivalent of 30 micrograms of As_2O_3. If the glass tube is used, do not insert it into the absorbing liquid until immediately after connecting the generator to the apparatus, thus preventing the absorbing liquid from backing up into the delivery tube.

ARSENIC ISOLATION PROCEDURE

Place in the generator flask a suitable aliquot from the solution to be analyzed but not exceeding 75 cc., containing the equivalent of 5-500 micrograms of As_2O_3; add sufficient concentrated HCl to make the total amount of acid (H_2SO_4+HCl) approximately 10 cc., 5 cc. of the KI reagent, and 1 cc. of the $SnCl_2$ reagent,and dilute to approximately 80–90 cc. Place 1 cc. of the absorbing solution (e) in the receiver and connect it to the apparatus. Add 4–5 grams of the Zn reagent to the generator flask and connect the flask to the apparatus immediately. Bring the solution to boiling in about 2 minutes and continue the heating at approximately 95° C. until the evolution has continued 5 minutes. Approximately 1500 cc. of hydrogen is evolved in 5 minutes, and this is sufficient to expel all arsenic up to the equivalent of at least 500 micrograms of As_2O_3.

IODINE STANDARDIZATION

Three concentrations of I_2 (0.001 *N*, 0.005 *N*, and 0.01 *N*), are used for the ranges 5–50, 50–250 and 250–500 micrograms of As_2O_3, respectively. Standardize each I_2 solution in the same manner, i.e., add to the receiver 3 cc. of the I_2 solution from a micro buret (5 cc. graduated to .01 cc.), 2 cc. of the buffer solution, and approximately 0.5 cc. of the starch indicator, and titrate to a colorless end point, as observed through length of tube, using that strength of As_2O_3 solution (from micro buret) that is approximately equivalent to the I_2 solution being titrated. Use the delivery tube as a stirring rod. As a check on the reagents, place in the receiving tube 1 cc. of the absorbing reagent, sufficient of the KI reagent (g) to precipitate and redissolve the HgI_2, 2 cc. of the buffer reagent, 0.5 cc. of the starch indicator, and 3 cc. of the I_2 solution and titrate to a colorless end point with the As_2O_3 solution. The two titrations should check within 0.01 cc. if the reagents are pure. Determine the factor needed to transform the As_2O_3 solution into the I_2 solution. Calculate the titer of the I_2 solution as follows:

$$\frac{\text{mmg. } As_2O_3/\text{cc.} \times \text{cc. } As_2O_3 \text{ soln.}}{\text{cc. } I_2 \text{ soln.} \times 4} = \text{mmg. } As_2O_3/\text{cc. of } I_2 \text{ soln.}$$

DETERMINATION

The description has been general up to this point for the range 5–500 micrograms of As_2O_3. The analyst must learn to estimate the amount of arsenic in the sample after isolation by the appearance of the suspended mercury arsenide so that the correct strength of I_2 may be used in the titration. For further descriptive purposes, it is assumed that the quantity of arsenic in the receiver tube after isolation is between the equivalent of 5 and 50 micrograms of As_2O_3.

After the 5 minute evolution, add to the receiver sufficient KI reagent (g) to precipitate and redissolve the HgI_2, and disconnect the receiver and delivery tube.

Add 5 cc. of 0.001 *N* I_2 solution from the micro buret through the delivery tube and stir with the delivery tube until the solution is well mixed; add 2 cc. of the buffer reagent and about 0.5 cc. of the starch indicator through the delivery tube to wash all I_2 solution into the receiver. Titrate the excess I_2 with As_2O_3 solution (1 cc. = 50 micrograms) to colorless end point. If the end point is over titrated, more I_2 solution may be added and this again back-titrated with As_2O_3 solution.

$$\text{mmg. } As_2O_3 = [(\text{cc. } I_2 - \text{cc. } As_2O_3 \times \text{factor}) - \text{Blank}] \times I_2 \text{ titer.}$$

NOTE: The blank is caused solely by the arsenic in the zinc and acid used in the generator and generally amounts to 1–2 micrograms of As_2O_3, depending upon the grade of reagents used.

DISCUSSION

If pentavalent arsenic is present in the aliquot to be analyzed, it is reduced to the trivalent form by potassium iodide, which is added to the acid solution. A preliminary reduction of the arsenic oxide is not necessary, as with the Gutzeit procedure, since the generator solution is heated close to boiling as quickly as possible.

The liberated arsine is absorbed quantitatively by the solution of mercuric chloride, for which it has an unusually great affinity. The arsine in its reactions with mercuric chloride first forms one or more arsenides, but no attempt has been made to identify these compounds. They are oxidized by the excess mercuric chloride, slowly when cold and rapidly on heating, forming arsenious acid and calomel. The iodine reduction brought about by the mixture before or after the transformation represents the oxidation of arsine to arsenic oxide in which 1 As is equivalent to 8I. Equations representing the reactions involved in this method are:

(1) $As_2O_3 + 6H_2 \rightarrow 2AsH_3 + 3H_2O$.
(2) $2AsH_3 + 12HgCl_2 + 3H_2O \rightarrow \text{arsenides} \rightarrow 12HgCl + As_2O_3 + 12HCl$.
(3) $12HgCl + 24KI \rightarrow 6Hg^\circ + 6K_2HgI_4 + 12KCl$.
(4) $6Hg^\circ + As_2O_3 + 8I_2 + 12KI + 2H_2O \rightarrow 6K_2HgI_4 + As_2O_5 + 4HI$.

FACTORS INFLUENCING THE METHOD

Since this method was intended to minimize the time required for a determination (5 minutes in its present form) sufficient hydrogen must be developed in that time to reduce the arsenic to arsine and sweep it through the apparatus into the absorption medium, where it must be completely absorbed. It has been found by experiment that 10 cc. of acid in a volume up to 90 cc. and 4–5 grams of zinc at 90°–100° C. will produce more than 1500 cc. of hydrogen in 5 minutes. This method does not limit the volume in the generator to 40 cc., as does the Gutzeit procedure, but 5–500 micrograms of arsenious oxide can be evolved from volumes up to 90 cc. It has also been demonstrated that 500 micrograms of arsenious oxide can be evolved out of a 300 cc. volume in 10 minutes by increasing the reagents proportionately. A water-cooled condenser is used in the apparatus to condense the water and acid vapor produced by boiling the solution.

Other workers have no doubt noticed the tendency of mercury arsenides to form on the inside of the glass delivery tube, but they have not commented on the strong adherence and resistance to oxidation of the deposited arsenides. Since the writers were seeking a rapid accurate method that would compete with the Gutzeit procedure, it was necessary to find some material that would not exhibit this phenomenon of sticking. The use of a tube formed from methyl methacrylate resin, known in commerce as Leucite,* obviated the trouble, probably because it is not wetted by aqueous solutions. Bakelite tubing has also been used satisfactorily. Gum arabic is added to the absorbing solution to keep the

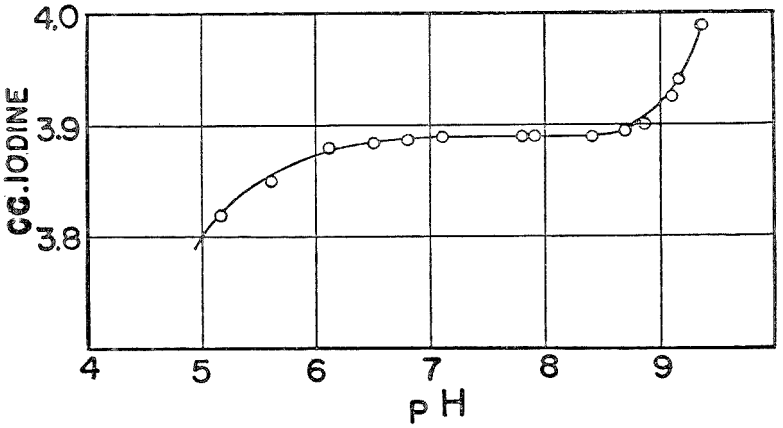

FIG. 1.—TITRATION CURVE OBTAINED BY TITRATING As_2O_3 WITH I_2 AT VARIOUS *p*H VALUES.

