FIRST DAY

MONDAY—MORNING SESSION

REPORT ON FEEDING STUFFS

By L. S. Walker (Agricultural Experiment Station, Burlington, Vermont), *Referee*

It is recommended*—

(1) That the method for galactan, **27.40,** be made first action.

(2) That the acid titration method, **27.49,** and alkaline titration method, **27.50,** for hydrocyanic acid formed by hydrolysis of glucosides in beans, be made first action.

(3) That the Knapheide-Lamb method for iodine in mineral mixed feeds, **27.54–27.56,** and the Elmslie-Caldwell method, **27.57,** be made first action.

(4) That work on the following be continued:

(a) Calcium and iodine in mineral mixed feeds.
(b) Lactose in mixed feeds.
(c) Adulteration of condensed milk products.
(d) Crude fat or ether extract.
(e) Fluorine.
(f) Protein evaluation in fish and animal products.
(g) Hydrocyanic acid glucosides.
(h) Sampling and analysis of condensed buttermilk.
(i) Microscopic examination of feeds.
(j) Tankage (hide, hoof, horn, and hair content).
(k) Fat in fish meal.

(5) That further study be made of the method for crude fibre as suggested by the Associate Referee.

(6) That the method for sulfaquinoxaline in feeds as recommended by the Associate Referee be adopted, first action, and that the work be continued and studies of methods for sulfaguanidine and other drugs be undertaken.

(7) That the method for determination of acid-soluble manganese be revised by changing the wording beginning "Heat nearly to boiling point" in **27.59,** to read "Heat nearly to boiling point, and with stirring or swirling add 0.3 g of KIO_4 for each 15 mg. of Mn present. Maintain at 90–100°C. for 30–60 minutes or until color development is complete. Cool, make to measured volume of 50 or 100 ml, and mix. Compare with standard $KMnO_4$ in a colorimeter or in a spectrophotometer at 530 mμ. Calculate p.p.m. of Mn in the sample."

(8) That the following statement " . . . In the case of wheat grains multiply the results by 5.7" be inserted as a note following **27.10.**

* For report of Subcommittee A and action of the Association see *This Journal*, **33**, 36 (1950).

(9) That the methods for calcium and phosphorus in feeds, as recommended by the Associate Referee for minerals in feeds, be adopted, first action; that the method adopted as official, first action, *This Journal*, **31**, 98, and as official, *Ibid.* **32**, 95, be dropped.

(10) That the procedure for sampling, **27.1**, be changed to agree with the new procedure for sampling fertilizers.

REPORT ON IODINE IN MINERAL MIXED FEEDS

By A. T. PERKINS (Kansas State College, Manhattan, Kansas), *Associate Referee*

A number of test analyses have been made in attempts to improve the Elmslie-Caldwell method for iodine. No improvements have been devised. It is recommended* that the Elmslie-Caldwell method be made first action. It is also recommended that investigations be continued.

REPORT ON FAT IN FISH MEAL

By M. E. STANSBY (Seattle Technological Laboratory, U. S. Fish and Wildlife Service, Seattle 2, Washington), *Associate Referee*

At the last report in which a collaborative assay of fat in fish meal conducted by several laboratories was included, a tentative method for fat in fish meal was recommended and has since been adopted by the Association. The tentative method was a great improvement over former methods for fat in fish meal but it was felt that the precision attained was not as great as might be desired. Accordingly, during the past year a series of experiments designed to find any possible improvements in precision have been carried out.

In the first series of tests, an attempt was made to determine whether the homogeneity of the fish meal samples circulated to collaborating laboratories was insufficient, thus causing a decrease in precision. A new series of fish meal samples was prepared in the same manner as those circulated to the collaborating laboratories and stored in half-pound tin cans in the same manner as those analyzed collaboratively. Seven replicate analyses were made on the contents of each of nine cans of herring meal. The results of these analyses are shown in Table 1. As will be seen, the averages of any individual can are not significantly different from that of any other can. Hence, differences found by individual laboratories cannot be attributed to a lack of homogeneity of the sample.

In another series of tests, the effect of varying the height of the extraction thimble was investigated. It had been noted that there was a tend-

* For report of Subcommittee A and action of the Association, see *This Journal*, **33**, 36 (1950).

ency for the fish oil to creep up the sides of the thimble. It was therefore thought possible that varying the height of the thimble might affect the results. In tests in which an alundum thimble 4.0 cm. high (which completely filled the glass siphon cup) was compared with a 2.6 centimeter high alundum thimble, no significant differences were found.

It was noted that when the usual 25 mm. diameter alundum thimbles were used, a continuous siphoning of solvent quite frequently took place instead of the usual intermittent filling and emptying of the glass sample cup. It was believed that this might be due to the fact that the alundum thimble completely filled the glass siphon cup and did not allow sufficient

TABLE 1.—*Data on total extractives from same sample herring meal individually vacuum sealed in 9 one-half pound cans*

IDENTITY OF SAMPLE CAN NUMBER	PER CENT TOTAL EXTRACTIVES (ACETONE EXTRACT OF MEAL PLUS EXTRACT OF ACID DIGESTED MEAL)							
	REPLICATE 1	REPLICATE 2	REPLICATE 3	REPLICATE 4	REPLICATE 5	REPLICATE 6	REPLICATE 7	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	17.59	17.74	17.72	17.62	17.43	17.96	17.24	17.61
2	17.40	18.04	17.61	17.87	18.11	17.90	17.19	17.73
3	17.83	17.66	17.66	17.74	17.89	17.50	17.00	17.61
4	17.91	18.02	18.21	17.92	18.09	18.06	17.47	17.95
5	17.27	17.81	17.38	17.36	17.77	18.04	17.18	17.54
6	17.30	17.53	17.78	17.61	17.81	17.80	17.53	17.62
7	17.60	17.49	17.49	17.63	17.61	17.58	17.28	17.53
8	17.41	17.88	17.51	17.20	17.60	18.05	17.44	17.58
9	17.53	17.33	17.25	17.85	17.63	17.91	17.33	17.55
Average	17.54	17.72	17.62	17.64	17.77	17.87	17.30	17.64

clearance for proper siphoning. Accordingly, some tests were made in which results were compared using 20 mm. and 25 mm. diameter alundum thimbles. The results showed no significant difference.

Another series of tests was made comparing results in which the samples were extracted in paper as well as in alundum thimbles. Here again the results showed no significant difference.

In another series, the effect of varying the sample size was determined. Samples ranging from one to seven grams of fish meal were extracted in the usual manner, using 30 ml. of solvent. No significant difference in results were obtained.

In the determination of fat in other products, such as fish flesh, acetone is sometimes used as the solvent. In the case of samples having fairly high moisture content (*i.e.*, about 80%) it has been found that the moisture which is extracted in the early stages of the extraction period sometimes interferes with the subsequent oil extraction. In such cases, it is

customary to make a short preliminary extraction with acetone. The solvent and extractives are then set aside and the extraction continued with fresh acetone. Finally, the two extractives are combined and the procedure continued in the usual manner. Some tests were carried out to determine whether such a procedure would have any beneficial effect in the oil extraction from fish meal. No significant differences were found when such a preliminary extraction, followed by a change of solvent, was used for herring meal.

Referring to Table 1, it will be noted that the average results for

TABLE 2.—*Effect of extraction time on per cent extractives from herring fish meal*

EXTRACTION PERIOD (EACH PERIOD 16 HOURS)	TOTAL TIME OF EXTRACTION (HOURS)	ACCUMULATIVE PER CENT ACETONE EXTRACTIVES						
		REPLICATE 1	REPLICATE 2	REPLICATE 3	REPLICATE 4	REPLICATE 5	REPLICATE 6	AVERAGE
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	16	17.47	17.00	16.93	16.91	16.33	15.98	16.77
2	32	17.93	17.58	17.41	17.44	16.80	16.46	17.27
3	48	18.31	17.96	17.79	17.87	17.16	16.95	17.67
4	64	18.50	18.27	18.02	18.13	17.49	17.31	17.87
5	80	18.70	18.56	18.38	18.51	17.65	17.52	18.22
6	96	19.01	18.76	18.55	18.78	17.91	17.67	18.45
7	112	19.29	18.89	18.81	19.04	18.03	17.99	18.68
8	128	19.39	19.06	18.93	19.19	18.19	18.18	18.82
9	144	19.55	19.20	19.10	19.38	18.39	18.42	19.00
10	160	19.63	19.35	19.16	19.46	18.50	18.51	19.10
HCl digest extraction after 10th acetone extraction period		0.95	0.94	0.92	1.04	0.92	0.93	0.95
Total extraction		20.58	20.29	20.08	20.50	19.42	19.44	20.05

samples extracted on extraction apparatus No. 7 were significantly lower than the results obtained in the other apparatus. It is apparent that for some reason this apparatus is giving erroneously low results. This same trend was found in all other experiments in which extraction equipment No. 7 was used. It was found that the rate of flow of acetone through the sample in this equipment was more erratic and in general slower than in the other equipment. This equipment was the last one on a block of seven, all using a common heating plate and water condensing system. It was observed that the heat at the right hand end of the heating block on which extraction flask No. 7 rested was somewhat less than of the center or left portion of the equipment and in many instances the rate of acetone distillation was likewise slower. It is also possible that some adverse effect resulted from the condenser on unit No. 7 being farthest removed from the water supply.

Referring to Table 2, a similar result seems to have occurred where the last two pieces of extraction equipment (No. 5 and No. 6) gave consistently lower results than the others. Tests are being continued to determine whether or not the use of individual extraction equipment with separate condensers and heating units give more consistent results.

These results indicated that possibly the rate of flow of the solvent through the sample, and very likely the total time of extraction might be of importance in obtaining maximum oil extraction. Accordingly, a series of tests was made in which successive 16-hour extraction periods were carried out on the same sample and the amount of oil extracted during each 16-hour period was determined. The meal, after the final extraction, was digested with hydrochloric acid and the residual oil after such hydrolysis was determined. Results are shown in Table 2. As will be noted, the apparent oil content (without HCl digest fraction) increased from 16.77 per cent after 16 hours to a value of 19.10 per cent after 160 hours total extraction time.

From these data it is not possible to determine whether the per cent of total extractives (acetone extraction of meal plus extraction of HCl digest fraction) increases after the initial 16-hour extraction or remains constant. It is possible that the HCl digest fraction is enough higher on the first 16-hour extraction meal to compensate for the lower value found. It is planned in future tests to investigate this point further.

Acknowledgement is made to Messrs. F. B. Sanford and William Clegg, Chemists, Seattle Fishery Technological Laboratory, who participated in the work.

REPORT ON MICROSCOPIC EXAMINATION

By J. A. SHRADER (Kentucky Agricultural Experiment Station,* Lexington, Ky.), *Associate Referee*

It has been rather difficult for me as a Referee on microscopic analysis to decide just what my duties are. Since apparently very little has been accomplished in the past in coordinating this form of analysis in the control laboratories of the States, there was not anything which I could "referee," if my understanding of the word is correct.

In view of the fact that in Kentucky for a number of years special emphasis has been placed upon microscopic analysis of feeds in our control work, and improvement has been noted in the quality of feeds sold in Kentucky, there is perhaps an obligation on our part to outline a system of microscopic analysis to serve as a pattern for this work in other States.

Having received a list of names of persons who would be willing to

* This report, made in connection with a project of the Kentucky Agricultural Experiment Station, is published by permission of the Director.

collaborate on microscopic examination, the Associate Referee wrote to all of them. Their response to the letters, outlining a course of action, has been satisfactory, and I am glad to report that at least a start has been made toward our goal.

As a result of these studies it has been agreed that some of the more important things needed in feed microscopy are:

(1) Definitions of all feed ingredients stated in terms to fit microscopic usage. These definitions should, of course, be reconciled with existing definitions of the A.O.A.C., A.A.F.C.O., and U. S. Grain Standards, so that one definition of each ingredient would have the same meaning to all phases of feed work. It may be that some definitions are satisfactory in their present form. Some definitely are not—U. S. Hay Standards, as compared with our definition of alfalfa meal, for example.

(2) A handbook containing these definitions and also descriptions of the appearance of all feed ingredients, chemical tests used in microscopic analysis, and recommended equipment for use of workers in microscopy in feed control and manufacturing laboratories. Such a handbook should include a series of color photographs illustrating each type of ingredient used in the compounding of feedstuffs.

(3) Sets of color slides to be used in training new microscopic analysts as well as acquainting interested persons in the industry with the appearances of ground feed ingredients. It has also been suggested that a training course in microscopy should be offered at some convenient place, similar to the class held in Washington by Miss Silberberg a number of years ago. It has been suggested that the Federal Government, or a State, or one of the associations concerned might be interested in sponsoring such a class.

(4) It is necessary to give more attention to the analysis of the results of the collaborative check sample sent out by Law & Co. for microanalysis. For example: Why did thirteen collaborators report finding distillers' grains in sample 3A? What part did the power of suggestion play in the finding of alfalfa meal, peanut oil meal, cottonseed meal, and coconut meal, by so many of the collaborators? (These four feedstuffs were listed as possible ingredients in the test sample.)

(5) Experimentation in the field of microscopic analysis is needed, to improve our technique and equipment.

(6) Improvement is necessary in our scheme of reporting analysis results to the manufacturer and consumer so that all concerned will receive the maximum benefit from our reports.

In Kentucky progress has been made toward fulfilling some of the needs listed above, especially in the field of reporting results of analysis to the manufacturer. When a feed is found to be out of line with the guarantee, not only is the manufacturer told what is wrong, he is shown

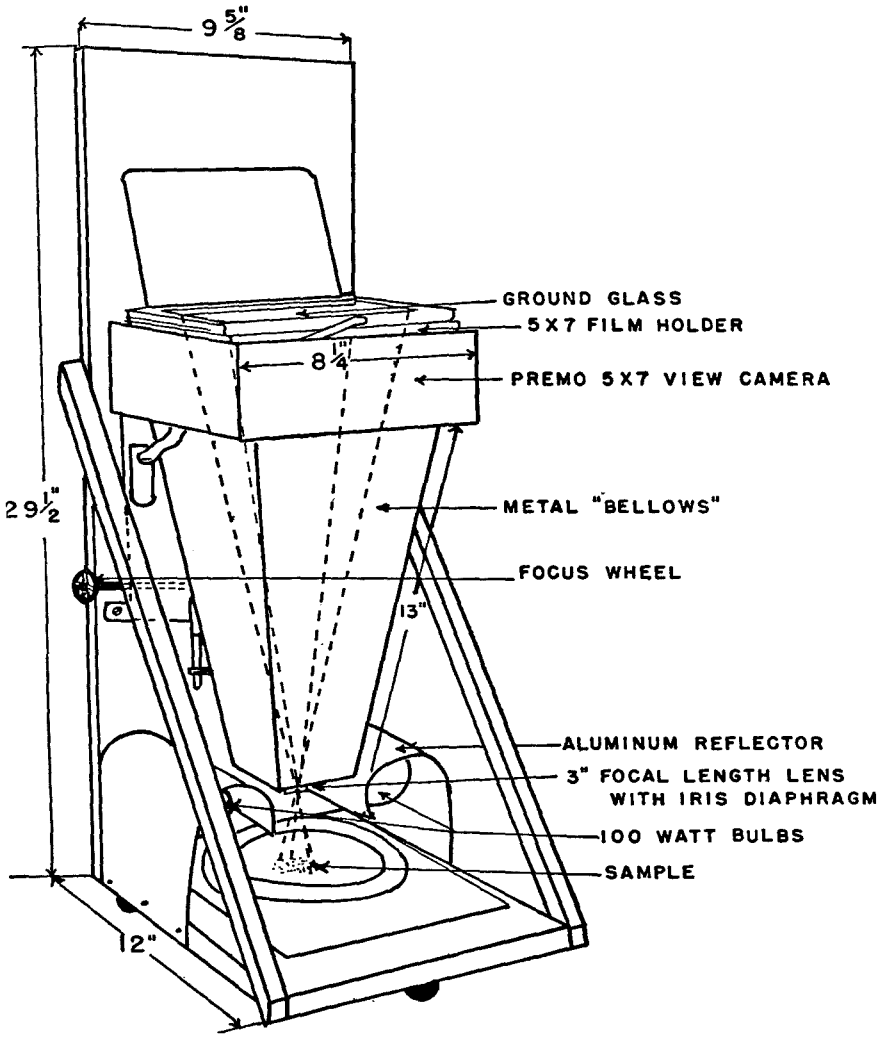

FIG. 1.—Direct positive enlarging camera.

by an enlarged picture, especially in the case of adulteration. This method may sound quite complicated, but really it is very simple and quick. A camera has been constructed which, using direct positive paper, will make a 5″×7″ enlargement (20×) directly from the sample. The whole process—exposing, developing, washing, and drying the print ready to go—requires approximately 15 minutes. (See Fig. 1.)

This laboratory has been using and improving this means of reporting for approximately a year, and has had numerous very favorable comments from the manufacturers. They really appreciate the extra effort made to send our reports in a clear and understandable form.

At present we are experimenting with a process, using the same camera, whereby the picture can be made in color at a cost which is not prohibitive.

Another camera attaches to the microscope and will make color transparencies or slides of feeds. With this it will be possible to make the sets of color slides mentioned in part three (3) above.

There has not been enough progress made on the other points of the program to warrant reporting here; but it is hoped to accomplish a large part of it in the coming year.

I wish to express my thanks to those collaborators and others who have sent helpful suggestions.

REPORT ON CRUDE FIBER

By WILLIAM L. HUNTER (State Department of Agriculture, Sacramento 14, California), *Associate Referee*

The 1948 report of the Associate Referee recommended study of shorter methods of determination of crude fiber. Accordingly, collaborative work was undertaken this year to compare the official method with one employing reagents of higher concentration with a shortened digestion period.

Three samples, selected for quantity and quality of crude fiber representative of feeding materials and uniformly ground in a hammer-type mill to pass a one-millimeter screen, were submitted to collaborators with the following instructions:

On each sample make three or more analyses by each of Methods A and B, reporting each result and not averages only. Replicate determinations on the same sample should not be made concurrently.

METHOD A: A.O.A.C., Official Method, Crude Fiber, *Methods of Analysis, A.O.A.C.*, 6th Ed. 1945.

METHOD B: Equipment and procedure are the same as specified in the official method except that the digestion time for acid and alkali treatments is reduced to 10 minutes each and the following solutions are employed:

Sulphuric Acid Solution.—0.64 *N* (ca 3.125%)

Sodium Hydroxide Solution.—0.78 *N* (ca. 3.125%)

The results obtained are contained in Tables 1, 2 and 3.

The results obtained by the short method are in very good agreement with those obtained by the official method. Tests of statistical significance indicate that only in the case of the sample of alfalfa meal is the difference noteworthy. It is believed that measurement of digestion and filtration time is much more critical where reagents of higher concentration are

TABLE 1.—*Poultry feed*

COLLABORATOR	METHOD A		METHOD B	
1	5.4		5.6	
	5.4		5.5	
	5.4		5.5	
Average		5.40		5.53
2	5.06		5.04	
	4.93		4.93	
	5.11		4.89	
	5.38		5.00	
Average		5.12		4.97
3	5.12		4.95	
	5.42		4.75	
	4.90		4.77	
	4.85		4.65	
Average		5.07		4.78
4	5.01		4.91	
	4.94		5.03	
	—		5.09	
Average		4.98		5.01
5	5.13		4.80	
	5.06		4.76	
	5.19		4.85	
	5.40		5.18	
Average		5.19		4.89
6	5.63		5.52	
	5.30		5.33	
	5.29		5.08	
	5.28		—	
Average		5.37		5.31
7	5.00		5.21	
	4.97		5.00	
	5.08		4.87	
	5.06		4.92	
Average		5.03		5.00
8	5.05		4.77	
	4.86		4.78	
	4.96		4.88	
	4.98		4.92	
Average		4.96		4.86
9	5.16		5.40	
	5.45		5.38	
	5.09		5.38	
	5.27		5.18	
Average		5.24		5.34
10	4.97		5.06	
	4.99		4.96	
	4.88		4.66	
Average		4.95		4.89
Mean, all results		5.14		5.04
Standard Deviation		0.20		0.26
Standard Error		0.033		0.044

used. Further work with closer attention to this portion of the method may reduce differences noted in this data.

Comments of collaborators were favorable and a definite interest in the method under study was expressed with the exception of those who have equipment designed to handle a group of 12–18 samples at one time. In their case, it was stated that the time saving in the short method may be canceled by having to handle a smaller group of samples.

The assistance of the following collaborators is gratefully acknowledged:

C. V. Marshall, Department of Agriculture, Ottawa, Canada.
Herbert L. Wilkins, U.S.D.A., Beltsville.
M. P. Etheredge and J. A. Ellard, Mississippi State Chemical Laboratory, State College.

TABLE 2.—*Dairy feed*

COLLABORATOR	METHOD A		METHOD B	
1	9.4		9.8	
	9.3		9.6	
	9.2		9.6	
Average		9.30		9.66
2	8.70		9.16	
	8.85		8.54	
	9.18		8.72	
	8.97		8.96	
Average		8.93		8.84
3	8.87		9.05	
	8.71		8.55	
	8.80		8.65	
	8.30		8.17	
Average		8.67		8.61
4	8.34		8.62	
	8.63		8.48	
	—		8.79	
Average		8.49		8.63
5	8.63		8.52	
	8.85		8.76	
	8.65		8.48	
	8.86		8.42	
Average		8.74		8.54
6	9.71		9.73	
	9.77		9.53	
	9.17		9.38	
	9.25		—	
Average		9.47		9.55
7	8.92		8.76	
	8.99		8.62	
	8.82		8.69	
	9.11		8.70	
Average		8.96		8.69
8	8.97		8.57	
	8.85		8.68	
	8.78		8.68	
Average		8.87		8.64
9	9.15		—	
	9.26		9.20	
	9.21		9.40	
	9.36		9.22	
Average		9.24		9.27
10	8.85		8.77	
	8.87		8.48	
	—		8.68	
Average		8.86		8.64
Mean, all results		8.98		8.88
Standard Deviation		0.33		0.43
Standard Error		0.056		0.074

Henry A. Davis, New Hampshire Agricultural Experiment Station, Durham.
Ernest Epps, Jr., and C. C. Moreland, Louisiana Agricultural Experiment Station, Baton Rouge.
P. R. Bidez, Alabama Department of Agriculture and Industries, Montgomery.
Stacy B. Randle, New Jersey Agricultural Experiment Station, New Brunswick.
Hobart Halloran and Gordon V. Nelson, Poultry Producers of Central California, Petaluma, California.
Tracy Barrett, M. F. A. Milling Company, Springfield, Missouri.
Feed Laboratory, California Department of Agriculture, Sacramento.

The order in which the above are listed does not conform to the numbers used in the table of results.

TABLE 3.—*Alfalfa meal*

COLLABORATOR	METHOD A		METHOD B	
1	24.6		24.6	
	24.5		24.1	
	24.5		24.2	
Average		24.53		24.30
2	23.53		23.58	
	23.70		23.35	
	24.59		24.13	
	23.77		23.39	
Average		23.90		23.61
3	23.80		23.55	
	24.52		23.18	
	23.80		23.50	
	23.25		22.90	
Average		23.84		23.38
4	23.78		23.40	
	23.58		23.31	
	—		23.02	
Average		23.68		23.24
5	24.01		23.46	
	24.13		23.33	
	24.29		23.85	
	24.24		23.76	
Average		24.16		23.60
6	24.65		24.63	
	24.13		24.67	
	24.29		24.37	
	24.48		24.28	
Average		24.39		24.49
7	24.45		23.91	
	24.48		23.92	
	24.74		23.42	
	24.65		23.54	
Average		24.58		23.70
8	23.80		23.15	
	23.98		23.14	
	23.60		23.12	
	—		23.12	
Average		23.79		23.13
9	23.88		23.86	
	24.20		23.88	
	24.13		23.40	
	24.12		23.79	
Average		24.08		23.73
10	23.89		23.48	
	23.66		23.54	
	23.13		23.27	
Average		23.56		23.43
Mean, all results		24.08		23.65
Standard Deviation		0.42		0.47
Standard Error		0.070		0.077

RECOMMENDATION*

It is recommended that further study be given to the crude fiber method employing acid and alkali of approximately 3.125% strength for a digestion period of 10 minutes in each to determine its possibilites as an alternate official method.

* For report of Subcommittee A and action of the Association, see *This Journal*, **33**, 37 (1950).

REPORT ON TANKAGE (HOOF, HORN, HIDE, HAIR, ETC.) IN PACKING HOUSE PRODUCTS

By A. T. PERKINS (Kansas State College, Manhattan, Kansas), *Associate Referee*

The Associate Referee has investigated several chemical and physical methods of determining the presence and amount of hoof, horn, hide, and hair in meat scraps and tankages. Enzymes, digestion, specific gravity separations, hydrolysis, and other methods that gave promise have been tried. To date no satisfactory method has been developed to make the separation, probably because of the great variation in the normal composition of such packing house products. Microscopic methods are being investigated by B. L. Smits of Kansas State College; not only microscopic methods as such, but the use of the microscope to check chemical and physical separations.

REPORT ON SULFA DRUGS IN FEEDS

By RICHARD T. MERWIN (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

The administration of sulfonamide drugs to other than human subjects has led during the past year to the use of sulfaquinoxaline in poultry feeds for the control of cecal and intestinal coccidiosis. Because the drug is considered toxic at higher levels, the feeds are usually processed to contain 0.0125 per cent of sulfaquinoxaline, although concentrated premixes containing 0.50, 5, and 10 per cent are available from feed manufacturers.

Methods for assay of the drug (2-sulfanilamidoquinoxaline) are based on the reaction proposed by Bratton and Marshall (1) for sulfanilamide in blood and urine. In sulfaquinoxaline, as in sulfanilamide, the arylamino group can be diazotized and coupled with *N*-(1-naphthyl)-ethylenediamine dihydrochloride to produce a purplish-red color the optical density of which can be measured by a spectrophotometer.

Dux and Rosenblum (2), Tyler and Brooke (3), Schlenker (4), and others have followed the Bratton and Marshall procedure. The Associate Referee has investigated the methods they have proposed and, with slight modifications, selected the method of Dux and Rosenblum, known as the zinc sulfate method, for collaborative study.

The high solubility of sulfaquinoxaline in dilute alkali hydroxide makes extraction of the drug from poultry feeds a simple procedure. It has been found that use of an organic solvent such as chloroform has not been altogether successful because of limited and slow solubility, although it does eliminate extraction of considerable protein. On the other hand, use of dilute alkali, although excellent for complete and rapid extraction,

poses the problem of protein separation from the extracting solution. Bratton and Marshall used trichloroacetic acid as a protein clarifying agent in their work on blood and urine, and Tyler and Brooke and Schlenker, using the same reagent, have obtained clear filtrates on poultry feed extracts containing sulfaquinoxaline. However, Dux and Rosenblum have successfully employed zinc sulfate solution, which has the advantage of producing nearly neutral filtrates, an important feature in *p*H control.

Several reasons influenced the Associate Referee to select the method of Dux and Rosenblum for collaborative study. The chief reason was the ease with which the correct *p*H of 1.3–1.4 could be effected for diazotization and coupling; another reason was the fact that the color complex was more stable in a hydrochloric acid solution than it was in a trichloroacetic acid solution. (Bratton and Marshall had observed that some reaction occurred between trichloroacetic acid and the coupling reagent, causing the solutions to show increasing color on exposure to light.) Other reasons were the ease and certainty of preparing reliable standards; the ability to apply the method to the assay of any percentage of sulfaquinoxaline likely to be found in feed, by varying the sample weight and final dilution; and the close theoretical accuracy of the results.

COLLABORATIVE METHOD FOR SULFAQUINOXALINE

REAGENTS

0.50 *N* NaOH.

0.50 *N* HCl.

1.00% soln of $ZnSO_4.7\ H_2O$.

0.10% soln of $NaNO_2$.—Prepare fresh each day.

0.50% soln of ammonium sulfamate.

0.10% soln of *N*-(1-naphthyl)-ethylenediamine dihydrochloride.—Store in amber-colored bottle.

DETERMINATION

Weigh 5 g of ground sample into 250-ml flask and add 100 ml of H_2O and 2.5 ml of 0.50 *N* NaOH. Heat in a water bath 15 min. with occasional swirling, cool, make to volume, and mix well. Let material settle and pipet 50 ml into 100-ml flask, add 10 ml of the $ZnSO_4$ soln, dilute to mark, mix well, and let stand one min. before filtering thru 18.5 cm Whatman No. 2 paper. Discard first 10 ml of filtrate. (Filtrate *must* be free of turbidity.)

Pipet 10 ml or 15 ml of clear filtrate into 25-ml volumetric flask, add 2.5 ml of the 0.50 *N* HCl and 2 ml of 0.10% $NaNO_2$ soln. Let stand 3 min. Add 2 ml of 0.50% ammonium sulfamate soln and wait an additional 2 min. Finally add 2 ml of the coupling reagent and dilute to mark. Swirl contents of flask after each addition of reagent. Shake vigorously. (If turbidity appears at this point, discard soln, pipet 5 ml instead of 10 or 15 ml aliquot into 25 ml volumetric flask, and repeat treatment with 0.50 *N* HCl, 0.10% $NaNO_2$ soln, etc.) Prepare blank using H_2O and same quantities of reagents made to volume of 25 ml.

Measure optical density of colored soln in spectrophotometer at wave length of 545 mμ against the reagent blank and determine quantity of sulfaquinoxaline present by reference to standard curve.

If visual colorimetric comparisons are made, use 15 ml sample aliquot and compare with standard containing 20 mmg of sulfaquinoxaline.

PREPARATION OF STANDARD CURVE

Dissolve 0.10 g of pure sulfaquinoxaline in 2 ml of 0.50 *N* NaOH in 500-ml volumetric flask and make to volume with H_2O. Transfer 25 ml of this soln to liter volumetric flask and dilute to volume. Each ml of this soln contains 5 mmg of sulfaquinoxaline. Dilute 1, 2, 3, 4, and 5 ml portions of this soln (corresponding to 5, 10, 15, 20, and 25 mmg of sulfaquinoxaline, respectively) separately to 10 ml with H_2O and treat each soln with 0.50 *N* HCl, 0.10% $NaNO_2$ soln, etc., as directed under "Determination." Measure optical densities of final solns against reagent blank, and plot density readings against mmg of sulfaquinoxaline.

Two samples of ground poultry feed of average formula, mixed in the laboratory with known quantities of 99.8 per cent pure sulfaquinoxaline, were sent to each collaborator. The samples were labelled No. 4 and No. 5 and contained 0.0100 and 0.0125 per cent of sulfaquinoxaline, respectively. Collaborators were asked to report four results on each sample, using the collaborative method. No instructions other than a copy of the method accompanied the request.

Table 1 presents the results of the collaborative study.

The close agreement between a large majority of the collaborators is adequate proof of the precision of the method. Individual variations are not greater than would be expected in routine analysis, and prove not only the accuracy of the method but the homogeneity of the samples. The figures reported are based on spectrophotometric measurement and represent evaluations obtained with several types of photoelectric equipment, mainly the Beckman Model DU and the Coleman Universal Model 11. The Associate Referee has found equal precision using a B. & L. Duboscq colorimeter.

COMMENTS OF COLLABORATORS

Comments on the method were favorable. A few collaborators suggested slight modifications, and the following comments in particular are noted:

No. 1 and No. 4. Both recommend discarding the first 10 ml of filtrate. This precaution was omitted from the method, as preliminary assays had not appeared to show that it was necessary, but as a good precautionary procedure it has been incorporated in the method as presented herewith.

No. 2 and No. 4. Smaller diameter, 11 cm. filter papers, were used to separate protein from the extracting solution. The larger paper was suggested because it speeds filtration, which is of advantage in the routine analysis of many feeds. Using large diameter doubled circles has been found helpful in filtering finely dispersed protein from some feeds, especially straight alfalfa meal.

No. 1. "It is our practice to use 15 ml instead of 10 ml of filtrate at the .01 per cent level. This increases the color intensity by 50 per cent and makes for a more accurate spectrophotometric reading." The point seems well taken. However, it may largely be a matter of choice. The 10 ml aliquot appears more desirable because it permits easier swirling of reagents as added within the 25 ml volume limitation.

TABLE 1.—*Analyses of feeds containing sulfaquinoxaline*

COLLABORATOR	SULFAQUINOXALINE, PER CENT			SULFAQUINOXALINE, PER CENT		
	PRESENT	FOUND	AVERAGES	PRESENT	FOUND	AVERAGES
1	.0100	.0099		.0125	.0127	
		.0102			.0130	
		.0109			.0130	
		.0099	.0102		.0122	.0127
2		.0105			.0124	
		.0103			.0118	
		.0098			.0124	
		.0103			.0122	
		.0103			.0118	
		.0103			.0128	
		.0103	.0103		.0128	.0123
3		.0103			.0129	
		.0107			.0125	
		.0104			.0123	
		.0105			.0123	
		.0102			.0123	
		.0105	.0104		.0123	.0124
4		.0105			.0129	
		.0104			.0132	
		.0104			.0126	
		.0096	.0102		.0122	.0127
5		.0118			.0135	
		.0118			.0140	
		.0120			.0140	
		.0120	.0119		.0138	.0138
R.T.M.		.0102			.0127	
		.0101			.0122	
		.0101			.0124	
		.0102	.0102		.0125	.0125
Average of all collaborators (omitting No. 5)			.0103			.0125

No difference in accuracy has been observed by the Associate Referee between the 10 ml and 15 ml aliquots. No. 1 used the 15 ml aliquot with the Associate Referee's approval for the purpose of comparison. All others used the 10 ml aliquot for both concentrations. A choice of aliquots, however, is now offered in the method, as follows: "Pipet 10 or 15 ml," etc.

No. 3. "The first four analyses of each sample are those of Miss Lois Decker who had analyzed only six previous samples for sulfaquinoxaline." Miss Decker's close results are pleasantly indicative of the method's simplicity and precision.

No. 2. "No difficulties were encountered in the technic. The standard curve was

an average from two lots of drug. There were small differences between the data for the two lots, but these include possible weighing and dilution errors. As I plotted the optical densities the curves were straight lines except for the 15 microgram point with Lot 8R5726 and that was not far out of line. The averages gave an excellent curve. I found that cuvettes must be exceptionally clean to prevent gas bubbles from clinging to the inner surfaces, but that is an ordinary precaution."

Several collaborators mentioned the formation of nitrogen bubbles on the cell walls and found it necessary to invert the cells slowly several times to liberate them. This tendency to bubble formation can usually be counteracted by vigorously shaking the diazotized and coupled sample after making to volume. The presence of only a few bubbles in the light-path of the cell will seriously affect results by influencing density readings. Frequent inspection of cells during readings is an ordinary precaution with all who are familiar with spectrophotometric work.

LATENT TURBIDITY

Sparklingly clear filtrates are necessary for accuracy in use of the method. Letting the filtrate stand one minute, after making to volume and shaking to recoagulate the protein, and use of a filter paper of good retentiveness, usually suffice in this respect. The average run of feeds will give no trouble, but those containing a high percentage of alfalfa may require refiltration or the use of double filter paper to obtain clear filtrates.

Even with the best precautions an occasional feed solution may develop a slight turbidity after color production, even though a clear filtrate was used. The usual method of correcting for turbidity is to deduct a sample blank. To check the validity of this procedure, a series of experiments was undertaken, using a Model DU Beckman spectrophotometer. Studies were made of the effects of extracted color, of turbidity, and of both on assay accuracy, by imposing these conditions on standard solutions. It was found that highly colored feed extracts added to standards did not interfere with theoretical density readings provided no turbidity was present, even though they produced off-color shades of the purplish-red complex. The point was well established that no blank deductions for color alone should be attempted. It was observed, however, that the use of turbid extract-colored solutions led to high results. Thus it was proved that turbidity only was the cause of assay error.

Corrections for varying degrees of turbidity imposed on standard solutions were attempted by blank deductions of corresponding turbidities. The range of turbidities was kept within that which might be encountered in actual assay. Under perfect conditions, the corrections yielded theoretical results. It was concluded that blank deduction for turbidity was a valid procedure, but only when the solutions had no extracted color.

When attempts were made to deduct for turbidity in the presence of extracted color, low results were obtained.

Summarizing the observations based on these experiments, the Associate Referee concludes that: (1) no blank correction should be made for extracted color alone; (2) no blank correction should be made on samples containing extracted color and turbidity; (3) blank deductions are valid when no extracted color is present.

Since it is rare that turbidity is not associated with extracted color, the Associate Referee believes the best solution to the latent turbidity problem lies in reducing the turbidity to a minimum or eliminating it entirely. This can usually be accomplished by taking an aliquot equal to one-half that in which the latent turbidity developed. In this way, dilution of sample will usually eliminate the turbidity or minimize its effect, even in the case of straight alfalfa meal, which experience has shown to be the most difficult type of sample to assay accurately. However, the occasion seldom arises for analysis of such a type of feed for sulfaquinoxaline.

The method is applicable to the assay of poultry feed premixes containing higher concentrations of sulfaquinoxaline. Thus, for a 5 per cent or 10 per cent premix only a 0.50 gram sample need be weighed, 5 ml aliquoted for protein precipitation, and 2 ml of filtrate taken for color development. The same standards may be used for evaluations.

ACKNOWLEDGEMENTS

The Associate Referee is greatly indebted to Charles Rosenblum and James P. Dux, of the Research Department, Merck & Co., Rahway, N. J., for a copy of their manuscript on the method entitled "The Spectrophotometric Determination of Sulfaquinoxaline and Its Application to Poultry Feeds and Feed Premixes." Thanks are also due the following for their collaboration: (1) Charles Rosenblum and James P. Dux; (2) James B. Smith, Agricultural Experiment Station, Rhode Island State College, Kingston, R. I.; (3) L. D. Matterson and Miss Lois Decker, Poultry Department, The University of Connecticut, Storrs, Conn.; (4) Stanley W. Tyler, Wirthmore Research Laboratory, Malden, Mass.; (5) W. R. Flach, Eastern States Farmers' Exchange, Buffalo, N. Y.; (6) L. E. Bopst, Association of American Feed Control Officials, Inc., Baltimore, Md.; and (7) L. E. Bartlett, Park and Pollard Co., Buffalo, N. Y.

RECOMMENDATIONS*

It is recommended—

(1) That the method be adopted as outlined (first action).

(2) That collaborative studies be continued.

(3) That collaborative studies of a method for sulfaguanidine be undertaken.

* For report of Subcommittee A and action of the Association, see *This Journal*, 33, 36 (1950).

REFERENCES

(1) A. C. BRATTON and E. K. MARSHALL, JR., *Jour. Biol. Chem.*, 128, 537 (1939).
(2) DUX and ROSENBLUM, mss., "The Spectrophotometric Determination of Sulfaquinoxaline and Its Application to Poultry Feeds and Feed Premixes."
(3) STANLEY W. TYLER and RICHARD O. BROOKE, mss., "The Determination of Sulfaquinoxaline and Sulfaguanidine in Commercial Feeds."
(4) F. S. SCHLENKER, mss., "Sulfaquinoxaline in Poultry Feeds."

REPORT ON MINERAL CONSTITUENTS OF MIXED FEEDS

METHOD OF SAMPLE PREPARATION FOR CALCIUM AND PHOSPHORUS IN FEEDS USING NITRIC-PERCHLORIC ACID

By J. L. ST. JOHN (*Associate Referee*) and EDITH ENG HUEY
(Division of Chemistry, Agricultural Experiment Stations and State Chemist's Laboratory, Pullman, Washington)

This report is based upon the work reported in *This Journal*, **30**, 606 (1947), **31**, 614 (1948), **32**, 650 (1949), and upon work on this method published elsewhere, as recorded in the above reports. It is also based upon Committee recommendations in *This Journal*, **31**, 41 (1948), and **32**, 43 (1949), the latter of which recommends that this method be made official, first action.

The method for sample preparation using nitric and perchloric acids is published in *This Journal*, **31**, 98 (1948). The present method contains certain editorial changes to dovetail it more closely with method **27.47.** It includes a method of sample preparation using dry ash method **27.9** for those who prefer a dry ash method. It also contains certain general directions, and recommends the calculation of the percentage of the elements as calcium (Ca) and as phosphorus (P), rather than a report of these elements in terms of the oxide (CaO and P_2O_5).

The need for precaution in the use of perchloric acid to guard against the danger of an explosion, which appears in previous reports (*This Journal*, **31**, 614, 1948), is repeated. It is emphasized that all of the easily oxidizable organic matter should be oxidized with nitric acid before the perchloric acid is added.

It is recommended* that this method be made official, as a method of sample preparation for calcium and phosphorus in feeds. It is also recommended that the nitric-perchloric acid method of sample preparation be further studied for use in the determination of calcium and phosphorus in mineral feeds for use with official method **27.47.**

The method as described is to be published in the 7th Edition, *Methods of Analysis*, 1950.

* For report of Subcommittee A and action of the Association, see *This Journal*, **33**, 36 (1950).

A contributed paper entitled "An Improved Arrangement of the Fiber Determination Apparatus," by V. C. Midkiff and J. A. Schrader, was published in the preceding number of the *Journal*, page 141.

Papers entitled "Stereoisomeric Analysis of Beta-Carotene, "by E. M. Bickoff, M. E. Atkins, G. F. Bailey, and Fred Stitt, and "Determination of Beta-Carotene Stereoisomers in Alfalfa," by E. M. Bickoff and C. R. Thompson, were published in the *Journal* for November, 1949, on pages 766 and 775, respectively.

No reports were given for lactose in mixed feeds; adulteration of condensed milk products; crude fat or ether extract; fluorine; protein evaluation in fish and animal products; sampling and analysis of condensed buttermilk.

REPORT ON CEREAL FOODS

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Referee*

The Referee concurs on the recommendations of the Associate Referees and wishes to thank them for their assistance to the Association.

RECOMMENDATIONS*

It is recommended—

(1) That the studies on the determination of starch in raw and cooked cereals be continued.

(2) That the method for the determination of fat acidity in grain and flour, secs. **20.18, 20.19, 20.20, 20.21,** and **20.76** be made official.

(3) That the study of the modification of the method, secs. **20.28, 20.29,** and **20.30,** for the determination of reducing and non-reducing sugars in bakery products, as recommended in the Associate Referee's report, be continued.

(4) That the method for benzoic acid in flour in the Associate Referee's report be made first action.

(5) That the methods for the determination of lactose in bread be further studied.

(6) That the method for the determination of fat and fat number in bread, *This Journal*, **32,** 85 (1949), be adopted as official.

(7) That the method for the determination of proteolytic activity of flour and malted wheat flour, *Ibid.*, **32,** 86 (1949), with the changes recommended in the Associate Referee's report, be adopted as official and that the work be continued as suggested in the report.

(8) That the methods for soybean flour, *This Journal*, **32,** 87 (1949), for

* For report of Subcommittee D and action of the Association, see *This Journal*, **33,** 63 (1950).

moisture (deleting "**20.2**"), ash, nitrogen, and oil or petroleum benzine extract, remain "first action" and study be continued.

(9) That the method for the determination of added inorganic material in phosphated flour (quantitative), *This Journal*, **32,** 88 (1949), be adopted as official.

(10) That the methods referred to in *This Journal*, **25,** 83 (1942), for the determination of unsaponifiable matter and sterols in noodles, be studied to determine their applicability to bakery products containing eggs.

(11) That the study of methods for the determination of albumen in noodles and macaroni products be conducted.

(12) That the study on the determination of moisture, fat, crude fiber, ash, and protein in bakery products be continued.

(13) That the study on the determination of moisture in flour products containing sodium bicarbonate as one of its constituents be continued.

(14) That the study on the determination of bromates in flour be continued.

The following recommendations refer to revision of Chapter 20, Cereal Foods:

That **20.39,** Water-soluble protein-nitrogen precipitable by 40% alcohol, be made first action.

That **20.44,** Starch, be dropped.

That **20.46–7,** Polarimetric method, be made first action.

That **20.48,** Chlorine in fat of flour, be made first action.

That **20.51,** Nitrite nitrogen, be made first action.

That **20.54,** Gasoline color value, be dropped. (This method is obsolete and no longer in general use.) Substitute as first action the method submitted by the Referee on "Pigments in flour expressed as carotene."

That **20.55–6–7–8–9,** Carotene, be made first action.

That **20.69,** Soybean flour in uncooked cereal products, be made first action.

That **20.80,** Crude fiber (soybean flour), be made first action.

That **20.81,** Crude fat or ether extract, be dropped.

That **20.87,** Citric acid, be made first action.

That methods for baked products other than bread (**20.98–20.103,** incl.) be made first action.

That **20.112,** Original ash in macaroni products containing added salt but not containing added eggs, be made first action.

In **20.113,** Chlorides in ash as sodium chloride, official, change "does not give" in line 4 to "gives only approximate."

That **20.117,** Water-soluble protein nitrogen precipitable by 40% alcohol, be made first action.

REPORT ON PIGMENTS IN FLOUR EXPRESSED AS CAROTENE

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington 25, D. C.), *Associate Referee*

The color of a gasoline extract of flour has long been used as an indication of a bleaching. The method designated as "Gasoline Color Value" Sec. **20.54,** *Methods of Analysis,* has served such a purpose. This method has poor precision and is no longer in general use. A new method, which is essentially the method using water-saturated normal butyl alcohol given in Cereal Laboratory Methods of the Association of American Cereal Chemists, gives good precision and can be used for the same purpose as the Gasoline Color Value.

Three samples of flour were sent out with a copy of the method with the instructions that the pigment content be determined on a definite date. The samples consisted of No. 1, a commercially bleached cake flour, No. 2, a commercial hard wheat flour, and No. 3, an unbleached soft wheat pastry flour. Five collaborators reported the following results:

Pigment in flour expressed as carotene in p.p.m.

COLLABORATORS	SAMPLE 1	SAMPLE 2	SAMPLE 3
1	0.5	1.6	2.8
2	0.7	1.6	2.9
	0.6	1.5	2.8
3	0.7	1.7	2.8
	0.7	1.7	3.0
4	0.7	2.0	2.9
	0.7	1.8	3.1
5	0.4	1.7	2.9
	0.5	1.7	2.8

One collaborator reported difficulty in obtaining a clear filtrate and results were not reported. These collaborative results appear to be in good agreement. It is recommended*—

(1) That the Gasoline Color Value method, **20.54,** be dropped.

(2) That the proposed method be adopted as first action.

The details of the method will be published in the 7th Edition, *Methods of Analysis, A.O.A.C.,* 1950.

* For report of Subcommittee D and action of the Association see *This Journal,* **33,** 64 (1950).

The valuable assistance of the following collaborators is acknowledged:

F. A. Collatz, General Mills Inc., Minneapolis, Minn.
Betty Sullivan, Russel Miller Milling Co., Minneapolis, Minn.
Hyman O. Silverberg, Robert D. Stanley, and L. C. Mitchell, all of the Food and Drug Administration.

REPORT ON BENZOIC ACID IN FLOUR

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Associate Referee*

This report is a continuation of the study made last year using the same method, rewritten to indicate more clearly the important steps believed

TABLE 1.—*Benzoic acid in flour in p.p.m.*

COLLABORATOR	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO. 1 PLUS 1 MG ADDED BENZOIC ACID BY EACH ANALYST
1	2.1	16.2	19.1
	1.0	16.0	17.0
2	2.0	13.2	13.6
	1.2	17.6	14.0
3	6.6	20.4	24.0
	6.8	20.6	26.6
4	1.5	19.0	19.9
	1.5	19.8	19.6
	1.4	19.5	20.0
5	4.2	22.0	21.2
	3.8	22.0	21.4
6	1.7	19.2	21.4
		18.2	
	1.3	18.3	21.4
		18.3	
7	1.6	19.5	18.0
	0.6	19.7	17.8
8	4.4	13.0	19.0
	5.6	12.6	21.0
9	2.3	15.4	—
10	2.4	17.8	—
	3.0	18.0	—

to be responsible for the variable results last year. A sample of untreated flour was used, and the same flour with 20.0 p.p.m. of benzoic acid added by mixing in a definite amount of a commercial bleaching mixture containing benzoyl peroxide. This value in p.p.m. is based on the analysis of the Associate Referee by a method for the determination of peroxides. In order to avoid any error in the amount of benzoyl peroxide added thru use of a commercial mixture, and also the possibility of any variation in distribution, the collaborators were asked to add to a 50-g portion of sample No. 1, the untreated flour, after weighing out into the flask a one-mg. aliquot of pure benzoic acid (0.5 mg benzoic acid per 1 ml acetone).

The details of the method will be presented in the 7th Edition, *Methods of Analysis*, 1950.

Results reported by ten collaborators are given in Table 1. Both the sample No. 2 and the sample treated with benzoic acid by the collaborator contained 20 p.p.m. of benzoic acid. These results are more uniform than last year and actually represent acceptable recoveries. While one of the collaborative results on each sample was a somewhat lower recovery than the others, it is thought that the method is satisfactory for this determination in flour. The assistance of the following collaborators is gratefully acknowledged:

K. L. Fortman, Wallace & Tiernan Co., Belleville, N. J.
Richard L. Gray, Novadel Agene Corp., Buffalo 5, N. Y.
E. Stegemeyer, Kroger Food Foundation, Cincinnati 4, Ohio.
R. C. Koehn, General Mills, Inc., Minneapolis, Minn.
W. L. Rainey, Commander-Larabee Milling Co., Minneapolis, Minn.
Hymen D. Silverberg, F. J. McNall, and Edward F. Steagall, all of Food and Drug Administration.

REPORT ON PROTEOLYTIC ACTIVITY OF PATENT FLOUR AND MALTED WHEAT FLOUR

By Byron S. Miller (Associate Chemist, Hard Winter Wheat Quality Laboratory, Manhattan, Kansas), *Associate Referee*

The modified Ayre and Anderson method[1] for the determination of proteolytic activity was studied collaboratively[2] and the method adopted as first action.[3] Extensive use of this method by the Associate Referee during the past year has shown the method to be entirely satisfactory; however, certain modifications appear to be desirable. These are designed to adapt the method to the analysis of extracts of active proteolytic materials.

[1] Miller, Byron S., *This Journal*, **30**, 659 (1947).
[2] Miller, Byron S., *Ibid.*, **32**, 261 (1949)
[3] *This Journal*, **32**, 86 (1949).

The following changes in the method for the determination of proteolytic activity of patent flour and malted wheat flour are recommended.

Page
87 line 2—Change to read, "Dissolve 36 g. trichloroacetic acid in 24 ml water. Use 3 ml aliquot."
87 line 9—Change "1 ml" to "2 ml."
87 line 11—Change "0.625 g" to "1.250 g."
87 line 19—Change "5 hours of digestion" to "5 hours and 15 minutes of digestion."
87 line 22—Change "5 ml" to "3 ml."
87 line 24—Change "1 ml" to "2 ml."
87 line 25—Change "4 ml" to "3 ml."
87 line 27—Change "5 ml" to "3 ml."
87 line 37—Change "5 hour digestion" to "long time digestion."

The following "NOTES" should be added to the method.

(1) Careful washing down of the trichloroacetic acid from the neck of the digestion flasks is mandatory.

(2) For some materials a turbid solution may remain after the final filtration. Such turbidity may be removed by boiling the centrifuged digestion mixture for a few seconds prior to final filtration. The liquid lost through evaporation should be replaced by the addition of water.

(3) More reproducible results will be obtained if the Kjeldahl determinations are completed without delay between digestion and distillation.

RECOMMENDATIONS*

It is recommended—

(1) That the method as proposed and modified be adopted as official, for the determination of proteolytic enzymes in patent flour and malted wheat flour.

(2) That further work be done on the application of the method to the analysis of proteolytic enzymes in materials other than patent flour and malted wheat flour.

(3) That other methods for the determination of proteolytic activity be investigated.

REPORT ON SOYBEAN FLOUR

By W. L. TAYLOR (General Mills, Inc., Minneapolis, Minn.),
Associate Referee

The changes made in the tentative method for soybean flour† at the 1947 meeting were studied last year and the results of collaborative work were reported on pages 267 and 268 of the May, 1949, issue of *This*

* For report of Subcommittee D and action of the Association, see *This Journal*, **33** ,63 (1950).
† *This Journal*, **31**, 81 (1948)

TABLE 1.—*Collaborative results*

LABORATORY	MOISTURE %		PROTEIN % (N×6.25)		OIL %		ASH %	
				Defatted Soy Flour				
D	8.65		53.56		.72		5.97	
	8.71		53.75		.70		6.00	
	8.72	8.69	53.75	53.69	.71	.71	6.08	6.02
E	8.77		53.79		.67		6.11	
	8.82	8.79	53.82	53.80	.63	.65	6.16	6.13
F	7.82	7.82	54.69	54.69	.74	.74	6.20	6.20
				Low Fat Soy Flour				
D	8.14		49.44		4.63		5.89	
	8.23		49.50		4.68		5.84	
	8.23	8.20	49.50	49.48	4.84	4.72	5.86	5.86
E	8.28		49.94		5.23		6.07	
	8.23	8.25	49.94	49.94	5.19	5.21	6.01	6.04
F	7.16	7.16	51.89	51.89	5.05	5.05	4.87	4.87
				Full Fat Soy Flour				
D	6.48		43.69		20.42		4.75	
	6.54		43.81		20.39		4.75	
	6.62	6.55	43.69	43.73	20.35	20.39	4.81	4.77
E	6.65		43.68		19.92		4.77	
	6.70	6.67	43.68	43.68	19.73	19.82	4.71	4.74
F	5.68	5.68	44.63	44.63	20.03	20.03	4.24	4.24

Journal. Supplementary collaborative work has been carried on this year. One-pound samples of the three leading types of soy flour, namely, defatted soy flour, low fat soy flour, and full fat soy flour, were taken from the same lots previously analyzed and were distributed to collaborators. The collaborators were again directed to follow the tentative methods for moisture, **20.4,** nitrogen, **2.26,** oil, **31.67,** and ash, **27.9,** as revised at the 1947 meeting.* Collaborative results are shown in Table 1.

COLLABORATORS

Laboratory D., Neustadt, M. H., Production and Marketing Administration, Standardization Research and Testing Division, Beltsville, Maryland.

* *This Journal* **31,** 81 (1948).

Laboratory E., Anderson, R. E., Archer-Daniels-Midland Company, Minneapolis, Minnesota.

Laboratory F., Freyer, Egbert B., Spencer Kellogg & Sons, Inc., Buffalo, New York.

REPORT ON SUGARS IN BAKED PRODUCTS

By R. P. SMITH (National Biscuit Co., New York, N. Y.),
Associate Referee

In pursuance of the recommendation of the Association for further study on the adaptation of the Alkaline Ferricyanide Method, **20.29** (1) for reducing and non-reducing sugars in flour to the determination of these sugars in baked products, work has been continued in furtherance of the work started by Walker (2) as reported to the Association in 1947.

The two evident weaknesses brought out in the preliminary work, *i.e.*, poor clarification of extract and unreliability of results, were investigated.

Blish and Sandstedt (3) have stated that only a high grade of sodium tungstate should be used in the clarification of flour extracts and further recommend Pfanstiehl's* grade reagent. Clarifications obtained with this reagent were somewhat better than those obtained with other reagent grades of sodium tungstate, but clarification still did not appear adequate.

It was felt that the best approach in determining the reliability of results was to investigate the percentage recovery of known mixtures of reducing and non-reducing sugars added to a standard cracker sample. (4) A low sugar content cracker was selected for the standard so that relatively large charges of sample could be used. Maltose and sucrose were used as the reducing and non-reducing sugars.

The following modifications of the official method, **20.28, 20.29, 20.30** for the determination of reducing and non-reducing sugars in flour were made:

(1) One hundred milliliter volumetric Bates flasks were used in the extraction in place of Erlenmeyer flasks.

(2) Corrections were made for space occupied by insoluble solids in volumetric flasks.

(3) Two milliliters of alcohol were used to wet charge to prevent immediate contact of the acid buffer solution.

Reducing sugars (calc. as invert) and sucrose were determined on the standard soda cracker using **20.29** with the adaptations enumerated.

Table 1 gives results of 5 determinations.

From results shown in Table 1 it might be concluded that the analyses, with the modifications of **20.29** as noted above, are reproducible within the limits of experimental error.

* Pfanstiehl Chemical Co., 104 Lake View, Waukegan, Ill.

TABLE 1.—*Results of analysis using adaptations of* **20.29**

DETERMINATION	REDUCING SUGARS AS INVERT	SUCROSE	REDUCING SUGARS AS MALTOSE	SUCROSE
	per cent	*per cent*	*mg/g*	*mg/g*
1	1.09	0.79	21.4	7.9
2	1.08	0.76	21.2	7.6
3	1.09	0.75	21.4	7.5
4	1.09	0.77	21.4	7.7
5	1.11	0.76	21.8	7.7
Average	1.09	0.77	21.4	7.7

Varying amounts of maltose and sucrose were added to samples of the standard soda cracker and the sugars determined to ascertain the average percentage recovery of each.

Results are shown in Table 2.

TABLE 2.—*Comparison of theoretical vs. actual recovery upon addition of maltose and sucrose to standard soda crackers*

DETERMINATION	MALTOSE ADDED	THEORETICAL RECOVERY MALTOSE	ACTUAL RECOVERY MALTOSE	RECOVERY MALTOSE	SUCROSE ADDED	THEORETICAL RECOVERY SUCROSE	ACTUAL RECOVERY SUCROSE	RECOVERY SUCROSE
	mg/g	*mg/g*	*mg/g*	*per cent*	*mg/g*	*mg/g*	*mg/g*	*per cent*
1	10	31.4	28.7	91.4	5	12.7	11.8	92.9
2	10	31.4	28.4	90.4	5	12.7	12.8	100.7
3	10	31.4	30.0	95.5	5	12.7	13.2	103.9
4	10	31.4	29.1	92.7	5	12.7	12.7	100.0
5	10	31.4	27.9	88.8	5	12.7	12.8	100.7
6	10	31.4	29.0	92.3	5	12.7	12.8	100.7
7	10	31.4	30.4	96.8	5	12.7	12.6	99.2
8	10	31.4	29.8	94.9	5	12.7	13.6	107.0
9	10	31.4	29.8	94.9	5	12.7	11.9	93.7
10	10	31.4	29.1	92.7	5	12.7	11.6	91.3

A study of Table 2 would seem to indicate the need for futher investigation of techniques and means by which the method might be altered to provide a satisfactory procedure for the accurate determination of sugars in baked products.

It was deemed necessary to further modify the procedure to minimize weaknesses which are in evidence when the alkaline ferricyanide method (**20.29**) is applied to the determination of sugars in baked products.

In the present study the following limitations were noted:

(1) Relatively small charges of sample must be used.

(2) Small volume of reagent (sodium thiosulphate) used in back titration necessitating the use of a micro-burette where a small excess of reagent, in the concentration employed, results in appreciable error.

(3) The necessity of a quantitative transfer of aliquot (15 ml) from reduction vessel to titrating vessel.

With a view to eliminating the afore-mentioned limitations the following changes were made in the method (**20.29**).

(1) Larger sample weights were used and made up to 500 ml and a 12.5 ml aliquot used in place of the 5 ml aliquot.

(2) A standard 50 ml burette was used in the back titration of 25 ml of the alkaline ferricyanide with the thiosulphate reagent.

(3) The reduction of ferricyanide by reducing sugars was carried out in 250 ml Erlenmeyer flasks in a boiling water bath eliminating the necessity of a quantitative transfer to the titration flask; back titration being performed in the reduction vessel.

(4) The inversion of sucrose was likewise carried out in a 250 ml Erlenmeyer flask as in (3) and the back titration performed similarly.

A preliminary comparison of the initial modified method and the method above to determine the effect of the modifications gave comparable results as shown below:

Reducing Sugars as Invert (per cent)	*Method I* (micro)	*Method II* (Macro)
1	1.04	1.02
2	1.04	1.09
3	1.03	1.08
4	1.07	1.01

On the basis of the above results a study was continued on a new standard soda cracker. Results of analysis using the micro method compared with the macro method are shown in Table 3.

TABLE 3.—*Comparison of micro and macro methods of analysis for reducing sugars and sucrose*
(all results in duplicate)

REDUCING SUGARS CALC. AS INVERT		SUCROSE	
MICRO METHOD	MACRO METHOD	MICRO METHOD	MACRO METHOD
per cent	*per cent*	*per cent*	*per cent*
0.63	0.59	0.44	0.38
0.66	0.60	0.40	0.44
0.62	0.62	0.48	0.46
0.63	0.57	0.53	0.48
0.69	0.56	0.55	—
0.72	0.55	0.62	—
Avg. 0.66	0.58	0.50	0.44

In view of the large volumes of acid buffer solution needed in performing the macro method it was felt that a concentrated acid buffer solution could be employed to circumvent the necessity of preparing large volumes of this reagent.

Analyses were performed using the following concentration of acid buffer solution. Make 164 g. anhydrous sodium acetate, 120 ml glacial acetic acid, 180 ml sulphuric acid to 1000 ml with water. In the analysis 23.3 ml of the concentrated acid buffer solution were added after dilution of the sample with about 250 ml of water, and finally diluted to volume with distilled water.

Results of analyses using the concentrated acid buffer solution are shown in Table 4.

TABLE 4.—*Results of analysis for reducing sugars and sucrose using concentrated acid buffer solution*
(all results in duplicate)

REDUCING SUGARS CALC. AS INVERT		SUCROSE	
MICRO METHOD	MACRO METHOD	MICRO METHOD	MACRO METHOD
per cent	*per cent*	*per cent*	*per cent*
0.70	0.55	0.43	0.47
0.67	0.59	0.48	0.50
0.65	0.59	0.48	0.44
Avg. 0.67	0.58	0.46	0.47

These results do not differ appreciably from those in Table 3 and indicate that this modification is practicable.

DISCUSSION

The results obtained in this study of the adaptation of the alkaline ferricyanide method (**20.29**) for the determination of reducing and non-reducing sugars in flour to the determination of reducing and non-reducing sugars in baked products are encouraging, but it is obvious that further study is needed to refine and apply the method to other baked products.

RECOMMENDATION*

It is recommended that collaborative study be made on this method and its application next year.

ACKNOWLEDGMENT

The writer acknowledges the cooperation of Messrs. W. V. Lyons, M. Bethge, and W. Woods of National Biscuit Company, who collaborated in this work.

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(4) SANDSTEDT, R. M., and FLEMING, J. C., *This Journal*, **30**, 550 (1947).

* For report of Subcommittee D and action of the Association, see *This Journal*, **33**, 63 (1950).

REPORT ON MOISTURE IN SELF-RISING FLOUR AND IN PANCAKE AND BISCUIT MIXES

By SIDNEY WILLIAMS (Food and Drug Administration, Federal Security Agency, Boston 10, Mass.), *Associate Referee*

The 1945 *Book of Methods* contains two methods for the determination of moisture in flour. Both of these methods were studied as means of determining "moisture," or loss of weight, in flours and prepared baking mixes containing sodium bicarbonate.

A sample of self-rising flour from a commercial mill and samples of the ingredients used in its manufacture were obtained from the mill.

TABLE 1.—*Results—moisture determination*

SAMPLE	A.O.A.C. 20.4, AIR OVEN		A.O.A.C. 20.3, VACUUM OVEN	
	per cent	*Ave.*	*per cent*	*Ave.*
Self-rising flour prepared in Lab.	12.94 12.91	12.93	12.76 12.76	12.76
Gloria Self-Rising Flour	12.98 12.99	12.99	12.96 12.97	12.97
Dorsels Self-Rising Flour	12.37 12.36 12.35 12.36	12.36	12.36 12.38 12.38 12.40	12.38
Sunnyfield Self-Rising Flour	12.21 12.22 12.20 12.25	12.22	12.26 12.28 12.30 12.30	12.28
Kroger Pancake Mix	9.24 9.21	9.23	9.23 9.22	9.23
Aunt Jemima Pancake Flour	9.39 9.41	9.40	9.38 9.35	9.36
Bisquick	9.06 9.07	9.07	9.15 9.12	9.13
Minit Mix	9.62 9.63	9.63	9.64 9.63	9.63
Pancake Mix prepared in Lab.	12.05 12.11	12.08	12.09 12.06	12.07
Biscuit Mix prepared in Lab.	10.54 10.62	10.56	10.55 10.49	10.52

Another sample of self-rising flour was prepared in the laboratory and then the "moisture" of each of the flours and its ingredients was determined by the two official methods. The values obtained by the two methods checked very well, although the actual loss in weight was in each case less than the total loss in weight of the ingredients going into the

TABLE 2.—*Self-rising flour prepared at mill*

LABORATORY & ANALYST		SAMPLE NO.	PER CENT LOSS IN WEIGHT			
			AIR OVEN A.O.A.C. 20.4		VACUUM OVEN A.O.A.C. 20.3	
				ave.		*ave.*
Cincinnati	A	34	12.68		12.64	
Cincinnati	A	46	12.70		12.72 12.32	12.52
Cincinnati	B	86	12.80		12.80	
Cincinnati	B	84	12.81		12.77	
Cincinnati	C	34	12.73		12.73	
Cincinnati	C	46	12.76		12.72	
Minneapolis	D	14	12.54		12.31	
Minneapolis	D	16	12.54		12.28	
Kansas City, Mo.	E	56	12.51 12.48	12.50	12.30 12.35	12.32
Kansas City, Mo.	E	64	12.69 12.68	12.68	12.18 12.25	12.22
Kansas City, Mo.	E	66	12.79 12.82	12.80	12.32 12.18	12.24
St. Louis	F	54	12.23		11.74	
New Orleans	G	26	12.04		12.22	
New Orleans	G	44	12.18		12.23	
Average			12.57		12.41	
Average deviation			0.19		0.25	
Maximum deviation			0.53		0.67	

flour. However, this was to be expected, since the flours were prepared by mixing in air and undoubtedly the moisture content changed during the mixing.

Other commercial flours and mixes were obtained and mixes were prepared in the laboratory. The results of the moisture determination on these are shown in Table 1.

Samples of authentic preparations with compositions comparable to commercial products were then sent out for collaborative study with the following directions:

(1) Mix samples by rolling unopened jars.
(2) Run one sub from each jar by A.O.A.C. **20.3**, and one sub from each jar by A.O.A.C. **20.4.**
(3) In following A.O.A.C. **20.3**, dry for 5 hours in an oven which had reached 100°C. before the sample was inserted.

TABLE 3.—*Self-rising flour prepared in laboratory*

LABORATORY & ANALYST		SAMPLE NO.	PER CENT LOSS IN WEIGHT			
			AIR OVEN A.O.A.C. 20.4		VACUUM OVEN A.O.A.C. 20.3	
				ave.		ave.
Cincinnati	A	52	12.64		12.62	
Cincinnati	A	35	12.66		12.63	
Cincinnati	B	82	12.67		12.71	
Cincinnati	B	85	12.72		12.78	
Cincinnati	C	52	12.65		12.41	
Cincinnati	C	35	12.69		12.62	
Minneapolis	D	62	12.53		12.31	
Minneapoiis	D	65	12.55		12.41	
Kansas City, Mo.	E	25	12.50 12.64	12.57	12.40 12.42	12.41
St. Louis	F	12	12.20		11.72	
St. Louis	F	32	12.29		11.80	
St. Louis	F	45	12.19		11.52	
New Orleans	G	22	11.88		12.24	
New Orleans	G	55	12.12		12.18	
Average			12.45		12.31	
Average deviation			0.23		0.30	
Maximum deviation			0.57		0.79	

TABLE 4.—*Pancake mix prepared in laboratory*

LABORATORY & ANALYST		SAMPLE NO.	PER CENT LOSS IN WEIGHT			
			AIR OVEN A.O.A.C. 20.4		VACUUM OVEN A.O.A.C. 20.3	
				ave.		ave.
Cincinnati	A	67	12.08		12.02	
Cincinnati	A	18	12.09		11.94 11.87	11.90
Cincinnati	B	38	12.05		12.09	
Cincinnati	B	77	12.11		12.06	
Cincinnati	C	67	12.07		11.99	
Cincinnati	C	18	12.08		12.10	
Minneapolis	D	78	12.03		11.63	
Minneapolis	D	37	12.07		11.70	
Kansas City, Mo.	E	57	11.99 12.05	12.03	11.44 11.41	11.42
Kansas City, Mo.	E	38	11.57 11.73 11.60	11.63	11.15 11.07 11.11	11.11
St. Louis	F	48	11.94		11.49	
St. Louis	F	87	11.74		11.03	
New Orleans	G	17	11.20		11.53	
New Orleans	G	28	11.42		11.55	
Average			11.90		11.69	
Average deviation			0.23		0.29	
Maximum deviation			0.70		0.66	

(4) In following A.O.A.C. **20.4**, the oven should be pre-heated to 130° and the sample then inserted. The one hour drying period begins when the oven temperature again reaches 130°C.
(5) Report results to two decimal places.

The results are summarized in Tables 2, 3, 4, and 5.

TABLE 5.—*Biscuit mix prepared in laboratory*

LABORATORY & ANALYST		SAMPLE NO.	PER CENT LOSS IN WEIGHT			
			AIR OVEN A.O.A.C. **20.4**		VACUUM OVEN A.O.A.C. **20.3**	
				ave.		*ave.*
Cincinnati	A	71	10.40		10.47	
Cincinnati	A	23	10.42		10.38	
Cincinnati	B	13	10.54		10.55	
Cincinnati	B	51	10.62		10.49	
Cincinnati	C	71	10.51		10.50	
Cincinnati	C	23	10.56		10.39	
Minneapolis	D	11	10.46		10.22	
Minneapolis	D	43	10.49		10.35	
Kansas City, Mo.	E	21	10.81 10.79	10.80	10.29 10.26	10.27
Kansas City, Mo.	E	53	10.54 10.33	10.44	10.14 10.14	10.14
St. Louis	F	41	10.71		9.61	
New Orleans	G	31	10.23		10.08	
New Orleans	G	63	10.05		10.15	
Average			10.48		10.28	
Average deviation			0.13		0.18	
Maximum deviation			0.43		0.67	

DISCUSSION

The variation of results between laboratories was surprising and may have been due to differences in equipment. A.O.A.C. method **20.4** merely calls for the use of an air oven. Cincinnati, St. Louis, and Minneapolis laboratories used forced draft ovens, whereas Kansas City used a Freas convection oven.

At Cincinnati the forced draft oven was operated with the ventilation opening permitting a continuous expulsion of hot air from the oven. The vacuum oven was run with the gage showing a vacuum of 30 inches and air entering the oven through sulfuric acid at the rate if 1–2 bubbles per second. The three analysts at Cincinnati, working independently and at different times but using the same ovens in the same manner, obtained very good checks, whereas the results between laboratories differed as much as 1 per cent on the same sample. Yet Kansas City, using the Freas convection oven, obtained results that checked well with Cincinnati's forced-draft oven results.

The average deviation on each sample was less by the air-oven method

than by the vacuum oven. This raises the question as to whether the vacuum ovens are operated in exactly the same manner in all the laboratories.

It is recommended* that the work be continued with more detailed directions to collaborators in an attempt to decrease the variations in results between laboratories.

The Associate Referee wishes to express his appreciation to the collaborating analysts, who were: H. C. Barry, F. H. Collins, G. Getchell, Wm. H. Munday, H. D. Silverberg, and H. C. Van Dame, all of the U. S. Food and Drug Administration.

REPORT ON BAKING POWDER

By V. E. MUNSEY (Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Referee*

There are no Associate Referee reports on this subject this year.

It is recommended†—

(1) That the qualitative test for phosphoric acid, **17.31**, be adopted as official.

(2) That the residual carbon dioxide methods, *This Journal*, **31**, 78 (1948), and *Ibid*, **32**, 83 (1949), be adopted as official.

In connection with revision of this chapter, views of the leading baking powder firms were solicited. On the basis of information available, it is recommended—

(1) That the gravimetric-method, **17.2** and **17.3**, total carbon dioxide, **17.7** residual carbon dioxide, and the phrase "Subtract residual CO_2 **17.7** from total CO_2 **17.3**," be dropped from **7.9**.

(2) That the quantitative determination of aluminum by precipitation with phenyl hydrazine, **17.24**, be dropped. These methods are no longer in general use.

It is recommended that the following methods be made first action—

(1) Neutralizing value of mono calcium phosphate, **17.11**.
(2) Neutralizing value of sodium acid pyrophosphate, **17.12**.
(3) Tartaric acid, free or combined (qualitative test), **17.13**.
(4) Aluminum (qualitative test), **17.22** and **17.23**.
(5) Arsenic, **17.34**.
(6) Fluorine, **17.35**.
(7) Lead, **17.36**.

No reports were given for milk solids and butterfat in bread; phosphated flour; bromates in flour; unsaponifiable matter and sterols in noodles and bakery products; albumen in noodles and macaroni products.

* For report of Subcommittee D and action of the Association, see *This Journal*, 33, 63 (1950).
† For report of Subcommittee D and action of the Association, see *This Journal*, 33, 64 (1950).

REPORT ON STANDARDIZATION OF MICROCHEMICAL METHODS

MICRO KJELDAHL NITROGEN DETERMINATION

By C. O. WILLITS, *Referee*, and C. L. OGG, *Associate Referee*
(Eastern Regional Research Laboratory* Philadelphia 18, Pennsylvania)

The 1949 collaborative studies on the standardization of microchemical methods were limited to determination of nitrogen by the Kjeldahl procedure, since it was necessary to establish an official method for inclusion in the seventh edition of the Association's *Book of Methods*. The 1948 studies proved that the micro Kjeldahl method (tentative) was unsatisfactory for refractory compounds, such as nicotinic acid, which contain ring nitrogen. However, these studies indicated that a satisfactory Kjeldahl method might be evolved for the analysis of these compounds, since several of the 1948 collaborators using different methods obtained nitrogen values for nicotinic acid in agreement with the theoretical value. Willits, Coe, and Ogg[1] reported a satisfactory procedure for macro samples, and later work at the Eastern Regional Research Laboratory indicated that the same procedure was adaptable to micro samples. In this adaptation, the authors stressed the importance of (a) the concentration of potassium sulfate in the digestion mixture, (b) the time of digestion, and (c) the temperature of the digestion mixture. This micro digestion mixture contained the same ratios of potassium sulfate and of mercuric oxide to sulfuric acid as that recommended by Willits *et al.* The minimum time of digestion was critical, requiring at least 3 hours. It is perhaps this latter condition, together with the temperature of the digest, that was largely responsible for the poor results obtained by the 1948 collaborators, who reported 36 values out of a total 75 which ranged from 0.25 to 9.75 per cent as compared with the theoretical value of 11.38 per cent. The method used in the 1949 collaborative study for the analysis of nitrogenous compounds other than those containing N-N, NO, and NO_2 recommended a digestion mixture containing a larger amount of potassium sulfate, with more exact limits for each milliliter of sulfuric acid. The amount of sulfuric acid to be used was based on the amount of organic matter to be digested. The collaborators were instructed to digest the sample for 4 hours, with the digestion mixture boiling vigorously enough to cause the acid to reflux halfway up the neck of the flask. The two samples submitted for analysis—nicotinic acid and tryptophane —are both refractory compounds.

Sixteen collaborators participated, reporting 87 analyses for nicotinic

* One of the Laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

[1] Willits, C. O., Coe, M. R., and Ogg, C. L., *This Journal*, **32**, 118 (1949).

acid and 74 for tryptophane. These analyses are shown as histograms in Figures 1 and 2.

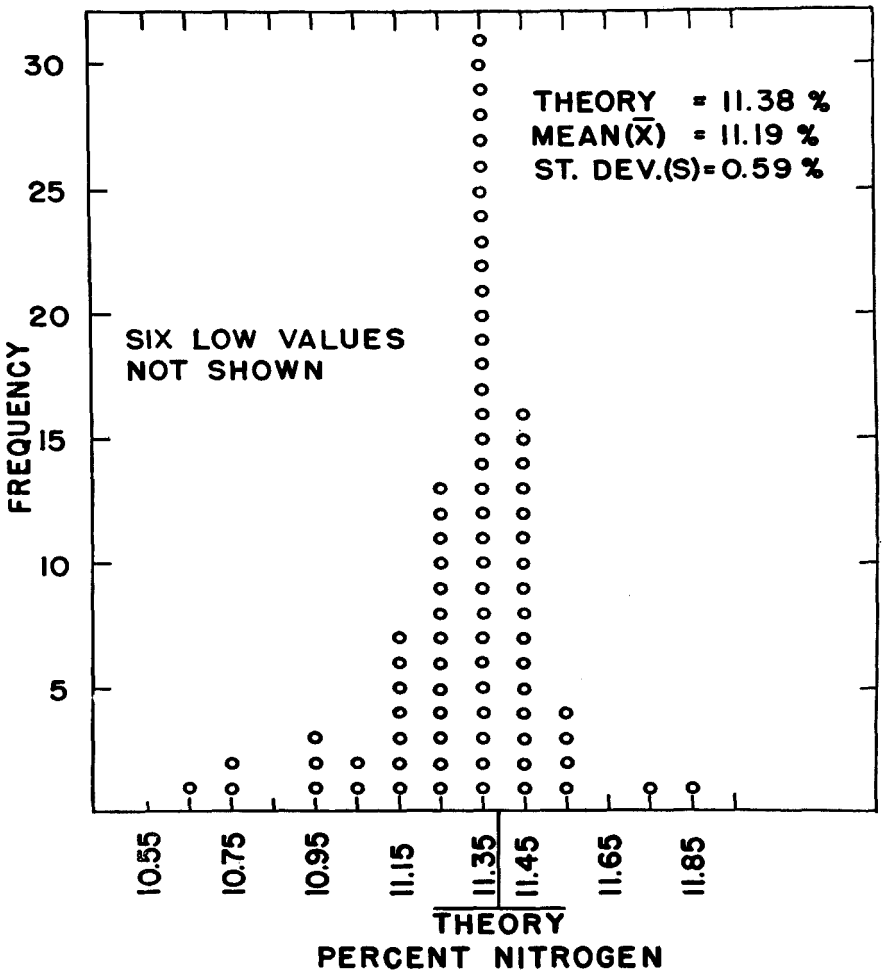

FIG. 1.—Kjeldahl nitrogen values for nicotinic acid.

The 87 nicotinic acid analyses ranged from a low of 9.16 per cent to a high of 11.86 per cent with a mean of 11.19, as compared with the theoretical value of 11.38 per cent. The chi square test, to determine whether or not these analyses represented a normal population, showed that 11 values were either too high or too low. The remaining 76 values, ranging from 10.96 to 11.56 per cent, had a standard deviation of 0.103 per cent

from a mean of 11.33 per cent, which is only 0.05 per cent below the theoretical value. Thus, 83 per cent of the total number of analyses had a standard deviation of only 0.103 per cent.

The 74 tryptophane nitrogen analyses gave values between 12.26 and 14.55 per cent, with a mean of 13.65 per cent, which when compared with

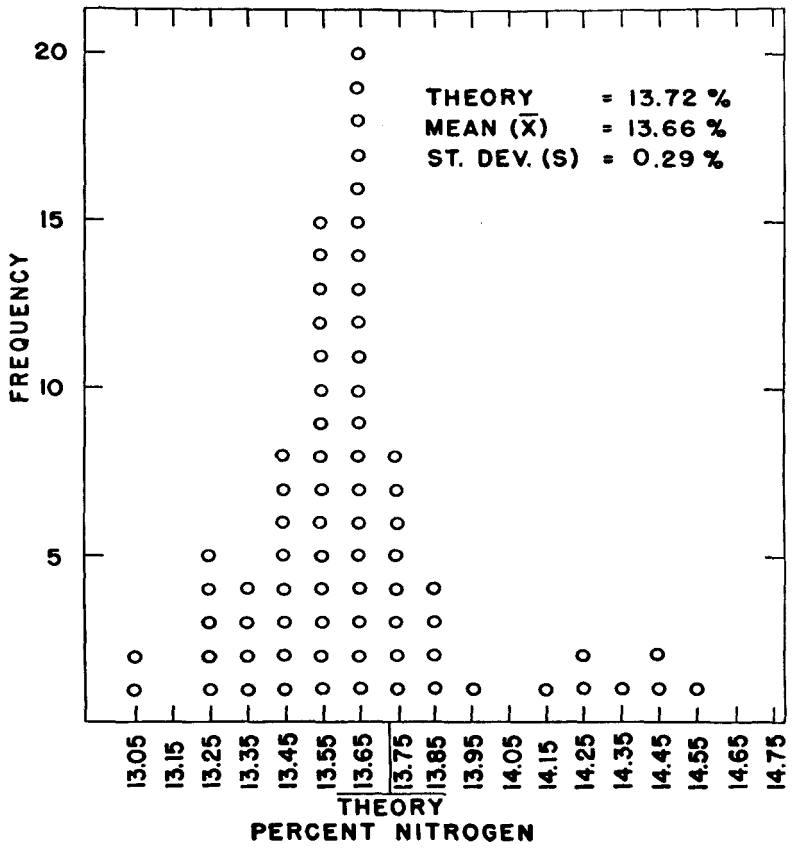

FIG. 2.—Kjeldahl nitrogen values for tryptophane.

the theoretical value of 13.72 per cent deviates by only 0.07 per cent. Of these 74 values, 3 were rejected by the chi square test. The remaining 71 values, constituting the normal population, ranged from 13.03 to 14.35 per cent, with a mean of 13.61 per cent, only 0.11 per cent below the theoretical value. Thus 96 per cent of the 74 tryptophane analyses had a standard deviation of 0.238 per cent.

In addition to the nitrogen analyses of these two compounds, each

collaborator reported the nitrogen obtained by the prescribed procedure for the particular compounds used by them as test or standard materials. There were 79 of these analyses of test materials, including 36 for acetanilide and 16 for cystine; the other 27 were distributed between benzyl-iso-thiourea, phenyl-thiourea, ammonium sulfate, nicotinic acid, glutamic acid, and tryptophane. Although these analyses were not done collaboratively, that is, each analyst's material was not an aliquot of the same lot, they were compounds of relatively well established purity. To permit comparison of the nitrogen data from these different compounds, it was necessary to treat the data in a somewhat different manner and to assume that these compounds were all pure. Thus, the values used represented the differences between that found and the theoretical values for nitrogen regardless of the percentage of the contained nitrogen, and they were treated either as an individual compound or as if all values were of a single compound.

The 36 Kjeldahl analyses of acetanilide gave nitrogen values which represented a normal population with a standard deviation of only 0.137 per cent. The values ranged from a low of 10.04 to a high of 10.52 per cent, with a mean of 10.27 per cent, which is only 0.09 per cent lower than the theoretical value of 10.36 per cent for nitrogen.

The 16 analyses of the standard compound cystine also yielded values representing a normal population with a small standard deviation of 0.078 per cent. The mean of 11.66 per cent is identical with the theoretical value.

The standard deviation for the entire 79 analyses, which included all the various standard compounds, was 0.127 per cent, and the mean of the differences from the theoretical value was −0.056 per cent.

These data indicate that the Kjeldahl method used in the 1949 collaborative studies can be applied successfully to both easily decomposed and refractory nitrogenous materials for the analysis of nitrogen.

The probability is that more than 95 per cent of all the results obtained by this method will fall within a normal population and that the standard deviation will be 0.17 per cent, the s value for all the analyses reported.

The Referees therefore recommend* that the following procedure be accepted as first action.

MICRO-KJELDAHL PROCEDURE

(not applicable to compounds with N-N, NO, or NO_2 linkages).

REAGENTS

(*a*) *Indicator:*

1. Methyl red-methylene blue. Mix 2 parts of 0.2 per cent methyl red with 1 part of 0.2 per cent methylene blue, both in 95 per cent ethanol.

or 2. Methyl red-bromcresol green. Mix 5 parts of 0.2 per cent bromcresol green with 1 part of 0.2 per cent methyl red, both in 95 per cent ethanol.

* For report of Subcommittee C and action of the Association, see *This Journal*, 33, 54 (1950).

(b) *Sodium hydroxide-sodium thiosulfate soln.*—Aqueous soln of 50 g of NaOH and 5 g of $Na_2S_2O_3 \cdot 5H_2O$/100 ml.

(c) *Boric acid soln.*—Dissolve 4 g of H_3BO_3 per 100 ml. of distilled water.

DETERMINATION

Weigh a 10- to 30-mg sample* on a micro or semimicro balance and transfer to

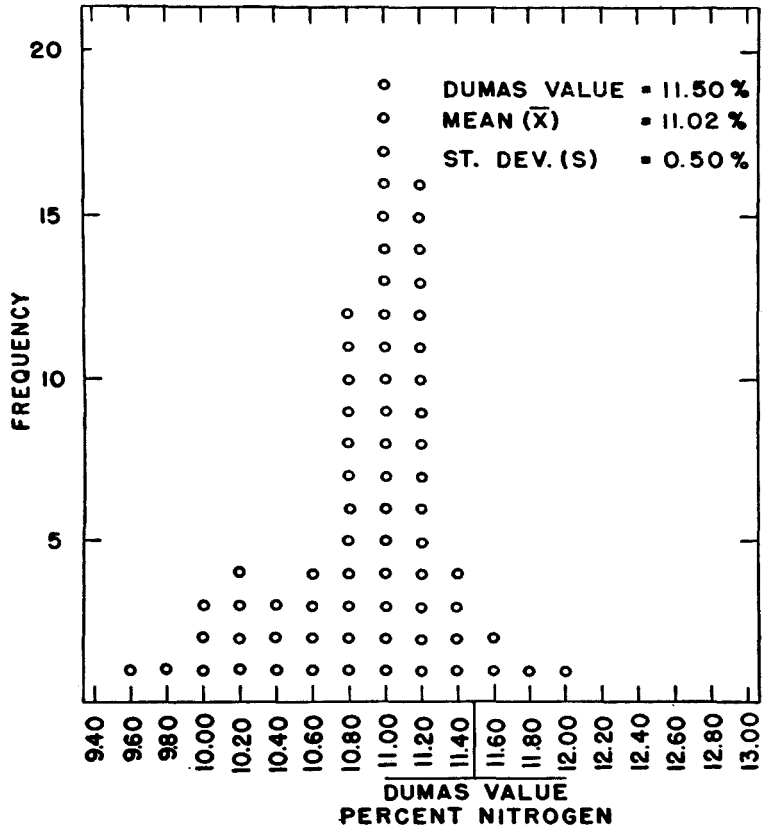

FIG. 3.—Kjeldahl nitrogen values for methyl orange.

a 30-ml Kjeldahl digestion flask. Weigh the sample either in a charging tube or on a piece of cigarette paper. Add to the sample 1.30 ± 0.05 g. potassium sulfate, 40 ± 5 mg mercuric oxide and 2.0 ml concentrated sulfuric acid. Add boiling chips and digest for 4 hours (digest for 1 hour if sample is known to contain only amines, amides, or other easily digestible materials) by boiling *vigorously*, with the acid condensing well up into the neck of the flask. Cool, add a minimum of distilled water (about 5 ml) to dissolve the solids, and place a thin film of vaseline on the lip of the flask.

* For samples of less than 10 mg, weigh on a micro balance and use one-half the specified amounts of the reagents except where the total organic matter in the digest (sample + cigarette paper, if used) exceeds 10 mg. Collect the distillate in a 50 ml Erlenmeyer flask, dilute to 25 ml and titrate with 0.01 *N* HCl.

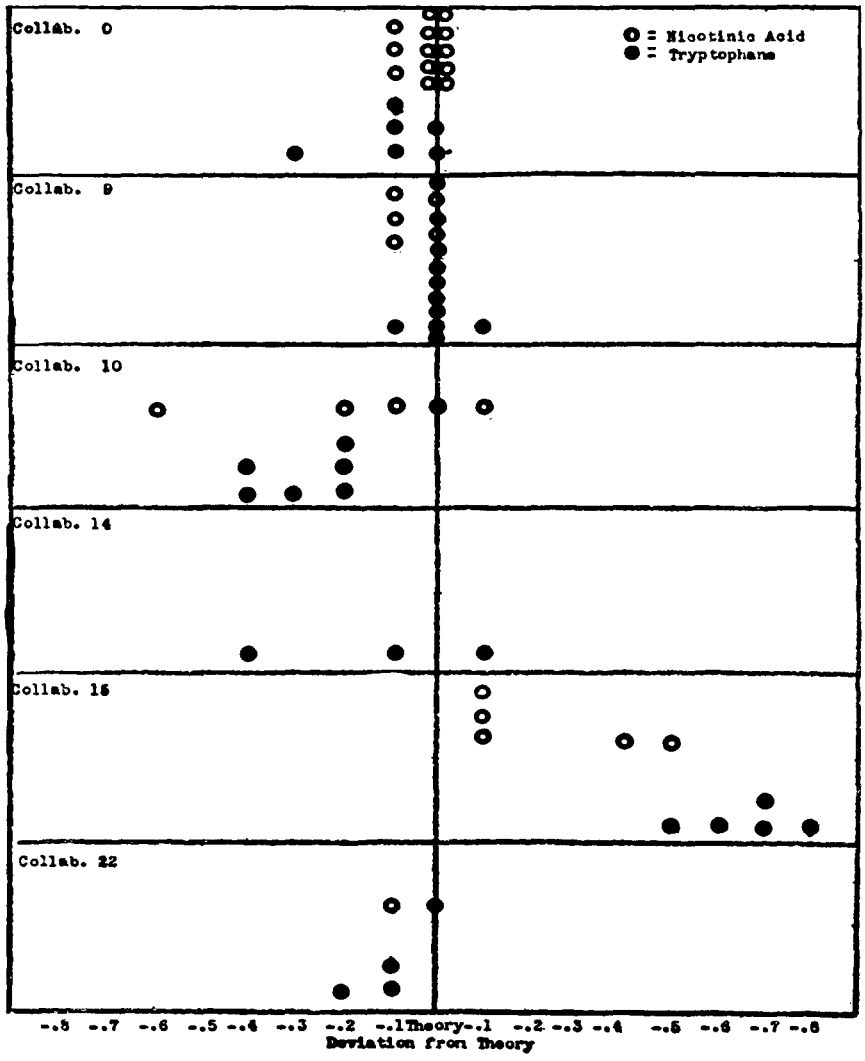

FIG. 4.—Distribution of Kjeldahl values. (Method I)

Transfer the digest including the boiling chips to a micro or semimicro steam distillation apparatus of standard design. To condition the apparatus after periods of disuse, pass steam through it for several minutes. Completeness of transfer of the digestion mixture can be tested by the addition of a drop of methyl orange indicator to the final rinse. Place the 125-ml Erlenmeyer receiver containing 5 ml of 4% boric acid and 4 drops of the indicator under the condenser with the tip extending below the surface. Add 8 ml of the sodium hydroxide-sodium thiosulfate reagent to the still, and steam distill until ca 15 ml of distillate is collected. Dilute the contents of

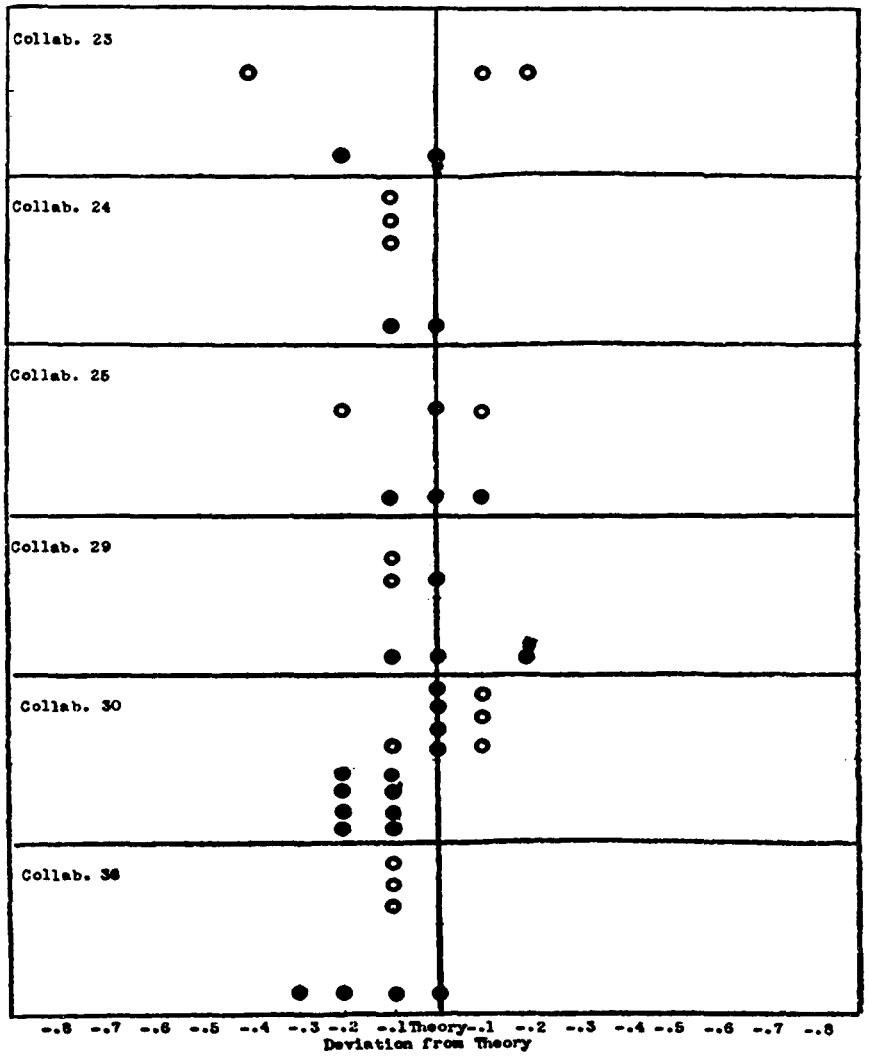

FIG. 4. (contd.)—Distribution of Kjeldahl values. (Method I)

the receiver to 50 ml with distilled water and titrate the ammonium hydroxide with 0.02 *N* HCl. Choose as the end point the grey color or the first appearance of the red. Determine the blank by using the same amount of reagents, rinsing the digestion flask in the same manner, and using the same digestion period as that for the determination. Calculate the percentage of nitrogen in the sample by the equation:

$$\frac{(\text{Vol. HCl}-\text{blank})\ (N)\ (\text{Eq. wt. nitrogen}) \times 100}{\text{Weight of sample, mg.}} = \%\ \text{nitrogen.}$$

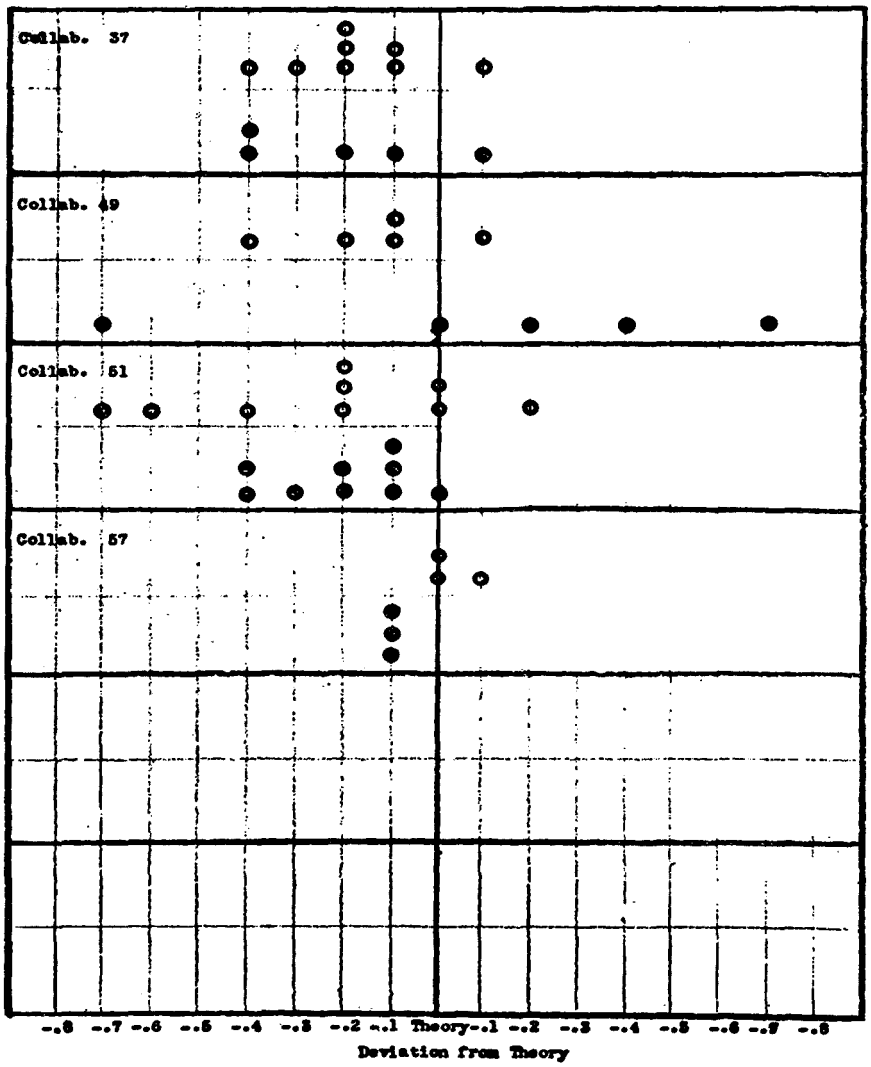

FIG. 4. (contd.)—Distribution of Kjeldahl values. (Method I)

MICRO KJELDAHL ANALYSIS OF COMPOUNDS CONTAINING N-N, NO, OR NO_2 LINKAGES

The collaborators were also requested to analyze a sample of methyl orange so that the tentative Friedrich method for nitrogen by the micro Kjeldahl method (for N-N, NO, and NO_2 linkages) could be appraised.

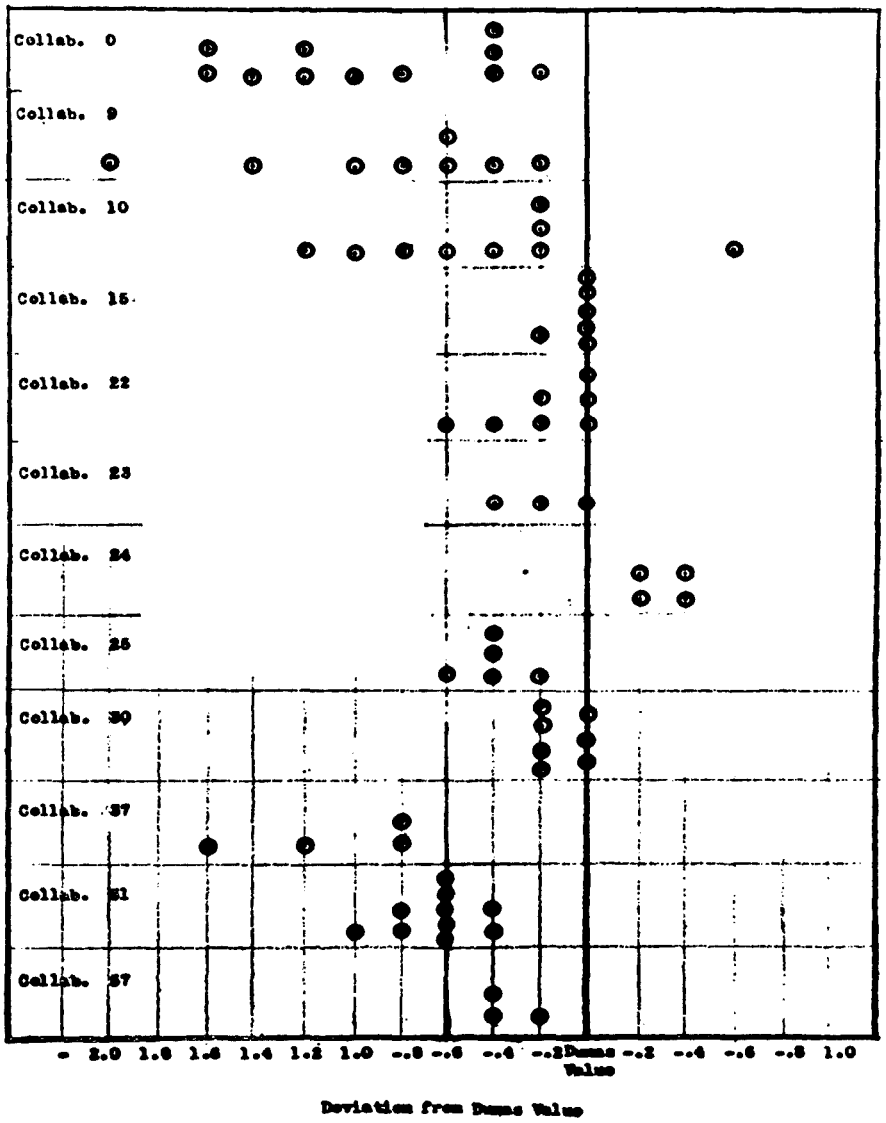

FIG. 5.—Distribution of Kjeldahl values for methyl orange. (Method II)

Seventy-nine micro Kjeldahl analyses were reported, ranging from a low of 9.57 to a high of 12.11 per cent, with a mean of 11.02 per cent. The analyses are shown in Figure 3.

Because the compound was known to be impure, this mean was com-

pared with a nitrogen value of 11.50 per cent obtained as an average of 10 analyses by the Dumas method rather than with the theoretical value of 12.84 per cent.

The standard deviation of these values, 0.496 per cent, is extremely high indicating poor precision. The confidence limits, ± 0.106 per cent ($2s/\sqrt{n}$), when added to the mean of 11.02 per cent, did not approach the Dumas nitrogen value; therefore, this method must be considered unreliable and unacceptable.

The Referees therefore recommend* that the Friedrich method (tentative) be deleted from the *Book of Methods* and that further work be done to develop a suitable method which can be recommended as first action for analysis of compounds having an N-N linkage for nitrogen by a micro Kjeldahl procedure.

The analytical values submitted by the various collaborators for nitrogen by the Kjeldahl method are presented graphically in Figures 4 and 5.

The following is the list of those collaborators who participated in these studies:.

Brown, L. E., Southern Regional Research Laboratory
Brunner, A. H., Ansco
Butler, A. Q., Mallinkrodt Chemical Works
Deering, A. W., Hunter College, N. Y.
Dutton, C. D., Picatinny Arsenal
Grodsky, J., Ortho Research Foundation
Hallett, L. T., General Aniline and Film Corp.
Hageman, B., The Texas Company
Jones, G. A., E. I. DuPont de Nemours and Co., Inc.
Ketchum, D. E., Eastman Kodak Company
Miller, L., University of Michigan
Ogg, C. L., Eastern Regional Research Laboratory
Steyermark, A., Hoffman-LaRoche, Inc.
Sundberg, O. E., Calco Chemical Div., American Cyanamid Co.
Van Etten, C. H., Northern Regional Research Laboratory
White, L. M., Western Regional Research Laboratory

METHOXYL AND ETHOXYL GROUPS—TENTATIVE

The procedure for the determination of methoxyl and ethoxyl groups **41.1** and **41.2**, 6th Ed., has been used successfully in the Associate Referee's and other laboratories. The Referee, therefore, recommends* that this method be adopted as official. The procedure has been reedited for publication in 7th Ed. *Methods of Analysis.*

A contributed paper "The Boiling Temperature of KJeldahl Mixtures," by C. L. Ogg and C. O. Willits, was published in the preceding number of *This Journal*, page 100.

* For report of Subcommittee C and action of the Association, see *This Journal*, **33**, 54 (1950).

REPORT ON VEGETABLE DRUGS AND THEIR DERIVATIVES

By PAUL S. JORGENSEN (Food and Drug Administration, Federal Security Agency, San Francisco 2, California), *Referee*

RECOMMENDATIONS*

Theobromine and Phenobarbital.—No report was received. It is recommended that the subject be continued.

Aminopyrine, Ephedrine, and Phenobarbital.—The Associate Referee states that work is in progress but that a written report cannot be issued at this time. It is recommended that the subject be continued.

Quinine.—The Associate Referee reports that a spectrophotometric method is in process of development for the determination of strychnine in combination with quinine. The strychnine is first precipitated as the ferrocyanide and extracted from alkaline solution. This procedure should be studied further and it is recommended that the subject be continued.

Rutin in Tablets.—No report was received. It is recommended that the subject be continued.

Ethylmorphine in Syrups.—A method was reported which is applicable to ethylmorphine in syrups when other alkaloids are absent. It is a simple immiscible solvent extraction procedure with titration of the extracted alkaloid. An average recovery in excess of 98 per cent was obtained by six collaborators.

It is recommended that the method be adopted as official and that the subject be closed.

In accordance with the plan to abolish the "tentative" classification in the forthcoming edition of the *Methods of Analysis* in 1950 the following report is submitted. It includes those methods now classified as "tentative" dealing with vegetable drugs, derivatives of vegetable drugs, and combinations of such drugs.

Acetophenetidin (Phenacetin) and Salol—39.20 & 39.21, *Acid Hydrolysis Method and Alkaline Hydrolysis Method*

Extensive collaborative work was done by the authors Emory, Spencer, and LeFebre in the development of this method (*J. Ind. Eng. Chem.*, **7**, 681, 1915). The method as published in *Methods of Analysis*, Sixth Edition, should be made first action without further study.

Acetophenetidin, Acetylsalicylic Acid, and Caffeine—39.35

Extensive collaborative work on this subject was reported in *This Journal*, **22**, 723, (1939). At the fifty-eighth annual meeting held in 1943 the method was made "official, first action," as reported, *Ibid.*, **27**, 111

* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 45 (1950).

(1944). This change in status was not made in the *Book of Methods*, Sixth Edition, 1945.

Aminopyrine, Acetophenetidin, Phenobarbital, and Caffeine—**39.45**

Collaborative work was reported in *This Journal*, **25**, 809 (1942). The results for acetophenetidin and phenobarbital varied considerably between collaborators but the averages were reasonably close to the theoretical. This is a useful method and should be made first action without further collaborative work.

Terpin Hydrate and Codeine in Elixers—**39.70**

Collaborative work was reported in *This Journal*, **23**, 757 (1940). At the fifty-ninth annual meeting held in 1944 the method was made "official, first action" as reported, *Ibid.*, **28**, 85 (1945), but the change was not made in *Methods of Analysis*, Sixth Edition.

Aconitine in Aconite Root—Qualitative Test—**39.72**

This is a microchemical test performed on an extract of the crude drug to determine the presence of aconitine. No collaborative work has been done. It is recommended that this subject be studied collaboratively before being made official.

Arecoline Hydrobromide—**39.73**

Collaborative work has been done as reported in *This Journal*, **25**, 817 (1942), with recoveries of 98 per cent or better. Without further study this method should be made first action.

Cocaine, Method II—**39.78**

Results of collaborative study are reported in *This Journal*, **11**, 328, (1928). This is a gravimetric method to serve as a check to Method I which is volumetric. The order of accuracy of the two methods is about the same. It is recommended that Method II be made first action.

Physostigmine Salicylate in Tablets—**39.99**

Collaborative work was reported in *This Journal*, **23**, 762 (1940), and **24**, 815 (1941). The method is sufficiently accurate to warrant first action status.

Separation of Quinine and Strychnine—**39.107**

It is recommended that this method be deleted from the forthcoming Seventh Edition of *Methods of Analysis* and the recommendation of the present Associate Referee be adopted—that of studying further the spectrophotometric method.

Cascara Sagrada—**39.114**

Collaborative work was reported in *This Journal* **12**, 276 (1929), and

15, 406 (1932). Results were not satisfactory and it was claimed that there was no correlation between the results obtained and physiological activity. It is recommended that this method be deleted and consideration be given to the development of a biological method of assay.

Identification of Gums—**39.116**

Omitted from consideration because covered by another Referee.

Total Alkaloids in Ipecac, Fluidextract—**39.121** & **39.122**

This subject was studied collaboratively during several years, *This Journal,* **8,** 529 (1925); **9,** 301 (1926); **10,** 359 (1927); **11,** 339 (1928). A method was developed using an automatic extractor and an alternate method using hand extraction. Both methods were recommended for tentative status, *Ibid.* **11,** 50 (1928). Both methods also differ from the USP XIII method. It is recommended that both be made first action.

Ipomea and Jalap—**39.123** & **39.124**

The method described for Ipomea is also applicable to Jalap. This method was studied collaboratively and reported in *This Journal,* **15,** 448 (1932), and **16,** 375 (1933), and made tentative, *Ibid.,* **16,** 53, 84 (1933). Methods for the determination of Ipomea and Jalap were deleted from the *Book of Methods* at the fifty-fifth annual meeting held in 1939, *Ibid.,* **23,** 90 (1940), because assay methods were included in the National Formulary monograph. However, they were reinstated as tentative methods at the fifty-ninth meeting held in 1944, *Ibid.,* **28,** 85 (1945). The A.O.A.C. method differs from the present National Formulary method. It is recommended that this method be made first action.

Podophyllum—**39.125**

Podophyllum was studied collaboratively during three years, *This Journal,* **15,** 452 (1932); **16,** 378 (1933); **18,** 555 (1935). The method finally adopted was made tentative by the committee, *Ibid.,* **18,** 89 (1935). Podophyllum is official in the N. F. VIII, but the assay method differs from the A.O.A.C. method. It is recommended that this method be made first action.

Belladonna and Stramonium Ointments
Method I and Method II—**39.126** & **39.127**

Collaborative results on these methods are reported in *This Journal,* **15,** 442 (1932). The Associate Referee recommended that both methods be made tentative. This recommendation was adopted by the Association, *Ibid.,* **15,** 83 (1932). The USP XIII has included an assay method for Belladonna ointment different from the A.O.A.C. methods. The N. F. VIII recognizes Stramonium Ointment but has not included an assay. It is recommended that both of the present methods be made first action.

Guaiacol—**39.154**

The method described is the Viebock and Schwappach method modified by E. P. Clark for the determination of alkoxyl groups. This is an extremely accurate method and gave excellent recoveries when applied to certain guaiacol compounds. It is recommended that collaborative study be applied to guaiacol with the view to making the method first action.

Quinine (Spectrophotometric Method)—**39.186**

A report on the quantitative determination of quinine by absorption spectrophotometry is given in *This Journal*, **25,** 524 (1942). Another report on the same subject is given, *Ibid.*, **26,** 238 (1943), under the general title, "Report on Spectrophotometric Methods." The method as published in this report was made tentative without collaborative study, *Ibid.*, **27,** 111 (1944). It is recommended that collaborative work be done before raising the method to first action status.

Santonin in Santonica (Levant Worm Seed)—**39.191**

Collaborative work was done as reported in *This Journal*, **19,** 517 (1936). The Referee concluded that agreement was satisfactory for this type of product and that further study of the subject be discontinued. It is recommended that the method be made first action.

Assay of Ergot—**39.240**

This is a biological test and is omitted from consideration as it is covered by another Referee.

Quinine Ethylcarbonate

A report of collaborative study is given in *This Journal*, **30,** 464 (1947). The Associate Referee recommended that the method be adopted as tentative and that the subject be closed. This method should now be made first action.

REPORT ON QUININE

By D. J. MILLER (Food and Drug Administration, Federal Security Agency, Buffalo, N. Y.), *Associate Referee*

It was recommended in 1948 (1) that the subject of the separation of quinine and strychnine be continued, with the present A.O.A.C. method (2) to be compared with the Herd method (3). The A.O.A.C. method, adopted as tentative in 1926, is based upon the original work of Simmonds (4) and depends on the precipitation of strychnine as the ferrocyanide with subsequent re-extraction and reprecipitation to complete

the separation from soluble quinine ferrocyanide. There are a number of objections to the A.O.A.C. method, the greatest of which is its lack of accuracy. Even by weighing the quinine, which gave the highest recovery, results of collaborators in 1926 (5) show a shortage in quinine which is not compensated for by a corresponding excess of strychnine, indicating a loss probably in the extraction. Another objection is the amount of time and manipulation involved. A third possible objection is the use of 10% sulfuric acid in the first precipitation of the A.O.A.C. method. This strength acid was specified in the original report by Simmonds, but an errata published the following month changed the percentage to 20%.

Because of the objection referred to above, it was thought desirable, before submitting the A.O.A.C. method and the Herd method to collaborative study, to study modifications of the A.O.A.C. method. The modification will not be described here in detail, since it will be the subject of a later report. Briefly, it consisted of an original precipitation in 20% sulfuric acid of strychnine as the ferrocyanide, purification of this strychnine (contaminated with quinine) by redissolving in 10% sodium hydroxide and reprecipitation as the pure ferrocyanide from acid solution, washing of the precipitate with a dilute hydrochloric acid solution and weighing of the precipitate as strychnine ferrocyanide. Using this procedure and with quinine-strychnine ratios of 10, 20, and 40 to 1, recoveries of quinine ranged from 99.0 to 100.1% per cent and of strychnine from 96.0 to 103.5 per cent (wt. of strychnine in all samples 40 mg.). The modification has the advantages of greater accuracy and less operations than the present A.O.A.C. method.

There was also considered for study a procedure which depended upon the present A.O.A.C. spectrophotometric method (6) as a measure of the quinine (strychnine has been shown not to interfere) and an unpublished spectrophotometric method devised by Analyst T. J. Klayder, of the Buffalo District, for the measurement of strychnine. Klayder has shown that quinine in an amount not greater than the strychnine does not interfere with its determination. Study of the quinine and strychnine ferrocyanide precipitation has shown that the quantity of quinine coprecipitated with the strychnine in the first precipitation is less than one-half the amount of strychnine. Accordingly, it appears feasible to determine the strychnine spectrophotometrically after a preliminary precipitation as the ferrocyanide and extraction from an alkaline solution. It is proposed to study this procedure.

No collaborative work was done on the modification of the A.O.A.C. method and no recommendation for adoption can be made. At the same time, the objections to the present A.O.A.C. method are considered serious enough to make inadvisable its inclusion as an official method in the forthcoming seventh edition of *Methods of Analysis*. Accordingly, it

is recommended* that the present tentative method for the separation of quinine and strychnine (7) be deleted from the new *Methods of Analysis*, and that the subject of the separation of quinine and strychnine be continued.

REFERENCES

(1) *This Journal*, **48**, 45 (1948).
(2) *Methods of Analysis, A.O.A.C.*, 1945, page 699.
(3) *J. Am. Pharm. Assoc.*, **31**, 9 (1942).
(4) *Analyst*, **39**, 81 (1914).
(5) *This Journal*, **9**, 310 (1926).
(6) *Methods of Analysis, A.O.A.C.*, 1945, page 722.
(7) *Loc. cit.*

REPORT ON ETHYLMORPHINE IN SYRUPS

By F. J. McNall (U. S. Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

Last year a preliminary report was given on ethylmorphine in syrups and in accordance with the recommendations of Subcommittee B of the Association (*This Journal*, **32**, 49) the work was continued this year and a method for assay was submitted for collaborative study.

A collaborative sample was prepared similar in composition to some of the commercial cough syrups on the market. The ethylmorphine hydrochloride used in making the synthetic mixture was assayed according to the method submitted to collaborators and was found to be 98.7% pure. Two liters of this sample was made according to the following formula with the theoretical concentration of ethylmorphine hydrochloride being 0.054 g per 100 ml.

Tolu balsam syrup	900 ml
Ethylmorphine hydrochloride	1.10 g
Tartar emetic	0.54 g
Simple syrup q.s. to	2000 ml

The following method was submitted to collaborators:

Ethylmorphine in Syrups

(In the absence of other alkaloids)

METHOD

Transfer by means of a pipette, 50 ml of the sample into a 250-ml separator, washing out the pipette with H_2O and dilute to ca 100 ml with H_2O. Make alkaline with a few drops of dilute NH_4OH. Extract alkaloid with 50-ml portions of $CHCl_3$-alcohol solvent (9+1). Test for complete extraction. (Usually 4 extractions are sufficient to completely remove alkaloids). Filter each portion of the solvent and combine in a 250-ml beaker. Evaporate solvent on the steam bath to ca 5 ml. Add 10 ml of 0.02 *N* H_2SO_4 and heat on the steam bath until the $CHCl_3$ has evaporated

* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 45 (1950).

and the alkaloids are in solution. Cool, add methyl red indicator, and titrate the excess acid with 0.02 *N* NaOH. 1 ml of 0.02 *N* H_2SO_4 = 0.00772 g of ethylmorphine hydrochloride $C_{19}H_{23}O_3$ *N*.HCl.$2H_2O$.

COLLABORATIVE RESULTS

Collaborator	*Ethylmorphine hydrochloride g/100 ml*	
1	0.054	0.053
2	0.053	0.053
3	0.053	0.052
4	0.053	0.052
5	0.054	0.054
6	0.052	0.052
Average	0.053	
Average per cent Recovery	98.1	

ACKNOWLEDGMENT

Appreciation is expressed to the following collaborators (all in the Food and Drug Administration) who participated in this study: R. L. Herd, Los Angeles, Sam D. Fine, Cincinnati, Gloria Getchell, Minneapolis, William H. Munday, Kansas City, H. P. Bennett, New Orleans.

RECOMMENDATIONS*

It is recommended that the method for ethylmorphine in syrups be made official and that the subject be closed.

No reports were given on theobromine and phenobarbital; aminopyrine, ephedrine, and phenobarbital; rutin in tablets.

REPORT ON SYNTHETIC DRUGS

By F. C. SINTON (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Referee*

RECOMMENDATIONS*

Butacaine.—An informal report was received indicating that collaborative studies of the methods for butacaine in ointments and butacaine in tablets yielded satisfactory results. The Associate Referee is recommending these methods for adoption as first action and that they also cover the simpler dosage forms as butacaine sulfate (crystals) and butacaine sulfate solutions. He also recommends that the subject be continued with respect to ointments having less than 2% butacaine. The Referee concurs.

Carbromal.—An informal report was submitted by the Associate Referee. He suggested two methods and recommends that collaborative study on samples of known composition be undertaken.

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 45 (1950).

Synthetic Estrogens.—No report. The Referee recommends that the subject be continued.

Methylene Blue.—No report. The Referee recommends that the subject be continued.

Phenolphthalein in Chocolate Preparations.—A report was submitted by the Associate Referee last year which was too late for consideration. This report is presented again; the Associate Referee recommends that the present A.O.A.C. method be amended to include certain improvements and that the subject be closed. The Referee concurs.

Propadrine Hydrochloride.—No report. The Referee recommends that the subject be continued.

Propylthiouracil.—The Associate Referee has submitted a report and recommends that the method which was studied collaboratively be adopted as first action and the subject be closed. The Referee concurs.

Pyribenzamine and Benadryl.—No report. The Referee recommends that the subject be continued.

Spectrophotometric Methods.—No report. The Referee recommends that the subject be continued.

Sulfanilamide Derivatives.—No report. The Referee recommends that the subject be continued.

REPORT ON PROPYLTHIOURACIL IN TABLETS

By GORDON SMITH (Food and Drug Administration, Federal Security Agency, New York), *Associate Referee*

A number of possible methods of assay have been considered or tried, and one has been submitted to collaborative study.

A quantity of propylthiouracil powder was received from the previous Referee. It was found to contain no moisture. Its melting range was found to be 218.5°–219.5°C., within the range 218°–220° specified in the NNR[1] monograph. A small quantity recrystallized from alcohol was found to melt at 219°–220°C. The original powder was therefore deemed sufficiently pure for the purposes of this work.

A tablet mixture of the following composition was made up.

	per cent
Propylthiouracil	50.0
Starch	35.5
Talc	8.5
Lactose	4.0
Stearic Acid	2.0

The following methods were considered. The first three were given some study in the laboratory.

[1] *New and Non-Official Remedies*, American Medical Association.

(1) *Simple titration with standard NaOH, in neutral alcohol solution, the N.N.R. method.* This was the method finally selected for collaborative study, largely because of its simplicity. It was slightly modified. It is discussed more fully farther on.

(2) *The British Pharmacopeia method for thiouracil.* This was found quite satisfactory for assaying propylthiouracil powder, but with the tablet mixture an interfering red color completely obscured the end point. It is possible that with sufficient study this interference might somehow be eliminated. The end point is normally sharper than that of the NaOH titration.

(3) *The Boie method for theobromine.* Attempt was made to adapt this method to propylthiouracil, but without success.

(4) *Bromination.* This method, if feasible, seemed unlikely to have any advantages over the simple NaOH titration. Further, M. Drucker[2] and A. Kramer,[2] who previously worked on propylthiouracil, both found that this method was not feasible.

(5) *A spectrophotometric method.* Such method has already been used successfully by the Cincinnati laboratory, and by at least one manufacturer. This present study, however, was intentionally confined to chemical methods not requiring special apparatus.

SIMPLE TITRATION METHOD

When the pure powder was assayed by the N.N.R. method, titrating to the first visible pink color, results were consistently low. When the titration was carried to the point where the pink color was definite, undoubted and permanent, the results were closer to theory. Similar results were obtained with the tablet mixture, after removal of its stearic acid. This removal is of course necessary for any mixture containing stearic acid. It was accomplished by successive extractions with petroleum benzine, in which propylthiouracil was found to be insoluble.

Thymolphthalein indicator, which changes in a higher *p*H range, was tried in place of phenolphthalein. Results were consistently high. The end point here was called at the first visible blue color. A higher concentration of indicator was used in attempt to bring the end point closer to theory, but results remained high.

The method was submitted to collaborators, the same tablet mixture being used as the sample. They were requested to make the titration with each of the two indicators. Results are shown in the following table, listed in the order received.

Thanks are expressed to the collaborators, here listed alphabetically.

M. E. Avery, Lederle Laboratories, Pearl River, New York
A. D. Felmeister, Food and Drug Administration, New York
E. M. Hoshall, Food and Drug Administration, Baltimore

[2] Food and Drug Administration, New York.

R. M. Hyatt, Food and Drug Administration, Cincinnati
H. Rogavitz, Food and Drug Administration, New York
A. J. Shingler, Food and Drug Administration, Atlanta

H. Rogavitz tried out the method before it was submitted to other collaborators. Slight changes were made as a result of this assistance on his part; therefore his results do not appear in the table.

As anticipated, there was comment on the lack of sharpness in the end

TABLE 1.—*Propylthiouracil in tablet mixture*

COLLABORATOR	PHENOLPHTHALEIN INDICATOR	THYMOLPHTHALEIN INDICATOR
	per cent	*per cent*
1	50.0	50.6
2	49.11	50.07
	49.01	50.11
3	49.2	49.0
	49.0	49.0
4	48.81	50.69
	48.89	51.03
5	49.57	50.21
	49.31	49.72
	49.93	49.72
Referee	50.09	50.86
	49.98	50.62
Average	49.41	50.14
Range, or spread	1.27	2.03

points, but of the four collaborators who mentioned this, two confined such comment to the thymolphthalein end point.

Propylthiouracil is so weakly acidic that a sharp end point cannot be had with either indicator, but the fact that the change is from colorless to colored might be expected to make the method practicable. The collaborative results indicate that such is the case. That the thymolphthalein average is closer to theory does not seem significant in view of the greater spread of results with this indicator. Collaborators apparently had more difficulty with the thymolphthalein end point. Phenolphthalein is deemed the indicator of choice, and in the published method it only is used. Details of the method are given below.

It is recommended* that the method for propylthiouracil be adopted, first action, and that the subject be closed.

* For report of Subcommittee B and action of the Association see *This Journal*, 33, 45 (1950).

(1) PROPYLTHIOURACIL IN TABLETS

Applicable in absence of stearic acid

REAGENT

Neutralized alcohol.—To each 100 ml of alcohol add 5 drops of phenolphthalein T. S. Add 0.1 *N* NaOH dropwise, to first visible pink color.

DETERMINATION

Weigh accurately a quantity of powder representing ca 0.4 g of propylthiouracil, and transfer to a beaker. Add 100 ml of neutralized alcohol and stir 1 min with glass rod to dissolve most of propylthiouracil. Titrate with 0.1 *N* NaOH. Near end point add NaOH slowly with constant stirring. Titrate to a definite, undoubted pink color that remains plainly visible one min.

1 ml 0.1 *N* NaOH = .017023 g of propylthiouracil.

(2) ALTERNATIVE METHOD

Applicable in presence of stearic acid

Fold a dry 9-cm filter paper with extra folds so as to fit fairly well down into a small funnel. Weigh accurately a quantity of powder representing about 0.4 g of propylthiouracil, and transfer to paper in funnel. Wash sample and paper with 6 successive portions of petroleum benzine, 5–10 ml each. Reject washings. Transfer paper and residue to a beaker. Heat on steam bath until odor of petroleum benzine disappears. Remove from steam bath and proceed as directed under (1), beginning "Add 100 ml of neutralized alcohol."

REPORT ON BENADRYL AND PYRIBENZAMINE

By HAROLD C. HEIM (University of Colorado College of Pharmacy, Boulder, Colorado), *Associate Referee*

Benadryl and pyribenzamine are both tertiary amino derivatives and exhibit few reactions which could be used in their quantitative determination. Gelvin and McGavack (1) reported a method for the determination of minute quantities of benadryl in blood and spinal fluid. The method used by these authors was a modification of the method developed by Brodie and Udenfriend (2) for the determination of organic bases and consisted essentially of the formation of a colored complex by treating benadryl base with an organic dye. The colored complex was extracted with an organic solvent and the color compared to that produced by known amounts of benadryl. A biological method for the determination of benadryl has been reported by Chen, Ensor, and Clarke (3). This method involved the measurement of the antagonistic effect of benadryl to histamine when the latter compound is applied to the isolated guinea pig ileum. *New and Nonofficial Remedies* (4) contains monographs for both benadryl and pyribenzamine and includes methods for their quantitative determination. Benadryl is determined by extracting the free base with ether from alkaline solution and subsequent titration. Pyribenzamine is determined by extracting the free base from alkaline solution

with ether and subsequent precipitation as the dipicrate. It was found that the free bases were slowly volatile so that they could not be extracted, dried, and weighed. Results obtained with colorimetric methods developed by the Associate Referee were not consistent and therefore it was decided to subject the precipitation and titration methods to collaborative study.

Collaborative study of a titration method for pyribenzamine was also carried out.

EXPERIMENTAL

Samples consisting of the antihistiminic drug, lactose, and starch were sent to collaborators with the following instructions:

METHOD FOR DETERMINATION OF BENADRYL

Sample No. 1

REAGENTS

Sodium hydroxide.—10% w/v.

Washed ether.—Shake U.S.P. ether with 2 portions of H_2O.

METHOD

Transfer an accurately weighed sample equivalent to approximately 0.15 g of benadryl.HCl to a 125-ml separatory funnel, add 40–50 ml of H_2O, and shake to disintegrate the sample. Add 2 ml of the NaOH soln and extract 4 times with 25 ml portions of washed ether. Combine the ether extracts in a second separatory funnel and wash twice with 10 ml portions of H_2O. Discard the washings.

Add, from burette, 20.0 ml 0.1 *N* H_2SO_4 to the ether soln in the separatory funnel and shake well. Allow to separate and draw off the H_2SO_4 layer into a 200-ml Erlenmeyer flask. Wash the ether soln 4 times with 20 ml portions of H_2O, adding the washings to the flask containing the H_2SO_4.

Titrate the excess H_2SO_4 with 0.1 *N* NaOH using methyl red indicator. Titrate to complete disappearance of the pink color. Report results as per cent benadryl.HCl.

1 ml 0.1 *N* H_2SO_4 is equivalent to 0.02918 g benadryl.HCl.

METHOD FOR DETERMINATION OF PYRIBENZAMINE

Sample No. 2

REAGENTS

Sodium Hydroxide.—10% w/v.

Sulfuric acid.—add 1 ml H_2SO_4 to 200 ml H_2O.

Picric acid soln.—Saturated at room temperature and filtered.

Method 1.—Transfer an accurately weighed sample equivalent to ca 0.15 g pyribenzamine.HCl to a 125 ml separatory funnel, add 40–50 ml H_2O, and shake to disintegrate sample. Add 2 ml of the NaOH soln and extract 4 times with 25 ml portions of washed ether. Combine the ether extracts in a second separatory funnel and wash twice with 10 ml portions of H_2O. Discard the washings.

Extract the ether soln 4 times with 15 ml portions of the 1 plus 200 H_2SO_4 and combine the H_2SO_4 layers in a 150-ml beaker. Add to the H_2SO_4 soln 50 ml of the saturated picric acid soln, stir well and allow to stand for 2 hours. Filter through a tared Gooch crucible, wash 3 times with 10 ml portions of H_2O, dry at 110°C., and weigh.

$$\text{Pyribenzamine dipicrate} \times 0.40896 = \text{pyribenzamine.HCl}$$

Method 2.—Follow the procedure for Benadryl, except that in the titration use

brom-thymol blue as the indicator and titrate to a green color (free from yellow tint).

1 ml 0.1 *N* H_2SO_4 is equivalent to 0.02918 g pyribenzamine·HCl

DISCUSSION

Collaborator's results for the benadryl assays are shown in Table 1. These results are in good agreement with the theoretical value. In Table 2 are set forth the results for the pyribenzamine assays. The results of three collaborators, using the dipicrate method, are in good agreement

TABLE 1.—*Collaborator's results for benadryl determinations*

ANALYST	PER CENT BENADRYL. HCL FOUND	PER CENT BENADRYL. HCL ADDED	PER CENT RECOVERY
Harold F. O'Keefe	39.29	38.97	100.87
Food & Drug Administration,	39.33		
Chicago, Ill.	Ave. 39.31		
Gordon Smith,	39.45		
Food & Drug Administration,	39.22		
New York, N. Y.	Ave. 39.34		100.95
Janice Carter,	38.50		
Food & Drug Administration,	38.80		
Cincinnati, Ohio	38.80		
	38.84		99.46
	Ave. 38.76		
H. C. Heim,	38.74		99.48
University of Colo.,	38.81		
Boulder, Colo.	Ave. 38.77		

with the theoretical values. The volumetric method for pyribenzamine yielded results which vary somewhat from the theoretical values. This may possibly be due to the fact that the directions called for continuing the titration until the indicator had assumed a green color free from yellowish tint. It is felt that such a procedure introduces a possible error, because it is difficult to decide just when this color is reached.

One collaborator commented as follows:

"The picrate precipitate formed some gummy lumps which were broken up with difficulty. The picric acid, or at least the first part of it, should probably be added slowly, dropwise, and with constant stirring.

In the shakeouts there was some tendency to form emulsions. It might be better to use a larger separator and shake gently."

CONCLUSIONS

(1) Collaborative study of methods for the determination of benadryl and pyribenzamine are herewith reported.

TABLE 2.—*Collaborator's results for pyribenzamine determinations*

ANALYST	PER CENT PYRIBENZAMINE FOUND	PER CENT PYRIBENZAMINE ADDED	PER CENT RECOVERY
Gravimetric Method			
Harold F. O'Keefe, Food & Drug Administration, Chicago, Ill.	31.09 30.95 Ave. 31.02	30.33	102.20
Gordon Smith, Food & Drug Administration, New York, N. Y.	30.25		99.73
Janice Carter Food & Drug Administration, Cincinnati, Ohio	28.15 28.25 Ave. 28.20		92.68
H. C. Heim University of Colo., Boulder, Colo.	30.23 30.15 Ave. 30.19		99.86
Volumetric Method			
Harold F. O'Keefe, Food & Drug Administration, Chicago, Ill.	31.07 31.18 Ave. 31.13	30.33	102.63
Gordon Smith Food & Drug Administration, New York, N. Y.	29.47		97.16
Janice Carter, Food & Drug Administration, Cincinnati, Ohio	29.16 29.54 Ave. 29.30		96.27
H. C. Heim, University of Colo. Boulder, Colo.	30.19 30.25 Ave. 30.22		99.63

(2) The results with the method for benadryl are in good agreement with the theoretical values. With the methods for pyribenzamine slightly greater deviation from the theoretical values was reported by collaborators.

(3) In view of the fact that there are at least six antihistamine drugs in common usage at present, the Associate Referee recommends* that this study be continued and broadened to include the investigation of methods which could be used for the determination of any of these drugs.

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 45 (1950).

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REPORT ON PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS

By HARRY ROGAVITZ (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

A method based on Hubacher's (1) modifications of the official method for phenolphthalein in chocolate preparations (2) was submitted to collaborative study.

Sample A consisted of 10% phenolphthalein and 90% chocolate. The mixture was prepared by thoroughly stirring the requisite amount of phenolphthalein with melted chocolate. Sample B was prepared to contain 5.50% phenolphthalein and 94.50% cocoa. Portions of each of these samples were sent to collaborators with these directions:

PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS

REAGENTS

(a) *Potassium hydroxide soln*—5 N ($\pm$0.1 N).

(b) *Iodine soln*—0.5 N.—Dissolve 12.7 g of KI in 10 ml of H_2O, add 6.35 g of iodine, and, when dissolved, add 12 ml of 5 N KOH. Dilute to 100 ml with H_2O.

(c) *Sodium sulfite soln.*—Dissolve 12.6 g of anhydrous Na_2SO_3 in H_2O and dilute to 100 ml with H_2O.

PREPARATION OF ALCOHOLIC EXTRACT

Chill the chocolate sample until it becomes hard and reduce it to a granular condition by grating, shaving, or grinding. Mix thoroly. Weigh accurately a quantity of the prepared sample to contain the equivalent of about 0.1 g of phenolphthalein into a Gooch crucible (with a thin asbestos mat or fritted glass disk). Extract the fat with 5+4+3 ml of CCl_4, using slight suction towards the end.

Place the crucible on a bell-jar arrangement. Extract the phenolphthalein from the sample with several portions of hot alcohol, collecting the filtrate in a 300-ml tall form beaker. Wash the underside of the crucible free from phenolphthalein with hot alcohol. (Ca 50 ml of hot alcohol is sufficient for extraction and washing.) Evaporate the combined alcoholic extract to dryness on a steam bath.

DETERMINATION

Dissolve the residue at room temp. in 1–1.5 ml of 5 N KOH. (The alkaline phenolphthalein soln is unstable in air and should be reacted to the tetraiodo compound within one hour.) Add a piece of ice (ca 40 g) and 7–8 ml of the iodine soln. Add conc. HCl dropwise from a buret, using a stirring rod, to complete precipitation. If sufficient iodine soln has been added, the precipitate, as well as the supernatant

TABLE 1.—*Collaborative results—per cent*

COLLABORATOR	SAMPLE A		SAMPLE B	
	PHENOLPHTHALEIN		PHENOLPHTHALEIN	
	FOUND	RECOVERY	FOUND	RECOVERY
1	10.41		5.48	
	10.41		5.55	
	10.38			
	Av. 10.40	104.0	Av. 5.52	100.4
2	9.94		5.69	
	10.49		5.53	
	10.45		5.51	
	Av. 10.29	102.9	Av. 5.58	101.5
3	9.81		5.35	
	9.68		5.43	
	Av. 9.75	97.5	Av. 5.39	98.0
4	9.97		5.55	
	10.09		5.47	
	Av. 10.03	100.3	Av. 5.51	100.2
5	9.86		5.40	
	9.79		5.49	
	9.84			
	10.26			
	Av. 9.94	99.4	Av. 5.44	98.9
6	10.00		5.52	
	10.12		5.61	
	Av. 10.06	100.6	Av. 5.56	101.1
General Av.	10.08	100.8	5.50	100.0

liquid will be brown from the excess iodine; if not, add more of the iodine soln to insure an excess. Dissolve the precipitate again by adding dropwise, with stirring, 5 *N* KOH from a buret. Any unreacted phenolphthalein adhering to the side of the beaker should be washed down with a little H_2O. (The resulting soln should be blue to blue-purple.) Repeat the process of precipitation with acid and re-solution with alkali three more times, adding a small piece of ice, if necessary, to keep the soln cold. Then add 1–1.5 ml of the Na_2SO_3 soln to the blue alkaline soln and filter thru a Gooch crucible (with a *thin* asbestos mat or a *coarse* fritted glass disk) into a 250-ml beaker. Wash the crucible several times with H_2O. Acidify the filtrate with conc. HCl, using a few ml in excess, and heat on the steam bath for 20–30 min., stirring occasionally. Decant the supernatant hot liquid thru a weighed Gooch crucible (with asbestos mat or medium fritted glass disk). Wash the white to cream-colored precipitate in the beaker by decantation with hot water a few times. Transfer the precipitate completely to the Gooch crucible and wash with hot H_2O until the filtrate is clear and gives a negative test for chlorides. When the apparatus has

cooled and the precipitate has been sucked fairly *dry*, wash the precipitate several times with petroleum ether, using suction towards the end. Dry the tetraiodophenolphthalein to constant weight at 110°–130° C.

Weight of the precipitate $\times 0.3872$ = weight of phenolphthalein.

Calculate the percentage of phenolphthalein and report the individual percentages to two decimal places.

Table 1 gives the results reported by collaborators.

One collaborator found it impossible to wash the final tetraiodophenolphthalein precipitate with petroleum ether in a reasonable time. The Associate Referee found that unless the precipitate was sucked fairly dry, difficulty in washing with petroleum ether may be encountered.

Some collaborators questioned the need for the final petroleum ether wash. In preliminary experiments with some cocoa-phenolphthalein mixtures, the Associate Referee obtained higher than theoretical recoveries unless the precipitate was washed with petroleum ether. The collaborative study showed that the modified method clarifies the directions for preparing the iodine solution, gives valuable precautionary suggestions, and results in satisfactory recoveries on samples containing relatively low percentages of phenolphthalein.

The Associate Referee gratefully acknowledges his indebtedness to the following collaborators:

Max Hubacher, Ex-Lax Inc., 423 Atlantic Ave., Brooklyn 17, N. Y.
Dr. Arthur Horner, Ex-Lax Inc., 423 Atlantic Ave., Brooklyn 17, N. Y.
Adelyn D. Felmeister, Food and Drug Adm., Federal Security Agency, N. Y.
Rupert Hyatt, Food and Drug Adm., Federal Security Agency, Cincinnati, O.
N. A. Carson, Food and Drug Adm., Federal Security Agency, St. Louis, Mo.

It is recommended* that the official method for Phenolphthalein in Chocolate Preparations be amended to incorporate some desirable modifications and that the directions, as submitted to collaborative study, be adopted, first action. It is recommended that the subject be closed.

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(1) M. L. HUBACHER, *This Journal*, **26**, 194 (1943).
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* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 45 (1950).

REPORT ON BUTACAINE SULFATE

By LLEWELLYN H. WELSH (Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

At the 1948 meeting of the Association it was reported[1] that preliminary work suggested the applicability of certain procedures to the determination of butacaine sulfate in its various dosage forms. The present report includes the results of a collaborative study of gravimetric, acidimetric, and bromometric methods.

Two samples were sent to collaborators. Sample I contained 2.00% butacaine sulfate and 98.00% of a mixture of 3 parts white petrolatum and 1 part anhydrous lanolin. This composition simulates that of a commercial ophthalmic ointment. Sample II had the following percentage composition: butacaine sulfate, 33.33; lactose, 61.57; sodium bisulfite, 5.00; epinephrine, 0.10. The formula is based on that of hypodermic tablets which are on the market. Collaborators were sent the following instructions:

REAGENTS

(a) 0.1 *N solns of sulfuric acid, bromide-bromate, and sodium thiosulfate,* standardized according to any accepted method.

(b) *Freshly prepared 20% KI soln.*

(c) *Starch indicator.*—Make a paste of 1.5 g of soluble starch in a few ml of water and add it slowly, with stirring, to 300 ml of boiling water.

(d) *Concentrated ammonium hydroxide.*

(e) *Picrolonic acid.*

(f) *Dibromobutacaine picrolonate.*

DIRECTIONS

Sample I.—Accurately weigh into a 125-ml separatory funnel about 2.5 g of ointment. Add 25 ml of benzene, swirl the funnel until the ointment base has dissolved, then add 10 ml of 5% HCl and shake gently ca 1 min. (Note 1, at end of Directions). After the layers have separated drain off as much of the aqueous phase as possible into a second separator, and repeat the shakeout 4 times with 10-ml portions of water. Wash the combined aqueous extracts with 5 ml of carbon tetrachloride and discard the wash. Basify the soln of butacaine salt with ammonium hydroxide, and extract out the butacaine base by 5 shakeouts with 15-ml portions of chloroform. Filter each extract thru a pledget of cotton into a 100-ml beaker and heat the beaker on the steam bath in a current of air until the residual oil has no odor of chloroform. Rinse down the wall of the beaker with 2 ml of alcohol delivered from a pipet, warm until the oily butacaine base has completely dissolved, then add one drop each of conc. HCl and methyl red T.S. (Note 2). Tilt and rotate the beaker so that the acidic soln will contact any liquid which has crept up the wall of the beaker prior to the addition of acid, dilute the soln with a few ml of water, and transfer it quantitatively to a 500-ml iodine flask with the aid of water. Pipet in 10 ml of 0.1 *N* bromide-bromate soln, dilute the mixture to 200 ml and add 10 ml of concd HCl. Immediately stopper the flask, swirl it, and allow it to stand 5 min. In the usual manner, introduce 5 ml of 20% KI soln into the flask, stopper the flask, and shake it vigorously.

[1] Welsh, L. H., *This Journal*, **32**, 548 (1949).

Rinse the stopper and neck of the flask with a little water and titrate the iodine with 0.1 *N* thiosulfate until the color is just discharged. Add 15 ml of starch soln and 20 ml of chloroform (Note 3), stopper the flask and shake it vigorously. Rinse the stopper and neck of the flask with water, and continue the titration. As the end point is approached, stopper the flask and shake it vigorously after each addition of thiosulfate. Each ml of 0.1 *N* bromide-bromate = 0.008887 g of butacaine sulfate. Calculate the percentage of the compound in the sample.

To isolate the bromination product, transfer the titrated mixture to a suitable separatory funnel, basify with ammonium hydroxide and shake it vigorously. Allow to settle a few minutes and break the chloroform-rich emulsion by filtering it with suction thru a quarter-inch layer of Hi-lo Super Cel supported on filter paper in a Büchner funnel (Note 4). Shake the aqueous phase in the separatory funnel with another portion of chloroform (ca 25 ml) and pass the chloroform emulsion thru the Büchner funnel. Transfer the filtrate to a separatory funnel and drain off the chloroform layer thru a pledget of cotton into a beaker. Evaporate the filtrate on the steam bath in a current of air, transfer the residual oil of dibromobutacaine to a 5-ml beaker, and evaporate as before until the chloroform is removed. To the residue add a soln of 50 mg of picrolonic acid in 2 ml of alcohol and stir well. Filter off the precipitate (Note 5) on a Hirsch funnel, wash it with 2–3 ml of alcohol, dry it at 105°, and determine the capillary melting point. Dibromobutacaine picrolonate melts at 158–160°.

Sample II.—Into a separatory funnel introduce about 0.6 g of sample, accurately weighed. Add 25 ml of water and swirl until the sample is dissolved. Basify with 2 ml of ammonium hydroxide and extract the butacaine base with six 15-ml portions of chloroform (Note 6). Shake each extract with 55 ml of water in a second separator before filtering thru a pledget of cotton into a beaker. Evaporate the combined extracts to a small volume to the steam bath in a current of air, transfer to a tared 50-ml beaker, and continue the evaporation until no odor of chloroform is detected. Heat the beaker in an oven at 105° for one-half hour, cool in a desiccator, and weigh. The weight of residue × 1.160 represents the equivalent of butacaine sulfate. Calculate the percentage of the latter in the sample.

By means of a pipet rinse down the wall of the beaker with 2 ml of neutral alcohol and warm on the steam bath until the butacaine base is dissolved. Add one drop of methyl red T.S., rinse down the wall with another 2 ml of alcohol and titrate with 0.1 *N* sulfuric acid almost to the point of color change. Rinse down the wall of the beaker with water, further dilute to ca 45 ml and complete the titration. Each ml of 0.1 *N* acid = 0.03555 g butacaine sulfate. Calculate the percentage of butacaine sulfate in the sample.

By the use of water quantitatively rinse the titrated soln into a 500-ml iodine flask, add 50 ml of 0.1 *N* bromide-bromate soln, dilute to 200 ml, and continue the determination by bromination as described for Sample I (Note 3). During the bromination period, swirl the flask frequently to coagulate the precipitate. Calculate the percentage of butacaine sulfate in the sample.

NOTES

(1) Vigorous shaking will probably cause an emulsion.

(2) The solution should be acid at this point.

(3) The bromination method is based on the procedure of Wells, *This Journal*, 25, 537 (1942). The bromination product forms a loose addition compound with iodine. The titration is not characterized by the sharp and rapidly attained end-point associated with iodimetric determinations chiefly because the iodine must distribute itself between the chloroform and aqueous phases. Also, the blue or violet color of the starch-iodine complex in the aqueous phase has superimposed on it the

TABLE 1.—*Results of collaborators*

COLLABORATOR	SAMPLE I			SAMPLE II					
				GRAVIMETRIC		ACIDIMETRIC		BROMOMETRIC	
	FOUND	RECOVERED	m.p. PICROLONATE, °C.	FOUND	RECOVERED	FOUND	RECOVERED	FOUND	RECOVERED
	per cent	*per cent*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1. Harold F. O'Keefe, U. S. Food and Drug Admin., Chicago, Ill.	1.98	99.0	159–160	33.19	99.6	32.99	99.0	33.42	100.3
	1.98	99.0		33.07	99.2	32.87	98.6	33.05	99.2
				33.04	99.1	32.97	98.9	32.79	98.4
2. Rupert Hyatt, U. S. Food and Drug Admin., Cincinnati, Ohio	2.03	101.5	158	33.0	99.0	32.5	97.5	34.3	102.9
	2.01	100.5		33.2	99.6	32.4	97.2	34.6	103.8
3. L. H. Welsh, U. S. Food and Drug Admin., Washington, D. C.	1.99	99.5	158–160	33.3	99.9	32.6	97.8	33.4	100.2
	2.00	100.0		33.1	99.3	32.7	98.1	33.2	99.6
				33.3	99.9	32.7	98.1	—	—
				33.2	99.6	32.7	98.1	—	—
4. J. C. Molitor, U. S. Food and Drug Admin., Washington, D. C.	2.00	100.0	159–161	33.31	99.94	32.71	98.12	33.68	101.05
	1.98	99.0		33.35	100.06	32.96	98.87	33.30	99.91
5. F. C. Sinton, U. S. Food and Drug Admin., New York, N. Y.	—	—	—	30.5	91.5	29.9	89.7	30.8	92.4
				29.9	89.7	29.6	88.8	30.5	91.5
6. Gordon Smith, U. S. Food and Drug Admin., New York, N. Y.	2.03	101.5	159–160	—	—	—	—	—	—
	1.97	98.5		—	—	—	—	—	—
Average	1.999	99.95		32.73	98.20	32.28	96.85	33.00	99.00

brown of the chloroform phase when the latter contains considerable iodine.

In the assay of the ointment, the titrated mixture gradually passes thru the following color changes before reaching the end point: dark blue→violet→light violet. In the assay of the powder these color changes may be anticipated: brownish green →dark gray→violet gray→pinkish gray→pinkish. In either case the end point is reached when, after the shaken mixture has stood a couple of minutes, the aqueous phase is white and the emulsified chloroform layer is practically white.

(4) More than one passage thru the filter may be necessary. If any other method of breaking the emulsion seems more convenient, it may be used.

(5) If the picrolonate does not crystallize out on stirring the soln and scratching the beaker wall, allow the soln to stand a couple of hours in a closed vessel before resorting to the use of seed crystals. Report what measures were necessary to induce crystallization. Do not open the tube containing the specimen of dibromobutacaine picrolonate until it is necessary to use it for seeding purposes, as it is desirable to have an idea as to how readily the compound will deposit in the absence of seed.

(6) Disregard any milky appearance of the aqueous phase and any haziness of the chloroform layer. It is important, however, that the phases be allowed to separate until the chloroform layer attains a volume approximately equal to that of the solvent added.

In Table 1 are listed the results of collaborators.

COMMENTS OF COLLABORATORS

Harold F. O'Keefe.—"It was not necessary to seed for formation of the dibromobutacaine picrolonate, and the crystals formed after the beaker was allowed to stand 15 minutes, then scratched with glass rod.

"It was found that after six extractions of the powder with 15-ml portions of chloroform the aqueous material was still very milky. The residues were weighed after the six extractions. Then 25 ml. of chloroform was added to each separator, shaken and allowed to set overnight, drawn off and passed through the wash water and added to the residue. Another shake-out was made with 25 additional ml of chloroform and this added to the residue in the beaker. An additional shake-out yielded no material, but it was found that from 25 to 40 mg of the butacaine base remained in the milky solution after the initial six shake-outs. This was removed in the subsequent shake-outs after the emulsion was broken. In view of this, it is believed that a stronger caution on complete extraction should be included in the directions."

Gordon Smith.—"Scratching beaker and stirring induced crystallization (of picrolonate) in 2 or 3 minutes. No other measures were needed."

F. C. Sinton.—"No difficulty was experienced (in assay of Sample II) except that in the gravimetric procedure a 100-ml beaker was used rather than the 50-ml specified since the subsequent titration required a dilution to 45 ml."

Rupert Hyatt.—"Sample I. *Speedex* was used to break the emulsion (formed in the shake-out of dibromobutacaine base). The picrolonate had not crystallized after one-half hour intermittent stirring and scratching, so it was poured into a 50-ml g.s. flask. During the pouring the crystals formed rapidly. It appears that the alcoholic solution was supersaturated and that a small amount of evaporation started the crystallization. This collaborator regards the value of this procedure limited by the fact that the analyst may conclude the test to be negative even though butacaine sulfate be present.

"Sample II. In the extraction of the powder the butacaine seemed to go into the $CHCl_3$ very slowly. The aqueous portion seemed full of precipitate even after the second shake-out."

J. C. Molitor.—"Sample I. No difficulty was observed in following the procedure for the ointment. The dibromobutacaine picrolonate crystallized from solution after about 15 minutes of occasional scratching of the beaker wall. Seeding was not required. The beginning of the melting range was quite definite, but decomposition followed.

"Sample II. During the extraction, it was observed that in the separation of the two layers the original volume of $CHCl_3$ added was not recovered. The addition of 0.5 g of Na_2SO_4 materially increased the separation of $CHCl_3$ from the water layer, apparently because of a lyotropic salt effect. Extraction was complete with six 15-ml portions of $CHCl_3$."

DISCUSSION

For Sample I, the percentage recoveries of all collaborators are within the range of 98.5–101.5, and average 100.0. These results are considered highly satisfactory.

Each collaborator was able to induce the picrolonate[2] to crystallize without the addition of seed crystals. Collaborator 2 appears to have experienced a little more difficulty than the others in inducing crystallization, and has commented on the limitations of the identification test. With respect to this comment, it must be pointed out that one should assume the test to be negative only if crystallization fails to occur in the presence of the solid phase. Possession of an authentic specimen of dibromobutacaine picrolonate is essential in order to avoid the possibility of a false negative test, and the substance may be regarded as a necessary "reagent."

In reporting results on Sample II, Collaborator 1 has commented on his inability to obtain complete extraction in using the six shake-outs called for in the procedure, and has recommended that a stronger caution concerning complete extraction be incorporated into the directions. Regarding this part of the original instructions, it seems appropriate to mention the experience of the Associate Referee in analyzing Sample II. Before submitting the sample to collaborators it was subjected to the described extraction procedure. It was noted that in the shake-outs the ammoniacal aqueous phase was persistently milky and that the chloroform layer was quite turbid prior to filtration through cotton. This result was perplexing, since work on pure butacaine sulfate yielded phases which were but slightly turbid, and the composition of Sample II did not appear to be one which might lead to the formation of emulsions in the procedure. The following percentage recoveries of butacaine were obtained on the sample: gravimetric, 99.9, 99.3; acidimetric, 98.1, 98.1. After noting the difficulties of collaborator 1, the procedure was again applied to the sample. It was observed that in the first shake-out about 20% of the added chloroform failed to settle out of the milky aqueous phase, but in the subsequent shake-outs the volume of the separated chloroform layer was approximately to equal to that of the solvent

[2] Welsh, L. H., *J. Am. Chem. Soc.*, **72**, 1863 (1950).

added. After ten shake-outs a test showed that extraction was incomplete. At this point ca 0.5 g. of anhydrous sodium sulfate was added to the milky aqueous phase; immediately a layer of chloroform began to deposit, and, after swirling the funnel and allowing it to stand a few minutes, about 5 ml. of the solvent settled out. The aqueous layer was somewhat turbid but no longer milky. A test after two additional shake-outs showed that extraction was complete. Recovery percentages on duplicates were: gravimetric, 99.9, 99.3; acidimetric, 97.8, 98.1; bromometric, 100.2, 99.6. This experience emphasizes (a), the need to include in the procedure a test for complete extraction as suggested by the experience of collaborator 1, and (b), that measures should be employed to break emulsions when it is apparent that the latter are preventing complete extraction. It would appear that the acceptable results obtained by collaborator 2 and by the Associate Referee in his first set of duplicates were due to the fortunate circumstance that the hold-up of chloroform was inappreciable in emulsions which were formed.

Collaborator 4 obtained good recoveries in analyzing Sample II. He reported a hold-up of chloroform in the top layer during the first shake-out, and that no further difficulty was experienced after adding sodium sulfate to the aqueous layer.

It is evident that the low recoveries of collaborator 5 were due to emulsion formation and resultant incomplete extraction.

On the whole, the collaborative results appear to warrant the conclusion that butacaine base may be extracted with chloroform from ammoniacal solutions of tablets and ultimately determined gravimetrically, by titration with standard acid, or by bromination. If one considers only the results of the four collaborators who are in agreement, the averages for the gravimetric, acidimetric, and bromometric determinations are, respectively, 99.58, 98.23, 100.60.

By the method employed collaboratively on Sample II, the Associate Referee obtained the following results on 200-mg samples of pure butacaine sulfate: gravimetric, 100.4, 100.3%; acidimetric, 99.7, 99.5%; bromometric, 100.0, 100.4%. During the extraction process there was no milkiness of the aqueous phase and no apparent tendency toward the formation of an emulsion. Samples of butacaine sulfate crystals and aqueous solutions thereof were not submitted to collaborative study. Since these constitute dosage forms of compositions simpler than that of Sample II, it seems reasonable to conclude that the method employed in analyzing that sample is equally applicable to these two dosage forms.

No work has been done on the analysis of ointments containing less than 2% butacaine sulfate. *New and Nonofficial Remedies* lists a 1% ointment to which the method employed on Sample I may well be applicable. It is believed, however, that the applicability should be tested in future work.

The methods which are proposed consist essentially of a rewording of the instructions sent to collaborators and include a test for complete extraction of butacaine.

BUTACAINE SULFATE

REAGENTS

(As described in instructions to collaborators)

DETERMINATION

A. Ointments having petrolatum or other grease as a base and containing about 2% butacaine sulfate.—Accurately weigh into a 125-ml separatory funnel a sample containing ca 50 mg of butacaine sulfate. Add 25 ml of benzene, swirl the funnel until the ointment base has dissolved, then add 10 ml of 5% HCl and shake the funnel gently ca 1 min. After the layers have separated, drain off the aqueous phase into a second separator, and repeat the extraction 4 times with 10-ml portions of water. Wash the combined aqueous extracts with 5 ml of CCl_4, and discard the wash. Basify the soln of butacaine salt with ammonium hydroxide, add 2-ml excess, and extract out the butacaine base by shaking 5 times with 15-ml portions of $CHCl_3$. Filter each extract thru a pledget of cotton into a 100-ml beaker and heat the beaker on a steam-bath in a current of air until the residual oil has no odor of $CHCl_3$. Rinse down the wall of the beaker with 2 ml of alcohol, delivered from a pipet, warm until the oily butacaine base has completely dissolved, then add one drop of conc. HCl. Tilt and rotate the beaker so that the acidic soln will contact any liquid which has crept up the wall of the beaker prior to the addition of acid. Add one drop of methyl red T.S. The soln should show a strongly acid reaction; if it does not, add conc. HCl *dropwise* until an acid reaction is shown, dilute with a few ml of water and transfer the soln quantitatively to a 500-ml iodine flask with the aid of water. Pipet in 10 ml of bromide-bromate soln, dilute the mixture to 200 ml, and add 10 ml of conc. HCl. Immediately stopper the flask, and, during a bromination period of 5 min., swirl the flask until the precipitate is coagulated. In the usual manner introduce 5 ml of KI soln into the flask, stopper the flask and shake it vigorously. Rinse the stopper and neck of the flask with a little water and titrate the iodine with thiosulfate soln until the color is discharged. Add 15 ml of starch soln and 20 ml of $CHCl_3$, stopper the flask, and shake it vigorously. Continue the titration by alternately adding thiosulfate and vigorously shaking the stoppered flask. As the end point is approached, the addition of thiosulfate should be dropwise. During titration, the mixture passes thru a series of color changes; the end point is reached when, after it has stood one or two min., the aqueous phase is white and the emulsified $CHCl_3$ layer is practically white.

To isolate the bromination product for identification purposes, transfer the titrated mixture to a separatory funnel, basify it with ammonium hydroxide, and shake it vigorously. Break the separated chloroform-rich emulsion by suction filtration thru ca 0.5 cm. layer of Hi-Flo Super Cel (or similar filter aid) supported on filter paper in a Büchner funnel. Shake the aqueous phase in the separatory funnel with another portion of $CHCl_3$ (ca 25 ml), and pass the chloroform phase thru the filter. Transfer the filtrate to a separatory funnel and drain off the $CHCl_3$ layer thru a pledget of cotton into a beaker. Evaporate the filtrate on the steam bath in a current of air. To the residual oil of dibromobutacaine add a soln of 50 mg of picrolonic acid in 2 ml of alcohol, and stir the mixture. Filter off the precipitate on a Hirsch funnel, wash it with 2–3 ml of alcohol, dry it at 105° and identify it by determining its capillary melting point, alone and in admixture with authentic dibromobutacaine picrolonate (m.p. 158–160° with decomp.). If a precipitate does not form on adding picrolonic acid to the bromination product, introduce a small crystal of dibromobu-

tacaine picrolonate; if precipitation fails to occur the absence of butacaine in the sample is indicated.

B. Tablets.—Accurately weigh 20 tablets and determine the average weight per tablet. Into a 125-ml separatory funnel introduce sufficient accurately weighed finely powdered tablet mixture to provide about 200 mg of butacaine sulfate. Add 25 ml of water and swirl the funnel until the sample is dissolved. Basify with 2 ml of ammonium hydroxide and extract the butacaine base by shake-outs with 15-ml portions of $CHCl_3$. Shake each extract with 5 ml of water in a second separator before filtering thru cotton into a beaker. If no emulsion is formed in the aqueous phase in the first funnel, six shake-outs should be sufficient. Test for complete extraction by evaporating a seventh extract on the steam-bath; the residue should be negligible. If an appreciable residue is obtained, dissolve it in chloroform, combine the soln with the previous extracts, and continue the shake-outs until extraction is complete. If the aqueous phase in the first funnel tends to emulsify, break the emulsion by suitable means (addition of Na_2SO_4 to the aqueous phase may prove effective). Evaporate the filtered $CHCl_3$ extracts to a small volume on the steam bath and complete the determination by one of the following procedures.

(1) By means of $CHCl_3$ quantitatively transfer the concentrated soln of butacaine base to a tared 50-ml beaker and remove the solvent by heating on the steam-bath in a current of air. Heat the residue of butacaine base in an oven at 105°, cool it in a desiccator and weigh. Weight of residue $\times 1.160$ represents the equivalent of butacaine sulfate. Calculate the per cent butacaine sulfate and the amount per tablet.

To check the gravimetric determination acidimetrically, rinse down the wall of the beaker with 2 ml of neutral alcohol delivered from a pipet. Warm the beaker on the steam bath until the butacaine base dissolves completely, add 1 drop of methyl red T.S., and rinse down the beaker wall with another 2 ml of alcohol. Titrate the soln with standard sulfuric acid almost to the point of color change. Rinse down the wall of the beaker with water, further dilute to ca 45 ml., and complete the titration. Each ml of 0.1 *N* acid = 0.03555 g butacaine sulfate.

(2) Determine butacaine sulfate gravimetrically as in (1). As described under *A*, dissolve the residue of butacaine base in alcohol, add conc. HCl dropwise until the soln is acid to methyl red, and quantitatively transfer it to a 500-ml iodine flask. Pipet in 50 ml of bromide-bromate soln and complete the bromometric determination as described under *A*.

(3) Continue the evaporation of the $CHCl_3$ extracts on the steam bath until the solvent is completely removed. Determine butacaine sulfate acidimetrically by dissolving the residue of base in alcohol and proceeding as directed in the second paragraph under (1).

By the use of water, quantitatively transfer the titrated soln to a 500-ml iodine flask, pipet in 50 ml of bromide-bromate soln, and complete the bromometric determination as described under *A*.

C. Crystals.—Accurately weigh a sample of ca. 200 mg of the crystals and transfer it to a 125-ml separatory funnel. Add 25 ml of water and proceed as directed under *B* (1), *B* (2), or *B* (3).

D. Solutions.—Transfer to a 125-ml separatory funnel an aliquot representing ca 200 mg of butacaine sulfate, and, if necessary dilute with water to 25 ml. If chlorobutanol is absent, proceed as directed under *B* (1), *B* (2), or *B* (3). If chlorobutanol is present, proceed as directed under *B* (3).

RECOMMENDATIONS*

It is recommended that the proposed general methods be adopted,

* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 45 (1950).

first action, for the assay of butacaine sulfate in the following dosage forms: crystals, tablets, solutions, and 2% ointments.

It is recommended that the subject be continued with respect to ointments containing less than 2% of the drug.

No report was given on methylene blue, sulfonilamide derivatives, propadrine hydrochloride, carbromal, spectrophotometric methods, and synthetic estrogens.

REPORT ON MISCELLANEOUS DRUGS

By I. SCHURMAN (Food and Drug Administration, Federal Security Agency, Chicago 7, Illinois), *Referee*

RECOMMENDATIONS*

Separation of Bromides, Chlorides, and Iodides.—The Associate Referee has submitted a report and recommends that further work be done and that samples be submitted for collaborative study. The Referee concurs.

Mercury Compounds.—The Associate Referee has left the Association to go into academic work. No report was received. The Referee recommends that this topic be reassigned.

No reports were received on the following topics: Microscopic Tests for Alkaloids and Synthetics; Estrone and Estradiol; Alkali Metals; and Methyl Alcohol in the Precense of Ethyl Alcohol. The Referee recommends* that these topics be continued.

REPORT ON ORGANIC IODIDES AND SEPARATION OF HALOGENS

By VINCENT E. STEWART (Food and Drug Laboratory, State of Florida, Department of Agriculture, Tallahassee, Florida), *Associate Referee*

The method recommended by Freeman[1] for the determination of chloride in the presence of large amounts of bromide and/or iodide has been investigated further by the Associate Referee and samples were submitted to collaborators.

Several changes have been made in the Freeman method and the Associate Referee has attempted to clarify the instructions. The revised method follows.

METHOD FOR THE DETERMINATION OF CHLORIDE IN THE PRESENCE OF LARGE AMOUNTS OF BROMIDE AND/OR IODIDE

CAUTION: The halogen acetone compounds produced in this procedure are ex-

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 46 (1950).
[1] Freeman, N. E., and McMullen, Beulah V., *This Journal*, 31, 550 (1948).

tremely irritating to the eyes and respiratory tract. All operations should be performed under hood with good draft and care taken to avoid getting these vapors into the eyes or inhaling them.

Select a sample containing preferably not more than 1.5 milli-moles (53 mg) of Cl^- and not more than 5 g of total halides. If organic matter is present ash at dull red heat with an excess of sodium carbonate (ca 5 times the amount required to combine with all halogens present).

Place sample in 200–300 ml flask (with lip), add water to make volume of ca 50 ml, 10 ml of $MnSO_4$ soln (5 g/100 ml), and 20 ml dil H_2SO_4 (1+1). Add in small portions and with swirling to permit solution and reaction, an amount of finely powdered $KMnO_4$ equal to 50–100 mg less than the quantity calculated to be necessary to convert all the Br^- and I^- to the free halogen; or, make a trial titration with 5% $KMnO_4$ soln and determine the amount of solid $KMnO_4$ to be used from this result. The weight of $KMnO_4$ required is equal to the weight of the respective alkali halide times the following factors: KBr 0.266, KI 0.190, NaBr 0.307, NaI 0.211.

Heat to boiling and simmer slowly until most of the free halogen has been volatilized and only a light yellow color remains. Add distilled water, if necessary, during the heating in order to maintain the original volume. Loss of Cl^- may occur if the volume decreases appreciably. Cool the solution, add ca 10 ml of acetone (free of reducing substances), dilute to 100 ml, and place in an ice-water bath. Add, dropwise, 5% $KMnO_4$ soln until the color of the soln changes through amber to a very deep wine or coffee color. Allow to remain in ice bath ca 15 min., adding more $KMnO_4$ from time to time as the color fades. The $KMnO_4$ must be present in slight excess at this point in the procedure. Two or more samples can be handled simultaneously up to this point but should be handled separately throughout the remainder of the procedure. While one sample is being completed, the others, to which $KMnO_4$ soln has been added, should remain in the ice bath.

Add 1% H_2O_2 soln, dropwise, slowly while swirling, to decolorize the soln and to dissolve any MnO_2, but avoiding any appreciable excess and keeping the liquid as cold as possible.

At once add 10 ml dil. HNO_3 (1+1), 10 ml ether, and then $AgNO_3$ soln (ca 0.1 *N*) with swirling until the opalescence decreases and the AgCl agglomerates and then ca 5 ml more (a total of 10–20 ml). Swirl ca 1 min., and immediately filter through a tared Gooch crucible. Wash the flask and precipitate thoroughly with water, then with a little alcohol, and finally with ether. Dry at 110°, cool and weigh.

1 g AgCl = 0.2474 g Cl^-

DISCUSSION

The results obtained by the collaborators with this method are shown in Table 1. Sample No. 1 consisted of an aqueous solution of sodium bromide, U.S.P., containing 12.0 g /100 ml. Sample No. 2 consisted of an aqueous solution of sodium, iodide, U.S.P., containing 12.0 g/100 ml. To this was added 0.150 g/100 ml. of sodium chloride, C.P., since the sodium iodide, U.S.P., was found to be practically free from Cl^-. Sample No. 3 consisted of Iso-Alcoholic Elixir (Low Alcoholic), N.F., containing, in each 100 ml: 5.0 g sodium bromide, U.S.P.; 5.0 g sodium iodide, U.S.P., and 2.0 g sodium chloride, C.P. This solution contained glycerin, sucrose, alcohol, water, and aromatics, and was colored with cochineal.

The principal change which has been made in the method is the use of sodium carbonate instead of calcium acetate as the fixative in ashing the

TABLE 1.—*Collaborative results on 3 samples*

ANALYST NO.				AVERAGE
	Sample No. 1. Mg Cl^-/25 ml			
1	20.2	23.9	21.7	21.9
2	13.1	13.2	12.6	13.0
3	37.6*	26.6	26.5	26.5
4	16.2	16.9	—	16.6
	Sample No. 2. Mg Cl^-/25 ml (Theoretical 22.7)			
1	22.7	22.7	22.4	22.6
2	20.5	20.9	17.9	19.8
3	23.3	23.1	24.3	23.5
4	None	None	None	None
	Sample No. 3. Per cent Cl^-			
1	0.916	0.966	0.929	0.937
2	0.888	0.955*	0.882	0.885
3	1.4	1.4	1.5	1.4
4	0.863	0.856	0.834	0.851
	Sample No. 3. Total halides (as g silver halide/g sample)			
1	0.1681	0.1678	0.1688	0.1682
2	0.1689	0.1689	0.1690	0.1689
3	0.1672	0.1673	0.1675	0.1673
4	0.1696	0.1696	0.1695	0.1696
Average (4 analysts)				0.1685

* Not included in average.

sample. Calcium acetate is unsatisfactory for this purpose since the ashed sample is dissolved in a solution containing sulfuric acid. Calcium sulfate is precipitated when a reasonable excess of calcium acetate is used, and cannot be removed readily. Sodium carbonate overcomes this difficulty and is equally efficient as a fixative for chlorides as calcium acetate, according to previous work by Freeman.[2] It is stated by Freeman in this report that sodium carbonate is not suitable as a fixative for iodides; since in the present method only chloride is determined, the loss of iodide is without consequence.

Analysts have considerable difficulty with that part of the procedure in which 5% potassium permanganate solution is added. There is some question in the analyst's mind as to when the proper amount has been added and it appears to be difficult to describe the color change more

[2] Freeman, N. E., *This Journal*, **25**, 833 (1942).

definitely. The color change depends in part upon the rate of addition of the potassium permanganate. Experience with the method is necessary in order to correctly judge the proper amount of potassium permanganate which should be added. If an insufficient amount is added there will be, of course, some Br^- or I^- remaining in the solution, which will be precipitated when silver nitrate is added. A considerable excess of potassium permanganate also appears to have deleterious effects upon the determination, although this has not been investigated sufficiently.

When the solution is boiled to remove excess bromine and/or iodine it is important that the original volume of the solution be maintained during the boiling by adding distilled water from time to time. If the volume is allowed to decrease appreciably a considerable loss of Cl^- may occur and the results of duplicate determinations will vary widely.

The previous Associate Referee, N. E. Freeman, and the present Associate Referee have been able to obtain fairly consistent checks with the method. The results of collaborators agree quite well in some instances but in others they vary widely. Collaborators whose results agree well on some samples disagree markedly on other samples, and vice versa. The results of all collaborators are in harmony on the determination of total halides by the method of Freeman.[2]

It is the opinion of the Associate Referee that the method is basically satisfactory. However, experience with the method and close adherence to all details are essential in order to obtain consistent results. It is obvious that still further investigation is necessary before the procedure can be recommended for adoption. It is believed that further study will reveal the causes of the inconsistent results which have been obtained and that the method can be further revised so as to eliminate these difficulties.

The Associate Referee sincerely appreciates the assistance of the following collaborators:

Frank G. Locker, Chemist, San Francisco Station, Food and Drug Administration, San Francisco, Calif.

Matthew Dow, Chemist, St. Louis Station, Food and Drug Administration, St. Louis, Mo.

N. J. Halbrook, Chemist, Food and Drug Laboratory, Florida Dept. of Agriculture, Tallahassee, Fla.

RECOMMENDATIONS*

It is recommended—

(1) That the revised acetone method for the determination of chloride in the presence of large amounts of bromide and/or iodide, as presented in this report, be investigated further by the Associate Referee and submitted again to collaborators.

(2) That the volumetric cyanide method for the determination of

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 46 (1950).

iodide and bromide in the presence of chloride be compared with the aeration absorption method and that these methods be submitted to collaborators.

REPORT ON GLYCOLS AND RELATED PRODUCTS

By HARRY ISACOFF (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

In a previous report[1] the Associate Referee gave the details of preliminary work on a method of separation and determination of the glycols in a mixture.

During the interim period a number of investigators have evidenced interest in the separation of glycols from mixtures, notably G. L. Métayer[2] who has used various hydrocarbons in a codistillation procedure. The glycols are entrained by the hydrocarbons and collected in a Dean and Stark type tube under a water or air condenser. If the collecting tube is fitted with a stopcock the glycol can be easily drawn off and identified.

The work this year was directed toward a determination of the glycols, utilizing the methods described in the literature.[2] The experimental method consisted of the following procedure; a quantity of propylene glycol ranging from 2 ml to 5 ml was measured from a buret into a 250-ml flat-bottom Florence flask. To this was added 80 ml to 100 ml of the hydrocarbon entrainer, and the flask was connected to a condenser (either water or air condenser, depending on the boiling point of the hydrocarbon used for the test) employing a Dean and Stark type tube fitted with a stopcock for drawing off the entrained glycol. The mixture was heated on an electric hot plate or in a sand bath by a Bunsen flame, again depending on the boiling point of the hydrocarbon. The condensate was collected in the Dean and Stark type tube and, when it was apparent that most of the glycol had been recovered, the condenser was washed 3 or 4 times with 10 ml. portions of the hydrocarbon. The glycol was then drawn off and identified by several general tests.[3]

Applying this method the following results were obtained using as entrainers the hydrocarbons—

Cyclohexane	C_6H_{12}	B.P. 81°C
Tetralin	$C_{10}H_{12}$	B.P. 206°C
Decalin	$C_{10}H_{18}$	B.P. 190°C

The results obtained were very encouraging and the method described above gave reasonably fast and clean recoveries of propylene glycol. The

[1] Isacoff, H., *This Journal*, 30, 484 (1947).
[2] Métayer, G. L., *Ann. Chim.* (12) 2, 790–843 (1947).
[3] Orchin, M., *This Journal*, 26, 99–101 (1943).

TABLE 1.—*Experimental results*

ENTRAINER	PROPYLENE GLYCOL ADDED	OTHER MATERIALS ADDED	RECOVERED	TOTAL DISTILLATION TIME	IDENTITY TESTS
	ml	*ml*	*ml*	*hours*	
Cyclohexane	5		5	5½	positive
Cyclohexane	2		2	1.3 ml—1 hr. 2 ml—1 hr. } 2	positive
Cyclohexane	2.5	H_2O—2.5	5	4 ml—2 hrs. 5 ml—4 hrs. } 6	positive
Cyclohexane	4	H_2O—1	5	5 hrs.	positive
Cyclohexane	5	Sugar—10 (gm) glycerin—10 H_2O—15	18	17 ml—2 hrs. 18 ml—1 hr. } 3	glycerin—neg. glycol—pos.
Tetralin	5		5	1 hr.	positive
Tetralin	5		5	1 hr.	positive
Decalin	5		5	1 hr.	positive

recoveries from the sugar-glycerin-water mixture would be typical of the conditions encountered in the work on a commercial sample.

It is recommended* that the method be given further study.

REPORT ON PRESERVATIVES AND BACTERIOSTATIC AGENTS IN AMPUL SOLUTIONS

By CHARLES N. JONES (Federal Security Agency, Food and Drug Administration, New York, N. Y.), *Associate Referee*

The work done this year on this topic was (A) to complete the qualitative outline given in a previous report (1) by developing a qualitative method for mercury and (B) to begin a quantitative survey of the individual preservatives.

(A) In lieu of identifying separately the several organomercurials used as preservatives, it was felt that a qualitative test for mercury would suffice as a general test for this type of preservative. The following test depends upon the amount of mercury present to completely saturate the dithizone complex to form the orange yellow mercury dithizonate.

Treat about 5 ml of the ampul solution in a small flask with 5 ml of dilute hydrochloric acid and 5 drops of bromine. Heat on steam bath until excess bromine has evaporated. Transfer to separator, and extract with 25 ml of ether. Add saturated permanganate to the aqueous phase until the pink color persists, and decolorize by the careful addition of 20% hydroxylamine hydrochloride soln. Now add 10 ml of dithizone soln (1 mg/100 ml chloroform) and shake for several minutes. An orange-yellow color in the chloroform indicates mercury.

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 46 (1950).

(B) Phenol can be separated from all interfering substances by steam distillation from a slightly acid solution, following which the phenol may be determined quantitatively by, preferably, Millons reagent. This reagent is quite sensitive, and, when read on a spectrophotometer, is capable of good checks.

A quantitative determination of cresol is quite different from that of phenol. The U. S. Pharmacopoeia definition of cresol is "a mixture of isomeric cresols obtained from coal tar," and gives a boiling range of 195–205°. This would include the meta (199°) and para (201°) isomers, plus lesser amounts of the ortho (190°) and perhaps traces af phenol (182°). In order to determine such a mixture, each isomer must react similarly and quantitatively to give a total additive effect to the mixture. If one isomer gives a different result than the other two, then the reaction cannot be used for determination of the mixture. Various reactions were tried; some were investigated more thoroughly than others, especially those which showed promise. Tabulated below are the results obtained from several of these reactions on controls of phenol, ortho, meta, and para cresols.

REAGENT	PHENOL	ORTHO	META	PARA
Bromination, equivalents of bromine (2)	6	4	6	variable
Coupling with diazotized p-nitroaniline (3)	orange	orange	orange	red
Coupling with diazotized napththionic acid	yellow-orange	orange	orange	reddish orange
Melzer, benzaldehyde reaction as modified by Deichmann (4)	orange	red	brown	greenish
Alkali extract of chloroform solution	violet	purple	colorless	colorless
Folin Dennis (5)	bluish green	blue-green	blue	blue
Folin Dennis, modified by Chapin (6)	blue	blue	blue	blue

Although the last reaction gives the same color, the intensity is variable between the individual isomers, the para being very dark. None of these reactions can be used alone to determine a mixture.

The Associate Referee recommends* that (1) the separation and determination of phenol be subjected to collaborative study and (2) that work continue on the quantitative determination of cresols.

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(4) DEICHMANN, W. B., *Ind. Eng. Chem., Anal. Ed.*, **16**, 37 (1944).

* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 46 (1950).

(5) SNELL, F. D., "Colorimetric Methods of Analysis," D. Van Nostrand, 1937, Vol. 2, p. 348.
(6) CHAPIN, R. M., *J. Biol. Chem.*, **47**, 309 (1921).

No reports were given for microscopic test for alkaloids and synthetics; alkali metals; estrone and estrodial; and methyl alcohol.

REPORT ON NAVAL STORES

By V. E. GROTLISCH, Naval Stores Division (Production and Marketing Administration, Federal Security Agency, Washington, D.C.), *Referee*

From the viewpoint of the official agricultural chemist, the subject of "Naval Stores" would appear to be of rather limited interest or importance. While that term generally comprises all those products of a chemical nature derived from the resinous secretion of the pine tree, the term, so far as A.O.A.C. is concerned, is limited to two products, spirits of turpentine and rosin.

Aside from its industrial use as a solvent, as a component of paint and varnish vehicles, and as a chemical raw material, turpentine has many uses about the home, as a paint thinner, insect repellant, cleansing agent, and, medicinally, as an antiseptic agent and counterirritant. It is quite widely distributed and available for counter display and sale in small attractive sealed cans and bottles. The packers, as a rule, take pride in the purity of their product and the accuracy of their label statements.

Rosin is almost wholly an industrial raw material, used in the manufacture of paint and varnish, synthetic resins, protective coatings, soap, paper size, adhesives, plastics, and a great many other compounded products. The general public has no immediate interest in rosin. Analysis usually is made to determine compliance with specifications.

Under certain conditions, turpentine packed and sold in small containers, because of the statements and directions for use on the label, may be deemed to be a medicinal product, in which case it comes within the scope of the Federal Food, Drug, and Cosmetic Act. Additionally, the Federal Naval Stores Act regulates the sale and shipment of all turpentine and rosin sold in interstate commerce and is administered by the Naval Stores Division, Production and Marketing Administration, of the U. S. Department of Agriculture.

A relatively small number of the States have laws governing the adulteration or mislabeling of turpentine sold wholly within the State, usually as part of a paint law. Only Georgia and Florida have laws applicable to rosin and these cover primary inspection and grading of such material. The State laws usually assign the work of enforcement to agen-

cies related to Agriculture or Agricultural Chemistry, since these are often the only ones having suitable chemical personnel and laboratory facilities.

The adulteration or gross misbranding of turpentine in interstate commerce has been discouraged and greatly reduced as a result of the work of the Government in enforcing the Federal statutes. There are, however, occasional instances of adulteration of turpentine sold in bulk to paint and hardware stores entirely in intrastate commerce. Perhaps because of the relative unimportance of such matters in comparison with other more pressing work, it has not always been possible to induce the responsible officials to take prompt steps necessary to develop cases against violators of the State law.

The Referee on Naval Stores has not conducted any collaborative test work on methods of analysis for these two products during recent years with members of A.O.A.C., since such work is also done continuously by the American Society for Testing Materials, through its Committee on Naval Stores, of which the Referee is chairman. Producers, consumers, and members having a general interest are represented on the Committee, which also includes in its scope such other naval stores products as pine oil, pine tar, terpene hydrocarbons, tall oil, and rosin oil.

The present A.O.A.C. methods of analysis for turpentine and rosin have been adapted from the methods of the A.S.T.M. In the case of turpentine, both associations include tests for color or grade, specific gravity, refractive index, distillation, and polymerization residue with sulphuric acid, the latter being the standard test for adulteration with petroleum (mineral) spirits. The A.S.T.M. methods are also being extended to include new methods for evaporation residue and for acidity, in terms of water-soluble and water-insoluble acid number. These tests have assumed importance in connection with the keeping quality of turpentine in bulk storage, and its resistance to discoloration when packed and distributed in steel drums.

Much study is being given by the A.S.T.M. Committee on Naval Stores to the methods applicable to rosin. Recent developments along that line include the addition of electrometric titration procedures for the acid number and saponification number methods, applicable to the analysis of darker colored rosins; modifications in the unsaponifiable matter test; and a new method for iron content of rosin.

The methods of analysis adopted by the American Society for Testing Materials are widely distributed in printed form. The A.S.T.M. Book of Standards is issued every three years, and includes all official and tentative methods then in effect. The recommendations on revisions in existing methods and the text of proposed new tentatives, after adoption by letter ballot of the standing committee having jurisdiction, are included in a printed annual report of the committee, and are presented for discussion

and acceptance at the Annual Meeting of the Society. Such action is subject to confirmation by letter ballot of all society members who feel qualified to cast a ballot thereon. A new Book of Standards is scheduled for issue this year.

In view of the expanded scope of the work of the Association of Official Agricultural Chemists and the great increase in the number of test methods applicable to products that are of major interest to its membership, the question arises whether it is necessary to continue the section in the A.O.A.C. Book of Methods covering Naval Stores, *i.e.*, turpentine and rosin.