mercury arsenides in a colloidal suspension that will permit almost instantaneous oxidation by the iodine. Only 1 cc. of absorbing solution is used per determination, in order to keep the volume in the receiver small enough for the necessary micro iodine titrations. That it is sufficient to absorb the arsenic quantitatively, even though the hydrogen is passing through the receiver at the rate of 300 cc. per minute, has been proved by the complete recoveries obtained, and also by the fact that no stain was produced by the gas after passing through the solution when a stopper fitted with a Gutzeit tube and strip was inserted in the top of the receiver.

After the 5 minute evolution sufficient potassium iodide is added to form the soluble double potassium mercuric iodide, and then a standard iodine solution representing about 25 per cent excess is added to dissolve the precipitate. At this stage all the mercury is oxidized, but the arsenic is not completely oxidized until the buffer solution of disodium phosphate

* Leucite is furnished in rod form by E. I. Du Pont de Nemours and Company and the delivery tube is machined from the rod and polished. At present the complete tube is not available commercially.

is added. The optimum pH value for this buffer solution was obtained by titrating arsenious oxide solutions with 0.001 N iodine at various pH

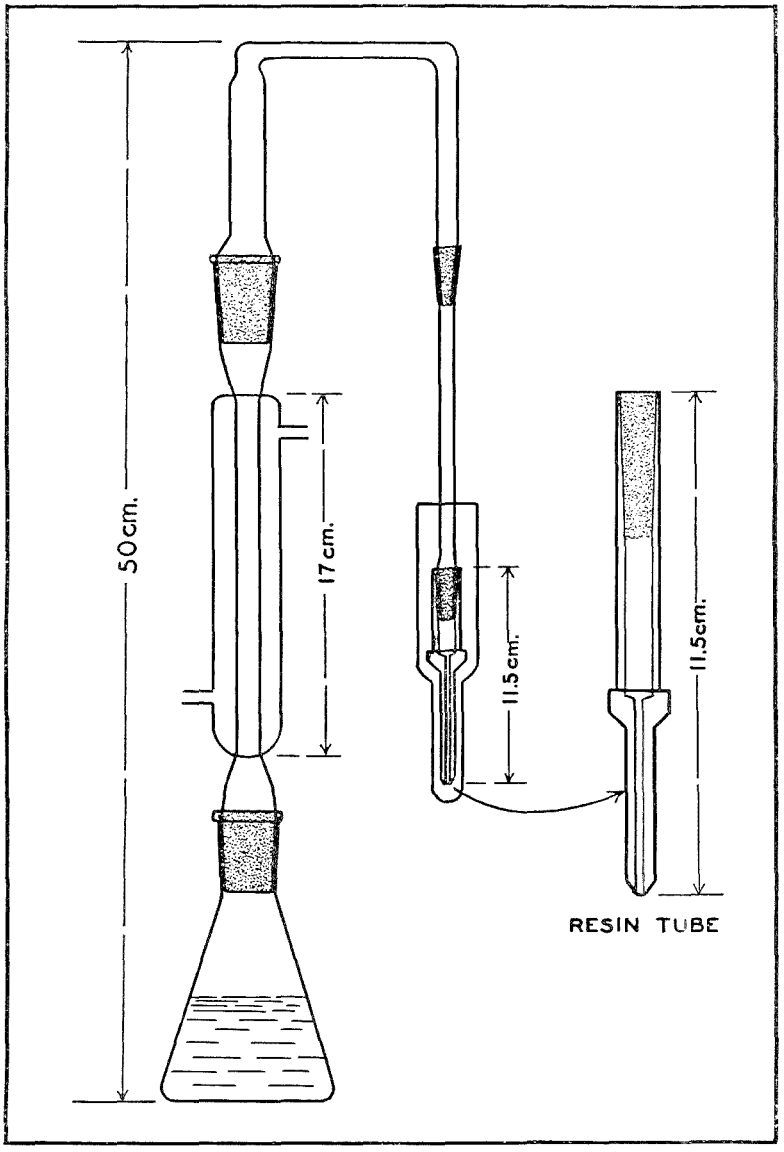

FIG. 2.—DIAGRAM OF APPARATUS USED FOR EVOLUTION, ABSORPTION, AND TITRATION OF ARSENIC.

levels. The pH values, obtained with a Beckman pH meter after each titration, were then plotted against cc. of iodine used. As can be seen

(Figure 1) the optimum *p*H value is centered between 7 and 8.5. The reaction is decidedly sluggish at *p*H values less than 7 and the end point continues to fade. The slope of the curve below *p*H 7 depends on the patience of the analyst. Above *p*H 8.7 too much iodine is used, and it forms hypoiodites and iodides with the excess base. Disodium phosphate in the concentration used will produce a *p*H of 8.5 when no arsenic is present in the determination, but owing to the production of hydrochloric acid in the reaction the *p*H value is reduced to 7.3 when quantities of arsenic equivalent to 500 micrograms of arsenious oxide are passed into the receiving solution. As soon as the buffer has been added and the mixture stirred, the excess iodine is back-titrated with a standard solution of arsenious oxide. The quantity of arsenic can then be calculated as shown in the experimental procedure.

INTERFERENCES

Any hydrogen sulfide that may be formed during the evolution is scrubbed out of the gas as it passes through the dry plugs of Pyrex glass wool impregnated with lead acetate. Phosphides, phosphites, or hypophosphites might well produce interference if placed directly in the generator but if previously oxidized to phosphate no interference will result. Selenates and selenites are both reduced, principally to metallic selenium, in the generator. If any hydrogen selenide is formed, it is either scrubbed out of the gas by the lead acetate or it does not have any effect on the absorbing solution, because experiments have shown that quantities up to 10 mg. of sodium selenate or ammonium selenite cause no interference. Sulfur dioxide is reduced to hydrogen sulfide in the generator and either compound would interfere if it passed the scrubber. Neither substance can be present after an oxidation. Ordinary concentrated sulfuric acid may contain sufficient sulfur dioxide in 10 cc. to saturate the scrubber if not previously submitted to a wet digestion. Blanks run on digestion mixtures have proved that sulfates and phosphates do not cause any interference. If mercury salts are present in the generator, mercury is plated out on the zinc and stops the evolution of hydrogen. The only element that could possibly interfere after a wet acid digestion is believed to be antimony, and up to the present time no effort has been made to guard against this interference. Smith[1] showed that arsenic could be separated from antimony by the familiar coprecipitation of magnesium ammonium arsenate with magnesium ammonium phosphate. This separation has not been confirmed, since the analyst is seldom confronted with the need for separating these two elements. Further studies on the separation of arsenic and antimony are in progress, and it is also hoped that the procedure described can be adapted to the quantitative evolution of stibine and the iodometric determination of antimony.