RECOMMENDATIONS*

Your Referee recommends that serious consideration be given by the Association to dropping "Naval Stores" from its agenda and eliminating the section on "Naval Stores" from the A.O.A.C. *Methods of Analysis.*

REPORT ON RADIOACTIVITY

By Louis Costrell (National Bureau of Standards, Washington, D.C.), *Referee*

The method **38.6–38.12** is widely accepted. It is recommended† that, as modified hereinafter, the method be adopted, first action. This modification eliminates the use of the electroscope constant and substitutes instead direct comparison between sample and standard, and will consist of taking readings on the standard and the sample in identical positions. This procedure will result in improved accuracy, since it makes the results independent of slow changes in the electroscope sensitivity and eliminates use of the inverse square law. The inverse square law does not hold up well at the close distances required for weak samples.

It is recommended† also that the standard deviation be used, instead of the probable error, in method **38.15.**

REPORT ON STANDARD SOLUTIONS

By H. G. Underwood (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Referee*

Potassium dichromate solutions.—The oxidimetric strengths of two commercial potassium dichromates (dried 2 hours at 100°C.) furnished by the Associate Referee were compared with that of the National Bureau of Standards standard sample (also dried 2 hours at 100°C.), by eight chemists in seven laboratories who titrated the iodine released from acidi-

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 44 (1950).
† For report of Subcommittee B and action of the Association, see *This Journal*, 33, 45 (1950).

fied potassium iodide solution with 0.1 *N* sodium thiosulfate. The values obtained for each commercial potassium dichromate checked within one part in one thousand. In view of the results, the Associate Referee recommends* that the procedure for preparing standard solutions of potassium dichromate by using the theoretical quantity of the National Bureau of Standards standard sample, or the equivalent amount of suitable commercial lots that have been compared for oxidimetric strength with the standard sample, be adopted as first action. The Referee concurs.

Sulfuric acid solutions.—The method for the standardization of sulfuric acid by the standard borax method (A.O.A.C. Methods **43.14** and **43.15**) was adopted as official, first action, by the Association last year. It is recommended* that the method be adopted as official.

Bromide-bromate solutions.—The procedure for standardization of bromide-bromate solutions against arsenious oxide reported in *This Journal*, **30,** 502 (1947), was adopted as official, first action, by the Association in 1947. It is recommended* that the method be adopted as official.

Standard buffer solutions.—No report was received and no further work is planned. The Associate Referee was closely associated with the work of the National Bureau of Standards which resulted in the preparation of three standard samples for *p*H value. These are standard sample 185, acid potassium phthalate, *p*H (approx.) 4.0, standard sample 186 consisting of potassium dihydrogen phosphate (186I) and disodium hydrogen phosphate (186II), *p*H (approx.) 6.8 when used together in equal molar proportions, and standard sample 187 borax, *p*H (approx.) 9.2. Since the work and theoretical considerations which serve as the background for the establishment of these *p*H standards do not lend themselves to collaborative study and since the National Bureau of Standards standard *p*H samples are recognized standards, it is recommended* that the methods for preparation of buffer solutions for calibration of *p*H equipment[1] be adopted as official. Details of the method are to be published in the 7th Ed., *Methods of Analysis* (1950).

*p*H VALUES FOR STANDARD BUFFER SOLUTIONS

The *p*H values of standard buffer solutions as a function of temperature are given in Table 1.

Hydrochloric acid solutions.—Since chemists have misinterpreted the intent of the last sentence in method **43.10**(d) of the sixth edition of *Methods of Analysis*, and since we are dealing with a hydrated salt, it is recommended* that "$Na_2B_4O_7$ in desiccator" in line 12 and "in desiccator" in line 15 of that section be replaced by "$Na_2B_4O_7.10H_2O$ in closed

* For report of Subcommittee A and action of Association, see *This Journal*, **33,** 43 (1950).

[1] *Proceedings, Am. Soc. Testing Materials*, **46,** 31–39 (1946); Nat. Bur. of Standards Certificates for Standard Sample 185, 186, and 187a.

* For report of Subcommittee A and action of the Association, see *This Journal*, **33,** 43 (1950).

TABLE 1

TEMPERATURE, °C.	0.05 *M* ACID POTASSIUM PHTHALATE	0.025 *M* PHOSPHATE	0.01 *M* BORAX
0	4.01	6.98	9.46
5	4.01	6.95	9.38
10	4.00	6.92	9.33
15	4.00	6.90	9.27
20	4.00	6.88	9.22
25	4.01	6.86	9.18
30	4.01	6.85	9.14
35	4.02	6.84	9.10
40	4.03	6.83	9.07
45	4.04	6.83	9.04
50	4.06	6.83	9.01
55	4.08	6.83	8.99
60	4.10	6.83	8.96

container" and "in closed container over soln satd with both sugar and NaCl," respectively.

Sodium thiosulfate solutions. Inasmuch as the National Bureau of Standards has issued a standard sample of potassium dichromate for oxidimetric value since method **43.29** of the sixth edition of *Methods of Analysis* was studied, it is recommended that the statement in line 1 of method **43.29** "(thrice recrystallized and dried at 200°)" be replaced by "(National Bureau of Standards standard sample dried 2 hrs. at 100°C.)."

New subjects.—Most of the standard solutions which are important and more frequently used in food, drug, and cosmetic analysis have been studied. It is recommended* that work on standard solutions be dropped until there is need for the standardization of additional solutions used in new or revised A.O.A.C. methods or there is reason for restudy of those already reported.

REPORT ON STANDARD POTASSIUM DICHROMATE

By GEORGE MCCLELLAN (U. S. Food and Drug Administration, Federal Security Agency, New Orleans, Louisiana),*
Associate Referee

In a previous A.O.A.C. report,[1] the Associate Referee presented a procedure for determining, within about one part in two thousand, the oxidimetric strengths of commercial analytical grade potassium dichromates in terms of the primary standard potassium dichromate supplied by the Bureau of Standards. Five such commercial dichromates selected at ran-

* Present address, San Juan, P. R., U. S. Collector of Customs.
[1] *This Journal*, 32, 587 (1949).

dom were analyzed by this procedure, and it was found that four of the five corresponded within 0.04 per cent with the strength of the Bureau of Standards product. It was concluded that these four dichromates were already so pure that any improvement by recrystallization probably could not be determined by volumetric assay. It was recommended that the analytical procedure be utilized in a collaborative study to determine the strengths of two or more commercial potassium dichromates in terms of the Bureau of Standards product, with the view that if collaborators should agree closely enough, a procedure for assaying laboratory stocks of potassium dichromate, rather than a routine procedure of recrystallization, might be incorporated in the *Methods of Analysis, A.O.A.C.* The recommended collaborative study has now been completed.

Details of the method are to be published in 7th Edition, *Methods of Analysis*, 1950.

COLLABORATIVE

To each of eight chemists in seven different laboratories were sent three 5-gram portions of $K_2Cr_2O_7$. The samples marked "B. of S." contained Bureau of Standards primary standard $K_2Cr_2O_7$. The samples marked "No. 1" and "No. 2" contained two brands of commercial analytical grade $K_2Cr_2O_7$. "No. 1" and "No. 2" had been assayed in duplicate by the Associate Referee in his previous study. Results at that time were:

ANALYST	PER CENT PURITY OF $K_2Cr_2O_7$ AS COMPARED WITH B. OF S. PRODUCT	
	No. 1	*No. 2*
George McClellan	99.94	100.00
New Orleans Distr.	99.90	100.00
Average	99.92	100.00

Six of the collaborators analyzed the samples in triplicate, the other in quintuplicate. All collaborators were members of the Food and Drug Administration.

CONCLUSIONS

Using the above method to assay commercial analytical potassium dichromates in triplicate, and averaging the three results, eight analysts in seven different laboratories were able to check each other within about eleven parts in ten thousand. Six of the analysts were in close agreement, checking each other within five parts in ten thousand. All of the analysts found Sample No. 1 to be significantly weaker than Sample No. 2.

Undoubtedly, the combination of pipet and buret used is an imperfect substitute for a properly constructed chamber buret.

In organizations having a central supply section, it might be advisable

COLLABORATORS	PER CENT PURITY OF $K_2Cr_2O_7$ AS COMPARED WITH B. OF S. PRODUCT			
	No. 1		*No. 2*	
George McClellan,	99.87		100.07	
New Orleans Distr.	99.96		100.00	
	99.92		99.95	
Average		99.92		100.01
Howard P. Bennett,	99.92		100.07	
New Orleans Distr.	99.97		100.01	
	99.94		100.00	
Average		99.94		100.03
Sam H. Perlmutter,	99.93		99.97	
Minneapolis District	99.94		99.98	
	99.93		99.98	
	99.92		99.98	
	99.91		99.99	
Average		99.93		99.98
Luther G. Ensminger,	99.81		99.91	
Cincinnati District	99.82		99.92	
	99.86		99.98	
Average		99.83		99.94
Floyd E. Yarnall,	99.91		100.04	
Kansas City District	99.88		99.99	
	99.90		99.99	
Average		99.90		100.01
Mrs. Phyllis B. Rokita,	99.89		99.92	
Atlanta District	99.89		99.94	
	99.88		99.91	
Average		99.89		99.92
Matthew L. Dow,	99.90		99.93	
St. Louis District	99.94		100.00	
	99.93		100.00	
Average		99.92		99.98
T. N. Bennett,	99.91		99.99	
New York District	99.90		100.00	
	99.93		99.96	
Average		99.91		99.98

to equip one laboratory with a custom-built chamber buret, and assign to a single analyst the task of assaying each batch of dichromate purchased. Acceptance of the dichromate could possibly be made conditional on its yielding an assay of between 99.95 and 100.05 per cent in terms of the Bureau of Standards article.

RECOMMENDATIONS*

It is recommended—

(1) That the proposed procedure be adopted, first action.

(2) That this subject be discontinued.

ACKNOWLEDGMENT

This idea of using a combination of pipet and micro-buret in lieu of a chamber buret is due to John A. Thomas, of the New Orleans District of the Food and Drug Administration.

* For report of Subcommittee A and action of the Association, see *This Journal*, 33, 43 (1950).

MONDAY—AFTERNOON SESSION

REPORT ON FERTILIZERS

By F. W. QUACKENBUSH (Purdue University Agricultural Experiment Station, Lafayette, Indiana), *Referee*

Acid and Base-forming Quality.—The Associate Referee did not report.

Boron.—The recommendations of the Associate Referee are approved as follows:

1. That the present official method 2.44 be deleted.
2. That the "Identical *p*H" Methods of the Associate Referee, as presented in the 1949 Report, be adopted as first action.

Copper and Zinc.—The Associate Referee did not report.

Inert Materials.—The Associate Referee did not report.

Magnesium and Manganese.—The recommendations of the Associate Referee are approved.

Moisture.—The recommendations of the Associate Referee are approved.

Nitrogen.—The recommendations of the Associate Referee are approved.

Phosphorus.—The recommendations of the Associate Referee are approved.

Potash.—The recommendations of the Associate Referee are approved.

Sampling.—Interest in sampling problems remained high during the year. Seven states have participated in the sampling project which was begun last year. The results of this study provide a statistical basis for the number of bags to be sampled from a lot as stated in the recommendations of the Associate Referee.

The recommendations of the Associate Referee are approved.

Sulphur.—The Associate Referee did not report.

REPORT ON SAMPLING FERTILIZERS*

By H. R. ALLEN (Kentucky Agricultural Experiment Station, Lexington, Kentucky), *Associate Referee*

This report consists chiefly of a preliminary investigation on reducing the inspection sample to the desired size and on preparation of sample for analysis. Very little work on this phase of sampling has been published. Ross, Rader, and Hardesty (1) reported on fineness of particle size for the laboratory sample but did not investigate preparation of different amounts of sample.

After a sample has been obtained from a number of bags it must be

* This investigation, made in connection with a project of the Kentucky Agricultural Experiment Station, is published by permission of the Director.

mixed and reduced to the desired size for the laboratory. Assuming this size is 2 pounds, or about 1 quart, this study attempts to furnish preliminary data to answer the following questions: What variation would result if another quart is obtained in place of the one selected? What variation would result from grinding for analysis varying amounts of the quart sample and what variation would be caused by grinding varying amounts of sample without sieving, in place of sieving through a 10-mesh sieve according to A.O.A.C. procedure?

EXPERIMENTAL

Two grades of mixed fertilizer, 5-10-10 and 4-12-8, were used in this study. The materials in the 5-10-10 fertilizer were nitrogen solution 2 A, ammonium nitrate, ammonium sulfate, muriate of potash, superphosphate, dolomitic limestone, and ground peanut hulls. In addition to the materials listed above, except ammonium sulfate, the 4-12-8 fertilizer contained sulfate of potash-magnesia, sulfate of potash, triple superphosphate, castor meal, and process tankage.

The 5-10-10 fertilizer consisted of 12 per cent of material coarser than 10-mesh, 15 per cent between 10 and 20-mesh, and 73 per cent finer than 20-mesh. The 4-12-8 grade contained 17 per cent of material coarser than 10-mesh, 22 per cent between 10 and 20-mesh, and 61 per cent finer than 20-mesh. The 5-10-10 grade contained 4.90 per cent moisture and the 4-12-8 grade contained 5.57 per cent, on the basis of 5 hours drying at 100°C.

About 20 pounds of each of these mixtures were in turn mixed on a large oil-cloth, the mixture was spread out evenly and portions obtained to fill 6 1-quart Mason jars. The portions were obtained for each jar by inserting an aluminum boat with flat bottom under the fertilizer at a number of places on the outside and at several places in the center of the mixture. After several jars were filled, the mixture was re-mixed, spread out, and the other jars filled.

Size of sample ground.—All samples were ground in the Mikro-Samplmill. Size of sample varied from 3 oz. to 1 pound ($\frac{1}{2}$ the quart sample). Material in quart No. 1 was mixed and two 1-pint portions obtained. The first pint sample was taken as the quart samples described above and the second pint sample was composed of the remainder. Quart No. 2 was a duplicate of No. 1. From quart No. 3 two $\frac{1}{2}$-pint samples were prepared. Material from quart No. 4 was mixed and divided into two equal parts. Each part was passed through a 10-mesh sieve, the coarser than 10-mesh material was ground in a mortar, mixed with the finer material, and a 3 oz. sample obtained for grinding. This is the A.O.A.C. procedure. From one-half of the fertilizer in quart No. 5, two 3 oz. samples were obtained. The other half was ground as a whole. Quart No. 6 was a duplicate of No. 5.

Samples were analyzed for nitrogen, total phosphoric acid, and potash.

Results are shown in Table 1. All nitrogen results and other results as indicated in the table are averages of two analyses.

Since potash results on samples from quarts Nos. 1 and 3 of the 5-10-10

TABLE 1.—*Comparison of analyses from different amounts of sample prepared from quart portions of mixed fertilizer*

(Results in per cent)

QUART NUMBER	SIZE OF SAMPLE GROUND	4-12-8 GRADE			5-10-10 GRADE		
		N*	TOTAL P_2O_5	K_2O	N	TOTAL P_2O_5	K_2O
1	1 lb.	4.20	13.30	7.97	5.26	11.57*	9.65*
1	1 lb.	4.18	13.25	8.02	5.30	11.55	9.86*
2	1 lb.	4.20	13.30	7.92	5.32	11.50	9.91
2	1 lb.	4.18	13.30	7.93	5.35	11.35	9.93
3	½ lb.	4.19	13.25	7.95	5.31	11.35	10.03*
3	½ lb.	4.18	13.25	7.96	5.25	11.50*	9.70*
4	3 oz.†	4.23	13.25	7.84	5.31	11.55	9.78
4	3 oz.†	4.20	13.27*	7.82	5.31	11.50	9.86
5	3 oz.	4.20	13.30	7.97	5.25	11.60	9.88*
5	3 oz.	4.20	13.40	7.88*	5.22	11.60	9.68*
5	1 lb.	4.22	13.40	7.90*	5.28	11.40*	9.90*
6	3 oz.	4.20	13.25	8.02	5.28	11.50	9.82*
6	3 oz.	4.20	13.30	7.95	5.27	11.50	9.91
6	1 lb.	4.23	13.35	7.99	5.29	11.50	9.82*
7	1 lb.						9.87
7	1 lb.						9.76
8	1 lb.						9.79
8	1 lb.						9.78
9	1 lb.						9.83
9	1 lb.						9.91
Maximum variation		0.05	0.15	0.20	0.10	0.25	0.38

* All nitrogen analyses, and others as indicated, are averages of two determinations.
† 1-pound sample thru 10-mesh, coarse material ground, mixed with fine material, and a 3-oz. sample taken.

grade were not in close agreement, three more quart portions of this grade were obtained. Quart No. 7 was divided into two 1-pint samples by the procedure used on quarts 1 and 2. Material in quart No. 8 was mixed, spread out, and divided into 2 equal parts to provide two 1-pint samples. Material in quart No. 9 was mixed and divided into two 1-pint samples

by the Jones Sample Riffle (2). Analyses of these samples are found in Table 1.

COMPARISON OF SINGLE-TUBE AND DOUBLE-TUBE SAMPLERS

Studies previously reported (3) showed no significant differences in analyses of samples obtained with the single-tube and double-tube samplers. It was pointed out that there should be no difference if the fertilizers sampled were uniform in composition. However, there was evidence of some non-uniformity in some of the samples studied.

This season several shipments were found in Kentucky of a 27-0-0 grade composed of urea with peanut hulls added. The urea varied in particle size from fine to coarser than 4-mesh. The peanut hulls also varied greatly in particle size. These conditions and the high nitrogen content made it difficult to keep this mixture uniform in composition, so it was decided to sample some of these shipments with both the single-tube and

TABLE 2.—*Nitrogen content of samples of 27-0-0, urea with peanut hulls, sampled with single-tube and double-tube samplers*

FERTILIZER STOCK	SINGLE-TUBE SAMPLER	DOUBLE-TUBE SAMPLER
Number	*per cent N**	*per cent N**
1	27.97	28.10
2	25.92	26.00
2†	25.10	25.07

* Analyses are averages of 2 or more analyses.
† Sample from another group of 10 bags in the same stock.

double-tube samplers. Two shipments were sampled, 10 bags being sampled in one shipment and two groups of 10 bags each in the other. The same bags were sampled with each sampler from approximately the same place in the bag. The order of the sampler used first was reversed after each bag was sampled. The whole sample (about 2 quarts) was reduced in particle size in a mortar or with a spatula, mixed, and a 1-pint sample ground for analysis. Results in Table 2 show no significant difference in use of the two samplers.

DISCUSSION OF RESULTS

Results cannot be conclusive because only two mixed fertilizers were studied. However, uniform results should be more difficult to obtain with these two fertilizers than with the average fertilizer because of the number of materials in each and because of wide differences in particle size.

Results indicate it is not difficult to obtain a quart or 2-pound sample from 15 or 20 pounds of a mixed fertilizer that is representative of that amount within the limits of chemical analysis, and that analyses obtained from grinding $\frac{1}{2}$ to 1 pound of sample without sieving are at least as ac-

curate as analyses obtained from first sieving whole sample through 10-mesh.

On the 4-12-8 grade all analyses from the different quart samples were very uniform except potash analyses from quart No. 4, where the A.O.A.C. procedure of sieving through 10-mesh was used. Again, on the 5-10-10 grade analyses are uniform except potash analyses from quarts 1 and 3 and from one 3 oz. sample of quart No. 5.

Quarts 7, 8, and 9 from the 5-10-10 grade were prepared after the analyses of the original quarts were obtained and results for potash on these samples were in line with results from the original sampling. It appears that the procedure for quart No. 8 may be preferable to that for quarts 1, 2 and 7. However, the latter procedure has been used in this laboratory for a year with very satisfactory results. On analyses that were below guarantee many re-check analyses have been made from the remainder of the quart reserve portion. Close duplication with original analyses resulted in nearly all cases.

Surprisingly, results from the 3 oz. samples checked well with results from samples where a larger amount of sample was prepared.

ACKNOWLEDGMENT

Analyses reported in this study were made by Lelah Gault, Elizabeth Swift, Wendell Kingsolver, and the writer. The fertilizers were obtained through the kindness of S. F. Thornton of the F. S. Royster Guano Company.

SUMMARY

This is a preliminary study of reducing the inspection sample to the desired size and of preparation of sample for analysis. Results from different quart portions of two grades of mixed fertilizer indicate it is not difficult to obtain a quart portion from 15 or 20 pounds of fertilizer that is representative of that amount. Results also show that analyses obtained from grinding ½ to 1 pound of sample without previous sieving are at least as accurate as analyses obtained from first sieving whole sample through 10-mesh, the A.O.A.C. procedure.

A comparison of single-tube and double-tube samplers was made by analyses of samples taken with each type of sampler of a 27-0-0 grade composed of urea with peanut hulls added. No significant differences were found.

RECOMMENDATIONS*

It is recommended—

(1) That in sec. **2.1,** line 3, after "material" the words "but preferably 2 pounds" be inserted.

(2) That in the procedure for sampling, **2.1,** the directions beginning

* For report of Subcommittee A and action of the Association, see *This Journal,* **33,** 37 (1950).

with "Remove a core" be changed to read: "Remove a core diagonally from end to end through the bag lying horizontally. Take cores as follows: From a lot of 1 to 10 bags, sample all bags; from a lot of 11 to 20 bags, sample 10 bags; from a lot of 21 to 40 bags, sample 15 bags; from a lot of 41 or more bags, sample 20 bags. Take one core from each bag sampled, except that for lots of 1 to 4 bags, take enough cores from each bag—following the same path—to total 5 or more cores."

(3) That the directions for sampling fertilizer in bulk lot and in small containers, recommended *This Journal*, **31**, 71, be retained.

(4) That the final sentence in section **2.1** be changed as follows: "Preferably send all cores to the laboratory for quartering. Thoroughly mix portions taken on clean oil-cloth or paper, reduce by quartering to quantity of sample required, and place in airtight container."

(5) That in sec. **2.2**, line 2, after the second sentence this sentence be inserted: "Alternate procedure—grind not less than ½ pound of the reduced sample without previous sieving."

(6) That investigation of sampling and preparation of sample be continued.

LITERATURE CITED

(1) Ross, William H., Rader, L. F. Jr., and Hardesty, John O., *This Journal*, **24**, 253 (1941).
(2) Fisher Scientific Company, catalog, p. 187.
(3) Allen, H. R., *This Journal*, **31**, 205 (1948), and **32**, 192 (1949).

REPORT ON PHOSPHORIC ACID IN FERTILIZERS: COMPARISON OF NEUTRAL AMMONIUM CITRATE AND TWO PER CENT CITRIC ACID SOLUTIONS AS SOLVENTS FOR ALPHA PHOSPHATE AND PHOSPHATE ROCK-MAGNESIUM SILICATE GLASS*

By K. D. Jacob, *Associate Referee*, R. M. Magness, and C. W. Whittaker (Division of Fertilizer and Agricultural Lime, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland)

Confirming the results of previous investigations (8, 10, 13, 14, 15, 26) the data of the collaborative study reported at the preceding meeting of this Association (17) show conclusively that the solubility of the P_2O_5 of basic slag in neutral ammonium citrate differs but little from its solubility in 2 per cent citric acid, as determined by the procedures outlined in the *Methods of Analysis*, 1945, pp. 23–25. In general, the difference is even less, sometimes markedly so, with continuous agitation during the citrate digestion (13, 14, 17)—an alternative procedure which is now of-

* Presented at the 63rd Annual Meeting of the Association of Official Agricultural Chemists, held in Washington, D. C., October 10–12, 1949.

ficial.[1] On the basis of this study it was recommended (17) and approved (first action)[2] that basic slag be evaluated by means of neutral ammonium citrate instead of 2 per cent citric acid.

Pursuant to recommendations approved at the 1948 and several preceding meetings of this Association, collaborative study of neutral ammonium citrate and 2 per cent citric acid as solvents for furnace-made phosphates was extended to alpha phosphate[3] and phosphate rock-magnesium silicate glass.[4]

PREVIOUS INVESTIGATIONS

Neutral ammonium citrate and 2 per cent citric acid are compared as solvents for numerous samples of alpha phosphate and related materials in the report on phosphoric acid presented at the 1946 meeting of this Association (16). In general agreement with other investigations (8, 10, 11, 13, 14, 15, 18, 20, 26), the results of this non-collaborative study usually show only small differences in the action of the two solvents on finely ground, low-fluorine products (Table 1, Items 13–20), *alpha* tricalcium phosphate (Item 7), and silicocarnotite (Item 8), while the differences are usually much greater in the case of partially defluorinated materials (Items 9–12), natural phosphates (Items 1–4), hydroxylapatite (Item 5), and *beta* tricalcium phosphate (Item 6). As with basic slag, there is evidence that continuous agitation during the citrate digestion, compared with shaking at 5-minute intervals, increases somewhat the solvent action of neutral ammonium citrate on alpha phosphate (13, 14, 19, 20).

Published information on the solubility of phosphate rock-magnesium silicate glass in ammonium citrate and citric acid solutions is limited. The data (8, 10, 14) indicate, however, that the factors influencing the action of these solvents on such material operate in much the same way as with basic slag and alpha phosphate.

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[1] Adoption of continuous agitation during the citrate digestion, as an alternative procedure, was recommended (14) and approved by Subcommittee A (first action) at the 1947 meeting of this Association (*This Journal*, **31**, 42 (1948), Fertilizers, Recommendation No. 3). It was recommended (17) and approved (final action) at the 1948 meeting (*This Journal*, **32**, 43 (1949), Fertilizers, Recommendation 7). Through an oversight the first-action recommendation was omitted from the published proceedings of the 1947 meeting (*This Journal*, **31**, 71 (1948), Fertilizers), and consequently the final action was incorrectly reported as first action in the published changes in methods made at the 1948 meeting (*This Journal*, **32**, 71 (1949), Fertilizers, Item 3).

[2] *This Journal*, **32**, 43 (Fertilizers, Recommendations 8–10), 72 (Fertilizers, Items 4–6) (1949).

[3] The term *alpha phosphate* (16) is used to denote the products obtained by heating natural calcium phosphates, usually phosphate rock, at temperatures above 1300°C in the presence of silica and water vapor for the purpose of volatilizing the fluorine and converting the P_2O_5 into plant available forms (2, 7, 15, 21, 25, 26, 30), chiefly *alpha* tricalcium phosphate. It does not include the product (all glass) obtained by fusing a mixture of phosphate rock and magnesium silicate or low-fluorine phosphate of the Rhenania type. The term now embraces three types of materials—fused, sintered, and calcined—all of which are frequently called "defluorinated phosphate rock" or "defluorinated phosphate." While fused alpha phosphate is currently called "fused tricalcium phosphate," it has also been designated as "fused rock phosphate," "fused phosphate rock," and "fused phosphate." Sintered alpha phosphate is marketed under the name of "Coronet defluorinated phosphate," but it has also been called "calcined phosphate." Calcined alpha phosphate has not been produced on a commercial scale; it has generally been called "calcined phosphate." The composition, properties, and solubility of the alpha phosphates are discussed in the report of the Associate Referee on Phosphoric Acid presented at the 1946 meeting of this Association (16), and in other papers (3, 8, 9, 21, 22).

[4] Also known as "calcium-magnesium phosphate," "Thermo-Phos," and "MP Phosphate." The preparation and properties of this type of material are discussed in several recent papers (4, 6, 8, 10, 23, 24, 29).

TABLE 1.—*Summary of some published solubility data for alpha phosphate and related materials*[a]

ITEM NO.	MATERIAL[b]	SAMPLES	FLUORINE		TOTAL P_2O_5		FRACTION OF TOTAL P_2O_5 EXTRACTED					
							BY NEUTRAL AMMONIUM CITRATE[c]		BY 2% CITRIC ACID		DIFFERENCE	
			RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE[d]	AVERAGE
		number	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	Fluorapatite, mineral[e]	2	2.83–3.08	2.95	39.30–39.50	39.45	1.7– 2.1	1.9	6.0– 8.4	7.2	4.3– 6.3	5.3
2	Phosphate rock, United States[f]	15	3.18–3.96	3.65	27.90–37.94	33.04	4.8–12.7	7.4	14.2–25.3	19.5	8.7–16.5	12.1
3	Land pebble and brown rock	7	3.18–3.92	3.75	30.17–36.47	32.96	5.3–12.7	8.5	17.4–25.3	20.1	8.7–14.1	11.6
4	Waste-pond phosphate, Florida[g]	2	2.01–2.20	2.11	19.83–25.31	22.57	9.7–11.1	10.4	27.2–40.3	33.7	17.5–29.2	23.3
5	Hydroxylapatite	2	0.00–0.00	0.00	41.40–42.10	41.75	21.8–24.3	23.0	58.1–58.1	58.1	33.8–36.3	35.1
6	*Beta* tricalcium phosphate	5	0.00–0.13	0.03	44.90–46.30	45.44	39.5–53.5	44.9	64.9–90.6	79.7	25.4–42.4	34.8
7	*Alpha* tricalcium phosphate	3	0.00–0.02	0.01	45.10–46.30	45.63	71.0–95.7	85.3	75.0–97.1	88.1	1.4– 4.0	2.8
8	Silicocarnotite	1	—	0.00	—	29.00	—	95.9	—	100.0	—	4.1
9	Partially defluorinated rock P_2O_5:F <22.4	6	1.02–2.08	1.42	21.05–33.88	24.15	5.3–27.0	13.0	16.8–36.8	24.4	9.6–13.7	11.4
10	Sintered material	4	1.02–1.56	1.30	21.05–21.53	21.31	5.3–27.0	13.6	19.0–36.8	25.8	9.8–13.7	12.2
11	Fused material	1	—	1.25	—	25.80	—	17.1	—	26.7	—	9.6
12	Calcined material	1	—	2.08	—	33.88	—	6.8	—	16.8	—	10.0
13	Alpha phosphate, P_2O_5:F >22.4	41	0.02–1.49	0.33	19.88–37.18	28.04	20.6–95.0	77.1	27.7–96.9	80.6	−0.9– 8.6	3.5
14	Sintered product	14	0.02–0.43	0.17	19.88–24.04	21.50	71.3–95.0	85.8	74.1–96.5	88.5	−0.9– 4.8	2.7
15	Fused product	14	0.02–1.11	0.38	25.75–30.50	28.59	29.8–95.0	72.5	36.0–96.9	76.8	−0.6– 8.6	4.3
16	Calcined product	13	0.09–1.49	0.43	32.95–37.18	34.49	20.6–92.7	72.6	27.7–94.3	76.2	1.6– 7.1	3.6
17	Alpha phosphate, F <0.5 per cent	35	0.02–0.49	0.22	19.88–37.18	27.28	63.4–95.0	82.9	71.3–96.9	86.1	−0.9– 8.6	3.2
18	Sintered product	14	0.02–0.43	0.17	19.88–24.04	21.50	71.3–95.0	85.8	74.1–96.5	88.5	−0.9– 4.8	2.7
19	Fused product	11	0.02–0.47	0.24	25.75–30.28	28.13	63.4–95.0	81.4	72.0–96.9	85.1	−0.6– 8.6	3.7
20	Calcined product	10	0.09–0.49	0.26	32.95–37.18	34.44	66.8–92.7	80.5	71.3–94.3	83.8	1.6– 7.0	3.3

[a] Results given by Jacob, Ward, Hill, and Pinkerton (16).

[b] The samples of fluorapatite, phosphate rock, and waste-pond phosphate were ground to pass the 100-mesh sieve, and the results are on the moisture free basis (105°C.). Of the 47 samples of partially defluorinated phosphate rock and alpha phosphates, 38 were ground to pass the 150-mesh sieve, 6 to pass the 100-mesh sieve, 2 to pass the 80-mesh sieve, and 1 to pass the 200-mesh sieve. The other samples were ground to pass the 100- or 150-mesh sieve.

[c] Manual shaking at 5-minute intervals during the citrate digestion.

[d] The minus sign denotes that the result for citric acid-soluble P_2O_5 is lower than that for citrate-soluble P_2O_5.

[e] Nelson County, Virginia, and the Kola Peninsula, U.S.S.R.

[f] Florida land pebble 4, Florida hard rock 2, Tennessee brown rock 3, Tennessee blue rock 2, Idaho 2, and Montana phosphate 2 samples.

[g] Commonly called "colloidal phosphate," and designated by the Association of Official Agricultural Chemists as "soft phosphate with colloidal clay" (*Methods of Analysis*, 6th Ed., p. 901).

SAMPLES

The samples submitted to the collaborators are listed in Table 2, which also shows the total P_2O_5 and the fluorine content of the materials. Table 3 gives the mechanical composition of the samples as used in the investigation. In the mechanical analyses 50-gram samples, in 8-inch sieves, were shaken for 30 minutes in the Ro-Tap machine.

TABLE 2.—*Samples for collaborative study of solubility of phosphorus in neutral ammonium citrate and 2 per cent citric acid solutions*

SAMPLE	MATERIAL	TOTAL P_2O_5		FLUORINE
		RANGE[a]	AVERAGE	
		per cent	*per cent*	*per cent*
1	Sintered alpha phosphate	19.95–20.99	20.41	0.05
2	Sintered alpha phosphate	19.44–20.30	19.89	0.25
3	Fused alpha phosphate	27.85–28.67	28.34	0.02
4	Fused alpha phosphate	28.92–29.87	29.53	0.30
5	Fused alpha phosphate	28.95–29.85	29.55	0.30
6	Phosphate rock-magnesium silicate glass	20.50–21.07	20.75	1.92
7	Phosphate rock-magnesium silicate glass	19.66–20.77	20.42	1.91
8	Phosphate rock-magnesium silicate glass	21.24–22.45	22.12	2.55

[a] Averages of triplicate determinations on each sample by 19 collaborators. Does not include Collaborators 1, 5, 8, and 15.

TABLE 3.—*Mechanical composition of samples as used in the investigation*

SAMPLE	COMPOSITION, MESH[a]						
	−35	−60	−80	−100	−150	−200	−300
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	100.0	100.0	100.0	99.9	89.6	74.6	50.5
2	100.0	100.0	100.0	99.9	89.1	64.4	45.7
3	100.0	99.7	98.9	96.9	88.7	76.8	61.0
4	100.0	90.4	64.2	50.0	39.2	27.1	16.3
5	100.0	100.0	100.0	99.4	78.9	60.4	43.1
6	53.6[b]	17.8	7.8	4.0	1.6	0.4	0.2
7	99.4	98.8	98.4	97.8	96.2	89.2	46.4
8	99.8	98.5	96.0	91.9	84.7	69.3	27.6

[a] Screen openings in sieve series are 420, 250, 177, 149, 104, 74, and 46 microns, respectively.
[b] Portions of sample passing screens of 6, 9, and 20 mesh (3,327, 2,000, and 840 microns) are 100, 99.9, and 95.1%, respectively.

Sintered Alpha Phosphate.—Samples 1 and 2 are from commercial products made from Florida land-pebble phosphate at Plant City, Fla., and West Conshohocken, Pa., respectively, by the Coronet Phosphate Company. Sample 1 was produced in 1946 and Sample 2 in 1944. Both samples were ground in the laboratory.

Fused Alpha Phosphate.—Those samples (Nos. 3–5) are from pilot-plant products made from Tennessee brown-rock phosphate in 1943 and

1944 by the Tennessee Valley Authority, Wilson Dam, Ala. Sample 3 is laboratory-ground material. Sample 4 is from −35-mesh material as distributed by the Authority. Sample 5 is a laboratory grind of Sample 4.

Phosphate Rock-Magnesium Silicate Glass.—These samples (Nos. 6–8) are from commercially produced and ground materials made from western phosphate rock. They were not subjected to further grinding or other treatment for use in the investigation. Samples 6 and 7 were made by the Permanente Metals Corporation, Permanente, Calif., in 1946 by fusing phosphate rock with serpentine. Sample 8 was made by Manganese Products, Inc., Seattle, Wash., in late 1948 or early 1949 by fusing phosphate rock with olivine.

COLLABORATORS' DIRECTIONS FOR ANALYSIS

(1) Determine total P_2O_5 by the volumetric method as directed in *Methods of Analysis, A.O.A.C.*, 1945, p. 23, sec. **2.12(a)** or **(b)**. Prepare the solution as directed on pp. 21–22, sec. **2.8(a)**.

(2) Determine citrate-insoluble P_2O_5 as directed on p. 24, sec. **2.16(b)**. Dissolve the citrate-insoluble residue as directed in sec. **2.8(a)** and determine P_2O_5 as directed in sec. **2.12(a)** or **(b)**.

(3) Repeat the determination of citrate-insoluble P_2O_5 as follows: Proceed as directed in sec. **2.16(b)** through the point where the flask is first shaken vigorously to reduce the filter paper to a pulp. Next place the flask in a continuous agitation apparatus provided with means for maintaining the contents of the flask at 65°C., agitate for exactly 1 hour from the time the sample was introduced into the flask. Then proceed with the determination as before. Only those collaborators having access to continuous agitation, constant temperature devices are requested to determine citrate-insoluble P_2O_5 by the procedure outlined in this paragraph.

As the materials, especially the phosphate rock-magnesium silicate glass, tend to have hydraulic properties, it is important that the sample for the citrate digestion be placed on a *dry* paper and that agitation of the flask's contents be such as to effect thorough dispersion of the sample in the citrate solution. Otherwise high values for citrate-insoluble P_2O_5 may be obtained as a result of caking of the sample.

(4) Digest the sample with citric acid solution as directed on p. 25, sec. **2.18**, using one of the following modifications. Filter immediately after the citric acid digestion and wash the insoluble residue with 200 ml. of water (room temperature) in small portions and with thorough draining between additions. Dissolve the insoluble residue as directed in sec. **2.8(a)**, and determine P_2O_5 as directed in sec. **2.12(a)** or **(b)**. In this way a direct comparison of the results for citrate-insoluble and citric acid-insoluble P_2O_5 is had independently of the values for total P_2O_5.

Prepare the citric acid solution to contain exactly 2 per cent of citric acid monohydrate ($C_6H_8O_7 . H_2O$) by weight. It is important that the temperature of the citric acid solution be adjusted to 17.5°C. just prior to use; that caking of the sample during addition of the citric acid solution be avoided; and that the citric acid extract be filtered immediately after the digestion is completed.

Modification I. Make the citric acid digestion with the aid of an end-over-end agitation apparatus (20–50 r.p.m.) and a wide-mouth, 230 ml. volumetric "fertilizer" flask, using a 2.5-gram sample, 2.5 ml. of alcohol, and sufficient citric acid solution to give a total flask content of 250 ml. As the 500-ml. Wagner flask specified by the official method is not commonly available in the fertilizer laboratories of the United States, this deviation from the official procedure is permissible because it has

been shown (18, 20) that with a constant ratio of weight of sample to volume of citric acid solution the results are not affected by variations in the sample weight. Also, it has been shown (18) that with end-over-end agitation in the range of 21–52 r.p.m. the results are not dependent on the speed of rotation of the flask.

Modification II. If an end-over-end agitation apparatus is not available, make the citric acid digestion (a) with the aid of continuous stirring or (b) with the use of a shaking apparatus, as, for example, the Ross-Kershaw machine or the Fisher "Gyrosolver." In such cases, add 1 ml. of alcohol and 99 ml. of citric acid solution to 1 gram of the sample in a 250-ml. beaker for (a) or a 250-ml. "fertilizer" flask for (b).

(5) Make all the determinations in triplicate, each on a separate portion of the sample as submitted, and report the individual results on the form enclosed with these Directions. If for any reason it is necessary to repeat a determination the repetition should be made in triplicate and the three results reported should be those obtained in simultaneous replications.

(6) Your comments and observations concerning this investigation are requested.

COLLABORATORS

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PROCEDURES

For comparison with results by manual shaking at 5-minute intervals during the citrate digestion, ten of the collaborators determined citrate-

TABLE 4.—*Type of continuous agitation apparatus and weight of sample used in determining citric acid-insoluble P_2O_5*

COLLABORATOR	APPARATUS		WEIGHT OF SAMPLE[a]
	TYPE	REVOLUTIONS OR OSCILLATIONS PER MINUTE	
			grams
2, 7, 12			
16, 19, 20	End-over-end[b]	20–22	2.5[c]
9	End-over-end	32	5.0
11	End-over-end	14	5.0
17	End-over-end	37	2.5
21	End-over-end	18	1.0
3	Stirring	1,150	1.0
4	Stirring[d]	670	1.0
10	Stirring[e]	600	1.0
22	Stirring[f]	300	1.0
14	Ross-Kershaw shaker[g]	180	1.0
6	Kahn-type shaker[h,i]	170	1.0
13	Kahn-type shaker[h]	45	1.0
18	Fisher "Gyrosolver"[j]	200	1.0
23	Burrell shaker[k]	240	1.0
5	Manual agitation	125	1.0

[a] In all cases the ratio of sample weight to solvent volume was 1 gram per 100 ml.
[b] MacIntire-Marshall-Meyer type (19). Catalog No. 5960, Precision Scientific Co., Chicago, Ill.
[c] Except Collaborator 16, 1 gram.
[d] Stirred with rod, 6 mm diameter with a right-angle bend 22–23 mm long, reaching close to bottom of beaker.
[e] Eberbach apparatus; Eberbach & Son, Ann Arbor, Mich.
[f] Blair apparatus; Catalog No. 9236, Arthur H. Thomas Co., Philadelphia, Pa., 1931.
[g] Eccentric rotary movement in the horizontal plane (28); Catalog No. 30873, Eimer & Amend, New York, N. Y., 1936.
[h] Straight-line oscillation in the horizontal plane.
[i] Catalog No. 8926-A, Arthur H. Thomas Co., Philadelphia, Pa., 1931.
[j] Movement in 3 planes; Catalog No. 14–258, Fisher Scientific Co., Pittsburgh, Pa., 1942.
[k] Catalog No. A75–770, Burrell Technical Supply Co., Pittsburgh, Pa., 1941.

insoluble P_2O_5 with the aid of continuous agitation, constant-temperature devices. For this purpose Collaborators 2, 7, 8, 12, 16, 19, and 20 used the end-over-end rotation (20–22 r.p.m.) apparatus described by MacIntire, Marshall, and Meyer (19). Collaborator 21 used an end-over-end apparatus made in the laboratory shop. Collaborator 3 employed con-

tinuous stirring at 1,525 r.p.m. Collaborator 23 used the Burrell "wrist action" shaker at 240 oscillations per minute.

The determinations of citric acid-insoluble P_2O_5 (not done by Collaborators 1, 8, and 15) were made with the aid of the several types of continuous agitation devices listed in Table 4 which also shows the weight of sample used. In this study citric acid-insoluble P_2O_5 was determined directly, rather than by difference between total and citric acid-soluble P_2O_5. Consequently, the results on a given sample, like those for citrate-insoluble P_2O_5, are not subject to the variations in the collaborators' figures for total P_2O_5.

COMMENTS OF COLLABORATORS

Collaborator 1.—It was not possible to obtain satisfactory agreement among replicate determinations of citrate-insoluble P_2O_5 (intermittent shaking during the citrate digestion) in Sample 6.

Collaborator 2.—With Samples 4 and 6 much difficulty was experienced in obtaining satisfactory agreement among replicate determinations of citrate-insoluble P_2O_5 with both intermittent and continuous agitation during the citrate digestion.

Collaborator 7.—The instructions were followed in all analyses. It was found that filtration of the insoluble residue after digestion with citric acid was very slow and that transfer of this material to the filter was difficult. Filtration of the samples after digestion with neutral ammonium citrate required a somewhat longer time than for samples of fused tricalcium phosphate (fused alpha phosphate) usually encountered in this laboratory.

For some samples the entire residue from citrate or citric acid digestion must be taken for the P_2O_5 determination. When this is necessary, filtration and washing of the wet-ashed material is extremely difficult because the colloidal silica blocks the filter. Dehydration of the silica with perchloric acid hastens the filtration.

We have noted, as have others, that acid digestion is not adequate for complete decomposition of fused tricalcium phosphate. For example, the acid-insoluble residue of Sample 4 contained 0.6 per cent of P_2O_5. Sodium hydroxide fusion followed by digestion with perchloric acid facilitates removal of silica and permits the determination of the true P_2O_5 content of both original samples and citrate-insoluble residues. We also prefer the fusion-acid digestion technique in the analysis of phosphate rock-magnesium silicate glass.

Collaborator 10.—In comparative determinations the same values for citric acid-insoluble P_2O_5 were obtained by continuous shaking as by continuous stirring.

Collaborator 11.—No caking of the samples was observed in either the citrate or the citric acid digestion.

Collaborator 12.—Adjustment of the initial temperature of the citric acid solution to 17.5°C. seems impractical. Sample 6 seems to lack uniformity of particle size. All filtrations were made by means of the Shimer suction filter and were very rapid without loss of material. When gravity filtration is used the time of contact of the sample with the solvent may be materially increased.

Collaborator 13.—It appears that acid digestion (30 ml. HNO_3 + 3–5 ml. HCl) does not effect complete decomposition of these types of phosphate. For example, analyses of acid-insoluble residues of Sample 2 showed 0.41–0.58 per cent of P_2O_5. An acid-insoluble residue of Sample 3 contained 0.58 per cent of P_2O_5.

Collaborator 22.—It was very difficult to obtain satisfactory replicate values for citrate-insoluble and citric acid-insoluble P_2O_5 in Samples 6 and 7.

TABLE 5.—*Effect of continuous agitation during citrate digestion on results for citrate-insoluble P_2O_5 in relation to corresponding results for citric acid-insoluble P_2O_5*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5					CITRIC ACID-INSOLUBLE P_2O_5[d]		DIFFERENCE[c]	
	BY INTERMITTENT AGITATION[a]		BY CONTINUOUS AGITATION[b]		DIFFERENCE, COL. III MINUS COL. V[c]				
	RANGE	AVERAGE	RANGE	AVERAGE		RANGE	AVERAGE	COL. III MINUS COL. VIII	COL. V MINUS COL. VIII
I	*per cent* II	*per cent* III	*per cent* IV	*per cent* V	*per cent* VI	*per cent* VII	*per cent* VIII	*per cent* IX	*per cent* X
					Sintered Alpha Phosphate				
					SAMPLE 1				
2	1.65– 1.70	1.68	1.65– 1.75	1.68	0.00[e]	1.44–1.48	1.45	0.23	0.23
3	1.44– 1.48	1.45	1.44– 1.48	1.45	0.00[e]	1.16–1.20	1.19	0.26	0.26
7	1.62– 1.71	1.68	1.30– 1.42	1.38	0.30	1.31–1.35	1.33	0.35	0.05[e]
8[f]	1.54– 1.56	1.55	1.46– 1.53	1.50	0.05[g]	—	—	—	—
12	1.60– 1.70	1.65	1.63– 1.65	1.64	0.01[e]	1.40–1.48	1.43	0.22	0.21
16	1.55– 1.60	1.58	1.75– 1.80	1.77	−0.19	1.15–1.45	1.32	0.26	0.45
19	1.85– 1.87	1.86	1.81– 1.87	1.83	0.03[e]	1.53–1.55	1.54	0.32	0.29
20	1.57– 1.57	1.57	1.58– 1.58	1.58	−0.01[e]	1.39–1.39	1.39	0.18	0.19
21	1.67– 1.80	1.72	1.67– 1.71	1.69	0.03	1.41–1.45	1.42	0.30	0.27
23	1.76– 1.86	1.82	1.64– 1.74	1.73	0.09[h]	1.24–1.48	1.37	0.45	0.36
Group[i]	1.44– 1.87	1.67	1.30– 1.87	1.64	0.03[h]	1.15–1.55	1.38	0.29	0.26
					SAMPLE 2				
2	3.70– 3.75	3.73	3.55– 3.65	3.62	0.11[e]	3.44–3.48	3.45	0.28	0.17[h]
3	2.84– 3.60	3.32	3.60– 3.68	3.64	−0.32	3.32–3.32	3.32	0.00[e]	0.32
7	3.69– 3.79	3.74	3.39– 3.41	3.40	0.34	3.36–3.40	3.38	0.36	0.02[e]
8[f]	3.72– 3.74	3.73	3.64– 3.67	3.66	0.07[g]	—	—	—	—
12	3.76– 3.81	3.79	3.74– 3.81	3.78	0.01[e]	3.43–3.51	3.47	0.32	0.31
16	3.70– 3.80	3.75	3.80– 3.90	3.87	−0.12[e]	3.20–3.35	3.28	0.47	0.59
19	4.03– 4.07	4.04	3.96– 3.99	3.98	0.06[e]	3.52–3.57	3.54	0.50	0.44
20	3.85– 3.90	3.88	3.60– 3.60	3.60	0.28	3.39–3.39	3.39	0.49	0.21[j]
21	3.88– 3.88	3.88	3.71– 3.73	3.72	0.16[h]	3.43–3.45	3.44	0.44	0.28
23	3.82– 3.94	3.87	3.70– 3.72	3.71	0.16[h]	3.50–3.58	3.54	0.33	0.17[h]
Group[i]	2.84– 4.07	3.78	3.39– 3.99	3.70	0.08[j]	3.20–3.58	3.42	0.36	0.28

TABLE 5.—*Continued*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5					CITRIC ACID-INSOLUBLE P_2O_5[d]		DIFFERENCE[e]	
	BY INTERMITTENT AGITATION[a]		BY CONTINUOUS AGITATION[b]		DIFFERENCE, COL. III MINUS COL. V[c]				
	RANGE	AVERAGE	RANGE	AVERAGE		RANGE	AVERAGE	COL. III MINUS COL. VIII	COL. V MINUS COL. VIII
I	*per cent* II	*per cent* III	*per cent* IV	*per cent* V	*per cent* VI	*per cent* VII	*per cent* VIII	*per cent* IX	*per cent* X
				Fused Alpha Phosphate					
				SAMPLE 3					
2	2.05– 2.15	2.10	2.00– 2.10	2.03	0.07[e]	1.16–1.16	1.16	0.94	0.87
3	2.48– 2.64	2.53	1.28– 1.32	1.31	1.22	0.84–0.88	0.85	1.68	0.46
7	2.51– 2.63	2.59	2.16– 2.19	2.18	0.41	0.58–0.61	0.59	2.00	1.59
8[f]	2.17– 2.25	2.22	1.72– 1.77	1.74	0.48[g]	—	—	—	—
12	2.88– 3.06	2.95	1.85– 2.11	1.99	0.96	1.06–1.14	1.11	1.84	0.88
16	1.65– 1.75	1.72	1.60– 1.65	1.63	0.09[e]	0.50–0.60	0.57	1.15	1.06
19	3.08– 3.21	3.16	2.35– 2.56	2.43	0.73	0.97–0.98	0.98	2.18	1.45
20	2.54– 2.56	2.55	2.54– 2.54	2.54	0.01[e]	1.38–1.41	1.40	1.15	1.14
21	2.45– 2.53	2.50	1.96– 2.16	2.08	0.42	0.84–0.88	0.86	1.64	1.22
23	3.90– 4.20	4.06	2.00– 2.48	2.17	1.89	1.12–1.12	1.12	2.94	1.05
Group[i]	1.65– 4.20	2.68	1.28– 2.56	2.04	0.64	0.50–1.41	0.96	1.72	1.08
				SAMPLE 4					
2	9.80– 9.90	9.87	9.20– 9.70	9.40	0.47	7.76–7.88	7.81	2.06	1.59
3	10.88–11.16	11.07	8.80– 8.92	8.87	2.20	8.04–8.76	8.29	2.78	0.58
7	10.66–10.81	10.71	9.55– 9.81	9.69	1.02	7.37–7.54	7.46	3.25	2.23
8[f]	10.39–10.46	10.42	9.11– 9.30	9.21	1.21[g]	—	—	—	—
12	10.55–11.14	10.78	9.04– 9.39	9.23	1.55	8.04–8.20	8.10	2.68	1.13
16	9.40– 9.60	9.53	8.30– 8.55	8.42	1.11	6.80–6.95	6.87	2.66	1.55
19	10.80–10.96	10.86	9.48– 9.57	9.53	1.33	6.17–6.30	6.25	4.61	3.28
20	10.79–10.81	10.80	9.20– 9.22	9.21	1.59	6.99–6.99	6.99	3.81	2.22
21	11.40–11.53	11.49	9.34– 9.38	9.36	2.13	7.24–7.28	7.26	4.23	2.10
23	13.60–14.10	13.84	9.52– 9.88	9.71	4.13	8.28–8.54	8.39	5.45	1.32
Group[i]	9.40–14.10	10.99	8.30– 9.88	9.27	1.72	6.17–8.76	7.49	3.50	1.78

TABLE 5.—*Continued*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5					CITRIC ACID-INSOLUBLE P_2O_5[d]		DIFFERENCE[c]	
	BY INTERMITTENT AGITATION[a]		BY CONTINUOUS AGITATION[b]		DIFFERENCE, COL. III MINUS COL. V[c]	RANGE	AVERAGE	COL. III MINUS COL. VIII	COL. V MINUS COL. VIII
	RANGE	AVERAGE	RANGE	AVERAGE					
I	*per cent* II	*per cent* III	*per cent* IV	*per cent* V	*per cent* VI	*per cent* VII	*per cent* VIII	*per cent* IX	*per cent* X
				SAMPLE 5					
2	6.55– 6.60	6.58	6.35– 6.45	6.38	0.20[e]	4.92–5.04	4.99	1.59	1.39
3	6.52– 6.72	6.60	6.00– 6.52	6.27	0.33[j]	4.84–4.88	4.85	1.75	1.42
7	6.60– 6.72	6.67	5.78– 6.21	5.99	0.68	4.82–4.92	4.89	1.78	1.10
8[f]	6.73– 6.83	6.79	6.04– 6.06	6.05	0.74[g]	—	—	—	—
12	6.87– 7.18	7.03	6.32– 6.53	6.39	0.64	5.00–5.36	5.17	1.86	1.22
16	6.10– 6.30	6.20	5.85– 5.90	5.88	0.32[j]	4.35–4.40	4.38	1.82	1.50
19	7.11– 7.30	7.23	6.43– 6.60	6.50	0.73	5.10–5.10	5.10	2.13	1.40
20	7.12– 7.15	7.13	7.05– 7.10	7.07	0.06[e]	4.19–4.19	4.19	2.94	2.88
21	7.12– 7.26	7.20	6.51– 6.61	6.56	0.64	4.67–4.73	4.69	2.51	1.87
23	8.52– 9.14	8.88	6.62– 6.96	6.78	2.10	5.06–5.48	5.25	3.63	1.53
Group[i]	6.10– 9.14	7.06	5.78– 7.10	6.42	0.64	4.19–5.48	4.83	2.23	1.59
			Phosphate Rock-Magnesium Silicate Glass						
				SAMPLE 6					
2	10.90–11.60	11.27	8.55– 8.75	8.63	2.64	4.24–4.56	4.41	6.86	4.22
3	10.76–11.84	11.45	7.08– 8.36	7.80	3.65	5.12–7.88	6.21	5.24	1.59
7	12.92–13.42	13.16	11.65–12.44	12.17	0.99[j]	4.65–4.97	4.76	8.40	7.41
8[f]	12.25–12.47	12.37	7.43– 8.92	8.13	4.24[g]	—	—	—	—
12	12.30–12.55	12.45	7.18– 7.63	7.41	5.04	4.28–4.35	4.32	8.13	3.09
16	9.40– 9.60	9.50	8.80– 8.90	8.85	0.65[h]	1.90–2.10	1.98	7.52	6.87
19	12.59–12.75	12.69	9.32–10.26	9.81	2.88	1.34–1.43	1.40	11.29	8.41
20	12.68–12.70	12.69	10.45–10.47	10.46	2.23	5.15–5.17	5.16	7.53	5.30
21	12.81–12.93	12.89	10.04–10.12	10.09	2.80	2.94–3.10	3.01	9.88	7.08
23	15.90–16.44	16.25	8.56– 8.78	8.64	7.61	6.40–6.66	6.47	9.78	2.17
Group[i]	9.40–16.44	12.48	7.08–12.44	9.32	3.16	1.34–7.88	4.19	8.29	5.13

TABLE 5.—*Continued*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5					CITRIC ACID-INSOLUBLE P_2O_5[d]		DIFFERENCE[e]	
	BY INTERMITTENT AGITATION[a]		BY CONTINUOUS AGITATION[b]		DIFFERENCE, COL. III MINUS COL. V[c]	RANGE	AVERAGE	COL. III MINUS COL. VIII	COL. V MINUS COL. VIII
	RANGE	AVERAGE	RANGE	AVERAGE					
I	*per cent* II	*per cent* III	*per cent* IV	*per cent* V	*per cent* VI	*per cent* VII	*per cent* VIII	*per cent* IX	*per cent* X
SAMPLE 7									
2	1.40– 1.50	1.43	1.15– 1.25	1.20	0.23[e]	0.48–0.52	0.49	0.94	0.71
3	2.24– 2.96	2.57	2.04– 2.92	2.48	0.09[e]	0.52–1.00	0.79	1.78	1.69
7	1.12– 1.42	1.31	1.14– 1.39	1.25	0.06[e]	0.45–0.46	0.46	0.85	0.79
8[f]	1.16– 1.42	1.27	1.00– 1.42	1.15	0.12[g]	—	—	—	—
12	2.43– 2.56	2.48	1.55– 1.75	1.63	0.85	0.44–0.50	0.47	2.01	1.16
16	1.40– 1.50	1.45	1.30– 1.45	1.38	0.07[e]	0.60–0.70	0.65	0.80	0.73
19	2.32– 3.20	2.76	1.69– 2.02	1.81	0.95	0.44–0.49	0.47	2.29	1.34
20	2.40– 2.40	2.40	2.13– 2.15	2.14	0.26[e]	0.69–0.69	0.69	1.71	1.45
21	2.12– 2.14	2.13	1.49– 1.51	1.50	0.63	0.82–0.94	0.87	1.26	0.63
23	2.86– 3.04	2.96	2.38– 2.48	2.43	0.53	0.40–0.46	0.42	2.54	2.01
Group[i]	1.12– 3.20	2.17	1.14– 2.92	1.76	0.41	0.40–1.00	0.59	1.58	1.17
SAMPLE 8									
2	2.80– 2.90	2.87	2.45– 2.55	2.50	0.37	1.24–1.24	1.24	1.63	1.26
3	3.96– 4.60	4.33	2.00– 2.44	2.16	2.17	1.76–1.92	1.84	2.49	0.32[j]
7	2.39– 2.68	2.57	2.69– 2.92	2.81	−0.24[h]	1.09–1.10	1.10	1.47	1.71
8[f]	2.49– 2.63	2.56	1.83– 2.08	1.98	0.58[g]	—	—	—	—
12	2.91– 3.36	3.09	2.01– 2.31	2.11	0.98	1.04–1.12	1.08	2.01	1.03
16	2.20– 2.25	2.23	1.90– 2.10	2.00	0.23[h]	1.10–1.15	1.13	1.10	0.87
19	3.26– 3.43	3.35	3.06– 3.26	3.16	0.19[e]	1.10–1.15	1.11	2.24	2.05
20	3.18– 3.19	3.19	3.04– 3.06	3.05	0.14[e]	1.63–1.63	1.63	1.56	1.42
21	3.12– 3.16	3.14	2.86– 2.94	2.90	0.24[h]	1.08–1.08	1.08	2.06	1.82
23	4.18– 4.41	4.31	3.70– 4.06	3.92	0.39	1.32–1.36	1.34	2.97	2.58
Group[i]	2.20– 4.60	3.23	1.90– 4.06	2.73	0.50	1.04–1.92	1.28	1.95	1.45

[a] Manual shaking at 5-minute intervals during the citrate digestion.
[b] Collaborators 2, 7, 8, 12, 16, 19, 20, and 21 used end-over-end rotation at 18–22 r.p.m.; Collaborator 3 used continuous stirring at 1,525 r.p.m.; and Collaborator 23 used the Burrell "wrist action" shaker at 240 oscillations per minute.
[c] Except as indicated otherwise, the differences are statistically significant at the 0.1% level.
[d] The agitation procedures during the citric acid digestion were the same as those indicated in Footnote *b* except that Collaborator 3 used continuous stirring at 1,150 r.p.m.
[e] Not statistically significant at the 5% level.
[f] This collaborator did not determine citric acid-insoluble P_2O_5.
[g] Not included in the statistical analysis.
[h] Statistically significant at the 5% level.
[i] Excluding Collaborator 8.
[j] Statistically significant at the 1% level.

TABLE 6.—*Citrate-insoluble and citric acid-insoluble P_2O_5 in alpha phosphate and phosphate rock-magnesium silicate glass*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5[a]		CITRIC ACID-INSOLUBLE P_2O_5		DIFFERENCE IN AVERAGE RESULTS[b]
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
			Sintered Alpha Phosphate		
			SAMPLE 1		
4	1.59– 1.63	1.61	1.31–1.35	1.32	0.29
5[c]	—	1.85	—	1.88	−0.03[d]
6	1.55– 1.60	1.57	1.30–1.35	1.33	0.24
9	1.38– 1.42	1.40	1.18–1.21	1.20	0.20
10	1.56– 1.56	1.56	1.20–1.26	1.23	0.33
11	1.60– 1.61	1.61	1.36–1.37	1.37	0.24
13	1.57– 1.65	1.61	1.53–1.72	1.62	−0.01[e]
14	2.00– 2.10	2.04	1.24–1.42	1.35	0.69
17	1.81– 1.81	1.81	1.19–1.21	1.20	0.61
18	1.61– 1.68	1.65	1.43–1.52	1.48	0.17
22	1.70– 1.72	1.71	1.43–1.48	1.45	0.26
Group[f]	1.38– 2.10	1.66	1.18–1.72	1.36	0.30
			SAMPLE 2		
4	3.68– 3.71	3.69	3.38–3.42	3.41	0.28
5[c]	—	4.15	—	3.89	0.26[d]
6	3.65– 3.75	3.69	3.38–3.50	3.43	0.26
9	3.61– 3.74	3.68	3.40–3.54	3.48	0.20
10	3.58– 3.60	3.59	3.30–3.42	3.37	0.22
11	3.88– 3.88	3.88	3.50–3.54	3.52	0.36
13	3.68– 3.85	3.74	3.38–3.48	3.44	0.30
14	3.71– 3.72	3.72	3.58–3.70	3.63	0.09[e]
17	3.80– 3.84	3.82	3.39–3.41	3.40	0.42
18	3.70– 3.81	3.75	3.49–3.54	3.51	0.24
22	3.72– 3.75	3.73	3.40–3.43	3.41	0.32
Group[f]	3.58– 3.88	3.73	3.30–3.70	3.46	0.27
			Fused Alpha Phosphate		
			SAMPLE 3		
4	3.27– 3.39	3.34	1.00–1.01	1.01	2.33
5[c]	—	2.74	—	1.54	1.20[d]
6	3.15– 3.35	3.26	0.98–1.02	1.00	2.26
9	2.48– 2.70	2.60	0.84–0.90	0.87	1.73
10	1.16– 1.24	1.20	1.20–1.24	1.21	−0.01[e]
11	2.44– 2.59	2.51	1.95–1.95	1.95	0.56
13	2.58– 2.84	2.68	1.67–2.12	1.91	0.77
14	1.60– 1.80	1.70	0.66–0.74	0.71	0.99
17	1.96– 1.99	1.97	0.85–0.88	0.86	1.11
18	3.37– 3.50	3.43	1.09–1.14	1.11	2.32
22	2.15– 2.16	2.16	0.90–0.93	0.91	1.25
Group[f]	1.16– 3.50	2.48	0.66–2.12	1.15	1.33

TABLE 6.—*Continued*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5[a]		CITRIC ACID-INSOLUBLE P_2O_5		DIFFERENCE IN AVERAGE RESULTS[b]
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
			SAMPLE 4		
4	11.92–12.22	12.10	7.94–7.95	7.95	4.15
5[c]	—	10.45	—	8.18	2.27[d]
6	12.98–13.05	13.01	7.60–7.65	7.62	5.39
9	10.38–10.62	10.49	8.08–8.15	8.11	2.38
10	9.94–10.02	9.97	9.24–9.42	9.32	0.65
11	11.01–12.04	11.63	8.37–8.44	8.40	3.23
13	9.69– 9.91	9.82	8.32–8.67	8.44	1.38
14	10.02–10.23	10.15	7.08–7.10	7.09	3.06
17	12.60–13.22	12.94	7.94–7.94	7.94	5.00
18	11.55–11.58	11.56	7.76–7.93	7.85	3.71
22	10.41–10.48	10.44	7.67–7.72	7.69	2.75
Group[f]	9.69–13.22	11.21	7.08–9.42	8.04	3.17
			SAMPLE 5		
4	7.90– 8.01	7.95	4.73–4.83	4.80	3.15
5[c]	—	6.95	—	5.14	1.81[d]
6	8.00– 8.05	8.02	5.50–5.50	5.50	2.52
9	7.18– 7.35	7.26	5.00–5.03	5.01	2.25
10	5.30– 5.36	5.34	5.20–5.36	5.30	0.04[e]
11	7.34– 7.40	7.37	4.93–5.12	5.00	2.37
13	7.07– 7.24	7.16	4.87–5.01	4.95	2.21
14	7.00– 7.08	7.05	4.96–5.04	5.00	2.05
17	8.71– 8.81	8.77	4.44–4.46	4.45	4.32
18	7.84– 8.10	7.95	5.06–5.15	5.11	2.84
22	6.89– 6.89	6.89	4.87–4.88	4.88	2.01
Group[f]	5.30– 8.81	7.38	4.44–5.50	5.00	2.38
			Phosphate Rock-Magnesium Silicate Glass		
			SAMPLE 6		
4	12.44–13.66	13.14	2.11–2.32	2.25	10.89
5[c]	—	8.79	—	2.12	6.67[d]
6	15.62–15.75	15.67	2.25–2.35	2.32	13.35
9	13.55–13.90	13.73	4.00–4.20	4.10	9.63
10	13.00–13.20	13.10	2.46–2.60	2.53	10.57
11	13.50–14.22	13.97	4.26–4.61	4.43	9.54
13	11.00–12.02	11.58	8.61–8.98	8.82	2.76
14	13.60–13.68	13.63	2.18–2.41	2.27	11.36
17	14.40–14.63	14.48	4.17–4.39	4.27	10.21
18	12.76–12.95	12.86	1.76–1.80	1.78	11.08
22	13.66–13.71	13.69	2.68–2.70	2.69	11.00
Group[f]	11.00–15.75	13.59	1.76–8.98	3.55	10.04

TABLE 6.—*Continued*

COLLABORATOR	CITRATE-INSOLUBLE P_2O_5[a]		CITRIC ACID-INSOLUBLE P_2O_5		DIFFERENCE IN AVERAGE RESULTS[b]
	RANGE	AVERAGE	RANGE	AVERAGE	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
			SAMPLE 7		
4	1.96– 2.56	2.20	0.42–0.50	0.46	1.74
5[c]	—	1.50	—	0.86	0.64[d]
6	1.92– 2.02	1.98	0.35–0.40	0.37	1.61
9	2.04– 2.10	2.06	0.53–0.55	0.54	1.52
10	2.50– 2.70	2.60	0.40–0.50	0.45	2.15
11	2.20– 2.32	2.28	0.47–0.49	0.48	1.80
13	1.58– 1.92	1.71	0.49–0.54	0.52	1.19
14	1.48– 1.64	1.55	0.54–0.58	0.56	0.99
17	1.57– 1.59	1.58	0.74–0.84	0.81	0.77
18	2.43– 2.53	2.48	0.43–0.52	0.48	2.00
22	1.89– 1.91	1.90	0.46–0.48	0.47	1.43
Group[f]	1.48– 2.70	2.03	0.35–0.84	0.51	1.52
			SAMPLE 8		
4	3.17– 3.28	3.21	0.94–0.96	0.95	2.26
5[e]	—	2.61	—	1.51	1.10[d]
6	3.02– 3.10	3.06	0.90–1.10	1.00	2.06
9	2.83– 2.85	2.84	1.02–1.04	1.03	1.81
10	3.40– 3.60	3.53	0.80–0.98	0.91	2.62
11	2.69– 3.01	2.85	1.12–1.13	1.12	1.73
13	2.36– 2.59	2.51	1.55–1.94	1.76	0.75
14	2.72– 2.84	2.77	1.16–1.20	1.17	1.60
17	2.99– 3.01	3.00	1.06–1.16	1.12	1.88
18	3.01– 3.10	3.05	0.99–1.04	1.01	2.04
22	3.06– 3.10	3.08	0.99–1.01	1.00	2.08
Group[f]	2.36– 3.60	2.99	0.80–1.94	1.11	1.88

[a] Manual shaking at 5-minute intervals during citrate digestion.
[b] The minus sign denotes that the value for citric acid-insoluble P_2O_5 is higher than that for citrate-insoluble P_2O_5. Except as indicated otherwise, the differences are statistically significant at the 0.1% level.
[c] This collaborator did not make replicate analyses.
[d] Not included in the statistical analysis.
[e] Not statistically significant at the 5% level.
[f] Excluding Collaborator 5.
[g] Statistically significant at the 5% level.

RESULTS OF COLLABORATIVE ANALYSES

The results of those collaborators who determined citrate-insoluble P_2O_5 with the aid of both continuous agitation and manual shaking at 5-minute intervals during the citrate digestion are shown in Table 5, which also gives their respective results for citric acid-insoluble P_2O_5.

Table 6 gives the results for citric acid-insoluble P_2O_5 and citrate-insoluble P_2O_5 obtained by those collaborators who used only manual shaking at 5-minute intervals during the citrate digestion.

Tables 5 and 6 omit the results of Collaborators 1 and 15, who did not receive complete sets of samples and who determined only total P_2O_5 and citrate-insoluble P_2O_5, the latter with intermittent shaking during the citrate digestion. Their results, not included in the statistical analysis, are as follows:

SAMPLE NO.	COLLABORATOR 1			COLLABORATOR 15		
	TOTAL P_2O_5	CITRATE-INSOLUBLE P_2O_5		TOTAL P_2O_5	CITRATE-INSOLUBLE P_2O_5	
		RANGE	AVERAGE		RANGE	AVERAGE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	20.99	1.60– 1.62	1.61	21.84	1.85– 1.94	1.90
2	20.29	3.80– 3.84	3.81	—	—	—
4	29.71	10.96–11.06	11.03	29.09	11.60–12.94	12.32
5	29.68	7.04– 7.21	7.14	29.67	8.18– 8.35	8.25
6	21.09	11.45–11.93	11.76	21.63	14.74–15.51	15.09
7	20.94	1.61– 1.71	1.67	20.39	3.81– 3.98	3.89
8	22.33	2.84– 2.96	2.90	21.77	3.28– 4.32	3.76

INTERPRETATION OF RESULTS

Intermittent Shaking vs. Continuous Agitation During Citrate Digestion.—The collaborative results of Table 5 and the supplemental data of Table 7 conform with previous observations in showing that, as in the case of basic slag (13, 14, 17, 19), continuous agitation during the citrate digestion—in comparison with manual shaking at 5-minute intervals—tends generally to give lower values for citrate-insoluble P_2O_5 in alpha phosphate (13, 14, 19, 20) and phosphate rock-magnesium silicate glass (14).

The differences between the group collaborative results for citrate-insoluble P_2O_5 by intermittent and continuous agitation (Table 5)—all in the direction of lower values by the latter procedure—are statistically significant for all the samples; they range from 0.03 to 3.16 per cent of the sample or 0.15 to 15.2 per cent of the group-average total P_2O_5. Significance was determined at the 5, 1, and 0.1 per cent levels by the F test in which variance within replicates was used as the error term. Inclusion of collaborator and interaction variance in the error term renders the differences between the group averages for Samples 1 and 2 nonsignificant and reduces the significance of the differences for Samples 7 and 8 from the 0.1 per cent to the 1 per cent level but has no effect on the significance levels for the other samples. Among the individual comparisons (excluding Collaborator 8) the distribution as regards statistical significance, de-

TABLE 7.—*Citrate-insoluble and citric acid-insoluble P_2O_5 in additional samples of alpha phosphate and phosphate rock-magnesium silicate glass*

SAMPLE NO.[a]	TOTAL P_2O_5[b]	FLUORINE[b]	CITRATE-INSOLUBLE P_2O_5[c]			CITRIC ACID-INSOLUBLE P_2O_5[c,e]	DIFFERENCE	
			BY INTERMITTENT AGITATION[d]	BY CONTINUOUS AGITATION[e]	DIFFERENCE[f]		COL. IV MINUS COL. VII	COL. V MINUS COL. VII
I	*per cent* II	*per cent* III	*per cent* IV	*per cent* V	*per cent* VI	*per cent* VII	*per cent* VIII	*per cent* IX
Sintered Alpha Phosphate								
2363	21.53	1.24	18.25	18.32	−0.07	16.20	2.05	2.12
2362	21.40	1.02	16.08	16.23	−0.15	14.90	1.18	1.33
2377	24.04	0.43	6.88	7.01	−0.13	6.27	0.61	0.74
2376	23.56	0.34	5.61	5.81	−0.20	4.91	0.70	0.90
2353	20.62	0.15	2.97	3.05	−0.08	2.68	0.29	0.37
Average	22.23	0.64	9.96	10.08	−0.12	8.99	0.97	1.09
Fused Alpha Phosphate								
2405	30.01	1.11	21.17	21.07	0.10	19.46	1.71	1.61
2404	30.36	0.92	18.46	18.27	0.19	16.12	2.34	2.15
2403	30.50	0.72	15.46	15.41	0.05	13.18	2.28	2.23
2496	27.68	0.34	5.13	5.11	0.02	4.53	0.60	0.58
2397	29.72	0.15	4.17	3.53	0.64	3.11	1.06	0.42
Average	29.65	0.65	12.88	12.68	0.20	11.28	1.60	1.40
Calcined Alpha Phosphate								
1351	36.58	0.68	11.33	11.66	−0.33	10.95	0.38	0.71
1324	33.49	0.42	10.69	10.47	0.22	9.31	1.38	1.16
1374[g]	35.17	0.15	3.85	3.59	0.26	2.86	0.99	0.73
Average	35.08	0.42	8.62	8.57	0.05	7.71	0.92	0.87
Phosphate Rock-Magnesium Silicate Glass								
2481[h]	23.61	2.21	8.11	6.27	1.84	4.75	3.36	1.52
2482[h]	23.33	1.98	6.94	5.29	1.65	3.84	3.10	1.45
2463[h]	19.75	1.65	6.70	6.01	0.69	0.64	6.06	5.37
Average	22.24	1.95	7.25	5.86	1.39	3.08	4.17	2.78

[a] Except as indicated, the samples were ground to pass the 150-mesh sieve.
[b] The results for the sintered, fused, and calcined alpha phosphates are those given by Jacob, Ward, Hill, and Pinkerton (16). The results for the phosphate rock-magnesium silicate glass Samples 2481 and 2463 are those given by Hill, Ward, Armiger, and Jacob (10).
[c] Average of triplicate determinations.
[d] Manual shaking at 5-minute intervals during the citrate digestion.
[e] End-over-end rotation (22 r.p.m.) in the MacIntire-Marshall-Meyer apparatus (19).
[f] Column IV minus Column V.
[g] −200 mesh.
[h] −80 mesh.

termined by the F test using the variance within replicates as the error term, is as follows:

COMPARISONS	NUMBER	FRACTION OF TOTAL
		per cent
Tested statistically	72	100
Statistically significant	50	69.4
5.0% level	7	9.7
1.0% level	3	4.2
0.1% level	40	55.5

Among the 72 individual collaborative comparisons between intermittent shaking and continuous agitation during the citrate digestion (Table 5), 65 (90 per cent) show lower results for citrate-insoluble P_2O_5 by continuous agitation, 5 by intermittent shaking, and 2 show no difference. The distribution of the differences, without regard for signs, is as follows:

DIFFERENCE	COMPARISONS	
per cent	*number*	*per cent of total*
<0.10	16	22.2
0.10–0.24	12	16.7
0.25–0.49	12	16.7
0.50–0.99	12	16.7
1.00–1.99	7	9.7
2.00–2.99	8	11.1
3.65–7.61	5	6.9
0.00–7.61	72	100.0

The group-average differences between the results for citrate-insoluble P_2O_5 determined respectively with the aid of intermittent and continuous agitation during the citrate digestion (Table 5) are quite small (0.03–0.08 per cent of the sample) with the sintered alpha phosphates (Samples 1 and 2), but are much larger (0.41–3.16 per cent of the sample) with fused alpha phosphates (Samples 3–5) and the phosphate rock-magnesium silicate glasses (Samples 6–8). In terms of the group-average results for total P_2O_5 the differences are as follows:

SAMPLE NO.	FRACTION OF TOTAL P_2O_5	SAMPLE NO.	FRACTION OF TOTAL P_2O_5
	per cent		*per cent*
1	0.15	5	2.2
2	0.4	6	15.2
3	2.3	7	2.0
4	5.8	8	2.3

For the same type of material of similar fineness these differences agree

fairly well with those reported in previous papers (13, 14, 19, 20) and with data of Table 7. It will be noted that the largest differences occur with the coarsely ground materials, namely, the −35-mesh fused alpha phosphate (Sample 4) and the −6-mesh phosphate rock-magnesium silicate glass (Sample 6), which also show the largest differences between the values for citrate-insoluble and citric acid-insoluble P_2O_5 (Tables 5 and 6).

In the comparison of intermittent shaking and continuous agitation during the citrate digestion (Table 5), 8 of the 10 collaborators (excluding Collaborator 8) accomplished agitation by means of end-over-end rotation, while the others used continuous stirring and the Burrell "wrist action" shaker, respectively. Owing to the very much greater use of end-over-end rotation and the considerable variation among the results of the collaborators who employed this type of apparatus, the data do not warrant conclusions as to the relative efficiencies and merits of the various continuous agitation procedures. Previous investigations (14, 17) indicate however, that the type of continuous agitation is not an important factor.

Citrate-Insoluble P_2O_5 vs. Citric Acid-Insoluble P_2O_5.—Previous work has indicated that with the present official methods the percentage of citrate-insoluble P_2O_5, as determined with the aid of either intermittent shaking or continuous agitation during the citrate digestion, can be expected, in general, to exceed that of the citric-acid insoluble P_2O_5 in the case of alpha phosphate (8, 10, 11, 13, 14, 15, 16, 18, 20, 26) and phosphate rock-magnesium silicate glass (8, 10, 14), as well as basic slag (8, 10, 12, 13, 14, 15, 17, 26, 27). With intermittent shaking during the citrate digestion this is true of 149 of the 152 individual replicated comparisons listed in Tables 5 and 6. With continuous agitation during the citrate digestion (72 comparisons) the values for citrate-insoluble P_2O_5 exceed those for citric acid-insoluble P_2O_5 in every instance (Table 5).

Statistical analyses were made of the data. Significance was determined at the 5, 1, and 0.1 per cent levels by the *F* test using the variance within replicates as the error term. Collaborator and interaction variances were excluded. With all the samples the group-average results for citric acid-insoluble P_2O_5 are significantly lower (0.1 per cent level) than those for citrate-insoluble P_2O_5, whether the latter was determined with the aid of intermittent shaking or continuous agitation during the citrate digestion (Tables 5 and 6).

Among the 152 individual replicated comparisons of citric acid-insoluble P_2O_5 with citrate-insoluble P_2O_5 as determined with the aid of intermittent shaking during the citrate digestion, only 4 show differences that are not statistically significant at the 5 per cent or a higher level, while 147 show differences that are statistically significant at the 0.1 per cent level. In the 72 comparisons involving continuous agitation during the citrate digestion, 66 show differences that are statistically significant at

the 0.1 per cent level; the differences lack statistical significance in only 2 cases.

In terms of percentage of the sample the distribution of the differences between the values for citrate-insoluble and citric acid-insoluble P_2O_5, without regard for signs, is as follows:

DIFFERENCE	COMPARISONS	
per cent	*number*	*per cent of total*
Intermittent Shaking During Citrate Digestion		
<0.25	15	9.9
0.25– 0.49	22	14.5
0.50– 0.99	15	9.9
1.00– 1.99	31	20.4
2.00– 2.99	37	24.3
3.00– 3.99	7	4.6
4.00– 5.99	8	5.2
6.86–13.35[a]	17	11.2
0.00–13.35	152	100.0
Continuous Agitation During Citrate Digestion		
<0.25	9	12.5
0.25– 0.49	11	15.3
0.50– 0.99	9	12.5
1.00– 1.99	27	37.5
2.00– 2.99	8	11.1
3.00– 3.99	2	2.8
4.00– 5.99	2	2.8
6.87– 8.41[a]	4	5.5
0.00– 8.41	72	100.0

[a] All differences in this range were obtained on the −6-mesh phosphate rock-magnesium silicate glass (Sample 6).

Among the comparisons involving intermittent shaking during the citrate digestion, 24.4 per cent show differences of less than 0.5 per cent between the values for citrate- and citric acid-insoluble P_2O_5, 34.3 per cent show differences of less than 1 per cent, and 54.7 per cent, less than 2 per cent. With continuous agitation the corresponding figures are 27.8, 40.3, and 77.8 per cent. With intermittent shaking all but 3 of the comparisons that show differences of more than 3 per cent are on the −35-mesh alpha phosphate (Sample 4) and the −6-mesh phosphate rock-magnesium silicate glass (Sample 6); with continuous shaking all such differences are on these two samples. Regardless of the agitation procedure during the citrate digestion all the differences that exceed 6 per cent are on Sample 6.

A better comparison of the differences between the values for citrate-

and citric acid-insoluble P_2O_5 is afforded by expressing the results in terms of the total P_2O_5 content of the sample, as in the following tabulation which is based on the weighted group-average data for the respective samples (Tables 5 and 6).

SAMPLE NO.	DIFFERENCE BETWEEN RESULTS FOR CITRATE- AND CITRIC ACID-INSOLUBLE P_2O_5 EXPRESSED AS FRACTION OF TOTAL P_2O_5	
	A[a]	B[b]
	per cent	*per cent*
1	1.4	1.3
2	1.6	1.4
3	5.3	3.8
4	11.3	6.0
5	7.8	5.4
6	44.4	24.7
7	7.6	5.7
8	8.6	6.6

[a] Intermittent shaking during citrate digestion.
[b] Continuous agitation during citrate digestion.

With intermittent shaking during the citrate digestion the differences between the collaborative results for citrate- and citric acid-insoluble P_2O_5 in the alpha phosphates (Samples 1–5) fall within the range of values for such materials of comparable grade and fineness as previously reported (Table 1) (8, 10, 11, 13, 14, 15, 16, 18, 20, 26) and as shown in Table 7, especially when the difference is expressed in terms of the total P_2O_5 content of the sample. Also, they are generally in good agreement with the published data for basic slag (8, 10, 12, 13, 14, 15, 17, 26, 27).

In the case of phosphate rock-magnesium silicate glass the differences between the values for citrate-insoluble P_2O_5 (intermittent shaking during the citrate digestion) and citric acid-insoluble P_2O_5 are generally wider than those obtained on alpha phosphate and basic slag of comparable grade and fineness, and the agreement of the collaborative results (Samples 6–8) with those of Table 7 and of previous investigations (8, 10, 14) is less satisfactory. The discrepancies in the results for this type of product may be due to several factors, including variability in the chemical and physical nature of the material and its rather marked tendency to cake upon addition of solvents with formation of agglomerates of particles which may fail of thorough dispersion during the subsequent citrate or citric acid digestion (8, 10). This caking tendency—also possessed in some degree by basic slag (17) and alpha phosphate—may have been an important factor in the difficulty reported by several of the collaborators in obtaining agreement among the replicate values for insoluble P_2O_5 in the samples of phosphate rock-magnesium silicate glass.

Use of continuous agitation during the citrate digestion usually reduces,

often markedly, the gap between the citrate-insoluble and the citric acid-insoluble P_2O_5 values. The reduction is greatest with fused alpha phosphate and phosphate rock-magnesium silicate glass, especially the coarsely ground materials (Samples 4 and 6).

The values for citric acid-insoluble P_2O_5 show no clearly defined effects of variations in the type of continuous agitation device or the speed of of agitation (Tables 5 and 6). This is in general agreement with previous findings (14, 17, 18).

Although this report is concerned chiefly with the differences in the respective values for citrate-insoluble and citric acid-insoluble P_2O_5, rather than with the actual values themselves, it should be noted that for a given sample the actual values for these determinations, as well as those for total P_2O_5, are generally less consistent than is desirable in studies of this kind, as is also true of the collaborative studies on P_2O_5 conducted in 1947 and 1948 (14, 17).

EFFECT OF PARTICLE SIZE

The effect of particle size on the results for citrate-insoluble and citric acid-insoluble P_2O_5 in alpha phosphate and phosphate rock-magnesium silicate glass has been reported in several papers (8, 10, 13, 15, 16, 20, 26). The data show that within certain limits the values for both citrate-insoluble and citric acid-insoluble P_2O_5, as well as the differences between these values, generally decrease with decrease in the particle size. Basic slag behaves similarly (8, 10, 17). Ford (5) has recently shown that, as compared with grinding in an old-style burr mill, finer grinding by means of a high-speed hammer mill results in slightly lower values for citrate-insoluble P_2O_5 in commerical mixed fertilizers.

For the different types of alpha phosphate a rigid interpretation of the published data points to a fineness of -100 mesh to -200 mesh in order to reduce the particle-size effect to zero or near zero proportions. In practice, however, the particle-size effect usually is substantially eliminated by grinding the sample to -100 mesh. The few data available for phosphate rock-magnesium silicate glass indicate that the values for citric acid-insoluble P_2O_5 are little affected by fineness beyond 80 mesh, whereas a fineness of more than 300 mesh may be necessary to eliminate completely the particle-size effect on the values for citrate-insoluble P_2O_5.

No special study of the effect of particle size on the results for insoluble P_2O_5 was made in the present investigation but evidence on this point is afforded by the data (Tables 5 and 6) for the -35-mesh fused alpha phosphate (Sample 4) and its -100-mesh grind (Sample 5). The effect of the finer grinding was to reduce very significantly not only the group-average values for citrate-insoluble and citric acid-insoluble P_2O_5 but also the differences between these values. Statistical analysis of the data shows that the finer grinding reduced the variances among the average results

of the collaborators but the differences in variability are not significant at the 5 per cent level.

The results on the −6-mesh phosphate rock-magnesium silicate glass (Sample 6) and the much finer material (Sample 7) from the same source afford an even more striking example of the effect of particle size on the values for insoluble P_2O_5. However, statistical analysis of the differences between the corresponding results is not permissible because of uncertainty that these samples differ only as regards particle size.

NUTRIENT VALUE OF ALPHA PHOSPHATE AND PHOSPHATE ROCK-MAGNESIUM SILICATE GLASS

Although the present investigation did not include plant-growth studies with alpha phosphate and phosphate rock-magnesium silicate glass, an indication of the results of such studies by other workers is of interest in this report.

Two collaborative studies (11, 15, 26), one of which was conducted by the Associate Referee on Phosphoric Acid in 1936 (15, 26), have been made of the value of alpha phosphate as a source of P_2O_5 for plants under greenhouse conditions. The finely ground (−80 mesh) material was usually as effective as superphosphate, monocalcium phosphate, or dicalcium phosphate in promoting growth of Sudan grass, millet, sorghum, wheat, sweet clover, tobacco, tomato, and cabbage on acid and neutral soils. Like dicalcium phosphate, however, it was not satisfactory for tomatoes on soil of *p*H 9.5. Coarser grinding (−20 or −40 mesh) reduced the efficiency of the alpha phosphate. In general, the effectiveness of the finely ground material was similar to that of high-grade basic slag.

Numerous plot and field tests have been made of alpha phosphate. The results indicate that, applied on the basis of equal quantities of available P_2O_5, high-quality alpha phosphate is generally as effective as superphosphate in promoting growth of legumes, pasture, and forage crops and of cereals in rotation, on acid and neutral soils, but the data are inadequate to assess properly the relative value of alpha phosphate for row crops in general. On the other hand, alpha phosphate, like dicalcium phosphate and other water-insoluble orthophosphates, appears to be less efficient than superphosphate in increasing crop yields on alkaline soils.

Aside from the results of a few greenhouse tests (10, 29), no data on the nutritive value of phosphate rock-magnesium silicate glass appear to have been published. In these tests the glass compared favorably with double superphosphate and fused alpha phosphate as a source of P_2O_5 for millet and Sudan grass on acid soils. On alkaline soils the results were inconsistent (10). Unpublished reports of plot and field experiments show considerable variation in the relative growth-response of crops to applications of phosphate rock-magnesium silicate glass on soils, chiefly alkaline soils, in the Pacific Coast States—the only area of domestic production of this

material. No such experiments on acid soils in eastern United States are known to the writers.

SUMMARY AND CONCLUSIONS

In previous reports it is shown for alpha phosphate and phosphate rock-magnesium silicate glass (14), as well as for basic slag (14, 17), that the results for citrate-insoluble P_2O_5 by continuous agitation during the citrate digestion are usually lower than those by shaking at 5-minute intervals. The current work, relating only to alpha phosphate and phosphate rock-magnesium silicate glass, supports these findings and shows that the differences may be much larger with coarsely ground materials.

Triplicate determinations of citrate-insoluble and citric acid-insoluble P_2O_5 in 2 samples of sintered alpha phosphate and 3 samples each of fused alpha phosphate and phosphate rock-magnesium silicate glass were made by 19 collaborators using manual shaking at 5-minute intervals during the citrate digestion. The samples included both commercially ground and laboratory ground materials, the latter being substantially −100 mesh.

With only 3 exceptions the values by the individual collaborators for citrate-insoluble P_2O_5—whether by intermittent or continuous agitation during the citrate digestion—are higher than those for citric acid-insoluble P_2O_5 in the same sample. The differences are statistically significant at the 5 per cent or a higher level in all but 4 of the 152 comparisons involving intermittent shaking during the citrate digestion and in all but 2 of the 72 comparisons where continuous agitation was used. For the individual samples the group-average differences are statistically significant at the 0.1 per cent level in all cases. With intermittent shaking during the citrate digestion the differences (weighted group-averages) for the finely ground (substantially −100 mesh) samples range from 0.30 to 2.31 per cent of P_2O_5 on the sample, or 1.4 to 8.6 per cent of the total P_2O_5, while with continuous agitation during the citrate digestion the respective ranges are 0.26 to 1.59 per cent and 1.3 to 6.6 per cent.

As compared with fine grinding (−100 mesh), coarse grinding (−6 or −35 mesh) increases markedly the values for citric acid-insoluble and citrate-insoluble P_2O_5, as well as the differences between these values, regardless of the agitation procedure during the citrate digestion.

The results of this study, together with those of previous investigations, point to the official neutral ammonium citrate method, preferably with continuous agitation during the citrate digestion, as a suitable procedure for the evalutation of the P_2O_5 in alpha phosphate and phosphate rock-magnesium silicate glass.

SUGGESTED EDITORIAL CHANGES IN METHODS FOR CITRATE-INSOLUBLE P_2O_5

In the *Methods of Analysis*, 1945, p. 24, sec. **2.16(b)**, the first sentence reads as follows: "Place 1 g of sample on 9 cm filter paper." It has been

pointed out in this report and elsewhere (8, 10, 17) that furnace-made phosphates, such as basic slag, alpha phosphate, and phosphate rock-magnesium silicate glass, tend to cake upon addition of solvents, with formation of agglomerates of particles which may fail of thorough dispersion during the subsequent citrate or citric acid digestion. Such agglomeration may also be caused by contact of the sample with wet filter paper prior to the digestion period. It is suggested, therefore, that the latter possibility be avoided by specifying that the sample be placed on a *dry* filter.

The need for rapid filtering and washing of the citrate-insoluble residue is generally recognized, and in practice these operations are always performed with the aid of suction—never by gravity alone. The official method for citrate-insoluble P_2O_5 is so worded, however, that the method of filtering, whether by suction or gravity, is optional. The wording of the method should be changed to eliminate the option of gravity filtration. These would seem to be permissible editorial changes.

ACKNOWLEDGMENT

The Associate Referee wishes to express his appreciation of the fine cooperation given by the collaborators and their respective organizations.

The samples were kindly supplied by the following organizations: sintered alpha phosphate, Coronet Phosphate Company, New York, New York; fused alpha phosphate, Division of Chemical Engineering, Tennessee Valley Authority, Wilson Dam, Alabama; phosphate rock-magnesium silicate glass, Manganese Products, Incorporated, Seattle, Washington, and Permanente Metals Corporation, Permanente, California.

Assistance in the various phases of the investigation was rendered by D. V. Bennett, Jr., P. P. Chichilo, E. J. Fox, W. L. Hill, and T. M. Sheets, all of the Division of Fertilizer and Agricultural Lime.

RECOMMENDATIONS*

It is recommended—

(1) That in the *Methods of Analysis, A.O.A.C.*, 1945, sec. **2.16** be altered by:

(1) Changing the phrase, in sec. **2.16**(**a**) first line, "*Acidulated samples*" to "*Acidulated samples and mixed fertilizers.*"

(2) Deleting the words, in sec. **2.16**(**b**) first line, "*other than basic slag*" (final action).

(2) That sec. **2.17** be changed by deleting the words, in second sentence, "in acidulated samples, dicalcium phosphate, precipitated bone phosphate, and precipitated bone" (final action).

(3) That the methods for citric acid-soluble phosphoric acid in basic slag, sec. **2.18, 2.19,** and **2.20,** be deleted (final action).

* For report of Subcommittee A and action by the Association, see *This Journal*, 33, 38 (1950).

(4) That the following editorial changes be made in the *Methods of Analysis*, Ed. 7, 1950:

(1) In the first sentence of sec. **2.16(b)** add the word "dry" after "on."
(2) In sec. **2.16(a)**, as published in the *Methods of Analysis*, 1945, change sentence 6 to read: "At expiration of exactly 1 hour from time filter and residue were introduced, remove flask from bath or apparatus and immediately filter contents by suction as rapidly as possible through Whatman filter paper No. 5 or other paper of equal speed and retentiveness using Büchner funnel or ordinary funnel with Pt or other cone." Delete sentence 7, parenthetical.[5]

(5) That alpha phosphate and phosphate rock-magnesium silicate glass be evaluated by the neutral ammonium citrate method, preferably with continuous agitation during the citrate digestion, and that collaborative work on determination of available P_2O_5 in these materials be discontinued.

(6) That work on methods for phosphoric acid in fertilizers be continued, with emphasis on:

(1) Aging of the molybdate solution used in the volumetric method to determine if a time limit should be put on its use or an addition made to preserve it.
(2) Use of perchloric acid in preparation of solutions for analysis.

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[5] These changes relate to sentences 4 and 5 of the revision of sec. 2.16(a), recommended for adoption (final action) in the 1948 report on phosphoric acid in fertilizers (ref. 17, p. 226).

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REPORT ON MOISTURE IN FERTILIZERS

By W. L. HILL (Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Md.), *Associate Referee*

Of the six recommendations pertaining to moisture in fertilizers reported by the Committee on Recommendations of Referees last year[1] two (Nos. 14 and 15) anticipated further experimental work looking towards the selection of a single stated temperature for the oven drying procedure and the establishment of the ranges of applicability of the procedures for determining water by oven drying, by vacuum desiccation and by the air-flow method. It was planned to deal with both objectives in an extensive study comprising the determination of rates of loss in weight by the three methods over several hours test of some 20 to 30 representative samples of fertilizers and fertilizer materials. This work, however, could not be completed in time for the meeting, which necessitates the continuance of these two recommendations another year.

In the published report of changes in Official and Tentative Methods of Analysis made at the meeting last year[2] two slightly differing procedures for vacuum desiccation were inadvertently included. The first was proposed in the 1947 Report on Moisture,[3] whereas the second was sent to the collaborators last year and recommended in the 1948 report.[4] As regards operations the two procedures appear to differ in two respects—sample size and pressure. The difference in sample size (4 and 2 grams) is only an apparent one, since 4 grams may be used with a 2-inch dish, whereas a 2-gram sample is specified for a smaller dish. A vacuum of 25 inches indicated in the first procedure turned out to be a little high for the

[1] *This Journal*, **32**, 44 (1949).
[2] *Ibid.*, **32**, 72, 73 (1949).
[3] *Ibid.*, **31**, 234 (1948).
[4] *Ibid.*, **32**, 228 (1949).

average laboratory installation; hence it was necessary to lower the specified vacuum to 20 inches. Accordingly, only one vacuum-desiccation method is involved, and the verbal order of the procedure in last year's report has been altered to embody the desirable features of both published procedures.

One other point, which relates to the apparatus for the air-flow method, should be discussed here. Oddly enough, the manifold made with the use of pipe fittings, which was suggested to the collaborators last year as a make-shift, turned out to be extremely practical and very satisfactory. Anyone can assemble the standard pipe fittings, whereas only a good tinner can make the box-type assembly[5] described in the official actions of last year.[2] Accordingly, a description of the simpler manifold is all that will be needed in the method as finally published.

The methods for water in fertilizers will appear in the revised *Methods of Analysis*, 7th Edition, 1950.

RECOMMENDATIONS*

It is recommended—

(1) That the air-flow method be made official for determining free water in fertilizers.

(2) That the vacuum-desiccation method with a drying period of 16 to 18 hours be made official for determining free water in fertilizers.

(3) That the oven-drying method be modified to state only one drying temperature, the selection of the temperature to be determined by study, not necessarily collaborative, during the coming year.

(4) That further study be made on the applicability of the foremention three methods.

(5) That the phraseology of the oven-drying method now in the *Methods of Analysis* be modified as follows: (a) Change parenthetical remark now worded "Not applicable to samples containing compounds other than H_2O that are volatilized at the temp. of drying" to read "Not applicable to samples that yield volatile substances other than H_2O at the temp. of drying." (b) Change first sentence of text now worded "Heat 2 g of prepared sample, **2.2**, for 5 hours in water oven at temp. of boiling H_2O (98–100°)" to read "Heat 2 g of prepared sample, **2.2**, for 5 hours in oven at temp. of 99–101°." (c) Change second sentence of text now worded "In case of potash salts, $NaNO_3$, and $(NH_4)_2SO_4$, heat at Ca 130° to constant weight" to read "In case of $NaNO_3$, $(NH_4)_2SO_4$, and potash salts heat to constant weight in oven at temp. of 129–131°." (d) Add the statement "Report the percentage loss in weight as water at the temp. used.† (e) Change section heading to read *Water* instead of *Moisture*.

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[5] *Ibid.*, **30**, 640 (1947).

* For report of Subcommittee A and action of the Association, see *This Journal*, **33**, 38 (1950).

† Not contained in recommendation last year.

REPORT ON NITROGEN IN FERTILIZERS

By M. P. ETHEREDGE (Mississippi State Chemical Laboratory, State College, Mississippi), *Associate Referee*

When the report was made to the Association last year *This Journal* **32,** 241 (1949), it was recommended that the rapid formaldehyde titration method for nitrogen in ammonium nitrate be adopted as official, first action. This method, however, carries no provision for an alternative electrometric titration; so it was decided to survey the collaborators on this point before final adoption this year.

Although the Devarda procedure seemed to give slightly higher results last year on nitrate of soda when compared to any variation of the Kjeldahl Method Modified to Include Nitrogen in Nitrates, it was thought well to give the old revived argument another trial this year.

The thing which was most unsettled in the report last year was the matter of high nitrate-chloride mixtures. So far no practical method has given all the nitrogen in these mixtures. It is true that we have worked with exaggerated cases; but later experiment might disclose what could be done with a type of high grade nitrate-chloride mixture that may carry a greater tonnage in the sales column. Also, it was decided to follow Method **2.27 (1)** and **(2),** or to *Ford's* variation of the latter.[1]

SAMPLES

This year only three samples were sent to thirty-three collaborators. These were not mailed until April, and the description follows:

(1) *C. P. Sodium Nitrate.*—A reagent grade product was sent out and, of course, the nitrogen content should approach 16.47%.

(2) *High Grade Nitrate-Chloride Mixture.*—Many experiment stations recommend a 1:1:1 mixture; therefore, we made a mixture in the Mississippi State Chemical Laboratory from nitrate of soda and superphosphate-muriate, and the analysis should be approximately 8.25–8.02–8.10 (Mississippi has an 8–8–8 grade for another year).

(3) *Low Grade Nitrate-Chloride Mixture.*—This mixture was prepared from (2) by cutting in half with superphosphate. The analysis should be 4.14–14.50–4.05. This, perhaps, is comparable to a grade in some of the western States.

DIRECTIONS FOR COLLABORATORS

(A) You perhaps remember that the formaldehyde titration of ammonium nitrate was adopted as official, first action.[2] Before adoption as final, perhaps we should set up a stoichiometric end point for those who expect to use a *p*H meter for the titration. Please give your views on this if you have not already done so. You might make a few titrations using any reagent grade ammonium nitrate.

(B) The results on the sodium nitrate by the Devarda procedure (**2.31**) were not conclusive when compared to Ford's suggested procedure. Therefore, we shall

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[1] *This Journal,* **32,** 244 (1949).
[2] *This Journal,* **32,** 71 (1949).

again send out a sodium nitrate sample for the same comparison. Dry at 130°C and consider dry.

Dr. Quackenbush is also interested in trying out the Willits-John bulb, and the Devarda procedure will be an excellent test for the bulb. Reference is made to this bulb in *This Journal*, **31**, 432 (1948). This bulb with a Goessman trap may be obtained by ordering from Harry J. John, 809 Glendalough Road, Chestnut Hill, Philadelphia, Pa.

(C) We shall send out two samples of nitrate-chloride mixture as a mixed fertilizer, one high and one medium in nitrogen content. You will please determine both by—

(1) Mr. Ford's variation of **2.27** (**2**).
(2) Method **2.27** (**1**), using 2 g of salicylic acid instead of 1 g (on June 21, 1949, this was changed to one, two, and three gram portions of salicylic).

RESULTS

An excellent response was received from the collaborators. Most names of last year will be seen in this year's report, and a few new ones are added. The persons whose names appear in the following list have made this report possible, and the Associate Referee feels deeply grateful to all.

R. L. Willis and A. C. Wark, New Jersey Exp. Sta., New Brunswick
F. W. Quackenbush and R. F. Serro, Indiana Exp. Sta., Lafayette
C. O. Willits, Eastern Regional Research Laboratory, Philadelphia, Pa.
C. A. Butt and W. H. Banks, Int. Minerals & Chem. Co., East Point, Ga.
Frances L. Bonner, Louisiana Experiment Station, Baton Rouge
R. D. Caldwell, Armour Fert. Works, Atlanta, Ga.
W. S. Thompson, Dept. of Agriculture, Columbus, Ohio
R. C. Koch and R. C. Rund, Swift and Co., Hammond, Ind.
Howard C. Hammond, State Laboratories, Bismarck, N. Dak.
W. A. Morgan, du Pont & Co., Wilmington, Del.
Charles V. Marshall, National Research Council, Ottawa, Can.
W. C. Geagley and Percy O'Meara, Mich. Dept. Agr., Lansing
A. T. Blackwell and Harry Velker, Davison Chem. Corp., Baltimore, Md.
Gordon Hart, Asst. State Chemist, Tallahassee, Fla.
D. M. Bates and Paul M. Goudelock, Smith-Douglas Co., Norfolk, Va.
P. R. Bidez, Alabama Polytechnic Institute, Auburn
J. B. Smith and Roland Gilbert, Rhode Island Exp. Sta., Kingston
Ralph D. Miller, Spencer Chemical Company, Pittsburg, Kan.
J. D. B. Ogilvie, North Am. Cyanamid, Niagara Falls, Canada
G. C. Bollinger, C. M. Fleming, C. E. Trimble, The Am. Agr. Chem. Co., Baltimore, Md.
W. R. Austin, Armour Fert. Works, Nashville, Tenn.
J. W. Kuzmeski and A. F. Spelman, Agr. Exp. Sta., Amherst, Mass.
Philip McG. Shuey, Shuey & Co., Savannah, Ga.
Louis Ballard, Mississippi State Chemical Lab., State College

Only a few collaborators sent in results or discussions on the electrometric end point when determining nitrogen in ammonium nitrate. With two exceptions they favored from 8.4 to 8.9 as a final *p*H. Perhaps if an approximate *p*H is set up it should be 8.6. This would make the complete method which is later to be recommended for final adoption read as follows:

NITROGEN IN AMMONIUM NITRATE

Formaldehyde Titration Method

(May also be adapted to Ammonium Sulfate)

Weigh out 7.004 or 14.008 g of sample and make up to 250 or 500 ml. Pull off 25 or 50 ml and put into a 300–500 ml Erlenmeyer (ca 1.5 g may be rapidly weighed and washed directly into flask). Add ca 1 ml of reagent formaldehyde for each 0.1 g of sample in aliquot. Make total volume 150–200 ml and allow 5 min. before titrating with 0.25–0.50 *N* sodium hydroxide, using 5 drops of phenolphthalein as indicator. Titrate until there is no perceptible color change at the point of contact, or until the proper color of pink persists. If electrometric titration is preferred, titrate to approximately *p*H 8.6. Run a blank on the formaldehyde.

$$\% \text{ Nitrogen} = \frac{\text{Net ml of NaOH} \times \text{Normality} \times 2.8016}{\text{Wt. of Sample}}$$

TABLE 1.—*Sample No. 1. Total nitrogen in C. P. sodium nitrate*

ANALYST	FORD METHOD	DEVARDA (*2.31*) WILLITS-JOHN BULB	DEVARDA (*2.31*) OTHER BULB	OTHER METHOD*
	per cent	*per cent*	*per cent*	*per cent*
1	16.32	—	16.48	—
2	16.51	16.48	16.61	—
3	16.49	16.50	—	—
4	16.36	—	16.50	—
5	16.32	—	16.39	—
6	16.23	—	—	16.45
7	—	—	16.44	—
8	16.12	—	16.20	—
9	16.45	16.41	16.22	—
10	16.23	16.32	—	—
11	16.18	—	16.39	—
12	16.29	—	16.30	—
13	16.31	—	16.38	—
14	—	—	—	16.40
15	15.99	—	16.43	—
16	16.32	—	16.42	—
17	16.29	—	16.40	—
18	15.77	16.09	16.11	—
19	15.81	16.14	16.34	—
20	16.41	16.46	16.44	—
21	16.43	—	—	16.43
22	16.02	—	—	16.06
23	—	—	16.48	—
24	16.35	16.37	16.43	—

* *Methods of Analysis, A.O.A.C.*, 6th Ed., 2.27 (1).

The results on the three samples which were sent out are shown in Tables 1, 2, and 3.

COMMENTS BY COLLABORATORS

Before going into a detailed discussion of the results it might be well to record a few comments of collaborators.

Analyst 1 thinks the Devarda method is superior to Ford's method for nitrate of soda.

Analyst 2 finds 8.4 as end point for formaldehyde titration.

Analyst 3 uses Willits-John bulb for all distillations.

Analyst 4 believes the high nitrate-chloride mixture was losing nitrogen.

Analyst 6 finds that with 14-0-14 mixtures the chlorides may well be precipitated out with silver sulfate.

Analyst 9 believes the Ford version of **2.27** (2) has merit; however, he comes to no conclusion on the Willits-John bulb. He titrates the formaldehyde to 8.5–9.0.

Analyst 10 thinks the formaldehyde end point should be 8.4.

TABLE 2.—*Sample No. 2. Total nitrogen in high grade nitrate-chloride mixture*

ANALYST	FORD METHOD	METHOD 2.27 (1)		
		1 g SALICYLIC	2 g SALICYLIC	3 g SALICYLIC
	per cent	*per cent*	*per cent*	*per cent*
1	8.15	8.23	8.30	8.21
2	8.22	—	8.01	—
3	8.17	8.21	8.24	8.12
4	8.17	8.12	8.13	8.13
5	8.10	7.55	8.08	8.11
6	8.04	7.94	8.09	8.14
7	8.11	7.99	—	8.80
8	7.95	—	8.03	—
9	7.94	7.95	7.95	8.03
10	7.71	7.70	7.78	7.95
11	8.15	8.13	8.26	8.26
12	7.99	7.90	8.01	7.93
13	7.90	7.76	8.02	7.98
14	—	7.73	7.99	—
15	7.98	7.80	7.97	7.98
16	7.97	7.93	7.98	8.00
17	8.43	—	8.30	—
18	8.33	—	8.34	—
19	8.22	7.75	7.99	8.20
20	8.23	8.16	8.21	8.36
21	8.12	7.85	8.16	8.14
22	7.84	7.82	7.85	—
23	8.05	7.99	8.13	—
24	8.15	8.03	8.18	8.24

TABLE 3.—*Sample No. 3. Total nitrogen in low grade nitrate-chloride mixture*

ANALYST	FORD METHOD	METHOD 2.27 (1)		
		1 g SALICYLIC	2 g SALICYLIC	3 g SALICYLIC
	per cent	*per cent*	*per cent*	*per cent*
1	4.27	—	4.37	—
2	4.20	—	4.15	—
3	4.17	—	4.21	—
4	4.09	—	4.08	—
5	4.11	—	4.10	—
6	4.04	4.07	4.16	4.16
7	3.99	—	4.03	—
8	4.03	—	3.97	—
9	4.08	4.00	4.08	4.11
10	4.04	—	4.05	—
11	4.17	4.16	4.22	4.10
12	4.17	—	4.09	—
13	4.01	4.02	4.07	4.08
14	—	3.99	4.00	4.07*
15	4.10	—	4.15	—
16	4.03	4.03	4.05	4.05
17	4.13	—	4.17	—
18	4.22	—	4.25	—
19	3.84	—	3.95	—
20	4.17	—	4.18	—
21	4.18	4.15	4.24	4.16
22	4.15	4.11	4.15	—
23	4.07	4.09	4.07	—
24	4.18	4.15	4.18	—

* Fuming sulfuric used.

Analyst 11 finds it necessary to use 40 ml of sulfuric when using 3 g salicylic.

Analyst 12 finds electrometric end point 9.1 with formaldehyde titration. He uses 2.28 for nitrogen; therefore, he is somewhat neutral on Ford's method. He believes, however, that the "hypo" method should be retained.

Analyst 13 prefers thiosulfate to zinc for reduction; however, he thinks Ford's suggestion of using 40 ml of sulfuric a good one.

Analyst 15 fails to get concurring results when using Ford's method on nitrate of soda and definitely favors Devarda procedure.

Analyst 16 believes each one will have to standardize his end point with formaldehyde titration to his own electrode. He very much prefers the Devarda procedure on nitrate of sodas, and likes the idea of 2 g of salicylic in 2.27 (1) over any other method.

Analyst 17 finds the ammonium nitrate end point to be 8.9–9.0. He suggests trapping the vapor-carried alkali with a Davisson bulb.

Analyst 19 finds the stoichiometric end point for the formaldehyde titration to be at least 8.5. He has trouble with the Devarda procedure; however, he obtains lower

results with Ford method. He feels that the Goessman trap and Willits-John bulb add nothing to the processes. He thinks **2.27** (1) with increased salicylic is much easier to handle than Ford's procedure.

Analyst 20 prefers Ford's method to the present **2.27** (2).

Analyst 22 has been using Ford's version for over twenty years and prefers it to **2.27** (2).

Analyst 23 prefers thiosulfate to zinc.

DISCUSSION OF RESULTS

If one refers to Table 1 he will obviously see that all collaborators except one who compared the Devarda method with the Ford procedure obtained higher or more nearly theoretical results by the former method. Certainly if one has hundreds of nitrate of sodas to analyze he will make more speed by the Devarda method. Unfortunately, just a few tried out the Willits-John bulb. A majority of these saw no benefits from its use.

Of course, Table 2 is the one in which we are most interested. It is obvious that Ford's version of **2.27** (2) gave higher results than **2.27** (1) as now written; however, when 2 g of salicylic is used the average is just as good by using thiosulfate. Actually, the 3 g of salicylic with thiosulfate gave highest of all. After taking out the extremely high result and one low result to offset, the average is 8.13 per cent. This mixture was made by using 50% C. P. nitrate of soda; therefore, with the bit of nitrogen contamination in the superphosphate the average should be 8.25 per cent. These data certainly indicate that the mass factor of the salicylic is very important.

Finally, there is little to discuss concerning the results in Table 3. It is obvious that in the case of a low percentage nitrogen, the quantitative amount can be approached by using 3 g of salicylic with either zinc or thiosulfate for reducing agent.

CONCLUSIONS

(1) For those who want to use potentiometric titrations in the formaldehyde procedure for total nitrogen in ammonium nitrate, they should probably standardize the end point with each electrode and they will find this to be approximately *p*H 8.6.

(2) The second year's revival of the Devarda procedure comparison with some variation of the Kjeldahl method to include nitrates corroborates the findings of last year and of approximately twenty-five years ago. In other words, the Devarda procedure definitely gives more nearly the theoretical value for nitrogen in nitrate of soda.

(3) The solution of the determination of the nitrogen in high nitrate-chloride mixtures is not reached; however, the mass of salicylic acid used seems to be a more potent factor than any trick of manipulation or of choice between zinc and thiosulfate for reduction. There is no problem with low percentage goods.

RECOMMENDATIONS*

It is recommended—

(1) That the formaldehyde titration method as incorporated in this report be adopted as official.

(2) That the comparison of the Devarda procedure with Kjeldahl procedures for the determination of nitrogen in nitrate of soda be discontinued.

(3) That **2.27** (1) be changed to read as follows:

. . . (1) Add 30–35 ml of H_2SO_4 containing 2 g of commercial salicylic acid, shake until thoroly mixed, allow to stand at least 30 min. with frequent shaking or until complete soln results, and then add 5–7 g of $Na_2S_2O_3.5H_2O$ and digest as directed below, . . .

(4) That further study be made of high percentage nitrogen in high nitrate-chloride mixtures.

REPORT ON POTASH IN FERTILIZERS

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

In accordance with the recommendations of the Association (*This Journal,* **32,** 42–43, 1949) "That a survey be made of the different types of mills being used for preparation of the sample and that some collaborative potash work on samples prepared by the different mills be conducted on a greater variety of samples," a questionnaire was circulated among control officials and fertilizer manufacturers. In addition, some collaborative work was done on a great variety of samples. At the request of some fertilizer manufacturers for a short-cut potash method, the collaborative work included a comparison of the Perrin "short-cut wet digestion method for potash" and the regular A.O.A.C. methods.

The Associate Referee in attempting to solve the problem of preparation of samples for analysis and the effect of preparation on analysis decided that the first step was to get a picture of what was actually being done in the various Laboratories both control and commercial. With this thought in mind the following questionnaire was sent to control and commercial laboratories.

QUESTIONNAIRE TO FERTILIZER MANUFACTURERS AND CONTROL OFFICIALS

1. Check means of preparation for routine analysis in your laboratory of fertilizer samples.

a. Burr mill _____	**d.** Bucking board _____
b. Hammer mill _____	**e.** Other means _____
c. Mortar & Pestle _____	Describe:

2. Check amount prepared for analysis 1, 2, 4, 8, 16 ounces or more.

* For report of Subcommittee A and action of the Association, see *This Journal,* **33,** 37 (1950).

3. State average time necessary for preparation of a 1-lb. sample.
4. Check laboratory handling of samples below:
   a. Sample is not remixed but weighed direct from bottle by chemist_____
   b. Sample is removed from bottle and remixed on paper by chemist before weighing _____
5. Could you submit one or two *10-lb.* samples of fertilizer ground for analysis by your method of preparation? (A complete fertilizer containing organic matter and one with one or more sources of mineral nitrogen such as 10–10–10, 5–10–10, 5–10–5, 6–12–6, 3–12–12, 4–12–8, 4–16–16, 4–24–12, or 3–18–9 would be ideal.) A rough estimate of the amounts of the individual ingredients used in the formulation of the sample would be of value. It may be possible that your sample will include both organic matter and one or more sources of mineral nitrogen, if so one sample will be enough. Yes _____ No _____.
6. List formulation of each sample.
   a. (0–0–0) b. (0–0–0)
7. Check below:
   a. I *will* collaborate on Potash in 1949 and will be able to report by August 1st. (Please send instructions and samples to _____ by (list date) _____
   b. I *will not* be able to collaborate in 1949 _____

A reply was not received from all to whom the questionnaire was sent nor were all questionnaires that were received completely filled out. Of those reporting, 26 indicated a laboratory practice of weighing the sample direct from the bottle, while 14 removed the sample from the bottle, mixing it on paper before weighing.

SUMMARY OF SURVEY OF DATA RELATIVE TO PREPARATION OF SAMPLES FOR ANALYSIS

	METHOD OF PREPARATION REPORTED IN USE								
	BURR MILL	BEATER CROSS MILL	HAMMER MILL	MORTAR PESTLE	BUCKING BOARD	WILEY INTERMEDIATE	WORM MILL	IMPACT MILL	GRIST MILL
No. Reported Using	7[1]	1[2]	21[3]	19	2	4	1	2	1[4]
	TIME REQUIRED IN PREPARATION OF SAMPLES FOR ANALYSIS								
	3–5 MIN.	5–10 MIN.	15 MIN.	20 MIN.	30 MIN.	45 MIN.	60 MIN.		
No. Reported Requiring	8	10	9	6	4	1	1		
	AMOUNT OF SAMPLE PREPARED FOR ANALYSIS								
	1 OZ.	2 OZ.	3 OZ.	4 OZ.	6 OZ.	8 OZ.	12 OZ.	16 OZ.	32 OZ.
No. Reporting Preparing	1	1	2	18	1	14	1	6	1

[1] Three Sturtevant, two Enterprise, and two makes not disclosed.
[2] Christie Norris Hammer-Mill made at Chelmsford, England. Hill has a "beater cross," instead of swing hammers.
[3] 10 listed as microsamplmill, 11 just as hammer mills or micro pulverizers.
[4] Grist Mill type—one rotary and one stationary block of carborundum, grooved to allow material to flow across the plates.

Many collaborators reported the use of more than one means of preparation of the sample, the method used being dependent upon the type of sample.

As about an equal number of control and commercial laboratories filled out the questionnaire and agreed to furnish a sample prepared in their laboratory for analysis, the original plan of sending out a mixture of known analysis and formulation prepared by several methods was changed. The wisdom of this was evidenced later when the samples were sent in without information as to formulation in many cases and without being too well prepared in the case of some samples.

1949 COLLABORATIVE WORK ON POTASH IN FERTILIZER

With the increasing changes in the formulation and composition of fertilizers today and with various methods of preparation of the samples for analysis, it is necessary to study the effect of these on the determination of potash by the Official Method. Since some manufacturers have expressed a need for a short-cut method for potash, we have included in this study a short-cut wet combustion method by Dr. C. H. Perrin, Canada Packers Limited, Toronto, Canada.

INSTRUCTIONS TO COLLABORATORS

It is recommended:

Part 1. Prepare enough solution for *six* determinations on each sample. (Make 3 determinations each as parts 2 and 3.)

Part 2. Using the Official Method for Potash, make *three* individual potash determinations on as many samples as time permits by each means of preparation of sample. (See Part 1.)

a. Micro-Samplmill samples 3, 4, 9, 12, and 15
b. Burr Mill samples 5, 6, and 17
c. Mortar and Pestle samples 10, 11, 14, and 8
d. Bucking Board samples 1 and 2
e. Phipps and Bird Impact Mill samples 7 and 18
f. Regular Hammer Mill samples 6 and 13.

Part 3. Using Dr. C. H. Perrin's wet combustion method, a copy of which is enclosed, make *three* individual potash determinations on the samples listed above by each means of preparation of samples. (See Part 1.)

Part 4. Make *three* individual insoluble phosphoric acid determinations by each means of preparation on as many samples as possible. Omit Sample 13.

Sample Number	*Grade*	*Sample Number*	*Grade*
1	3-12-6	10	4-12-8
2	10-10	11	4-9-6
3	5-10-10	12	4-12-8
4	10-10-5	13	0-0-60
5	5-10-5	14	4-12-8
6	2-10-8	15	3-12-12
7	3-9-6	16	3-12-12
8	3-9-6	17	3-12-12
9	3-12-12	18	7-7-7

COPY OF DETAILS OF THE RAPID WET-DIGESTION METHOD FOR POTASH IN FERTILIZER

C. H. Perrin

Since the following proposed method is a modification of the official A.O.A.C. method, the style of the latter has been followed.

METHOD

REAGENTS

(a) *Platinum soln.*—Use a Pt. soln. containing the equivalent of 0.5 g of Pt. (1.05 g H_2PtCl_6) in every 10 ml.

(b) *Diglycol stearate soln.*—Dissolve 20 g of diglycol stearate tech. in 1 liter of equal parts of benzene and ethyl alcohol.

PREPARATION OF SOLUTION

Place 2.5 g or the factor weight 2.422 g of sample in 250-ml volumetric flask, and add 125 ml of H_2O and 50 ml of saturated NH_4 oxalate soln, also 1 ml of diglycol stearate soln when necessary to prevent foaming. Boil 30 min, add slight excess of NH_4OH and, after cooling, dilute to 250 ml, mix, and pass thru dry filter.

DETERMINATION

Place a 50 ml. aliquot of soln (or a 25 ml aliquot and 25 ml H_2O, if sample contains over 20% K_2O) in a 500 ml Kjeldahl flask. Add 10 ml HNO_3 and a silica granule (about 1 cm long) previously weighed along with a prepared Gooch or medium fritted crucible (Pyrex M porosity). Boil 2 min and add 10 ml HCl. Boil down to approximately 25 ml and add 5 ml HCl and excess Pt soln. Boil down to 10–15 ml, rotating flask occasionally, and then add 5 ml HCl. Reduce heat and boil down to 3–5 ml (depending on amount of precipitate), rotating flask frequently near the end of the evaporation. Remove flask from heat and swirl to dissolve any soluble residue on walls. After cooling, immediately add 25 ml of 95% alcohol so that it washes down neck of flask. Chill under tap, swirl and allow to stand for at least 5 min. Decant into the tared crucible and transfer precipitate and granule with the aid of a stream of 95% alcohol. Dry crucible to constant weight (5–7 min. usually are sufficient) in an aluminum plate (having depressions to fit crucibles loosely) maintained at 130°. Weigh and subtract weight of crucible plus the silica granule. $K_2PtCl_6 \times 0.19376 = K_2O$.

As the samples submitted for analysis appeared visually to be quite different in degree of fineness although all were supposed to have been prepared for analysis, the Associate Referee decided that a sieve analysis of about 500 grams of each would throw some light on the kind of job that is being done in preparation of the sample for analysis in the various laboratories. After thoroly mixing the sample, about 500 grams was set aside for sieving and analysis of the sieve portions before bottling the collaborative samples.

Since the sieve analysis indicated variation of particle size, potash values on the various sieve portions were made to see what happens to the potash under this treatment. It is evident from the sieve test that many laboratories are not doing as well in preparation of the samples for analysis as they think they are.

Visual examination of the portions left on the sieves during the sieve test showed in many causes a preponderance of coarse potash salt. It would appear from this that in a mixture, unless the potash salt is previously ground very fine, it tends to resist grinding more than the other ingredients, with a result that the higher K_2O may often be found in the coarser portion of a fertilizer mixture. A very good example of this is

TABLE 1.—*Sieve data on the eighteen 1949 collaborative potash samples*

SAMPLE NUMBER	ANALYSIS	METHOD OF PREPARATION	WEIGHT OF SAMPLE IN GRAMS				
			SIEVED	LEFT ON 10 MESH	LEFT ON 40 MESH	LEFT ON 70 MESH	PASSING 70 MESH
1	3-12-6	Bucking Board	480	—	40	220	220
2	10-10-10	Bucking Board	485	—	135	200	150
3	5-10-10	Microsamplmill	495	—	110	100	295
4	10-10-5	Microsamplmill	485	—	65	150	270
5	5-10-5	Burr Mill[1]	480	—	230	135	115
6	2-10-8	Regular Hammer Mill	485	—	110	150	225
7	3-9-6	Impact Mill (Phipps Bird)	495	—	90	200	205
8	3-9-6	Mortar & Pestle	495	—	155	130	210
9	3-12-12	Microsamplmill	490	—	50	145	295
10	4-12-8	Mortar & Pestle	490	—	210	195	85
11	4-9-6	Mortar & Pestle	490	—	135	150	205
12	4-12-8	Microsamplmill	495	30	180	80	205
13	0-0-60	Regular Hammer Mill	515	—	355	130	30
14	4-12-8	Mortar & Pestle	510	75	220	110	105
15	3-12-12	Microsamplmill	495	—	50	180	265
16	3-12-12	Burr Mill[2]	485	—	200	135	160
17	3-12-12	Burr Mill[2]	500	—	250	150	100
18	7-7-7	Impact Mill (Phipps Bird)	490	—	150	130	210

[1] Sturtevant Burr Mill.
[2] Enterprise #2 Burr Mill.

sample No. 14. This does not mean that finer grinding will not give the correct value and the most uniform result, since a check on the sieve test, and a comparison of the K_2O values of the samples that were the best prepared, showed the best uniformity of results.

As previously stated, the original plan, of having a few standard fertilizer mixtures of known formulation prepared for analysis by the various methods of preparation, was changed. The potash values on the samples in the following tables are a comparison of the A.O.A.C. and the Perrin Method, since all of the determinations were supposed to have been made on aliquots of the same solution, although the samples were ground for analysis by different methods.

COMMENTS ON THE POTASH RESULTS

If one disregards the methods of preparation of the 18 samples it is noted that in 2 cases the average per cent of K_2O was the same by both A.O.A.C. and the Perrin method of analysis; in 13 cases it ranged from .03 to .14% higher by the A.O.A.C., and the other 3 cases from .03 to .2% less by the A.O.A.C. than by the Perrin method. Since all determinations for the 2 methods were run on aliquots of the same solution, it would seem that the 2 methods could be rated to have about the same degree of accuracy.

Taking into consideration the variation in K_2O content shown by the

TABLE 2.—*Comparison of the average K_2O found and the K_2O content of the sieve portions*

SAMPLE NUMBER	AV. K_2O BY A.O.A.C.	% K_2O OF SAMPLE ON 10 MESH	% K_2O OF SAMPLE ON 40 MESH*	% K_2O OF SAMPLE ON 70 MESH†	% K_2O OF SAMPLE PASSING 70 MESH†
1	6.19	—	8.22	6.76	6.16
2	8.13	—	9.72	8.38	6.62
3	10.37	—	11.24	10.78	10.72
4	5.25	—	3.44	4.94	6.74
5	5.40	—	5.56	6.04	6.20
6	8.23	—	8.00	8.58	9.04
7	6.52	—	5.18	8.14	7.42
8	6.81	—	6.68	9.92	6.42
9	11.72	—	9.10	15.64	10.62
10	8.26	—	9.02	8.66	7.18
11	6.88	—	7.22	8.02	6.58
12	8.69	6.18	8.00	10.40	9.52
13	60.86	—	61.76	61.84	60.56
14	13.60	13.48	15.90	14.52	9.76
15	12.81	—	14.76	14.98	11.28
16	12.39	—	12.00	10.58	10.16
17	12.42	—	13.02	14.28	10.62
18	7.27	—	6.54	9.60	7.14

* Portions on 10 and 40 mesh were reground by Bucking Board, put through sieve with 1 mm opening, and mixed before analyzing.
† Portions on 70 and passing 70 mesh were mixed before analyzing.

different methods of preparation, it is to be noted that one of the samples showing the least as well as one showing the greatest variation was in the samples prepared by the use of the Mortar and Pestle, the range of variation being 1.33 to 11.46. This would indicate that the preparation of the sample by this method is more dependent on the operator than the equipment. As one collaborator pointed out, we should not confuse preparation of the sample with the method of preparation. He stated that just as good a sample can be prepared by the use of the Mortar and Pestle as by any other method, but it might take more time and re-

TABLE 3.—*A.O.A.C. vs Perrin method for potash*

A Comparison of Uniformity of K_2O Results. Samples Prepared by Microsamplmill

COLLABORATOR NUMBER	NO. OF ANALYSES	A.O.A.C.					PERRIN				
		SAMPLE 3	SAMPLE 4	SAMPLE 9	SAMPLE 12	SAMPLE 15	SAMPLE 3	SAMPLE 4	SAMPLE 9	SAMPLE 12	SAMPLE 15
1	3	10.30	5.14	—	—	12.73	10.16	4.97	—	—	12.44
2	3	10.26	5.15	11.49	8.53	12.69	10.07	5.12	11.45	8.34	12.62
3	3	10.20	5.05	11.42	8.36	12.51	10.29	5.12	11.30	8.33	12.53
4	3	10.37	5.24	11.84	8.69	12.79	10.23	5.16	11.89	8.58	12.65
5		10.36	5.23	11.64	8.66	12.68	10.23	5.07	11.52	8.54	12.77
6	3	10.08	4.93	11.25	8.55	12.35	10.15	4.94	11.51	8.69	12.88
7	3	10.41	—	—	—	—	10.19	—	—	—	—
8	4	10.32	5.15	11.62	8.18	12.70	10.00	4.65	11.30	7.77	12.41
9	3	10.81	—	11.61	—	—	10.34	—	11.51	—	—
10	3	10.12	5.16	11.53	8.61	12.35	10.03	4.96	11.43	8.57	12.55
11	1	—	—	10.82	—	—	—	—	11.41	—	—
12	3	11.17	6.07	13.62	9.74	14.38	11.22	5.84	13.29	9.58	14.13
13	3	10.40	5.20	11.68	8.52	12.80	10.37	5.10	11.68	8.49	12.73
14	3	10.47	5.24	11.73	8.56	12.73	10.37	5.00	11.49	8.48	12.66
15	3	10.23	—	—	—	—	10.30	—	—	—	—
16	3	10.19	—	—	—	—	10.31	—	—	—	—
17	3	10.17	—	—	—	—	10.39	—	—	—	—
18	3	10.34	5.19	11.50	8.68	12.73	10.45	5.22	11.63	8.68	12.80
19		10.61	—	—	—	—	10.31	—	—	—	—
20	3	10.35	5.20	11.80	8.95	12.92	10.18	5.03	11.38	8.59	12.77
21	1	10.39	5.46	11.40	8.57	13.17	10.49	5.12	11.92	8.63	13.46
23	3	10.24	5.38	12.62	9.05	12.67	10.32	5.29	11.94	8.51	12.76
24*	3	10.24	5.07	—	—	—	10.29	5.09	—	—	—
25*	3	10.34	5.18	11.56	8.37	12.30	10.21	5.04	11.18	8.28	11.88
26*		10.33	5.16	11.49	8.49	12.69	10.21	5.21	11.59	8.48	12.71
Average		10.37	5.25	11.72	8.69	12.81	10.30	5.11	11.66	8.55	12.81
High		11.17	6.07	13.62	9.74	14.38	11.22	5.84	13.29	9.58	14.13
Low		10.08	4.93	10.82	8.18	12.35	10.00	4.94	11.30	8.34	12.44
Maximum variation		1.09	1.14	2.80	1.56	2.03	1.22	.90	1.99	1.24	1.69

* Reported late; not included in averages. Number 24 samples reground before analysis.

TABLE 4.—*A.O.A.C. vs Perrin method for potash*

A Comparison of Uniformity of K_2O Results. Samples prepared by Burr Mill

COLLABORATOR NUMBER	NO. ANALYZED	A.O.A.C.			PERRIN		
		SAMPLE 5	SAMPLE 16	SAMPLE 17	SAMPLE 5	SAMPLE 16	SAMPLE 17
1	3	5.36	—	—	5.13	—	—
2	3	5.32	12.48	12.57	5.22	12.37	12.39
3	3	5.15	11.97	12.25	5.23	11.77	12.15
4	3	5.41	12.21	12.27	5.37	12.20	12.36
5	3	5.39	12.19	12.11	5.26	12.21	12.11
6	3	5.13	11.92	12.26	5.28	12.22	12.57
7	3	5.32	12.43	—	5.34	12.53	—
8	4	5.39	12.08	12.27	5.06	11.99	12.09
9	3	5.70	12.31	12.60	5.42	12.09	12.41
10	3	5.31	12.33	12.45	5.21	12.14	12.56
11	1	—	11.66	11.61	—	11.84	11.90
12	3	6.15	13.76	13.78	5.97	13.64	13.91
13	3	5.39	12.46	12.63	5.39	12.42	12.65
14	3	5.36	12.34	12.52	5.16	12.39	12.41
15	3	5.48	11.93	—	5.23	11.89	—
16	3	5.32	12.10	—	5.24	12.25	—
17	3	—	—	12.42	—	—	12.29
18	3	5.29	12.77	12.94	5.31	12.83	12.97
19	3	5.35	12.42	—	5.29	12.38	—
20	3	5.47	12.37	11.89	5.07	12.61	12.47
21	1	5.46	13.68	12.15	5.12	13.17	12.28
23	3	5.35	12.42	12.47	5.41	12.34	12.84
24*	3	—	12.23	12.37	—	12.24	12.32
25*	3	5.41	12.08	12.20	5.18	12.06	11.85
26*		5.40	12.27	12.45	5.39	12.32	12.63
Average		5.40	12.39	12.42	5.29	12.36	12.49
High		6.15	13.76	13.78	5.97	13.64	13.91
Low		5.13	11.92	11.61	5.07	11.77	11.90
Maximum variation		1.02	1.84	2.17	.90	1.87	2.01

* Collaborator Number 24 reground samples before analysis. Nos. 24, 25, and 26 reported late; not included in average.

quire more care. All of the mechanical methods of preparation reduced the maximum variation in analysis when a comparison is made with the variation found in analysis of Bucking Board and Mortar and Pestle prepared samples. This variation ranged from 1.02 to 2.8 on samples prepared by some mechanical means of preparation, but was considerably less than for non-mechanical means of preparation (Mortar-Pestle and Bucking Board). It would seem, since the variation of K_2O is less when the sample is prepared by some mechanical means, and since samples prepared in this way required less time for preparation, that more studies

TABLE 5.—*A.O.A.C. vs Perrin method for potash*

A Comparison of Uniformity of K_2O Results. Samples Prepared by Mortar & Pestle

COLLABORATOR NUMBER	NO. ANALYZED	A.O.A.C.				PERRIN			
		SAMPLE 8	SAMPLE 10	SAMPLE 11	SAMPLE 14	SAMPLE 8	SAMPLE 10	SAMPLE 11	SAMPLE 14
1	3	—	8.36	—	—	—	8.28	—	—
2	3	6.80	8.26	6.98	14.32	6.67	8.09	6.82	14.38
3	3	6.71	8.10	6.87	12.73	6.72	8.09	6.84	12.44
4	3	7.02	8.50	6.94	14.49	6.90	8.51	6.91	14.38
5	3	6.88	8.29	6.88	13.18	6.70	8.19	6.74	13.11
6	3	6.49	7.99	6.72	13.09	6.64	8.28	6.92	13.24
7	3	—	7.43	—	—	—	8.08	—	—
8	4	6.71	8.23	6.95	15.12	6.24	8.01	6.66	15.84
9	3	6.79	8.23	—	—	6.60	8.23	—	—
10	3	6.78	8.66	6.84	14.05	6.88	8.25	6.66	13.38
11	1	6.18	7.71	—	15.33	6.56	7.90	—	15.81
12	3	7.97	9.53	7.85	—	7.23	9.47	7.25	—
13	3	6.92	8.27	6.94	13.35	6.90	8.24	6.89	13.23
14	3	6.74	8.39	6.79	12.37*	6.72	8.43	6.95	12.37
15	3	—	—	6.98	—	—	—	6.81	—
16	3	—	—	6.73	—	—	—	6.88	—
17	3	—	8.18	—	—	—	7.96	—	—
18	3	6.75	8.37	6.81	13.31	6.82	8.45	6.84	12.99
19	3	6.93	—	—	—	6.78	—	—	—
20	3	7.04	8.19	6.67	17.77†	6.71	8.46	6.99	17.35
21	1	6.56	8.07	6.67	6.31	6.71	8.16	6.58	6.96
23	3	6.55	8.29	6.52	15.04	6.89	8.48	6.92	15.00
24‡		—	8.18	—	13.66	—	8.22	—	13.58
25	3	6.84	8.31	6.89	10.57	6.87	8.23	6.64	10.64
26		6.56	8.38	6.73	13.93	6.70	8.36	6.71	13.72
Average		6.81	8.26	6.88	13.60	6.74	8.29	6.85	13.60
High		7.97	9.53	7.85	17.77	7.23	9.47	7.25	17.35
Low		6.18	7.42	6.52	6.31	6.24	7.96	6.58	6.96
Maximum Variation		1.79	2.10	1.33	11.46	.99	1.31	.67	10.39

* A repeat new weighing gave 13.75% K_2O. Ironically sample No. 14 which showed the greatest maximum variation in analysis gave identical average K_2O values by the A.O.A.C. and Perrin Method.

† A new weighing from top gave a 14.04% K_2O value by A.O.A.C. By remixing again another weighing gave 13.42% K_2O.

‡ Reported late, not included in the average. No. 24 reground samples before analysis.

should be made along this line of approach. This is particularly appropriate since the trend in preparation of samples is toward a mechanical means of preparation and away from that method of preparation where the personal element may be a factor (like Bucking Board or Mortar and Pestle). Almost without exception, the method of preparation of sample had little effect on the insoluble phosphoric acid. Very consistent values were obtained on practically all samples, the maximum variation being small in most cases.

TABLE 6.—*A.O.A.C. vs Perrin method for potash*

A Comparison of Uniformity of K_2O Results. Samples Prepared by Bucking Board

COLLABORATOR NUMBER	NUMBER DETERMINATION	A.O.A.C.		PERRIN	
		SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2
1	3	6.09	—	6.04	—
2	3	6.12	7.97	6.15	8.00
3	3	5.95	7.91	5.89	7.80
4	3	6.21	8.10	6.16	8.08
5	3	6.06	7.98	6.01	7.93
6	3	5.91	7.69	5.94	7.81
7	3	—	7.90	—	7.95
8	4	6.10	7.98	5.83	7.70
9	3	6.32	—	6.02	—
10	3	6.02	7.88	5.85	7.90
12	3	7.26	9.21	6.79	9.21
13	3	6.18	8.06	6.14	7.98
14	3	6.24	8.00	6.06	7.99
15	3	6.13	—	6.05	—
16	3	5.97	—	5.95	—
17	3	6.10	—	5.96	—
18	3	6.09	7.98	6.11	8.22
19	3	6.22	—	6.11	—
20	3	6.30	7.95	6.17	7.52
21	3	6.36	7.99	6.25	8.18
23	3	6.18	10.03	6.26	8.15
25*	3	6.26	8.11	5.58	7.58
26*	3	6.04	7.95	6.04	7.99
Average		6.19	8.13	6.09	8.03
High		7.26	10.03	6.79	9.21
Low		5.91	7.69	5.83	7.52
Maximum variation		1.35	2.34	.96	1.69

* No. 25—Average from individual weighing of original sample. Nos. 25 and 26 reported late; not included in average.

FLAME PHOTOMETER

Collaborator No. 15 inclosed Flame Photometer values for samples 1, 3, 5, 6, 7, 11 and 16. These values agreed with a range of .1 to .2% K_2O of the values for these samples as reported by both the A.O.A.C. and Perrin methods. Collaborator No. 16, using the same type of Flame Photometer (Perkins-Elmer) has been cooperating with their soil department and reports favorably on the use of the instrument. In the laboratory of the Dept. of Agric. Chemistry, Purdue University, studies are being made of the adaptability of the Flame Photometer with a Beckman hook up to determination of potash in fertilizers by collaborator No. 20. It is regretted that these studies were not complete enough to be included in this report. Since there is an evident growing interest in the use of the

TABLE 7.—*A.O.A.C. vs Perrin method for potash*

A Comparison of Uniformity of K_2O Results. Samples Prepared by Phipps Bird Impact Mill

COLLABORATOR NUMBER	NUMBER DETERMINATION	A.O.A.C.		PERRIN	
		SAMPLE 7	SAMPLE 18	SAMPLE 7	SAMPLE 18
1	3	6.76	—	6.66	—
2	3	6.66	7.14	6.52	7.02
3	3	6.66	7.21	6.58	7.16
4	3	6.98	7.35	6.97	7.21
5	3	6.79	7.16	6.68	7.16
6	3	6.61	7.30	6.40	7.03
7	3	7.11	—	7.03	—
8	3	6.79	7.06	6.43	6.93
9	3	6.90	—	6.57	—
10	3	6.68	7.20	6.89	7.07
11	1	6.18	—	6.46	—
12	3	7.44	8.15	6.90	8.27
13	3	6.79	7.32	6.79	7.28
14	3	6.73	7.30	6.71	7.23
15	3	6.85	—	6.67	—
16	3	7.12	—	7.30	—
17	3	6.89	—	6.60	—
18	3	6.76	7.24	6.80	7.27
19	3	7.44	—	6.70	—
20	3	6.91	7.37	6.59	6.98
21	1	6.51	7.17	6.73	7.32
23	3	6.57	6.87	6.95	7.32
25*	3	6.37	7.26	6.00	7.11
26*		6.41	7.72	6.74	7.43
Average		6.52	7.27	6.72	7.23
High		7.44	8.15	7.30	8.27
Low		6.18	6.87	6.40	6.93
Maximum variation		1.26	1.28	.90	1.34

* No. 25. Average from individual weighings of original sample. Nos. 25 and 26 reported late; not included in average.

Flame Photometer as a means of measuring the potash content of fertilizers it seems advisable that collaborative studies using this instrument should be initiated.

COMMENTS OF THE COLLABORATORS

(1) Noting that the wet combustion method gave lower results, I carried the evaporation almost to dryness (moist condition). After weighing crucible and precipitate, ammonium chloride and alcohol washings were made as in the official method, crucible dried and reweighed. Loss of weight due to the ammonium chloride wash varied from .9 to 2.7 mg. on the 5 samples treated this way. This procedure increased the K_2O value found from .2% on the samples of 5% guarantee to .4% on those of 12% guarantee.

TABLE 8.—*A.O.A.C. vs Perrin method for potash*

A Comparison of Uniformity of K_2O Results. Samples Prepared by Regular Hammer Mill

COLLABORATOR NUMBER	NUMBER DETERMINATION	A.O.A.C.		PERRIN	
		SAMPLE 6	SAMPLE 13	SAMPLE 6	SAMPLE 13
1	3	8.28	—	8.17	—
2	3	8.10	60.75	8.08	60.76
3	3	8.04	60.76	8.08	60.68
		—	60.16*	—	60.35*
4	3	8.24	60.74	8.17	60.93
5	3	8.22	60.83	8.11	60.76
6	3	7.93	60.80	8.08	60.67
7	3	8.13	—	8.26	—
8	4	8.44	61.01	7.60	60.38
9	3	8.79	—	8.10	—
10	3	8.21	60.51	8.14	61.09
11	1	7.85	—	7.99	—
12	3	9.07	67.42†	9.04	67.34†
13	3	8.10	60.95	8.17	60.68
14	3	8.27	60.44	8.16	60.70
15	3	7.90	—	8.03	—
16	3	8.08	—	8.04	—
17	3	8.02	—	8.17	—
18	3	8.19	61.14	8.24	60.68
19	3	8.41	—	8.15	—
20	3	8.49	60.83	8.27	61.07
21	3	8.18	61.08	8.28	60.86
23	3	8.20	61.68	8.46	62.09
24[1]	3	—	60.88	—	61.46
25[2]	3	8.16	60.99	8.07	—
26[3]	3	8.22	60.85	8.26	60.62
Average		8.23	60.86	8.17	60.83
High		9.07	61.68	9.04	62.09
Low		7.85	60.16	7.60	60.35
Medium variation		1.22	1.52	1.44	1.74

* 2nd run, made on a second solution; curiosity aroused by coarse grinding of sample.
† Omitted from average. Theoretically impossible with Nuriate of Potash.
[1] No. 24—Sample reground before analysis.
[2] Nos. 24, 25, and 26 received late; not included in average.
[3] No. 25—Each figure average of 3 separate weighings.

(2) Pass organic material through Wiley mill and then mix with the Mortar & Pestle portion.

(3) We like the Perrin method, with adaptations of equipment to fit the individual laboratory it should be okay.

(4) The two potash methods give the same results in our laboratory. One fact should be definitely established from the various laboratory results on these samples; namely, the importance of sample preparation. I hope we do not, however, confuse "Method of Preparation" with "Preparation." A sample can be properly prepared

even with Mortar & Pestle. I must add a few of these samples appear not ground at all. You will note 2 sets of results on sample #41. This sample was so poorly prepared that we hoped to really show the sample preparation up.

(5) Sample #14 appears to have had no preparation. A few particles in this sample failed to pass a 10 mesh screen. In addition to the results we have tabulated, we made six determinations for an average of 14.16 per cent potash. An additional set of six gave an average of 15.14 per cent potash.

(6) We use four methods of preparation for samples, namely, Mortar & Pestle, Bucking Board, Wiley mill and Micro-samplmill.

TABLE 9.—*A comparison of uniformity of insol. P_2O_5*

Samples Prepared by Various Methods

COLLABORATOR NUMBER	NUMBER DETERMINATION	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5	SAMPLE 6	SAMPLE 7	SAMPLE 8
1	3	1.25	—	.38	.80	1.07	.37	.58	—
2	3	1.23	.59	.43	.89	.75	.37	.75	.76
3	3	1.00	.47	.37	.80	.46	.34	.59	.67
4	3	1.32	.54	.50	.86	.53	.41	.77	.74
5	3	1.50	.67	.52	.87	.52	.38	.72	.75
6	3	1.03	.53	.50	.89	.62	.44	.76	.74
7	3	—	.66	.63	—	.72	.36	.75	—
9	3	1.26	—	.46	—	.92	.41	.72	.80
10	3	1.61	.76	.70	1.14	1.02	.78	.96	1.00
11	1	—	—	—	—	—	.48	.76	.80
12	3	.93	.46	.38	.83	.49	.35	.65	.68
13	3	1.23	.56	.46	.92	.58	.43	.73	.79
14	2	1.10	.55	.41	.89	.64	.37	.68	.70
15	3	1.37	—	.46	—	.69	.39	.73	—
17	3	1.29	—	.53	—	—	.47	.78	—
22	3	1.39	.62	.57	.94	1.00	.59	1.00	1.01
25*	2	1.25	.55	.40	.84	.74	.45	.60	.65
26*	3	.90	.52	.43	.76	.61	.43	.56	.67
Average		1.25	.58	.45	.89	.71	.43	.74	.79
High		1.61	.76	.70	1.14	1.07	.78	1.00	1.01
Low		.93	.46	.37	.80	.46	.35	.58	.67
Maximum variation		.68	.30	.33	.24	.61	.43	.42	.34

* Reported late; not included in average.

(7) We were much impressed by the rapidity and accuracy of the Perrin Method of potash analysis.

(8) Our analyst complained about the preparation of the samples and a cursory examination indicates there was some justification for his complaint. We believe, however, some of them were supposed to be poorly prepared, so no doubt they were sent as you intended. Our analyst did not like the Perrin method. It might be all right for two or three samples, but for routine determinations, we believe it unsatisfactory.

(11) Sample #14 was a very coarse grind or preparation. A repeat analysis on

this did not check very well the first sample preparation. Hence the values reported indicate the comparative value of the methods of analysis rather than the true potash content. We will be interested in hearing the future status of the Perrin method, as we feel it has certain advantages over the present A.O.A.C. method.

(12) Results on sample #14 have been omitted on the two potash methods because they were too erratic.

(13) Single determinations of potash were completed in less time by the Perrin method than with the A.O.A.C. method. However, in using this method for routine analysis, we have not found any saving of time. The elimination of platinum and

TABLE 9.—*A comparison of uniformity of insol. P_2O_5—Continued*

Samples Prepared by Various Methods

COLLABORATOR NUMBER	NUMBER DETERMINATION	SAMPLE 9	SAMPLE 10	SAMPLE 11	SAMPLE 12	SAMPLE 14	SAMPLE 15	SAMPLE 16	SAMPLE 17	SAMPLE 18
1	3	—	1.30	—	—	—	.71	—	—	—
2	3	.68	1.13	.78	1.28	.36	.90	1.06	1.00	.62
3	3	.65	.81	.59	.93	.27	.71	.90	.66	.55
4	3	.67	1.05	.53	.88	.35	.70	.96	.85	.49
5	3	.67	1.07	.73	1.15	.42	.80	1.02	.90	.62
6	3	.73	.97	.75	1.11	1.24	.71	1.03	.82	.63
7	3	—	1.33	—	—	—	—	1.18	—	—
9	3	.77	1.33	—	—	—	—	1.06	.91	—
10	3	.80	1.67	1.10	1.56	.82	1.19	1.22	1.17	.90
11	1	.70	1.15	—	—	.40	—	1.03	.88	—
12	3	.60	1.00	.67	1.09	.45	.68	.90	.83	.53
13	3	.75	1.09	.74	1.25	.46	.85	1.03	.80	.60
14	2	.75	.99	.76	.83	.96*	.79	.85	.85	.61
15	3	—	—	.70	—	—	—	1.03	—	—
17	3	—	1.22	—	—	—	—	—	.90	—
22	3	.84	1.69	2.27†	3.21†	.94	1.22	1.20	—	—
25‡	2	.68	1.07	.72	1.06	.65	.78	1.01	.82	.60
26‡	3	.61	1.20	.76	.90	.42	.77	.98	.73	.72
Average		.72	1.20	.74	1.12	.61	.77	1.03	.88	.61
High		.84	1.69	1.10	1.56	1.24	1.22	1.22	1.17	.90
Low		.65	.81	.53	.83	.27	.68	.90	.63	.49
Maximum variation		.19	.88	.57	.73	.97	.54	.32	.54	.41

* Sample contained 4–5 mm lumps (values of .36 and 1.56 obtained on 2 different weighings.
† Not included in the average.
‡ Reported late; not included in the average.

silica dishes and the ammonium chloride wash in this method has its advantage. Samples 12, 13 and 14 did not pass the prescribed fineness as specified by the method of the A.O.A.C.

(14) Samples 12, 14 and 17 were so poorly ground that in our laboratory under most ordinary conditions we would not attempt to analyze them. Particle size definitely shows that it would be an impossibility to weigh out a representative sample for analysis.

(15) The Perrin method has already been adopted by a number of Canadian

laboratories and I am hopeful it will prove a boon to the fertilizer industry (where rapid analysis is so necessary to improve control of production).

The Flame Photometer results will, no doubt be of interest to you. We believe that we have learned how to get reliable and fairly accurate results with this instrument and we think that it has a bright future.

(16) We think the Perrin method less complicated than the A.O.A.C. and requires less time. We would like to suggest that consideration be given to the adoption of the Perrin method.

We think an interesting comparison could have been made of the methods of sample preparation if one grade had been prepared by the seven different methods. In order to avoid as many variables as possible, the grade should come from one batch. We would like to suggest that you consider such a sample next year.

(18) The Perrin method has some good points. But it would be rather hot down here standing over a hot plate keeping close watch of a set on routine work. It seems to check well, results seem a bit higher which should please the fertilizer companies. Method is fool proof, would save time in analysis. I would like to see this method included in the book of methods as an optional method as it could be of considerable help where a few samples must run in a short space of time.

(20) In trying to run 32 to 48 samples per day the time saved by the method would be expended in the constant attention required in treating the samples on the hot plate. Time could be saved over the regular A.O.A.C. in running a few samples at a time. This method entails an excessive amount of washing to transfer the chloroplatinate from the flask to the fritted crucible.

(21) Due to illness, unable to do more than 1 potash determination by each method of analysis.

(22) Due to reorganization of our department, unable to do any potash work, We are now known as "State of Colorado Department of Agriculture" instead of "Office Director of Markets."

(23) As reported to you previously some of the samples sent out were in a very rough condition and close checks were hard to obtain.

(24) The Perrin method seems to be promising with minor modifications. Is as accurate as the A.O.A.C., is more rapid in elapsed time, although it requires more of the operator's time. Method should be valuable in plant control work with KCl sample—when 24 ml aliquot was used, there was an apparent incomplete oxidation of the oxalate. For percentages of potash above 40 the method should specify the use of a 10 ml aliquot. Suggest that cattle manure and high organic fertilizer be included in next year's work.

(25) Samples 1, 2 and 13 gave us some trouble. On sample No. 13 we were unable to obtain a figure of value using Dr. Perrin's method. Each figure reported is the result of separate weighing from the sample.

(26) On the Perrin wet digestion method we obtained several occasional results which were 1% or more high and were considered out of line so a replacement was tested. On the muriate potash sample #13 we got several results 5 to 6% above theoretical, these were of course discarded and retests were made but still did not get good agreement. Part of this trouble may have come from coarsely ground sample as to individual agreement, but do not know cause of heavy insoluble precipitate observed in off results. All results on potash are after leaching back crucibles, of course, for blank, or insoluble residues. Found very little residue except on some 60% muriate, however.

In our opinion the Perrin wet digestion method offers quite a saving of time and apparently with practice it should give results comparable to Official A.O.A.C. procedure. No doubt it could be accepted as an optional method for A.O.A.C. recognition. We intend to do further work here with it on routine control work. Right now

we still want to know just what can cause a straight muriate determination to give 5 to 6.6% above theoretical. We plan to do quite a bit of work along on it, if we find anything special will advise you, and if you have the answer to the one point worrying us, let us know.

LIST OF COLLABORATORS

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(2) D. B. Bates and P. M. Goudelock, Smith-Douglass Company, Inc., 304 East Plume Street, Norfolk, Virginia.
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(4) A. T. Blackwell, Mr. Biship and Mr. Hoffmaster: Davison Chemical Corporation, Baltimore 3, Maryland.
(5) P. R. Bidez, Alabama Polytechnic Institute, Auburn, Alabama.
(6) R. C. Charlton, P. A. Lang, H. L. Holland and A. Mudrak, The American Agricultural Chemical Company, 50 Church Street, New York 7, New York.
(7) R. G. Eklund, Darling & Company, P. O. Box 271, East St. Louis, Illinois.
(8) M. P. Etheredge, Mississippi State Chemical Laboratory, State College, Mississippi.
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(10) H. Kocaba, and H. J. Fisher, The Connecticut Agric. Expt. Station, New Haven, Connecticut.
(11) L. A. Koehler and R. O. Baird, State Laboratories Department, Lock Box 900, Bismarck, North Dakota.
(12) G. C. Mowery and M. Escue, Dept. of Agric., State of Tennessee, Nashville 3, Tennessee.
(13) R. O. Powell, R. M. Morris and H. L. Moxon, Virginia-Carolina Chemical Corporation, Fertilizer Laboratory, P. O. Box 623, Richmond 5, Virginia.
(14) P. Ijams and A. T. Perkins, Dept. of Chemistry, Kansas State College, Manhattan, Kansas.
(15) C. H. Perrin, Canada Packers Limited, Toronto, Canada.
(16) S. B. Randle, L. Faneuf, and K. Helrich, Rutgers University, New Jersey Agric. Expt. Station, New Brunswick, New Jersey.
(17) W. A. Smith, Smith-Douglass Company, Inc., Streator, Illinois.
(18) R. M. Smith, Box 212, Tallahassee, Florida.
(19) H. Silverman, Arizona Feed and Fertilizer Control Office, Officer of State Chemist, Tucson, Arizona.
(20) B. E. Tripp, Agric. Chem. Department, Purdue University, Lafayette, Indiana.
(21) H. Gilbertson, 543 State Office Bldg., St. Paul, Minnesota.
(22) R. S. Harding, State of Colorado Dept. of Agric. Laboratory, Section, 1370 Krameris Street, Denver 7, Colorado.
(23) L. E. Bopst, State of Maryland, Inspection and Regulatory Service, College Park, Maryland.
(24) C. Tyson, Mr. Smith and John W. Kuzmeski, University of Massachusetts Agric. Expt. Station, Amherst, Massachusetts.
(25) R. C. Kock, Swift & Company, Hammond, Indiana.
(26) W. R. Austin and Mrs. N. C. Buford, Armour Fertilizer Works, Nashville, Tennessee.

RECOMMENDATIONS*

It is recommended—

(1) That additional collaborative work be done in comparing the present A.O.A.C. method for potash with the "Perrin—wet digestion," looking toward a recommendation of the latter as an optional method for potash.

(2) That additional collaborative potash work be done to study the effect of the method of preparation on the uniformity of potash results. (To help in the formulation of plans for next year, the Associate Referee would like to meet with as many collaborators as possible while at the 1949 A.O.A.C. Meeting)

(3) That a comparative collaborative study of the potash content of selected samples be made using the flame photometer.

(4) Editorial Correction—That page 31, *Methods of Analysis*, sixth edition, 1945, line 2 part (b) under **2.41** (Preparation of Solutions), now reading "Dissolve 2.5 g or the factor weight 2.425 g," be changed to read "Dissolve 2.5 g or the factor weight 2.422 g."

(5) Since 2 years' collaboration on the effect of fineness of grinding on the insoluble phosphoric content of fertilizers showed liittle if any differences, that these studies be discontinued.

ACKNOWLEDGMENT

The thanks of the Associate Referee are extended to F. W. Quackenbush of the Agricultural Chemistry Department, Purdue University, for suggestions and criticisms in the development of this report. In addition, thanks are extended to the collaborators for their cooperation.

REPORT ON MAGNESIUM AND MANGANESE IN FERTILIZERS†

By JOHN B. SMITH (Rhode Island Agricultural Experiment Station, Kingston, R. I.), *Associate Referee*

Since the last formal report in *This Journal*, **27**, 500 (1944), no collaborative work has been attempted. A questionnaire sent to former collaborators in 1947 disclosed no important difficulties with the methods, but suggested improvements in minor details, and indicated certain inconsistencies. These suggestions point to several desirable changes for the 7th Edition of the *Book of Methods* that appear acceptable without further collaborative analyses.

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* For report of Subcommittee A and action of the Association, see *This Journal*, **33**, 37 (1950).
† Contribution No. 751 of the Station.

MAGNESIUM

The gravimetric method for magnesium **2.51,** although it omits several orthodox safeguards, appears to be satisfactory. The treatment of the magnesium ammonium phosphate precipitate should be made similar to that for the same precipitate in the gravimetric method for phosphoric acid, **2.9,** substituting ammonium hydroxide (1+9) for water for washing the precipitate, and decreasing the final ignition temperature from 1100°C. to 950–1000°C. The higher temperature injures electric furnaces. The provision for a longer precipitation period for samples low in magnesium should be retained.

Directions have been requested for the preparation of the bromothymol blue indicator. A satisfactory solution may be made by disolving 0.1 g of the indicator in 1.6 ml of 0.1 *N* sodium hydroxide and adding water to make a volume of 25 ml.

The more rapid volumetric method **2.52** is preferred in many laboratories. Hardin and MacIntire[1] suggested an essentially similar procedure, with filtration through paper pulp on a Shimer filter tube rather than through asbestos on a Gooch crucible, and used bromocresol green as the indicator in place of methyl orange. They also substituted successfully tax-free 1+9 methyl-ethyl alcohol corresponding to "Formula 30," for the 95% ethyl alcohol prescribed in the Official Method. These modifications may have advantages in some laboratories, although filter paper may interfere in the subsequent oxidation of manganese for the necessary correction for that element. Comparisons with the official volumetric method were not included.

A mixed indicator suggested by Marsden[2] was found to have advantages over methyl orange for the titration of magnesium ammonium phosphate[3] changing sharply from orange through a gray color to blue at *p*H 4.4. This observation has been verified by P. McG. Shuey.[4] The indicator is made by dissolving 0.02 g neutral red and 0.2 g bromocresol green in 100 ml of 50% alcohol. It should be included as an alternate choice.

Corrections for manganese pyrophosphate or manganese ammonium phosphate in the final precipitates are important for materials containing more than traces of manganese. Procedures for the determination of manganese corrections should be more uniform and related more closely to the Official Methods for manganese. The differences arose because of adoption of the methods at different times. These changes require collaborative study. One correction should be made immediately in method **2.52,** providing for the addition of the potassium periodate to the acid solution heated to the boiling point. Initiation of the oxidation in the cold

[1] *This Journal,* **32,** 139 (1949).
[2] *J. Soc. Chem. Ind.,* **60,** 20 (1941).
[3] *This Journal,* **27,** 500 (1949).
[4] Private communication.

often produces a persistent precipitate, principally from solutions high in manganese.

There are two methods for water-soluble magnesia, **2.53, 2.54.** The Associate Referee concurs with a suggestion of the Editors of the *Book of Methods* that these be combined as separate paragraphs under a single heading. Official method **2.54** applies only to water-soluble carriers of magnesium. Method **2.53,** applies to mixed fertilizers, and if retained must be advanced to first action. This method has been in use for a number of years and should be included to insure uniformity by laboratories where it is employed. It should not be interpreted as a complete measure of activity or availability, since it excludes much useful magnesium in dolomite, and possibly in other carriers. The present description contains directions that have been made unnecessary by changes in method **2.51,** to which it is related, and these should be deleted. Combination of the two methods into a single section with first action status demotes method **2.54,** now official, but it is intended to recommend official status for the entire section in the succeeding year.

ACID-SOLUBLE MANGANESE

The colorimetric method for manganese, **2.55,** was written for the visual type of colorimeter that has been replaced in most laboratories by various types of the spectrophotometer. No fundamental changes in the directions are required, but brief additions will save trouble for the analyst and promote uniformity in use of the procedure. A provision for holding the temperature of the solution near the boiling point for 30 minutes after addition of potassium periodate was deleted from the original method, because of a belief that oxidation to permanganate is so rapid that the waiting period is unnecessary. Subsequent work has shown that this is not true, and that the provision should be restored. The statement that the maximum concentration of manganese is 200 p.p.m. is too high for the visual colorimeter unless equipped with a special filter, and is much too high for the spectrophotometer. Each instrument may have different limits for maximum concentrations, and the selection should be left to the analyst. The wave length suggested by Peach[5] for measuring light transmittance through potassium permanganate solutions is 530 mμ. This value has been verified in this laboratory.

The directions refer to the method for manganese in feeds, **27.59,** for completion of the analysis. This saves very little space, and could lead to confusion if the feed methods were changed. It seems desirable to publish the complete method under section **2.55,** except for the directions for standard potassium permanganate which are the same for both procedures.

[5] Soil Sci. **59,** 25 (1945).

RECOMMENDATIONS*

It is recommended—

(1) That the official method for acid-soluble magnesium **2.51** include the statement, "(0.1 g. bromothymol blue dissolved in 1.6 ml 0.1 *N* NaOH and diluted to 25 ml with H_2O)." That directions for treatment of the magnesium ammonium phosphate precipitate after filtration be changed to read, "Wash with NH_4OH (1+9) and ignite slowly in crucible at temp. below 900°C. (preferably in muffle furnace with pyrometric control) until C is burned and then at 950–1000°C. for 1–2 hours; cool in, desiccator, and weigh as $Mg_2P_2O_7$."

(2) That the volumetric method for acid-soluble magnesium include as an indicator alternative with methyl orange, a mixed indicator made by dissolving 0.02 g neutral red and 0.2 g bromocresol green in 100 ml 50% alcohol, titrating to a blue end point. That the directions for correction for $Mn_2P_2O_7$ be changed to read, "Add 5 ml H_3PO_4, heat nearly to the boiling point, with stirring or swirling, add 0.3 g KIO_4 for each 15 mg Mn and maintain at 90–100°C. for 30–60 minutes or until color development is complete."

(3) That methods **2.53, 2.54** be combined under the heading Water-Soluble Magnesium, making method **2.54** paragraph **(a)**, retaining the restriction, "Applicable to sulfate of potash magnesia, sulfate of magnesia and kieserite," but changing the directions to read, "Weigh 1 g samples into 250 ml volumetric flask, add 200 ml of H_2O, and boil for 30 min.; cool, and dilute to volume with H_2O. Proceed as directed in **(b)** beginning "Transfer to beaker, an aliquot containing less than 12 mg of Mg." That method **2.53** be made paragraph **(b)** labeled "In other materials, including mixed fertilizers," and that the description be simplified by the deletion of all directions after the words, "Partially neutralize with NH_4OH." That with these editorial changes, the methods for water-soluble magnesium be made first action.

(4) That the final directions of the colorimetric method for acid-soluble manganese, **2.55,** be changed to read—

"Heat nearly to b.p., with stirring or swirling, add 0.3 g KIO_4 for each 15 mg Mn present and maintain at 90–100°C. for 30–60 min., or until color development is complete. Cool and dilute to a measured volume to provide a satisfactory concentration for colorimetric measurement by the instrument chosen, usually less than 20 p.p.m. Mn. With $KMnO_4$, prepared as directed in **27.58,** as the standard, compare in a colorimeter, or in a spectrophotometer at 530 mμ. Calculate to Mn."

(5) That the study be continued.

* For report of Subcommittee A and action of the Association, see *This Journal,* **33,** 37 (1950).

REPORT ON BORON IN FERTILIZERS

By G. N. TYSON, JR. (Pacific Coast Borax Company, Division of Borax Consolidated, Limited, Pasadena, California), *Associate Referee*

General dissatisfaction with the current method for boron analysis ("Water Soluble Boron," Method **2.44**, page 32 of *Methods of Analysis*, Sixth Ed., 1945) led to two collaborative studies of new methods, both of which were developed by D. S. Taylor.

PART A. COLLABORATIVE STUDY OF D. S. TAYLOR'S BARIUM CARBONATE METHOD

This first method was given collaborative study primarily during 1948. The samples were prepared according to the following:

Collaborative test samples were prepared from materials passing 65-mesh Tyler screen, and thoroughly mixed. The Special Quality borax used was analyzed and calculated to be 100.5% $Na_2B_4O_7 \cdot 10\ H_2O$ based on its B_2O_3 content.

Fertilizer No. 1 (1.00% borax added):

19% Ammonium sulfate (Ford Motor Company).
16% KCl (supplied by F. S. Royster Guano Company, Norfolk, Va.).
35% Superphosphate (F. S. Royster Guano Company, Norfolk Va.).
30% Pulverized dolomite (U. S. Lime Products Corporation, Los Angeles, Calif.)
Special Quality borax to make 1.00%.

Fertilizer No. 2 (No borax added):

19% Ammonium sulfate (Ford Motor Company).
16% KCl (supplied by F. S. Royster Guano Company, Norfolk, Va.).
40% Superphosphate (F. S. Royster Guano Company, Norfolk, Va.)..
25% Ground limestone

Fertilizer No. 3 (5.00% borax added):

Same as No. 2 + 5.00% Special Quality borax.

Fertilizer No. 4 (3.00% borax added):

90% Red Star Gro Master V (said to contain blood meal, bone meal, cottonseed meal, superphosphate, KCl, K_2SO_4), manufactured by Downey Fertilizer Company, Downey, Calif.
10% Superphosphate
Special Quality borax to make 3.00%.

The collaborators were sent copies of the Taylor Barium Carbonate method, which has subsequently been published.[1] The request was made for quadruplicate analyses by the Taylor Barium Carbonate method on the four samples submitted and described above, and for analyses also using the official method.[2]

The following collaborators generously responded:

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[1] D. S. Taylor, "Determination of Borax in Mixed Fertilizers," *This Journal*, **32**, 422 (1949).
[2] "Water Soluble Boron," method **2.44**, *Methods of Analysis, A.O.A.C.*, Sixth Edition (1945), p. 32.

1. W. R. Austin, Armour Fertilizer Works, Nashville 2, Tenn.
2. Rodney C. Berry, Elmer T. Miller, and John M. Van Pelt, Department of Agriculture and Immigration, State of Virginia, Richmond 19, Va.
3. C. R. Byers, Armour Fertilizer Works, Carteret, N. J.
4. R. D. Caldwell, Armour Fertilizer Works, Atlanta, Ga.
5. W. R. Flach, Eastern States Farmers Exchange, Buffalo 5, N. Y.
6. O. W. Ford, Purdue University Agricultural Experiment Station, Lafayette, Ind.
7. W. C. Geagley, Percy O'Meara, Michigan Department of Agriculture, Lansing 13, Mich.
8. C. R. Godwin, Pacific Coast Borax Company, Pasadena, Calif.
9. F. Leslie Hart, M. J. Gnagy, Food and Drug Administration, Los Angeles, Calif.
10. R. L. Jones, Armour Fertilizer Works, Navassa, N. C.
11. F. O. Lundstrom, U. S. Dept. Agriculture, Beltsville, Md.
12. J. Morrison, Armour Fertilizer Works, Chicago Heights, Ill.
13. Stacy B. Randle, New Jersey Agricultural Experiment Station, New Brunswick, N. J.
14. W. A. Ryder, F. S. Royster Guano Company, Norfolk, Va.
15. G. F. Tubb, Armour Fertilizer Works, Jacksonville, Fla.

All of the collaborators listed ran the samples by the Barium Carbonate method. Additionally, a majority responded to the request to run the "Official Water Soluble Boron" Method **2.44.** Table 1 shows the results using the official method.

The results using the Taylor Barium Carbonate method were confusing, in that some results were excellent, while other values were of the same order of inaccuracy as those obtained with the official method. In general, the collaborative results did not substantiate the excellent results of the preliminary evaluation by Dr. Taylor and his colleagues, and are not being reported.

No recommendation for the Taylor Barium Carbonate method is made at this time. Some further exploratory work will be attempted to determine the cause of the variant results.

The erratic results obtained by the "Water Soluble Boron" Official Method **2.44** indicate that the method should be deleted, and it is so recommended.*

PART B. COLLABORATIVE STUDY OF D. S. TAYLOR'S "IDENTICAL *p*H" METHOD

During 1949, another analytical method developed by D. S. Taylor was subjected to collaborative study.

The test samples sent out to collaborators were identical with those described above.

The collaborators were asked to run the four samples in quadruplicate, by the method given in *This Journal,* **33,** 135 (1950).

The collaborators who most generously responded were as follows:

* For report of Subcommittee A and action of the Association, see *This Journal,* **33,** 37 (1950).

15. W. R. Austin, T. G. Brandon, Armour Fertilizer Works, Nashville, Tenn.
16. A. W. Avens, Associate Professor of Chemistry, George Rickey, N. Y. State Agricultural Experiment Station, Cornell University, Geneva, N. Y.
17. Rodney C. Berry, State Chemist, Elmer T. Miller, Department of Agriculture and Immigration, Division of Chemistry, Richmond 19, Va.
18. F. Leslie Hart, M. Gnagy, U. S. Food and Drug Administration, Los Angeles, Calif.
19. John W. Kuzmeski, A. F. Spelman, Agricultural Experiment Station, University of Massachusetts, Amherst, Mass.
20. George H. Marsh, P. R. Bidez, Department of Agriculture, Auburn, Ala.

TABLE 1.—*Percentage borax reported by collaborators using official water-soluble boron method* **2.44**

COLLABORATOR	NO. 1 (1.00% BORAX)	NO. 2 (0.00% BORAX)	NO. 3 (5.00% BORAX)	NO. 4 (3.00% BORAX)
1	1.25	0.65	5.38	2.93
	1.23	0.89	5.91	2.87
	1.19	0.83	5.95	
	Av. 1.22	Av. 0.79	Av. 5.75	Av. 2.90
3	1.01	0.12	4.95	1.59
4	1.53	0.06	4.77	2.86
5	0.85	0.0	4.59	2.71
6	1.30	0.19	4.45	3.0
7	0.89	0.11	4.75	2.98
	0.89	0.11	4.87	2.76
			4.90	
	Av. 0.89	Av. 0.11	Av. 4.84	Av. 2.87
9	0.79	0.00	4.39	2.65
	0.79		4.13	2.55
	Av. 0.79		Av. 4.26	Av. 2.60
10	1.08	0.04	5.03	3.01
	1.01	0.07	5.21	3.21
	Av. 1.05	Av. 0.06	Av. 5.12	Av. 3.22
13	1.03	0.00	4.42	2.86
	1.01	0.05	4.43	2.63
	1.01	0.00	4.20	2.75
	1.04	0.00	4.35	2.86
	Av. 1.02	Av. 0.01	Av. 4.35	Av 2.77
Maximum Av.	1.53	0.79	5.75	3.22
Minimum Av.	0.79	0.00	4.26	1.59
Av. of Avs.	1.07	0.15	4.68	2.73

TABLE 2.—*Percentage borax reported by collaborators using Dr. Taylor's "Identical pH" method*

COLLABORATOR	NO. 1 (1.00% BORAX)	NO. 2 (0.00% BORAX)	NO. 3 (5.00% BORAX)	NO. 4 (3.00% BORAX)
15	1.09	0.013	4.41	3.16
	1.07	0.013	4.47	3.11
	1.11	0.025	4.44	3.16
	1.09	0.013	4.47	3.08
	Av. 1.09	Av. 0.02	Av. 4.45	Av. 3.13
16	0.879	0.012	4.96	2.95
	0.840	—	5.04	3.00
	0.908	0.027	4.94	3.05
	Av. 0.88	Av. 0.02	Av. 4.98	Av. 3.00
17	0.95	None	5.12	3.08
	0.95	None	5.07	3.03
	1.01	None	4.92	3.05
	0.95	None	5.09	3.07
			5.14	3.02
			4.95	
	Av. 0.96	Av. None	Av. 5.05	Av. 3.05
18	1.04	0.05	5.14	3.06
	1.06	0.04		3.05
		0.06		
	Av. 1.05	Av. 0.05	Av. 5.14	Av. 3.05
19	1.05	0.04	5.10	2.95
	1.07	0.04	5.05	2.98
	0.98	0.04	5.17	2.93
	1.00	0.00	5.12	2.98
	Av. 1.03	Av. 0.04	Av. 5.11	Av. 2.96
20	1.05	0.02	5.00	3.01
	1.03	0.02	5.03	3.03
	1.03	0.02	5.03	3.07
	Av. 1.04	Av. 0.02	Av. 5.02	Av. 3.04
21	0.93	0.05	4.97	3.01
	0.93	0.00	5.00	3.01
	0.96	0.00	5.00	2.99
	Av. 0.94	Av. 0.02	Av. 4.99	Av. 3.00
22*	3.74	2.03	10.05	5.91
	2.07	0.67	11.87	6.40
	4.62	1.62	11.76	6.25
	4.33	0.59	10.24	5.60
Maximum Av.	1.09	0.05	5.14	3.13
Minimum Av.	.88	0.00	4.45	2.96
Av. of Avs.	1.00	0.02	4.96	3.03

* These values were not included in averages.

21. F. W. Quackenbush, O. W. Ford, R. F. Serro, Agricultural Experiment Station, Purdue University, Lafayette, Ind.
22. Laboratory X requested withdrawing of names and values, and values are listed but not included in averages.

The results obtained by the collaborators, using the Taylor "Identical *p*H" method on the four samples as described above, have been given in Table 2.

Comments of the collaborators on the Taylor "Identical *p*H" method were, with one exception, that this method seemed both fast and accurate. The results reported by the collaborators, with one exception, apparently vindicate the favorable judgement. The unfavorable comment did not concern the accuracy, but only the time requirement for the analysis. (Collaborator 22 requested withdrawal of values and recognition; the latter only has been withheld. The values from "laboratory X" have not been included in the averages.)

RECOMMENDATIONS*

In view of the general excellence of the results obtained by the Taylor "Identical *p*H" method, as contrasted with the values obtained by the current official **2.44** method (previously recommended for deletion), it is recommended that the Taylor "Identical *p*H" method as herein reported and collaboratively studied be adopted and accepted as first action.

No report was given an acid- and base-forming quality, copper and zinc, or inert materials.

A contributed paper "The Identical *p*H Method for Determination of Borax in Mixed Fertilizers" by Donald S. Taylor, was published in *This Journal*, in February, page 132.

A paper on "Precision of Samples and Analysis of Fertilizers and Feeds," by S. R. Miles and F. W. Quackenbush, is published in *This Journal*, page 424 and a paper entitled "A Permanganate Method for Nitrogen Availability," by K. G. Clark and V. L. Gaddy, is given in *This Journal* on page 480.

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* For report of Subcommittee A and action of the Association, see *This Journal*, **33**, 37 (1950).

REPORT ON SUGARS AND SUGAR PRODUCTS

By CARL F. SNYDER, (National Bureau of Standards, Washington, D.C.), *Referee*

The Associate Referees for the following subjects have submitted reports which will be presented at this meeting: Confectionery, Reducing Sugars, Honey, and Micro-Sugar Methods. In addition last year's Associate Referee on Unfermented Reducing Sugars in Molasses, Dr. F. W. Zerban, has submitted a final report closing this subject and recommending the final adoption of the method which has been extensively studied collaboratively over the past several years.[1] In regard to the recommendations made in these subjects, the Referee concurs.

The Associate Referee on Honey, George P. Walton, will be unable to continue his work in this capacity and it is with sincere regret that he is relieved of these duties. Mr. Walton has served the Association for many years and has contributed generously to its activities. It is fortunate that his associate, Dr. J. W. White, Jr. has consented to serve as Associate Referee on this important subject.

Of particular interest to all engaged in the subject of sugar analysis was the recent meeting of the International Commission for Uniform Methods of Sugar Analysis held in Brussels, August 15 to 20, 1949. The membership of the International Commission consists of National Committees representing the various countries. Each National Committee designates delegates to represent it at the official sessions. Three members of this Association were among the delegation of six representing the U.S. National Committee. They were F. W. Zerban, F. J. Bates, and C. F. Snyder, who presented reports at the technical sessions of the Commission. In addition, a report by this Association's Associate Referee on Reducing Sugars, Dr. Emma J. MacDonald, was presented.

The agenda of the Brussels meeting included reports on twenty-one subjects covering the various phases of sugar analysis. It is proposed to undertake the preparation of the complete proceedings including the texts of all of the reports, together with a digest of the discussions and to make copies available to all interested parties.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the determination of lac on confectionery, *This Journal*, **32**, 102 (1949), official, first action, as amended by the Associate Referee be adopted as official, and the subject closed.

(2) That the study of methods for the determination of moisture be continued.

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[1] *This Journal*, **32**, 103 (1949).

* For report of Subcommittee D and action of the Association, see *This Journal*, **33**, 68 (1950).

(3) That the study be continued on tables of density of solutions of sugar at various temperatures.

(4) That the official method for the determination of free acid in honey, **34.99,** be modified as suggested in this year's report.

(5) That the study of methods for the detection of adulteration of honey be continued.

(6) That micro methods for reducing sugars be studied.

(7) That the method for unfermented reducing substances in molasses adopted as official, first action, in 1948, *This Journal,* **32,** 103 (1949) be adopted as official.

(8) That the Zerban and Martin values for refractive indices of dextrose and invert sugar solution, *This Journal,* **27,** 295 (1944), be adopted as first action.

(9) That the tentative methods, **34.133–34.155,** inclusive, be adopted as first action and that they be further studied.

(10) That the procedures for the measurement of transmittancy of solution of commercial sugar products be studied.

(11) That the micro method for reducing sugars, **34.63, 34.64,** and **34.65** be modified as suggested by the Associate Referee and adopted as first action after changing the words "Reducing Sugars" in the title to "Dextrose."

(12) That Ofner's method for the determination of invert sugar in the presence of sucrose be made official, (first action, *This Journal,* **32,** 103).

(13) That the method, **34.8,** for solids by refractometer be amended by adding: "In liquid products containing invert sugar, correct the per cent solids obtained from **44.7** by adding 0.022 for each per cent invert sugar present in the sample." (Final recommendation.)

(14) That the following methods be made first action:

34.2 Moisture direct drying.
34.16 and **34.73** Mineral adulterants in ash.
34.78 Starch.
34.79 Ether extract, continuous extraction.
34.80 Ether extract, Roese-Gottlieb.
34.81 Paraffin.
34.92 Direct polarization.
34.93 Invert polarization.
34.96 Levulose.
34.97 Dextrose.
34.102–34.103 Commercial invert sugar, resorcinal test.
34.104–34.105 Commercial invert sugar, aniline chloride test.
34.130 Malic acid value.
34.131–34.132 Sucrose, hot water digestion, Methods I and II.
34.133–34.155 Starch conversion products methods.

(15) That the following methods be dropped:

34.12 Mineral constituents.
34.36–34.37 Invert sugar II. Scales method.

34.46 Invert Sugar IV. Herzfield gravimetric method.
34.69 Mineral constituents.
34.83 Coloring matters.
34.84, 34.115 Metals.

(16) That the following methods be adopted as official:

34.43 Determination of reduced copper by titration with potassium permanganate.
34.38 Invert sugar, Munson-Walker general method.
34.50 Dextrose, Lane-Eynon general volumetric method.

(17) That the following methods for the preparation of sample, maple syrup, be designated as procedures:

34.107 a(1) and **a(2)** Maple syrup.

(18) That the following methods for the preparation of sample for maple sugar and other solid or semi-solid products be designated as procedures:

34.107, b(1) and **b(2).**

(19) That the following methods be made procedures:

34.31–34.32 Commercial glucose (approximate), polarimetric Methods I and II.
34.77 Commercial glucose.
34.98 Dextrin.
34.100 Commercial glucose, qualitative.
34.101 Commercial glucose, quantitative.
34.106 Diastase.
34.121 Commercial glucose.

REPORT ON CONFECTIONERY

By CHARLES A. WOOD (Food and Drug Administration, Federal Security Agency, New York, N. Y.), *Associate Referee*

Experience with the official lac method, as approved by this Association indicates a final check on the purity of the lac residue to be advisable. Therefore, the following precautions were added to the method.[1] "After weighing, check for complete removal of sugars by rinsing dish and surface of shellac thoroly with hot water, warming on steam bath, decanting, rinsing down with alcohol, and evaporating with care to give uniform film on dish. Dry and reweigh."

Two samples were made up for collaborative testing:
Sample A—Sugar candy with 0.37% lac added.
Sample B—Sugar candy with 0.56% lac added.
Results are shown in Table 1.

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[1] *This Journal*, **32**, 103 (1948).

TABLE 1.—*Collaborative results*

COLLABORATOR	LAC FOUND	
	(A)	(B)
	per cent	*per cent*
S. Shendleman	0.40	0.59
	0.39	0.59
C. A. Wood	0.36	0.54
	0.37	0.53
Ave.	0.38	0.56

Results appear reasonably good for this type of determination.