Gross[5] showed that 20 mg. of pyridine or nicotine, when digested with nitric and sulfuric acids, could not be completely destroyed, and that the residue inhibited the evolution of arsine in the Gutzeit method. Cassil[6] was able to destroy these interferences by using perchloric acid in addition to nitric and sulfuric acids in the digestion. Pure pyridine or nicotine, present to the extent of 1 gram in the generator flask, exhibits the same darkening effect as that observed in the Gutzeit bottle, but on being heated the solution clears and the arsenic recovery is within the limits

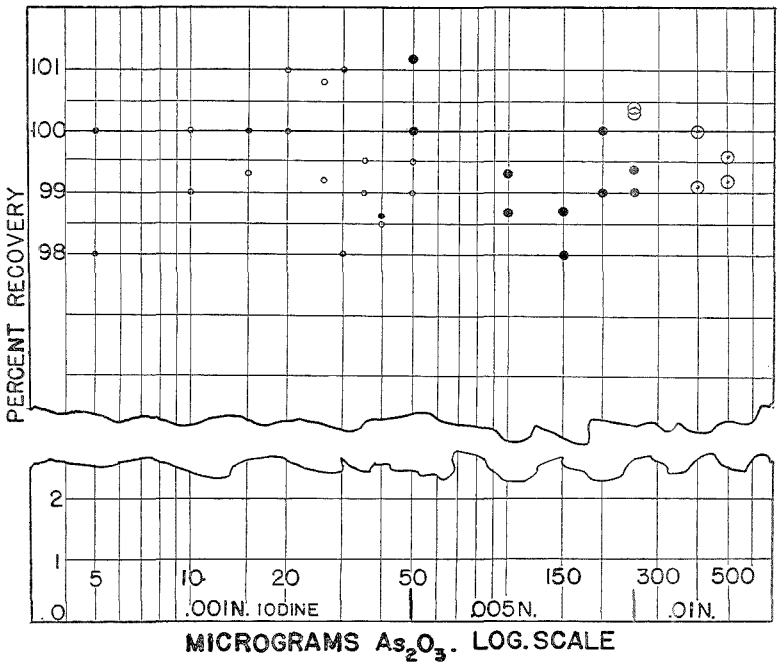

FIG. 3.—RECOVERIES OBTAINED ON QUANTITIES OF As_2O_3 RANGING FROM 5 TO 500 MICROGRAMS.

of accuracy. However, when 0.4 gram of pyridine or nicotine is partially digested with sulfuric and nitric acids and placed in the evolution flask, the arsenic recovery is only 80–90 per cent. It is advisable to digest any organic material with nitric, sulfuric, and perchloric acids unless it can be shown that the organic matter does not interfere with the method. Rose leaves and apple plugs placed directly in the generator do not interfere with this procedure, but other kinds of organic material might produce volatile reducing constituents, not condensed or scrubbed out

[5] *Ind. Eng. Chem. Anal. Ed.*, 6, 327 (1934).
[6] *This Journal*, 20, 172 (1937).

under the conditions of the method. Analysts should, therefore, be cautious in accepting results obtained on new products without digestion unless there is proof that interfering substances are absent.

ACCURACY AND PRECISION

The results of 34 recovery experiments are given in Figure 3. The titration of the first 18 determinations on the left (small open circles) were made with 0.001 *N*, the next 10 (solid circles) with 0.005 *N*, and the last 6 (large open circles) with 0.01 *N* iodine. All these experiments were made with arsenious oxide that had been oxidized to arsenate by means of iodine before being placed in the generator flask. Equally satisfactory results have been obtained with known amounts of arsenic that had been submitted to an acid digestion. The average recovery shown in Figure 3 is 99.5 per cent with a standard deviation of 0.85 per cent. The minimum quantity that can be determined is set at 5 micrograms, because the titration error, 0.01 cc. of 0.001 *N* iodine, causes an error of more than 2 per cent on any quantity below 5 micrograms. If the analyst finds that his recoveries are consistently less than 99.5 per cent, he should check the amount of hydrogen evolved in 5 minutes and, if necessary, add more stannous chloride, heat more rapidly, or extend the evolution time a minute or two.

TIME AS A FACTOR IN CONTROL WORK

Approximately 10 minutes is required for a single determination. In routine work it is possible to run 12–15 samples per hour with two sets of apparatus, if the runs are staggered so that one is ready for titration every 4 minutes. This would amount to about 100 determinations per day. While it is true that more than 100 Gutzeit determinations can be run in a single day, considerable time is wasted in setting up standards for each set of determinations, many repeats must be made owing to the inability to choose an aliquot containing 5–30 micrograms of As_2O_3, and it is not unusual for duplicate strips not to check. Furthermore, the Gutzeit determination has a standard deviation of about 10 per cent,[7] whereas with the proposed method the standard deviation is not more than 0.85 per cent.

APPLE STRIP SOLUTION

This rapid method gives satisfactory results with apple strip solutions as prepared by the tentative A.O.A.C. method. Results obtained on the alkali-oleate strip solution, and also on a portion of the same digested with sulfuric, nitric, and perchloric acids, are shown in Table 1. For confirmatory work a group of unwashed apples and a group of washed apples purchased from a local store were used. They appear in the table as lots I and II, respectively. The oleic acid must be removed from the strip solution by the usual acidification and filtration, because its presence in

[7] Neller, J. R., *This Journal*, 12, 332 (1929).

the generator either retards or stops the evolution of hydrogen. The waxes and other organic material present in the strip solution do not interfere with the method when compared with a digested portion of the same, as shown in Table 1.

TABLE 1.—*Analyses obtained on alkali-oleate strip solutions of apples and on acid-digested portions of the same*

	STRIP SOLUTION	DIGESTED SOLUTION
	gram As_2O_3/lb.	*gram As_2O_3/lb.*
	0.0637	0.0649
	0.0631	0.0634
LOT I	0.0634	0.0631
Unwashed Apples	0.0637	0.0643
	0.0628	0.0640
Mean	0.0633	0.0639
	0.00402	0.00404
LOT II	0.00414	0.00404
Washed Apples	0.00404	0.00404
Mean	0.00407	0.00404

SUMMARY

A rapid volumetric method for determining from 5 to 500 micrograms of arsenious oxide has been developed. The complete determination after necessary sample preparation can be carried out in less than 10 minutes. The 34 results given show an average recovery of 99.5 per cent with a standard deviation of 0.85 per cent. Results are also presented to prove that the procedure is satisfactory for apple strip solutions. The main factors that make this rapid method possible are: (1) heating of the evolution solution, (2) a resin tube that prevents the mercury arsenides formed at one stage from adhering to the inside of the delivery tube, (3) the addition of gum arabic to the absorbing solution to keep the arsenides in suspension, (4) adjustment of *p*H for complete rapid oxidation, (5) the development of an apparatus and the use of an extraordinarily efficient arsine absorbent, which permits the use of the small volumes so necessary for micro titration.

THE LIMIT OF ACCURACY OF THE A.O.A.C. CHICK ASSAY FOR VITAMIN D

By BERNARD L. OSER (Food Research Laboratories, Inc., New York City)

The collaborative study of the vitamin D chick assay method of the Association of Official Agricultural Chemists, conducted in 1937 under

the associate refereeship of W. B. Griem,[1] had for its primary object the comparison of average bone ash values of tibiae of individual chicks with those obtained by analysis of composite samples representing whole groups of chicks. The results of this study have justified the practice of basing these assays on composite bone ash determinations.

The data submitted by the collaborators provided an excellent opportunity for ascertaining the practical limit of accuracy of the A.O.A.C. procedure, inasmuch as the same oils were assayed in all the laboratories and (it may be assumed) with probably more than ordinary care. It is essential that attention be focused on the statistical variation of individual bone ash values, since composite ash figures may tend to assume an absolute significance not warranted by the facts.