Since there appears to be no immediate need for further revision or addition to the confectionery methods at this time, it is recommended.*—

(1) That the official, first action, method for shellac as published in *This Journal*, **31**, 196 (1948), be amended by the addition indicated above and be adopted as official.

(2) That the subject be closed.

REPORT ON REDUCING SUGAR METHODS

By EMMA J. MCDONALD (National Bureau of Standards, Washington, D.C.), *Associate Referee*

Many copper carbonate solutions have been used for the determination of invert sugar in refined sucrose. Of these methods, that proposed by Striegler is frequently used by industry and in the preparation of standard samples. The method is not included in the A.O.A.C. *Methods of Analysis*, since its main function has been that of a tool by which the analyst determines when sucrose has been recrystallized to a point where a minimum of invert sugar appears to be present. However, the storage problem of copper reagents is of general interest.

Striegler's reagent contains 150 grams of potassium bicarbonate, 101 grams of potassium carbonate, and 6.928 grams of cupric sulfate per liter. Bates and Jackson[1] make the statement "this reagent is not of general service because of its lack of stability on long standing." Invert sugar analysis of high grade sugars are made in a laboratory at infrequent intervals and hence the keeping qualities of the reagent are an important factor in determining the use of a given method. The reagent is prepared and stored as two solutions by some analysts and is said to be more stable under these storage conditions. However, no data has been available by

* For report of Subcommittee D and action of the Association, see *This Journal*, **33**, 68 (1950).

[1] F. J. Bates and R. F. Jackson, Scientific Paper of the Bureau of Standards, No. 268, page 80.

which one could judge the relative stability of the reagent when stored either as a single solution or as two solutions.

A study has been made in which a comparison was made of the results obtained by analyzing the same sugar by Striegler's reagent that was freshly prepared, and by a reagent that had been stored both as a single solution and as two solutions. The following results were obtained:

TABLE 1.—*Comparative results*

SUCROSE SAMPLE NO. 1				SUCROSE SAMPLE NO. 2
DATE OF ANALYSIS	REAGENT FRESHLY PREPARED	REAGENT PREPARED 8-11-48 AND STORED AS TWO SOLUTIONS	REAGENT PREPARED 3-22-46 AND AND STORED AS ONE SOLUTION	REAGENT FRESHLY PREPARED
	mg	*mg*	*mg*	*mg*
9-20-48	20.6	20.0	20.1	28.2
	19.8	20.0	20.1	28.5
	Av. 20.2	Av. 20.0	Av. 20.1	Av. 28.4
2- 8-49	23.9	23.0	22.5	
	23.3	22.8	23.0	
	Av. 23.6	Av. 22.9	Av. 22.8	
7-11-49	26.6	25.3	25.0	28.9
	26.5	25.1	24.5	28.5
	Av. 26.6	Av. 25.2	Av. 24.8	Av. 28.7

Duplicate analysis differed by an average of 0.3 mg with a maximum of 0.8 mg. A slight amount of copper appeared to have settled in the reagent that had been standing for three years; however, this seemed to be negligible in its effect on the results of the analysis. From this work it is concluded (1) that for practical purposes the reagent is stable over a considerable period of time and (2) that no benefit is gained in making up the reagent in two parts.

The Reducing Sugar methods of Chapter 34 on Sugar Analysis have been carefully studied in anticipation of changes that should be made before the publication of the 7th edition of the *Book of Methods*. The following recommendations are made as a result of this work.

RECOMMENDATIONS*

It is recommended—

(1) That the word "official" be placed after Munson and Walker general method **(34.38)**,

(2) That the Herzfeld method be deleted, since it is obsolete.

* For report of Subcommittee D and action of the Association, see *This Journal*, **33**, 68 (1950).

(3) That Lane and Eynon method be made official for the determination of dextrose.

(4) That Somogyi micro method for the determination of dextrose be made official.

(5) That the permanganate method for the determination of copper be made official.

(6) That Zerban and Sattler modification of Steinhoff's method be designated as first action.

(7) That Sichert and Bleyer method be designated as first action.

(8) That Scales method be deleted from the *Book of Methods*.

REPORT ON HONEY*

By GEORGE P. WALTON (Eastern Regional Research Laboratory[1] Philadelphia 18, Pennsylvania), *Associate Referee*

After the 1948 meeting, Committee D recommended further study of the official method for determination of free acid in honey, **34.99,** to establish the end point more accurately; also a study of methods for detection of adulterants in honey, particularly commercial sirups.

A brief survey to aid in planning work for the second study indicated that the primary objective should be collaborative testing of two methods developed in the Federal Food and Drug Administration, one by W. O. Winkler, Washington, D. C., the other by George McClellan, Houston, Texas. Both are methods for detecting adulteration of honey with invert sugar sirup; neither one has been published. Since the time available for the work was limited, and details of the methods were not readily available to persons outside of the Food and Drug Administration, collaborative work was not attempted. However, a heavy invert sugar sirup was carefully prepared from pure sucrose and invertase with added organic acids to simulate the composition of honey, and this and authentic samples of natural honeys will be available to next year's Referee for collaborative work.[2]

Determination of the free acid in light-colored honeys can be accomplished satisfactorily by the present official method; but concordant titration values are less likely to be obtained with increasing depth of color in the honeys. For example, three lots of honey ranging in color grades from "white" to dark were each carefully mixed and subsampled for collaborative work. Table 1 gives the titration results obtained by Patricia J. Dougherty, the one collaborator reporting, and this Referee.

It is apparent that a charge of 10 grams is too large for dark honeys.

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* Report of a study made under the Research and Marketing Act of 1946.

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

[2] Because of the limited time available for A.O.A.C. work, the writer has requested that he not be reappointed Associate Referee.

TABLE 1.—*Titration of A.O.A.C. Samples*

(At room temperature; after 10 seconds of phenolphthalein "pink" coloration)

SAMPLE NO.	COLOR	COLLABORATOR		
		A	B	B (BY *p*H METER)
		ML 0.1 *N* NaOH REQUIRED PER 100 G HONEY AT—		
		20°C.	*27°C.*	*25°C.*
A.O.A.C. 1	White	16.62	16.65	16.80
A.O.A.C. 2	Dark	31.05	50.15[1]	49.95
A.O.A.C. 3	White	22.11	21.89	21.75

[1] Determined on one-half usual weight of honey.

The dark color of the solution titrated masks the change in the color of the phenolphthalein indicator.

Some advantage was noted by conducting the titration according to the official method for determining the acidity of wines **15.22(a),**

In order to determine the true titration end points for the three honeys, the progress of the neutralization was followed with a *p*H meter. The values in the last column of Table 1, correspond to *p*H 8.3. Evidently the pink tint of phenolphthalein was discernible in the white honey at a slightly lower *p*H than 8.3.

At *p*H 7.0, the respective titration figures were 10.7, 35.5, and approximately 16 ml. of 0.1 *N* alkali. Evidently the constituents of honey exert the equivalent of a strong buffer effect on alkali added after the *p*H 7 point has been passed.

Titrating at 37°–38°C., instead of at 20°, or at 27°, gave significantly higher results only for sample 1. The increase ranged from 0.16 to 2.35 ml.

When the titrations at room temperature were continued until the pink coloration due to the indicator, or a *p*H of 8.3, had persisted for 30 seconds and again for 90 seconds, instead of approximately 10 seconds, the interval for the recorded titration, increases were recorded in every case. Collaborator A used an average of 1.36 ml additional alkali for 30 seconds' persistence of the pink color and further additional 1.25 ml for 90 seconds. Maintaining the *p*H at approximately 8.30 (by *p*H meter) for 30 seconds required an average of 1.03 ml more alkali than for the 10-second titration, but only 0.1 to 0.2 ml additional to maintain that state for 90 seconds. Collaborator B's titration with phenolphthalein agreed with that by *p*H meter. At 37°–38°C., the increments of alkali required to maintain the end-point concentration were of about the same magnitude as at 20° to 27°.

RECOMMENDATIONS*

On the basis of this work, in titrating the free acids in honey, it is recommended—

* For report of Subcommittee D and action of the Association, see *This Journal*, 33, 68 (1950).

(1) That four to six drops of a carefully neutralized, so-called 1 per cent alcoholic solution of phenolphthalein be used as indicator, and a "blank" determination on water and indicator be used to correct the titration.

(2) That the weight taken for titration of honeys grading "Amber" or darker should not exceed 5 to 6 grams.

(3) That the charge of honey should be entirely dissolved in 75 ml. or more of CO_2-free water before starting the titration.

(4) That the recorded end point of the titration should fulfill the following requirements: (a) the pinkish coloration due to the indicator should persist for at least 10 seconds; and (b) mixing should be adequate to produce a homogeneous solution.

(5) That titration to *p*H 8.30 (corrected) measured by *p*H meter shall be considered equivalent to titration using phenolphthalein indicator.

REPORT ON MICRO METHODS FOR SUGAR ANALYSIS

By BETTY K. GOSS (National Bureau of Standards, Washington 25, D. C.), *Associate Referee*

Somogyi's micro method for reducing sugar analysis is now a tentative method of the A.O.A.C. This method is used extensively in biochemical and medical laboratories.

Collaborative work has been carried on during the past year toward the end of recommending that the method be made official.

The results of this collaboration are given in the following table:

TABLE 1.—*Tabulation of results*

DEXTROSE PRESENT	.20	.50	1.0	1.5	2.0
	mg	*mg*	*mg*	*mg*	*mg*
Analyst A	.18	.470	.960	1.405	1.890
	.18	.485	.960	1.440	1.930
Analyst B	.202	.485	1.014	1.503	1.963
	.205	.502	1.004	1.497	1.961
Analyst C	.164	.498	1.024	1.522	1.993
	.169	.499	.973	1.541	2.056
Averages	.18	.49	.99	1.48	1.96
Average deviation	.019	.011	.026	.036	.053
Maximum deviation	.036	.030	.040	.095	.110

Determinations were run on National Bureau of Standards Standard Sample of dextrose. The results of the analysis of 0.2 mg of dextrose showed an average deviation of .019 mg with a maximum deviation of

.036 mg. Five-tenth, 1.0, 1.5, and 2.0 mg samples gave respective deviations of .011, 0.26, .036, and .053 mg., and maximum deviations of .036, .030, .040, .095, and .110 mg of dextrose. The agreement between duplicate analyses of an individual operator was found to be greater than the total average agreement.

It is customary in most laboratories for the analyst to set up a copper-sugar relationship curve for each new quantity of copper reagent, and to refer subsequent sugar analyses to this curve rather than to the table. In this case, it can be expected that the analyst will be able to determine the amount of sugar present within .05 mg.

Even in cases where the results of an analysis are referred directly to the table, as in this collaborative work, the operator can expect any error to be less than 0.1 mg.

It is recommended that the following statement pertaining to the standardization of the sodium thiosulfate solution be included under reagents:

"Dissolve 1.24 g $Na_2S_2O_3.5\ H_2O$ in water and make to a volume of one liter. Standardize against copper according to the instructions under **34.41,** using 0.01 g accurately weighed copper instead of 0.2–0.4 g. Despite the use here of very dilute copper soln, the KI concentration of 4.2 to 5.0 g per 100 ml soln must be carefully observed. One ml of 0.005 *N* thiosulfate soln is equal to 0.3179 mg of copper."

It is recommended* that Somogyi's micro method for reducing sugar analysis be made first action.

REPORT ON UNFERMENTED REDUCING SUBSTANCES IN MOLASSES

By F. W. ZERBAN (New York Sugar Trade Laboratory, Inc., New York, N.Y.), *Associate Referee*

Collaborative studies on this subject were initiated, and a first report was rendered in 1939,[1] followed by further reports in 1940,[2] 1942,[3] 1945,[4] 1947,[5] and 1948.[6] It was first attempted to use for the determination of the reducing power after fermentation the Munson and Walker[7] and the Quisumbing and Thomas[8] methods, commonly employed for the determination of reducing sugars, in order not to add still another method to the large number already adopted by the Association. But both of them proved unsatisfactory for the purpose, and the Lane and Eynon[9] method was not found to be any better. It was therefore decided to study the

* For report of Subcommittee D and action of the Association, see *This Journal,* **33,** 68 (1950).

[1] *This Journal,* **23,** 562 (1940).
[2] *Ibid.,* **24,** 656 (1941).
[3] *Ibid.,* **26,** 112 (1943).
[4] *Ibid.,* **29,** 242 (1946).
[5] *Ibid.,* **31,** 192 (1948).
[6] *Ibid.,* **32,** 184 (1949).
[7] *Methods of Analysis, A.O.A.C.,* 6th Ed., 1945, p. 571.
[8] *Ibid.,* 6th ed., 1945, p. 133.
[9] *Ibid.,* 6th Ed., 1945, p. 570.

iodometric method used in Java.[10] The results obtained with this method in 1945, and again in 1947, were so encouraging that the method was adopted as tentative.[11] Repetition of the work in 1948 confirmed the previous results, and the method was made official, first action.[12] The evidence obtained in 1945, 1947, and 1948 was so convincing that the Referee felt no further collaborative work was necessary on this subject. He therefore recommends* that the method which was made first action in 1948, be adopted as official at the 1949 meeting of the Association.

No reports were given on drying methods, densimetric and refractometric methods, corn sirup and corn sugar, color and turbidity in sugar products, or starch conversion products.

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By JOHN B. WILSON (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Referee*

Mindful of the forthcoming seventh edition of the *Book of Methods*, the Referee concluded that the greatest progress toward putting the chapter on flavoring extracts in order, with respect to the new policy on classification of methods, would result from a study of methods for alcohol and essential oil, since these methods are tentative.

Several experienced analysts had expressed their opinions as to the inadequacy of the methods for alcohol, in which essential oil is occluded upon magnesium carbonate or salted out with sodium chloride and washed away with petroleum ether.

The Referee considered that it might be possible to extend to other extracts the specific gravity method **(25.24)**, which is official for lemon, lime, and orange extracts.

In this procedure the specific gravity of the extract is determined and corrected for the effect of the essential oil by means of a methematical formula to give the true specific gravity of the vehicle. This calculation is possible only when the extract consists only of alcohol, water, and essential oil, which is very often the case.

The Referee prepared extracts using the following essential oils: almond, clove, cinnamon (cassia), peppermint, spearmint, and wintergreen (methyl salicylate) and submitted them to three collaborators. As no data were available regarding the expansion of solutions of these oils in dilute ethyl alcohol, the collaborators were requested to determine

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[10] Methoden van Onderzoek, 6th Ed., 1931, p. 365.
[11] *This Journal*, 31, 61 (1948).
[12] *Ibid.*, 32, 64 (1949).
* For report of Subcommittee D and action of the Association, see *This Journal*, 33, 68 (1950).

specific gravity at 15.56°/15.56° and at 20°C./20°C. and to determine oil by the methods in common use by the Alcohol Tax Unit, which are **25.63** in the case of cinnamon and clove and **25.75** in case of the remaining extracts.

To prepare the extracts the approximate quantity of absolute alcohol required to dissolve a volume of oil, in keeping with the advisory standards issued under the Federal Food and Drug Act of 1906, was weighed and diluted to a liter at 20°C. The specific gravity of the vehicle was determined at 20°/20° and the alcohol content found from the alcohol table. The requisite quantity of oil was then pipetted into a liter volumetric flask, the appropriate vehicle was added to dissolve the oil, and finally, after standing overnight in the 20° room, each was filled to the mark with the same vehicle.

Table 1 contains the specific gravities of the extracts as reported by the collaborators, the specific gravities of the vehicles as determined originally and those calculated by the formula in **25.24,** using the volume and weight of essential oil used in their preparation.

In view of the first sentence in **25.54** concerning calculation of alcohol content from the specific gravity of almond extract without correction, the alcohol contents corresponding to all determinations of the specific gravity of almond extract are also given.

The data in Table 1 show that the specific gravity method, **25.24,** is applicable to extracts of almond, clove, cinnamon, peppermint, spearmint, and wintergreen (methyl salicylate), with a fair degree of accuracy, provided the oil content is known. Since most of the figures for alcohol content obtained from the uncorrected specific gravity of the almond extract are approximately correct, the word "approximate" should be inserted in any statement similar to that in **25.54** referred to above.

When alcohol is calculated from the specific gravity of the extract at 15.56°C./15.56°C., 24 of 32 results are within 0.5% of the actual alcohol content and all are within 1%. When calculated from the specific gravity of the extract at 20°C./20°C., 28 of 32 results are within 0.5% of the actual alcohol content and 30 are within 1%. These results warrant the adoption of this method, first action.

The oil content was determined by the method **25.63** in the case of cinnamon and clove extracts, and by the method **25.75** in the case of the remaining extracts. These data are given in Table 2, together with the alcohol contents of the various extracts calculated from the specific gravities in Table 1 but using the oil content as determined by the various analysts. In this table the average specific gravity was used when the analyst reported more than one determination of specific gravity.

The data in Table 2 warrant adoption of the methods for oil, **25.63** for cinnamon and clove, **25.75** for peppermint, spearmint, and winter-

TABLE 1.—*Alcohol*[1] *in extracts, by specific gravity method*[2]
Used weight and volume of oil used in calculations

	EXTRACT	ALMOND		CLOVE		CINNAMON (CASSIA)		PEPPERMINT		SPEARMINT		WINTERGREEN (METHYL SALICYLATE)	
	ANALYST	SP. GR.	ALCOHOL	SP. GR.	ALCOHOL	SP. GR.	ALCOHOL	SP. GR.	ALCOHOL	SP. GR.	ALCOHOL	SP. GR.	ALCOHOL
Vehicle 20°/20°	Wilson	0.9604	32.66	0.9053	62.30	0.8865	70.16	0.8501	83.66	0.8594	80.41	0.9132	58.78
Extract 15.56°/15.56°	Wilson	0.9629	32.02	0.9104		0.8935		0.8552		0.8644		0.9251	
		0.9630	31.93	0.9110		0.8935		0.8554		0.8646		0.9252	
		0.9631	31.85	0.9110		0.8934		0.8552		0.8645		0.9253	
	Marder	0.9627	32.17	0.9103		0.8931		0.8556		0.8644		0.9249	
						0.8937							
						0.8931							
	Mathers	0.9629	32.02	0.9102		0.8930		0.8548		0.8642		0.9248	
Extract 20°/20°	Wilson	0.9612	32.05	0.9074		0.8904		0.8521		0.8614		0.9217	
		0.9611	32.13	0.9074		0.8903		0.8519		0.8612		0.9219	
		0.9611	32.13	0.9074		0.8904		0.8520		0.8612		0.9217	
	Marder	0.9603	32.73	0.9072		0.8900		0.8520		0.8613		0.9224	
						0.8900							
						0.8900							
	Mathers	0.9604	32.66	0.9067		0.8894		0.8511		0.8605		0.9213	
Vehicle calc.[2] Sp. Gr. 15.56°15/56°	Wilson	0.9619	32.80	0.9086	62.10	0.8902	69.86	0.8531	83.70	0.8623	80.47	0.9162	58.70
		0.9620	32.72	0.9092	61.83	0.8903	69.82	0.8532	83.66	0.8625	80.40	0.9163	58.66
		0.9621	32.65	0.9092	61.83	0.8901	69.90	0.8531	83.70	0.8624	80.44	0.9163	58.66
	Marder	0.9617	32.96	0.9085	62.14	0.8900	69.94	0.8534	83.59	0.8623	80.47	0.9159	58.84
						0.8904	69.78						
						0.8898	70.02						
	Mathers	0.9619	32.80	0.9084	62.18	0.8897	70.06	0.8527	83.84	0.8620	80.58	0.9159	58.84
Average			32.79		62.02		69.99		83.70		80.47		58.74
Vehicle calc.[2] Sp. Gr. 20°/20°	Wilson	0.9602	32.81	0.9055	62.22	0.8871	69.92	0.8498	83.76	0.8592	80.48	0.9130	58.89
		0.9601	32.88	0.9055	62.22	0.8870	69.96	0.8496	83.83	0.8590	80.55	0.9128	58.98
		0.9601	32.88	0.9055	62.22	0.8870	69.96	0.8497	83.79	0.8590	80.55	0.9125	59.12
	Marder	0.9593	33.47	0.9053	62.30	0.8867	70.08	0.8498	83.76	0.8591	80.52	0.9134	58.71
						0.8867	70.08						
						0.8866	70.12						
	Mathers	0.9593	33.47	0.9048	62.48	0.8861	70.32	0.8488	84.10	0.8582	80.84	0.9122	59.25
			33.01		62.29		70.06		83.85		80.59		58.99

[1] Per cent by volume at 15.56°C.
[2] *Methods of Analysis*, 6th ed., 25.24.

TABLE 2.—*Alcohol in extracts by specific gravity method*[1]

Determined Volume of Oil Used in Calculations[2]

	EXTRACT	ALMOND		CLOVE		CINNAMON		PEPPERMINT		SPEARMINT		WINTERGREEN	
	ANALYST	OIL	ALCOHOL	OIL	ALCOHOL	OIL	ALCOHOL	OIL	ALCOHOL	OIL	ALCOHOL	OIL	ALCOHOL
Present		1.25	32.66	2.00	62.30	2.50	70.16	3.50	83.66	3.00	80.41	3.50	58.78
Found using Sp. Gr. 15.56°/15.56°	Wilson	0.60	32.36	2.00	62.42	2.60	70.03	3.50	83.55	2.60	80.22	3.60	58.96
		0.80	32.56									3.50	58.79
	Marder	0.80	32.74	2.00	62.64	2.06	69.50*	4.40	83.54	3.00	80.42	3.60	59.10
												3.60	59.10
	Mathers	0.65	32.20	2.10	62.68	2.20	69.98	3.80	83.71	3.10	80.33	3.30	58.72
	Av.		32.46		62.51		69.84		83.60		80.32		58.93
Found using Sp. Gr. 20°/20°	Wilson	0.60	32.52	2.00	62.71	2.60	70.12	3.50	83.65	2.60	80.32	3.60	59.31
		0.80	32.66									3.50	59.17
	Marder	0.80	33.25	2.00	62.78	2.06	69.70	4.40	83.74	3.00	80.08	3.60	58.16
												3.60	58.16
	Mathers	0.65	32.29	2.10	63.00	2.20	70.26	3.80	83.93	3.10	80.59	3.30	59.13
	Av.		32.68		62.86		70.03		83.77		80.33		58.79

[1] *Methods of Analysis, A.O.A.C.*, 6th ed., **25**, 54.
[2] In cases where analyst reported more than one Sp. Gr. the average was used in these calculations.
* Average.

green; and for alcohol, **25.24,** in clove, cinnamon (cassia), peppermint, spearmint, and wintergreen (methyl salicylate) as first action at this time.

Because of the low recoveries of almond oil which are customarily obtained in the analysis of almond extract by the use of section **25.75,** the Referee requested that collaborators determine benzaldehyde by section **25.55.** The results obtained are given in Table 3, together with the calculated specific gravities of the vehicle using the formula given in **25.24,** and the benzaldehyde found by this method. Such use of the benzaldehyde content is justified by the fact that almond oil consists almost entirely of benzaldehyde.

TABLE 3.—*Alcohol in almond extract by the specific gravity method*[1]
Benzaldehyde found used in calculations

BENZALDEHYDE		SPECIFIC GRAVITY		ALCOHOL FROM SP. GR.	
		15.56°C./15.56°C.	20°C./20°C.	15.56°C./15.56°C.	20°C./20°C.
Present	1.300 g		0.9604	32.66	32.66
Found:					
Marder	1.301	0.9616	0.9592	33.04	33.54
	1.297	0.9616	0.9592	33.04	33.54
Mathers	1.297	0.9619	0.9593	32.80	33.47
	1.305	0.9618	0.9592	32.88	33.54
Wilson	1.286	0.9621	0.9602	32.65	32.81
	1.309	0.9618	0.9599	32.88	33.03
Average	1.299	0.9618	0.9598	32.84	33.32

[1] Per cent by volume at 15.56°C.

The data in Table 3 show excellent results for benzaldehyde by all collaborators; the alcohol content as calculated, using benzaldehyde content for oil, shows that this compound forms at least as good a basis for calculation as the oil. Figures obtained from the specific gravity at 15.56°C./15.56°C. are all within 0.5% of the true alcohol content. The variance of those calculated from the sp. gr. at 20°C./20°C. can probably be accounted for by the difference in the expansion coefficient of this mixture as compared with that of a mixture of alcohol and water only.

Because of certain criticism of the salting out methods for alcohol as given in sections **25.23** and **25.54,** the Referee decided to investigate these procedures before submitting them to collaborative study. By the time the study had been made it was impractical to have other analysts do the work also. The samples used for the work reported above were analyzed by the modified procedures given in the report for 1947, *This*

Journal, **31** (202–203). The results obtained by the Referee are given in Table 4.

The results in Table 4 indicate a considerable shortage in recovery of alcohol in lemon and orange extracts after treatment with magnesium carbonate. Since the method for determination of alcohol by the specific gravity method which is already official for lemon and orange extracts, was found to give excellent results, *This Journal* **4,** 472 (1921); **5,** 308 (1922), it seems best to drop the method using magnesium carbonate. This method is more generally applicable to lemon and orange extracts which require vehicles containing 85 to 90% alcohol by volume and are

TABLE 4.—*Alcohol content of extracts by salting out methods*

	CALC.	FOUND	DIFFERENCE
Lemon	85.38	81.40	−3.98
Orange	90.20	86.00	−4.20
Almond	31.76	31.96	+0.20
Clove	61.05	60.82	−0.23
Cinnamon (cassia)	68.41	68.01	−0.40
Peppermint	80.73	79.39	−0.34
Spearmint	78.00	77.46	−0.54
Wintergreen (methyl salicylate)	56.22	54.70	−1.52

much less likely to contain sugar, glycerol, or other substances of higher density than water, than to extracts containing less alcohol.

In the case of the remaining extracts the results in Table 4 are comparable to the specific gravity method and in several cases closer to the calculated alcohol content. In view of the fact that these extracts may also contain sugar, glycerol, etc., which would alter the density, the specific gravity method would be applicable in a smaller proportion of cases than for lemon and orange extracts, and in view of the results reported in Table 4, it is the conclusion of the Referee that the salting out method as given in *This Journal,* **31,** 203 (1948) should be substituted for the present methods in **25.54, 25.60,** and **25.68** and adopted as first action.

The Referee concurs in the recommendation of R. D. Stanley, Associate Referee on Organic Solvents in Flavors, that the method for isopropyl alcohol in the absence of acetone be adopted as first action, with the change specified, and that this study be continued.

RECOMMENDATIONS*

It is recommended—

(1) That the methods listed below (which consist chiefly of the application to non-alcoholic beverages, of official methods in other chapters)

* For report of Subcommittee D and action of the Association, see *This Journal,* **33,** 65 (1950).

be adopted, to have the same status as they have in the chapter referred to.

13.19—Tartaric acid—First Action.
13.20—Citric acid—First Action.
13.21—Malic acid—First Action.
13.29—Benzaldehyde—Official.
13.30—Gamma undecalactone—Official.

(2) That the methods for glycerol listed below be made first action, since to drop them would leave the chapter without any method for this important constituent of flavors.

25.3—Glycerol in Vanilla Extract.
25.29—Glycerol in Lemon, Orange, and Lime Extracts and Flavors.

(3) That **25.15**—Vanilla Resins Quantitative Method, be made first action. This conclusion was reached after a reconsideration of the collaborative data reported in *This Journal,* **9,** 446 (1926).

(4) That the sections listed below be adopted as procedures:

25.16—Qualitative Tests (Vanilla Resins).
25.18—Color Value.
25.19—Residual Color.

(5) That **25.23,** Method I, for alcohol in lemon, orange, and lime extracts and flavors, be deleted.

(6) That the tentative method for isopropyl alcohol in lemon extract in the absence of acetone, **25.26, 25.27, 25.28,** be adopted as first action, with the following change in section **25.27,** first sentence: "To 2 ml of distillate add 0.5 ml of 5% . . . . " This conclusion was reached after a reconsideration of the collaborative data reported in *This Journal,* **25,** 693 (1942).

(7) That the tentative method for oils of lemon, orange, or lime in oil base flavors, **25.35,** Method II by Polarization, be made first action. This conclusion was reached after a reconsideration of the collaborative data reported *This Journal* **10,** 496 (1927).

(8) That **25.44,** Lemon and Orange Peel Color—Tentative, be dropped, since in our experience we have never met with an extract giving the test.

(9) That **25.54,** Alcohol—Tentative (in almond extract) be made first action.

(10) That the following method for alcohol in almond extract be adopted as first action:

Determine specific gravity of the extract at 15.56°C./15.56°/C. or at 20°C./20°C. as directed in **16.4,** and the benzaldehyde content as in **25.55.** Apply the formulas given in **25.24** using the benzaldehyde content as *p,* percentage of oil found.

(11) That **25.55,** Benzaldehyde—Tentative, be made first action.

(12) That **25.56,** Benzoic Acid—Tentative, be made first action.

(13) That **25.58,** Hydrocyanic Acid, Quantitative Method—Tentative, be made first action.

(14) That the following method for alcohol in cassia, cinnamon, and clove extracts be adopted as official, first action:

Determine the specific gravity of the extract at 15.56°C./15.56°C. or at 20°C. /20°C. as directed in **16.4,** and the oil content as directed in **25.63** and apply the formulas given in **25.24.**

(15) That **25.62,** Oil Method I—Tentative, be dropped. This procedure has been retained for some years in deference to pharmaceutical chemists who used it. A new method similar to **25.63** has now been adopted by them. After reconsideration of the data given in *This Journal,* **15,** 539, and **16,** 542, it is recommended that **25.63** be adopted as first action.

(16) That the methods for Ginger Extract listed below, be adopted as first action

25.64—Alcohol.
25.65—Solids.
25.66—Ginger (Qualitative Test).
25.67—Capsicum (Qualitative Test).

(17) That the following method for alcohol in peppermint, spearmint, and wintergreen extracts be adopted as first action:

Determine the specific gravity of the extract at 15.56°C./15.56°C. or at 20°C. /20°C. as directed in **16.4,** and the oil content as directed in **25.63** and apply the formulas given in **25.24.**

(18) That **25.70,** Oil—Tentative, be dropped because of its cumbersomeness and limited use among flavor chemists.

(19) That **25.72,** Methyl Salicylate in Wintergreen Extract—Tentative, be dropped as a cumbersome method.

(20) That **25.73,** Oil—Tentative, be adopted as first action.

(21) That **25.74,** Oil—Method II, be adopted as first action. This conclusion was reached after a reconsideration of the data reported in *This Journal,* **15,** 539 and **16,** 542.

(22) That **25.75,** Essential Oil—Tentative, be adopted as first action. This conclusion reached after reconsideration of data reported in *This Journal,* **15,** 539 and **16,** 52.

(23) That the collaborative study of the reflux method for determination of peel oil in citrus fruit juices and the use of the modified oil separation trap be continued.

(24) That collaborative work be continued on the method for determination of beta-ionone where small amounts are present.

(25) That collaborative studies on the Ripper method for determination of aldehydes in spirits as applied to lemon oils and extracts be continued.

(26) That collaborative studies of the methods proposed by the

Referee for determination of esters in lemon extract be continued.

(27) That collaborative studies on the Seeker-Kirby Method for determination of esters in lemon and orange oils (Dept. of Agri. Bull. 241) be continued.

(28) That collaborative studies of extract methods containing both isopropyl alcohol and acetone be continued.

(29) That collaborative study of the photometric method for determination of vanillin and coumarin be continued.

(30) That work be continued on the determination of glycerol, vanillin, and coumarin in vanilla and imitation vanilla extracts, with special reference to the automatic extraction of vanillin and coumarin.

(31) That the study of emulsion flavors be continued.

(32) That studies on maple concentrates and imitations be continued.

(33) That study of the method for determination of diacetyl, published in *This Journal*, **25**, 255 (1942), be continued.

(34) That the methods for vanilla resins in vanilla extract, **25.15** and **25.16**, be studied collaboratively.

REPORT ON ORGANIC SOLVENTS IN FLAVORS

By ROBERT D. STANLEY (U. S. Food and Drug Administration, Federal Security Agency, Chicago, Ill.), *Associate Referee*

In accord with the policy of this Association, as outlined in the Reports of the Committee on Classification of Methods, the following comments and/or recommendations are made for the isopropyl alcohol methods described in Chapter 25 of the *Book of Methods*, 6th Edition; as well as the work now in progress and as yet unreported.

Referring to the qualitative test for acetone **(25.27)**, the Associate Referee has found that the sensitivity of this test, as well as the ease of manipulation, is increased by using a lesser amount of the *o*-nitrobenzaldehyde reagent.

RECOMMENDATIONS*

It is recommended.—

(1) That the first sentence of **25.27**, be changed to read: "To 2 ml. of distillate add 0.5 ml. of 5% . . . . "

(2) That on the basis of collaborative work previously reported (*This Journal*, **25**, 693) methods **25.26**, **25.27**, **25.28**, **25.61**, and **25.69** be adopted as first action.

(3) That collaborative studies of methods for extracts containing both isopropyl alcohol and acetone be continued.

* For report of Subcommittee D and action of the Association, see *This Journal*, 33, 65 (1950).

A contributed paper, "Determination of Propylene Glycol in Vanilla Extract," by C. F. Bruening, was published in the February number of *This Journal*, page 103.

No report was given on beta-ionone, lemon oils and extracts, emulsion flavors, maple flavor concentrates and imitations, diacetyl, or vanilla extracts and imitations.

REPORT ON ALCOHOLIC BEVERAGES

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

The report this year will consist of the recommendations of the Associate Referees and the Referee, with particular reference to those dealing with revision of *Methods of Analysis, A.O.A.C.*, 7th ed., and of comments thereunder.

The recommendations* and comments follow:

MALT BEVERAGES, BREWING MATERIALS, AND ALLIED PRODUCTS

It is recommended—

(1) That study of methods for determination of essential oil and resins in hops be continued.

(2) That the dye color method for the estimation of color in wort and beer, described in *This Journal*, **32**, 81 (1949), be adopted as official.

(3) That work on photometric beer color evaluations be continued.

(4) That study of beer turbidity methods be continued.

(5) That the Mathers test for caramel, *This Journal*, **31**, 76 (1948), be adopted for beer, as first action.

(6) That the method for carbon dioxide in beer, described in the report of the Associate Referee this year, be adopted as first action.

(7) That work be continued on polarographic and spectrographic methods for tin in beer.

(8) That the method for total solids in yeast, **14.112–14.115**, as revised in *This Journal*, **31**, 174 (1948), and *Book of Methods A.S.B.C.*, 5th ed., 1949, be adopted as official.

(9) That the following methods which are dealt with in this year's report of the Associate Referee on Malt Beverages, etc., be adopted as first action:

14.16 Dextrin.
14.21–14.25, incl. Carbon dioxide.
14.28–14.30 Iron.
14.33 End fermentation.

* For report of Subcommittee D and action of the Association, see *This Journal*, **33**, 58 (1950).

14.42 Alternate bushel weight.
14.57 Extract in caramel malt.
14.58 and **14.59.** Color in caramel and black malts.
14.94 Diastatic power of malt sirups.
14.116–14.124 Spent grains.

and the following new methods:

(a) Dextrose, under Brewing Sugars and Sirups. (*Book of Methods, A.S.B.C.*, 5th Ed. (1949), p. 57.)
(b) Protein, under Malt. (*Ibid.*, p. 109.)
(c) Wort Nitrogen, under Malt. (*Ibid.*, p. 110.)

(10) That the methods for mold, **14.47,** length of acrospire, **14.43,** mealiness, **14.44,** assortment, **14.46,** foreign seeds and broken kernels, **14.48,** be classed as procedures.

(11) That the official (*This Journal,* **31,** 56 (1948)) method for hops, **14.80 (b),** and **14.81** be revised as described in the report of the Associate Referee for malt beverages, etc., and by the Associate Referee on hops, and adopted as first action (except, **14.81 (b),** color and luster; and **(e)** aroma, which should be deleted).

(12) That studies be continued on the determination of iron in beer, giving attention to both the wet-ashing orthophenanthraline procedure and the direct non-ashing procedure as suggested by the Associate Referee.

WINES

It is recommended—

(1) That chromatographic studies of wine be continued.

(2) That the tentative method for volatile acidity—exclusive of SO_2, **15.25**—be dropped and that the tentative method II, **15.26,** for the same constituent be made first action,

(3) That the tentative method for citric and malic acids, **15.30,** be made first action, for the reasons set forth in the report of the Referee on Fruits and Fruit Products for this year.

(4) That the methods for nitrates, **15.36 (a)** and **(b),** be dropped, as they are not now used to detect the addition of water to wine as was originally intended.

(5) That the general reference to Chapter 21, "Coloring Matters—Tentative" **(15.37),** be dropped.

(6) That **15.15** be made a procedure.

DISTILLED LIQUORS

It is recommended—

(1) That the study of methods of analysis with reference to the aging or maturing of whisky in laminated (plywood) barrels be continued.

(2) That the study of colorimetric methods for fusel oil be continued.

(3) That the official method **16.29,** for methanol by the immersion

refractometer method be studied in the light of the findings of Beyer and Reeves, *This Journal*, **28**, 800 (1945).

(4) That the rapid method for proof of distilled spirits as described in the report of the Associate Referee for 1948, *This Journal*, **32**, 154 (1949), be adopted as official.

(5) That study be continued of the official Denigés method for methanol, **16.25**, and the tentative method for methanol in **39.161** and **39.162** to bring about uniformity in these procedures as far as possible.

(6) That the tentative method for "Detection of Acetone, Ketones, Isopropanol, and Tertiary Butyl Alcohol" **(16.20** and **16.21)**, be made first action after changing "one or more of the above-mentioned compounds" to "acetone, other ketones, or tertiary butyl alcohol."

(7) That the tentative tetramethylammonium iodide method for methanol, **16.31** to **16.34**, incl., be dropped.

(8) That the method for water-insoluble color, **16.36**, be made first action, after revision as recommended by the Associate Referee this year.

(9) That the methods listed below be adopted as first action, after revision as described in the Associate Referee's report this year.

16.13 Esters—Official.
16.16 Aldehydes Volumetric Method—Official, first action.
16.22 Fusel oil—Official.

(10) That the tentative method for color insoluble in amyl alcohol, **16.37**, be dropped.

(11) That the following tentative methods be made first action:

16.38 Marsh test, caramel.
16.40 Cyclohexanol test, caramel.
16.42, 16.43 Tannin.

(12) That the last sentences in Ch. **XVI, 5**, 5th Ed., "The alcohol content of distillate may be checked by determining immersion refractometer reading and obtaining percentage of alcohol from Table 20, **XLIII**," which was omitted from the 6th Edition by oversight, be included in the 7th Edition.

(13) That the Fulton test for caramel, *This Journal*, **31**, 77 (1948), be adopted, first action, for distilled liquors and for cordials and liqueurs.

CORDIALS AND LIQUEURS

It is recommended—

(1) That **16.44**—Physical Examination be designated a procedure.

(2) That the caption "**16.63**—Preliminary Procedure*** Acids" be changed to "**16.63**—Characteristic Acids—Preparation of Sample—Procedure."

(3) That the following methods be adopted as official.

16.45 Specific Gravity.
16.46 Alcohol.

16.47 Methanol.
16.49 Aldehydes.
16.49 Furfural.
16.50 Fusel Oil.

(4) That the method for "Total solids—from the specific gravity of the dealcoholized sample," as given in this year's report, be adopted as first action to replace **16.51(a).**

(5) That the method for "Total solids by evaporation," as given in this year's report, be adopted as first action to replace **16.51(b),**

(6) That "Mather's Test for Caramel," as given in the Report of the Associate Referee on caramel, be adopted as first action.

(7) That the method for "Total Acidity," **16.62,** be adopted as first action.

(8) That the qualitative method for Thujone, **16.73,** be adopted as first action.

(9) That the methods listed below, which consist chiefly of the application to cordials and liqueurs of official methods in other products whose composition differs from that of cordials and liqueurs only in the substitution of alcohol for more or less of the water present, be given the same status as they have in their respective chapters.

Official

16.52 Glycerol.
16.53 Sucrose by Polarization.
16.54 Sucrose by Reducing Sugars
16.55 Ash.
16.56 Soluble and Insoluble Ash.
16.57 Alkalinity of Soluble Ash.
16.58 Alkalinity of Insoluble Ash.
16.59 Phosphoric Acid.

First Action

16.64 Tartaric Acid, deleting reference to **26.35.**
16.65 Citric Acid.
16.66 Active Malic Acid.
16.67 Inactive Malic Acid.

REPORT ON ALDEHYDES, ESTERS, AND FUSEL OIL IN DISTILLED SPIRITS

By GEORGE F. BEYER (Bur. of Internal Revenue, Alcohol Tax Unit, Laboratory Division, Washington, D.C.), *Associate Referee*

A large number of experiments have shown that it is unnecessary to make separate distillations for each of the above-mentioned congeners, as outlined in the present volume of the *Methods of Analysis*, A.O.A.C.

The suggested changes are as follows:

Esters and Aldehydes

Place 200 ml of the sample in a 500 ml Erlenmeyer flask, add about 35 ml H_2O, distill and collect nearly 200 ml of the distillate in a 200 ml vol., flask using ground glass connections. Make to volume, mix, pipette off 100 ml for ester determination, and use the remainder for aldehyde determination.

Determine the esters according to section **16.13,** and the aldehydes according to **16.16** and **16.17.** Save soln from ester detn. for fusel oil detn.

Fusel Oil Determination

Transfer the soln from the ester determination to a 200 ml vol. flask, make to mark, mix, pipette 100 ml of this soln to any convenient cylinder, add ca 50 ml H_2O, and saturate with NaCl. Extract this soln 4 times with CCl_4, using 40, 30, 20, and 10 ml, respectively, in a separatory funnel shaking about 1 min. Then wash the combined CCl_4 extracts 3 times with saturated NaCl soln, and twice with saturated Na_2SO_4 soln. (Shaking for one min. has been found sufficient.) Transfer the CCl_4 layer to a flask containing 50 ml of the oxidizing soln made according to section **16.22** and boil for 2 hours under a reflux condenser.

Allow to cool about 15 to 20 min.; then add 20–30 granules of 20 *M* carborundum and 50 ml H_2O thru the condenser, and distill until about 50 ml is left. Then add 50 ml H_2O again and continue the distillation until about 50 ml remains. (Use extreme care while distilling to prevent oxidizing mixture from burning or baking on sides of distilling flask. Distillate should be water white.) Titrate the distillate with 0.1 *N* NaOH, using 1% soln phenolphthalein as an indicator and calculate as amyl alcohol.

It has been the experience of this laboratory that the grade of CCl_4 listed in General Schedule A.C.S. needs no purification for this purpose, as the blank on 100 ml of this grade is seldom more than 0.2 ml 0.1 *N* NaOH.

TABLE 1.—*Comparison of new and old procedures*

DISTILLERY	NO. SAMPLES ANALYZED OLD PROCEDURE	VARIATION	AVERAGE	NO. SAMPLES ANALYZED NEW PROCEDURE	VARIATION	AVERAGE
A	42	124–141	132	28	120–140	131
B	12	127–138	133	8	130–139	135
C	12	194–206	200	8	188–205	193
D	11	143–155	147	8	144–153	150
E	36	144–158	152	23	144–157	151
F	6	185–195	190	4	183–192	187
G	14	111–125	116	12	113–125	118
H	30	102–110	106	9	102–107	104
I	5	190–214	202	4	195–208	201
J	12	204–215	209	4	205–211	206
K	10	183–192	188	4	186–197	192
L	12	180–195	190	4	185–202	192
M	8	228–240	236	4	237–250	243
N	8	168–180	174	8	173–175	174

The above conclusion regarding the new procedure was reached as a result of a large number of determinations on whisky from 14 distilleries, as well as collaborative work performed by 6 branch laboratories of the Alcohol Tax Unit.

The preceding table will give a graphic illustration of how the two procedures compare with each other, in so far as the fusel oil content is concerned.

Reports from the laboratories collaborating in this work are as follows:

Baltimore

No. Sample	*Present Method*	*Proposed Method*
Esters—Grams per 100 l		
1	56.3	54.6
2	56.3	55.4
3	66.0	65.1
4	59.0	58.1
5	59.0	57.2
6	56.3	55.4
Aldehydes—Grams per 100 l		
1	11.2	11.2
2	11.9	11.7
3	11.7	11.2
4	13.9	13.7
5	11.9	12.1
6	11.7	11.7
Fusel Oil—Grams per 100 l		
1	200.6	198.9
2	191.8	191.0
3	195.4	194.5
4	186.6	185.7
5	183.0	181.3
6	195.4	193.6
Water Insoluble Color—Per cent		
1	68.8	74.3
2	67.7	74.2
3	67.7	74.2
4	66.4	72.7
5	67.5	74.2
6	68.4	73.5

Louisville

No. Sample	*Present Method*	*Proposed Method*
Esters—Grams per 100 l		
1	54.6	53.7
2	51.0	51.0
3	61.6	60.7
Aldehydes—Grams per 100 l		
1	8.0	8.2
2	9.8	9.5
3	7.8	8.6
Fusel Oil—Grams per 100 l		
1	99.4	99.4
2	101.2	98.6
3	123.2	120.6
Water Insoluble Color—Per cent		
1	53.3	50.0
2	56.3	59.4
3	56.7	53.3

New York

Fusel Oil—Grams per 100 l		
1	170	167
2	120	125
3	195	200
4	264	266
5	146	150
Water Insoluble Color—Per cent		
1	65	61
2	65	61
3	60	50
4	53	53

Philadelphia

Esters—Grams per 100 l		
1	87	80
Aldehydes—Grams per 100 l		
1	22	20
Fusel Oil—Grams per 100 l		
1	290	240

No. Sample	Present Method	Proposed Method
No comparison was made on Water-Insoluble Color.		
Kansas City		
Esters—Grams per 100 l		
1	59.8	59.7
2	65.0	64.3
Fusel Oil—Grams per 100 l		
1	117	119
2	99	97
Water Insoluble Color—Per cent		
1	68	70
2	68	70
Aldehydes—Grams per 100 l		
1	7.0	6.9
2	11.7	12.0

COMMENTS BY COLLABORATORS

Baltimore stated that the results obtained by using the two methods for esters, aldehydes, and fusel oil were within the limits of experimental error, but the proposed method for determining water-insoluble color produced higher results than the official method.

Louisville had the following comments to make:

(1) *Aldehydes.*—More consistent results and closer checks were obtained with the proposed method than with the A.O.A.C. method.

(2) *Fusel Oil.*—The saving in time resulting from the use of the proposed method is a very definite improvement.

New York stated that no satisfactory results were obtained with the proposed aldehyde method as all results were too low. It is further stated that no satisfactory explanation can be given for the low results produced by the volumetric method.

Fusel Oil.—The proposed fusel oil method is considered satisfactory and an improvement of the older method.

Esters.—Since the ester method is substantially the same, no comparison was made.

Water Insoluble Color.—Since comparative results are desired and that spontaneous evaporation requires considerable more time, no advantage could be seen in the proposed method.

Philadelphia's comments were that the proposed changes give satisfactory agreement with the present methods. It is further stated that considerable difficulty was experienced during the distillation of the oxidized mixture because of bumping and charring. This can be avoided by adding some granular boiling stones (carborundum). It is noted that this precaution was omitted in the method. It is also concluded that the proposed changes are advantageous in that they considerably shorten the time of analysis with resulting savings in equipment when these methods are adopted for routine use.

Chicago did not submit any figures for the two sets of methods; however, the following comments were submitted:

Esters.—No appreciable difference in the results was found by the use of the two methods.

Aldehydes.—In every determination, lower results were obtained by the proposed method than by the present, but if the distillate is collected in ice-cooled containers the loss is only about 5%. However, it is felt that the present method should be retained.

Fusel Oil.—In most of the determinations, the amount of fusel oil found was larger by the proposed method (4–40 per cent) than by the present. The laboratory

states that it is unable to account for the high results obtained on the whiskey samples by the proposed method, because, when a standard fusel solution was treated with both methods the present method gave about 2.0% higher results than the proposed one.

Water Insoluble Color.—Small differences in the results were obtained depending upon the manner in which the whiskey was evaporated to dryness and the manner in which the color was read. It is further stated that since it is difficult to duplicate the conditions regarding the evaporation of the whiskey, that a water extract of the solids, after its determination, could be used to an advantage as more consistent results could be obtained thereby. It is also stated that the Tintometer and Duboscq colorimeter used in reading the color gave the most satisfactory results. In this connection I desire to state that the Duboscq colorimeter was first used for this purpose in the Washington laboratory, discarded as unsatisfactory, then the Neutral Wedge Photometer was used and also discarded for the same reason. Finally the Photoelectric Spectrophotometer was used and found to give the most satisfactory color readings.

Kansas City.—No comments were submitted by this laboratory; however, from the results submitted, it is evident that the proposed changes give results equally as good as the present A.O.A.C. methods.

In view of the amount of work performed by the Associate Referee and the results and comments submitted by the collaborators, it is recommended that the proposed changes for the determination of Esters, **16.13,** Aldehydes, **16.16, 16.17,** Fusel Oil, **16.22,** be adopted first action.

Although there seemed to be some objection to the method for water-insoluble color, it is the opinion of the Associate Referee that the reason for the unsatisfactory results was principally due to reading the color; at least that was the trouble in the Washington laboratory. Another cause may have been the grade of filter paper used. The grade found most satisfactory was that similar to Whatman's #42.

Since the color of spirits is generally reported or referred to in terms of Lovibond numbers, it is suggested that in using the spectrophotometer for this purpose, the instrument be checked for correctness by finding the correct wave length at which the optical density multiplied by 10 equals the Lovibond number. This is conveniently done by setting the spectrophotometer wave-length dial around 430 millimicrons and balance the instrument against water as a reference solution. Then insert a Lovibond slide, number 3, 4, or 5 in the light beam and read the transmittance, "T." If $-\log T$ multiplied by 10 does not equal the number of the slide used, then change the wave-length setting until the correct result is obtained.

REPORT ON CHROMATOGRAPHIC ADSORPTION OF WINES

By PETER VALAER, JR. (Bureau of Internal Revenue, Alcohol Tax Unit, Washington 25, D.C.), *Associate Referee*

On November 29, 1948, the following information was received. "The Association wishes to have you continue as associate referee on the chromatic adsorption of wines."

As far as the Associate Referee is aware there has not been any later or more extensive work along the lines set out by Hamil several years ago in his "Detection of Grape Wine in Blackberry Wine."[1]

Although this principle is employed in a routine manner by a number of laboratories working with food materials, it well deserves more study and application.

The laboratory of the Bureau of Internal Revenue, Alcohol Tax Unit, in Washington, D.C., and branches or field laboratories in various cities, have made use of the Hamil procedure in a simplified or modified form since that time. The Hamil method consists briefly of passing diluted wine (5 or 10 ml wine to 50 ml distilled water) through a narrow tube about $\frac{1}{4}$ inch in diameter and about 8 inches long, packed with various mixtures of dry aluminum oxide and filter cell (1 to 1 or 2 to 1 by weight). Although Hamil used a much more detailed process, all of it was not considered necessary by later operators.

After the wine is drawn through the tubes by suction, about the same amount of water was run through it to wash the powder and a 30-(or less) minute suction was continued to partially dry the powder. The damp powder was then rammed out of the tubes into a petri dish and examined under ultra-violet light. All berry wines were found to give a deep blue fluorescence under the U.V. light, while grape, peach, apple, cherry, and some other fruits gave a yellow fluorescence. A number of other wines gave various special colors, and a mixture of the above mentioned fruit with berry wines showed an influence of yellow to an extent that one could roughly estimate the amount of foreign wine that had been mixed with the berry wine.

In recent years Paul Simonds inverted, or reversed the process, by passing the adsorptive material (washed and dried aluminum oxide) into wine instead of passing the wine through the powder as in the Hamil process. This Simonds procedure is still simpler in operation than the Hamil test and is briefly as follows:

EXPERIMENTAL TEST

Into a cylindrical glass tube (like a colorimetric tubing) about $\frac{3}{4}$ of an inch in diameter and about 25 centimeters long stoppered with a rubber stopper at one end,

[1] George K. Hamil and Paul W. Simonds, *This Journal*, 25, 220 (1942).

is introduced 25 ml of wine and 25 ml of water and mixed. Into this mixture is sifted slowly, dry, washed, activated alumina powder (aluminum oxide) until a deposit is built up at the bottom of the tube to about ½ inch. It should require about 30 minutes to build up. Usually ten or more tubes could be used at the same time during the said 30 minutes. A small pledget of cotton is now stuffed in the top of the tube and rammed down, using a long solid glass rod flattened at right angles on its end, against the built up layer of alumina and packed well and the upper layer of liquid poured off. The ramrod with its flattened top is then pressed hard against the cotton and the deposited alumina until most of its liquid is squeezed out. The rubber stopper is now removed and the deposit pushed out with the glass rod onto a small Büchner funnel containing a flat filter paper. The suction is turned on and the deposit sucked quite dry after washing well with absolute alcohol (about 25 ml). The alumina pack during washing loosens into a fine powder which flows or pours easily into a small beaker or petri dish, the latter serving merely as a place to study the powder conveniently under the ultraviolet light. About the same results are obtained as described under the Hamil test. No further use is made of the cotton used as packing. The powders keep their colors under the light for weeks but become altered to some extent on long standing. Just when they change has not been determined. Without going into detail, a number of fruit wines which exhibit delicate fluorescence under the ultra-violet light easily show the presence of any cheaper fruits such as apples, peaches, grapes, et cetera. Adulteration and rectification have often been detected in honey wines, where grape and other fruits have been added to give the wine more character or to assist in its usual sluggish fermentation. This material that causes the various colors under the ultra-violet light has nothing to do with the acids of the fruits, such as lactic, malic, tartaric, citric, and other acids found in fruits naturally or formed during their fermentation.

No doubt an A.O.A.C. method based on the principle of chromatic adsorption would be quite useful but the Associate Referee is not ready to offer any method at the present time.

It is recommended* that the matter be further studied and a report made next Fall as to the possibilities of selecting a suitable A.O.A.C. method based on chromatic adsorption.

REPORT ON CARAMEL IN ALCOHOLIC BEVERAGES

By PETER VALAER, JR. (Bureau of Internal Revenue, Alcohol Tax Unit, Washington 25, D.C.), *Associate Referee*

The following recommendations were adopted last year by Committee D, *This Journal* **32,** 59 (1949).

That the Milos test for caramel, **14.35** and **15.38,** be deleted (final action).

That the Mathers test for caramel, *This Journal,* **31,** 76 (1948), be studied collaboratively with respect to its application to beer.

That the Mathers test for caramel, *This Journal* **31,** 76 (1948), be adopted as official (final action), for distilled liquors and wine and that

* For report of Subcommittee D and action of the Association, see *This Journal,* **33,** 59 (1950).

it be studied collaboratively, with respect to its application to cordials and liqueurs before adoption as final action.

During 1948, the Mathers test had been collaboratively studied and was accepted as an official test for caramel in wines and distilled liquors and it was recommended to take the place of the Milos test for caramel, which was to be deleted. The confirmatory portion of the Milos test however (**15.39,** p. 189), was not to be removed, but is intended to become the confirmatory part of the Mathers test. The purpose is to strengthen the test. A few minor changes and removals were made in the confirmatory test in which a number of small tests were deleted and the 2,4-dinitrophenylhydrazine reagent was used to confirm caramel in the final brown residue or its aqueous solution obtained by the confirmatory test.

Although the Mathers test for caramel was recommended in 1948 by the Associate Referee to be applied to beer, vinegar, cordials, and flavoring extracts, this could not be done under the requirements of the A.O.A.C. rules unless the recommendations for its acceptance as tests for caramel in these four classes of food products be supported by successful collaborative evidence and experiments. To this end the Associate Referee during the early months of 1949 following the Fall meeting of 1948 of the A.O.A.C., prepared substantial quantities of nine separate samples, and 8-ounce portions of each were submitted to a number of chemist collaborators, particularly those who by virtue of their analytical duties would be experienced in these classes of food substances. For the 20 actual sets of samples that were sent out, more than 25 separate chemists were involved and more than 200 actual tests were made.

Of the nine separate samples sent to each collaborator, samples 1, 2, 3, 7, 8, and 9 were analyzed correctly. About 80% reported correctly on samples 4 and 5 (beers) and about 90% reported correctly on No. 6, cordials; samples 1 and 2 were vinegar, sample 7 was beer, and 8 and 9 were vanilla extracts.

From the good results and the generally favorable comments of the collaborators, it is recommended that the Mathers test for caramel be included in the official methods of analysis of beer, vinegar, cordials and liqueurs, and flavoring extracts, but not to appear in detail as recommended in the analysis of wine, but by reference to the wine methods only. The method enclosed with the samples was as follows:

"*Mathers Test:* To 10 ml of the above samples previously introduced into a Babcock cream bottle, or any convenient small centrifuge bottle, add 1 ml of pectin solution (made by dissolving 1 gram of pectin in 75 ml of water and adding 25 ml of alcohol for preserving it). Shake well before using. Add to the material to be tested in the centrifuge bottle about 3 drops of concentrated HCl and fill bottle with alcohol (about 50 ml or more). Shake well and centrifuge for 5 to 10 minutes or more, and decant carefully the supernatant liquid off of the gelatinous residue. Dissolve the residue in the bottle by adding 10 ml water and shaking well. To this residue dissolved in water add about 3 drops of concentrated HCl and 50 ml or more of alcohol; shake

well and again centrifuge. Repeat this process until the upper alcoholic layer is quite clear and colorless. After the final decantation of the water-white supernatant alcohol, the gelatinous residue is dissolved in 10 ml of hot water. A colorless solution would show that no caramel is present. A clear brown solution is usually indicative of caramel coloring. To further confirm caramel, add 1 ml of the following reagent (made by dissolving 1 gram of 2,4-di-nitro-phenyl-hydrazine in 7.5 ml of concentrated sulphuric acid and bringing the volume up to 75 ml with 95% ethyl alcohol. Keep in a glass-stoppered bottle in which it will stay clear and stable for several months) to the residue dissolved in the 10 ml of hot water. Put the bottle in the beaker of boiling water for 30 minutes. In the presence of substantial quantities of caramel a precipitate forms almost at once. Smaller amounts show as a precipitate before the 30 minutes are up. Even the smallest amount of caramel will show a precipitate under the conditions described but if the analysis has been conducted as described above, no precipitate will appear if caramel is absent. In order to be sure a precipitate has formed, pour the hot test solution from the bottle on a small filter paper and wash any residue with hot water. A reddish brown precipitate will be clearly seen on the filter. This precipitate although amorphous is quite characteristic and will always be the same if caramel is present. A low-power microscope affords a fine examination of the precipitate. If caramel is found to be present by the above test, check the sample by the confirmatory test which involves the use of KOH and $ZNCl_2$ etc. If no precipitate is obtained by 2,4-di-nitro-phenyl-hydrazine reagent, report caramel as absent."

The following letter was written to each collaborator on or after March 29, 1949:

"There will be transmitted to you in the near future 9 samples consisting of beer, cordial, vanilla flavoring, and vinegar, which may or may not contain caramel coloring matter. You are requested to have these samples examined in your laboratory using the Mathers' method for the detection of caramel.

At the A.O.A.C. meeting in Washington last Fall, it was recommended that this method be accepted as an official method for the detection of caramel coloring matter in wine and spiritous liquors. It was suggested at that time that it might also prove satisfactory for the detection of caramel in beer, cordials, flavoring extracts, and vinegars; but before it is adopted as an official method, collaborative work should be performed for the purpose of ascertaining whether it will be satisfactory for detecting caramel in this class of product.

After you have tested the samples you will receive with the Mathers' method, compare the results obtained with ether methods which may be available or are now being used for the detection of caramel in the products mentioned above. It would simplify matters if the Mathers' method could be used for detecting caramel in all of the products mentioned.

Kindly forward your report to the Washington laboratory not later than the first of September, with your comments and recommendations in such form that it may be presented by Mr. Valaer, the Associate Referee, at the next Fall meeting. A similar set of samples is being transmitted to each branch laboratory.

The Mathers' method is to be incorporated in the 7th Edition of the A.O.A.C. methods of analysis, which will be published in 1950 and will be used for the detection of caramel in all the products mentioned, if the collaborative work proves it to be satisfactory."

REPORT ON HOPS

By D. E. BULLIS (Chemist, Oregon Agriculture Experiment Station, Corvallis, Oregon), *Associate Referee*

No study on methods for hop analysis has been conducted during the past year. Only one chemist has indicated interest in this subject and his offer of collaboration came too late in the year to complete any work in time for the 1949 report.

The physical and chemical methods for analysis of hops listed in sections **14.80** to **14.89,** inclusive, of Chapter 14, *Methods of Analysis*, sixth edition, were studied collaboratively by the American Society of Brewing Chemists for a number of years. This set of methods developed after several years' work under the leadership of Dr. Louis Ehrenfeld and Dr. Stephen Laufer was adopted as official by the A.S.B.C. and later included in the *A.O.A.C. Methods*, sixth edition. It is therefore recommended* that these methods be adopted by the A.O.A.C. as first action.

It is further recommended* that the following addition be made to section **14.81.**

"If large numbers of samples are to be analyzed for seed, leaf and stem, time will be saved by following the procedure given in the Hop Inspectors Manual issued by Pacific Coast Headquarters, Grain Branch, Production and Marketing Administration, U.S.D.A., 345 U. S. Court House, Portland 5, Oregon."

This agency supervises hop inspection for California, Oregon, Idaho, and Washington and has mechanized the seed, leaf, and stem tests by use of the Bates air aspirator and a small Clipper cleaning mill so that many samples can be analyzed in a short time. This procedure has been employed for the past four or five years by the Federal inspection service and the results are accepted by the hop industry and brewing trade in the merchandising of hops.

REPORT ON CARBON DIOXIDE IN BEER

By IRWIN STONE (Wallerstein Laboratories, 180 Madison Avenue, New York 16, N.Y.), *Associate Referee*

In the last report on Carbon Dioxide in Beer,[1] it was noted that the Subcommittee on Carbon Dioxide in Beer of the American Society of Brewing Chemists were revising the CO_2 methods after an active program of collaborative work covering a period of several years. The revisions have been completed and the new methods will appear in the forthcoming new edition of *Methods of Analysis of the A.S.B.C.*

In line with the policy of maintaining uniformity of methods of both societies, the revision is now submitted to the A.O.A.C. for inclusion in their 1950 *Book of Methods.* The chemical method for Carbon Dioxide, **14.21–14.23,** is unchanged. The most significant change in the manometric or pressure method, **14.24–14.25,** is the use of "head space air" (*i.e.*, the

* For report of Subcommittee D and action of the Association, see *This Journal*, **33**, 59 (1950).
[1] *This Journal*, **30**, 222 (1947).

air coming over with the first 40 ml of evolved gas) in the formula, instead of the present "total" air value, which tended to over-correct for this factor. There are also many minor changes in the present text. The revised method will appear in the 7th Edition, *Methods of Analysis*, 1950.

RECOMMENDATION*

It is recommended that the revised method for "Dissolved Carbon Dioxide—Pressure Method" be adopted, first action, and be included in the new edition of *Methods of Analysis*.

REPORT ON INORGANIC ELEMENTS IN BEER

By W. C. STAMMER (Continental Can Company, Inc., Chicago, Illinois), *Associate Referee*

IRON

INTRODUCTION

The wetashing cellosolve-thiocyanate procedure for determining iron in beer was adopted as a tentative method on the basis of work by Clifcorn (7). This procedure left much to be desired in the way of speed and convenience and certain inherent difficulties led to erroneous results with inexperienced analysts. About this time direct iron methods or non-ashing procedures were being given serious consideration. The first direct comparison method was proposed by Gray & Stone (11) using the bipyridyl reagent for the iron color complex, with a visual color comparison. Not long after this, with spectrophotometers coming into general use, Nissen (10) introduced a direct non-ashing procedure employing orthophenanthroline as the color agent along with a spectrophotometric calibration. Subsequent users of direct iron procedures made modifications of their own (4) with equal success.

The results of a survey by Bendix (4) of laboratories running iron in beer indicated that a direct non-ashing procedure sensitive to a fraction of a p.p.m. of iron would be preferred. The study of the direct comparison method in 1948 (5) brought out certain minor uncertainties and preferences of the collaborators. The present study was intended to eliminate these discrepancies.

Points to be Considered:

1. *Standard iron solutions:* There are two accepted ways of preparing standard iron solutions. The first employs pure iron wire dissolved in hydrochloric acid with provisions for keeping the iron in the ferric state. The second uses ferrous ammonium sulfate hexahydrate dissolved in water containing hydrochloric acid.

* For report of Subcommittee D and action of the Association, see *This Journal*, 33, 58 (1950).

2. *Standard curve for beer:* The standard curve can be prepared using beer with appropriate amounts of iron added or with iron in aqueous solutions with or without buffer. The range selected for bottle beer is 0–1 p.p.m. and for canned beer 0–3 p.p.m.

3. *Color development reagent:* Two reagents are commonly used in the direct determination of iron in beer, namely, bipyridyl and orthophenanthroline. Moss and Mellon (6) have reported that there is little basis for recommending one reagent over the other. Recent communications with Moss and Mellon indicate no change in their opinions. Dr. Moss based a personal preference on his desire to see some unification, in response to the variety of reagents for iron proposed in recent years. There has been a trend toward the use of phenanthroline in many laboratories, and it now is in the official methods of both the ASTM and APHA.

4. *Sample size:* The sample size selected depends upon the instrument and cells used in evaluating the colored beer solutions. Samples of 25, 50 and 100 ml are common.

5. *Reducing agent:* It is generally believed that the iron in beer is present as ferrous iron. Many users of the direct iron determination have operated without a reducing reagent and found little or no effect on the final results. Nevertheless, there is always present the element of doubt as to whether the iron in the beer may become oxidized during sampling or standing for color development. It is probably good insurance for these rare cases to have a reducing agent present. There are several excellent reducing agents available for this determination; for example, hydroquinone, ascorbic acid, thioglycollic acid, and hydroxylamine hydrochloride. The latter is in more frequent use. It has been noted that hydroxylamine often contains sufficient iron contamination so as to seriously interfere when low iron beers are run. Methods currently used specify an addition of the hydroxylamine reducing agent far in excess of the amount actually needed to reduce the iron that may be present, and this leads to unnecessarily high iron contamination.

6. *Instrumentation:* The colored iron complexes of phenanthroline and bipyridyl may be read from 500 to 515 millimicrons. The resulting standard curve is usually a straight line (obeys Beer's law 1–5 p.p.m. iron) but does not necessarily have to be so because of the lack of linearity in response of certain spectrophotometers. The samples can be read in 10, 25, 40, or 50 centimeter cells. Meter readings can be made in % transmission or density.

From the foregoing discussion it should be apparent that an analyst could select a number of procedures for the direct determination of iron in beer, varying only in minor details. The Associate Referee has carefully considered these variable points and has outlined two procedures that he believes will produce the desired results and will lead to uniformity among analysts running direct iron in beer determinations.

SAMPLES

Five samples of a commercial beer were submitted for analysis. Ferrous iron was added to certain samples to bring the iron content to the desired range. Two of the samples were especially prepared to contain an abnormal amount of air.

STANDARD IRON SOLUTIONS

A. *Iron Wire*

Dissolve 0.500 grams of reagent grade iron in 5 ml of 20% HCl plus 1 ml of nitric acid. Heat to dissolve and evaporate to dryness, add water, and evaporate to dryness again. Take up with conc. HCl, cool, and rinse into 500-ml volumetric flask. Add 2 drops bromine water and dilute to volume. One ml of the soln is equivalent to one mg of iron.

B. *Ferrous Ammonium Sulfate Hexahydrate*

Weigh out 3.512 grams of the above salt, dissolve in distilled water, add 5 ml hydrochloric acid, and dilute to 500 ml. One ml of this soln is equivalent to one mg of iron.

METHOD NO. 1

REAGENTS

(1) 1% *Hydroxylamine hydrochloride.*—Dissolve 5 g hydroxylamine hydrochloride in 500 ml distilled water.

(2) .3% *Orthophenanthroline.*—Dissolve 1.5 g orthophenanthroline in 500 ml distilled water heated to 70°C.

Standard Curve.—Depending upon the sample size of beer to be taken prepare a series of 25 or 50 ml water standards containing 0, 0.5, 1.0, 2.0, and 3.0 p.p.m. iron. Develop the color in these standards according to the procedure outlined below. Transmission values may be read and plotted against p.p.m. Fe on semi-log paper; or densities may be read and the data used to calculate a suitable factor for converting densities to p.p.m. Fe.

Procedure: Pipette two portions of beer (25 or 50 ml) into 125-ml Erlenmeyer flasks. Add 5 ml. 1% hydroxylamine hydrochloride to each and allow to stand 30 minutes. Then to one add 5 ml 0.3% orthophenanthroline and to the other 5 ml distilled water. Allow to stand another 30 minutes. Compare the solutions in the photometer against distilled water at a wave length of 505 mm (or using a blue-green filter) employing a suitable-sized cell.

METHOD NO. 2

REAGENTS

(1) *Acetate buffer.*—Dissolve 16.6 g sodium acetate in distilled water. Add 24 ml glacial acetic acid and dilute to 200 ml.

(2) *Thioglycollic acid solution.*—Add 10 ml thioglycollic acid (72%) to 500 ml. distilled water and mix well.

(3) *Orthophenanthroline-thioglycollic acid mixed reagent.*—Dissolve 1.5 g orthophenanthroline in 500 ml distilled water heated to 70°C. Cool and add 10 ml thioglycollic acid (72%).

Standard Curve: Depending upon sample size of beer to be taken prepare a series of 25 or 50 ml water standards buffered with 5 ml of acetate buffer to contain 0, 0.5, 1.0, 2.0 and 3.0 p.p.m. of iron. Develop color with the mixed reagent as outlined in procedure below. Final volume should be 30 or 55 ml. Read transmissions or densities on photometer as per Method No. 1.

Procedure: Pipette two portions of beer (25 or 50 ml.) into 125-ml Erlenmeyer flasks. To one portion add 5 ml of thioglycollic acid solution and to the second portion add 5 ml orthophenanthroline-thioglycollic acid mixed reagent. Allow to stand 10 minutes and read the solutions in the photometer against distilled water at a wave length of 505 mm (or using a blue-green filter) employing a suitable-sized cell.

CALCULATIONS

The calculations are made in the following manner:

If transmission values are used, the iron values for the colored and the uncolored samples are read directly from the standard curve. The difference is the iron content of the beer in p.p.m.

If densities are read, the difference between the colored and the uncolored sample is multiplied by a calculated factor to give the p.p.m. Fe in the beer.

DISCUSSION OF RESULTS

From Table 1 it is apparent that the two methods selected for this study gave good duplication and excellent agreement among the collaborators. Table 2, which indicates the variation among the collaborators in regard to instrumentation and sample size, shows that these variables had little effect on the consistency of the results. Samples No. 4 and No. 5 containing more than the usual amount of included air presented no difficulties with respect to the reducing agents present.

Method No. 2 gave slightly higher results than did Method No. 1. Neglecting this slight difference between the two procedures Method No. 2 is preferred because of the fewer reagent additions and shorter reaction time. Ferrous ammonium sulfate is preferred as an iron standard because of its convenience.

The beer samples were made to contain varying amounts of iron by adding ferrous iron to a low iron beer. As a check on the amounts added the Referee's laboratory determined iron by a wet ash phenanthroline procedure and found the samples to contain the specified amounts of iron. The direct procedures did not completely recover the amounts of iron added to this series of samples. The low recoveries may be attributed to the way in which the direct procedures are standardized, namely, the water-iron standardization. An examination of direct procedures appearing in the literature (3), (10), (12) will show that when a direct non-ashing procedure is standardized against iron-in-beer, or iron-in-water solutions, good agreement can be obtained with wet ashing procedures for beers containing 0.6 p.p.m. iron or less. When the iron present in beer exceeds this amount the recoveries by the direct methods are generally 0.1 to 0.2 p.p.m. low.

The current direct methods as presently standardized are not adequate as an A.O.A.C. official method for the determination of iron in beer. The Associate Referee suggests that the General Referee on alcoholic beverages

TABLE 1.—*Results of collaborative study*

COLLABORATOR	METHOD #1—P.P.M. IRON										METHOD #2—P.P.M. IRON									
	SAMPLE										SAMPLE									
	1		2		3		4		5		1		2		3		4		5	
	DUPL.	AVE.	DUPL.	AVE.	DUPL.	AVE.	DUPL.	AVE.	DUPL.	AVE.	DUPL.	AVE.	DUPL.	AVE.	DUPL.	AVE.	DUPL.	AVE.	DUPL.	AVE.
No. 1	0.67		0.08		1.43		0.78		0.05		0.73		0.07		1.81		1.00		0.04	
	0.69	0.68	0.07	0.08	1.48	1.46	0.78	0.78	0.04	0.05	0.74	0.74	0.05	0.06	2.06	1.93	0.95	0.98	0.11	0.08
No. 2	0.66		0.07		1.46		0.82		0.04		0.75		0.09		1.77		1.00		0.06	
	0.64	0.65	0.07	0.07	1.48	1.47	0.79	0.80	0.04	0,04	0.75	0.75	0.08	0.09	1.77	1.77	0.99	1.00	0.06	0.06
No. 2*	0.69		0.08		1.48		0.80		0.04		0.73		0.07		1.68		0.93		0.04	
	0.70	0.70	0.08	0.08	1.48	1.48	0.79	0.80	0.04	0.04	0.73	0.73	0.07	0.07	1.66	1.67	0.92	0.93	0.05	0.05
No. 3	0.77		0.09		1.57		0.67		0.03		0.72		0.08		1.87		0.75		0	
	—	0.77	0.09	0.09	1.57	1.57	0.68	0.68	0.05	0.04	0.72	0.72	0.09	0.09	1.85	1.86	0.75	0.75	0	0
No. 4†	—	0.71	—	0.08	—	1.87	—	0.68	—	0.02	—	0.72	—	0.08	—	1.74	—	0.77	—	0.02
No. 5‡																				
Average		0.70		0.08		1.57		0.75		0.04		0.73		0.08		1.79		0.88		0.04
Max. deviation		0.07		0.01		0.30		0.07		0.02		0.02		0.02		0.14		0.13		0.04
Ave. deviation		0.03		0.004		0.12		0.05		0.006		0.01		0.01		0.08		0.10		0.003
Iron added		0.80		0		2.0		1.0		0										
Iron found by wet ash phenanthroline method		0.90		0.15		1.95		1.15		0.10										

* Two sets of samples were run by this collaborator.
† Single determinations.
‡ Results of one collaborator omitted.

consider the adoption of a wet-ash orthophenanthroline method, as an official method, and a direct non-ashing procedure (with appropriate standardization) as an alternate method for determining iron in beer after suitable collaborative study.

COLLABORATORS

O. R. Alexander, American Can Company, Inc., Maywood, Illinois.
R. E. Bacon, Texas Liquor Control Board, Austin, Texas.
B. H. Nissen, Anheuser-Busch, Inc., St. Louis, Missouri.
I. Stone, Wallerstein Laboratories, New York, New York.
B. Buerger, Continental Can Company, Inc., Chicago, Illinois.

COPPER

Two dithizone procedures for the determination of copper in foods, namely, the Greenleaf mixed color method and the Bendix-Grabenstetter

TABLE 2.—*Instrumentation and sample size*

COLLABORATOR	INSTRUMENT	CELL SIZE	IRON STANDARD	SAMPLE SIZE	WAVELENGTH
		mm		*ml*	*mμ*
No. 1	Coleman Model 11	40	B	25	505
No. 2	Coleman Model 14	13	B	25	505
No. 3	Cenco-Sheard-Sanford	50[1] 10[2]	A	50	515 Blue-green filter
No. 4	Klett-Sommerson	20	B	50	500–570 Klett green filter

[1] Method No. 1.
[2] Method No. 2.

single color method, have been studied collaboratively (6). Either of the methods can be applied to beer. The Greenleaf method is considered the more sound theoretically but is rather cumbersome to carry out. From a practical standpoint the Bendix single color method is sufficiently reproducible in unknowns and standards to warrant its use as a general procedure, for the determination of copper in beer.

TIN

An unpublished polarographic procedure as developed by Henry & Strodtz (8) in this Referee's laboratory and a spectrographic method of O. R. Alexander (1) for the determination of tin in beer have been studied by the Referee. The tin contents of normal beer were found to be 0.01 to 0.10 p.p.m. tin. The Alexander spectrographic method appears to be the logical choice because of the very low concentrations of tin involved. The

polarographic method functions at the lower limits of its detection when applied to beer having less than 0.05 p.p.m. tin.

RECOMMENDATIONS*

Based on the results of the foregoing study, it is recommended:

IRON

(1) That a wet-ashing orthophenanthroline procedure be selected and submitted to collaborative study with the intent of its becoming the official A.O.A.C. method.

(2) That a suitable means of standardizing the direct non-ashing procedure be selected and submitted to collaborative study with the intent of a direct procedure becoming an alternate A.O.A.C. method.

COPPER

(3) That the Bendix-Grabenstetter single color dithizone method for the determination of copper in foods be submitted to collaborative study for the determination of copper in beer.

TIN

(4) That the Alexander Spectrographic method and/or the Henry-Strodtz polarographic method for the determination of tin in beer be considered by the General Referee for possible collaborative study.

ACKNOWLEDGMENT

The Associate Referee wishes to express his appreciation for the excellent consulting and laboratory work of Miss Beata Buerger.

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* For report of Subcommittee D and action of the Association, see *This Journal*, **33**, 59 (1950).

REPORT ON MALT BEVERAGES, SYRUPS, AND EXTRACTS AND BREWING MATERIALS

By ROBERT I. TENNEY (Wahl-Henius Institute, 64 E. Lake St., Chicago, Ill.), *Associate Referee*

RECOMMENDATIONS*

It is recommended that the following first action methods be adopted as official, final action, with reference to the listed reports of collaborative study:

That the Dye Color method for the estimation of color in wort and beer, described in *This Journal*, **32**, 81 (1949), be adopted as official.

That the method for total solids in yeast, **14.112–14.115**, as revised in *This Journal*, **31**, 174 (1948), and *Book of Methods, A.S.B.C.*, 5th Ed. (1949), be adopted as official. (First action, *This Journal*, **32**, 59 (1949).

It is also recommended that the following tentative methods be adopted as first action, with reference to *Book of Methods, A.S.B.C.*, 5th edition, 1949:

14.16 Dextrin.
14.21–14.25, incl. Carbon dioxide.
14.33 End fermentation.
14.42 Alternate bushel weight.
14.57 Extract in caramel malt.
14.58 and **14.59** Color in caramel and black malts.
14.116–14.124, incl. Spent grains.

It is recommended that **14.94**, diastatic power in malt syrups, now listed as a tentative method, be adopted as official, upon the basis of its identity with method **14.61–14.64** which is being recommended to this same status; also because of its inclusion in the *Book of Methods, A.S.B.C.*, 5th edition, 1949.

It is recommended that the following tentative methods be classified as Procedures, for inclusion in the 7th Edition of *A.O.A.C. Methods of Analysis*:

14.43–14.48, incl., Acrospire, mealiness, assortment, mold, and foreign seeds and broken kernels in malt.

It is recommended that methods corresponding to the following references to the *Book of Methods, A.S.B.C.*, 5th Edition, 1949, be adopted as first action. Collaborative study was accomplished in similar items upon methods well understood and widely used.

Dextrose, under Brewing Sugars and Syrups—**13**, p. 57.
Protein, under Malt—**9**, p. 109.
Wort Nitrogen, under Malt—**10**, p. 110.

It is recommended that the section on hops starting with **14.80** be re-

* For report of Subcommittee D and action of the Association, see *This Journal*, **33**, 60 (1950).

vised to take into consideration the controversial nature of some of these tests and to make practical use of the collaborative studies made upon the determination of seeds, leaves and stems, by the Federal-State Hop Inspection service, which has been operating in close agreement for several years.

These changes are in general agreement so far as principle is concerned with the recommendations of Dr. D. E. Bullis, Associate Referee on Hops, whose report will be found elsewhere.

14.80 (b) should be changed to read substantially as follows:

Use Oregon sampling device or sharp knife to cut samples of 100–200 g each from 10% of the bales in the shipment, avoiding sampling at the press seam. Place the individual bale samples in separate containers but do not use cartons, paper bags, wrapping paper, or wooden boxes if chemical tests are to be made. For this purpose use tin cans having friction or screw-top.

14.80 (c) *Compositing of samples*

Take not less than 25 g from each individual bale sample to make up a gross composite sample of the lot. Carefully loosen up the cones from one another, break up all coarse stems and divide to size required for tests to be made. (The Bates Divider is the most accurate method for doing this.) Sample may be split into 20-g size for convenience in handling, and the results of the separate portions averaged to get the results.

14.81 *Physical Examination*

(*a*) *Leaves & Stems.*—Sift the composite sample lightly thru nested 8/64 and 4/64 round hole sieves of 12″ diameter to facilitate separation of smaller leaf fragments. Separate all leaf and stem fragments by visual examination of that portion remaining on larger screen. Separate all leaf and stem fragments of that portion remaining on the smaller screen by visual examination, or preferably by the use of the Bates Aspirator, which method lends both accuracy and speed to the results. Portion should be aspirated until visual examination shows complete separation of leaves and stems from petals. Material passing thru smaller screen consists largely of lupulin and very fine hop particles which cannot be further separated by physical means. It is considered as hops in the leaf and stem determination. Separate and discard the seeds and strigs in the aspirated portions by the use of a 4×20 wire mesh screen. Combine and weigh all leaf and stem portions. Report leaves and stems to nearest ½%.

(*b*) *Seeds.*—Dry a separate 20–40 g sample, split from the original composite by means of the Bates Divider, in an oven at 100–115°C. for 1¾ to 2½ hours (sufficient time to eliminate stickiness) or enclose the sample in a muslin cloth and wash in methyl alcohol or ethylene trichloride for one minute. Press out and dry in air or over steam radiator. Rub the dried sample enclosed in muslin between the hands to crush the petals completely and empty the fully pulverized material onto a 4×20 wire mesh screen to separate the petal substance. Continue this process until the portion left on the screen consists mainly of seeds and rachillae. Separate these by rolling the seeds off a large sheet of sandpaper into a tared dish. Weigh and report to the nearest ½% by weight.

Section **14.81 (c)**, relating to size and condition of cones and **(d)** lupulin, should receive only procedure status. The former would be improved by including a statement that a 30/64″ round hole screen will facilitate removal of whole cone.

It is recommended that methods **14.81 (e)**, Aroma, and present **(b)**, Color and Luster, be dropped from the 7th Edition as undeserving of inclusion as first action and too dependent upon individual opinion to be listed as procedures.

REPORT ON CORDIALS AND LIQUEURS

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

Last year's report on cordials and liqueurs contained recommendations for the collaborative study of (1) methods for caramel; (2) total solids by evaporation; (3) total solids from the specific gravity of the dealcoholized sample, and (4) total acidity.

The study of methods for caramel in cordials and liqueurs was carried out by Peter Valaer, Associate Referee on caramel in alcoholic beverages,[1] who concluded that the Mathers test for caramel was satisfactory; and the Associate Referee on cordials and liqueurs is recommending its adoption as first action, for cordials and liqueurs.

For the study of the two methods for total solids, and that for acidity, two synthetic cordials were prepared using the formulas given below and submitted to three collaborators.

	Creme de Menthe	*Imitation Raspberry*
Sugar	480 g	370 g
Citric acid	3.5 g	7.5 g
H_2O	250 ml	200 ml
Mint color	q.s.	q.s.
Raspberry color	—	q.s.
Peppermint oil flavor	1 ml	—
Imitation raspberry oil	—	3 ml
Alcohol	300 ml	340 ml
H_2O	q.s.	q.s.
	1000 ml	1000 ml

Details of the methods used by collaborators are given below.

(1) TOTAL SOLIDS BY EVAPORATION

Fill a 25-ml volumetric flask with the sample at 20°C., adjust the meniscus by means of a capillary tube or narrow strips of filter paper while the flask is immersed in a bath held at this temperature for about 30 minutes. Quantitatively transfer the contents of the 25-ml flask to a 100-ml volumetric flask with water and fill to the mark with water at a convenient temperature. At the same temperature pipet 10 ml of the diluted sample into a dish containing sand, and dry as described in **34.5.** Wt. of residue multiplied by 40 = g total solids per 100 ml of sample.

[1] *This Journal*, page 321.

(2) TOTAL SOLIDS FROM SP. GR. OF DEALCOHOLIZED SAMPLE

Transfer the residue from the alcohol determination **16.46(b)** to the original pycnometer with H_2O, make up to the mark with water at 15.56°, and mix. Now adjust the temperature of the pycnometer and contents to 20°C.; adjust the meniscus to the mark using a capillary tube or narrow strips of filter paper to remove any excess liquid while in the 20°C. bath. Weigh and calculate the specific gravity of the liquid. From **44.3** ascertain the percentage of dry substance and the corresponding specific gravity at 20°/4°C. Multiply specific gravity at 20°/4° by the percentage dry substance to obtain grams total solids per 100 ml.

(3) TOTAL ACIDITY

Determine as directed in **16.62** and report as grams crystallized citric acid per 100 ml. The results obtained are given in Table 1.

TABLE 1.—*Analysis of cordials*
(grams per 100 ml)

	CREME DE MENTHE			IMITATION RASPBERRY		
	TOTAL SOLIDS		ACID	TOTAL SOLIDS		ACID
Method,	(1)	(2)	(3)	(1)	(2)	(3)
Present	48.35	50.75	0.350	37.75	39.60	0.750
Found by collaborator:						
Marder	48.53	50.24	0.360	37.47	39.55	0.750
			0.360			
Mathers	48.92	50.69		38.76	39.59	
Wilson	48.15	50.53	0.343	37.41	39.73	0.749
	48.16		0.357	37.47		0.756

In the application of Method 1, little if any change occurs in the sucrose contained in the sample, but when subjected to distillation as in Method 2, practically all of the sucrose is inverted, causing a corresponding increase in weight of the solids. It seems desirable to retain this procedure since it is convenient for analysts when alcohol determinations are made. It it is desired to obtain the original solids content, correct the figure obtained as follows: Obtain the sum of the acid and any other non-sucrose solids and subtract from the total solids found. Multiply the remaining percentage of invert sugar by 0.95 and again add non-sugars to obtain original total solids.

In the Associate Referee's opinion, the results obtained warrant the adoption of all three methods as first action. Further collaborative work should be done before final action.

The recommendations submitted this year in addition to those tied up with the collaborative studies reported are largely connected with the new policy of the Association regarding classification of methods.

Methods included under items 1 and 2 below are concerned with changes of designation in the interest of uniformity.

Methods under item 3 consist mainly of references to official methods in the main body of the chapter on distilled spirits.

Methods under items 4 to 7, inclusive, have been studied this year.

The method for thujone (item 8) has been subjected to study by the writer[2] and by several European chemists[3] and is recognized throughout the world as suitable for detection of thujone. The writer found that it can be depended upon to give positive tests when 5 mg of thujone is present. The method also offers an opportunity to recognize the ketone by its odor.

The remaining recommendations consist of giving official status in the present chapter to equal that given in their respective chapters in which the same methods are applied to foods differing in composition only in the substitution of alcohol for a part of the water content.

RECOMMENDATIONS*

It is recommended—

(1) That **16.44**—Physical Examination be designated a procedure.

(2) That the caption "**16.63**—Preliminary Procedure*** Acids" be changed to "**16.63** Characteristic Acids—Preparation of Sample—Procedure."

(3) That the following sections be made first action.

16.45 Specific gravity.
16.46 Alcohol.
16.47 Methanol.
16.48 Aldehydes.
16.49 Furfural.
16.50 Fusel oil.

(4) That the method for "total solids—from the specific gravity of the dealcoholized sample" as given in this year's report, be adopted as first action.

(5) That the method for "total solids by evaporation" as given in this year's report be adopted as first action.

(6) That Mather's Test for Caramel, as given in the report of the Associate Referee on caramel, be adopted as first action.

(7) That the method for total acidity, **16.62,** be adopted as first action.

(8) That the qualitative method for thujone, **16.73,** be adopted as first action.

This method usually gives positive results when 5 mg or more of thujone is present. However, a negative test does not preclude the possibility that pharmacologically significant quantities of thujone are contained in the

[2] *Ibid.*, 19, 120 (1936).
[3] *Schweiz. med. Wochschr.* 49, 337, 507 (1911); *Ann. chim. anal.*, 13, 227 (1908).
* For report of Subcommittee D and action of the Association, see *This Journal*, 33, 60 (1950).

sample. It is the best procedure known for thujone and is substantially the same as the methods used throughout the world for its detection at this time.

(9) That the methods listed below, which consist chiefly of the application to cordials and liqueurs of official methods in other products whose composition differs from that of cordials and liqueurs only in the substitution of alcohol for more or less of the water present, be given the same status as they have in their respective chapters.

Official

16.52 Glycerol.
16.53 Sucrose by polarization.
16.54 Sucrose by reducing sugars.
16.55 Ash.
16.56 Soluble and insoluble ash.
16.57 Alkalinity of soluble ash.
16.58 Alkalinity of insoluble ash.
16.59 Phosphoric acid.

First Action

16.64 Tartaric acid.
16.65 Citric acid.
16.66 Active malic acid.
16.67 Inactive malic acid.

No report was given on yeast, total and yeast solids (see report on malt beverages, surups, and extracts in brewing materials); color and turbidity in beer; and methanol.

A contributed paper entitled "Rapid Determination of Sulfur Dioxide in Wine," by A. P. Mathers, was published in *This Journal*, in November, page 745.

REPORT ON CACAO PRODUCTS

By W. O. WINKLER (U. S. Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Referee*

Investigations have been carried on this year on four phases of the cacao products program: fat, lactose, maltose, and shell. Of these projects studied, a report will be made only on two, namely, fat and pectic acid. The other studies were not yet ready to report.

Last year, the Associate Referee on lactose reported a method which gave good recoveries and would no doubt be a good reference method but it was too time-consuming for control work. The Associate Referee is now endeavoring to shorten the time necessary for a determination.

Shell by Pectic Acid.—It is felt that a pronounced advance has been made in the determination of pectic acid (for shell estimation) particularly in products containing milk solids. In most previous work the problem has been approached by trying to precipitate out the pectins without precipitating the milk protein or by careful precipitation of the protein from the pectin solutions without removing the latter. These attempts were only partially successful.

This year the effort has been directed toward removing or dissolving out the protein without removing the pectin. The Referee has been able to do this in two different ways: (1) by a mixture of 100 parts alcohol and 75 parts of conc. hydrochloric acid, and (2) by a mixture of acetone (110 parts) and an aqueous (18%) solution of triethanolamine (100 parts). After some experimentation the first procedure was discarded as being too drastic, and the second one was adopted. A new washing and filtering procedure for the final pectic acid precipitate was also devised which greatly speeded and simplified this operation.