Through the courtesy of Mr. Griem the writer was privileged to calculate from the individual bone ash data for the assays of the U.S.P. Reference Cod Liver Oil the standard error of the mean values reported. All but one of the thirteen collaborators submitted individual, as well as composite, bone ash analyses on negative control groups and on groups receiving 10, 15, 20, and 25 A.O.A.C. units per 100 grams of feed, in the form of the Reference Oil. Included in this statistical survey are the data obtained in the writer's laboratory (designated as Lab. No. 14), which was not among those in the original collaborative study. In addition to these data, the collaborators reported the results of an assay of another sample of oil which are not included in the present analysis.

In the accompanying table are compiled the number assigned to each collaborator and, for each level of assay, the number of chicks per group, the mean ash content of the tibiae (dry, fat-free basis), the standard deviation (σ) of the individual values, and the probable error of the means (PE_m).* It will be seen that the size of the groups varied from 8 to 23 chicks, averaging 13.7; of the entire 64 groups, about two-thirds ranged in size from 12 to 16 chicks.

The maximum differences observed in individual laboratories in mean bone ash values between the negative controls and the groups receiving the highest dose of Reference Oil (25 units per 100 grams) ranged from 5.6 to 15.3 per cent; the average of these differences was 10.2 per cent.

The weighted mean bone ash values for each dosage level, also included in Table 1, spread from 33.47 per cent for the negative controls to 44.04 per cent for the 25 unit group. It is interesting to note that these weighted means (representing combined groups of 155–186 chicks) fall almost exactly on a straight line instead of on a parabolic curve such as is usually

[1] *This Journal*, 21, 607 (1938).

* Calculated from the following equations:

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}};$$

$$PE_m = 0.6745\frac{\sigma}{\sqrt{n}}.$$

TABLE 1.—*Statistical variation in the averages of the individual tibia analyses as reported in 1937 collaborative study of the A.O.A.C. chick assay for vitamin B*
(Results expressed in percentage)

LAB. NO.*	NEGATIVE CONTROL				10/95% REF. C.L.O.				15/95% REF. C.L.O.				20/95% REF. C.L.O.				25/95% REF. C.L.O.				LAB. AVERAGES		
	NO. OF CHICKS	MEAN BONE ASH	σ	PE_m	NO. OF CHICKS	MEAN BONE ASH	σ	PE_m	NO. OF CHICKS	MEAN BONE ASH	σ	PE_m	NO. OF CHICKS	MEAN BONE ASH	σ	PE_m	NO. OF CHICKS	MEAN BONE ASH	σ	PE_m	CHICKS PER GROUP	σ	MEAN PE_m
1	14	34.06	3.08	0.55	15	39.59	2.20	0.38	15	42.16	2.38	*0.42*	15	41.51	2.34	0.41	13	43.98	2.03	0.38	14.4	2.406	0.428
2	15	35.99	1.31	.23	15	40.29	3.16	*.55*	14	43.06	2.22	.40	15	44.31	1.30	.23	14	44.74	1.05	.19	14.6	1.808	.320
3	15	36.36	1.96	.34	15	44.43	2.91	.51	15	45.10	2.91	*.51*	15	46.80	1.43	.25	15	47.36	0.84	.15	15.0	2.010	.352
5	16	34.39	2.53	.44	11	36.23	2.74	.56	9	39.68	3.46	*.78*	13	42.49	5.41	1.01	12	43.23	2.82	.55	12.2	3.392	.668
6	13	32.13	2.02	.38	13	34.86	1.87	.35	15	40.16	3.36	*.59*	15	43.27	1.69	0.30	15	44.59	2.24	.39	14.2	2.236	.402
7	15	33.30	2.25	.39	15	35.43	2.40	.42	15	35.61	2.13	.37	15	38.88	3.34	*.58*	14	38.90	3.87	.70	14.8	2.798	.492
8	12	31.58	2.40	.47	11	35.50	2.79	.57	11	37.14	3.37	*.68*	10	38.87	2.15	.46	10	37.59	2.01	.43	10.8	2.544	.522
9	13	36.18	3.73	.69	10	44.52	2.42	.52	10	46.57	2.16	*.46*	11	47.55	1.75	.36	10	47.66	1.25	.27	10.8	2.262	.460
10	11	34.70	2.72	.55	13	38.90	2.54	.47	23	40.68	2.52	*.36*	11	41.79	2.12	.43	13	42.84	1.62	.30	14.2	2.304	.422
11	16	32.74	1.74	.30	13	37.71	2.66	.50	13	40.18	4.12	.77	13	42.06	4.77	*.89*	14	48.05	2.55	.46	13.8	3.168	.584
12	8	35.08	3.31	.79	9	44.91	2.21	.50	10	45.74	1.35	*.29*	9	46.31	0.81	.18	10	46.71	1.59	.34	9.2	1.854	.420
13	17	29.02	2.21	.36	15	38.79	2.57	.45	14	38.74	2.85	*.51*	15	42.69	2.73	.48	15	42.43	1.69	.30	15.2	2.410	.420
14	13	30.68	2.79	.52	22	33.12	2.44	.36	22	34.39	3.39	.50	18	40.19	4.10	*.65*	—	—	—	—	18.8	3.180	.510
Totals	178	—	—	—	177	—	—	—	186	—	—	—	175	—	—	—	158	—	—	—	—	—	—
Weighted Averages	—	33.47	—	—	—	38.38	—	—	—	40.29	—	—	—	42.70	—	—	—	44.04	—	—	—	—	—
Averages	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	13.69	2.490	0.462

* Collaborator No. 4 reported composite bone ash values only. Lab. No. 14 is the writer's laboratory, not included among the original collaborators.

obtained from data of this kind. Evidently the region of diminishing slope is above the 25 unit dosage level.

It is not the purpose of the writer to attempt to account for the variations reported by these collaborators, but rather to point out that such factors as strain of chicks, nutritional heredity, housing conditions, temperature control, dissection technic, etc., are undoubtedly contributing factors.

In the last three columns of Table 1 are given for each laboratory the average number of chicks per group, the mean standard deviation of the individual bone ash values, and the corresponding mean PE_m for all dosage levels. The latter figures fall within rather narrow limits (0.320–0.668) considering the variations in the average size of the groups. No consistent trend was noted in the direction of less variation within groups at either the higher or lower dosage levels. From these mean values, the standard deviation of individual bone ash values for all laboratories at all feeding levels of vitamin D was found to be ± 2.490, which would indicate that the chances of a bone ash value for an additional chick in any group falling within ± 2.49 per cent of the previously determined mean for that group are about 2 to 1. The mean PE_m for all laboratories at all levels was ± 0.462, the average group consisting of 13.69 chicks.

However, rather than use these figures as the basis for calculating the minimum significant difference in mean bone ash between two groups of chicks, it is perhaps preferable to recognize the existence of a region of maximum sensitivity in the curve of response relating bone ash to vitamin D dosage. Most of the collaborators found the 15 unit level to fall within this range. In Table 1 the figures for σ and PE_m at the point of greatest slope on the curves of response are italicized. From these values the mean was found to be ± 3.056. For convenience it may be assumed that in ordinary assays 10 chicks per group are used. In that case the standard error (or the standard deviation of the mean) would be $3.056/\sqrt{10} = 0.966$, indicating that the chances of the mean bone ash value of a second group of 10 chicks falling within ± 0.966 of the original mean are approximately 2 to 1.

Using this value for the standard error (ϵ), the analyst may compute the significance of differences between mean or composite bone ash values. The standard error of such a difference is obtained from $\sqrt{\epsilon_1^2+\epsilon_2^2}$, in which ϵ_1 and ϵ_2 are the standard errors of the individual groups. Hence in groups of 10 chicks, where ϵ_1 and ϵ_2 are 0.966, $\epsilon_{\text{difference}} = 1.366$. When a difference is equal to its standard error, it is statistically significant with a degree of certainty of 68.26 per cent, i.e. the chances favoring significance are about 2 to 1. Obviously a conclusion as to an assay can hardly rest secure on a 1 in 3 chance of such a difference being without significance. It is customary in quantitative bioassays to adopt some degree of certainty, usually based on the nature of the tests, as the criterion for evaluation of data. As stated by Dunn:[2] "The boundary lines of signifi-

[2] *Physiol. Rev.*, 9, 275 (1929).

cance should be determined by the individual experimenter with reference to his particular problem. For instance, suppose he were interested in establishing the dosage of a lethal drug, he would not wish to come within one chance in a million of giving a lethal dose to a patient due to the sampling variation in drug effect. However, if he were gambling on a horse at the race track he might be only too glad to have a chance of 60 to 40 in his favor." For a difference to be significant with a degree of certainty of 95 per cent or greater, it should be at least twice its standard error. If 99 per cent certainty is required, the difference must be 2.576 times its standard error; in this case there would be only one chance in a hundred that the difference was not significant. This somewhat severe criterion has been adopted by the British Pharmacopoeia for the official interpretation of vitamin assay data.

However, in the present writer's opinion, the less conservative degree of certainty represented by 2ϵ is better suited to the interpretation of vitamin assays and, in fact, has wider usage in this country. On this basis, then, it may be stated that the minimum significant difference in bone ash between two groups of 10 chicks each would be 2 times 1.366 or 2.73 per cent.

The increase in precision that results from using larger assay groups stands in inverse relationship to the square root of the size of the group; for example, in order to double the precision (or cut in half the minimum significant difference) the assay groups must be quadrupled. This is illustrated in the following table, which shows, for example, that in order for a difference of one per cent in composite bone ash between an assay and reference control group to have statistical significance the groups should consist of at least 75 chicks.

NUMBER OF CHICKS PER GROUP	MINIMUM SIGNIFICANT DIFFERENCE IN MEAN (OR COMPOSITE) BONE ASH
	per cent
10	2.73
20	1.93
30	1.58
40	1.37
50	1.22
75	1.00
100	0.86

It may be added that if the mean ϵ for all groups were used in this computation instead of the mean ϵ for groups at the level of maximum sensitivity, the minimum significant difference for groups of 10 chicks would fall close to 2.0 per cent bone ash. Furthermore, while the statistical analysis here presented is in the nature of a cross-section of the variability prevailing in the laboratories that participated in the collaborative study, for accurate interpretation individual assayers should subject their own data to a similar analysis.

THE QUANTITATIVE ADAPTATION OF THE CODEINE TEST TO THE COLORIMETRIC DETERMINATION OF SELENIUM IN PLANT MATERIALS*

By JEHIEL DAVIDSON (Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.)

Selenium as a plant constituent belongs to the so-called "rare" elements. While in certain limited areas the selenium content of plants is relatively high, in some plants reaching a concentration of 0.5 per cent,[1] this element is generally present in plants, if at all, in minute quantities, which are commonly estimated as parts per million. In such cases colorimetric methods are preferable to gravimetric or volumetric methods.

Two color tests are available for the qualitative detection of selenium, viz., the reaction using codeine and that using pyrol,[2] but thus far no colorimetric method for the quantitative determination of selenium has been developed.†

The codeine test, which is well known, has long been used as a qualitative procedure for the detection of selenium in sulfuric acid[3] and in glass,[4] and vice versa, selenium has been used by pharmacologists as a qualitative test for codeine.[5] When codeine is added to a solution of selenium in concentrated sulfuric acid there is produced a characteristic color varying from green to blue.

Horn[6] adapted the codeine test to the qualitative detection of selenium in plant materials and protein fractions. The material is digested in Kjeldahl flasks with sulfuric acid and mercuric oxide, as in the procedure for nitrogen determinations, and to the clear sulfuric acid digest codeine sulfate is added. It was the object of this work to adapt Horn's procedure to the quantitative determination of selenium in plant materials.

DIGESTION OF MATERIAL

It is generally a simple matter to digest 1 or 2 grams of plant material in the Kjeldahl procedure for the determination of nitrogen, but in the determination of selenium, due to the small quantity present, it is often necessary to digest as much as 10 grams, and as a result there is often serious difficulty with foaming. This difficulty was overcome (1) by using unground material whenever possible, as in the case of grain; (2) by cutting off for a short time the external source of heat after the digestion was

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* Food Research Division Contribution No. 401.

† This manuscript was submitted for publication November 8, 1938. Since then a paper describing the same fundamental procedure for photometric estimation of selenium has appeared in *Analytical Edition of Industrial and Engineering Chemistry*, Vol. 11, 198, April, 1939.—The Editor.

[1] Miller and Byers, *J. Agr. Research*, 55, 59–68 (1937).

[2] Berg and Titelbaum, Festschrift zum 70 Geburtstag von Hofr. Prof. Dr. Friedrich Emich, Emil Haim & Co., Wien and Leipzig, 1930, pp. 23–28.

[3] *J. Pharm. Chim.*, 11, 261–62 (1900).

[4] *J. Soc. Glass Tech.*, 11, 386–93 (1927).

[5] U. S. Pharmacopoeia, 1926, p. 114.

[6] *Ind. Eng. Chem. Anal. Ed.*, 6, 34–5 (1934).

well started (in exceptional cases it was necessary to cut off the external heat several times); and (3) by using large Kjeldahl flasks (800 cc.).

VOLATILITY OF SELENIUM

The volatility of selenium is influenced by two factors, its concentration and the form in which it is present. In boiling 10 mg. of selenium with hydrochloric acid in an Erlenmeyer flask on an open flame for 3 hours Brückner[7] observed a loss of 27 per cent. By continued dropwise addition of hydrogen peroxide during the boiling with hydrochloric acid the loss of selenium was prevented. Similarly, by boiling for 6 hours 10 mg. of selenium with nitric acid, which is itself an oxidizing agent, no loss of selenium resulted. By boiling 20 mg. of selenium with sulfuric acid under similar conditions the same author observed a loss of 4 per cent in 2 hours and of 10 per cent in 3 hours.

Under the conditions of the present experiments no loss could be detected when 0.05–0.2 mg. of selenium (the concentrations commonly used for the standard solutions) was digested with sulfuric acid and mercuric oxide in Kjeldahl flasks for 2.5–3.5 hours, but there were indications of some loss, not greatly exceeding the limits of experimental error, when 0.8–1.0 mg. was digested under the same conditions. It was found that the mercuric oxide used as a catalyst to hasten digestion also reduced the volatility of selenium, probably by keeping it in an oxidized state.

To prevent or minimize errors due to the possible volatilization of selenium, however, the standard solutions should be as nearly as possible of the same concentration as that of the unknown samples and should be digested for the same length of time. It is also advisable to adjust the quantities of sulfuric acid used so that the residual quantity of acid after digestion will be about the same in all determinations.

SELENIUM-CODEINE COLOR

A serious difficulty in the development of the codeine test into a quantitative procedure was the changeability of the selenium-codeine color.