As a result of these investigations and previous work, methods were drafted for the various types of cacao products, incorporating the necessary new operations in the various procedures.

Samples of sweet chocolate and of milk chocolate were prepared, containing added quantities of cacao shell. These were submitted to collaborators for analysis by the new procedures for a comparative study. The findings of the various analysts are recorded in Table 1.

TABLE 1.—*Collaborative results*

COLLABORATOR: WOOD	SWEET CHOCOLATE. PECTIC ACID IN DRY FAT-FREE CACAO INGREDIENT	CACAO RESIDUE AFTER EXTRACTION OF 25 GRAM SAMPLE
	per cent	*grams*
Sweet chocolate:		
(1)	0.96, 0.96	2.728, 2.734
(2)	0.98, 0.88	2.899
(3)	0.88, 0.84	
(4)	0.84, 0.95	2.8218, 2.854
Milk chocolate:		
(1)	0.67, 0.67	2.806
(2)	0.62, 0.55	2.876
(3)	0.63, 0.65	
(4)	0.53, 0.55	

The results show what is considered very good agreement for this type of determination and are the most satisfactory results obtained by any of the procedures so far tried. The procedures will be given in detail in the 7th Edition, *Methods of Analysis.*

Fat in Refractory Samples.—The Associate Referee, C. B. Stone, has conducted collaborative study of three methods for fat determination or extraction for various refractory types of samples such as chocolate malted milks, beverage bases, and some milk chocolate. The methods used were the A.O.A.C. method, the dioxane method proposed by L. W. Ferris, Associate Referee on Fruit Acids, and a modification of the unified acid hydrolysis method formerly proposed by F. Hillig. He has obtained some interesting results and the Referee concurs in the recommendation that the modified acid hydrolysis (Hillig) method be adopted for use on such samples.

RECOMMENDATIONS*

It is recommended—

(1) That the methods for pectic acid given in this report for various cacao products be adopted as first action, and that the present tentative method **19.16** be dropped. It is also recommended that the method be further tested and applied to liquor of known shell content.

(2) That the procedure (modified acid hydrolysis—Hillig) for fat determination in milk chocolate, referred to in the report of the Associate Referee, be adopted as first action, and that the method be further tested.

(3) That **19.24** be deleted and **19.23(b)(c)** limited to non-refractory products.

(4) That the work on the determination of lactose in cacao products in the presence of other reducing sugars be continued.

(5) That the work on the determination of maltose in cacao products be continued.

(6) That the work on cacao constituents be continued.

The following recommendations pertaining to the Cacao Chapter are also made:

19.1 (a) and **(b)**—Preparation of Sample.

Inasmuch as both of these paragraphs are sampling procedures, it is recommended that the designation be changed to Procedure. Paragraph **(a)** has been the official method of preparation for some time. However, various versions of procedure **(b)** have also been used by chemists for many years. This method of sampling is particularly advantageous in the case of samples collected in jars and when it is difficult to break up the material and grate it. This is especially true if the sample has been collected in a liquid condition and has been allowed to solidify slowly, with partial separation of the fat. The first procedure "**(a)** Official" requires considerable work to prepare the material for sampling, but this is not the case with the second procedure **(b)**.

19.2—Moisture—Tentative.

When the present method was proposed, a considerable amount of

* For report of Subcommittee D and action of the Association, see *This Journal*, 33, 61 (1950).

data was presented (*This Journal* **14,** 529 (1931)), which showed the comparison of this with other methods. This comparison was favorable. Moisture determinations by drying at 100°C. are common in many types of foods. It is recommended that the tentative method for moisture be made first action.

19.9—Coloring Matters—Tentative.

It is recommended that this section be dropped. It is the Referee's opinion that this section is unnecessary, since an analyst would proceed to the chapter on coloring matters if such a determination were found necessary. No collaborative work was found on this paragraph.

19.10 to **19.14**—Shell in Cacao Nibs—Tentative.

It is recommended that this method be made first action. Some collaborative work was done on it at the time it was proposed at the cacao standards hearing. Report on a part of this work is contained in *This Journal* **23,** 593 (1940), and the remainder in the hearing records as an exhibit. The method was given legal standing in the standards.

19.16—Pectic Acid—Tentative.

It is recommended that this method be dropped and that the method for pectic acid given in this report be adopted as first action.

19.22—Chocolate Liquor—Tentative.

The status of this method should be made first action. The factor 1.43 which is an essential part of the method, was obtained in the course of extended work on crude fiber, using a similar extraction, and data to support it were reported in *This Journal,* **14,** 530 (1931). The factor 2.2 is also an essential part of the method and was based on extended work leading up to the standards for cacao products, reported in the records of the hearings on those standards.

19.23—Fat—Quantitative Determination—Method I.

The following parenthetical statement should be inserted immediately following the caption "Quantitative Determination": "Not applicable to sweet cocoa, sweet milk cocoa, or other similar products prepared by cooking or mixing with water or milk and drying."

19.24—Fat—Method II—Tentative.

This method should be dropped and the acid hydrolysis method, recommended by the Associate Referee, should be substituted as first action, based on the work in this year's report.

19.25—Separation and Preparation of Fat—Tentative.

Because of the difficulty of removing all fat, particularly milk fat, from many samples of a refractory type, as shown in the Associate Referee's report this year, a new method for separation and preparation of fat, to be designated as "applicable to cacao products containing milk ingredients or those prepared by cooking with sugar and water and drying," and the present directions, **19.25,** designated as not applicable to such products, are recommended for adoption as procedures.

19.30—Milk Fat in Milk Chocolate—Tentative.

It is recommended that this method be changed to first action under suspension of the rules. The method is of many years standing, going back to the old Bureau of Chemistry Bulletin 107. It was used in a particular form previous to the publication of the sixth edition of the *Book of Methods,* but in the sixth edition was changed to the general algebraic form to allow substitution of more recent data.

19.32—Detection of Coconut and Palm Kernel Oils in Cacao Butter and Fat Extracted from Milk Chocolate—Tentative.

It is recommended that this method be made first action. It is qualitative and has been found to distinguish between pure cacao butter and that containing foreign oils. Collaborative work was done, with good agreement, when the method was proposed as tentative. See *This Journal,* **9,** 486 (1930).

19.33–19.34—Silver Number for Detection of Coconut and Palm Kernel Oils—Tentative.

It is recommended that this method be made first action. The method gives a fairly quantitative measure of the amount of such oils or their stearins when the original fat added is known. Good collaborative results were obtained on this method when it was made tentative. See *This Journal* **17,** 375 (1934).

19.35–19.36—Critical Temperature of Dissolution of Fat in Acetic Acid—Tentative.

It is recommended that this method be made first action. It is capable of detecting a considerable number of foreign oils or fats in cacao fat. Collaborative work was done formerly, with reasonably good agreement. See *This Journal,* **7,** 152 (1923). Fats detected were cottonseed oil, coconut oil or stearin, palm kernel oil or stearin, peanut oil, butterfat, etc.

19.37—Acetone-Carbon Tetrachloride Test of Fat—Tentative.

It is recommended that this method be dropped. The test is rather ambiguous in its directions regarding time and is probably not used much at present.

19.38—Milk Fat in Milk Chocolate—Tentative.

This should be retained as previously stated and made first action, as it is a method of many years standing.

19.41—Milk Ash (from calcium)—Tentative.

It is recommended that this method be dropped, as sufficient supporting data are not available.

19.42—Sucrose (14)—Official.

There is an error in the transcript of the method as contained in the Book. The letters "P" and "I" are used on the third and second from the last lines of the present paragraph to represent direct and invert polarization, respectively, and are so used in the formula. Actually, the

readings obtained must be multiplied by 2 to obtain these polarizations on a normal solution. The letters "P" and "I" are so placed that they represent the readings of the dilute solution ($N/2$) rather than that of the normal or inverted normal solution. It is therefore proposed to change the last three lines to read: "Volume of H_20, mix, and polarize in 200 mm tube at 20°. Obtain the invert reading at 20° as directed under **34.24(b)**. Multiply both readings by 2 to obtain the direct and invert polarizations 'P' and 'I,' respectively. From the data obtained calculate per cent sucrose 'S' from following formulas:". With this correction it is recommended that the method be retained as official.

19.43—Dextrose—Tentative.

It is recommended that the method for dextrose be made first action The method is quite specific for monosaccharides of which dextrose is the principal one used in foods.

19.44—Corn Sirup—Tentative.

It is recommended that this method be dropped. It is applicable to only one type of corn sirup (that having solids polarizing at about 211°V) and is not applicable in the presence of added dextrose or lactose. Because of the numerous brands of corn sirup, high and low conversion, it may give erroneous results.

19.45—Starch—I. Direct Acid Hydrolysis Method—Tentative.

It is recommended that this method be made first action. The method follows the one used in grains and stock feeds **(27.33)**, except for preliminary sample treatment. The latter method is official and the one used here should give comparable results as a method of many years standing.

19.46—Starch—II. Diastase Method—Tentative.

It is recommended that this method be first action, and for the same reasons as those given regarding section **19.45**.

19.47—Theobromine (15)—Tentative.

It is recommended that this method be made first action, and that the heading be followed by the notation: "Not applicable to products containing more than 10% of sweetening ingredients." The method yields a very pure form of theobromine as found by Microbiological Division of the Food and Drug Administration, but the presence of appreciable quantities of sweeteners causes the material to gum and interferes with theobromine extraction.

It is recommended that further work be done on the method for tannins and pigments, reported two years ago, before any action is taken on this method.

For report on Pectic Acid, see Referee Report, page 336.

REPORT ON FAT IN MILK CHOCOLATE AND REFRACTORY CHOCOLATE PRODUCTS

By CARL B. STONE (U. S. Food and Drug Administration, Federal Security Agency, Cincinnati, Ohio), *Associate Referee*

Committee D recommended that the method of separation of fat, **19.25**,[1] when used on milk chocolate, be studied and compared with the method for refractory sample proposed by Ferris, *This Journal*, **31**, 728 (1948). The Associate Referee decided to include the Modified Hillig Method, *This Journal*, **28**, 482 (1945) in this study.

A series of products purchased on the open market were examined by the three methods and the results are tabulated below.

TABLE 1.—*Comparative results*

NO.	PRODUCT	19.25 METHOD	FERRIS METHOD	MODIFIED HILLIG METHOD
		per cent	*per cent*	*per cent*
A	Chocolate Malted Milk	3.16	3.33	5.86
B	Malted Milk	2.31	2.43	4.88
C	Milk Chocolate	36.88	37.41	37.55
D	Milk Chocolate	30.96	31.29	31.49
E	Sweet Chocolate Coating	37.46	37.79	38.08

As a result of the information secured on the various commercial samples the Associate Referee obtained the assistance of John Bornmann of the Chicago District, and E. C. Aderholdt and W. J. Keating of the Cincinnati District, all of the Food and Drug Administration, in the preparation of authentic samples of chocolate malted milk and milk chocolate for collaborative study. Each collaborator was furnished two subdivisions of the chocolate malted milk, identified as A and B, and two subdivisions of the milk chocolate, identified as C and E. The collaborators were requested to make single determinations on each subdivision by each of the three methods.

The instructions to collaborators suggested as an optional procedure the Modified Hillig method:

> A 150-ml Büchner fritted glass funnel may be used in place of the Knorr tubes specified. Overlay the disc with a layer of asbestos and cover the asbestos with a qualitative filter paper. Place a circle of wire screen (ca 20 mesh) over the paper. This arrangement facilitates the stirring of the mixture after each addition of ether, insuring complete extraction. Either coarse or medium porosity funnels may be used.

Results obtained by the collaborators are shown in Tables 2 and 3.

[1] *Methods of Analysis, A.O.A.C.*, 6th edition, page 229.

TABLE 2.—*Fat content of chocolate malted milk*

ANALYST	19.25 METHOD	ARITHMETIC DEVIATION	FERRIS METHOD	ARITHMETIC DEVIATION	MODIFIED HILLIG METHOD	ARITHMETIC DEVIATION
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
(1)	2.34	−0.04	2.88	+0.14	4.52	−0.17
	2.18[1]	—	2.78	+0.04	4.56	−0.13
(2)					4.68*	−0.01
					4.77	+0.08
(3)	2.38	0.00	2.93	+0.19	4.67*	−0.02
	2.39	+0.01	2.77	+0.03	4.61	−0.08
(4)	2.39	+0.01	2.92	+0.18	4.50	−0.19
	2.45	+0.07	2.81	+0.07	4.52	−0.17
(5)	2.37	−0.01	2.47	−0.27	4.89*	+0.20
	2.42	+0.04	2.53	−0.21	4.90	+0.21
(6)	2.42	+0.04	2.58	−0.16	4.79*	+0.10
	2.36	+0.02	2.54	−0.20	4.77	+0.08
(7)	2.35	−0.03	2.63	−0.11	4.67	−0.02
	2.33	−0.05	2.60	−0.14	4.63	−0.07
(8)	2.38	0.00	3.08	+0.34	4.76	+0.07
	2.39	+0.01	3.16	+0.42	4.71	+0.02
(9)	2.41	+0.03	2.70	−0.04	4.78*	+0.09
	2.35	−0.03	2.69	−0.05	4.66	−0.03
(10)	2.35	−0.03	2.57	−0.17	4.72	+0.03
	2.37	−0.03	2.59	−0.15	4.70	+0.01

[1] Results rejected.
* Used fritted funnel.

COLLABORATORS' COMMENTS

Bornmann, Chicago.—The extract obtained after dioxane treatment appears to contain brown cacao extractives which I believe are not fat. Perhaps the extract should be dissolved in petroleum benzine rather than ether before filtering. The brown material appears to be insoluble in petroleum benzine.

McRoberts, San Francisco.—The dried fat residues in Ferris method were not completely soluble in petroleum ether. This was especially true of the fatty residue after dioxane treatment. Difficulty was encountered in drying the dioxane extract to constant weight.

McNall, Cincinnati.—The Modified Hillig procedure appears to be the one method which will work on all chocolate products, particularly refractory products such as malted milk. The material extracted is clear fat and not contaminated with other material. The dioxane treatment followed by an ether extraction does not give a clear fat extract but a mixture of ether extractives which are not soluble in petroleum ether and which decompose on heating.

TABLE 3.—*Fat content of milk chocolate*

ANALYST	19.25 METHOD	ARITHMETIC DEVIATION	FERRIS METHOD	ARITHMETIC DEVIATION	MODIFIED HILLIG METHOD	ARITHMETIC DEVIATION
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
(1)	31.21	+0.11	31.48	−0.07	31.21	−0.24
	31.18	+0.08	31.46	−0.09	31.46	+0.01
(2)	31.07	−0.03	31.55	0.00	31.59*	+0.14
	31.13	+0.03	31.55	0.00	31.62	+0.17
(3)	31.31	+0.21	31.71	+0.16	31.56*	+0.11
	31.07	−0.03	31.68	+0.13	31.34	−0.11
(4)	31.30	+0.20	31.80	+0.25	32.26[1]	—
	31.49	+0.39	31.93[1]	—	32.18[1]	—
(5)	31.33	+0.23	31.70	+0.15	31.44*	−0.01
	31.31	+0.21	31.67	+0.12	31.52	+0.07
(6)	31.20	+0.10	31.47	−0.08	31.57*	+0.12
	31.13	+0.03	31.42	−0.13	31.51	+0.06
(7)	30.87	−0.23	31.42	−0.13	31.44	−0.01
	30.76	−0.34	31.38	−0.17	31.35	−0.10
(8)	31.01	−0.09	31.61	+0.06	31.66	+0.21
	31.01	−0.09	31.56	+0.01	31.51	+0.06
(9)	30.96	−0.14	31.50	−0.05	31.31*	−0.14
	30.93	0.17	31.46	−0.09	31.21	−0.24
(10)	30.75	−0.35	31.37	−0.18	31.29	−0.16
	30.99	−0.11	31.62	+0.07	31.46	+0.01

[1] Results rejected.
* Used fritted funnel.

INTERPRETATION OF RESULTS

In order to properly evaluate the methods, a statistical analysis was made of the data. The results of this analysis are shown in Table 4.

In the final analytical interpretation of the data obtained, four individual results of chemists were rejected. 2.18% by chemist 1 for A.O.A.C., 19.25, 31.93% by chemist 4 for the Ferris method, and 32.26% and 32.18% also by chemist 4 for the Modified Hillig method were eliminated as not representative of the respective methods by the Pierce-Chauvenet criterion.

For the Modified Hillig method on either product, the fritted funnel gave an average for 5 cases about 0.05 per cent more extract than did the

TABLE 4.—*Comparison of methods for chocolate malted milk and milk chocolate*

PRODUCT	METHOD	ARITHMETIC MEAN	STANDARD DEVIATION	PROBABLE ERROR, SINGLE OBSERVATION	PROBABLE ERROR OF THE MEAN	COEFFICIENT OF VARIATION	RANGE, PER CENT			
							OBSERVED		PIERCE-CHAUVENET CRITERION	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*max.*	*min.*	*max.*	*min.*
Chocolate Malted Milk	A.O.A.C. **19.25**	2.38	0.03	0.02	0.005	1.34	2.45	2.33	2.45	2.31
	Ferris	2.74	0.20	0.13	0.03	7.19	3.16	2.47	3.18	2.30
	Modified Hillig	4.69	0.11	0.08	0.02	2.41	4.90	4.50	4.94	4.44
Milk Chocolate	A.O.A.C. **19.25**	31.10	0.20	0.14	0.03	0.65	31.49	30.75	31.55	30.65
	Ferris	31.55	0.12	0.08	0.02	0.39	31.80	31.37	31.83	31.27
	Modified	31.45	0.14	0.09	0.02	0.43	31.66	31.21	31.75	31.15

method without its use. Since only 5 results in each instance are given, not much weight can be given the greater amount of extract, and hence no differentiation was made in the computations.

The Modified Hillig method consistently and significantly obtained more extract from the chocolate malted milk, while the A.O.A.C. **19.25,** method gave the least, about one-half the Modified Hillig method. Results by **19.25** method were quite consistent with very small precision, dispersion, and variation measures, which illustrate uniform low maximum and limited fat extraction for this method (see Table 4). The percentage range to be normally expected by **19.25** for chocolate malted milk is 2.31-2.45, as compared to 2.30–3.18 by the Ferris method and 4.44–4.94 by the Modified Hillig method. The latter not only obtains a much more complete extraction of fat from the above product than the other methods, but also has about one-third the variation of the Ferris method.

For milk chocolate, the method **19.25** still gave less extract than did the other two methods, and also gave about 1.5 to 2 times the variability. The precision and dispersion measures of the Ferris and Modified Hillig methods for this product are small and compare favorably, but with the latter giving slightly more variability, which is insignificant. As discussed by the collaborators, the Ferris method seemed to extract certain substances non-fat in nature which probably accounts for its extracting somewhat more (0.10%) than did the Modified Hillig method. The Modified Hillig method gave a clear extract, soluble in petroleum benzine. The ranges permitted by the Pierce-Chauvenet criterion for the milk chocolate sample are 31.27–31.83 by the Ferris method and 31.15–31.75 by the Modified Hillig method, indicating that this latter method will continue to give lower results than the Ferris method.

Examining Table 4 further, it is noted that the Modified Hillig method also was more consistent in comparing results between the two products. In other words, the dispersion and precision measures were about the same for milk chocolate and chocolate malted milk.

Considering the clean fat extract obtained by the Modified Hillig method, the relatively low precision, dispersion, and variation measures obtained for both products, and the more complete extraction of fat from chocolate malted milk, this is the most reliable method to be used on such products.

ACKNOWLEDGMENT

The Associate Referee gratefully acknowledges the assistance of Luther Ensminger for the statistical study of the results submitted by the collaborators. The assistance of the following collaborators is appreciated: L. W. Ferris, John F. Weeks, L. H. McRoberts, M. L. Offut, Halver VanDame, Mary A. McEniry, John Bornmann, F. J. McNall, and Fred Hillig, all members of the Food and Drug Administration.

RECOMMENDATIONS*

It is recommended—

(1) That method **19.24,** *Methods of Analysis,* be deleted.

(2) That the Modified Hillig method for fat be made first action for milk chocolate and refractory chocolate products, with the optional procedure of using the Büchner fritted funnel in place of the Knorr tube, and providing a 10–20 g sample for milk chocolates.

(3) That the method **19.23,** be restricted to non-refractory products.

No report was given on lecithin, malt solids, cacao ingredient, lactose (see Referee report).

REPORT ON FRUITS AND FRUIT PRODUCTS

By R. A. OSBORN (Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Referee*

Last year the committee on Recommendations of Referees made six recommendations for fruit products (*This Journal,* **32,** 63 (1949)). The following recommendations deal with each of these in the same order as given in the committee report.

RECOMMENDATIONS†

(1) The Associate Referee made no report on methods for determining fruit and sugar content of frozen fruits. It is recommended that the subject be continued.

(2) The Associate Referee made no report on the method for the electrometric titration of acidity. In a communication (Sept. 7, 1949) to the Referee, the Associate Referee, H. M. Bollinger, suggests that the glass electrode method **26.29–26.30** be changed in status to first action. The Referee so recommends. The collaborative results are given in the report of 1944 (*This Journal* **28,** 507 (1945)).

(3) The report of the Associate Referee on fruit acids recommends the change of status from tentative to first action for **26.32–26.33** and **26.36–26.43** and deletion of **26.34–26.35.** This recommendation is concurred in and it is further recommended that study be continued on the retained methods and those proposed by B. G. Hartman (*This Journal,* **26,** 444 (1943)).

(4) The Referee has subjected the regular and the rapid tentative procedures, **26.7,** and *This Journal,* **32,** 94, 95 (1949), for determination of water-insoluble solids and seeds of berry fruits to collaborative study and recommends adoption as first action.

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* For report of Subcommittee D and action of the Association, see *This Journal,* **33,** 61 (1950).
† For report of Subcommittee D and action of the Association, see *This Journal,* **33,** 66 (1950).

(5) It is recommended that the changes adopted last year in **26.18(a)** (*This Journal*, **32**, 94 (1949)) be made official.

(6) It is recommended that the sentence beginning "If recoveries are low . . . " in **26.19**, note (3) be deleted (final action).

The following changes are recommended on other sections of Chapter 26 for purposes of revision of *Methods of Analysis:*

(1) **26.8.** The procedure was subjected to collaborative study (*This Journal*, **15**, 384 (1932)). It is recommended that the title be revised by inserting "by Refractometer" between "Solids" and "in" and that it be adopted as first action. It is recognized that the refractometer scale for soluble solids is based on sucrose and fruits and fruit products contain soluble constituents in addition to sugar. To this extent the values obtained are relative rather than absolute. However, it is the general practice in the commercial manufacture of jams, jellies, marmalades, and fruit butters to determine the degree of concentration by use of the refractometer. The definition and standard of identity for preserves, jellies, and fruit butters relates to the direct reading of soluble solids by refractometer without correction for insoluble solids.[1]

(2) **26.12.** Total Sulfur.—This procedure received collaborative study (*This Journal*, **6**, 35 (1922)). It is recommended that it be made first action.

(3) **26.13.** Chlorine in Ash.—The procedure is official for Plants, **12.41–12.44.** It received collaborative study (*This Journal*, **11**, 216 (1928)), and it is recommended that it be made first action.

(4) **26.19.** For purpose of clarification only, it is recommended that the phrase "using N/10 HNO_3" be inserted after "if necessary" in line 5.

(5) **26.20–26.21.** Manganese.
26.22–26.23. Calcium.
26.24. Magnesium.

Each of the above procedures was subjected to collaborative study in the same report (*This Journal*, **14**, 463 (1931)). See also, *Ibid.*, **12**, 362 (1929). It is recommended that these procedures be adopted as first action.

(6) **26.25.** Alcohol precipitate.
26.26. Pectic acid.

These two procedures were subjected to collaborative study (*This Journal*, **6**, 36 (1922)). Since then they have been used extensively. It is recommended that both procedures be adopted as first action.

(7) **26.28(b).** Titratable acidity, highly colored solutions.—This procedure received collaborative study (*This Journal*, **28**, 507 (1945)). It is a satisfactory procedure and it is recommended that it be adopted as first action.

(8) **26.29–26.30.** Since buffer standards suitable for checking the elec-

[1] Service and Regulatory Announcements F.D.C. No. 2, Food and Drug Administration "Definitions and Standards for Food," January, 1949, pp. 47–54.

trometer and glass electrode are to be contained in Chapter 43, "Standard Solutions," it is recommended that **26.29** be deleted and a reference to such standards be made.

(9) **26.51–26.53.** Free mineral acids. See **33.85–33.87.**—It is recommended that the status of these procedures be changed to conform with that recommended by the Referee on Spices and Other Condiments.

(10) **26.56.** Dextrin—Tentative.—This procedure has not received sufficient study in recent years to justify recommendation for adoption as official. It is recommended that it be dropped.

(11) **26.58** Gelatin.—This tentative procedure is not specific for gelatin. It is recommended that it be dropped.

(12) **26.59–26.60.** Agar-agar.—These procedures have not been subjected to collaborative study. It is recommended that they be dropped.

(13) **26.61.** Added water in grape juice (applicable to white juices only).—This procedure has a limited application. It is recommended that the procedure be dropped.

REPORT ON WATER-INSOLUBLE SOLIDS AND SEEDS OF FRUITS AND FRUIT PRODUCTS

By R. A. OSBORN (Division of Food,* Food and Drug Administration, Federal Security Agency, Washington, D.C.) *Associate Referee*

This report deals chiefly with a collaborative study of methods for the determination of water-insoluble solids and seeds, as published in *This*

TABLE 1.—*Commercial peach preserves*

% Water insoluble solids (25 g aliquots)

ANALYST	15 CM WHATMAN NO. 4 PAPER ON 12½ CM BUCHNER		7 CM WHATMAN NO. 4 PAPER ON 7 CM BUCHNER		7 CM WHATMAN 41 H ON 7 CM BUCHNER		12½ CM COTTON ON BUCHNER		12½ CM COTTON ON 60° FUNNEL	
Strange	0.24		—		—		0.23		0.23	
	0.24	0.24	—		—		0.23	0.23	0.23	0.23
Edge	0.23		—						0.24	
	0.26	0.25	—						0.24	0.24
Osborn	0.22		0.23		0.22					
	0.23	0.22	0.23	0.23	0.23	0.22				
Hatmaker	0.20				0.21					
	0.21	0.21			0.22	0.21				
Av.		0.23		0.23		0.22		0.23		0.23

* W. B. White, Chief.

TABLE 2.—*Commercial boysenberry preserve*

% Water-insoluble solids (25 g aliquots) and seeds (50 g aliquots)

ANALYST	15 CM WHATMAN NO. 4 ON 12½ CM BUCHNER		12½ CM COTTON ON BUCHNER		12½ CM COTTON ON 60° FUNNEL		7 CM WHATMAN NO. 4 ON 7 CM BUCHNER		SEEDS		NUMBER SEEDS	AVERAGE WEIGHT OF SEED
											/100 g	*mg*
Strange	1.50		1.47						1.40		827	1.7
	1.60	1.55	1.50	1.49					1.41	1.41		
Edge	1.44				1.54				1.18*		738	1.6
	1.60	1.51			1.60	1.57						
Osborn	1.42						1.50		1.25		746	1.85
	1.67	1.54					1.57	1.53	1.52	1.38		
Hatmaker	1.51											
	1.56	1.53					1.54		1.33		738	1.87
							1.58	1.56	1.43	1.38		
Av.		1.53		1.49		1.57		1.54		1.39	762	1.76

* Single determination; excluded from Av.

TABLE 3.—*Commercial blackberry preserve*

% Water insoluble solids (25 g aliquots) and seeds (50 g aliquots)

ANALYST	15 CM WHATMAN NO. 4 ON 12½ CM BUCHNER		MOISTURE TELLER RAPID PROCEDURE		7 CM WHATMAN NO. 4 OR 41 H ON 7 CM BUCHNER		SEEDS		NUMBER SEEDS	AVERAGE WEIGHT SEED
									/100 grams	*mg*
Walde	2.59		2.57							
	2.62		2.61	2.59	—		2.01		688	2.9
	2.65									
	2.77	2.66								
Osborn	2.76		2.66		2.67		2.16		702	3.15
	2.76	2.76	2.78		2.80	2.74	2.26	2.21		
			3.16	2.87						
Hatmaker	2.55		2.76		2.64		2.12		671	3.26
	2.77	2.66	2.82	2.79	2.67	2.66	2.26	2.19		
Av.		2.69		2.75		2.70		2.20	687	3.10
Commercial red raspberry preserve										
Walde	2.06		2.05							
	2.08	2.07	2.07	2.06	—		1.67		1440	1.17
Osborn	2.08		2.06		1.98		1.45		1382	1.10
	2.13	2.10	2.16	2.11	2.01	2.00	1.60	1.52		
Hatmaker	2.06		2.23		2.04		1.65		1470	1.17
	2.14	2.10	2.25		2.11		1.78			
			2.40	2.29		2.08		1.72		
Av.		2.09		2.15		2.04		1.62	1431	1.15

TABLE 4.—*Commercial blackberry preserve*

% Water-insoluble solids (25 g aliquots) and seeds (50 g aliquots)

ANALYST	WHATMAN 15 CM & 12½ CM BÜCHNER		MOISTURE TELLER RAPID PROCEDURE		SEEDS		NUMBER SEEDS	AVERAGE WEIGHT SEED
							/100 g	*mg*
Meschter	—		2.35 2.35 2.39 2.49 2.50 2.53	2.43	1.92		593	3.2
Marder	2.42 2.45 2.45 2.45	2.44	2.07 2.10 2.28 2.32	2.19	1.71		575	3.0
Hatmaker	2.47 2.63 2.82	2.64	2.40 2.50 2.73	2.54	1.73		610	2.8
Osborn	2.20 2.28	2.24	2.13 2.33	2.23	1.66		582	2.9
Av.		2.44		2.35	1.76		590	3.0
Commercial red raspberry preserve								
Meschter	—		2.10 2.14	2.12				
Marder	2.08 2.32	2.20	2.06 2.36	2.21	1.67		1376	1.22
Hatmaker	2.23 2.37	2.30	2.28 2.33	2.30	1.75		1461	1.20
Osborn	2.02 2.11	2.07	2.19 2.36	2.27	1.69		1474	1.14
Av.		2.19		2.22	1.70		1437	1.19
Strawberry preserves								
Meschter	—		1.27 1.29 1.34	1.30	0.74 0.77 0.78	0.76	1206	0.63
Marder	1.24 1.25	1.25	—		0.76 0.83	0.79	1386	0.57
Hatmaker	1.34 1.34 1.42	1.37	—		0.76 0.86	0.81	1229	0.62
Osborn	1.25 1.27	1.26	—		0.70 0.75	0.73	1237	0.59
Av.		1.29		1.30		0.77	1265	0.60

TABLE 5.—*Youngberry preserve*

% Water-insoluble solids (25 g aliquots) and seeds (50 g aliquots)

ANALYST	15 CM WHATMAN NO. 4 ON 12½ CM BÜCHNER		12½ CM E & D FOLDED NO. 192		SEEDS		NUMBER SEEDS	AVERAGE WEIGHT SEED
							/100 g	*mg*
Minsker	2.20		2.22		2.00		523	3.85
	2.21	2.21	2.23	2.22	2.01	2.00		
Hatmaker	2.55							
	2.60							
	2.65		—		1.88		521	3.61
	2.69	2.62			—			
Av.		2.42		2.22		2.00	522	3.73
Commercial grape preserve								
Minsker	0.30		0.30					
	0.29	0.29	0.31	0.31				
Hatmaker	0.26							
	0.26							
	0.28	0.27	—					
Av.		0.28		0.31				

Journal, **32**, 177 (1949) and *Methods of Analysis. A.O.A.C.*, 6th ed., **26.7** (1945).

Collaborative results were obtained from T. E. Strange and Richard Edge, U. S. Food and Drug Administration, Portland, Oregon; W. Lowe Walde, National Preservers' Association, Washington, D.C.; E. Everett Meschter, American Preserve Company, Philadelphia, Pa.; F. C. Minsker U. S. Food and Drug Administration, Philadelphia, Pa.; and Jacob Marder, C. G. Hatmaker, and R. A. Osborn, U. S. Food and Drug Administration, Washington, D.C. Samples of commercial preserves were analyzed after preparation of the samples by mixing in a Waring blendor. Results of the analyses and the procedures and variations employed are contained in the accompanying tables.

In general, the collaborative results are in good agreement. There are occasional instances where the agreement between duplicates and between collaborators is not as close as may be desired. Such differences, in the opinion of the Associate Referee, are to be expected in consideration of the heterogeneous nature of small fruit samples and the difficulty of sample preparation.

The collaborators indicated that the use of relatively large (15 cm) circles of loose-textured (Whatman #4 or 41 H) filter paper on a Büchner

funnel simplified the filtration and washing operations. Difficulty was experienced by some of the collaborators when using the small (7 cm) circles of the filter paper. Based on these tabulations and data previously published, *This Journal*, **30**, 260 (1947), it appears desirable to permit the use of either filter paper or cotton of any convenient size with either a Büchner or 60° funnel. The collaborative results indicate that the rapid method is comparable with the regular procedure.

RECOMMENDATION*

It is recommended—

That with the slight changes as indicated in this report, the regular method, *Methods of Analysis*, 6th ed., **26.7**, and the rapid procedure, *This Journal*, **32**, 94 (1949), be made first action.

REPORT ON FRUIT ACIDS

By L. W. FERRIS (Food and Drug Administration, Federal Security Agency, Buffalo, N.Y.), *Associate Referee*

A preliminary study of methods for fruit acids was conducted this year. It included those proposed by Hartmann.[1] No opportunity was found to conduct collaborative study. Recommendations follow for the present tentative methods.

RECOMMENDATIONS†

26.32–26.33, Tartaric Acid, Bitartrate Method.—This method appeared in the 3rd edition (1930) and was proposed in a paper by Hartmann and Hillig.[2] It involves the principles of precipitation of potassium and tartrate for many years official for tartaric acid in wine. There is no other simple method established to take its place and it is recommended that it be changed to first action.

26.34–26.35, Tartaric Acid, Kling Method.—This method is longer and requires use of a not too readily available reagent, ammonium laevotartrate. Hartmann and Hillig,[2] in comparing these two methods stated: "Of these the acid potassium tartrate method is preferable to the calcium racemate method. The calcium racemate method requires a reagent that is expensive and not readily available, two precipitations are necessary, and the oxidation with potassium permanganate requires close attention. The acid potassium tartrate method requires less time, and the titration is easily accomplished." There would appear to be no need for the two methods, and it is recommended that the Kling method be dropped.

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* For report of Subcommittee D and action of the Association, see *This Journal*, **33**, 67 (1950).
† For report of Subcommittee D and action of the Association, see *This Journal*, **33**, 66 (1950).
[1] *This Journal*, **26**, 444 (1943).
[2] *Ibid.*, **13**, 103 (1930).

26.36–26.37, Citric acid.—This method has been in *Methods of Analysis* since the 3rd edition and involves the principles of the pentabromacetone precipitation reaction of Strahle, which was originally employed in the method for citric acid in the 1st edition (1919). Collaborative results are reported in 1931[3] and support a recommendation for change to first action.

26.38–26.40, Laevo-malic acid.—The collaborative results reported at the time this method was adopted as tentative[4] support the recommendation for change to first action.

26.41–26.43, Inactive malic acid.—This method has not been studied collaboratively. It was proposed by Hartmann and Hillig in 1933.[5] It has been in use since that time and is the only method which has been employed for the determination of this acid. It is recommended that it be retained as a first action method.

No reports were given on titration of acids or fruit and sugar in frozen fruit.

REPORT ON WATERS, BRINE, AND SALT

By C. G. HATMAKER (Food and Drug Administration, Federal Security Agency, Washington 25, D.C.), *Referee*

Following the advice of Harry J. Fisher, Chairman of the Committee on Revision, J. W. Sale, Chief of the Beverage Branch, Food and Drug Administration, and others who are actively engaged in water analysis, the Referee feels justified in recommending deletion of sections dealing primarily with water sanitation, irrigating waters, and industrial waters. These subjects are treated more completely in "Standard Methods for Examination of Water and Sewage" published by the American Public Health Association and their retention in this chapter without broad revision and additions would serve no useful purpose. With these sections deleted it is recommended that the chapter be entitled "Mineral Waters and Salt."

Methods of analysis for metals in water should be studied with a view to adopting more modern colorimetric methods.

The Referee recommends adoption of a quantitative procedure for determination of boron in water, to replace the present Gooch Method. It is believed this is a great improvement over the present method and it has been adopted as a standard method by the American Public Health Association, and by the laboratories of Quality of Water Branch, U. S. Geological Survey.

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[3] *Ibid.*, 14, 459 (1931).
[4] *Ibid.*, 15, 645 (1932), and 18, 198 (1935).
[5] *Ibid.*, 16, 277 (1933).

Details of the method will be published in the 7th Edition, *Methods of Analysis*, 1950.

There is no report on Fluorine in Salt at this time.

RECOMMENDATIONS*

It is recommended—

(1) That title of Chapter 37 be changed from "Water, Brine, and Salt" to "Mineral Waters and Salt."

(2) That section captions "Potable Water," "Industrial Water," "Irrigating Water," and "Brine" be deleted.

(3) That the following methods be deleted:

Turbidity, **37.1, 37.2.**
Color, **37.3, 37.4.**
Odor, **37.5.**
Suspended matter, **37.9.**
Nitrogen in form of free and albuminoid ammonia, **37.10** (except that reagents (a) and (c) be placed under **37.18**)—**37.11, 37.12, 37.13,** and **37.48.**
Nitrogen in form of nitrite, **37.14, 37.15,** and **37.49.**
Oxygen required, **37.27–37.30,** incl.
Dissolved oxygen, **37.31–37.34,** incl.
Lead, **37.35–37.40,** incl.
Copper, **37.41–37.42.**
Zinc, **37.43–37.44.**
Free carbon dioxide, **37.55.**
Industrial water, **37.84–37.97,** incl.
Irrigating water, **37.98–37.100,** incl.

(4) That method for total solids **37.6,** solids in solution **37.7,** and ignited residue **37.8** be transferred to the section on "Mineral Waters" after "Specific gravity—Official," in place of **37.46** and **37.47;** and that present methods **37.46** and **37.47** be dropped.

(5) That methods for nitrogen in form of nitrate **37.16** thru **37.19** and chloride **37.20** and **37.21** be transferred to the section on "Mineral Water" in place of **37.50** and **37.51,** and that present methods **37.50** and **37.51** be dropped.

(6) That colorimetric methods using dithizone for the determination of lead, copper, and zinc in Mineral Waters be studied.

(7) That methods for fluorine **37.22** thru **37.26** be transferred to the section on "Mineral Water" in place of **37.52** and that present method **37.52** be dropped.

(8) That the following be deleted from method **37.19:** "Nesslerize, and compare with standards as in determinations of free NH_3, **37.11.** Subtract quantity of N found in blank from that found in sample. Calculate to mg/liter of N." and substitute therefor the following from method **37.11:** "at rate of ca 1 tubeful in 10 min. into 50 ml. Nessler tubes until

* For report of Subcommittee D and action of the Association, see *This Journal*, 33, 69 (1950).

NH_3 ceases to be given off (4 to 5 tubes are usually sufficient). Add 2 ml of Nessler reagent to each tube and let stand 10 min. From a small buret measure definite quantity of the NH_4Cl soln into Nessler tubes. Dilute to 50 ml with NH_3-free H_2O, add 2 ml of the Nessler reagent, and compare depth of color with Nesslerized distillate. Report as mg/liter of N."

(9) That method **37.59** line 3 be changed from "add 5% $(NH_4)SCN$ soln" to "add 3 ml. 5% $(NH_4)SCN$ soln."

(10) That method **37.60,** line 1, be changed from "with fused $KHSO_4$" to "with about 1 gram fused $KHSO_4$."

(11) That the following methods be adopted as official:

Strontium, **37.63.**
Bromide in presence of chloride and iodide, **37.105–37.107,** incl.

(12) That in Method **37.71,** "Phosphoric Acid" line 5, "freshly prepared" be inserted between words "add" and "molybdate soln."

(13) That method **37.81(a)** Boric Acid, *qualitative procedure* be retained, deleting only at the end, "**32.17.**"

(14) That the present reference to the quantitative method for boric acid under method **37.81** be deleted and the method recommended by the Referee in this year's report be adopted as first action.

(15) That in title of method **37.83** "Equivalent Combining Weights and their Reciprocals Based on International Atomic Weights, 1943," date be changed to "1948."

(16) That the following methods be designated as procedures:

Boric acid, qualitative test, **37.81(a),** as revised.
Reporting of results for water, **37.82.**
Preparation of sample (salt), **37.108.**
Preparation of solution for sulfate, **37.112.**
Reporting results, **37.119.**

(17) That the following methods be adopted as first action:

Iodide and bromide, **37.77–37.78.**
Bromide in presence of chloride but not iodide, **37.101–37.104,** incl.
Salt, **37.109–37.111,** incl.
Sulfate, **37.113.**
Calcium, **37.114.**
Magnesium, **37.115.**

REPORT ON COSMETICS

By G. ROBERT CLARK (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Referee*

RECOMMENDATIONS*

Deodorants.

The Referee concurs in the recommendation for the adoption of the proposed method for zinc in deodorants, first action. The Referee further recommends that the methods for total zinc and aluminum, **4.1, 4.2,** and **4.3(a)** and **(b),** be adopted, first action.

The Referee further recommends that the following paragraph be inserted and adopted, first action:

Total aluminum.—Multiply the weight of the zinc present (reference to zinc method) by 5.408 to obtain equivalent weight of 8-hydroxy-quinoline precipitate. Multiply by appropriate factor for aliquot taken and subtract from weight of the precipitate obtained as directed in **4.3(a)** or **(b).** The difference multiplied by 0.05872 is the weight of aluminum.

The Referee concurs in the recommendation for continuance of this topic.

Cosmetic Creams.

The Referee concurs in the recommendation that the proposed methods for determining type of emulsion, water, ash, and chloroform-soluble matter be adopted, first action, and that the topic be continued.

Cosmetic Powders.

The Referee concurs in the recommendation that the proposed methods for the determination of boric acid and zinc be adopted, first action. The Referee recommends that the topic be reassigned and continued.

The Referee concurs in the recommendations of the Associate Referees for continuance of the following topics:

Cosmetic Skin Lotions
Mascaras, Eyebrow Pencils, and Eyeshadows
Sun Tan Preparations
Hair Dyes and Rinses

The Referee recommends reassignment and continuance of the topic "Depilatories."

The Referee recommends discontinuance of the topic "Moisture in Cosmetics."

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* For report of Subcommittee B and action of the Association, see *This Journal,* **33,** 46 (1950).

REPORT ON MASCARAS, EYEBROW PENCILS, AND EYE SHADOWS

By PAUL W. JEWEL (Max Factor & Co., 1666 North Highland Avenue, Hollywood, California), *Associate Referee*

The method for the analysis of Mascara which was submitted to collaborative study in 1948 has been reinvestigated with a view to improving its relative precision, and to correct the deficiencies which were made apparent by this study.

A new technique for extracting the base from the pigments has been devised which is applicable to all three of the types of cosmetics in this section, and which has the advantage of being much less time consuming. The method is as follows:

Weigh a sample, ca 3.0000 grams, place in a tared centrifuge tube, add 20 ml of benzene, and digest in a water bath at the boiling point of the solvent for 30 min. Add 15 ml of benzene, heat just to boiling, and centrifuge for 10 min or until the supernatant liquid is free from pigment. Decant the clear liquid and repeat the extraction. Be sure the pigment is completely dispersed in the solvent each time, rotating the tube gently if necessary. Repeat the extraction until no further base can be extracted.

Dry the pigments in the oven at 100°C. for one hour, cool, weigh, and report as total pigments.

Combine the extracts, evaporate in a tared dish, dry in the oven for one hour at 100°C., cool, weigh, and report as total base.

The major deficiencies which were shown by up the collaborative study referred to have been given considerable attention, but up to date no changes of any import are ready for publication. This method will require a considerable amount of additional study before it reaches a degree of precision which would warrant its inclusion in the official methods.

REPORT ON COSMETIC POWDERS

By HELEN C. BARRY (U. S. Food and Drug Administration, F.S.A., New Orleans, La.), *Associate Referee*

THE ANALYSIS OF TALCUM POWDERS

The analysis of talcum powder is based essentially on the methods for face powders published as official, first action, in *This Journal*, **32**, 76 (1949). With the exception of boric acid, talcums contain the same ingredients as face powder, although usually fewer. A formula may call for talc and perfume; or it may combine talc, boric acid, calcium or magnesium carbonate, zinc stearate, and perfume. The face powder method easily adaptable to the other constituents, makes no provision for boric acid which, unless separated, will interfere with the zinc determination.

The method as devised separates boric acid from other talcum powder ingredients and applies the face powder method to the remainder. Boric acid is removed by a simple water extraction and titrated; zinc is determined in the ashed residue. Acid-soluble and acid-insoluble constituents are estimated in a separate sample of smaller size from which boric acid and zinc have been removed by leaching with Wulfing's precipitant. The residue from the quantitative separation of boric acid and zinc is not used because experiments have shown that the hot water extraction interferes with calcium and magnesium determinations. In the absence of starch (the usual case) boric acid is extracted with hot water. When starch is present, a cold water extraction with prolonged shaking must be used.

METHODS

Boric Acid

(Starch absent)

Weigh accurately ca 4 g of powder and transfer to a 500-ml glass-stoppered Erlenmeyer flask. Add 50 ml H_2O, stopper flask, and shake vigorously. With stopper out, heat just to boiling, and when cool enough to handle, stopper flask, shake, and filter thru 12.5 cm medium quantitative paper. Transfer residue quantitatively to paper by shaking with small portions of water. Wash residue with water and reserve for determination of Zn. Acidify filtrate to methyl orange with 0.5 *N* H_2SO_4, adding ca 1 ml in excess. Continue the analysis as directed under section **32.18** (*Methods of Analysis, A.O.A.C.*, 1945, p. 530) beginning, "Boil ca 1 min . . . ." 0.1 *N* NaOH may be used in place of the 0.2 *N* specified.

(Starch present)

Extract sample with 50 ml of cold water by shaking flask vigorously 100 times at intervals of five minutes for half an hour. Filter and continue the analysis as directed above, beginning, "Transfer residue quantitatively . . . ."

Total Zinc

Transfer residue from boric acid determination to a platinum dish and continue the analysis as directed under Total Zinc, *This Journal*, **32**, 77 (1949) beginning, "Ignite to a light gray ash . . . ."

Acid-soluble and Acid-insoluble Components

Weigh accurately ca 2 g of powder into a platinum dish, ash, and treat with Wulfing's precipitant according to method for Total Zinc. Discard filtrate and analyze residue for acid-soluble and acid-insoluble constituents by face powder method, *This Journal*, **32**, 77 (1949).

COLLABORATIVE

Duplicate samples of a typical talcum powder containing talc, boric acid, zinc stearate, calcium (as an impurity in the talc), and magnesium carbonate, were sent to collaborators to be analyzed for boric acid and zinc.

TABLE 1.—*Analytical results*

COLLABORATORS	BORIC ACID 3.00% PRESENT		TOTAL ZINC AS ZINC OXIDE 1.40% PRESENT	
	A	B	A	B
George McClellan New Orleans District	3.01	3.03	1.40	1.40
Marion McGuire Louisiana State Dept. of Health	2.99	2.98	1.36	1.32
George E. Keppel Minneapolis District	3.06 3.09	3.07 3.09	1.33 1.34	1.33 1.35
Henry Kramer Cosmetic Division	3.09 3.10	3.03 2.99	1.36 1.37	1.39 1.40
Helen C. Barry New Orleans District	3.00	3.02	1.38	1.36

Mr. McClellan continued the separation and reported these results:

Constituent	*% present*	*% found*	
		A	*B*
Acid-Soluble CaO	0.26	0.26	0.26
Acid-Soluble MgO	2.99	3.13	3.05

To determine recoveries of boric acid from talcum powder containing starch, 0.4 g of corn starch, USP, was added to 4.000 g of the test talcum. Boric acid was extracted by shaking at 5-min. intervals for approximately a half-hour, with 50 ml. of cold water

Boric acid—3.00% present

Sub	*% found*
1	2.99
2	3.02
3	3.00
4	3.03

CONCLUSIONS

Using the methods described, five analysts were able to recover 99.3–103.3% boric acid and 94.3–100.0% zinc as zinc oxide from a typical talcum powder. Laboratory experiments indicate that the face powder methods for acid-soluble calcium and magnesium are applicable to talcum powders after boric acid and zinc are removed and that boric acid can be quantitatively separated in the presence of starch by cold water extraction.

RECOMMENDATIONS*

It is recommended—

(1) That the above methods be made first action.

(2) That the subject of cosmetic powders be continued.

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 46 (1950).

ACKNOWLEDGMENT

Acknowledgement is gratefully extended to George McClellan, the former Associate Referee, who suggested the topic and participated in all phases of the work, and to all collaborators whose names are listed in the report.

REPORT ON COSMETIC SKIN LOTIONS

By HENRY R. BOND (U. S. Food and Drug Administration, Federal Security Agency, Kansas City, Mo.), *Associate Referee*

DETERMINATION OF GLYCEROL, ETHYLENE GLYCOL, AND PROPYLENE GLYCOL IN A CLEAB TYPE SKIN LOTIOO

This year's collaborative study was undertaken for the purpose of clarifying the conditions resulting from the information obtained in 1948. Last year's collaborative results were inconclusive, inasmuch as two of the four reports submitted listed excellent recoveries, while the other two were indecisive.

Accordingly, two samples, exactly alike in composition, were sent to each of eight collaborators for the purpose of determining the suitability of the method.

METHOD

REAGENTS

Potassium periodate.—0.02 *M*. Dissolve 4.6 g. of KIO_4 in ca 500 ml of hot water. Dilute to ca 900 ml with water, cool to room temp., and make to 1000 ml. (Filter thru sintered glass or asbestos if any filter fibers are present in the KIO_4.)

Potassium arsenite.—0.02 *N*. Dilute 100 ml of U.S.P. 0.1 *N* $KAsO_2$ to 500 ml with water.

Iodine.—0.02 *N*. Dilute 100 ml of U.S.P. 0.1 *N* iodine to 500 ml with water.

Sodium hydroxide.—0.02 *N*. Dilute 100 ml of U.S.P. 0.1 *N* NaOH to 500 ml with water.

Sodium bisulfite.—5%. Dissolve 5 g of reagent $NaHSO_3$ in 100 ml of water.

Borax-sodium carbonate mixture.—Dissolve 4 g of $Na_2B_4O_7 \cdot 10\ H_2O$ and 5 g of Na_2CO_3 in 100 ml of water.

Aminoacetic acid.—U.S.P. grade.

Starch indicator.—Mix 0.5 g of soluble starch with 10 ml of cold water and add 90 ml of boiling water. Heat to boiling for ca 5 minutes. Cool before use.

Sodium bicarbonate.—Saturated soln.

OXIDATION WITH PERIODATE

Make a 20-ml sample neutral to methyl red, carrying color change to a definite yellow.

Transfer the neutralized sample to a glass-stoppered 100-ml volumetric flask, and add 50 ml of 0.02 *M* KIO_4. Make to 100 ml with water, mix well, and allow to stand ca 1 hour.

Use aliquots of this oxidized mixture in each of the following determinations.

DETERMINATION

(1) *Formic acid.*—Transfer a 20 ml aliquot of the oxidized mixture to a titration flask. Add a drop of methyl red indicator soln and titrate with the NaOH to a clear yellow. Reserve the soln for determination of excess periodate.

Apply appropriate corrections for any acidity in the 0.02 *M* KIO_4.

1 ml of 0.02 *N* NaOH = 0.92 mg of formic acid
= 1.84 mg of glycerol.

(2) *Excess periodate.*—After the titration with NaOH dilute the soln to ca 50 ml with water. Add ca 0.5 g of $NaHCO_3$, 0.2 g of KI, and 5 ml of the starch indicator. Titrate immediately with the $KAsO_2$ to the disappearance of the blue color. Allow ca 5 minutes for the reaction to reach equilibrium and to determine the final end point.

Standardize 10 ml of the KIO_4 by the same titration procedure used with $KAsO_2$. 10 ml of 0.02 *M* KIO_4 = 20 ml of 0.02 *N* $KAsO_2$.

The difference between the two titrations is a measure of the amount of periodate reduced.

1 ml of 0.02 *N* $KAsO_2$ = 2.3 mg of KIO_4
= 0.46 mg of glycerol
= 0.62 mg of ethylene glycol
= 0.76 mg of propylene glycol.

(3) *Total aldehyde (formaldehyde and acetaldehyde).*—Pipet a 20-ml aliquot of the oxidized mixture into ca 5 ml of the $NaHSO_3$ soln. Let stand for 30 min. at room temp. Dilute with water to ca 50 ml. Add 5 ml of starch indicator, and sufficient strong (ca 0.5 *N*) iodine soln to destroy excess sulfite. Discharge the blue color with a drop of the $NaHSO_3$ soln and make a careful adjustment to the starch end point with the iodine soln.

Add 10 ml of the borax-Na_2CO_3 mixture and titrate with the iodine soln to an end point that is stable for 1 minute or longer. The latter titration is a measure of the sulfite bound as aldehyde complexes, *i.e.*, a measure of total aldehydes present.

1 ml of 0.02 *N* iodine = 0.30 mg of formaldehyde.
(from total aldehyde)
= 0.44 mg of acetaldehyde
= 0.31 mg of ethylene glycol
= 0.38 mg of propylene glycol
= 0.46 mg of glycerol.

(4) *Acetaldehyde.*—Assemble a gas absorption train, consisting of four 6 × 1-inch test tubes fitted with 2-hole rubber stoppers, each stopper carrying one short piece of glass tubing and one long enough to reach to the bottom of the test tube. Connect with rubber tubing so that CO_2 gas under pressure will bubble thru any liquid in the first tube and then thru the liquids in the remaining tubes.

Transfer a 20 ml aliquot of the oxidized mixture of the sample to Tube 1 and add ca 2 g of $NaHCO_3$. In Tube 2 place ca 0.2 g of aminoacetic acid, 1 g of $NaHCO_3$, and 10 ml of water. Place ca 1 ml of the $NaHSO_3$ soln and 15 ml of water in each of Tubes 3 and 4.

Attach a CO_2 supply to Tube 1 and force the gas thru the train at the rate of ca 1½ liters per min. Continue the aspiration for ca 1 hour, then transfer the contents of Tubes 3 and 4 to a titration flask. The volume, including wash water, should be ca 50 ml.

Add starch indicator, then the approximate 0.1 *N* iodine until a blue color per-

TABLE 1.—*Collaborative results*

All results shown as milligrams of constituent per 20 ml of sample

SAMPLE 1	COLLABORATOR							THEORETICAL	AVE. PER CENT OF THEORETICAL
	A	B	C	D	E	F	G		
Glycerol Method 1 Using Methyl Red	18.86	17.02 17.02	18.40 18.40	17.02 17.02	18.25	17.30	19.0	18.51	96.40
Glycerol Method 2 Using Bromcresol-Purple	19.01	18.0 17.8	18.4 18.4	18.03	17.13	17.56	16.34	18.51	96.27
Propylene Glycol	15.58	14.75 15.10	15.58 16.15	15.20 15.58	17.16	17.10	11.34 12.12	16.11	95.55
Ethylene Glycol Using Calculation (1)	13.33	15.56	14.73 14.26	17.67 17.36	13.43	14.23	17.76 19.44	13.73	111.49
Ethylene Glycol Using Calculation (2)	13.38	15.10	15.35 13.80	15.35 15.19	15.25	11.58	14.97 16.29	13.73	104.60
SAMPLE 2									
Glycerol Method 1 Using Methyl Red	18.86	18.40 18.40	18.40 18.40	17.48 17.48	18.77	17.30	16.19	18.51	96.78
Glycerol Method 2 Using Bromcresol-Purple	18.86	17.7 17.7	18.58 18.51	18.40 18.03	17.17	17.56	15.97 15.97	18.51	95.72
Propylene Glycol	15.50	14.75 14.35	16.15 15.58	15.96 15.96	16.53	17.00	10.6 13.26	16.11	95.18
Ethylene Glycol Using Calculation (1)	13.52	14.15	14.11 14.57	16.43 16.43	12.90	14.17	21.92 21.30	13.73	111.83
Ethylene Glycol Using Calculation (2)	13.53	14.45	14.42 14.96	14.73 14.57	14.17	11.22	18.61 16.58	13.73	104.36

sists, shaking the flask continually and avoiding the addition of a large excess at any one time. Discharge the blue color with a drop of 5% $NaHSO_3$. After 5 min., add 0.02 *N* iodine dropwise to the blue starch-iodine end point. Add 10 ml saturated $NaHCO_3$ soln and again titrate with 0.02 *N* iodine to the blue color. Before taking the final end point add 10 ml of the borax-carbonate buffer soln. Record the total volume of 0.02 *N* iodine used after removal of excess $NaHSO_3$ by the first addition of 0.02 *N* iodine, and calculate the per cent by weight of 1, 2 propylene glycol.

1 ml. of 0.02 *N* iodine = 0.44 mg of acetaldehyde
(from acetaldehyde) = 0.76 mg of propylene glycol.

CALCULATIONS

The determination of acetaldehyde is used as the basis for the estimation of propylene glycol. In the presence of glycerol, ethylene glycol, and propylene glycol, the glycerol is calculated from the formic acid titration and propylene glycol from the acetaldehyde titration. Ethylene glycol may then be calculated in two ways:

(1) Subtract 4 times the formic acid titer and the acetaldehyde titer from the arsenite titer. The difference multiplied by 0.62 gives mg of ethylene glycol, or (2) subtract 4 times the formic acid titer and 2 times the acetaldehyde titer from the total aldehyde titer. The difference multiplied by 0.31 gives mg of ethylene glycol.

SUPPLEMENTARY DETERMINATION OF GLYCEROL

REAGENTS

Bromcresol purple indicator soln.—Dissolve 0.1 g in 100 ml of alcohol.

Potassium periodate.—0.02 *M*. Dissolve 4.6 grams KIO_4 in about 500 ml hot water. Dilute to about 900 ml with water, cool to room temp., and make to 1000 ml.

Sodium hydroxide.—0.02 *N*. Dilute 1 volume of 0.1 *N* NaOH to 5 volumes with water.

Propylene glycol.—Dilute 0.5 ml of commercial product to 25 ml with H_2O. Add 25 ml of 0.02 *M* KIO_4 and allow to stand for 10 min. Titrate with 0.02 *N* NaOH using 3 drops of the indicator soln. If not more than 0.05 ml of the base is consumed, the product is suitable for use.

PROCEDURE

Transfer a 20 ml sample to a 100 ml volumetric flask. Add 1 drop of bromcresol purple indicator soln and neutralize. Make the final adjustment to a light purple color with 0.02 *N* NaOH. Add 50 ml of 0.02 *M* KIO_4, dilute to volume with water, mix and allow to stand for one hour.[1] Pipet a 50 ml aliquot into a flask, add 10 drops of propylene glycol (ca 0.5 ml), mix well, wash down sides of flask with water, and allow to stand for 10 min. Add 3 drops of indicator and titrate with 0.02 *N* NaOH to a light purple end point. The color change is from yellow, thru neutral gray, to a light purple color.

1 ml 0.02 *N* NaOH = 1.84 mg glycerol.

COLLABORATORS' COMMENTS

E. M. Hoshall: "Sample No. 2 contained appreciable stringy suspended matter;

[1] Excess periodate must be present. Test for periodate by adding $NaHCO_3$ and KI to test portions; if excess is present, iodine will be liberated.

was assayed 'as is.' Instructions somewhat confusing but adequate. Most unsatisfactory part of method is that for acetaldehyde . . . ."

M. L. Dow: "Instructions for calculating periodate used in 'excess periodate' appear to be confusing . . . . I experienced no trouble with the methods used."

George McClellan: "No difficulties were encountered. . . . Greatest defect is the short initial formic acid titration . . . . Any slight misinterpretation of end point in the formic acid titration under I(1) will enormously affect the ethylene glycol results . . . ."

DISCUSSION

The methods employed for this year's collaborative work were virtually identical with those used last year. The results in general paralleled those of last year, with about 50 per cent of the collaborators reporting excellent recoveries (within 5% of the theoretical) and with the remaining reports at larger variance.

The majority of the collaborators, while not selected by the Associate Referee, are personally known by him to be chemists of excellent reputation, yet the results submitted by some were at considerable variance with the theoretical. On the other hand, results submitted by some of those collaborators personally unknown to the Associate Referee were in close agreement with the theoretical.

While the Associate Referee is convinced of the fundamental value of the method, it is obvious from the results reported that a revision is necessary to preclude the possibility of error. As stated by McClellan, an error in the small titration volume of the formic acid determination will drastically affect the calculation of ethylene glycol, as will also a similar error in the acetaldehyde determination, described by Hoshall as the most unsatisfactory portion of the assay method. The Associate Referee concurs in this belief, which is substantiated by several high results reported for ethylene glycol (chiefly through erroneous results obtained in the acetaldehyde titration). In general, the titrations for formic acid (glycerol) were fairly close to the theoretical.

CONCLUSION AND RECOMMENDATION*

It is concluded that a revision of the methods for glycerol and propylene glycol is necessary. Therefore, it is recommended that the topic be subjected to further study.

ACKNOWLEDGMENT

The Associate Referee wishes to express his appreciation of the efforts of J. L. Thomson and his assistant, Food and Drug Divisions, Department of National Health and Welfare, Ottawa, Ontario, Canada, and of the following members of the U. S. Food and Drug Administration: A. G. Buell, San Francisco; M. L. Dow, St. Louis; George McClellan, New Orleans; W. J. McCarthy, Cincinnati; and E. M. Hoshall, Baltimore.

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 46 (1950).

REPORT ON COSMETIC CREAMS

By CHARLES F. BRUENING* (U.S. Food and Drug Administration, Baltimore, Md.), *Associate Referee*

ANALYSIS OF VANISHING CREAMS

This year a collaborative study was made of four methods used in the analysis of vanishing creams. The four methods studied were: Type of Emulsion, Water, Ash, and Chloroform Soluble Material. The methods studied are important because the results obtained using them must be known in order to ascertain the composition of a vanishing cream. In previous reports, (1, 2), methods for determining glycerol in vanishing creams were studied and supplement the four methods presented in this report, forming a group of methods for the analysis of vanishing creams.

The four methods chosen for collaborative study have been previously tested by the Associate Referee on a vanishing cream of known composition (3). In that study all the methods proved to be satisfactory.

The collaborative sample prepared for the purpose of testing the proposed methods was a typical vanishing cream of approximately the same composition as that previously used by the Associate Referee and as also used in the collaborative study of the glycerol methods (1). The composition of the cream is given below:

Composition of Vanishing Cream

	per cent
Stearic acid	20.30
Water	68.31
Glycerol	10.15
Potassium Hydroxide	1.14
Propyl *p*-hydroxy benzoate	0.10
Ash, Potassium carbonate, calculated	1.41
Chloroform soluble material (stearic acid and propyl *p*-hydroxy benzoate)	20.40

The following methods were used by the collaborators:

ANALYSIS OF VANISHING CREAM

Type of emulsion.—Dust small amounts of a finely ground oil-soluble and a water-soluble dye on separate portions of the cream. If the color of the oil-soluble dye spreads rapidly a water-in-oil emulsion is indicated, while if the color of the water-soluble dye spreads, an oil-in-water emulsion is indicated.

Water.—Transfer 5–20 g of the sample to an Erlenmeyer flask, add 50 ml toluene, a few glass beads, ca 2 g of lump rosin, and connect to a Dean and Stark distilling tube receiver (*see Methods of Analysis, A.O.A.C.*, 6th Ed., 6.100).

Distill until no more water collects in the receiver. Allow contents of the tube to cool to room temperature, read the volume of water under the toluene, and from this volume calculate the percentage of water.

Ash.—Place 2–10 g of sample in a flat bottom platinum dish and remove the water and volatile material by placing dish on a steam bath or in a 100°C. oven.

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Ignite the sample at low temp. and finally at 600°C. until constant weight is obtained.

Chloroform-soluble material.—Place 2–10 g of sample in a separatory funnel, add 25–50 ml of water, acidify slightly with dilute H_2SO_4 (10 g/100 ml), and extract with successive portions of chloroform. Usually 4–5 portions of chloroform, each ca 35 ml, are sufficient to remove all chloroform soluble material. Wash the combined chloroform extract with 10 ml of water. Filter the combined extract thru a cotton plug placed in the stem of a funnel and collect filtrate in a tared dish. Wash the water with a small amount of chloroform and add washings to combined chloroform extract. Evaporate off the chloroform on a steam bath and dry residue at 15-min. intervals at 100°C. until constant weight is obtained.

DISCUSSION OF METHODS

The test for the type of emulsion was selected because of its simplicity and general reliability. Electrical conductivity methods which are based on the insulating properties of oils, may be superior to the method used, but because special equipment is needed which may not be generally available, these methods were not proposed. All collaborators correctly identified the vanishing cream as an oil-in-water emulsion using the proposed method. The Associate Referee suggested the use of F.D.&C. Blue No. 1 as the water-soluble dye and D&C Red No. 18 as the oil-soluble dye, because these dyes give brilliant colors, although many other dyes may be just as suitable.

In the water determination a 10-g sample was suggested which would give a water volume of approximately 7 ml. It was anticipated that the collaborators may encounter some difficulties in weighing and transferring the sample for this determination, as well as the chloroform-soluble material determination, because cosmetic creams are quite viscous and difficult to handle. It was thought advisable to give collaborators a method of weighing and transferring cosmetic creams that has been found to be successful. In sampling this cream it was found convenient to draw up by gentle suction or to pack the cream, using a spatula, into a 10–12 mm. internal diameter glass tubing about 15–20 cm. in length and open at both ends. The filled tube was weighed and then held over the Erlenmeyer flask in the water determination or over the separatory funnel in the chloroform soluble determination until a cylinder of cream of the desired weight had dropped in the container. The tubing was again weighed to obtain the sample weight. In some cases, with very viscous creams, a solid glass rod slightly smaller in diameter than that of the tube and previously weighed with the filled tube, can be used as a piston to push out the desired amount of cream. One of the collaborators, E. C. Fearns, obtained samples for the chloroform-soluble material determinations by discharging a weighed amount of cream from a 2 ml. piston pycnometer, described by Tuckley (4). This instrument consists of a Luer hypodermic syringe cut off square at the zero mark. Using such a pycnometer as a weighing device enables the simultaneous determination of cream density with preparation of chloroform soluble samples.

Rosin is used in the water determination to prevent foaming. Normally all the water comes over in about ½ hour. To check for complete recovery the volume can be read at 15 minute intervals until no change in volume is noted. One collaborator, H. Kramer, suggested that after distillation is complete the condenser should be washed with a small amount of toluene to dislodge drops of water that may be clinging to the sides of the condenser.

In the ash determination a sample of about 5 g was suggested which would give an ash of approximately 70 mg.

A 2-g sample was suggested in the chloroform soluble determination. The method is essentially the same as that previously used for the isolation of glycerol in the glycerol methods (5), in which it was necessary to remove chloroform-soluble material. Thus in a vanishing cream the aqueous solutions remaining after the removal of the chloroform-soluble material could be used for the determination of glycerol.

DISCUSSION OF RESULTS

The results obtained by the various collaborators are shown in Table 1.

TABLE 1.—*Analysis of vanishing cream*

COLLABORATOR	WATER (PER CENT) (THEORY 68.31%)		ASH (PER CENT) (THEORY 1.41%)		CHLOROFORM SOLUBLE MATERIAL (PER CENT) (THEORY 20.40%)	
	Found	*Recovery*	*Found*	*Recovery*	*Found*	*Recovery*
1	68.8	100.7	1.3	92.2	20.3	99.5
2	67.24	98.4	1.37	97.2	19.36	94.9
	67.21	98.4	1.37	97.2	19.44	95.3
3	68.7	100.6	1.47	104.3	20.49	100.4
	69.8	102.2	1.52	107.8	20.42	100.1
4	70.20	102.8	1.42	100.7	19.65	96.3
	70.29	102.9	1.44	102.1	20.30	99.5
					19.92	97.6
					20.00	98.0
5	71.0	103.9	1.56	110.6	20.0	98.0
	71.0	103.9	1.57	111.3	20.1	98.5
6	66.79	97.8	1.32	93.6	19.37	94.95
	66.60	97.5	1.30	92.2	19.14	93.80
7	67.3	98.5	1.40	99.3	20.34	99.7
	67.3	98.5	1.41	100.0	20.42	100.1
Av.*	68.6	100.4	1.41	100.0	19.97	97.9

* This average was obtained from the individual collaborator's averages.

As previously stated, all collaborators correctly identified the type of emulsion.

In the water determination the recoveries varied from 95.7% to 103.9% with an average recovery of 100.4%. Considering the manner in which the water is collected in the apparatus, and the difficulty in determining accurately the small volume of water obtained, the results are considered satisfactory.

In the ash determination the recoveries varied from 92.2% to 111.3% with an average recovery of 100.0%. Three collaborators reported a high ash which might been caused by incomplete ashing. The ash actually present was calculated assuming that the potassium hydroxide used in preparing the cream was eventually converted into potassium carbonate in the ash. Considering the small amount of ash involved, and the generally limited significance of ash in most cosmetic analyses, the results are considered satisfactory.

In the chloroform-soluble material determination the recoveries varied from 93.8% to 100.4% with an average recovery of 97.9%. Five of the seven collaborators obtained average results very close to the average or the theoretical amounts. Because of this response by the majority, the results are considered satisfactory.

COLLABORATORS

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Shirley M. Walden, U. S. Food & Drug Administration, Baltimore, Md.
Henry Kramer, U. S. Food & Drug Administration, Cosmetic Division, Baltimore, Md.
E. C. Fearns, C. W. Haefele, J. Wolter, Lever Brothers Co., Cambridge, Mass.
A. T. Schramm, National Aniline Division, Allied Chemical and Dye Corp., Buffalo, N. Y.
Louis B. Dobie, Bristol Myers Co., Hillside, N. J.
C. F. Bruening, U. S. Food & Drug Administration, Baltimore, Md.

ACKNOWLEDGMENTS

The Associate Referee expresses his gratitude to the collaborators for their generous efforts.

RECOMMENDATIONS*

It is recommended that the following methods for the analysis of vanishing creams be made official, first action.

(1) Type of Emulsion, (2) Water, (3) Ash, (4) Chloroform Soluble Material.

REFERENCES

(1) *This Journal*, **30**, 507 (1947).
(2) *Ibid.*, **31**, 580 (1948).
(3) *Ibid.*, **25**, 903 (1942).
(4) *Proceedings of the Scientific Section of the Toilet Goods Association*, **10**, 1 (1948).
(5) *This Journal*, **31**, 72 (1948).

* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 46 (1950).

REPORT ON DEODORANTS AND ANTI-PERSPIRANTS

By HENRY KRAMER (Cosmetic Division, Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

This study concerns the determination of zinc in deodorants by the use of the reagent 8-hydroxyquinaldine. Previously, J. H. Jones (1) ascertaining that zinc and aluminum salts are the two most common "active" ingredients in deodorants, developed an analytical method for the determination of the metallic ions which involves the precipitation of the two by 8-hydroxyquinoline. This reagent is non-selective—that is, the precipitate may consist of the zinc salt, the aluminum salt, or a combination of both salts.

Merritt and Walker (2) have published a method by which zinc can be selectively determined in the presence of aluminum and magnesium by the use of 8-hydroxyquinaldine. Zinc is separated from aluminum and magnesium ions by precipitation with this reagent in an acetic acid-acetate solution containing ammonium tartrate. The zinc can be determined gravimetrically by weighing the precipitate or volumetrically by bromination. The gravimetric method was chosen because it seemed to require less manipulation. The Associate Referee was able to obtain satisfactory recoveries of known amounts of zinc by this procedure. (See Tables 1 and 2.)

ANALYTICAL RESULTS

TABLE 1.—*Determination of zinc in aqueous solutions*

WEIGHT OF Zn TAKEN	WEIGHT OF Zn PRECIPITATE	Zn RECOVERED
mg	*grams*	*per cent*
25	0.1452	99.48
25	0.1446	99.07
	Av. 0.1449	(99.27)
50	0.2912	99.75
50	0.2905	99.51
	Av. 0.2909	(99.65)

TABLE 2.—*Determination of zinc in the presence of aluminum*

WEIGHT OF Zn TAKEN	WEIGHT OF Al TAKEN	WEIGHT OF Zn PRECIPITATE	Zn RECOVERED
mg	*mg*	*grams*	*per cent*
50	48.32	0.2926	100.23
50	48.32	0.2929	100.34
50	60.39	0.2938	100.64
50	60.39	0.2942	100.78
		Av. 0.2934	(100.51)

The procedure used was essentially that of Merritt and Walker and is as follows:

REAGENTS

8-Hydroxyquinaldine.—A technical product was purified. Six grams were recrystallized from a soln of 24 ml of alcohol and 12 ml of water. The product is a light tan powder. M.P. 71–72°C. Literature 74°C. (3).

Ammonium Acetate Soln. 2 N.—This was prepared by dissolving 154 grams of C.P. ammonium acetate in water and diluting to 1 liter. It may be necessary to filter the soln thru a glass wool pledget.

Ammonium Hydroxide (1+4).

8-Hydroxyquinaldine soln.—Weigh out 5.0 g of 8-hydroxyquinaldine and dissolve in 12 ml glacial acetic acid. Dilute to 100 ml with water. Filter if the soln is not clear. The soln is stable for about a week.

DETERMINATION

Pipet an aliquot containing 20–50 mg of zinc into a 400-ml beaker. In the presence of aluminum add 1 g of ammonium tartrate to the clear, slightly acid soln. Add 2 ml of the 8-hydroxyquinaldine soln for each 10 mg of zinc present. Dilute the soln to 200 ml and heat to 60–80°C. Neutralize the excess acid by adding dilute ammonium hydroxide (1+4) until the zinc complex salt which forms on the addition of each drop just redissolves on stirring. Add 45 ml of 2 *N* ammonium acetate slowly with stirring, and allow the mixture to come to room temp. Determine the *p*H of the soln; if the *p*H is not between 5.7–5.9 make the necessary adjustment with dilute ammonium hydroxide (1+4). If necessary to adjust the *p*H, allow the soln to stand 10–20 min. longer to achieve equilibrium. Decant the supernatant liquid thru a tared Gooch crucible. Wash the precipitate in the beaker twice with hot water, decanting each time thru the Gooch crucible. Finally transfer the precipitate to the Gooch crucible and again wash with hot water. Total volume of washings should exceed 200 ml. Dry the precipitate for 2 hours at 130–140°C. Cool and weigh. Repeat the heatings at 130–140°C. for 30 min. intervals until constant weight is attained.

Weight of precipitate × 0.17128 = Weight of zinc

The experimental work resolved itself into two phases:

(1) Zinc was determined in a number of aqueous solutions.

(2) A solution containing zinc and a number of other materials most likely to be found in deodorants was submitted for collaborative study.

The composition and preparation of the solutions used for analysis follow:

SOLUTION I— ZINC

Zinc (Bureau of Standards #109 Slab Zinc Special High Grade 99.99582% Zinc) ..... 20.0000 grams

The zinc was dissolved in hydrochloric acid and diluted to 2000 ml with water.

SOLUTION II—ALUMINUM

Aluminum (Bureau of Standards Sample #44c Aluminum Ingot Freezing point 660.1F) ..... 24.1548 grams

The aluminum was dissolved in hydrochloric acid and diluted to 2000 ml with water.

SOLUTION III—MASTER SOLUTION FOR COLLABORATIVE STUDY

Zinc (from solution I)	4.0000	grams
Aluminum (from solution II)	2.4155	grams
Glycerine Tech Grade	10.0	grams
Magnesium Oxide C.P.	10.0	grams
Calcium Carbonate C.P.	10.0	grams
Borax	2.0	grams

The mixture was dissolved in dilute hydrochloric acid and diluted to 2000 ml.

DETERMINATION OF ZINC IN SOLUTION SUBMITTED FOR COLLABORATIVE STUDY

TABLE 3.—*Analysis by Associate Referee*

SIZE OF ALIQUOT	WEIGHT OF Zn PRECIPITATE	Zn RECOVERED
ml	*grams*	*per cent*
10	0.1177	100.80
10	0.1178	100.88
10	0.1173	100.46
	Av. 0.1176	(100.71)
20	0.2353	100.75
20	0.2345	100.41
20	0.2357	100.93
	Av. 0.2352	(100.71)

TABLE 4.—*Analysis by collaborators*

COLLABORATOR	SIZE OF ALIQUOT	WEIGHT OF Zn PRECIPITATE	Zn RECOVERED
	ml	*grams*	*per cent*
1	20	0.2342	100.28
	20	0.2339	100.16
2	20	0.2349	100.58
	20	0.2352	100.71
3	20	0.2341	100.24
	20	0.2334	99.94
	20	0.2325	99.56
	20	0.2327	99.64
4	20	0.2338	100.11
	20	0.2328	99.68
5	20	0.2309	98.87
	20	0.2321	99.39
6	20	0.2295	98.27
	20	0.2295	98.27
		Av. 0.2328	(99.69)

The above results were submitted by the following collaborators:

C. F. Bruening, Food and Drug Administration, Baltimore, Md.

L. S. Harrow, Cosmetic Div., Food and Drug Administration, Washington, D. C.

K. S. Heine, Jr., Cosmetic Div., Food and Drug Administration, Washington, D. C.
J. H. Jones, Cosmetic Div., Food and Drug Administration, Washington, D. C.
S. R. Kraus, Bristol-Myers Company, Hillside, N. J.
S. Walden, Food & Drug Administration, Baltimore, Md.

COLLABORATORS' COMMENTS

J. H. Jones reports that on addition of hot water to one aliquot a suspension was obtained. This was filtered and washed with hot water and the determination continued on the filtrate. No other collaborator reported this phenomenon.

K. S. Heine, Jr., suggests that the use of an indicator covering the desired *p*H range would simplify the procedure.

COLLABORATORS' RESULTS

The results of the collaborators vary from 100.71% recovery to 98.27%. The average of the results obtained by the six analysts was 99.69% and the largest deviations from this value were −1.42% and +1.02%. The average deviation calculated from these results is 0.77%.

The collaborative work reported here was confined to the analysis of solutions. However, if the product is a cream or paste, the procedure can still be applied if the sample is prepared as described in *Methods of Analysis, A.O.A.C.*, Sixth Edition (1945), page 46.

RECOMMENDATIONS*

The Associate Referee recommends—

(1) That the proposed method for zinc in deodorants be adopted first action.

(2) That the study of deodorants and anti-perspirants be continued.

REFERENCES

(1) JONES, J. H., *This Journal*, **28**, 734 (1945).
(2) MERRITT, L. L., JR., and WALKER, J. K., *Ind. Eng. Chem. Anal. Ed.*, **16**, 387 (1944).
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REPORT ON HAIR DYES AND RINSES

By S. H. NEWBURGER, *Associate Referee*, and J. H. JONES (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.)

p-Phenylenediamine and 2,5-diaminotoluene, either singly or together, are frequently used in hair dyes. Analytical procedures for the determination of these dyes when they occur individually have already been published (1). The literature (2) (3) also records several efforts to solve the problem of determining each diamine when both are present; but these methods are not very satisfactory.

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 46 (1950).

In a study made to find a more suitable procedure for the analysis of mixtures of both these diamines, the spectrophotometric properties of solutions of the diamines and their diacetyl derivatives were determined. It was found that the spectra of alcoholic solutions of the diacetyl derivatives were sufficiently different to permit the spectrophotometric analysis of mixtures of the two diamines with a fair degree of accuracy. The spectrophotometric analysis has been combined with an extraction procedure for separating the diamines from the other components in hair dyes to give a reasonably accurate method for the determination of the amount of each diamine present when they occur together in hair dyes.

METHOD

APPARATUS

A spectrophotometer capable of isolating a wave band of 5 mμ, or less, in the region 230–300 mμ. (A Beckman Model DU was used in this study.)

REAGENTS

Ethyl ether, C.P.

Chloroform, C.P.

Alcohol, 95%.—The alcohol used should have a high transmission from 210 mμ to 300 mμ.

Standard solns of the diacetyl derivatives (5 mg per liter in alcohol).—Dissolve 100 mg of the purified diacetyl derivative in exactly 200 ml of alcohol. Dilute 10 ml of this soln to one liter with alcohol.

ISOLATION OF DIAMINES

Dissolve the sample in 6 ml of (1+5) HCl and transfer the soln to a separatory funnel with the aid of 15 ml of water. Extract with five 20-ml portions of ether and discard ether extracts. Transfer the remaining aqueous soln to a continuous extractor, add 10 ml of 50% NaOH and 5 ml of water containing 50 mg of Na_2SO_3. Rinse the separatory funnel with 5 ml of water and add the rinse to the continuous extractor. Extract for four hours with ether. Test for complete extraction by extracting for 15 min. with a fresh portion of ether. (The ether when filtered thru cotton should show no appreciable residue upon evaporation.) Filter the ether extract thru a cotton plug into a tared beaker containing 2 ml of acetic anhydride. Wash the extraction flask and cotton plug with several small portions of chloroform. Evaporate the volatile solvents on the steam bath and dry the residue of diacetyl compounds at 100°C. until constant weight is obtained.

SPECTROPHOTOMETRIC DETERMINATION

Dissolve the isolated diacetyl derivatives of the diamines in a measured amount of alcohol (use sufficient alcohol to give a final concentration of about 5 mg per liter).

Determine the optical density of the two standards and the unknown at 250 and 270 mμ, respectively (see discussion).

Calculate the concentration of each diacetyl derivative in the unknown solution from the equations:

$$Du^{250} = xd_p^{250} + yd_t^{250}$$
$$Du^{270} = xd_p^{270} + yd_t^{270}$$

Where x is the concentration of diacetyl *p*-phenylenediamine and y is the concentration of diacetyl-2,5-diaminotoluene; Du^{250} and Du^{270} are the optical densities

of the unknown at 250 and 270 mμ, respectively; and d_p^{250}, d_p^{270} are the optical densities, per unit concentration of diacetyl-*p*-phenylenediamine at the respective wave lengths; d_t^{250} and d_t^{270} are similar values for the diacetyl diaminotoluene.

Calculate the weights of the diacetyl derivatives from the concentration in final soln and the dilution factor. To obtain the weights of the diamines multiply by the conversion factors.

Diacetyl-*p*-phenylenediamine × 0.563 = *p*-phenylenediamine.
Diacetyl-2,5-diaminotoluene × 0.592 = 2,5-diaminotoluene.

EXPERIMENTAL

The diacetyl derivative of the *p*-phenylenediamine used as a spectrophotometric standard was prepared from a sample of the diamine (M.P. 140.5–141.5°C. N: Found, 25.82%; Theor., 25.91%) as follows:

Dissolve about 0.5 g of *p*-phenylenediamine in 35 ml of chloroform and add 2 ml of acetic anhydride. Volatilize the chloroform and excess acetic anhydride on the steam bath and dry the remaining diacetyl derivative of *p*-phenylenediamine to constant weight at 100°C. The product is a very light gray powder.

The standard diacetyl derivative of 2, 5-diaminotoluene was prepared from the dihydrochloride of the base (N: Found, 14.17%; Theor., 14.36%; Chlorine: Found, 36.43%; Theor., 36.34%) as follows:

Dissolve about 1 g of the dihydrochloride in 25 ml of water, transfer to a separatory funnel, add 2 ml of 50% NaOH containing 75 mg Na_2SO_3, and extract with five 25-ml portions of chloroform. Wash the combined chloroform extracts with three 5-ml portions of water, discard washings, and filter washed chloroform extract thru a cotton plug. Add 3 ml of acetic anhydride, evaporate the volatile solvents on the steam bath, and dry the diacetyl derivative of 2,5-diaminotoluene to constant weight at 100°C. The product is a very light tan crystalline powder; M.P. 222–222.5°C.

A number of synthetic mixtures of the two diamines were prepared and analyzed by the proposed procedure (with one exception ca 100 mg of resorcinol was also added to the mixture). The results are tabulated in Table 1.

DISCUSSION

Solutions of the free diamines are not very stable and are, therefore, not suitable for the spectrophotometric determination. Alcoholic solutions of the diacetyl derivatives, however, appear to be quite stable.

The absorption spectra of alcoholic solutions of the diacetyl derivatives *p*-phenylenediamine and *p*-toluenediamine are shown in Figure 1. It is apparent that in the region 230–250 mμ, the unit absorption for diacetyl-2, 5-diaminotoluene is slightly greater than that for diacetyl-*p*-phenylenediamine, but that in the region 270–290 the unit absorption of diacetyl-*p*-phenylenediamine is several times that of diacetyl 2, 5–diaminotoluene. Solutions of either of the compounds follow Beer's law to within ±1%.

Application of simultaneous equations to the spectrophotometric data obtained at 250 and 270 mμ from a solution of a mixture of the two

compounds should, therefore, give a reasonably accurate estimate of the amount of each compound present.

Since it is necessary to make one of the absorption measurements on the steep portion of the curve, it is essential for accurate results that the densities of the standards and unknowns be determined at as nearly the same wave length and slit width as possible. With the Beckman spectrophotometer the most convenient way of doing this is to run the standards and sample successively without changing the wave length and slit width adjustments. The two wave lengths specified are the most suitable

TABLE 1.—*Composition and analysis of diamine mixtures*

EXP. NO.	COMP. OF SAMPLE*			FOUND (SPECTROPHOTOMETRICALLY)				WEIGHT OF COMBINED DIACETYL DERIVATIVES		
	p-PHENYLENEDIAMINE	2,5-DIAMINOTOLUENE†	TOTAL DIAMINES	*p*-PHENYLENEDIAMINE	2,5-DIAMINOTOLUENE	TOTAL DIAMINES		THEOR.	FOUND	
	mg	*mg*	*mg*	*mg*	*mg*	*mg*	*per cent*	*mg*	*mg*	*per cent*
1	104.4	9.5	113.9	99	7.1	106.1	93.2	201.6	192.5	95.5
2	10.1	98.9	109.0	10	92	102	93.6	184.9	179.1	96.9
3	51.2	50.8	102.0	50	42	92	90.2	176.8	169.0	95.6
4	49.9	51.7	101.6	52	47	99	97.4	176.0	175.7	99.8
5	0.0	98.3	98.3	Nil	91	91	92.6	165.9	156.0	94.0
6	102.1	9.4	111.5	99	5.5	104.5	93.7	197.4	189.9	96.2
7	59.8	40.0	99.8	56	40	96	96.2	173.8	167.0	96.1
8	40.4	60.5	100.9	37	57	94	93.2	173.9	165.3	95.1
9	59.7	40.6	100.3	57	36	93	92.7	174.6	167.3	95.8

* Each sample except No. 4 also contained 100 mg of resorcinol. Sample No. 4 contained no resorcinol.
† The 2,5-diaminotoluene, added as the dihydrochloride, was calculated as the free base.

for the determination but other wave lengths may be used (for example, 280 instead of 270 mμ).

The data in Table 1 show that the recoveries of total diamines by the spectrophotometric method varied from 90.2 to 97.4% with an average recovery of 93.6%. The average recovery calculated from the weight of combined diacetyl derivatives was 96.1%, or 2.5% greater than that obtained spectrophotometrically.

The recoveries of the individual amines in the separate determinations varied from 83–104%, excluding those cases in which one amine was present in relatively small amounts. In such cases, as would be expected from the nature of the calculations, the relative error in the determination of the minor component is frequently quite high.

The recovery of 2,5-diaminotoluene appears to be somewhat lower than that for *p*-phenylenediamine. The reason for the lower recoveries of 2,5-diaminotoluene has not been definitely determined, but may be due to incomplete extraction or greater decomposition during the experimental manipulation.

Since many commercial diamine hair dyes contain a polyhydric phenol, resorcinol was incorporated into the mixtures analyzed to simulate commercial practice. The presence of resorcinol does not appear to affect the results.

In the analysis of commercial samples the presence of other diamines,

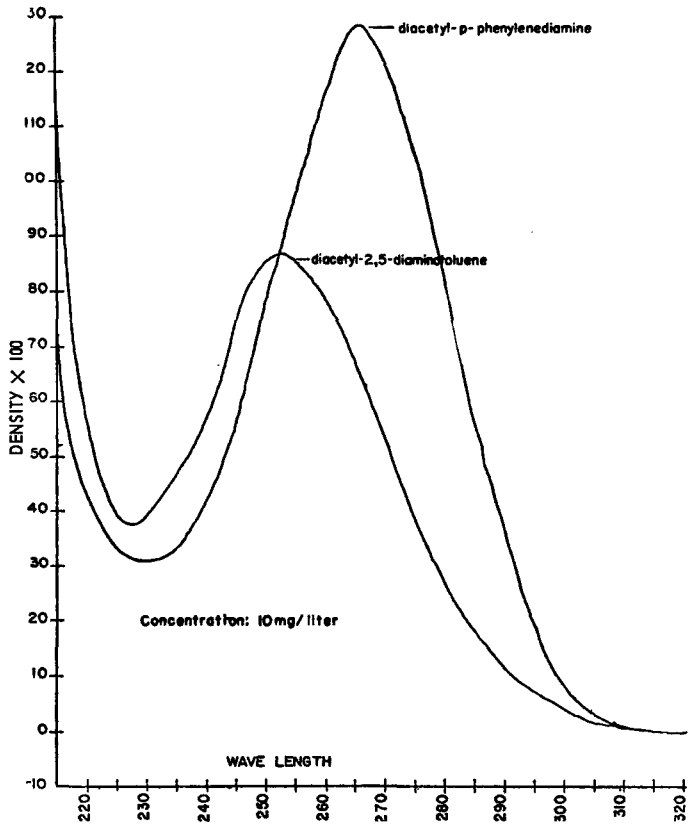

FIG. 1.—Ultraviolet absorption curves of diacetyl-*p*-phenylenediamine and diacetyl-2,5-diaminotoluene.

or other interfering materials, would be expected to cause considerable variation between the gravimetric and spectrophotometric results. As an additional check for interfering materials, the results calculated from the readings at 250 and 270 mμ may be compared with the results calculated from the readings at 250 and 280 mμ. The results should agree if substances that interfere with the spectrophotometric determination are absent.

SUMMARY AND RECOMMENDATIONS*

A spectrophotometric method of fair accuracy has been developed for the analysis of mixtures of *p*-phenylenediamine and 2, 5-diaminotoluene. Any resorcinol which may be present is first removed by an ether extraction from acid solution.

It is recommended that work on this subject be continued in an attempt to develop a method of greater accuracy.

LITERATURE REFERENCES

(1) *Methods of Analysis, A.O.A.C.*, 6th Ed. (1945), pp. 47–49.
(2) C. GRIEBEL and F. WEISS, *Z. Unters. Lebensm.*, **65**, 419 (1933), and **67**, 86 (1934).
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No reports were given for depilatories, moisture in cosmetics, or sun tan preparations.

REPORT ON COAL-TAR COLORS

By KENNETH A. FREEMAN (Food and Drug Administration, Federal Security Agency, Washington D.C.), *Referee*

Acetates, Carbonates, Halides, and Sulfates in Certified Coal-Tar Colors

The Referee concurs in the recommendation that the proposed method for sodium acetate in FD&C Blue No. 1 be adopted as first action. The Referee further recommends continuation of this topic.

Buffers and Solvents in Titanium Trichloride Titration

The Referee recommends discontinuation of this topic.

Ether Extract in Coal-Tar Colors

The Referee concurs in the recommendation that in the method for D&C Red No. 39 a Soxhlet extractor be substituted for the Dunbar extractor specified (A.O.A.C. method **21.34**). The Referee further concurs in the recommendation that the topic be continued.

Halogens in Halogenated Fluoresceins

The Referee concurs in the recommendation that the proposed method for chlorine plus bromine in coal-tar colors be adopted as official. The Referee further concurs in the recommendation that the proposed method for iodine in coal-tar colors be adopted as official. The Referee concurs with the recommendation that the topic be continued.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 46 (1950).

Identification of Coal-Tar Colors

The Referee recommends continuation of this topic.

Volatile Amine Intermediates in Coal-Tar Colors

The Referee concurs in the recommendation that the proposed method for the determination of xylidine in FD&C Red No. 32 be adopted as official. The Referee further concurs with the recommendation that the topic be continued.

Non-Volatile Unsulfonated Amine Intermediates in Coal-Tar Colors

The Referee concurs with the recommendation that the proposed method for the determination of 2-nitro-4-methoxyaniline in Ext. D&C Orange No. 1 and 2-chloro-4-nitroaniline in D&C Red No. 36 be adopted as official. The Referee further concurs in the recommendation that the topic be continued.

Sulfonated Amine Intermediates in Coal-Tar Colors

The Referee recommends continuation of this topic.

Unsulfonated Phenolic Intermediates in Coal-Tar Colors

The Referee concurs in the recommendation that the proposed method for the determination of β-naphthol in D&C Red No. 35 and D&C Orange No. 17 he adopted as first action. The Referee further recommends continuation of this topic.

Sulfonated Phenolic Intermediates in Coal-Tar Colors

The Referee recommends continuation of this topic.

Intermediates Derived from Phthalic Acid

The Referee concurs in the recommendation that the topic be continued.

Mixtures of Coal-Tar Colors for Drug and Cosmetic Use

The Referee recommends continuation of this topic.

Lakes and Pigments

The Referee recommends continuation of this topic.

Spectrophotometric Testing of Coal-Tar Colors

The Referee concurs in the recommendation that the topic be continued.

Subsidiary Dyes in D&C Colors

The Referee recommends that the proposed method for the determination of subsidiary dye in D&C Red No. 6 or 7 be adopted as first action. The Referee concurs in the recommendation that the topic be continued.

Hygroscopic Properties of Coal-Tar Colors

The Referee recommends that this topic be continued.

Subsidiary Dyes in FD&C Colors

The Referee concurs in the recommendation that the proposed method for the determination of Orange II in FD&C Orange No. 1 be adopted as official. The Referee further concurs in the recommendation that the topic be continued.

The Determination of Arsenic in Coal-Tar Colors

The Referee concurs in the recommendation that the topic be continued.

Boiling Range of Amines Derived from Coal-Tar Colors

The Referee concurs in the recommendation that the topic be continued.

The Determination of Heavy Metals in Coal-Tar Colors

The Referee concurs in the recommendation that the topic be continued.

Many of the methods contained in the section on "Commercial Coal-Tar Colors" in Chapter 21, "Coloring Matters," are no longer useful and should be deleted. On the other hand, there are numerous useful methods that have never appeared in the chapter, and these should be added. It appears that the simplest way to accomplish a revision with a minimum of confusion, is to delete this entire section of the chapter, and after appropriate revision, to propose the revised version for adoption in its entirety. Therefore, the Referee recommends deletion of the entire section on "Commercial Coal-Tar Colors," which includes methods **21.17** through **21.63,** and **21.68,** and recommends adoption of the revision, for the 7th Edition, *Methods of Analysis*, 1950.

All methods previously adopted by the Association as first action or official have been retained, as have those tentative methods for which such status is recommended. However, they may appear in a different order.

REPORT ON HALOGENS IN HALOGENATED FLUORESCEINS

By JOHN H. JONES (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Previous reports (2, 4, 5) on this topic have given the results of collaborative studies of the Clark and Jones (3) procedure for bromine and/or

chlorine in halogenated fluoresceins. As a result of these studies, this procedure is now the official A.O.A.C. (1) procedure for bromine in halogenated fluoresceins.

In this method of analysis, the compound is decomposed with a chromic-sulfuric acid mixture and the evolved halogen is absorbed in alkaline hydrazine solution. Bromine is then determined by the bromine-cyanide titration of Lang (8) and the chlorine plus bromine by precipitation as the silver halides.

Recently, a volumetric method has been developed for the determina-

TABLE 1.—*Collaborative results on chlorine*

COLLABORATOR	CHLORINE*	
	VOLUMETRIC	GRAVIMETRIC
	mg/10 ml	*mg/10 ml*
1	32.8	—
	32.8	—
2	33.10	32.80
	33.04	32.80
3	33.6	32.7
	33.6	32.5
4	32.81	32.85
	32.76	32.83
5	32.77	32.70
	32.71	32.60

* The solution was prepared from a sample of purified tetrachloro fluorescein: the calculated value, assuming that the compound was 100% pure, is 33.19%.

tion of bromine plus chlorine in such compounds, based on a chromic acid digestion followed by determination of the halide by Sendroy's silver iodate titration (7). Since the titration procedure is more convenient than the precipitation method, particularly when a number of samples are to be analyzed, a collaborative study of this procedure was undertaken.

A solution of a chlorinated fluorescein was submitted to the collaborators with the request that it be analyzed by both the gravimetric and volumetric procedures. Reports were received from the following collaborators:

Max Factor, Inc., Paul Jewel reporting.
National Aniline Division, Allied Chemical & Dye Corp., A. T. Schramm, reporting.
Ansbacher-Siegle Corporation, H. Holtzman, reporting.
Cosmetic Section, Canadian Department of National Health and Welfare, J. L. Thomson, reporting.

Color Certification Branch, Division of Cosmetics, Food and Drug Administration, Meyer Dolinsky, and Charles Graichen, reporting.

The results submitted by two collaborators (from the same laboratory) were quite variable. The results of the other collaborators are shown in Table 1.

The agreement between the results obtained by the gravimetric procedure is quite good; the average deviation being less than 0.5% and the maximum deviation less than 1%. The precision is somewhat better than that obtained in last year's study using essentially the same procedure,

TABLE 2.—*Collaborative results on iodine*

COLLABORATOR	IODINE*	COLLABORATOR	IODINE*
	mg/100 ml		*mg/100 ml*
1	266.2	4	265.6
	267.2		264.6
2	267.25	5	260.4
	265.65		
	266.70	6	266.05
			266.35
3	266.3		
	266.9	7	266.95

* Solution prepared from a sample of purified FD&C Red No. 3. Calculated value, based on the color acid content of the solution, 265.6 mg/100 ml.

probably because most of the analysts had taken part in the previous study. With one exception the results obtained by the volumetric procedure are in good agreement with the gravimetric results.

If the volumetric and gravimetric results are grouped together 16 of the 18 results fall within $\pm 1\%$ of the average value and 13 of the 18, within $\pm 0.5\%$. The average result obtained by either procedure is about 99% of the calculated value.

It appears, therefore, that precise results for the chlorine content of halogenated fluoresceins can be obtained by both procedures. Since the volumetric method is as precise and accurate for bromine as for chlorine (7), it should give an accurate value for the bromine plus chlorine content of the sample.

The present tentative A.O.A.C. method for iodine in halogenated fluoresceins (1) has been studied collaboratively on two occasions (4, 6). In each study, the results were generally satisfactory, but one or more analysts had difficulty with the procedure. The main source of error in the procedure appears to be in the step where the excess permanganate is reduced with nitrite. The directions for this step in the procedure was, therefore, rewritten, giving more detailed directions for carrying out the

reduction, and the method again submitted for collaborative study. Reports were received from the collaborators listed above.

All results submitted are tabulated in Table 2.

The results obtained by six of the seven collaborators are in excellent agreement with one another and the calculated value. The average value obtained by these collaborators is 100.3% of the calculated value, the lowest, 99.6% and the highest, 100.6%. The seventh analyst's result is almost 2% below the calculated value.

Despite the fact that most of the collaborators obtained very satisfactory results, several of the collaborators noted that the directions for the reduction were not very clear and that some practice was required before consistently satisfactory results were obtained.

RECOMMENDATIONS*

It is recommended.—

(1) That the proposed volumetric method for chlorine plus bromine be adopted as official.

(2) That the proposed method for iodine be adopted as official.

(3) That the topic be continued.

REFERENCES

(1) *Methods of Analysis, A.O.A.C.*, Sixth Edition (1945), p. 295, 296.
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(3) CLARK, G. R. and JONES, J. H., *Ibid.*, **26**, 433 (1943).
(4) GORDON, N., *Ibid.*, **31**, 589 (1948).
(5) ——, Report on Halogens in Halogenated Fluoresceins, *Ibid.*, **32**, 609 (1949).
(6) JONES, J. H., *Ibid.*, **26**, 313 (1943).
(7) JONES, J. H., and GORDON, N., *Ibid.*, **32**, 680 (1949).
(8) LANG, R., *Z. Anorg. Chem.*, **144**, 75 (1925).

REPORT ON VOLATILE AMINE INTERMEDIATES IN COAL-TAR COLORS—XYLIDINE

By ALICE B. CAEMMERER (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*†

This year samples of D&C Red No. 32 containing 0.2% by weight of xylidine were prepared and sent to five collaborators for analysis following the method reported previously for aniline[1] in coal-tar colors. The factor 1 ml. of 0.1 *N* $TiCl_3 = 0.003$ grams of xylidine was used in calculating results. The results in the order in which they were received, are shown in Table 1.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 46 (1950).
† Present address: U. S. Department of the Interior, Geological Survey, Washington, D. C.
[1] *This Journal*, **31**, 81 (1948), under "Changes in Methods of Analysis."

TABLE 1.—*Collaborative results*

COLLABORATOR	XYLIDINE FOUND	COLLABORATOR	XYLIDINE FOUND
	per cent		*per cent*
1	0.20	4	0.20
			0.20
2	0.18		
	0.20	5	0.17
			0.17
3	0.22		
	0.23	Average	0.20

These results are all within the expected experimental error.

The Associate Referee wishes to acknowledge with thanks the assistance of the following collaborators:

Ansbacher-Siegle Corporation, H. Holtzman, reporting.
Calco Chemical Division—American Cyanamid Company, William Seaman, reporting.
H. Kohnstamm & Company, Inc., Robert Milligan, reporting.
U. S. Food and Drug Administration, Division of Cosmetics, Charles Graichen, and Keith S. Heine, Jr., reporting.

RECOMMENDATIONS*

It is recommended—

(1) That the proposed method for the determination of xylidine in FD&C Red No. 32 be adopted as official.

(2) That the topic be continued.

REPORT ON ACETATES, CARBONATES, HALIDES, AND SULFATES IN CERTIFIED COAL-TAR PRODUCTS

By J. SCHIFFERLI and A. T. SCHRAMM (*Associate Referee*), (National Aniline Division, Allied Chemical and Dye Corporation, Buffalo 5, N. Y.)

SODIUM ACETATE IN FD&C BLUE NO. 1

In the Associate Referee's report of last year, *This Journal*, **32**, 614 (1949), a method for the determination of sodium acetate in FD&C Blue No. 1 was described. Briefly, the method involves esterification of sodium acetate to ethyl acetate in the presence of *p*-toluenesulfonic acid and silver toluenesulfonate, distillation, and saponification of the distilled ester with a measured excess of standard sodium hydroxide.

Following the recommendation the previous report that the method be subjected to collaborative study, four samples of FD&C Blue No. 1 of known sodium acetate content were sent to each collaborator for analysis. The results are recorded in Table 1.

* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 46 (1950).

TABLE 1.—*Collaborative data for sodium acetate in FD&C Blue No. 1*

COLLABORATOR	SODIUM ACETATE ADDED	SODIUM ACETATE FOUND*	DIFFERENCE
	per cent	*per cent*	*per cent*
No. 1	1.03	1.00	−0.03
	1.03	0.99	−0.04
	3.02	2.89	−0.13
	3.02	2.92	−0.10
No. 2	1.03	1.05	+0.02
	1.03	1.01	−0.02
	3.02	2.90	−0.12
	3.02	3.04	+0.02
No. 3	1.03	1.01	−0.02
	1.03	1.03	0.00
	3.02	2.99	−0.03
	3.02	2.94	−0.08
No. 4	0.07	0.08	+0.01
	0.07	0.08	+0.01
	2.94	2.82	−0.12
	2.94	2.81	−0.13
No. 5	0.07	0.08	+0.01
	0.07	0.07	0.00
	2.94	2.94	0.00

* Average deductions for reagent blanks were made.

DISCUSSION OF RESULTS

The samples submitted for collaborative study were prepared to fall within the range of sodium acetate contents usually present in FD&C Blue No. 1. Federal Coal-Tar Color Regulations impose a maximum limit of 3.0% for this impurity.

As indicated in Table 1, the maximum difference between sodium acetate added and that found by collaborators is −0.13% which corresponds to a minimum recovery of 95.6% of the amount of sodium acetate added. The average recovery based upon collaborative findings is 97.9%. This is regarded as sufficient indication of satisfactory accuracy and precision of the method as written.

ACKNOWLEDGMENTS

The Associate Referee expresses his gratitude to the following collaborators for their generous cooperation:

M. Dolinsky, U. S. Food & Drug Administration, Washington, D. C.
L. S. Harrow, U. S. Food & Drug Administration, Washington, D. C.
R. N. Sclar, U. S. Food and Drug Administration, Washington, D. C.
W. H. Kretlow, Wm. J. Stange Co., Chicago, Illinois.
W. C. Bainbridge, H. Kohnstamm & Co., Inc., Brooklyn, N. Y.

RECOMMENDATION*

It is recommended that the method for sodium acetate in FC&C Blue No. 1 be adopted as first action.

REPORT ON UNSULFONATED PHENOLIC INTERMEDIATES IN COAL-TAR COLORS

By H. HOLTZMAN (*Associate Referee*) and H. GRAHAM (Ansbacher-Siegle Corporation, Staten Island, N. Y.)

BETA-NAPHTHOL IN D&C RED NO. 35

In last year's work,[1] there was submitted for collaborative study a method involving successive aqueous extractions of the beta-naphthol from the pigment with dilute aqueous hydrochloric acid, followed by coupling with diazotized sulfanilic acid and titanous chloride titration of the dyestuff formed. Varied analytical results were obtained by different collaborators, although each collaborator duplicated his own results closely. It appeared that the rather cumbersome extractions must involve a personal equation. Furthermore, account must be taken of possible presence of other titratable substances in the extract, including residual amines.

In order to simplify the extraction procedure, continuous extraction methods were studied, employing various solvents including water. It was found necessary to take into account problems of adsorption by the pigment, as well as of co-extraction of excessive amounts of the dyestuff by the solvent employed. Petroleum ether of boiling range 35–60° C. provided the best all-round solvent among those studied. The presence of other titratable substances has been corrected for by running an uncoupled blank in each determination.

It was determined that eight hours of continuous extraction sufficed to recover amounts of beta-naphthol of the order of 1.0% on the weight of pigment. Longer extraction times are preferable for higher amounts of beta-naphthol. A series of analyses were run on samples of D&C Red No. 35, to which pure beta-naphthol was added in amounts between 0.2% through 0.6%; excellent recoveries were obtained.

The following method was evolved:

METHOD

Weigh 10 g of color into 25×80 mm seamless extraction thimble and place in a soxhlet extractor of suitable size. The extractor is then connected to a 500-ml flask containing 250 ml of petroleum ether (B.R. 35°–60°), and extracted for 8 hours. (Some dye is extracted along with the beta and may collect on the sides of the flask.)

Disconnect and add 150 ml 1:10 HCl to the extraction flask. Gently boil off the

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* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 46 (1950).

[1] Holtzman, H., and Graham, H., *This Journal*, **32**, 617 (1949).

ether on a hot plate. The insoluble dye is then filtered off by passing thru glass wool. Rinse the flask several times with small amounts of the 1:10 HCl, and make to 250 ml volume. Mix soln thoroly and divide into two equal portions. The *p*H of each portion is adjusted to neutrality with dilute NaOH (using phenolphthalein indicator). Reserve one soln for a blank.

Add 10 g Na acetate to the remaining portion. Cool to 5°C. in ice bath.

PREPARATION OF DIAZO

Make a soln of ca 0.05 *N* sulfanilic acid by dissolving 4.779 g of sulfanilic acid in 500 ml H_2O, to which has been added 5 ml of 12 *N* HCl. Make a soln of ca 0.05 $NaNO_2$ by dissolving 1.04 g $NaNO_2$ in 300 ml H_2O. Place 40 ml of the sulfanilic acid in 100-ml vol. flask. When cooled to 5°C., add 44 ml of the $NaNO_2$ soln and allow to diazotize, testing for excess nitrous acid. Destroy excess with few mg of sulfamic acid. Dilute to volume.

Add slowly 25 ml of the diazo soln, stir 5 min. and test for excess diazo (with alkaline beta-naphthol soln on spot paper). If not positive, add additional diazo until positive test is obtained. Let stand for one hour (or longer).

Heat on water bath for one-half hour to decompose diazo. Test. Add 10 g sodium bitartrate (which has been dissolved in 50 ml hot water) to each of the coupled and the uncoupled portions. Titrate the uncoupled blank with $TiCl_3$ to a direct and colorless end point.

Titrate the coupled portion until the dye reduces to yellow. Add 1–2 ml excess $TiCl_3$ and back titrate *immediately* with Me Blue, or some other suitable dye. A back titration is necessary for the coupled portion.

Subtract the ml required for the blank from the ml required for the coupled portion, and calculate as % beta.

1 ml of 0.1 *N* $TiCl_3$ = .0036 g Beta-Naphthol

After this method had been worked out, a tentative procedure for beta-naphthol in D&C Red No. 35 set up by the Cosmetic Division, Food and Drug Administration, and subsequently included in their new issue of "Methods of Analysis Applicable to Certified Coar-Tar Colors" (May, 1949), was sent to the authors by L. S. Harrow of the Division. This procedure was similar in outline to that of our laboratory, and involved a continuous extraction with isopropyl ether, followed by separation with dilute caustic soda, coupling with diazotized para-nitroaniline. This procedure was found to be rather difficult to carry out, because of (a) the large amount of dyestuff extracted by the solvent; (b) dilute caustic soda extraction carried through appreciable amounts of dyestuff, making it difficult to reach an end point for the extractions; (c) a suitable spectrophotometer is not always available.

This method was submitted to collaborative analyses, on a commercial sample of D&C Red No. 35. Results are given in Table 1.

The following collaborators cooperated in these analyses.

Calco Chemical Division, American Cyanamid Company, William Seaman reporting.

Food and Drug Administration, Cosmetic Division, N. Ettelstein and Lee S. Harrow reporting.

National Aniline Division, A. T. Schramm reporting.
Ansbacher-Siegle Corporation, Robert Messina reporting.

The results obtained by the various collaborators were fairly close, the maximum deviation being ±0.05–0.07% based on the pigment samples.

The proposed method is fairly similar to a procedure sent us by Kenneth Freeman, of the Cosmetic Division, Food and Drug Administration, in connection with our collaborative analyses for 3-nitro-para-anisidine in Ext. D&C Orange No. 1. It should be possible to combine the determination of amines as well as beta-naphthol in D&C Red No. 35 by using the procedure, slightly modified.

BETA-NAPHTHOL IN D&C ORANGE NO. 17

It was found possible to apply the same method as used for the D&C Red. No. 35, with a slight modification involving the size of sample required. Extraction times and recovery data were similar to those obtained with the D&C Red No. 35.

TABLE 1.—*Collaborative analyses*

COLLABORATOR	% BETA-NAPHTHOL IN D&C RED NO. 35	DEVIATION FROM AVERAGE	% BETA-NAPHTHOL IN D&C ORANGE NO. 17	DEVIATION FROM AVERAGE*
1	0.18	−0.03	0.28	−0.02
	0.20	−0.01	0.30	0.00
2	0.184	−0.03	0.14	—
3	0.205	−0.00	0.28	−0.02
4	0.16	−0.05	0.20	−0.10
	0.20	−0.01	0.28	−0.02
5	0.16	−0.05	0.20	−0.10
Associate	0.25	+0.04	0.35	+0.05
Referee	0.28	+0.07	0.38	+0.08
Average	0.21		0.28†	

* Omitting low value of 0.14.
† Omitting the low 0.14 value average was 0.30.

A sample of commercial D&C Orange No. 17 was then submitted for collaborative analysis, using same method as for D&C Red No. 35, except start with 8 grams of color.

COLLABORATIVE ANALYSES

The collaborative analyses are recorded in Table 1. Fairly good results were obtained, except for one low value reported by one of the collaborators. The average deviation, obtained by omitting the low value, was ±0.08–0.10% based on pigment sample.

The following comments on the method were offered by one of the collaborators:

(1) Low values may result from this method because β-naphthol may be occluded and lost in the dye precipitate when the insoluble dye is filtered off from the 1:10 HCl solution.

(2) It is recommended that the neutralization of the acid filtrate be made with 30% NaOH to keep the volume of solution low.

We find that little dye is carried through when employing the solvent specified, and do not find any serious errors due to adsorption. The volume of solution did not present a problem.

It is of interest that our results were somewhat higher than those of the collaborating analysts. This may possibly be due to a high rate of reflux. We have found that while practically all of the beta-naphthol is extracted within the 8-hour reflux period, very small amounts may be extracted by still longer reflux times. These amounts we found to be of a low order, however, and they did not appreciably affect the results.

RECOMMENDATIONS*

We recommend that the methods for D&C Red No. 35 and D&C Orange No. 17 be adopted, first action.

REPORT ON NON-VOLATILE UNSULFONATED AMINE INTERMEDIATES

By Lee S. Harrow (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.),
Associate Referee

In previous reports, methods were proposed for the separation and quantitative determination of *p*-nitroaniline and 3-nitro-4-aminotoluene in certifiable coal-tar colors in which they may be encountered (1, 2). This report deals with the separation and quantitative determination of five more intermediates of this class.

Separation and determination of 2-nitro-4-methoxyaniline in Ext. D&C Orange No. 1.—This intermediate is determined by the method described for 3-nitro-4-aminotoluene in D&C Red No. 35 and D&C Red No. 38 (2) except that the extraction time is increased to 10–12 hours. In this procedure, the intermediate is extracted from the dye with petroleum benzin and the nitroamine titrated with standard titanium trichloride solution.

1 ml 0.1 *N* $TiCl_3$ = 2.8 mg 2-nitro-4-methoxyaniline.

A sample of Ext. D&C Orange No. 1 was extracted with petroleum benzin until no more intermediate could be detected by this method. Known quantities of 2-nitro-4-methoxyaniline were added to 10-gram

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 46 (1950).

portions of the intermediate-free color and the mixtures were analyzed by the proposed method. The results are shown in Table 1.

TABLE 1.—*Recovery of 2-nitro-4-methoxyaniline from Ext. D&C Orange No. 1*

2-NITRO-4-METHOXYANILINE		RECOVERY
ADDED	RECOVERED	
grams	*grams*	*per cent*
0.00	0.00	
0.0243	0.0230	94.7
0.0197	0.0184	93.6
0.0211	0.0202	95.7
0.0422	0.0404	96.2
0.0430	0.0408	94.8
0.0388	0.0387	99.8
0.0612	0.0587	95.9
0.0590	0.0570	96.7
0.0601	0.0580	96.6
Average Recovery		96.0

Separation and determination of 2-chloro-4-nitroaniline in D&C Red No. 36.—2-Chloro-4-nitroaniline in D&C Red No. 36 is determined by the same procedure described for the 2-nitro-4-methoxyaniline in Ext. D&C Orange No. 1.

1 ml 0.1 N $TiCl_3$ = 2.9 mg of 2-chloro-4-nitroaniline.

A composite sample of D&C Red No. 36 was subjected to continuous

TABLE 2.—*Recovery of 2-chloro-4-nitroaniline in D&C Red No. 36*

2-CHLORO-4-NITROANILINE		RECOVERY
ADDED	RECOVERED	
grams	*grams*	*per cent*
0.000	0.000	
0.0204	0.0199	97.3
0.0211	0.0205	97.0
0.0207	0.0201	97.3
0.0400	0.0394	98.6
0.0397	0.0378	95.2
0.0412	0.0396	96.1
0.0630	0.0638	101.2
0.0602	0.0595	98.8
0.0611	0.0585	95.7
Average Recovery		97.5

extraction with petroleum benzin until no more intermediate could be detected by the proposed method. Known quantities of intermediate were added to 10-gram samples of dye, and the mixtures were analyzed by the proposed method. The results are shown in Table 2.

SEPARATION AND DETERMINATION OF AMINO-AZO-BENZENE IN D&C RED NO. 17

APPARATUS

Spectrophotometer suitable for measurements at 500 mμ.

REAGENTS

Standard soln (5 mg/liter) of amino-azo-benzene.—Dissolve 0.100 gram of amino-azo-benzene in 100 ml of alcohol and dilute to exactly 500 ml with 4 *N* HCl. Dilute 5 ml of this soln to exactly 200 ml with 4 *N* HCl.

Acetone, c.p.

NaOH solution, 30% (*w/v*).

HCl (1+3)(*v/v*).

HCl (1+49)(*v/v*).

Place 1 g of D&C Red No. 17 in a 500-ml wide-mouth Erlenmeyer flask, suspend the dye in 100 ml of acetone, and heat to boiling on a steam bath. Stir in slowly 100–200 g of crushed ice and allow the mixture to stand for 15 min.; most of the color will precipitate. Filter the mixture thru a large fluted paper into a 1-liter separatory funnel and wash the residue once with 50 ml of (1+49) HCl. Return the filter paper to the wide-mouth Erlenmeyer flask, add 100 ml of acetone, heat to boiling on a steam bath, and repeat the precipitation with ice and filtration. Make the combined filtrates alkaline to litmus with 30% NaOH and extract with 50-ml portions of petroleum benzin until the extracts are colorless. Wash the combined petroleum benzin extracts with 20 ml of 2% NaOH and then extract the petroleum benzin layer with 10 ml portions of 4 *N* HCl until the acid extracts are colorless. Heat the combined acid extracts for 15 min. on a steam bath, cool, and dilute to 250 ml with 4 *N* HCl. Determine the extinction of the unknown soln and the standard amino-azo-benzene soln at 500 mμ.

$$\% \text{ Amino-azo-benzene} = \frac{E_{\text{Unknown}}}{E_{\text{Standard solution}}} \times \frac{\text{Conc. standard solution}}{\text{(in mg/liter)}} \times \frac{1}{40}.$$

If the final solution is too concentrated for accurate measurements, dilute an aliquot with 4 *N* HCl and multiply the result calculated from the above formula by the dilution factor.

RESULTS

Crude amino-azo-benzene was purified by four recrystallizations from (55–45) alcohol-water solution.

Known amounts of the purified amino-azo-benzene were added to 1 gram samples of D&C Red No. 17 and the mixtures analyzed by the method given above. The results of the analyses are shown in Table 3.

SEPARATION AND DETERMINATION OF AMINO-AZO-XYLENE IN D&C RED NO. 18

The method for this intermediate is similar to that used for the determination of amino-azo-benzene in D&C Red No. 17.

APPARATUS

Spectrophotometer suitable for measurements at 335 mμ.

TABLE 3.—*Recovery of amino-azo-benzene in D&C Red No. 17*

AMINO-AZO-BENZENE		RECOVERY
ADDED	RECOVERED	
mg	*mg*	*per cent*
0.00	0.048	
2.10	2.35	89.2
2.14	2.49	93.9
2.10	2.36	89.5
4.00	4.08	90.1
4.00	4.12	91.3
4.00	4.11	90.8
5.44	5.60	94.1
5.44	5.81	95.2
5.44	5.83	95.6
Average Recovery		92.2

REAGENTS

Standard soln (5 mg/liter) of amino-azo-xylene.—Dissolve 0.100 g amino-azo-xylene in 100 ml of chloroform, stir well, and dilute to 500 ml with chloroform. Dilute 5 ml of the soln to 200 ml with chloroform.

Chloroform, U.S.P.

Other reagents as described in the method for the determination of amino-azo-benzene in D&C Red No. 17.

DETERMINATION

Proceed as directed for amino-azo-benzene in D&C Red No. 17 until the intermediate is extracted with 4 *N* HCl. Then make the combined acid extract alkaline to litmus with 30% NaOH, cool to room temp., and extract with 20 ml portions of chloroform until the chloroform extracts are colorless. Filter the combined chloro-

TABLE 4.—*Recovery of amino-azo-xylene in D&C Red No. 18*

AMINO-AZO-XYLENE		RECOVERY
ADDED	RECOVERED	
mg	*mg*	*per cent*
0.00	0.14	
2.11	2.16	96.0
2.11	2.13	94.9
2.11	2.68	92.5
4.22	4.09	94.1
4.22	4.14	95.3
4.22	4.33	99.5
6.33	6.52	101.5
6.33	6.10	94.4
6.33	5.97	92.4
Average Recovery		95.6

form extracts thru a cotton pledget into a 250 ml volumetric flask and dilute to volume with chloroform. Determine the extinction of the standard and unknown soln at 335 mμ.

$$\% \text{ Amino-azo-xylene} = \frac{E_{\text{Unknown}}}{E_{\text{Standard solution}}} \times \text{Conc. standard solution (in mg/liter)} \times \frac{1}{40}.$$

RESULTS

Crude amino-azo-xylene was purified by four recrystallizations from alcohol. Known quantities of the purified intermediate were added to 1 gram samples of D&C Red No. 18 and the mixtures analyzed by the method given above. The results of the analyses are given in Table 4.

Determination of 2,4-dinitroaniline in D&C Orange No. 17.—Evenson (3) has proposed a method for the determination of 2,4-dinitroaniline in D&C Orange No. 17 which depends upon the formation of a colored compound when 2,4-dinitroaniline is treated with sodium hydroxide solution. This reaction has been used as a qualitative test for aromatic nitro compounds. [The accuracy of a method based on a measurement of the color of the complex depends on the stability of the color complex and the sensitivity of the complex to slight variations of reagent quantities.] Further investigation of this procedure showed that satisfactory results were not obtained; the colors produced are not stable, and the color intensity varies with slight variations of the quantities of the reagents.

The proposed method is a modification of the Evenson method for the separation of the intermediate from the dye followed by spectrophotometric determination of the intermediate in acid solution.

Determination of 2, 4-dinitroaniline in D&C Orange No. 17

APPARATUS

Spectrophotometer suitable for measurements at 290–390 mμ.

REAGENTS

Alcoholic hydrochloric acid (ca. 0.1 N).—Place 9.0 ml of conc. HCl in a liter volumetric flask and dilute to volume with 95% alcohol.

Standard 2,4-dinitroaniline solution (10 mg/liter).—Dissolve 10 mg of purified 2,4-dinitroaniline in 20 ml of the alcoholic hydrochloric acid soln. Transfer to a 100 ml volumetric flask and dilute to the mark with the alcoholic hydrochloric acid soln. Dilute a 10-ml aliquot of this soln to 100 ml with the same solvent.

DETERMINATION

Mix 1.0 g of color and 10 ml of conc. H_2SO_4 in a 500-ml tall form beaker until a uniform mixture is obtained. Carefully add small quantities of alcohol, stirring after each addition until about 100 ml has been added, then add 150 ml of water and mix. Evaporate on a steam bath until about 100 ml of soln remains, using a gentle air stream to hasten the process. Transfer the contents to a 250-ml volumetric flask, cool, and dilute to the mark with water. Mix and filter thru a small filter paper. Transfer a 100-ml aliquot of the filtrate to a 500-ml separatory funnel. Add 30% NaOH (ca 20 ml) until the soln is alkaline to litmus, cool, and extract with three 50-ml portions of ethyl ether. Wash the combined ether extracts once with 20 ml

of water. Transfer the ether soln to a beaker, rinse the funnel with 10 ml of ether, and add to the main extract. Evaporate the extract to dryness on steam bath using gentle air stream to hasten the process. Dissolve the residue in 20 ml of warm alcoholic HCl and transfer the soln to a 100-ml volumetric flask. Rinse the beaker with two 20-ml portions of the alcoholic HCl and add the washings to the volumetric flask. Cool, dilute to the mark with alcoholic HCl, and determine the extinction of the standard and unknown solns at 290 mμ, 335 mμ, and 380 mμ.

$$\% \text{ 2,4-dinitroaniline} = \frac{E_{335\ m\mu} - \frac{E_{380\ m\mu} + E_{290\ m\mu}}{2}\ \text{Unknown solution}}{E_{335\ m\mu} - \frac{E_{380\ m\mu} + E_{290\ m\mu}}{2}\ \text{Standard solution}} \times \text{Conc. of standard solution (in mg/liter)} \times \frac{1}{40}.$$

Aliquots of an alcoholic solution of 2,4-dinitroaniline were added to 0.1 gram and 1.0 gram samples of D&C Orange No. 17 and the mixtures were analyzed by the proposed method. The results of the analyses are shown in Table 5.

TABLE 5.—*Recovery of 2,4-dinitroaniline from D&C Orange No. 17*

RECOVERY	WEIGHT DYE	INTERMEDIATE ADDED	FOUND	RECOVERY
	g	*mg*	*mg*	*per cent*
1	0.1	0.00	0.0827	
2	0.1	0.156	0.223	89.6
3	0.1	0.156	0.233	96.2
4	0.1	0.156	0.229	93.6
5	1.0	1.565	1.510	96.6
6	1.0	3.130	3.300	105.5
7	1.0	1.565	1.559	99.7
Average Recovery				96.9

2-Nitro-4-methoxyaniline.—A collaborative study of the method proposed for the determination of 2-nitro-4-methoxyaniline in Ext. D&C Orange No. 1 has been made. A composite sample of Ext. D&C Orange No. 1 was prepared and sent to the following collaborators for study:

H. Kohnstamm and Company, Inc., Eugene F. Wojt reporting.
National Aniline Division, Allied Chemical and Dye Corporation, A. T. Schramm reporting.
Ansbacher-Siegle Corporation, H. Holtzman reporting.
Calco Chemical Division, American Cyanamid Company, Wm. Seaman reporting.
Division of Cosmetics, Food and Drug Administration, Keith S. Heine, Jr. reporting.

The results, in the order in which they were received, are given in the following table:

COLLABORATOR	2-NITRO-4-METHOXYANILINE
	per cent
1	0.228
2	0.296
3	0.220
4	0.233
	0.204
5	0.217
	0.250
Associate Referee	0.236
	0.241
Average for collaborators	0.236
Average for Associate Referee	0.238

SUMMARY

Methods have been developed for the separation and determination of 2-nitro-4-methoxyaniline in Ext. D&C Orange No. 1, 2-chloro-4-nitroaniline in D&C Red No. 36, amino-azo-benzene in D&C Red No. 17, amino-azo-xylene in D&C Red No. 18, and 2,4-dinitroaniline in D&C Orange No. 17. Satisfactory recoveries of known amounts of intermediates were obtained.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the determination of 2-nitro-4-methoxyaniline in Ext. D&C Orange No. 1, and 2-chloro-4-nitroaniline in D&C Red No. 36 be adopted as official.

(2) That the methods for amino-azo-benezene in D&C Red No. 17, and 2,4-dinitroaniline in D&C Orange No. 17 be studied collaboratively.

(3) That the topic be continued.

REFERENCES

(1) HARROW, LEE S., "Non-volatile Unsulfonated Amine Intermediates in Coal-Tar Colors," *This Journal*, **31**, 594 (1948)

(2) HARROW, LEE S., *Ibid.*, **32**, 624 (1949).

(3) EVENSON, O. L., "Separation and Determination of 2,4-Dinitroaniline in D&C Orange No. 17," *Ibid.*, **27**, 131 (1944).

* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 46 (1950).

REPORT ON ETHER EXTRACT IN COAL-TAR COLORS

By S. S. FORREST (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

Since the last report[1] the use of isopropyl ether instead of ethyl ether has been investigated. Ethyl ether is unsatisfactory because of the greater solubility of water in this solvent, with the consequent possibility of contamination of the ether extract with water-soluble substances. This investigation indicated that results are more uniform and reproducible when isopropyl ether is used instead of ethyl ether. It was, therefore, decided to study the use of isopropyl ether collaboratively.

Samples of each of the following colors were prepared and submitted to the collaborators for analysis: FD&C Orange No. 1, FD&C Yellow No. 5, D&C Green No. 5, and D&C Orange No. 5. The collaborators were asked to analyze the D&C Orange No. 5 by the method given in the last report[1] and the others by the method given in *Methods of Analysis A.O.A.C.*, 6th Ed. (1945), Sec. **21.31** and **21.32,** substituting isopropyl ether for ethyl ether. In the case of D&C Green No. 5, a five-gram sample was specified instead of a ten-gram sample. It was further specified that each liter of isopropyl ether was to be washed before use with two 100 ml portions of 0.5 *N* NaOH, and then by three 150 ml portions of distilled water.

The Associate Referee wishes to acknowledge with thanks the cooperation of the following collaborators:

Ansbacher-Siegle Corporation, H. Holtzman, reporting.
Bates Chemical Company, Inc., C. O. Beecher, reporting.
Calco Chemical Division, American Cyanamid Company, Wm. H. Seaman reporting.
National Aniline Division, Allied Chemical & Dye Corp., A. T. Schramm reporting.
H. Kohnstamm & Company, Inc., Israel Hanig, reporting.
Food and Drug Administration, Baltimore District, Douglas D. Price, reporting.

The results in the order in which they were received are shown in Table 1. Each figure is the average of duplicate analyses.

As may be seen the results differ considerably. Some collaborators reported difficulty with emulsion formation, and with the separation of the solvents at the interface. These difficulties probably account for the varying results.

RECOMMENDATIONS*

It is recommended—

(1) That the methods be studied further, revised where necessary, and resubmitted to collaborative study.

[1] *This Journal*, **31** 588 (1948).
* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 46 (1950).

TABLE 1.—*Collaborative results on ether extracts*

COLLABORATOR	A	B	C	D
	per cent	*per cent*	*per cent*	*per cent*
1	0.50	0.07	0.12	0.07
2	0.47	0.16	0.12	0.06
3	0.27	0.19	0.03	0.004
4	0.30	0.15	0.03	0.08
5	0.36	0.25	0.09	0.20
6	0.75	0.50	—	0.28
Associate Referee	0.45	0.27	0.08	0.08

A = D&C Green No. 5.
B = FD&C Orange No. 1.
C = D&C Orange No. 5.
D = FD&C Yellow No. 5.

(2) That in the official method for D&C Red No. 39 a Soxhlet extractor be substituted for the Dunbar extractor which can no longer be obtained from the usual sources.

(3) That the topic be continued.

REPORT ON INTERMEDIATES DERIVED FROM PHTHALIC ACID

By CHARLES GRAICHEN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

A survey of the literature showed that a number of methods have been developed for the determination of phthalic acid in resins. None of these procedures, however, seemed adaptable to the determination of small quantities of unreacted phthalic acid in fluorescein or halogenated fluoresceins. The Associate Referee, has, therefore, developed a method for determining small amounts of phthalic acid in fluorescein colors.

In the proposed procedure, the major portion of the dyestuff is removed by filtration after precipitation from aqueous solution by addition of mineral acid; the remaining dyestuff is then extracted from the resulting filtrate with ethyl acetate. The final determination of phthalic acid is made by spectrophotometric measurements upon the aqueous solution.

Phthalic acid is quite soluble in dilute mineral acids, fluorescein is slightly soluble, while halogenated fluorescein compounds are almost completely insoluble. Since fluorescein is present to some extent in all commercial samples of halogenated fluoresceins, it is necessary to separate the phthalic acid from unprecipitated fluorescein before the spectrophotometric examination can be made.

It has been found that extraction of the acid filtrate with ethyl acetate

satisfactorily removes the fluorescein. Resorcinol, which also may be present, is removed by extraction with the solvent to an extent sufficient to prevent interference unless present in quantities over one per cent of the sample. All samples of fluorescein dyes examined have been found to contain unidentified water-soluble impurities that absorb light of the same wave lengths as does phthalic acid. These are partially, but not entirely, extracted by the ethyl acetate. Although phthalic acid is also soluble in ethyl acetate, recoveries of known amounts of phthalic acid treated according to the extraction procedure described have been 90 to 91 per cent.

Since it is impossible to eliminate the "background" absorption due to extraneous material, the concentration of phthalic acid in the aqueous extract obtained by the procedure cannot be directly calculated from measurements at any one wave length, but is estimated from optical densities obtained at 230, 262, and 276 mμ. In so doing, the assumption is made that the "background" absorption is linear between 230 and 276 mμ.

METHOD

REAGENTS

Sodium hydroxide, 10% (w/v).—Dissolve 100 g NaOH in water and dilute to 1 liter.

Hydrochloric acid (1+9).—Dilute 100 ml of conc. HCl to 1 liter.

Ethyl acetate, absolute, C.P.

Standard phthalic acid soln.—Accurately weigh 0.130–0.135 gram of C.P. potassium acid phthalate, dissolve in water, and dilute to exactly 500 ml. Dilute 10 ml of this soln to 200 ml with ca 0.1 *N* HCl. Calculate the concentration of phthalic acid in this soln as follows:

$$\text{Conc. phthalic acid (mg/100 ml)} = \text{Mg of } KHC_8H_4O_4 \times 0.00813$$

(*a*) *Water-soluble salts.*—Wash a 2-g sample (accurately weighed) of the dye into a 250-ml beaker with ca 100 ml of distilled water. Heat nearly to boiling and add slowly with stirring (1+9) HCl until precipitation appears complete. Add an additional 8.5 ml of (1+9) HCl, dilute to about 150–160 ml and digest on the steam bath 1–2 hours. Cool to room temp., wash into a 200-ml volumetric flask, and dilute to volume with distilled water. Filter thru a dry filter paper.

Pipette 50 ml of the filtrate into a 125-ml separatory funnel (use no grease on the stopcocks) and extract with 30 ml of ethyl acetate. Transfer the aqueous phase to another separatory funnel and extract with 25 ml of ethyl acetate. Again transfer the aqueous phase to a third separatory funnel and extract with 20 ml of solvent. Pass three successive 50 ml portions of distilled water thru the funnels in the same order that the extractions were made. Discard the ethyl acetate, combine the aqueous extracted soln, and evaporate to dryness. (It is convenient to reduce to a small volume on a hot plate with the aid of an air jet and then evaporate to dryness on a steam bath.) Dissolve the residue in distilled water and transfer to a 100-ml volumetric flask. Add 8.5 ml of (1+9) HCl and dilute to volume. Filter thru a dry filter paper and determine the extinction of the soln at 230, 262, and 276 mμ on a Beckman ultraviolet spectrophotometer against 0.1 *N* HCl as the blank. (If the soln is too concentrated for accurate readings, dilute an aliquot to a more suitable concentration with 0.1 *N* HCl and multiply the final result by the dilution factor.)

Measure the extinction of the standard phthalate soln at the same wave lengths.

(*b*) *Color acids.*—To 2 g of the color, add 6 ml 10% NaOH, a few ml of water, and mix until the color is dissolved. Dilute to ca 100 ml and proceed as directed above beginning with "Heat nearly to boiling . . . ."

CALCULATIONS

Calculate the quantity A for both sample and standard as follows:

$$A = [E_{230} - (E_{230} - E_{276}(0.7)] - E_{262}$$

then

$$\% \text{ Phthalic acid} = \left(\frac{\text{A sample}}{\text{A standard}}\right) \times \frac{\text{Conc. standard solution}}{\text{(in mg/100 ml)}} \times 0.2.$$

EXPERIMENTAL

A commercial sample of D&C Yellow No. 7 (Fluorescein) was dissolved in base and precipitated with acid. This procedure was repeated until analysis by the proposed method showed a very low phthalic acid content. The "background" absorption was also reduced by this treatment. A commercial sample of D&C Red No. 21 (Tetrabromofluorescein) was treated in the same manner. Known amounts of standard solutions of potassium acid phthalate and resorcinol were added to 2-gram portions of the purified dyes and the phthalic acid content determined by the proposed method. The results are shown in Table 1. The recovery of phthalic acid varied from 80 to 100 per cent with an average recovery of about 90 per cent. There was no noticeable interference with 1 per cent or less added resorcinol; with 2.2 per cent added resorcinol, the results were high but the error was less than 0.1 per cent as phthalic acid.

TABLE 1.—*Recovery of phthalic acid*

DYE	RESORCINOL ADDED	PHTHALIC ACID		RECOVERY*
		ADDED	FOUND	
	per cent	*per cent*	*per cent*	*per cent*
D&C Yellow No. 7	—	—	0.019	—
	—	0.203	0.215	96.7
	—	0.196	0.215	100.0
	—	0.196	0.199	91.8
	—	0.207	0.224	99.0
	—	0.433	0.371	81.3
	0.53	0.433	0.371	81.3
	1.06	0.433	0.392	86.2
	2.20	0.433	0.457	105.7
	—	0.866	0.704	79.1
	—	2.07	1.74	83.1
D&C Red No. 21	—	—	0.026	—
	—	0.165	0.176	90.9
	—	0.412	0.368	89.3

* Corrected for "blank."

There will be a postive error in all spectrophotometric determinations due to the "background." Since a number of commercial samples have been found to contain less than 0.1 per cent of phthalic acid when analyzed by the proposed procedure, this error does not appear to be significant.

Several samples showed a high (over 0.5%) phthalic acid content upon analysis by the proposed method; the complete absorption curves were determined on the final solutions from these. In each case, the shape of the absorption curve indicated that the major portion of the absorption was due to phthalic acid.

SUMMARY

A method is presented for the determination of phthalic acid in fluorescein and halogenated fluorescein. The determination depends upon spectrophotometric examination of an aqueous solution from which the dyes have been removed by precipitation and extraction.

The method cannot be said to give great precision or accuracy. It is, however, satisfactory for the determination of phthalic acid in the quantities ordinarily found in samples of fluorescein colors.

RECOMMENDATIONS*

It is recommended that the proposed method be submitted to collaborative study, and that the topic be continued.

REPORT ON SPECTROPHOTOMETRIC TESTING OF COAL-TAR COLORS

By JOHN H. JONES (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D.C.), *Associate Referee*

Several reports are available on the spectral range covered, effective slit width, accuracy, and reliability of various spectrophotometers. This type of data is very useful to anyone who must decide on which instrument is most suitable for his purpose. Other studies have compared the values obtained on the same sample by various supposedly identical instruments. These results indicate that in general, the absolute data obtained on any instrument will differ significantly from that obtained on any other instrument.

However, from the analyst's point of view, a more fundamental consideration would appear to be whether or not he can, with a given standard and sample, obtain the same result for the sample as do other analysts. Considerable information on this point can be obtained from the various studies mentioned. It was felt, however, that a collaborative study of this point by the A.O.A.C. was desirable.

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 46 (1950).

Four certifiable colors were selected for this study and a quantity of each color was prepared and/or purified for use as a "standard." Part of the standard was then mixed with a colorless diluent to obtain a "sample" of known "purity." Each collaborator was furnished a supply of the "standard" and "sample." They were requested to determine the wave length of maximum absorption of the "standard," whether or not solutions of this material obeyed Beer's law, and the specific absorption coefficient of the color. Using the data obtained on the standard they were requested to determine the "pure dye" content of the sample.

The dyes selected for study were:

(1)—D&C Orange No. 4, a typical water-soluble azo dye. The standard was diluted with salt.

(2)—FD&C Orange No. 2, an oil-soluble azo dye. Napththalene was used as a diluent.

(3)—D&C Yellow No. 7. Selected to test the effect of fluorescence. Salt was the diluent.

(4)—Ext. D&C Red No. 2. This dye was selected as a representative dye of the type usually submitted as lakes. The sample was a lake which had been analyzed collaboratively by $TiCl_3$ titration.

For the first three samples, solution in the appropriate solvent was the only manipulation required other than the spectrophotometric determination. It was necessary to treat the Ext. D&C Red No. 2 Lake with sulfuric acid and filter to eliminate the substratum.

Reports were received from the following collaborators.

National Aniline Division, Allied Chemical & Dye Corporation, A. T. Schramm, reporting.

Calco Chemical Division, American Cyanamid Company, Wm. Seaman, reporting.

Harmon Color Works, Inc., V. C. Vesce, reporting.

Warner-Jenkinson Manufacturing Company, Inc., J. C. McCormack, reporting.

Food and Drug Administration, Los Angeles District, L. C. Weiss, reporting.

Food and Drug Administration, Color Certification Branch, Division of Cosmetics, J. H. Jones, Associate Referee, M. Dolinsky and C. Stein, reporting.

Four of the collaborators used the General Electric recording spectrophotometer, three the Beckman Model DU, and the other a Coleman Model 10S. All of these instruments have an effective slit width of 10 mμ or less.

The results for the "pure dye" content of the samples obtained by the collaborators are shown in Table 1.

Six of the eleven results on the sample of D&C Orange No. 4 are within 1% of the calculated value; the other results deviate from the calculated value by 1.6%, 1.7%, 2.2%, 3.75%, and 4.9%, respectively.

The choice of naphthalene as a diluent for the sample of FD&C Orange No. 2 appears to have been an unfortunate one. Most of the results obtained more than four weeks after the sample was prepared are high;

TABLE 1.—*Collaborative results for "pure dye"*

COLLABORATOR	FD&C ORANGE NO. 4	FD&C ORANGE NO. 2	D&C YELLOW NO. 7	EXT. D&C RED NO. 2 LAKE
	per cent	*per cent*	*per cent*	*per cent*
Calculated	83.3	87.0	77.8	29.5
1	82.0	88.9 86.9	78.0	28.4
2	83.1	86.2 87.0	77.7 77.7	27.8
3	81.5	92.6	75.4 76.7	30.3
4	82.8	89.5	77.8	29.8
5	87.4	93.5	75.7	25.8
6	83.2	90.5	77.9	29.2
7	86.4 83.9 82.6	85.2	77.7	29.4
8	83.0 84.7	89.6 89.3	78.5 77.9	31.0 31.3

this probably means that there was some evaporation of the diluent.

Eight of the eleven results on the sample of D&C Yellow No. 7 are within 1% of the calculated value, the other results differ from the calculated value by 1.4%, 2.7% and 3.1% respectively. It appears, therefore, that the fluorescence of the sample does not interfere with its spectrophotometric determination with these instruments.

The relative errors of the results on the sample of Ext. D&C Red No. 2 Lake are rather high; however, eight of the nine results would give values 28–31% for the "pure dye" content of the sample. This does not appear to be an excessive variation for this type of sample. The average value for these eight results is very close to the calculated value.

On the whole, the results on the two water-soluble samples and the color lake appear to be quite satisfactory.

The wave length of maximum absorption (as reported by the collaborators or read from the curves submitted) together with the calculated value of extinction per milligram per liter for the standards are shown in Table 2.

All of the instruments were in fairly good agreement as to the wave length of maximum absorption.

TABLE 2.—*Wave length of maximum absorption and specific extinction of standard samples*

COLLABORATOR	D&C ORANGE NO. 4		FD&C ORANGE NO. 2		D&C YELLOW NO. 7		EXT. D&C RED NO. 2 LAKE	
	mμ	E*	mμ	E*	mμ	E*	mμ	E*
1	485	6.60	492	7.02	489	25.0	495–500	4.68
2	485	6.66	490	7.00	489	25.2	495–500	4.67
3	486–8	6.53	492	7.15	490	25.0	500 ± 5	4.56
4	—	6.42	—	7.20	—	24.0	—	4.52
5	484	6.56	490	7.00	492	25.5	492	4.52
6	487	6.45	493	6.94	492	25.0	496	4.60
7	486	6.33	490	6.90	492	24.9	494	4.33

* Extinction per milligram per liter ×100.

The two orange colors and the red dye have very broad absorption peaks which should eliminate differences in the specific extinction due to minor variations in the effective slit widths of the instrument and the wave length selected for the measurement. There are, however, significant differences in the specific extinction of the standards as determined by by the various instruments. The difference between two supposedly identical instruments, however, is just as great as that between two different instruments. These results emphasize the conclusion reached in other studies that, for accurate results, the standard and sample must be compared on the same instrument, and preferably at the same time.

No reports were received from collaborators using filter photometers on spectrophotometers with wide effective slit widths. It would have been of considerable interest to compare the results obtained on such instruments with those reported here and an attempt will be made to secure such data in the future.

SUMMARY

A collaborative study of the spectrophotometric determination of the "pure dye" content of four samples of certifiable colors has been made. The collaborator was furnished with a "standard" and a "sample" of each color and asked to determine the "pure dye" content of the sample.

Reports were received from eight collaborators, four of whom used General Electric spectrophotometers, three Beckman spectrophotometers, and one a Coleman Model 10S spectrophotometer.

The results reported for three of the four samples were reasonably satisfactory; more than half of the reported results on these samples were within 1% of the calculated value.

The fourth sample apparently changed composition between the time it was prepared and the time several of the collaborators analyzed it so that the results in this sample could not be evaluated.

Results obtained from each of the spectrophotometers used in this

study showed reasonably good agreement as to the wave length of maximum absorption of solutions of the colors. There were, however, significant differences in the specific extinction coefficients of the colors as determined upon the various instruments.

RECOMMENDATION*

It is recommended that the topic be continued.

REPORT ON SUBSIDIARY DYES IN D&C COLORS (D&C RED NOS. 6 OR 7)

By L. Koch (H. Kohnstamm & Co., Ind., Brooklyn, N.Y.),
Associate Referee

In the last report[1] on the estimation of 4-toluene-azo-2-naphthol-3-carboxylic acid in D&C Red No. 6, consistent low *p*-toluidine recoveries, from the acid reduction of the dye, were reported by the collaborators. It was therefore deemed advisable to modify the reduction conditions to an alkaline medium, to ascertain whether or not the change would yield more quantitative results.

This variation has yielded analyses that indicate the new procedure has greater merit, the findings reported being more truly indicative of the actual subsidiary dye content. The results are outlined in Table 1.

At this point, the Associate Referee wishes to express his appreciation to the following collaborators, without whose cooperation this report would not have been possible. They are:

F. Howard Hedger, Chas. Pfizer & Co., New York, N. Y.
A. T. Schramm, National Aniline Division, Allied Chemical & Dye Corp., Buffalo, N. Y.
J. M. Remsen, E. I. du Pont de Nemours & Co., Wilmington, Del.
M. Dolinsky, Food and Drug Administration, Washington, D. C.
Charles Graichen, Food and Drug Administration, Washington, D. C.
Lee S. Harrow, Food and Drug Administration, Washington, D. C.
Virginia Schmuckli, H. Kohnstamm & Co., Brooklyn, N. Y.

METHODS

REAGENTS

Sodium Hydrosulfite.—Solid.

Sodium Hydroxide.—10%.

Methyl Cellosolve.

Potassium bromide-bromate soln.—0.05 *N* containing 1.3920 g of C.P. $KBrO_3$ and 10 g of C.P. KBr per liter.

Sodium Thiosulfate.—12.5 g of $Na_2S_2O_3 . 5\ H_2O$ per liter. Standardize against the $KBrO_3$-KBr soln as follows: Place 100 ml of water, 25 ml of conc. HCl, and 100 g

* The report of Subcommittee B and action of the Association, see *This Journal*, 33, 467(1950).
[1] *This Journal*, 31, 603 (1948).

of crushed ice into a 500 ml iodination flask. Add ca 20 ml of the $KBrO_3$-KBr soln, from a buret, as rapidly as possible. Stopper, and let stand in an ice bath for 10 min. Continue as in the Procedure, beginning "Add 2–3 grams of KI," Calculate the value of the $Na_2S_2O_3$ soln in terms of the $KBrO_3$-KBr soln.

Starch Indicator.—0.5% soln.

Potassium Iodide.—Solid.

PROCEDURE

Place 2.0 g of the D&C Red No. 6 into the 1-liter round bottom flask of the distillation apparatus, and add a few anti-bumping pellets. (Boileezers, from the Fischer Scientific Co., are suggested.) Cover the dyestuff with 100 ml of a 10% NaOH soln,

TABLE 1.—*Collaborative results*

SUBSIDIARY DYE ADDED	SUBSIDIARY DYE FOUND—MG						
	ANALYST						
	A	B	C	D	E	F	G
mg.							
0	4.85	8.00	7.46	2.00	2.77	2.00	0.00*
10	11.25	9.40	8.73	9.35	—	10.90	10.03
15	15.45	10.20	14.24	14.35	—	14.20	15.04
20	20.35	18.60	16.16†	19.00	—	18.10	20.05
25	26.45	24.30	25.14	23.80	23.70	23.70	24.98
50	53.25	45.20	48.34	48.30	45.15	45.50	50.07
	SUBSIDIARY DYE FOUND—PER CENT						
per cent							
0	0.24	0.40	0.37	0.10	0.14	0.10	0.00*
0.50	0.56	0.47	0.44	0.47	—	0.55	0.50
0.75	0.77	0.51	0.71	0.72	—	0.71	0.75
1.00	1.02	0.93	0.81‡	0.95	—	0.91	1.00
1.25	1.32	1.22	1.26	1.19	1.19	1.19	1.25
2.50	2.66	2.26	2.42	2.42	2.26	2.28	2.50

* Figures in 0 column represent the subsidiary dye content of the unadulterated D&C Red No. 6. Figures in the other columns represent the total subsidiary dye found, less the original content of the D&C Red No. 6.

† 19.2 mg of subsidiary dye added.

‡ 0.96 per cent of subsidiary dye added.

25 ml of methyl cellosolve, and then add 10 g of $Na_2S_2O_4$. Attach the adapter to the condenser, leading the tip below the surface of 25 ml of a 1 + 4 HCl soln, and heat the mixture, to effect simultaneous reduction and distillation.

When ca 50 ml of distillate has been collected, allow 150 ml of water to drip into the heated flask at rate ca equal to that of the distillation. Continue, until 300 ml has been caught in the receiving vessel. Transfer the liquid to a 500-ml iodination flask, and concentrate to 100 ml. Cool, add 20 ml of conc. HCl, and 100 g of crushed ice.

Run the $KBrO_3$-KBr soln into the iced concentrate, from a buret, until the soln remains yellow for at least one-half minute. Then add ca 5 ml more. Stopper and let stand in an ice bath for 10 min. Add 2–3 g of KI, and titrate immediately with the $Na_2S_2O_3$ soln, using starch indicator internally, near the end point.

1 ml of 0.05 *N* $KBrO_3$ soln is equal to 4.11 mg of subsidiary dye—Na

1 ml of 0.05 *N* $KBrO_3$ soln is equal to 4.07 mg of subsidiary dye—Ca/2

COMMENTS BY COLLABORATORS

A. T. Schramm.—The distillation was continued in each case until 225 ml had been collected. It was found that further distillation gave additional subsidiary dye. It is recommended therefore, that a definite volume of distillate be indicated. We found that a distillation of 375 ml gave 6.0 mg found for none added, and 55.5 mg for 50 mg added. Apparently 375 ml distillate gives better recovery at that particular concentration.

J. M. Remsen.—Our analytical section reports that no difficulties were experienced in running this analysis in accordance with your submitted apparatus sketch and procedure.

M. Dolinsky.—The method appears to give consistent results. However, since the method depends upon the recovery of a volatile amine dye component, any free amine intermediate, or its oxidation product, would also be recovered, and tend to give high results.

Lee S. Harrow.—The method is rather time consuming. Suggest use of 500 ml flask for greater distilling efficiency.

DISCUSSION

This year's collaborative results indicate that the proposed procedure yields quantitative analyses, whose accuracy parallels that of other methods for the estimation of coloring matters. However, the erratic percentages reported for the unadulterated D&C Red No. 6 indicate that the comment of A. T. Schramm bears investigation. Only after this point has been clarified, can the method be termed acceptable.

Regarding the comment by M. Dolinsky, the probability of free unsulfonated amine being present is relatively small, and the oxidation products of amines are usually non-volatile from an alkaline medium.

It is therefore recommended* that the study of this topic, collaboratively, should be discontinued, but that experimental investigation of the results termed "erratic" should be pursued.

REPORT ON SUBSIDIARY DYES IN FD&C COLORS

By MEYER DOLINSKY (Division of Cosmetics, Food and Drug Administration, Federal Secuirty Agency, Washington, D.C.), *Associate Referee*

At the annual meeting of the A.O.A.C. last year, a spectrophotometric method was presented for the determination of D&C Orange No. 4 in samples of FD&C Orange No. 1. This method has now been studied collaboratively.

Samples of purified D&C Orange No. 4 and FD&C Orange No. 1 were prepared, and a known mixture of these purified colors containing 4.1% of D&C Orange No. 4 was made. Samples of this mixture were submitted to seven collaborators for analysis by the proposed method. The collaborators reporting results are:

* For report of Subcommittee B and action of the Association, see *This Journal*, 33, 46 (1950).

National Aniline Division, Allied Chemical & Dye Corp., A. T. Schramm, reporting.
Warner-Jenkinson Manufacturing Company, John C. McCormack, reporting.
Harmon Color Works, Inc., Vincent C. Vesce, reporting.
Calco Chemical Division, American Cyanamid Company, Wm. Seaman, reporting.
Food and Drug Administration, Los Angeles District, Louis C. Weiss, reporting.
Food and Drug Administration, Cosmetic Division, Charles Graichen and Charles Stein, reporting.

The results are shown in Table 1, in the order in which they were received.

Of the eighteen individual results, ten fall within ±0.5% and fourteen

TABLE 1.—*Spectrophotometric determination of D&C Orange No. 4 in FD&C Orange No. 1*

COLLABORATOR	FOUND	AV.	TYPE OF INSTRUMENT
	per cent	*per cent*	
1	4.7, 4.2	4.45	General Electric
2		5.0[1]	Beckman
3	4.3, 4.2	4.25	General Electric
4	6.6, 3.7, 6.7, 4.7, 4.2, 4.5	5.1	General Electric
5	6.65,[2] 5.5,[2] 4.3,[2] 3.3	4.9[2]	Coleman—10S (5 mμ slit)
6	5.0, 3.7	4.35	Beckman
7	3.8, 3.9	3.85	General Electric and Beckman
	Av.	4.56	
	Mean Deviation	0.38	
	Max. Deviation	0.71	

[1] Based on average extinction values.
[2] Using cylindrical cells.

within ±1.0% of the correct amount of D&C Orange No. 4. The maximum absolute error in the average value reported by any collaborator is 1.0%; the average absolute error is 0.5%. In terms of relative error, these would be 24% and 12%, respectively. These figures agree closely with those reported by the Associate Referee at last year's A.O.A.C. meeting. In this type of determination it is apparent that slight deviations in the density or wave length readings will cause appreciable errors in the final results. For accurate results it is advisable, therefore, to make several determinations, using in each case the average of a number of readings taken at each wave length. It is interesting to note that three types of spectrophotometers were used for these analyses.

SUMMARY

Collaborative analyses of samples of FD&C Orange No. 1 containing a known amount of D&C Orange No. 4 have been carried out. The results indicate that analysts using the proposed procedure may be expected to

determine the amount of D&C Orange No. 4 present to within one per cent of the sample weight.

RECOMMENDATIONS*

Since the certifiable color D&C Orange No. 4 has the same constitution as does Orange II, namely 1-*p*-sulfophenylazo-2-naphthol, the method herein described is entirely applicable to the determination of Orange II as a subsidiary dye in FD&C Orange No. 1. The following recommendations are therefore made:

(1) That the proposed method be adopted as official for the determination of Orange II in FD&C Orange No. 1.

(2) That the topic be continued.

REPORT ON HEAVY METALS IN COAL-TAR COLORS—MERCURY

By CHARLES STEIN (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

Of the methods proposed for the determination of micro quantities of mercury, those involving the use of dithizone appear most desirable from the standpoint of speed, accuracy, and minimum requirements for special equipment. These methods have been reviewed by Welcher (4). This report covers the work done to find a dithizone procedure applicable to the determination of micro amounts of mercury in coal-tar colors.

The procedure employed is essentially that described by Laug and Nelson (2) for biological materials; their article should be consulted for a discussion of the theory. The present method differs from their procedure chiefly in that perchloric acid is used in the digestion step and that ammonium acetate is used as the buffer in place of a disodium phosphate-potassium carbonate solution.

APPARATUS

The digestion apparatus consists of a two-neck, 500 ml flask fitted with a Friedrichs condenser and a 50 ml dropping funnel. Pyrex glassware and standard taper joints are used throughout. As many as six condensers can be connected in series.

REAGENTS

(1) *Sulfuric acid, analytical reagent.*

(2) *Nitric acid, analytical reagent.*

(3) *Digestion mixture.*—Equal parts by volume of H_2SO_4, HNO_3, and distilled H_2O.

(4) *Hydrochloric acid, C.P.*—Prepare 0.25 *N* acid as required.

(5) *Perchloric acid,* analytical reagent, 70–72%.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 46 (1950).

(6) *Hydroxylamine hydrochloride soln, 20% w/v.*—Remove heavy metals from the soln by shaking with 50-ml portions of 100 mg/liter dithizone in $CHCl_3$. Wash with several portions of $CHCl_3$, to remove excess dithizone.

(7) *Potassium bromide soln, 40% w/v.*—Free the soln from heavy metals as described above. Make alkaline with 1–2 drops of 10% NaOH soln before storing.

(8) *Ammonium acetate soln,* (1+1).—This is conveniently prepared by dissolving the entire contents of a 1 or 2 pound container of $NH_4C_2H_3O_2$ in the required volume of water. Remove heavy metals as described above.

(9) *Chloroform, U.S.P. grade,* containing about 0.75% alcohol.

(10) *Dithizone soln.*—The reagent supplied by Eastman Kodak Company can be used without further purification. Prepare a stock soln containing 1 mg per ml of dithizone dissolved in $CHCl_3$, and a soln containing 6 mg per liter by dilution of the stock soln. (The concentrated soln is best prepared in relatively small volumes. The dilute soln can be prepared in liter lots.)

(11) *Mercury standards.*—Use C.P. HgO and dissolve in H_2SO_4 according to the A.O.A.C. procedure. (1) Prepare a stock soln containing 1000 mmg of Hg per ml, an intermediate dilution of 100 mmg of Hg per ml, and a working standard of 5 mmg of Hg per ml.

PROCEDURE

Transfer a 1-g sample (0.5 g in the case of triphenylmethane and oil-soluble dyes) to the digestion flask, add 10 ml of the digestion mixture and allow to react in the cold for ca 5 min. Heat gently for ca 5 min., add 2 ml of $HClO_4$ and continue the digestion at full heat for 2 hours (3 hours for triphenylmethane and oil-soluble dyes; about 1 hour for lakes having a relatively low percentage of dye). Run two blanks concurrently containing all the reagents used, but no dye.

Allow to cool. Wash the condenser and funnel thoroly, using sufficient water to bring the volume to about 100 ml. Add 10 ml of $NH_2OH \cdot HCl$ soln and reflux for 10 min. When cool, wash the condenser and funnel again with 20–30 ml of water and transfer the soln to a 250-ml separatory funnel (filter, if necessary). Dilute to 200 ml. Add 10 mmg of Hg to one of the blanks. This is the standard; the second blank is the reagent blank.

Add 10 ml of dithizone soln (6 mg/liter) and shake vigorously for 1 min. Allow the $CHCl_3$ to settle and transfer it quantitatively to a second 250-ml separatory funnel containing 50 ml of 0.25 *N* HCl. Pass 5 ml of $CHCl_3$ thru the first funnel and combine with the dithizone extract. Repeat the extraction with successive 10-ml portions of dithizone until the green color of the dithizone remains unchanged (usually 2 portions will be sufficient when the amount of Hg present does not exceed 15 mmg). Wash the contents of the first funnel by shaking with 10 ml of $CHCl_3$ and combine the $CHCl_3$ with the dithizone extracts.

Shake the second funnel vigorously for 1 min., allow the $CHCl_3$ to settle and transfer to a third 250 ml funnel containing 50 ml of 0.25 *N* HCl and 5 ml of KBr soln. Wash the contents of the second funnel with 10 ml of $CHCl_3$ and add the $CHCl_3$ to the third funnel.

Shake the third funnel vigorously for 1 min. Drain and discard the $CHCl_3$ phase. Wash the aqueous phase by shaking with successive 10-ml portions of $CHCl_3$ until both the $CHCl_3$ and aqueous layers are colorless. Drain and discard the $CHCl_3$ layer. Add 10 ml of $CHCl_3$ and 20 ml of $NH_4C_2H_3O_2$ soln. Shake vigorously for 10 seconds. Remove the cover of the funnel to allow the film of $CHCl_3$ on the surface to evaporate (ca 10 min.). Drain the $CHCl_3$, allowing the aqueous phase to fill the stopcock bore. No droplets of $CHCl_3$ should be present in the funnel at this point.

Add exactly 10 ml of dithizone soln and shake vigorously for 1 min. Dry the

stem of the funnel with absorbent cotton and insert a fresh pledget of cotton. Collect the $CHCl_3$ phase in a clean test tube, discarding the first ml of the filtrate. Determine the extinction (optical density) of the $CHCl_3$ solns at 490 mμ. If a Beckman spectrophotometer is employed use 10 mm cells and a $CHCl_3$ blank.

Calculate the amount of Hg present by the following relationship:

$$\text{mmg Hg} = \frac{(\text{E sample} - \text{E reagent blank})\ (10)}{\text{E standard} - \text{E reagent blank}}$$

where E = extinction of the respective solutions at 490 mμ.

The final extraction of Hg should be carried out in the absence of direct sunlight and the optical measurements made within 1 hour after the Hg has been extracted.

DISCUSSION

The quantity of acids and digestion periods specified gave a satisfactory digest in all cases studied. Triphenylmethane and oil-soluble dyes are especially difficult to digest and for this reason the sample taken is reduced to 0.5 gram. An incompletely digested dye yields a turbid solution when diluted. A slight turbidity does not interfere, but a heavy precipitate results in the formation of a troublesome emulsion in the chloroform layer. The solution obtained by digesting dyes containing insoluble inorganic matter must be filtered. No evidence of retention of mercury in the precipitate by either organic or inorganic matter has been noticed.

A small blank is usually present, probably due to traces of metals in the reagents. In order to obtain a uniform blank it is essential that all reagents added to the third funnel be carefully measured. To avoid contamination, the glassware must be carefully washed with dilute nitric acid and distilled water. The absorbent cotton and stopcock grease (if any is used) must be free of metals reacting with dithizone. In addition, the stopcock grease must not absorb light at a wave length of 490 mμ.

Most dithizone procedures specify the use of C.P. or redistilled chloroform. U.S.P. chloroform was employed in this work with satisfactory results. However, there is probably considerable variation in the quality of this grade of chloroform, and certain brands may be unsuitable.

Reith (3) has shown that a 5–10 per cent excess of dithizone is sufficient to insure the complete extraction of mercury. Ten ml. of dithizone solution (6 mg/liter) should contain sufficient dithizone to combine with about 20 micrograms of mercury. Since the dithizone is not pure, and because of the presence of a small blank, not more than 15 micrograms of mercury can be safely determined with the quantity of dithizone employed. The presence of a sufficient excess of dithizone can be determined by measuring the extinction of the final solution at 610 mμ, since at this wave length neither the yellow oxidized form of dithizone nor the mercury dithizonate has any absorption.

The results obtained in nineteen series of recoveries are shown in Table 1. It appears that an accuracy of ±1 microgram of mercury can reasonably be expected.

TABLE 1.—*Recovery of known amounts of mercury*

MERCURY ADDED	0 MMG	5 MMG	10 MMG	15 MMG
	Mercury Found			
FD&C Blue No. 2	0.0	5.5	10.2	14.6
FD&C Red No. 32	0.1	5.0	10.0	14.7
FD&C Yellow No. 3	0.3	5.3	10.5	15.5
FD&C Red No. 1	−0.4	4.9	10.5	16.0
FD&C Red No. 4	−0.5	4.7	10.2	15.6
FD&C Green No. 1	0.0	4.7	10.2	14.9
FD&C Orange No. 1	0.1	5.3	10.0	14.5
FD&C Yellow No. 6	−0.1	4.8	10.4	15.8
FD&C Green No. 2	0.0	5.6	10.0	15.0
FD&C Green No. 3	−0.6	5.3	9.8	15.6
FD&C Blue No. 1	−0.2	5.3	9.8	15.1
D&C Red No. 19	0.5	4.9	10.1	15.1
D&C Red No. 9, Ba Lake Gloss white	0.0	5.0	9.9	14.6
D&C Red No. 34, Lake Talc. & rosin	0.0	4.4	9.7	14.2
D&C Blue No. 1, Al Lake ca 90% alumina	0.1	4.9	10.8	15.6
D&C Yellow No. 5, Zirconium Lake	0.0	4.9	10.5	14.8
Ext. D&C Green No. 1	−0.2	4.7	10.5	15.0
D&C Yellow No. 10	0.6	5.8	10.0	14.4
D&C Red No. 10 Na Lake ca 10% gloss white	0.0	4.9	10.0	14.8

The proposed procedure appears to be suitable for straight colors and lakes. It is not applicable, however, to dyes containing iodine or bromine, since at the low *p*H of the first extraction, mercury cannot be extracted with dithizone in the presence of iodides or bromides.

RECOMMENDATIONS*

It is recommended—

(1) That the proposed method be studied collaboratively.

(2) That the topic be continued.

ACKNOWLEDGMENT

The author wishes to express his appreciation to A. K. Klein for suggesting the use of hydroxylamine hydrochloride under reflux conditions.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 46 (1950).

REPORT ON ARSENIC IN COAL-TAR COLORS

By LEE S. HARROW (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

During the past year a preliminary study has been made for the purpose of selecting a method suitable for the determination of arsenic in coal-tar colors. A review of the literature indicated that the Gutzeit[1] and the Cassil-Wichman[2] methods would probably be most adaptable for this purpose. Accordingly, these two methods were investigated.

It was found that considerable experience in the interpretation of the stained strips is required before the Gutzeit method may be relied upon. It is felt that this feature of the method makes its use of questionable value; few chemists make this determination often enough to be called "expert."

A "wet ashing" of the coal-tar colors is required before arsenic can be determined, the process employing about 10 ml. of conc. sulfuric acid. The Cassil-Wichman method accommodates this amount of acid with ease, while the Gutzeit method does not. Furthermore, the Cassil-Wichman method is not as dependent on the experience of the analyst. This method was, therefore, chosen for more extensive study. The results thus far are inconclusive, but indicate that the method shows promise of giving accurate and reproducible results. It is the intention of the Associate Referee to study this method further and if the results warrant such action, to submit it to collaborative study.

It is recommended* that the topic be continued.

REPORT ON BOILING RANGE OF AMINES DERIVED FROM COAL-TAR COLORS

By LEE S. HARROW (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D. C.), *Associate Referee*

At the annual meeting of the Association last year, a method was proposed for the determination of the boiling range of pseudocumidine obtained by the reduction of FD&C Red No. 1. (1) This method has now been studied collaboratively. A composite sample of FD&C Red No. 1 was prepared and samples of this submitted to the following collaborators:

Ansbacher-Siegle Corporation, H. Holtzman reporting.
Bates Chemical Company, Inc., Cyrus O. Beecher reporting.
Calco Chemical Division, American Cyanamid Company, Wm. Seaman reporting.
H. Kohnstamm and Company, Inc., Louis Koch reporting.

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[1] *Methods of Analysis, A.O.A.C.*, 6th Ed., 29.1–29.5.
[2] Cassil, C. C., and Wichman, H. J., *This Journal*, **22**, 436 (1939).
* For report of Subcommittee B and action of the Association, see *This Journal*, **33**, 46 (1950).

The results, in the order reported, are shown in the following table:

COLLABORATOR	BOILING RANGE FOUND
	Degrees C.
1	220.8–239.6
2	226.0–238.0
3	225.5–226.0
4	220.0–237.0
Associate Referee	219.6–238.5

Two of the collaborators are in good agreement with the Associate Referee and a third differs only in respect to the initial boiling point. The Associate Referee can offer no explanation for the results of collaborator No. 3. It is believed that these results, in general, indicate that the directions are not sufficiently specific to insure agreement by various workers in reporting the "initial boiling point."

BOILING RANGE OF XYLIDINE OBTAINED BY REDUCTION OF FD&C RED NO. 32

One of the specifications for FD&C Red No. 32 listed in the Coal-Tar Color Regulations (2) states, "Boiling range of xylidine, obtained by reduction of the dye, 95 per cent between 212–232°C."

The method for reducing FD&C Red No. 1 is not applicable to FD&C Red No. 32 because of its insolubility in water. Sodium hydrosulfite is not an effective reducing agent for large quantities (40–100 grams) of oil-soluble dyes because a large quantity of dye is dissolved and held in the amine layer which forms on top of the reaction mixture. FD&C Red No. 32 may be reduced effectively in acid solution with 20 per cent titanium trichloride solution, but the cost of the titanium trichloride is prohibitive. Reduction with zinc and hydrochloric acid is time-consuming but appears to be the most practical method.

The determination of the portion of amine boiling between 212 and 232°C. presents a unique problem because of the material necessarily "held up" in the column and the residue in the distilling flask. In order to overcome this difficulty, a material was sought that could be added to the amine in the flask to allow the amine to be distilled almost quantitatively. In order to avoid interference with the boiling range determination, such a material must have a boiling point a few degrees higher than that of the highest boiling fraction of the amine, and must not form an azeotropic mixture with the amine. It was found that a petroleum fraction boiling between 260–270°C served well for this purpose.

METHOD

APPARATUS

Reduction and steam distillation.—A 3-liter, 3-neck, round-bottom flask, one neck fitted with a dropping funnel, a second with a mechanical stirrer, and the third with a reflux condenser.

Fractionating apparatus.—As described under **13.1,** but fitted with a graduated receiver of 15–25 ml capacity accurate to 0.1 ml.

REAGENTS

Zinc.—Either fine granular or dust.

Aliphatic hydrocarbon fraction.—Boiling range 260–270°C. Distill a high boiling aliphatic hydrocarbon mixture, such as light mineral oil, and collect the fraction boiling at 260–270°C.

DETERMINATION

Dissolve ca 60 g of color in 400 ml of hot dioxane contained in the reduction apparatus, add 120 g of zinc, and reflux with stirring for 10 min. While refluxing and stirring, add conc. HCl (ca 200 ml) dropwise thru the dropping funnel until the soln shows no more color change (red–orange–yellow). Reflux the mixture one hour, cool the reaction flask in ice, and add 30% NaOH (ca 200 ml) slowly until the soln is distinctly alkaline to litmus. Add 1 liter of water and steam distill the mixture until the distillate shows the first signs of cloudiness. Discard distillate collected up to this point. Continue the distillation until no more oil drops are apparent in the condenser. Transfer the distillate to a separatory funnel and extract with three 50-ml portions of ether and wash the combined extracts twice with 10-ml portions of water. Evaporate the major portion of the ether on a steam bath and dry the residue over sodium or potassium hydroxide pellets. Filter the residual soln into the 25-ml round-bottom flask of the fractionating apparatus, add 5 ml of the petroleum fraction, and heat cautiously with a heating mantle or a water bath until all of the ether has been removed, then distill the xylidine (raising the temp. slowly) using a Wood's metal bath or an equivalent even-temperature bath. The final boiling point is taken as the last point of temp. constancy before the sudden temp. rise to 260°C. Note the volume percentage distilling between 212–232°C.

EXPERIMENTAL

A commercial sample of "mixed xylidines" was distilled and all material boiling over 180°C. was reserved. This material was kept for 24 hours over sodium hydroxide pellets, and then divided into two portions. The boiling range of one portion was determined using the apparatus suggested under "Method."

A quantity of FD&C Red No. 32 was prepared from the second portion employing standard procedure. This color was then reduced according to the proposed method and the boiling range of the resulting xylidine was determined. The results are shown in Table 1.

TABLE 1.—*Xylidine recovery*

BOILING RANGE	DISTILLED ORIGINAL	RECOVERED MATERIAL
	per cent	*per cent*
Initial–213.0	4	6
213.0–213.5	40	36
213.5–215.0	50	50
215.0–Upper Limit (217.0)	6	8

The boiling range of the original material was 203.9°C. to 217.0°C. and that of the recovered material was 205.0°C. to 217.8°C.

SUMMARY

A method is proposed for the evaluation of the boiling range of xylidine in FD&C Red No. 32. The intermediate is recovered from the color by reduction with zinc and hydrochloric acid, and the boiling range determined by means of a semi-micro fractionating apparatus fitted with an Anschutz thermometer.

A collaborative study of the proposed method for the determination of the boiling range of pseudocumidine indicated that additional studies should be made.

RECOMMENDATIONS*

It is recommended—

(1) That the method for the determination of the boiling range of pseudocumidine in FD&C Red No. 1 be revised and recommitted to collaborative study.

(2) That the method for the determination of the boiling range of xylidine obtained by reduction of FD&C Red No. 32 be studied collaboratively.

(3) That the topic be continued.

REFERENCES

(1) FREEMAN, K. A., and HARROW, L. S., *This Journal*, **32**, 127 (1949).
(2) S.R.A. F.D.C. 3 Coal-Tar Color Regulations, U. S. Foodand Drug Administration.

Two contributed papers, studies in Coal-Tar Colors, D&C Reds Nos. 14, 15, 16, and 31, by K. A. Freeman and C. Stein, and D&C Red No. 34, By K. A. Freeman and C. Graichen, were published in the November number of *This Journal*, 1949, pages 718 and 726, respectively.

Another of the studies on Coal-Tar Colors, "Spectrophotometric Analysis of Coal-Tar Colors: D&C Orange No. 17," by Rachel Sclar, is published in *This Journal*, page 418.

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* For report of Subcommittee B and Action of the Association, see *This Journal*, **33**, 46 (1950).

ANNOUNCEMENTS

Referee Assignments, Changes, and Appointments

NITROGEN IN FERTILIZERS:

Henry A. Davis, Agricultural Experiment Station, Durham, New Hampshire, has been appointed Associate Referee, in place of M. P. Etheredge.

ECONOMIC POISONS:

Lloyd Kierstadt, Associate Referee on Oil Emulsions, has resigned.

EXCHANGEABLE POTASSIUM:

The name of A. Mehlich should be substituted for that of J. F. Reed, as Associate Referee.

PHOSPHORUS IN SOILS:

L. E. Ensminger, Alabama Experiment Station, Auburn, Alabama, has been appointed Associate Referee, to succeed L. A. Dean.

FLUORINE IN SOILS

W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn., has been appointed as Associate Referee for experimental study of this subject.

OILS, FATS, AND WAXES:

Albert B. Karasz, State Food Laboratory, Department of Agriculture and Markets, Albany 1, N. Y., has been appointed Associate Referee on Peanut Oils.

GUMS IN FOODS:

Sutton Redfern, Associate Referee on Starchy Salad Dressings, has resigned.

CORRECTIONS IN FEBRUARY JOURNAL

Page 3. F. I. Edwards, Associate Referee for Parathion, was incorrectly given as in the Production and Marketing Administration, instead of in Bureau of Entomology and Plant Quarantine, Beltsville, Md.

Page 5. Maxwell L. Cooley, Associate Referee on Vitamin A in Animal Foods, change Larraine Division to Larrowe Division, of General Mills, Inc.

CONTRIBUTED PAPERS

SPECTROPHOTOMETRIC ANALYSIS OF COAL-TAR COLORS—D&C ORANGE NO. 17

By RACHEL N. SCLAR (Division of Cosmetics, Food and Drug Administration, Federal Security Agency, Washington, D.C.)

This report is a continuation of the study of spectrophotometric analyses of certifiable oil-soluble dyes. Previous reports gave similar data for Ext. D&C Yellow No. 5 (1), D&C Red No. 35 and D&C Red No. 36 (2), Ext. D&C Orange No. 1 (3), D&C Violet No. 2 (4), and D&C Green No. 6 (5).

Conformity to Beer's law, location of the absorption peaks, and the ratio of extinction values at suitable wave lengths, were tested on chloroform solutions of D&C Orange No. 17. In addition the color of the dye in basic alcoholic solution was investigated.

EXPERIMENTAL

The spectrophotometric data were obtained with a General Electric recording spectrophotometer which has an automatic slit adjustment for an 8 millimicron wave length band.

Preparation of D&C Orange No. 17 (Permanent Orange)

2,4-Dinitroaniline (m.p. 177° on Fisher block) was diazotized and coupled with β-naphthol (m.p. 119°) in acid solution. The product was washed thoroughly with hot water, dried, and recrystallized three times from glacial acetic acid; it melted at 304°C. Recrystallization from dioxane or benzene did not change the melting point. Literature m.p. is 302°C. (6).

A small sample of the purified material was adsorbed from benzene on a column of activated alumina and developed with benzene; the chromatogram appeared homogeneous. Spectrophotometric examination of a commercial sample indicated a purity of 96 per cent. This sample, when chromatographed in the above manner, gave two bands: one brown and one orange. When a portion of this material was washed with hot water and recrystallized once from glacial acetic acid, it was spectrophotometrically identical with the laboratory preparation and gave a homogenous chromatogram. Further recrystallization from dioxane did not change its spectrophotometric characteristics. The sample prepared in this laboratory was therefore considered sufficiently pure to serve as a standard for D&C Orange No. 17.

SPECTROPHOTOMETRIC DATA

The dye, weighed on a semimicro balance sensitive to 0.02 mg., was dissolved in about 75 ml. of U.S.P. chloroform by refluxing on a steam

bath. The solution was cooled, transferred to a volumetric flask and made to volume with chloroform. Aliquot portions were diluted to concentrations similar to those shown in Table 1 and the spectrophotometric curves drawn. Solutions were made to volume at ambient temperature.

TABLE 1.—*Extinction values of solutions of D&C Orange No. 17 in chloroform*

Typical Data

CURVE NO. (CHART 1)	CONCENTRATION MG/LITER	EXTINCTION			$E_{479m\mu}$ / CONCENTRATION	$E_{465m\mu}$ / $E_{495m\mu}$
		465 mμ	479 mμ	495 mμ		
1	3.51	.246	.272	.256	.0768	.961
2	7.03	.492	.542	.510	.0765	.965
3	10.54	.738	.816	.768	.0768	.961
4	14.06	.986	1.088	1.024	.0768	.963
Av.					.0767	.96

A set of typical extinction curves for chloroform solutions of D&C Orange No. 17 is shown in Chart 1. The curves are non-symmetrical with a large absorption in the blue area. The absorption peak is at 479 ± 2 mμ. (All wave lengths were corrected to ± 2 mμ with the aid of didymium glasses calibrated by the National Bureau of Standards; see footnote to Charts 1 and 2.) The average extinction per milligram per liter at this wave length, calculated from the results of 48 determinations (at various concentrations) made from twelve portions of the dye, is .0767. (These extinction values were corrected to a standardized extinction value for the signal lunar white glass, to compensate for slight changes in the operation of the instrument.) The average deviation from the mean was 0.4 per cent, and the maximum deviation 1.0 per cent.

The ratio of extinction values was calculated from point readings taken at 465 mμ and 495mμ, which lie on opposite sides of the absorption peak. This ratio ($E_{465m\mu}/E_{495m\mu}$) is $0.96 \pm .01$ (see Table 1).

A chloroform solution of D&C Orange No. 17, stored for three days in the dark, gave spectrophotometric data identical with that of freshly prepared solutions.

APPLICATION TO COMMERCIAL SAMPLES

Two samples of D&C Orange No. 17 (straight colors) and one sample of D&C Orange No. 17 (barium lake) were analyzed spectrophotometrically. Weighed samples were dissolved in chloroform by refluxing on the steam bath. For the lake, the dye solution was filtered through a fine sintered glass crucible to remove the substrate, transferred to 200 ml. volumetric flasks and made to volume when at room temperature. Extinction measurements were made on suitably diluted aliquots. The curves are shown in Chart 2, and the data given in Table 2.

DISCUSSION

Table 1 shows that the extinction per milligram per liter for chloroform solutions of D&C Orange No. 17 at 479 mμ, containing 3 to 16 mg. of

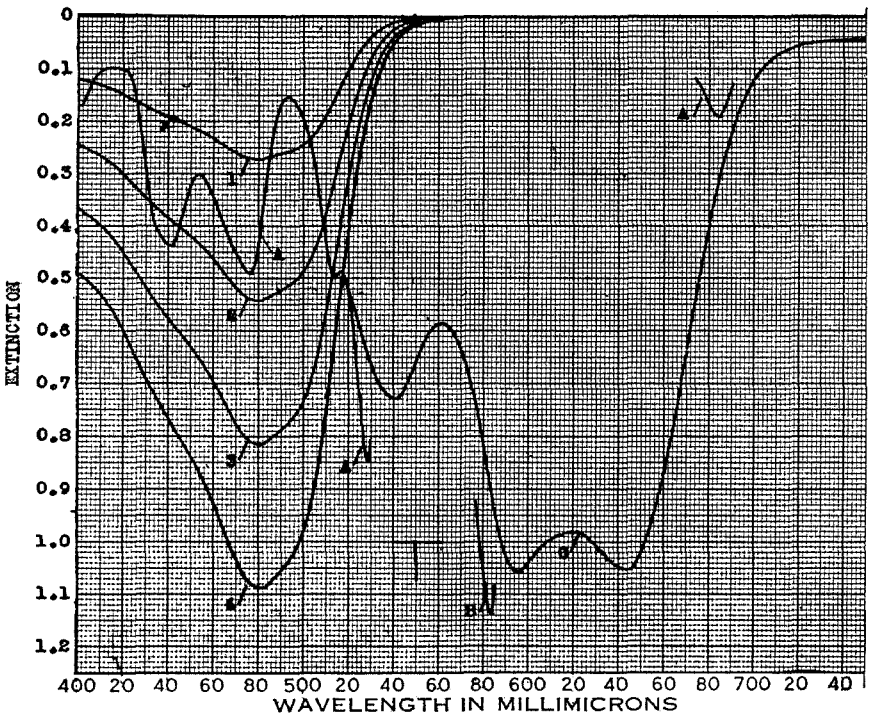

CHART 1.—Solutions of D&C Orange No. 17 in Chloroform.