The presence of too much water, for example, prevented the initial development of the color or destroyed it when the water was added after the color development. When solid codeine sulfate was added to a sulfuric acid solution of selenium the development of the color was delayed and the shade was irregular, but when the codeine was added in solution a normal characteristic color began to develop immediately.

The shade of the selenium-codeine color is variously described in the literature as green or blue or as passing from blue to green. An explanation of this discrepancy suggested itself in the course of this investigation. When codeine was added in excess of the quantity required to react with the selenium in the solution the color was blue and it lasted for a compara-

[7] *Z. anal. Chem.*, 94, 306–22 (1933).

tively long time without undergoing much change, but if insufficient codeine was added to react with all the selenium the color was green and less stable.

When the codeine was added to the sulfuric acid shortly after digestion, the color did not develop properly and was not stable, but turned to varying shades of olive. This difficulty, which was probably due to the incomplete precipitation of the mercuric sulfate, was overcome by allowing the digestates to stand overnight. The greater part of the mercuric sulfate precipitate came down immediately after cooling of the digestate, but when allowed to stand overnight an additional fine precipitate settled.

The mineral elements present in the plant material may also affect the shade of the selenium-codeine color. This difficulty was overcome as follows:

1. The standard solutions were digested with samples of a selenium-free material identical or similar in nature to the one that was to be analyzed, equal in weight to the corresponding unknown samples.

2. The standard solutions were digested with the selenium-free ash of weights of the same material that was to be analyzed equal to those of the corresponding unknown samples. Practically all the selenium was expelled during the combustion of substances that yielded an ash with an acid balance. Substances that yielded an ash with an excess of bases over acids retained a considerable part of their selenium content. It was found that when, previous to ashing, sufficient monopotassium phosphate was added to the material to give the ash an excess of acid-forming over base-forming elements, and when the ashing temperature was raised to 700° C., selenium was completely expelled from all materials. (The selenium-free ash should be added to the standard solutions toward the end of their digestion period, as it generally causes considerable bumping.)

3. Approximately as much iron and potassium phosphate (potassium and phosphorus generally constitute the greater part of most plant ashes) were added to the standard as were present in the samples of the material that was to be analyzed. Iron affected the shade of the selenium-codeine color to a greater extent than did any other ash constituent.

It was found in this investigation that iron not only does not interfere with the codeine test for selenium, as was shown by Horn,[6] but that it is essential to the proper development and stability of the color, as it is essential in the colorimetric qualitative test for selenium with pyrol.[2] It was also found to be necessary to add iron to plant materials of low iron content, such as wheat, when smaller samples were used. In all cases reported here the addition of phosphate and iron was sufficient to obtain normal results. The other two ways of overcoming interference of mineral elements (1 and 2 above) did not prove to be of particular advantage.

Occasionally for some unknown reason, such as a contamination with some organic substance, a solution failed to develop the normal color.

The solution was then returned to the Kjeldahl flask, redigested till clear, and treated anew with the codeine reagent.

Care must be taken to add sufficient codeine reagent, because it was found that several times as much codeine as selenium is required for the reaction.

INTERFERENCE OF VANADIUM

Of the mineral elements that may be present in plants only vanadium interferes with the determination of selenium under the conditions of this procedure, as then vanadium develops with codeine a color similar to that developed by selenium. Vanadium, however, seldom has been found in plants.[8] Moreover, vanadium was found to be about one-tenth as sensitive to codeine as selenium. For example, 100 micrograms of vanadium gave but a feeble test with codeine in 25 cc. of concentrated sulfuric acid. Accordingly, a plant material containing 10 p.p.m. of vanadium will cause a slight interference (about 1 p.p.m.) only when 10 grams of sample is used.

If, under exceptional conditions, the occurrence of vanadium in plants in relatively large quantities is suspected, its interference with the colorimetric determination of selenium may be overcome by making use of the fact that under the conditions under which vanadium remains in the ash selenium is expelled, as described above. It was done in two ways:

1. A weighed quantity of the material was ashed with monopotassium phosphate at about 700° C. to expel the selenium. The vanadium in the ash was determined in terms of selenium (with a selenium standard) and its value subtracted from the selenium value obtained in the ordinary procedure.

2. A sample of the same weight as the one to be used in the ordinary procedure was ashed with monopotassium phosphate and the ash containing the vanadium was added to the standard solution just before digestion was completed. (In this case the selenium concentration of the standard solution must be approximately the same as that of the sample of the substance that is analyzed.)

INTERFERENCE DUE TO TURBIDITY

Grains as a rule give a clear solution after the digestates are allowed to stand overnight. The clear liquid can then be decanted or pipetted off without disturbing the precipitate. On the other hand, the digestates of the vegetative parts of plants often remain turbid for a long time and the precipitate is readily disturbed during decantation or pipetting. In such cases filtering is necessary. A Gooch plate was placed in a small funnel and covered with wet asbestos. The stem of the funnel was put through a one-holed rubber stopper, which was placed in a suction flask. The digestates filtered readily with the aid of suction and the filtrates were per-

[8] Robinson, Steinkoenig, and Miller, U. S. Dept. Agr. Bull. 600, 1917.

fectly clear. (The same asbestos may be used two to three times after being washed each time by suction with sulfuric acid. Eventually the asbestos becomes clogged with mercuric sulfate and with the precipitated plant constituents and filtration becomes very slow.)

At times the solutions were apparently clear and did not seem to require filtration, but when placed in the colorimeter they were found to have a slight turbidity, which interfered with the readings. The solutions were then removed from the colorimeter, filtered, and returned to the colorimeter for comparison, as the filtration did not affect the selenium-codeine color.

Preparation of Standard Solutions

A selenium stock solution was prepared as follows: 1 gram of elemental selenium was dissolved in concentrated nitric acid, evaporated nearly to dryness on a steam bath, dissolved, and made up to one liter with water. The standard solutions containing 20–200 micrograms were prepared from the stock solution by dilution with distilled water.

Choice of Colorimeter

While Nessler tubes may be used for comparison of the unknown with the standard solutions, the use of a colorimeter was found to be more convenient and to give more accurate results.

Only a colorimeter whose receptacles are not attacked by sulfuric acid should be used. In good daylight a Schreiner colorimeter was found to be very convenient. A Klett colorimeter has the advantage in that it may be used with artificial light.

Range of Concentrations

When the sulfuric acid digestion of materials and standards was completed there remained in the Kjeldahl flasks 20–25 cc. of sulfuric acid solution, and by the time all digestates were made up to volume the latter reached 25–30 cc. Under these conditions the smallest quantity of selenium that could be determined by this method with a reasonable degree of accuracy was 10 micrograms. To overcome this limitation with materials of low selenium content large samples may be used. With 10 grams of material, for example, 10 micrograms of selenium would make 1 p.p.m. No upper limit need be set as the sulfuric acid digestates may be freely diluted with concentrated sulfuric acid before or after the development of the color. Too large quantities of selenium in the samples, however, may increase the danger of possible loss by volatilization and should be avoided.

The concentrations most convenient for color comparison in a colorimeter are from 2 to 8 parts of selenium per million parts of sulfuric acid.

Procedure

The plant material was digested with sulfuric acid and mercuric oxide (0.7 gram) in Kjeldahl flasks as in the nitrogen procedure. (When small samples of a material poor in iron are used it is advisable to add iron to the Kjeldahl flasks before digestion as stated under digestion of standards.) The weight of the samples depended upon the selenium content of the material. (If possible, the samples should contain not less than 20 micrograms of selenium, as those containing less are difficult to handle. A convenient sample should contain 50–200 micrograms.)

Ten grams of plant material (the maximum quantity that can be conveniently digested in this procedure) required about 75 cc. of sulfuric acid in order to have a residue of 20–25 cc. of sulfuric acid solution when digestion was complete; 5 grams of plant material required about 60 cc. and two grams about 25 cc. of sulfuric acid. The running over of the Kjeldahl flask was prevented by using unground material, by using large flasks, or by cutting off at intervals the source of external heat. The digestion was continued till digestates were colorless when cooled (2.5–3.5 hours).

The standard solutions (dilutions of the stock solution containing 20–200 micrograms of selenium) were digested with samples of selenium-free material similar in kind and equal in weight to those in which selenium was to be determined. (If such selenium-free material is not available, the ash of samples of the material that is to be analyzed, comparable in weight to that of the unknown samples but freed from selenium by ashing the material with monopotassium phosphate at about 700° C., is added to the standard solution shortly before the close of the digestion period.)

In most cases, however, it was sufficient to add to the standard solution before digestion 1 cc. of a 0.5 per cent solution of ferrous sulfate and 1 cc. of a molecular solution of mono- or di-potassium phosphate. The ferrous sulfate was dissolved with the aid of a few drops of sulfuric acid. In all cases the standard solutions were digested with sulfuric acid for the same length of time as were the unknown samples.

When selenium-free plant material was used with the standard solutions they were digested with the same quantity of sulfuric acid as was used for the samples of the analyzed materials; in the other cases they were digested with 25 cc. of sulfuric acid.

When digestion was complete the residual contents of the Kjeldahl flasks were cooled and made up to the same volume (about 30 cc.) with concentrated sulfuric acid, returned to the same flasks, stoppered, and allowed to stand overnight. Aliquots of about 20 cc. were then pipetted off into small Erlenmeyer flasks without disturbing the precipitate. If the supernatant liquid was not clear or could not be pipetted off without disturbing the precipitate, it was filtered in the manner described previously. Five to six drops of a 2 per cent aqueous solution of codeine sul-

fate or of the codeine alkaloid (the latter dissolved with the aid of a few drops of sulfuric acid) were added slowly, with constant shaking, to the solutions in the Erlenmeyer flasks, which were then stoppered and allowed to stand for about 2 hours. (For selenium solutions containing more than 200 micrograms more codeine must be added. The quantity of codeine added must be not less than 30 times the quantity of selenium present in the solution.)

The unknown solutions were compared with the standards in a Schreiner or Klett colorimeter.

RESULTS

The method was tried with varying quantities of selenium added to selenium-free wheat. Some of these results are given in Table 1. The

TABLE 1.—*Comparative colorimetric readings of duplicate standard solutions and recovery of selenium added to wheat*

SELENIUM ADDED TO STANDARDS AND WHEAT	COLORIMETRIC READINGS OF STANDARDS			RECOVERY OF SELENIUM FROM WHEAT				
				COLORIMETRIC READINGS			RECOVERY	RECOVERY AVERAGE
	1	2	AGREEMENT	STANDARD		WHEAT		
micrograms			*per cent*				*per cent*	*per cent*
				a*	20	20	100	
150	21	20	95	b*	20	20	100	100
				a	30	31	97	
100	31	30	97	b	30	32	94	95.5
				a	36	32	112	
50	39	36	93	b	36	32	112	112
				a	40	39	97	
20	40	40	100	b	40	38	95	96

* Duplicate determination.

agreement between duplicate standard solutions as well as between duplicate determinations of selenium added to wheat is good. The recovery of selenium added to wheat fluctuates around 100 per cent, above as well as below, which indicates that there was no loss of selenium from wheat as compared with the standard solutions. On the basis of 10 grams of wheat the concentrations given in this table will make 15, 10, 5, and 2 p.p.m., respectively.

In Table 2 are given results obtained with varying quantities of a selenium-containing flour. The variation in the quantities of selenuim found

TABLE 2.—*Selenium in varying quantities of a selenium-containing flour*

WEIGHT OF SAMPLES	COLORIMETRIC READINGS		WEIGHT OF SELENIUM	AVERAGE	RELATIVE WEIGHTS OF SELENIUM
	STANDARD[1]	UNKNOWN			
grams			*mmg.*	*mmg.*	
10.0	30	16	190		
10.0	30	16	190	190	100
7.5	30	20	150		
7.5	30	19	160	155	81
5.0	—	—			
5.0	30	30	100	100	53
2.0	10	26	38		
2.0	10	27	37	37.5	20

[1] Standard = 0.1 *mg.*

by analysis is fairly closely related to the variation in the size of the samples.

In Table 3 are given results obtained on four samples of wheat grown under field conditions on a soil containing selenium. The analysis was repeated twice on two of the samples and thrice on two others. The duplicates and repetitions agree fairly well.

TABLE 3.—*Selenium in different samples of wheat*

SAMPLES	1[1]		2[1]		3[2]	
	P.P.M.	AV.	P.P.M.	AV.	P.P.M.	AV.
A	28				26	
A	24	26			24	25
B	24				22	
B	22	23			24	23
C	14		14		14	
C	16	15	18	16	12	13
D	22		22		22	
D	22	22	—	22	22	22

[1] 10 grams used.
[2] 5 grams used.

In Table 4 results by the present method are compared with those obtained by a method developed by Robinson and co-workers.[9]

The Robinson method is turbidimetric for the lower concentrations and gravimetric for the higher concentrations. The material for analysis, wheat and wild vegetation, was obtained from areas in which the soil contains selenium in varying concentrations. The selenium content of

[9] Robinson, Dudley, Williams, and Byers, *Ind. Eng. Chem. Anal. Ed.*, 6, 274 (1934).

the material varied from 0 to 600 p.p.m. The results by the Robinson method were obtained by R. A. Osborn in single determinations. The results by the proposed method are all averages of duplicates. These results are offered simply as a basis of comparison of those by the proposed method with those on a series of samples available at the time the investigation was conducted.

TABLE 4.—*Comparison of results obtained by the proposed method with those obtained by Robinson and co-workers' method*

MATERIAL	PROPOSED METHOD	ROBINSON & CO-WORKERS' METHOD[1]
	p.p.m.	*p.p.m.*
Winter Wheat (plants) A	2.5	None
Winter Wheat (plants) B	13.5	10
Winter Wheat (plants) C	6.0	3
Wheat (heads)	10.0	8
Wild Aster A	137.0	110
Wild Aster B	440	350
Wild Aster C	201	174
Wild Aster D	620	620
Aster (Kuhnia)	None	None
Prairie Shoestring	12	7
Not Identified A	115	111
Not Identified B	123	156

[1] Results obtained by R. A. Osborn.

SUMMARY

The qualitative codeine test was adapted to the quantitative colorimetric determination of selenium in plant materials. It was found that the presence of iron is essential for the development of a stable and relatively lasting color.

Consistent results were obtained with this method in recovering selenium added to wheat and in determining selenium in varying weights of a selenium-containing flour. Consistent results were also obtained in determining selenium in different wheats grown in an area in which the soil contains selenium.

Results obtained with this method on wheat and on various wild vegetation grown on a seleniferous area compare satisfactorily with results obtained with another method developed by Robinson and co-workers.[9]

BOOK REVIEWS

Humus—Origin, Chemical Composition, and Importance in Nature. By SELMAN A. WAKSMAN. 526+VIX pps. 44 text. figs. 63 tables. 2nd ed. 1938. Williams and Wilkins. Baltimore. Price $6.50.

The author attempts, with considerable success, to present a comprehensive discussion of humus. He reviews the historical development of concepts concerning "humus and humic acids," explains in some detail the formation and chemical characteristics of humus in soils, composts, peat, and coal, and discusses its relation to plant nutrition, soil genesis, and soil conservation. The argument is supported by many analytical data and copious quotations from other investigators.

The author has inserted a number of improvements, although in the main this edition is not greatly different from the first edition that appeared in 1936. There is still a tendency to repetition, e.g., the chapter "Humus and Soil Conservation" is composed largely of material given in previous chapters.

As one might expect from such a distinguished microbiologist, the chemical and especially the biological aspects of humus formation and decomposition have been treated most comprehensively. In properly stressing the important role of the micro-organisms, he may underestimate the importance of certain significant chemical reactions that accompany the main decomposition processes. It is likely that his specialized experience in the laboratory has determined his approach to the subject, predominately analytical. Thus some of his concepts will probably not be entirely acceptable to soil scientists dealing with soils as geographic bodies supporting native plants or as fields and ranches producing crops and pastures. One point of probable disagreement lies in the concept of humus itself. The author states, for example, " . . . it (humus) should be used to designate the organic matter of the soil as a whole", whereas most soil scientists consider humus to be a relatively stable product of decomposition. Even the author himself states elsewhere, "Humus is a product of decomposition of plant and animal residues. . . . " The author's not always definite use of these two entirely different concepts—(1) humus is the total organic matter of the soil, and (2) humus is a special portion of the total resultant from microbial decomposition—makes it difficult for the reader to determine when he means one and when the other.

The author mildly criticizes the soil scientist for not giving soil organic matter proper weight in soil classification and genesis. He then places the "Podzols, brown forest, red, and yellow soils" in the same class as far as "abundance and nature of humus" are concerned and the "chestnut soils" with the "serozems," although the "brown grassland soils" are included with "chernozem"!! Such generalizations emphasize the difficulty of specialty coordination.

While the author has not been entirely successful in coordinating his data with those from other branches of soil science, his emphasis upon the need is timely and cogent. It is hoped that in subsequent editions particular attention may be given to the nature and functions of humus in different soils and to "humus" or organic soil amendments and their effects on soil structure and plant growth.

Points where differences of opinion may occur, while possibly serious to the individual reader, should not be overemphasized. The book is clearly the best on the subject, serves to bring together a large body of literature dealing with organic matter, and will be indispensable as a reference book for students and investigators. The data on the constituents of various types of humus are especially valuable. The bibliography includes 1,608 references and there is an author and subject-matter index.—CHARLES E. KELLOGG.

Plant Growth-Substances. By HUGH NICOL, 108 pp. Distributed by Chemical Publishing Company of New York. 1938. Price $2.00.

The author describes experiments that led to the discovery of naturally occurring plant hormones and presents a brief and technical account of chemical methods used in the synthesis of some compounds known to be effective as plant growth-regulators. Methods used by various workers in applying growth-regulating compounds to plants are discussed, together with a detailed description of how some synthesized compounds can be used to stimulate root growth of cuttings. A chapter is devoted to the natural occurrence of growth substances and a none too clear account of the chemistry of some synthetic compounds in relation to plant growth. The final chapter deals briefly with the identification of some substances known to be effective as plant growth-regulators and clearly shows the need for more adequate chemical methods of detecting and estimating the amounts of these compounds in plant tissues.

The book should be of particular interest to those desiring a brief account of the chemistry of natural and synthetic growth-regulating substances and of how some of these compounds have been utilized in horticultural practices.—JOHN W. MITCHELL.

The Standardization of Volumetric Solutions. By R. B. BRADSTREET, with a Foreword by HARRY L. FISHER. 119 pp. Chemical Publishing Co. of New York, Inc. Price $3.00.

While there is a large number of comprehensive volumes on volumetric analyses, there are fewer books that deal with only the standardization of volumetric solutions. From the foreword it is learned that the purpose of this volume is to have available a ready reference of concise procedures.

The directions for standardization in this volume are contained in four chapters. Under each solution directions are given for its preparation and standardization. The reaction involved and the necessary calculations are also presented.

In all well accepted practices for the standardization of a volumetric solution the substance used in determining the strength of the solution is weighed. In this book too many of the procedures for standardization direct that the normality be determined by checking with another standard solution. In those instances where the primary standard is weighed, too small a weight is directed to be used.

For example, in the standardization of sodium thiosulfate (p. 53), 0.12 gram of potassium bromate is weighed. It is obvious that if only weights of Class S accuracy are used the errors in weighing might be greater than 0.1 per cent.

It is also fundamental practice that the primary standard substance by which the strength of the standard solution is determined must be pure or its degree of purity must be known. In only few cases does the author present detailed directions for the purification, or methods for testing the purity, of the primary substance used in the standardization.

Under the standardization of sodium hydroxide with acid potassium phthalate (p. 38), no mention is made that this salt may be obtained from the National Bureau of Standards especially prepared for use in standardizations.

Further, with respect to sodium hydroxide solution, the directions do not include the protection of the solution from carbon dioxide from the air. The caution that standard alkali solutions should be kept in alkali-resistant glass is also omitted.

Even though the literature citations appear to be entirely adequate, it is the reviewer's opinion that the worth of this book to the chemist is not commensurate with its cost.—R. L. VANDAVEER.

The Structure and Composition of Foods. By ANDREW L. WINTON and KATE BARBER WINTON. Volume IV. Sugar, Sirup, Honey, Tea, Coffee, Cocoa, Spices, Extracts, Yeast, Baking Powder. 580 pp., 134 illustrations, John Wiley & Sons, Inc., New York. 1939. Price $9.00.

This fourth and final volume completes a series on the structure and composition of foods by these authors. Reviews of the previous volumes have appeared in *This Journal*, Vol. XV, p. 500. Vol. XVIII, p. 647; and Vol. XXI, p. 157. The same treatment is afforded the subjects as was followed in the preceding volumes. A general discussion of the physical and chemical properties of sugars introduces Part I on saccharine products. The products of sugar cane and beet, sorghum and maple are treated in this section, together with honey, invert sirup, starch sugar, and glucose, and caramel, to mention the chief subjects. What are called the alkaloidal products—maté, tea, cocoa and chocolate, coffee, and other minor plants—are the subjects of Part II. Spices and extracts, so inclusive as to be too numerous to list, comprise Part III, to which one-half of the volume has been devoted. Those interested in the chemistry of spices have long awaited such a comprehensive compilation. In Part IV in the presentation of leaven, yeast is dealt with from its modern chemical aspects.

The sense of accomplishment must be most satisfying to these authors with the completion of the whole work, by which they have urged "recognition of food histology as the logical, although novel, approach of the student to food science and of the trained chemist to food research of a more fundamental character than the mere diagnosis of commercial doubtfuls and unknowns, in which field the authors long labored." This work is a monument to those labors, representing, as it does, the only American compilation of its kind, and rivaling works of renowned standing from European sources. While much of the data and information is of American sources, the choice does not neglect that from the world at large.

The reviewer can always add comment on some subjects which, to his mind, have not been so completely treated as he believes they might have been, but why do so when these authors have so obviously shown, by their addenda on vitamins, an appreciation of the impossibility of bringing together in the pages of a single book the ever-changing development of knowledge.

As would be expected from Dr. Winton, who for so long was engaged in the work of enforcement of the Federal Food and Drugs Act, the problems of the regulatory chemist are not overlooked, even to the concept of the newer Food, Drug, and Cosmetic Act of 1938, as evidenced by the quotation in legal phraseology in the discussion of caramel: " . . . make the product appear better or of greater value than it is."—HENRY A. LEPPER.