Curve 1— 3.51 mg/liter
Curve 2— 7.03 mg/liter
Curve 3—10.54 mg/liter
Curve 4—14.06 mg/liter
Cells—1 cm

A = Corning Didymium Glass 512, 6.0 mm. (Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ.)
B = Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 mμ.)
C = Signal Lunar White Glass-H-6946236.

color, is constant. The dye solution obeys Beer's law within this range of concentration. The "pure dye" content of samples of this color may be obtained from the extinction of chloroform solutions of the samples at 479 mμ, by the use of the extinction per milligram per liter as determined for the pure dye.

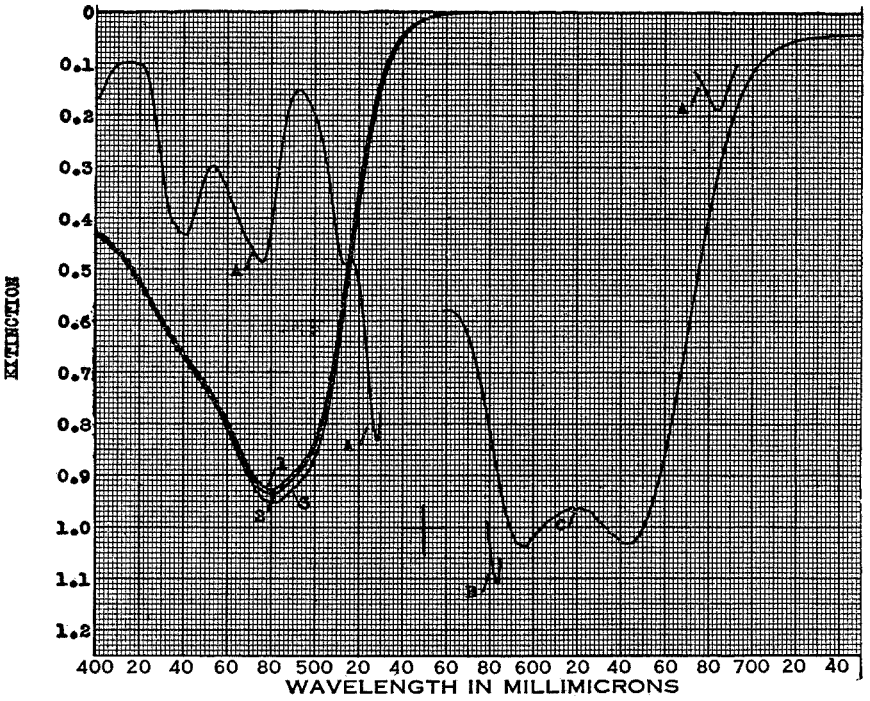

CHART 2.—Certified Samples of D&C Orange No. 17.

Curve 1—13.48 mg/liter
Curve 2—13.02 mg/liter
Curve 3—13.06 mg/liter
Cells—1 cm

A = Corning Didymium Glass 512, 6.0 mm. (Absorption peaks at 400.4, 441.4, 477.1, 529.0, and 684.8 mμ.)
B = Corning Didymium Glass 592, 4.02 mm. (Absorption peak at 583.7 mμ.)
C = Signal Lunar White Glass-H-6946236.

TABLE 2.—*Analysis of certified samples of D&C Orange No. 17*

SAMPLE NO.	M.P.	CONCENTRATION OF SAMPLE	$E_{479\,m\mu}$	DYE SPECTROPHOTOMETRICALLY	DYE FROM NITROGEN CONTENT
	°C.	*mg/liter*		*per cent*	*per cent*
1	Lake*	13.48	.928	91.0	90.0
2	300–1	13.02	.938	95.2	96.2
3	302–3	13.06	.952	96.3	94.4

* Substratum—barium sulfate.

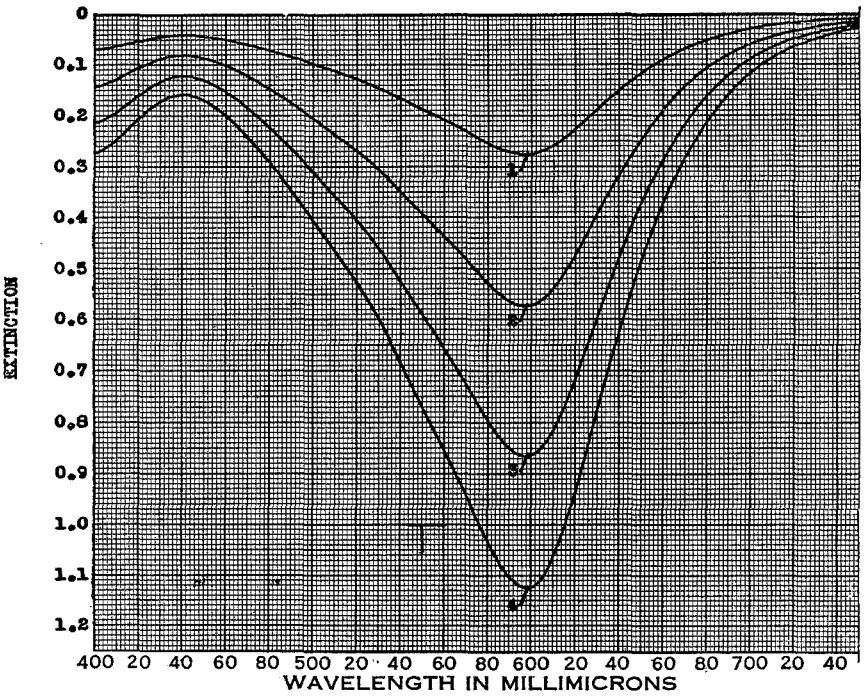

CHART 3.—D&C Orange No. 17 in Alcoholic Base.

Curve 1— 3.38 mg/liter
Curve 2— 6.76 mg/liter
Curve 3—10.14 mg/liter
Curve 4—13.52 mg/liter
Cells—1 cm

TABLE 3.—*D&C Orange No. 17—Alcoholic KOH*

Typical Data

CURVE NO. (CHART 3)	CONCENTRATION MG/LITER	EXTINCTION 596 mμ	$\frac{E_{596\,m\mu}}{\text{CONCENTRATION}}$
1	3.38	not used	
2	6.76	.574	.0865
3	10.14	.866	.0870
4	13.52	1.124	.0847
Av.			.0861

D&C Orange No. 17 in Alcoholic Base

Basic alcohol solutions of D&C Orange No. 17 are bluish-purple. The presence of two nitro groups in the dye molecule probably causes this color. To check the conformity of these solutions to Beer's law 5, 10, 15, and 20 ml aliquots of a solution containing about 13 mg of pure dye in 100 ml of chloroform were diluted to 20 ml with chloroform, made to 200 ml. with 0.02 *N* alcoholic potassium hydroxide; the solutions were permitted to age for fifteen minutes, and the spectrophotometic curve was then determined.

A representative set of extinction curves for the four solutions above is shown in Chart 3; data for these curves are given in Table 3. The major absorption peak is at 596 ± 2 mμ. Aging the solution for one hour does not affect the curve qualitatively; the extinction, however, decreases continuously with time.

Chloroform was used to dissolve the dye, since the dye is insufficiently soluble in alcohol. However if more than 10% chloroform is used, the shape of the curve, as well as the extinction, changes in a period of less than one hour.

The data for the least concentrated solutions were much more variable than data for the remaining solutions, and were therefore discarded. The average extinction per milligram for solutions prepared in the above manner is .0866 (based on the result of 15 determinations). The results for the extinction values of D&C Orange No. 17 in basic solution are not very precise; the extreme variations in the extinction value were $\pm 5\%$. Identification and fairly accurate quantitative determination of D&C Orange No. 17, when mixed with other certifiable oil-soluble orange dyes, appears practicable when alkaline alcoholic solutions are used.

SUMMARY

Spectrophotometric data for chloroform solutions of pure D&C Orange No. 17 are presented. Chloroform solutions of the color follow Beer's law. The absorption peak is at 479 ± 2 mμ. The extinction per milligram per liter at this wave length is $0.0767 \pm .0004$. The extinction ratio, $E_{465m\mu}/E_{495m\mu}$ is $0.96 \pm .01$. Chloroform solutions of the dye are stable for at least three days if stored in the dark.

Basic alcoholic solutions of the dye have a bluish color. In this medium, the dye follows Beer's law to within ± 5 per cent, if the concentration range and time limit indicated are employed.

The data obtained on chloroform solutions of the color was applied to the determination of the "pure dye" content of commercial samples of the color. Typical results are given.

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PRECISION OF SAMPLES AND ANALYSES OF FERTILIZERS AND FEEDS

By S. R. MILES and F. W. QUACKENBUSH* (Purdue University Agricultural Experiment Station, Lafayette, Indiana)†

This report concerns a study of factors which affect the precision of samples and chemical analyses of feeds and fertilizers. The sampling in which we are interested at present is done to determine whether a particular lot of fertilizer or feed meets the manufacturer's guarantee. Estimates are given of the precision attained when cores are drawn from various numbers of bags in lots of several sizes.

A review of literature led to the conclusion that present sampling methods were developed in the absence of much basic information concerning factors which affect representativeness and precision. Jones, Proulx, and Robertson (2) constituted a committee which recommended in 1921: "That cores shall be taken from not less than 10 per cent of the bags present, unless this necessitates cores from more than 20 bags, in which case a core shall be taken from one bag from each additional ton represented. If there are less than 100 bags, not less than 10 bags shall be sampled, provided that in lots of less than 10 bags all bags shall be sampled." This recommendation was adopted as official by the Association of Official Agricultural Chemists (4). This official intensity of sampling remained unchanged until 1947 when, by official, first action (5), larger samples than heretofore are to be taken from lots of less than 200 bags. The minimum number of bags to sample is 20, unless the lot contains fewer than 20 bags, in which case a core is to be taken from each bag. We have been unable to find data to support either intensity of

* We gratefully acknowledge the active participation, in the project reported, of the feed and fertilizer control officials and their staffs in California, Canada, Indiana, Kentucky, Maryland, New Jersey, Ohio, Oregon, and Texas.
† Journal paper 376, Purdue University Agricultural Experiment Station. Presented at the annual meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October 10 to 12, 1949.

sampling. Haigh (1), in 1927, published the analysis of the fertilizer in each of 10 bags from each of 10 different fertilizers, but no statement was made regarding the number of bags which should be sampled. Munch and Bidwell (3) stated that "adequate samples should consist of such a number of individuals as is proportional to the square root of the number of individuals in the different lots," and they added: "For the general run of material, such as flour and feeds, the square root gives an adequate sample." These writers did not define *adequate*. The present research provides basic information for deciding how many bags to sample.

To simplify the presentation, we will first discuss fertilizers only. Later we will consider feeds. Persons interested in feeds should, however, read all the fertilizer discussion, because it includes much information which applies to feed also.

FERTILIZERS

Procedure

Fertilizer was sampled in 7 States, namely: Indiana, Kentucky, Maryland, New Jersey, Ohio, Ontario (Canada), and Texas. Official inspectors sampled 106 batches of fertilizer at 41 plants. In all factories, the mixed fertilizer was taken from curing piles of hundreds or thousands of tons and was ground and bagged. In some plants, the fertilizer was remixed before bagging, while in others it was not. The batches contained 0 to 8% nitrogen, 8 to 20% available phosphoric acid (P_2O_5), and 5 to 20% soluble potash (K_2O). The inspector selected 3 bags from each batch as they came from the bagger. The interval between bags varied from 1 to 700 bags. By an interval of 1 bag, we mean that a second bag was taken immediately after the previous one. Both 80-pound paper bags and 100-pound cloth bags were sampled.

In most States, sampling was done with a standard single-tube trier which was pointed at one end, was slotted along one side, and had an inside diameter of three-quarters inch. It was pushed the length of the bag with the slot down while the bag was lying flat. The slot was then turned up and the tube rotated slightly several times to fill it. Each core so obtained was kept and analyzed separately. From all 106 batches, a single core was taken from 1 top corner to the diagonally opposite bottom corner of each bag. From 21 bags in 7 batches, cores were taken from 3 positions: the first diagonally, the second lengthwise through the center of the bag, and the third lengthwise of one side of the bag. In 33 instances, duplicate cores were taken from a position by inserting the trier a second time along the same path.

In the Indiana laboratory, each core was ground in a high-speed mill so that 96% passed a 0.5-mm sieve, 98% passed a 1-mm sieve, and 100% passed a 2-mm sieve. The ground fertilizer was poured onto a clean paper, mixed by rolling, piled, and quartered. Quarters 1 and 3 were combined,

put into a bottle, and stoppered. Quarters 2 and 4 were put into a second bottle. No reduction in size of the sample core was necessary because cores weighed only 4 to 6 ounces. In Indiana, duplicate analyses were made, one from each bottle. The 2 analyses were made on different days and sometimes by different analysts. If duplicates did not agree reasonably well—by an indefinite and varying standard—analysis was repeated. Several samples were analyzed 4 times and one was analyzed 8 times.

In States other than Indiana, laboratory procedures were somewhat different, but were essentially similar to those described.

All cores were analyzed for soluble potash (K_2O), and many cores were analyzed for nitrogen and available phosphoric acid (P_2O_5), also.

Standard statistical procedures were used in analyzing the data.

Discussion and Results

Determination of suitable methods and intensities of sampling depends on a study of (1) sources of variation and (2) amounts of variation. In some statements, amount of variation is expressed as differences. Usually, however, the standard deviation will be the measure of variation, since it is the best measure. In a large group (or population) of values—of chemical analyses, for example—about two thirds of the values will differ from the average value by less than 1 standard deviation, and about 95% will differ from the average by less than 2 standard deviations. For example, if the standard deviation of the potash analysis of many bags of fertilizer is 0.3% potash, and the true average is 12.0%, we expect approximately two thirds of the analyses of individual bags to be between 11.7% and 12.3%, and we expect about 95% of the bags to analyze between 11.4% and 12.6%. (In wording statistical statements, we sometimes sacrifice absolute rigor for simplicity.)

Two major sources of variation which will be considered are (1) variation due to sampling from a batch or lot and (2) variation in chemical analyses. Accuracy in the analysis found for a lot is contingent upon—and is limited by—the sample submitted to the analyst. If a sample does not have approximately the same chemical composition as the lot sampled, no amount of chemical skill and repeated analysis can give a determination which is accurate for the lot. Therefore, we first consider matters pertinent to the accuracy of the sample.

Sampling variation

If several composite samples were taken from a lot of fertilizer consisting of a few or of many bags, these samples would vary in their true analysis, unless the entire lot were completely homogeneous. This variation would result from: (1) a gradual trend or change in the analysis of successive bags in a batch, if this trend exists, (2) random variation from bag to bag, (3) variation among cores from the same position in a bag, (4) vari-

ation from position to position within bags, and (5) lack of perfection in the process of reducing the sample. In our investigation, this last factor was not at work because each sample was only one core and all of it was given to the chemists after grinding.

If there is a trend in analysis in a batch, one should expect the difference in analysis between 2 bags to be greater if the bags are farther apart as they come from the bagger than if they are close together. Examination of the data indicates practically no relation between the difference in analysis of 2 bags and the interval between those bags. A slight exception is that bags with an interval of 5 or less are somewhat more nearly alike in analysis than bags with a greater interval. The trend is negligible: thus the bag-to-bag variation appears to be almost entirely random. The practical conclusion is that even a small number of bags, say 10 or 20, are about as likely to be representative of a lot of many bags when the small number are successive bags from the bagger as when they are widely scattered in the lot. The effect of the slight tendency for the bags close together in the production line to have similar analyses will probably be almost entirely dissipated by the shuffling of the bags which takes place in shipment. To conclude more specifically: if, in a warehouse, an inspector finds only a few bags, which are a remnant of a large lot, these bags are representative of the large lot. The precision of a sample taken from the remnant bags depends on how many bags are sampled.

Having concluded that bag-to-bag variation is almost entirely random, we are interested in examining the random variation. There is no appreciable relation between bag-to-bag variation and the percentage of a fertilizer constituent. This is true for all three constituents: nitrogen, phosphoric acid, and potash. Therefore, it is reasonable to use a single average value of variation in estimating the precision of samples taken from various numbers of bags, regardless of the percentage of a constituent.

The number of bags sampled from a lot should be sufficient to give an accurate composite sample of fertilizer that is reasonably well mixed. However, no one should expect sampling to be done at an intensity great enough to give accurate samples of poorly mixed fertilizer. A few batches of very poorly mixed fertilizer were excluded in calculating the average bag-to-bag variation which was used in determining how many bags to sample for an accurate composite sample. One batch was omitted from the nitrogen average. In this batch, 2 of the 3 bags sampled differed by 1.2% nitrogen. This is 44% of the average nitrogen content, 2.7%. Two batches were omitted from the phosphoric acid average. In one of these batches, 2 bags differed by 2.0% phosphoric acid; in the other batch, the range was 3.7% phosphoric acid. Seven batches were omitted from the potash average. For these, the range between 2 bags within a batch was 2.1% potash in the least poorly mixed batch, and was 5.2%

potash in the most poorly mixed. Imagine a batch of fertilizer so poorly mixed that one bag contained only 4.3% potash while another bag had 9.7%, a range of 5.2%! This range was 73% of the average composition, 7.1% potash.

Appreciable variation was found between 2 cores taken from the same path. This source of variation must be taken into account in calculating the accuracy of samples. In 3 cases, 2 cores from the same path differed by 1.0%, 1.2%, and 1.7% potash. Obviously, this fertilizer was very poorly mixed.

Cores taken from the 3 positions in bags varied no more than can be accounted for by the variation of duplicate cores from the same path. The positions were: (1) diagonal, (2) lengthwise through the center of bags, and (3) lengthwise along the side. If there were segregation of ingredients in filling bags, we would expect that the center and side positions would differ most in analysis. The data indicate that such segregation did not occur. The positions from which cores were taken did not permit investigation of cross stratification. A bag of fertilizer is filled in 2 or 3 seconds. It seems unlikely that segregation could occur so quickly. The data confirm this expectation.

What is the best position from which to draw the core from a bag? Logic leads to the conclusion that a full-length diagonal core should represent the average analysis of a bag if there is either horizontal or vertical stratification. Our evidence substantiates this conclusion, for the average analysis of all diagonal cores was identical with the average for all center and side cores from the same bags. If there is no separation of ingredients, a diagonal or any other core would be representative.

Variation in analysis

Variation in chemical analysis may result from myriad variations in details of the technic of a chemical determination. Such variation affects the precision of a reported analysis but does not affect the accuracy of a sample.

Amount of variation

The discussion this far has stressed sources of variation, but has also said something about amount of variation, usually measured by differences. Table 1 summarizes the major sources of variation and the amount of variation for each, expressed as a standard deviation. Each value is an estimate of the variation due to the single source named. The variation due to other sources has been removed. These values are the basis of subsequent calculations. For bags and chemical analysis, nitrogen is least variable and potash is most variable. For all three constituents, bag-to-bag variation is greater than that due to other sources. The variation of chemical analysis is large for potash.

Application of the results

It is necessary to distinguish between a true analysis and an analysis from a chemical determination. The true analysis is just what the term says. The analysis from a chemical determination we call the analysis *found*. The true analysis of a lot—or even of a small sample—can never be known exactly because of imperfection in the chemical procedure of analysis. (The true analysis is approached closely, however, by the average of several chemical determinations.) While the true analysis of a sample,

TABLE 1.—*Three fertilizer constituents: Net standard deviation associated with 4 sources of variation*

SOURCE OF VARIATION	STANDARD DEVIATION[1]		
	NITROGEN	PHOSPHORIC ACID	POTASH
A	B	C	D
	per cent	*per cent*	*per cent*
Bags within batch	0.12	0.19	0.32
Cores from same path	.06	.15	.13
Positions within bag	—[2]	—[2]	.00
Chemical analysis	.06	.08	.28

[1] Square root of component of variance.
[2] No data are available.

bag, or lot cannot be determined, we can ascertain what fraction of the true analyses of many samples, bags, etc. will lie between any specified limits.

Table 2 shows the precision of *composite samples* for various intensities of sampling from lots of several sizes. This table pertains to the precision of true analyses of composite samples. The precision is expressed as *1-in-20 one-way deviations*. An illustration will explain the meaning of these deviations. For a lot of 21 bags (column A), of which 15 are sampled (column B), one core being taken per bag sampled (column C), we find 0.04% nitrogen in column D. The interpretation is that we expect only one composite sample in 20 (on the average) to contain as much as 0.04% nitrogen *less* than the entire lot of 21 bags; also, we expect only one composite sample in 20 to contain as much as 0.04% nitrogen *more* than the entire lot. Two times in 20, or 1 in 10, we expect the sample to contain either 0.04% less or 0.04% more than the lot. Other entries in Table 2 are interpreted in a parallel manner.

RECOMMENDATION

From a study of Table 2, we recommend that the following be considered for adoption as a part of the official sampling procedure for fertilizers.

From a lot of 1 to 10 bags, sample all bags;
From a lot of 11 to 20 bags, sample 10 bags;

TABLE 2.—*Three fertilizer constituents: Estimates of deviations—positive only or negative only—of the true percentage of the composite sample from the true lot-average percentage, which will be exceeded in 5% of cases (1 time in 20)*

BAGS IN LOT	BAGS SAMPLED	CORES PER BAG SAMPLED	COMPOSITE SAMPLE DEVIATION FOR[1]—		
			NITROGEN	PHOSPHORIC ACID	POTASH
A	B	C	D	E	F
number	*number*	*number*	*per cent*	*per cent*	*per cent*
1	1*	1	0.09	0.25	0.21
1	1†	5	.04	.11	.10
2	2†	3	.04	.10	.09
3	3†	2	.04	.10	.09
4	4†	2	.03	.09	.08
5	5*†	1	.04	.11	.09
10	5	1	.07	.15	.19
10	10*†	1	.03	.08	.07
11	10†	1	.03	.08	.08
20	5	1	.09	.17	.23
20	10†	1	.05	.11	.14
20	20*	1	.02	.05	.05
21	15†	1	.04	.08	.09
40	10	1	.06	.12	.16
40	15†	1	.05	.09	.12
40	20*	1	.04	.08	.10
41	20†	1	.04	.08	.10
60	10	1	.06	.12	.17
60	20*†	1	.04	.08	.11
100	10	1	.07	.12	.17
100	20*†	1	.05	.08	.12
200	20*†	1	.05	.09	.12
400	30*	1	.04	.07	.10
1,000[2]	5	1	.10	.18	.25
1,000[2]	10	1	.07	.13	.18
1,000[2]	15	1	.05	.10	.15
1,000[2]	20†	1	.05	.09	.13
1,000[2]	30	1	.04	.08	.10
1,000[2]	50	1	.03	.06	.08
1,000[2]	60*	1	.03	.05	.07
1,000[2]	1,000	1	.003	.008	.007

[1] These deviations for composite samples apply to true values, not to analyses found from chemical determinations.

[2] Deviations are the same for lots of more than 1,000 bags as for lots of that size.

* Present official sampling intensity. For lots of more than 200 bags, the number to sample applies strictly to 100-lb bags only.

† Recommended sampling intensity. This does not depend on bag weight.

NOTE: To estimate corresponding standard deviations, divide above values by 1.65 or multiply by 0.606. To estimate corresponding 1-in-100 deviations, multiply above values by 1.4.

From a lot of 21 to 40 bags, sample 15 bags;
From a lot of 41 or more bags, sample 20 bags.

Take one full-length diagonal core per bag sampled, except that for lots of 1 to 4 bags, take enough cores per bag—from the same path—to total 5 or more cores from the lot.

Table 3 shows the same recommendations in table form.

TABLE 3.—*Recommended sampling intensities*

BAGS IN LOT	BAGS TO SAMPLE	CORES PER BAG SAMPLED
number	*number*	*number*
1	1	5
2	2	3
3	3	2
4	4	2
5	5	1
6 to 10	All	1
11 to 20	10	1
21 to 40	15	1
41 or more	20	1

DISCUSSION OF RECOMMENDATION

The recommended sampling is indicated by daggers in column B of Table 2, which also shows the precision attained by such sampling. Since the potash samples are usually least precise, let us discuss them first. For a lot of 1,000 bags and the recommended sample from 20 bags, the 1-in-20 deviation is 0.13% potash. We think this is precise enough. The previous official sampling method required sampling 60 bags—3 times as many—yet reduced the deviation only 0.06% potash. The gain in precision is not worth the work of sampling 40 additional bags.

For the recommended method, the deviation does not exceed 0.14% potash for a lot of any size. Phosphoric acid deviations are slightly smaller than potash deviations when only part of the bags in a lot are sampled. Nitrogen deviations are less than half those for potash.

As stated previously, a few bags which are a remnant of a large lot are representative of the large lot; and the precision of a sample taken from the remnant bags depends on the number of bags sampled. Table 4 shows the precision attained by composite samples of certain sizes from large lots: 400 bags up, without limit. This table applies whether the entire lot or only the number of bags sampled is present. Table 2 gives the precision for certain smaller lots. Table 4 (like Table 2) indicates precision as deviations of the true composite sample analysis from the true lot-average analysis. Both 1-in-20 one-way deviations and 1-in-100

one-way deviations are given. (Here and elsewhere, 1-in-100 deviations are 1.41 times 1-in-20 deviations.) Small samples from large lots give surprisingly precise composite samples.

The best and only information about the composition of a lot of fertilizer is the analysis *found.* Therefore, we should consider the reliability

TABLE 4.—*Three fertilizer constituents: Precision of samples from lots of 400 or more bags*

BAGS SAMPLED	CORES PER BAG SAMPLED	COMPOSITE SAMPLE DEVIATION[1]	
		1-IN-20	1-IN-100
number	*number*	*per cent*	*per cent*
	Nitrogen		
60	1	0.03	0.04
20	1	.05	.07
10	1	.07	.10
5	1	.10	.14
1	5	.20	.28
	Phosphoric acid		
60	1	.05	.07
20	1	.09	.13
10	1	.13	.18
5	1	.18	.26
1	5	.34	.48
	Potash		
60	1	.07	.10
20	1	.13	.18
10	1	.18	.25
5	1	.25	.35
1	5	.54	.76

[1] These deviations are explained in the text and in the title of Table 2.

of such analyses by inspecting Tables 5, 6, and 7, columns E, F, and G. These data show the precision of analyses *found* when 1, 3, or 5 chemical analyses are made of a composite sample. The values in column D apply to the precision of the *true* analysis of the composite sample; these data are copied from Table 2. The deviations in columns E, F, and G include the variation in composite samples plus the variation in chemical determinations.

Let us again compare the recommended with the previous official sampling intensities by means of Table 7, for potash. For a lot of 1,000 bags, a *sample* of 60 bags (previous official method) has a precision of 0.07%

TABLE 5.—*Nitrogen: Estimates of deviations—positive only or negative only—from the true lot-average percentage, which will be exceeded in 5% of cases (1 time in 20)*

BAGS IN LOT	BAGS SAMPLED	CORES PER BAG SAMPLED	DEVIATION OF—			
			COMPOSITE SAMPLE[1]	CHEMICAL ANALYSIS FOUND FROM[1]—		
				1 ANALYSIS	3 ANALYSES	5 ANALYSES
A	B	C	D	E	F	G
number	*number*	*number*	*per cent*	*per cent*	*per cent*	*per cent*
1	1*	1	0.09	0.14	0.11	0.10
1	1†	5	.04	.11	.07	.06
2	2†	3	.04	.11	.07	.06
3	3†	2	.04	.11	.07	.06
4	4†	2	.03	.10	.07	.05
5	5*†	1	.04	.11	.07	.06
10	5	1	.07	.12	.09	.09
10	10*†	1	.03	.10	.06	.05
11	10†	1	.03	.10	.07	.05
20	5	1	.09	.13	.10	.10
20	10†	1	.05	.11	.08	.07
20	20*	1	.02	.10	.06	.05
21	15†	1	.04	.11	.07	.06
40	10	1	.06	.12	.08	.08
40	15†	1	.05	.11	.07	.06
40	20*	1	.04	.11	.07	.06
41	20†	1	.04	.11	.07	.06
60	10	1	.06	.12	.09	.08
60	20*†	1	.04	.11	.07	.06
100	10	1	.07	.12	.09	.08
100	20*†	1	.05	.11	.07	.06
200	20*†	1	.05	.11	.07	.06
400	30*	1	.04	.11	.07	.06
1,000[2]	5	1	.10	.14	.11	.11
1,000[2]	10	1	.07	.12	.09	.08
1,000[2]	15	1	.05	.11	.08	.07
1,000[2]	20†	1	.05	.11	.08	.07
1,000[2]	30	1	.04	.11	.07	.06
1,000[2]	50	1	.03	.10	.07	.05
1,000[2]	60*	1	.03	.10	.06	.05
1,000[2]	1,000	1	.003	.10	.06	.04

[1] The deviations for composite samples (column D) apply to true values, while the deviations in columns E to G apply to analyses found from chemical determinations.

[2] Deviations are the same for lots of more than 1,000 bags as for lots of that size.

* Present official sampling intensity. For lots of more than 200 bags, the number to sample applies strictly to 100-lb bags only.

† Recommended sampling intensity. This does not depend on bag weight.

NOTE: To estimate corresponding standard deviations, divide above values by 1.65 or multiply by 0.606. To estimate corresponding 1-in-100 deviations, multiply above values by 1.4.

TABLE 6.—*Phosphoric acid: Estimates of deviations—positive only or negative only—from the true lot-average percentage, which will be exceeded in 5% of cases (1 time in 20)*

BAGS IN LOT	BAGS SAMPLED	CORES PER BAG SAMPLED	DEVIATION OF—			
			COMPOSITE SAMPLE[1]	CHEMICAL ANALYSIS FOUND FROM[1]—		
				1 ANALYSIS	3 ANALYSES	5 ANALYSES
A	B	C	D	E	F	G
number	*number*	*number*	*per cent*	*per cent*	*per cent*	*per cent*
1	1*	1	0.25	0.28	0.26	0.26
1	1†	5	.11	.18	.14	.13
2	2†	3	.10	.17	.13	.12
3	3†	2	.10	.17	.13	.12
4	4†	2	.09	.17	.12	.11
5	5*†	1	.11	.18	.14	.13
10	5	1	.15	.21	.17	.16
10	10*†	1	.08	.16	.11	.10
11	10†	1	.08	.16	.12	.10
20	5	1	.17	.22	.19	.18
20	10†	1	.11	.18	.13	.12
20	20*	1	.05	.15	.10	.08
21	15†	1	.08	.16	.11	.10
40	10	1	.12	.18	.14	.13
40	15†	1	.09	.17	.12	.11
40	20*	1	.08	.16	.11	.10
41	20†	1	.08	.16	.11	.10
60	10	1	.12	.19	.15	.14
60	20*†	1	.08	.16	.11	.10
100	10	1	.12	.19	.15	.14
100	20*†	1	.08	.16	.12	.10
200	20*†	1	.09	.17	.12	.11
400	30*	1	.07	.16	.11	.10
1,000[2]	5	1	.18	.23	.20	.19
1,000[2]	10	1	.13	.19	.15	.14
1,000[2]	15	1	.10	.17	.13	.12
1,000[2]	20†	1	.09	.17	.12	.11
1,000[2]	30	1	.08	.16	.11	.10
1,000[2]	50	1	.06	.15	.10	.09
1,000[2]	60*	1	.05	.15	.10	.08
1,000[2]	1,000	1	.008	.14	.08	.06

See footnotes to Table 5.

TABLE 7.—*Potash: Estimates of deviations—positive only or negative only—from the true lot-average percentage, which will be exceeded in 5% of cases (1 time in 20)*

BAGS IN LOT	BAGS SAMPLED	CORES PER BAG SAMPLED	DEVIATION OF—			
			COMPOSITE SAMPLE[1]	CHEMICAL ANALYSIS FOUND FROM[1]—		
				1 ANALYSIS	3 ANALYSES	5 ANALYSES
A	B	C	D	E	F	G
number	*number*	*number*	*per cent*	*per cent*	*per cent*	*per cent*
1	1*	1	0.21	0.51	0.34	0.30
1	1†	5	.10	.47	.28	.23
2	2†	3	.09	.47	.28	.22
3	3†	2	.09	.47	.28	.22
4	4†	2	.08	.47	.28	.22
5	5*†	1	.09	.47	.28	.23
10	5	1	.19	.50	.33	.28
10	10*†	1	.07	.46	.27	.22
11	10†	1	.08	.47	.28	.22
20	5	1	.23	.51	.35	.30
20	10†	1	.14	.48	.30	.25
20	20*	1	.05	.46	.27	.21
21	15†	1	.09	.47	.28	.22
40	10	1	.16	.49	.31	.26
40	15†	1	.12	.47	.29	.24
40	20*	1	.10	.47	.28	.23
41	20†	1	.10	.47	.28	.23
60	10	1	.17	.49	.31	.26
60	20*†	1	.11	.47	.29	.23
100	10	1	.17	.49	.32	.27
100	20*†	1	.12	.47	.29	.24
200	20*†	1	.12	.47	.29	.24
400	30*	1	.10	.47	.28	.23
1,000[2]	5	1	.25	.52	.37	.33
1,000[2]	10	1	.18	.49	.32	.27
1,000[2]	15	1	.15	.48	.30	.25
1,000[2]	20†	1	.13	.48	.29	.24
1,000[2]	30	1	.10	.47	.28	.23
1,000[2]	50	1	.08	.47	.28	.22
1,000[2]	60*	1	.07	.46	.27	.22
1,000[2]	1,000	1	.007	.46	.27	.21

See footnotes to Table 5.

potash, while a *sample* of 20 bags (recommended method) has a precision of 0.13% potash. The difference is 0.06% potash. But the analysis *found* is only 0.02% potash less from sampling 60 bags than from 20. This is true whether 1, 3, or 5 analyses are made. This negligible improvement in precision is further evidence—and strong evidence—that the recommended sampling is adequate.

The futility of sampling many bags is shown further on the last line of Tables 5, 6, and 7. A sample practically free of error is secured by sampling every bag in a lot of 1,000. Yet the deviations of analyses *found* are only slightly smaller than when only 20 bags are sampled.

Table 8 presents additional information about the variation reported

TABLE 8.—*Deviations due to sampling variation only, to analytical variation only, and to both combined*[1]

CONSTITUENT	CHEMICAL ANALYSES MADE MADE	1-IN-20 ONE-WAY DEVIATION DUE TO—		
		SAMPLE VARIATION ONLY	ANALYTICAL VARIATION ONLY	BOTH SAMPLING AND ANALYTICAL VARIATION
A	B	C	D	E
	number	*per cent*	*per cent*	*per cent*
Nitrogen	1	0.05	0.10	0.11
Nitrogen	3	.05	.06	.08
Nitrogen	5	.05	.04	.07
Phosphoric acid	1	.09	.14	.17
Phosphoric acid	3	.09	.08	.12
Phosphoric acid	5	.09	.06	.11
Potash	1	.13	.46	.48
Potash	3	.13	.26	.29
Potash	5	.13	.21	.24

[1] Data apply to a sample drawn from 20 bags in a lot of 400 or more bags, but samples and lots of other sizes would show similar trends.

in Tables 5, 6, and 7. The data in Table 8 are specific for a sample taken from 20 bags in a lot of 400 or more bags, but samples and lots of other sizes would show similar trends. All the data are 1-in-20 one-way deviations. The values in column C are copied from column D of Tables 5, 6, and 7. These deviations are due to variation in sampling *only*. Column D data have not been shown before. These deviations are due to variation in chemical analysis *only*. Column E data are copied from columns E, F, and G, of Tables 5, 6, and 7. These deviations are due to the combination of sampling and analytical variation. The table shows that the combined effect is less than the sum of the 2 separate effects. When only one analysis is made, the variation associated with analysis contributes the major portion of the total. Additional analyses reduce the contribution of the analytical variation but have no effect on the deviations due to sampling

only. A large number of analyses will reduce the deviation due to analysis to insignificance; sample variation then accounts for practically all of the variation in an analysis *found* (column E). The deviations due to analysis only (column D) are considerably larger for potash than for nitrogen and phosphoric acid. This fact reflects the low average precision of potash analyses.

Each datum in column E of Tables 5, 6, and 7 is a good estimate of the precision when only one chemical analysis is made. However, the estimates in columns F and G—for 3 and 5 analyses, respectively—are probably slightly too large. This is because when 2 or more determinations are made, the chemist can discover gross errors. He discards these, and so usually improves the analytical precision. (But the chemist should realize that values which are discarded because they are poor checks are occasionally more nearly correct than the good checks which are kept.)

The deviations of composite samples in the tables are calculated from the average bag-to-bag variation found in the investigation reported herein. If a lot is less variable than this average, the precision of samples will be greater than indicated. On the contrary, if variation is greater than average, the precision will be less. Analyses of fertilizer from a manufacturer whose product varies greatly will be reported high (or low) more often than if the product varied less. To avoid injury to his reputation and the paying of refunds for deficiencies, a manufacturer who has great bag-to-bag variation must do one of two things. He must mix more thoroughly or produce fertilizer which averages well above guarantee. In this investigation, the fertilizer averaged 0.1% nitrogen above guarantee, 0.1% phosphoric acid below guarantee, and 0.6% potash above guarantee.

The data in the tables depend on the assumption that the true analysis of the reduced sample supplied the chemist is identical with that of the entire composite sample. To realize this condition, great care is essential in mixing and reducing the sample. Bias due to imperfection in this procedure will reduce the precision of both the reduced sample and the analysis found.

FEEDS

The discussion of feeds will parallel that of fertilizers. The principles of sampling for precision are similar for the two products, and many of the results are similar in a general way, though different in degree. Only those matters will be touched on which are more or less peculiar to feeds.

Procedure

Feeds were sampled in 6 States, namely: California, Indiana, Kentucky, Maryland, Ohio, and Oregon. Official inspectors sampled 128 batches of feeds of many kinds in 63 plants.

Samples were drawn from bags as they came from the bagger, but after they were sewed or tied shut. Most bags contained 100 pounds. Several

of the feeds were mixed in "runs" by continuous mixers, but most of them were mixed in batch mixers. The batches and runs varied in size from 400 pounds to 30 tons. Most batches were bagged directly from the mixer. However, at a few plants the mixed feed was run from batch mixers into a bin from which the bagging was done. This procedure resulted in more or less mixing of different batches. Three or more bags were sampled from each batch or run. In a few cases, 2 successive batches of the same feed were sampled. The interval between successive bags sampled varied from 1 to 320 bags. One full-length, diagonal core was drawn from each bag sampled. A second core was taken from the same path in a few bags. Cores were not drawn from different positions. Cores were drawn with the same trier used for fertilizer.

Cores were left separate and were ground for chemical analysis. All samples were analyzed for protein, and some were analyzed for fat, fiber, and ash. Duplicate determinations were made on different days and often by different chemists. In some cases more than 2 analyses were made. The feeds contained 9% to 45% protein, 2.2% to 6.3% fat, 4% to 14% fiber, and 0.7% to 14% ash.

Results and Discussion

Sampling variation

For feed, as for fertilizer, it was learned that bags close together as they come from the bagger are about as variable as bags far apart. This was true for all 4 constituents studied. As for fertilizers, the practical conclusion is that if, in a warehouse, an inspector finds only a few bags, which are a remnant of a large lot, these bags are representative of the large lot.

A single average value of bag-to-bag variation for each constituent is used to estimate the precision of samples, because there was not much greater variation in feeds of high analysis than in those of low analysis of a constituent. For the reason given for fertilizer, a very few batches were omitted in computing the average bag-to-bag variation. No protein analyses were omitted. Two batches were omitted from the fat average. In one batch, the fat of 2 bags differed by 1.4 percentage points and in the other by 1.1 points. These differences were 25% of the average fat content in each case. One batch was omitted from the fiber average. Two bags in that batch differed by 2.0 percentage points. This was 16% of the average fiber in the batch. One batch was omitted for ash. Two bags differed by 6.1 percentage points, which was 45% of the average ash.

Amount of variation

Table 9 shows the net standard deviation for bag-to-bag variation within batches and for chemical analysis. This is similar to Table 1 for

fertilizer. Table 9 gives no measure of variation among positions within bags because all cores were from a single position. Also, variation among cores from the same path is omitted because so few duplicate cores were drawn that the estimate of variation was unreliable. The variation among bags within batch for feed includes the variation among cores from the same path.[1]

Table 9 gives standard deviations for the 4 constituents protein, fat,

TABLE 9.—*Four feed constituents: Net standard deviation associated with 2 sources of variation*

SOURCE OF VARIATION	STANDARD DEVIATION FOR[1]—				
	PROTEIN	NITROGEN[2]	FAT	FIBER	ASH
A	B	C	D	E	F
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Bags within batch	0.60	0.10	0.06	0.21	0.25
Chemical analysis	.33	.05	.16	.19	.31

[1] Square root of component of variance.
[2] Nitrogen values are the protein values divided by 6.25. Protein percentage in a feed is determined by making a chemical analysis for nitrogen and multiplying the percentage nitrogen by 6.25.

fiber, and ash. In addition, data are shown for nitrogen. This is done because to determine protein percentage, the chemical analysis is for nitrogen; then the nitrogen percentage is multiplied by 6.25 to obtain the protein percentage. Any imperfections in the chemical technic for determining nitrogen are multiplied by 6.25 in the protein. Therefore, we are not surprised that the standard deviations for protein are higher than for any other constituent of feed or fertilizer. The standard deviation of nitrogen analyses is 0.06% in fertilizer and 0.05% in feed. These values are so similar that we can conclude that the accuracy of chemical analysis for nitrogen is equal in the two products.

Table 9 shows that bag variation is greater than chemical analysis variation for protein, nitrogen, and fiber. For fiber, the difference is small. On the contrary, for fat and ash the chemical analysis variation is greater than bag variation.

RECOMMENDATION AND APPLICATION OF THE RESULTS

Table 10, which is similar to Table 2, shows the precision of *composite* feed samples for various intensities of sampling from lots of several sizes. From a study of this table, the authors recommend the same sampling

[1] The inclusion of variation for bags and cores in 1 value causes no difficulty in estimating the precision of composite samples when cores are drawn from fewer than about 5% of the bags in a lot. When a larger proportion of the bags is sampled, the precision of composite samples would be overestimated (i.e., the variation underestimated) unless the core variation and the bag variation are separated. Therefore, it was necessary to estimate core variation. The component of variance for cores was taken as 33% of that for bags. The figure for fertilizer was 30%. The 33% may be an overestimate. If so, the 1-in-20 deviations in Tables 10 to 16 are slightly too large for cases in which more than 5% of the bags in a lot are sampled. In other words: for these cases, the correct 1-in-20 deviations are slightly less than those in the tables.

TABLE 10.—*Four feed constituents: Estimates of deviations—positive only or negative only—of the true percentage of the composite sample from the true lot-average percentage, which will be exceeded in 5% of cases (1 time in 20)*

BAGS IN LOT A	BAGS SAMPLED B	CORES PER BAG SAMPLED C	COMPOSITE SAMPLE DEVIATION FOR[1]— PROTEIN D	NITROGEN[2] E	FAT F	FIBER G	ASH H
number	*number*	*number*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	1*	1	0.50	0.08	0.05	0.17	0.21
1	1†	5	.22	.04	.02	.08	.09
2	2†	3	.20	.03	.02	.07	.08
3	3†	2	.20	.03	.02	.07	.08
4	4†	2	.18	.03	.018	.06	.07
5	5*†	1	.22	.04	.02	.08	.09
10	5	1	.35	.06	.04	.08	.15
10	10*†	1	.16	.03	.02	.05	.07
11	10†	1	.18	.03	.02	.06	.07
20	5	1	.40	.06	.04	.08	.17
20	10†	1	.25	.04	.02	.09	.10
20	20*	1	.11	.02	.01	.04	.05
21	15†	1	.18	.03	.02	.06	.07
40	10	1	.28	.04	.03	.10	.12
40	15†	1	.22	.04	.02	.08	.09
40	20*	1	.18	.03	.02	.06	.07
41	20†	1	.18	.03	.02	.06	.07
60	10	1	.29	.05	.03	.10	.12
60	20*†	1	.19	.03	.02	.07	.08
100	10	1	.30	.05	.03	.11	.12
100	20*†	1	.20	.03	.02	.07	.08
200	20*†	1	.21	.03	.02	.07	.09
400	30*	1	.18	.03	.02	.06	.07
1,000[3]	5	1	.44	.07	.04	.15	.18
1,000[3]	10	1	.31	.05	.03	.11	.13
1,000[3]	15	1	.26	.04	.03	.09	.11
1,000[3]	20†	1	.22	.04	.02	.08	.09
1,000[3]	30	1	.18	.03	.02	.06	.07
1,000[3]	50	1	.14	.02	.01	.05	.06
1,000[3]	60*	1	.12	.02	.01	.05	.05
1,000[3]	1,000	1	.016	.003	.002	.005	.007

[1] These deviations for composite samples apply to true values, not to analyses found from chemical determinations.

[2] Nitrogen deviations are the protein deviations (to 4 decimal places) divided by 6.25. Chemical *analysis* is made for nitrogen, and protein percentage is calculated by multiplying the percentage nitrogen by 6.25.

[3] Deviations are the same for lots of more than 1,000 bags as for lots of that size.

* Present official sampling intensity. For lots of more than 200 bags, the number to sample applies strictly to 100-lb bags only.

† Recommended sampling intensity. This does not depend on bag weight.

NOTE: To estimate corresponding standard deviations, divide above values by 1.65 or multiply by 0.606. To estimate corresponding 1-in-100 deviations, multiply above values by 1.4.

intensities for feed as for fertilizer. For nearly all lot sizes and sampling intensities, all feed constituents, excepting protein but including nitrogen, are more precisely sampled than are potash and phosphoric acid in fertilizer. Because of the high factor for conversion of nitrogen to protein, it seems reasonable to sample for high precision in feed nitrogen but not to expect such precision for protein. To achieve as high precision for protein as for nitrogen *in samples* would require sampling about 40 times as many bags. The number would be prohibitive for large lots. To achieve as high precision for protein in feed samples as for potash in fertilizer samples would require sampling about 3.5 times as many bags.

Table 11 for feed is similar to Table 4 for fertilizer. Both tables show that small samples from large lots give surprisingly precise composite samples.

Tables 12 to 16 for feed are similar to Tables 5 to 7 for fertilizer. Most of the discussion of Tables 5 to 7 applies to Tables 12 to 16. These last tables re-emphasize the major contribution of variation in chemical analyses to the variation of analyses *found,* which are influenced by both sampling and analytical variation.

We previously mentioned the difficulty of obtaining high precision of protein *in samples.* However, this fact is not as important as it may seem, because the precision of analyses *found* for protein may be increased by additional chemical analyses. For example: (See Tables 7 and 12.) If duplicate analyses for protein are made of a feed sample, the 1-in-20 deviation for analysis *found* is about the same as that for potash when only one analysis is made of a fertilizer sample. Also, if 5 analyses for protein are made of a feed sample, the 1-in-20 deviation for analysis *found* is about the same as that for potash when 3 analyses are made of a fertilizer sample.

For the feeds sampled in this investigation, the average protein found was 1.9 percentage points above guarantee, which was 108% of guarantee; the fat averaged 0.5% above guarantee or 109% of guarantee; and the fiber averaged 1.6% below guarantee or 78% of guarantee. By count, 9% of the feeds were below guarantee in protein, 32% were below guarantee in fat, and 20% contained more than the maximum fiber guaranteed.

SUMMARY

An investigation was conducted to determine how many bags of feed or fertilizer should be sampled to obtain a reliable sample. Nine states collaborated in sampling a total of 106 batches of fetilizer at 41 plants and 128 batches of feed at 63 plants and making chemical analyses on the samples taken. Sources and amounts of variations were investigated, and these provide the basis for detailed sampling recommendations.

It was found that a sufficiently precise sample of a lot is usually obtained by taking full-length diagonal cores from a maximum of 20 bags, regardless of how large a number of bags is in the lot. The bags sampled

TABLE 11.—*Four feed constituents: Precision of samples from lots of 400 or more bags*

BAGS SAMPLED	CORES PER BAG SAMPLED	COMPOSITE SAMPLE DEVIATION[1]—	
		1-IN-20	1-IN-100
number	*number*	*per cent*	*per cent*
Protein			
60	1	0.12	0.18
20	1	.22	.31
10	1	.31	.44
5	1	.44	.63
1	5	.99	1.40
Nitrogen in feed			
60	1	.02	.03
20	1	.04	.05
10	1	.05	.07
5	1	.07	.10
1	5	.16	.22
Fat			
60	1	.01	.02
20	1	.02	.03
10	1	.03	.04
5	1	.04	.06
1	5	.10	.14
Fiber			
60	1	.05	.07
20	1	.08	.11
10	1	.11	.15
5	1	.15	.22
1	5	.34	.49
Ash			
60	1	.05	.08
20	1	.09	.13
10	1	.13	.18
5	1	.18	.26
1	5	.41	.58

[1] These deviations are explained in the title of Table 2 and in the text discussion of Table 2.

TABLE 12.—*Protein: Estimates of deviations—positive only or negative only—from the true lot-average percentage, which will be exceeded in 5% of cases (1 time in 20)*

BAGS IN LOT	BAGS SAMPLED	CORES PER BAG SAMPLED	DEVIATION OF—			
			COMPOSITE SAMPLE[1]	CHEMICAL ANALYSIS FOUND FROM[1]—		
				1 ANALYSIS	3 ANALYSES	5 ANALYSES
A	B	C	D	E	F	G
number	*number*	*number*	*per cent*	*per cent*	*per cent*	*per cent*
1	1*	1	0.05	0.73	0.59	0.55
1	1†	5	.22	.58	.38	.33
2	2†	3	.20	.58	.37	.32
3	3†	2	.20	.58	.37	.32
4	4†	2	.18	.57	.36	.30
5	5*†	1	.22	.58	.38	.33
10	5	1	.35	.64	.47	.43
10	10*†	1	.16	.56	.35	.29
11	10†	1	.18	.57	.36	.30
20	5	1	.40	.67	.51	.47
20	10†	1	.25	.59	.40	.35
20	20*	1	.11	.55	.33	.27
21	15†	1	.18	.57	.36	.30
40	10	1	.28	.61	.42	.37
40	15†	1	.22	.58	.38	.33
40	20*	1	.18	.57	.36	.30
41	20†	1	.18	.57	.36	.30
60	10	1	.29	.62	.43	.38
60	20*†	1	.19	.57	.37	.31
100	10	1	.30	.62	.43	.39
100	20*†	1	.20	.58	.37	.32
200	20*†	1	.21	.58	.38	.32
400	30*	1	.18	.57	.36	.30
1,000[2]	5	1	.44	.70	.54	.51
1,000[2]	10	1	.31	.62	.44	.40
1,000[2]	15	1	.26	.60	.40	.35
1,000[2]	20†	1	.22	.58	.38	.33
1,000[2]	30	1	.18	.57	.36	.30
1,000[2]	50	1	.14	.56	.34	.28
1,000[2]	60*	1	.12	.55	.34	.27
1,000[2]	1,000	1	.016	.54	.31	.24

[1] The deviations for composite samples (column D) apply to true values, while the deviations in columns E to G apply to analyses found from chemical determinations.

[2] Deviations are the same for lots of more than 1,000 bags as for lots of that size.

* Present official sampling intensity. For lots of more than 200 bags, the number to sample applies strictly to 100-lb bags only.

† Recommended sampling intensity. This does not depend on bag weight.

NOTE: To estimate corresponding standard deviations, divide above values by 1.65 or multiply by 0.606. To estimate corresponding 1-in-100 deviations, multiply above values by 1.4.

TABLE 13.—*Nitrogen in feed: Estimates of deviations—positive only or negative only—from the true lot-average percentage, which will be exceeded in 5% of cases (1 time in 20)*

BAGS IN LOT	BAGS SAMPLED	CORES PER BAG SAMPLED	DEVIATION OF—			
			COMPOSITE SAMPLE[1]	CHEMICAL ANALYSIS FOUND FROM[1]—		
				1 ANALYSIS	3 ANALYSES	5 ANALYSES
A	B	C	D	E	F	G
number	*number*	*number*	*per cent*	*per cent*	*per cent*	*per cent*
1	1*	1	0.08	0.12	0.09	0.09
1	1†	5	.04	.09	.06	.05
2	2†	3	.03	.09	.06	.05
3	3†	2	.03	.09	.06	.05
4	4†	2	.03	.09	.06	.05
5	5*†	1	.04	.09	.06	.05
10	5	1	.06	.10	.08	.07
10	10*†	1	.03	.09	.06	.05
11	10†	1	.03	.09	.06	.05
20	5	1	.06	.11	.08	.08
20	10†	1	.04	.09	.06	.06
20	20*	1	.02	.09	.05	.04
21	15†	1	.03	.09	.06	.05
40	10	1	.04	.10	.07	.06
40	15†	1	.04	.09	.06	.05
40	20*	1	.03	.09	.06	.05
41	20†	1	.03	.09	.06	.05
60	10	1	.05	.10	.07	.06
60	20*†	1	.03	.09	.06	.05
100	10	1	.05	.10	.07	.06
100	20*†	1	.03	.09	.06	.05
200	20*†	1	.03	.09	.06	.05
400	30*	1	.03	.09	.06	.05
1,000[2]	5	1	.07	.11	.09	.08
1,000[2]	10	1	.05	.10	.07	.06
1,000[2]	15	1	.04	.10	.06	.06
1,000[2]	20†	1	.04	.09	.06	.05
1,000[2]	30	1	.03	.09	.06	.05
1,000[2]	50	1	.02	.09	.05	.04
1,000[2]	60*	1	.02	.09	.05	.04
1,000[2]	1,000	1	.003	.09	.05	.04

See footnotes to Table 12.

TABLE 14.—*Fat: Estimates of deviations—positive only or negative only—from the true lot-average percentage, which will be exceeded in 5% of cases (1 time in 20)*

BAGS IN LOT	BAGS SAMPLED	CORES PER BAG SAMPLED	DEVIATION OF—			
			COMPOSITE SAMPLE[1]	CHEMICAL ANALYSIS FOUND FROM[1]—		
				1 ANALYSIS	3 ANALYSES	5 ANALYSES
A	B	C	D	E	F	G
number	*number*	*number*	*per cent*	*per cent*	*per cent*	*per cent*
1	1*	1	0.05	0.27	0.16	0.13
1	1†	5	.02	.27	.15	.12
2	2†	3	.02	.27	.15	.12
3	3†	2	.02	.27	.15	.12
4	4†	2	.02	.27	.15	.12
5	5*†	1	.02	.27	.15	.12
10	5	1	.04	.27	.16	.12
10	10*†	1	.02	.27	.15	.12
11	10†	1	.02	.27	.16	.12
20	5	1	.04	.27	.16	.13
20	10†	1	.02	.27	.15	.12
20	20*	1	.01	.27	.15	.12
21	15†	1	.02	.27	.15	.12
40	10	1	.03	.27	.16	.12
40	15†	1	.02	.27	.15	.12
40	20*	1	.02	.27	.15	.12
41	20†	1	.02	.27	.15	.12
60	10	1	.03	.27	.16	.12
60	20*†	1	.02	.27	.15	.12
100	10	1	.03	.27	.16	.12
100	20*†	1	.02	.27	.15	.12
200	20*†	1	.02	.27	.15	.12
400	30*	1	.02	.27	.15	.12
1,000[2]	5	1	.04	.27	.16	.13
1,000[2]	10	1	.03	.27	.16	.12
1,000[2]	15	1	.03	.27	.15	.12
1,000[2]	20†	1	.02	.27	.15	.12
1,000[2]	30	1	.02	.27	.15	.12
1,000[2]	50	1	.01	.27	.15	.12
1,000[2]	60*	1	.01	.27	.15	.12
1,000[2]	1,000	1	.002	.27	.15	.12

See footnotes to Table 12.

TABLE 15.—*Fiber: Estimates of deviations—positive only or negative only—from the true lot-average percentage, which will be exceeded in 5% of cases (1 time in 20)*

BAGS IN LOT	BAGS SAMPLED	CORES PER BAG SAMPLED	DEVIATION OF—			
			COMPOSITE SAMPLE[1]	CHEMICAL ANALYSIS FOUND FROM[1]—		
				1 ANALYSIS	3 ANALYSES	5 ANALYSES
A	B	C	D	E	F	G
number	*number*	*number*	*per cent*	*per cent*	*per cent*	*per cent*
1	1*	1	0.17	0.36	0.25	0.22
1	1†	5	.08	.33	.20	.16
2	2†	3	.07	.32	.20	.16
3	3†	2	.07	.32	.20	.16
4	4†	2	.06	.32	.19	.15
5	5*†	1	.08	.33	.20	.16
10	5	1	.08	.33	.20	.16
10	10*†	1	.05	.32	.19	.15
11	10†	1	.06	.32	.19	.15
20	5	1	.08	.33	.20	.16
20	10†	1	.09	.33	.20	.17
20	20*	1	.04	.32	.19	.15
21	15†	1	.06	.32	.19	.15
40	10	1	.10	.33	.21	.17
40	15†	1	.08	.33	.20	.16
40	20*	1	.06	.32	.31	.15
41	20†	1	.06	.32	.31	.15
60	10	1	.10	.33	.21	.17
60	20*†	1	.07	.32	.19	.16
100	10	1	.11	.33	.21	.18
100	20*†	1	.07	.32	.20	.16
200	20*†	1	.07	.32	.20	.16
400	30*	1	.06	.32	.19	.15
1,000[2]	5	1	.15	.35	.24	.21
1,000[2]	10	1	.11	.33	.21	.18
1,000[2]	15	1	.09	.33	.20	.17
1,000[2]	20†	1	.08	.33	.20	.16
1,000[2]	30	1	.06	.32	.19	.15
1,000[2]	50	1	.05	.32	.19	.15
1,000[2]	60*	1	.05	.32	.19	.15
1,000[2]	1,000	1	.005	.32	.18	.14

See footnotes to Table 12.

TABLE 16.—*Ash: Estimates of deviations—positive only or negative only—from the true lot-average percentage, which will be exceeded in 5% of cases (1 time in 20)*

BAGS IN LOT	BAGS SAMPLED	CORES PER BAG SAMPLED	DEVIATION OF—			
			COMPOSITE SAMPLE[1]	CHEMICAL ANALYSIS FOUND FROM[1]—		
				1 ANALYSIS	3 ANALYSES	5 ANALYSES
A	B	C	D	E	F	G
number	*number*	*number*	*per cent*	*per cent*	*per cent*	*per cent*
1	1*	1	0.21	0.56	0.36	0.31
1	1†	5	.09	.52	.31	.25
2	2†	3	.08	.52	.31	.25
3	3†	2	.08	.52	.31	.25
4	4†	2	.07	.52	.31	.24
5	5*†	1	.09	.52	.31	.25
10	5	1	.15	.54	.33	.27
10	10*†	1	.07	.52	.31	.24
11	10†	1	.07	.52	.31	.24
20	5	1	.17	.54	.34	.28
20	10†	1	.10	.53	.32	.25
20	20*	1	.05	.52	.30	.24
21	15†	1	.07	.52	.31	.24
40	10	1	.12	.53	.32	.26
40	15†	1	.09	.52	.31	.25
40	20*	1	.07	.52	.31	.24
41	20†	1	.07	.52	.31	.24
60	10	1	.12	.53	.32	.26
60	20*†	1	.08	.52	.31	.24
100	10	1	.12	.53	.32	.26
100	20*†	1	.08	.52	.31	.25
200	20*†	1	.09	.52	.31	.25
400	30*	1	.07	.52	.31	.23
1,000[2]	5	1	.18	.55	.35	.30
1,000[2]	10	1	.13	.53	.33	.27
1,000[2]	15	1	.11	.53	.32	.25
1,000[2]	20†	1	.09	.52	.31	.25
1,000[2]	30	1	.07	.52	.31	.24
1,000[2]	50	1	.06	.52	.30	.24
1,000[2]	60*	1	.05	.52	.30	.24
1,000[2]	1,000	1	.007	.52	.30	.23

See footnotes to Table 12.

may be any bags in the lot; they need not be widely separated. Therefore, a small number of bags which are a remnant of a large lot are practically as representative of the entire lot as an equal number of bags selected in any way from the entire lot.

The accuracy of the analysis found for a lot is limited by the accuracy of the sample, but is influenced to a considerable extent by variation in chemical analysis.

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NUTRITIVE EVALUATION OF DEFLUORINATED PHOSPHATES AND OTHER PHOSPHORUS SUPPLEMENTS

IV. FURTHER UTILIZATION EXPERIMENTS WITH RATS

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In previous papers of this series based on collaborative studies, Hill, Bird, Ellis, and their associates (1, 2, 3) have reviewed the recent literature and described experiments on the utilization of various phosphatic compounds. The interest in defluorinated phosphates as a source of phosphorus for animal feeds was mainly responsible for focusing attention on the problem. Postwar conditions have increased supplies of bonemeal to near previous levels, but there is still a demand for substitute phosphate carriers.

The results of rat feeding tests presented by Ellis, *et al.* (3) showed that the availability of calcium phosphate in different preparations varied considerably with its form not only as to ortho, meta, and pyro forms, but within certain of these. These findings were interpreted as explaining in considerable part the variations in availability of the defluorinated phosphates which were tested. Black, *et al.* (4) have shown that disodium phosphate is comparable to bonemeal when used as a phosphorus supplement for cattle feeding. This experiment and those of many others with soluble phosphates suggest the desirability of examining various products which have recently become available in quantity such that they could be used for animal feeding. A product that will give good results when

used in drinking water is desirable under some conditions for this purpose.

Although many reports have been made on the availability of various soluble phosphates to rats, variations in diets and methods of assay used make it difficult to obtain satisfactory comparisons. The present experiments were accordingly undertaken to obtain more exact information on the relative biological availability of (1) recently manufactured defluorinated phosphates and (2) newly developed soluble phosphates, by comparing these products with known standards and those phosphates which have been used extensively in animal feeding.

MATERIALS AND METHODS

In previous studies, Ellis, *et al.* (3) have pointed out possible methods of improving biological activity of thermally treated phosphates. Accordingly, the samples of defluorinated phosphates chosen for these tests were selected with the hope of finding improvement in commerical products. The four samples of defluorinated superphosphate tested in series A and D, Table 2, were obtained from different manufacturers of commercial products and represent somewhat different manufacturing conditions. Fox, *et al.* (5) have shown that varying procedures and conditions in producing defluorinated superphosphates affect the character of the finished product. The process used in manufacture of the defluorinated phosphate rock sample included in this study has been described recently by Whitney and Hollingsworth (6). The procedure is based on the laboratory investigation of Reynolds, *et al.* (7). According to this process, ground phosphate rock and silica are fed into a kiln and heated to 2700° to 2900°F. in the presence of water vapor for a few minutes. This removes fluorine and the resulting product is high in silica in the form of cristobalite. Methods for removing the silica with a resulting increase in P content of the product have been developed. However, in this particular sample the silica was allowed to remain in the mixture.

Included in the samples are a number of soluble phosphates which have not been tested biologically and which have become available as possible feeding materials. Those which are relatively new on the market as industrial products, such as sodium tripolyphosphate, sodium acid pyrophosphate, and vitreous sodium metaphosphate, are economically in about the same class as disodium phosphate, which is used as a phosphorus supplement for stock either in drinking water (4) or in dry form. The chemistry, crystalline structure, and nomenclature of these products have been discussed by Partridge, *et al.* (8), and Partridge (9).

The Rhenania phosphates tested are representative of material made by a special calcination process in which soda ash is employed to assist in producing solubility. Development and production of Rhenania phosphate have occurred principally in Germany and have been discussed by Franck, *et al.* (10) and Hawes and Lea (11).

TABLE 1.—*Results of analytical and solubility studies of phosphorus supplements*

SAMPLE NO.	TYPE OF MATERIAL	CONTENT OF—			PHOSPHORUS SOLUBLE IN—	
		P	F	Ca	0.4% HCl	NEUTRAL AMMONIUM CITRATE
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	Steamed bonemeal	14.8	0.07	32	89	78
2	Defluorinated phosphate rock (commercial)	8.7	.16	21	95	87
3	Defluorinated superphosphate (commercial)	13.0	.06	36	78	44
4	Defluorinated superphosphate (commercial)	13.4	.12	32	81	23
5	Defluorinated superphosphate (commercial)	18.9	.04	32	55	39
6	Defluorinated superphosphate (commercial)	13.1	.09	31	88	18
7	Rhenania phosphate (commercial)	10.8	2.76	29	67	96
8	Rhenania phosphate (experimental)	13.5	.10	32	67	90
9	Monocalcium phosphate $Ca(H_2PO_4)_2 \cdot H_2O$[1]	24.6		16	100	100
10	Dicalcium phosphate $CaHPO_4 \cdot 2H_2O$[1]	18.0		23	100	100
11	Dipotassium phosphate K_2HPO_4[1]	17.8			[3]	
12	Disodium phosphate $Na_2HPO_4 \cdot 12H_2O$[1]	8.7			[3]	
13	Sodium acid pyrophosphate $Na_2H_2P_2O_7$	27.7			[3]	
14	Sodium tripolyphosphate $Na_5P_3O_{10}$	25.1			[3]	
15	Vitreous sodium metaphosphate $Na_6P_6O_{18}$	29.3			[3]	
16	Tetra sodium pyrophosphate $Na_4P_2O_7$	23.2			[3]	
17	Insoluble sodium metaphosphate $NaPO_3$	30.1			29	99
18	Dry sugar acid $CaH_4(PO_4)_2 \times H_3PO_4 \times H_2O$	23.2			100	100
19	Phosphoric acid ($75\% H_3PO_4$) + rust inhibitor[2]	23.7				
20	Urea phosphate $(CONH_2)_2H_3PO_4$	19.6			[3]	
21	Monoammonium phosphate $NH_4H_2PO_4$[1]	26.9			[3]	
22	Diammonium phosphate $(NH_4)_2HPO_4$[1]	23.5			[3]	

[1] Regent grade material, Ca and P calculated from molecular formula.
[2] Gave a colorless solution with all proportions of water.
[3] Completely dissolved in water when 1 g was shaken with 100 ml of water.

Phosphorus content and other analytical and solubility data determined on these and other samples are shown in Table 1. Calcium, phosphorus, and fluorine were determined analytically except in case of the reagent grade materials.

The methods employed in evaluating the phosphates were the same as those described previously by Ellis, *et al.* (3). The rats were fed the same basal diet composed of the following ingredients expressed in per cent: blood fibrin 18, lard, 10, dextrinized starch 68.7, agar 2, and salt mixture minus calcium and phosphorus, 1.3. Chemical analysis of this basal diet showed 0.04 per cent phosphorus and 0.06 per cent calcium. Bonemeal or other phosphates of like composition, when added to this diet in amounts of 2 per cent, produced a diet mixture containing approximately

0.30 per cent of phosphorus and 0.60 per cent of calcium. Excellent growth, blood phosphorus within normal limits, and excellent bone development were obtained with rats fed this diet combination, supplemented with all necessary vitamins. Calcium carbonate was added, in an amount necessary to provide twice as much calcium as phosphorus in all diets.

The rats used were weanling albino males and females similar to those described in the former experiments by Ellis, *et al.* (3). Furthermore, the same procedures were followed for feeding, care, and distribution of the animals fed the various diets and for determination of inorganic serum phosphorus and femur ash. Again, because of the number of materials tested, four trials or series of tests were run and are designated in Table 2 by letters. In each of these trials control groups of rats were fed levels of phosphorus known to be available in the form of the normal salt mixure ranging from the full level (0.26 per cent) to one-fourth the full level (0.065 per cent).

RESULTS

In Table 2, the four series of tests referred to in the previous paragraph are designated as A, B, C, and D. Direct comparisons of growth or other criteria should be made only within a series as the rats showed considerable variation from one series to another. To simplify comparison, the right-hand column of the table, designated "response grade," has been provided. It is evident from statistical study of the data that the least variation from one series to another and within each series occurs in the femur ash determinations. Accordingly, this criterion was given the most weight in evaluating the phosphates.

In the results of evaluation of the defluorinated phosphates given in Table 2, it is noteworthy that there is improvement in phosphorus availability over earlier samples of similar products. This is apparent from comparisons of the data on femur ash content, weight gains, and blood phosphorus values in Table 2 with corresponding data in earlier work reported by Ellis, *et al.* (3). It is true that on the average they were found to be less available than the standards. This statement applies especially to the samples in series D, designated 5 and 6. Neither of these appears to be much more than approximately 50 per cent as efficient in availability as the standard salt mixture. However, at least two of the defluorinated superphosphates, numbers 3 and 4, appear to be almost up to the standard of bonemeal or potassium phosphate in availability. The one sample of defluorinated phosphate rock tested in series A also appears to be more available than earlier samples, in fact, the data show it to be about equal to bonemeal in biological availability. These findings indicating general improvement in defluorinated phosphates are in agreement with reports of other workers making practical feeding tests—Hodgson, *et al.* (12) with cattle, and Gillis, *et al.* (13) with chickens.

TABLE 2.—*Availability of different phosphorus supplements for rats*

TEST SERIES	MINERAL SUPPLEMENT SOURCE OF PHOSPHORUS	P ADDED TO DIET	EXPERIMENTAL GROUP MEANS—8 ANIMALS PER GROUP GAIN IN WEIGHT		FEMUR ASH		S.I.P.[3]		RESPONSE GRADE
		per cent	*grams*	*S.E.*[2] ±	*per cent*	*S.E.* ±	*mg/100 ml*	*S.E.* ±	
A	Salt mixture[1] (full level of P)	0.26	132	10.9	61.3	0.21	11.09	0.52	Excellent
	Salt mixture (½ level of P)	.13	128	11.0	55.3	.43	9.05	.36	Fair
	Salt mixture (¼ level of P)	.065	110	7.2	40.6	1.15	7.96	.94	Poor
	Steamed bonemeal	.26	132	9.1	60.6	.35	10.74	.41	Excellent
	Defluorinated phosphate rock	.26	129	10.3	60.4	.12	10.39	.40	Excellent
	Defluorinated superphosphate No. 3	.26	132	10.1	58.1	.68	9.10	.47	Very good
	Defluorinated superphosphate No. 4	.26	130	13.8	59.4	.66	9.17	.37	Very good
B	Salt mixture (full level of P)	.26	91	8.2	64.4	.43	9.63	.48	Excellent
	Salt mixture (½ level of P)	.13	96	6.3	59.2	.55	7.50	.22	Fair
	Salt mixture (¼ level of P)	.065	78	8.0	46.9	1.01	6.36	.19	Poor
	Sodium acid pyrophosphate	.26	71	5.4	62.5	.88	9.53	.29	Very good
	Sodium tripolyphosphate	.26	85	8.1	63.2	.62	9.07	.30	Excellent
	Vitreous sodium metaphosphate	.26	93	6.3	63.2	.28	8.73	.31	Excellent
	Tetrasodium phosphate	.26	81	3.4	63.2	1.00	8.57	.24	Excellent
	Insoluble sodium metaphosphate	.26	52	3.1	43.5	1.50	5.35	.20	Poor
	Dry sugar acid	.26	83	5.2	64.6	.45	9.01	.25	Excellent
C	Salt mixture (full level of P)	.26	105	8.6	63.1	.46	10.13	.19	Excellent
	Salt mixture (¼ level of P)	.065	71	5.9	43.1	.83	5.88	.17	Poor

TABLE 2.—*Continued*

TEST SERIES	MINERAL SUPPLEMENT SOURCE OF PHOSPHORUS	P ADDED TO DIET	EXPERIMENTAL GROUP MEANS—8 ANIMALS PER GROUP						RESPONSE GRADE
			GAIN IN WEIGHT		FEMUR ASH		S.I.P.[3]		
		per cent	*grams*	*S.E.*[2] ±	*per cent*	*S.E.* ±	*mg/100 ml*	*S.E.* ±	
C	Disodium phosphate	.26	75	3.0	62.7	.41	11.01	.27	Very good
	Dipotassium phosphate	.26	102	4.9	61.4	.79	9.02	.20	Very good
	Phosphoric acid	.26	80	3.7	63.2	.27	10.46	.20	Excellent
	Monoammonium phosphate	.26	85	2.1	63.2	.40	9.71	.28	Excellent
	Diammonium phosphate	.26	100	7.3	63.0	.53	9.57	.18	Excellent
	Urea phosphate	.26	76	5.4	62.7	.77	10.54	.37	Very good
	Monocalcium phosphate	.26	94	6.9	63.3	.47	9.09	.26	Excellent
	Dicalcium phosphate	.26	94	9.3	62.9	.56	10.06	.13	Excellent
D	Salt mixture (full level of P)	.26	93	6.1	65.2	.33	9.05	.70	Excellent
	Salt mixture ($\frac{1}{2}$ level of P)	.13	93	9.6	54.2	1.17	8.28	.19	Fair
	Salt mixture ($\frac{1}{4}$ level of P)	.065	78	7.6	44.6	1.97	6.84	.32	Poor
	Rhenania phosphate	.26	19	3.1	57.2	1.13	7.72	.36	Poor
	Rhenania phosphate (experimental)	.26	79	4.5	57.0	1.18	7.80	.35	Good
	Defluorinated superphosphate No. 5	.26	74	5.0	48.6	.60	7.25	.44	Fair
	Defluorinated superphosphate No. 6	.26	71	4.4	53.6	.93	7.65	.37	Fair

[1] Phosphorus was supplied in this salt mixture as monopotassium phosphate, and calcium as calcium carbonate. In the tests containing less than 0.26 per cent phosphorus, both phosphate and carbonate were reduced proportionately to supply the levels of phosphorus indicated.
[2] S.E. = standard error of the mean.
[3] S.I.P. = serum inorganic phosphorus.

Rhenania phosphate has been tested as a source of phosphorus for animals in Germany (14). Results were in accord with those found for the experimental material reported in Table 2, series D, which show availability probably in excess of 50 per cent. (See control containing ½ or 50 per cent level of P in Table 2, series D). However, the commerical product as tested in series D was high in fluorine and consequently toxic to rats as shown by poor growth of the animals. The relatively good femur ash and blood phosphorus are probably not indicative of good response under these circumstances.

All the soluble sodium phosphates tested gave high femur ash and blood phosphorus values which are probably normal, indicating excellent availability of the phosphorus in these salts. Insoluble sodium metaphosphate was found, as in earlier tests, to be relatively unavailable to the rats. However, vitreous sodium metaphosphate tested approximately equal in availability to the other soluble phosphates. This is contrary to results obtained with chicks by Gillis, *et al.* (13), indicating that the two species may vary in utilization of P from this salt.

Dipotassium and diammonium phosphates, as well as the samples of commercial phosphoric acid, dry sugar acid, and urea phosphate appear, from the tests, to be readily available forms of phosphate. Judged by availability of the phosphorus to rats, the results on soluble phosphates show that all the products tested are probably suitable for use either in drinking water or in dry feeding. Mono- and dicalcium phosphates gave results approximately equivalent to the control salt mixture. By comparison with previous results, it appears that these calcium salts are equal, in availability for rats, to the samples of tricalcium phosphate tested previously by the same methods.

The solubility data given in Table 1 for purposes of comparison with the results on availability to rats in Table 2 indicate that the agreements between the chemical and the biological methods of appraisal of feeding value are similar to those reported in previous work by Ellis, *et al.* (3). When the samples are grouped according to their degree of availability as shown in the rat feeding tests, the solubility figures for 0.4 per cent hydrochloric acid and neutral ammonium citrate, on the whole, show a fairly good correlation with availability.

Of the two procedures of Hill, *et al.* (1) for measuring solubility, the one employing 0.4 per cent hydrochloric acid appears to give the better results. When the samples rated excellent and good are considered as one group and those rated poor and fair as another group, the division point of 65 per cent solubility appears to segregate 80 per cent or more in each group when hydrochloric acid is used as a solvent, and 75 per cent or more when ammonium citrate is employed. If the water soluble samples are excluded from consideration, hydrochloric acid solubility of at least 65 per cent still segregates about 75 per cent in each group. Ammonium

citrate fails to show as definite a correlation in the case of these samples, however. This is in agreement with previous findings with ammonium citrate by Ellis, *et al.* (3). Although the solubility data generally confirm earlier results, the relative insolubility of vitreous sodium metaphosphate is in contrast to the report of Gillis, *et al.* (13) that the alkali metaphosphates are soluble though unavailable to chicks. Actually, the material reported to be only 29 per cent soluble in hydrochloric acid is one of the crystalline forms of sodium metaphosphate and is readily produced by crystallizing vitreous sodium metaphosphate (more often called sodium hexametaphosphate) in the appropriate temperature range. This crystalline product is less soluble in hydrochloric acid and from Table 2 it appears to be even more unavailable, less than 25 per cent available, to the rats. This follows the general correlation between solubility in hydrochloric acid and availability. That is, 0.4 per cent hydrochloric acid appears to be a generally better solvent medium for phosphates than conditions found in the digestive tract of animals, and products insoluble in this reagent are generally unavailable to animals. It seems evident, however, that further studies are necessary before methods for solubility determination can be relied upon to predict availability with confidence.

SUMMARY

The availability of various phosphorus supplements for bone formation in rats was measured by adding the phosphates at the same phosphorus level (0.26 per cent) to a low-phosphorus diet

Results obtained with commercial batches of defluorinated phosphates show that products of recent manufacture are generally improved in availability for rats. Some products were no more available than those tested earlier but others were almost equivalent to bonemeal in availability. Rhenania phosphates were slightly less available and the one commercial sample with high fluorine content was toxic to rats.

Commercial phosphoric acid, urea phosphate, dry sugar acid, mono- and diammonium phosphate, mono- and dicalcium phosphate, dipotassium phosphate, sodium tripolyphosphate, vitreous sodium metaphosphate, tetrasodium phosphate, and sodium acid pyrophosphate were all found to be equal to or only slightly less available than the standard bonemeal and monopotassium phosphate. Insoluble sodium metaphosphate was relatively unavailable.

If the availability ratings as determined by the rat feeding tests are compared with the data on solubility in 0.4 per cent hydrochloric acid or neutral ammonium citrate, good correlation from a group standpoint may be noted. However, lack of agreement on individual products in some cases make solubility data appear unreliable for accurately predicting availability.

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DETERMINATION OF FLUORINE CONTENT OF FUSED PHOSPHATE FERTILIZERS

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TVA is producing fused tricalcium phosphate and calcium metaphosphate fertilizers in demonstration plants (2, 3) and has developed, on a pilot-plant scale, a process for the production of fused calcium-magnesium phosphate fertilizer (21). All these products contain some residual fluorine from the phosphate rock used in their production.

Hignett and Hubbuch (3), in a description of the TVA process for the production of fused tricalcium phosphate, have pointed out that the present requirement for the product limits the residual fluorine content, as determined by a modification (1, 6, 18) of the Willard and Winter (22) method, to 0.4 per cent. The modified method has the advantage of being relatively rapid and is used for control in the TVA demonstration plant near Columbia, Tennessee. A similar method has been used for control of the calcium metaphosphate process and in the study of the fused calcium-magnesium phosphate. As recognized by Hignett and Hubbuch (3), however, the method gives somewhat less than the actual fluorine content of fused tricalcium phosphate. Difficulties also have been encountered in the determination of fluorine in fused calcium-magnesium phosphate (4). The inaccuracies of the methods arise from incomplete separation of fluorine from the samples and not from the determination of fluorine once it is isolated.

With respect to fused tricalcium phosphate, the state of combination of the residual fluorine is particularly important. In initial studies of the defluorination of phosphate rock at the Bureau of Chemistry and Soils (12, 16, 17, 19), it was observed that the solubility of the phosphate in a neutral solution of ammonium citrate increased only after removal of all the fluorine in excess of that required to satisfy the composition, $Ca_{10}(PO_4)_6OHF$; further removal of fluorine was accompanied by a linear increase in the citrate solubility of the phosphate. Trömel and Ehrenberg (20) reached the same conclusion from experiments in which pure fluorapatite was the source of phosphorus. MacIntire and co-workers (9, 10) considered the residual fluorine to be in the form of fluorapatite.

Jacob and co-workers (7), in a study of the solubilities of the alpha phosphates (fused, sintered, and calcined), observed that materials produced in large-scale equipment differed from laboratory preparations and suggested that these differences should be considered before attempting to assign combinations from fluorine content or solubility. The products from large-scale equipment show higher citrate solubility for the same degree of defluorination, but also contain much larger quantities of glass

which often must be regarded as a principal phosphatic constituent. They suggested that the glass phase may contain some of the fluorine, as well as some of the phosphorus.

This paper describes a study of methods for the separation of fluorine from the fused phosphates in a form suitable for determination by a standard method, and touches briefly upon the manner in which the fluorine is held by the phosphates.

MATERIALS

Five samples of fused tricalcium phosphate, produced in an experimental shaft furnace at Wilson Dam, Alabama, and in the demonstration

TABLE 1.—*Composition of fused tricalcium phosphate*

SAMPLE	PERCENTAGE COMPOSITION					
	P_2O_5	CaO	SiO_2	Fe_2O_3	Al_2O_3	F[a]
Std. No. 1	29.5	41.9	20.1	3.7	5.2	0.66
FPD-6-7	29.2	41.7	21.3	3.3	4.9	0.47
8-AE	26.3	37.7	24.0	5.1	6.0	0.33
1-AE	29.9	42.6	19.6	4.1	3.5	0.31
3-AE	28.9	40.6	21.5	5.0	3.7	0.09

[a] Determined by method A of present work.

plant near Columbia, Tennessee, were studied intensively. Table 1 shows their chemical composition. Their x-ray diffraction patterns showed the presence of α-tricalcium phosphate, apatite, and cristobalite. The apatite was identified as fluorapatite by optical methods. In addition to the five samples that were studied intensively, several other samples of fused tricalcium phosphate from the same sources were used in testing the adequacy of experimental procedures.

TABLE 2.—*Composition of fused calcium-magnesium phosphate*

SAMPLE	PERCENTAGE COMPOSITION				
	P_2O_5	CaO	MgO	SiO_2	F[a]
PSE-29	27.1	38.3	8.0	16.8	2.60
PSE-11	23.5	38.3	10.5	18.6	2.42
PSE-14	24.5	35.9	12.1	19.4	2.07
PSE-9	21.3	31.5	17.4	23.4	1.85
PSE-33	20.3	28.6	21.1	23.5	1.91

[a] Determined by method A of present work.

Fused calcium-magnesium phosphate was obtained from the production of a pilot plant in which mixtures of olivine and phosphate rock were fused in an electric furnace (21). Chemical analyses of the products are

shown in Table 2. Apatite was identified in all the samples by x-ray and optical methods; its proportion ranged from that of a major constituent in the first two samples to a trace in the last two. Only the last two samples in Table 2 should be regarded as typical; the others were prepared with less than the optimum proportion of olivine.

TABLE 3.—*Composition of calcium metaphosphate*

SAMPLE	PERCENTAGE COMPOSITION					
	P_2O_5	CaO	SiO_2	Fe_2O_3	Al_2O_3	F[a]
MP-31	62.4	26.8	6.9	1.9	1.8	0.35
MP-3	62.2	24.6	8.6	1.9	1.7	0.41
MP-6	62.0	22.0	11.8	1.7	1.6	0.40

[a] Determined by method A of present work.

Calcium metaphosphate was taken from production in the demonstration plant at Wilson Dam. The composition of the samples, Table 3, varied sufficiently to be representative of typical production. The samples were essentially glasses, although x-ray diffraction patterns indicated minor amounts of β-calcium metaphosphate in sample MP-31 and cristobalite in the two samples of higher silica content (MP-3 and -6).

EXPERIMENTAL STUDY OF FUSED TRICALCIUM PHOSPHATE

Fluorine in solution was determined by the titration method recommended by Reynolds and Hill (15). Standard sodium fluoride was prepared from hydrofluoric acid and sodium bicarbonate.

Approximately 0.05 *N* thorium nitrate was standardized by titration of solutions containing various quantities of sodium fluoride. The titration curve deviated from linearity with amounts of fluorine less than 0.2 mg. Subsequent analyses were made by comparison of titers with the experimentally determined curve.

Distillations were made in a multiple-unit electrically heated steam-distillation apparatus which was a modification of a design that has been illustrated (11). Each unit was regulated to yield 200 ml of distillate per hour. The distilling flasks were of a modified Claissen type, each having a spray trap. Steam was introduced through a 1-mm capillary that was curved to impart a swirling motion to the solution.

REMOVAL OF FLUORINE BY ACID DIGESTION

Willard and Winter (22) have shown that fluorine in raw phosphate rock is separated through digestion with perchloric acid at 135°C. and collection of 50 to 75 ml of distillate. Reynolds (14) has indicated that the method is applicable to various kinds of phosphate rocks and slags

containing acid-decomposable silicates, if 150 ml. of distillate is collected. To determine the relative rate of release of fluorine from fused tricalcium phosphate and other materials, samples were subjected to successive 1-hour distillations in which 200 ml of distillate was collected each time. Blank determinations showed that the reagents contained negligible quantities of fluorine. The results of the successive distillations are shown in Table 4.

TABLE 4.—*Recovery of fluorine from various materials by the modified Willard-Winter distillation technique*

MATERIAL	FLUORINE ADDED	FLUORINE RECOVERED[a]		
		FIRST DISTN.	SECOND DISTN.	THIRD DISTN.
	mg	*mg*	*mg*	*mg*
Standard NaF soln	2.0	2.03 ± 0.07	0.00	—
Bureau of Standards phosphate rock 56a (0.2 g)	7.12	7.20 ± 0.03	0.00	—
Bureau of Standards phosphate rock 56a (1.0 g)	35.60	36.23 ± 0.15	0.06 ± 0.01	—
Fused tricalcium phosphate (1.0 g)	5.44[b]	4.20 ± 0.24	0.55 ± 0.09[c]	0.36 ± 0.06[d]

[a] Each value represents an average of six analyses.
[b] Total fluorine recovered.
[c] This distillate contained phosphorus equivalent to from 0.007 to 0.06 mg of fluorine.
[d] Analyses of the residues after fusion with NaOH showed that an average of 0.33 mg of fluorine remained after three distillations.

To test the influence of the particle size of the sample upon rate of volatilization of fluorine, a portion of minus 16-mesh fused tricalcium phosphate from the TVA demonstration plant was crushed to pass an 80-mesh screen, and fractions of the minus 80-mesh material were crushed to minus 150 and minus 325 mesh, respectively. Analyses of the material in the three degrees of fineness indicated little, if any, improvement in fluorine volatilization with decrease in particle size below 80 mesh.

Tests also were made in which 0.5-gram samples were digested with sulfuric acid, and the distillates, after evaporation in an alkaline medium, were redistilled with perchloric acid. Although higher results were obtained in this manner than by a single digestion with perchloric acid, significant amounts of fluorine were obtained in a subsequent digestion, and recovery was still incomplete.

EFFECT OF FUSION ON FLUORINE REMOVAL

In other tests the residues from three successive perchloric acid distillations of fused tricalcium phosphate were fused with sodium hydroxide and subjected to two more distillations. A significant amount of fluorine was obtained in the fifth distillation.

All the results indicated that a fusion is essential for complete recovery

of fluorine from fused tricalcium phosphate. Since the fusion of a residue from repeated acid digestions is impractical for a control method, tests were made in which 1-gram samples were fused with sodium hydroxide prior to their digestion with perchloric acid. Results were variable on the first digestion, and some were lower than those obtained by a single digestion with acid without a prior fusion. Since the samples were thoroughly disintegrated, the low fluorine recovery was attributed to the large amount of gelatinous silica derived from a 1-gram sample.

Tests were then made on 0.5-gram samples. The samples first were fused with sodium hydroxide or sodium carbonate or were sintered with calcium peroxide (8, 13), and then were subjected to repeated distillations until recovery of fluorine virtually ceased. Three distillations for the low-fluorine samples and four distillations for samples of higher fluorine content proved necessary, regardless of the prior treatment. The lowest results for total fluorine were obtained from the samples that were sintered with calcium peroxide, presumably because of incomplete decomposition of the samples.

Similar tests on 0.25-gram samples that had been subjected to the same three types of treatment prior to the perchloric acid digestion revealed that virtually complete recovery of fluorine could be obtained in three distillations. The amount of fluorine in the third distillate was insignificant with low-fluorine samples, but amounted to as much as 0.03 per cent with samples high in fluorine.

In other tests the sample was fused with sodium carbonate and digested with sulfuric acid. The procedure, which is recommended by the Bureau of Standards (5) for the separation of fluorine from phosphate rock, gave higher recovery of fluorine in the first distillate than any of the other techniques. A second distillation with sulfuric acid appeared adequate for recovery of the residual fluorine. Apparently complete separation of the fluorine in the first distillate was attained by extending the time of distillation and thereby increasing the volume of distillate to 500 ml. The distillate had to be evaporated in an alkaline medium and redistilled with perchloric acid prior to titration of the fluorine.

Fusion with sodium carbonate and disintegration of the melt are tedious. Substitution of sodium hydroxide as the flux not only speeded up the analyses but eliminated the necessity of using platinum crucibles.

EVALUATION OF METHODS

Of the methods tested, only two appear to yield quantitative separation of fluorine from fused tricalcium phosphate. Method A involves fusion of a 0.5-gram sample with sodium hydroxide or sodium carbonate, and digestion of the fusion with sulfuric acid at $165° \pm 5°$C. until 500 ml of distillate is collected. The distillate is then evaporated in alkaline medium and redistilled with 10 ml of perchloric acid at $135° \pm 5°$C. prior to titration with 0.05 N thorium nitrate solution. In method B, a 0.25-gram sample

is fused with sodium hydroxide and distilled with perchloric acid at 135° ± 5°C. until 500 ml of distillate is collected. An aliquot of the distillate is titrated directly with 0.01 *N* thorium nitrate solution.

In an experimental evaluation of the two procedures in terms of accuracy and time required per determination, analyses were made in multiple, and in each analysis duplicate aliquots of the distillate were titrated by two people. The results of these tests are shown in Table 5.

TABLE 5.—*Comparison of two methods for the determination of fluorine in fused tricalcium phosphate*

METHOD A			METHOD B		
NUMBER OF ANALYSES	AVERAGE F, %	MAXIMUM DEVIATION, % F	NUMBER OF ANALYSES	AVERAGE F, %	MAXIMUM DEVIATION, % F
4	0.06	0.00	5	0.08	±0.01
3	0.07	+0.01	5	0.09	0.00
4	0.29	−0.04	5	0.29	±0.01
3	0.30	+0.02	5	0.27	±0.01
3	0.30	−0.02	5	0.29	±0.01
3	0.31	−0.02	5	0.28	±0.02
3	0.32	+0.02	5	0.29	±0.01
3	0.33	+0.01	5	0.29	±0.01
3	0.35	−0.02	5	0.33	±0.01
3	0.37	±0.01	5	0.35	+0.03
3	0.41	−0.02	4	0.37	±0.01
3	0.59	−0.03	5	0.52	−0.03

The data in Table 5 indicate that the precision of the two methods is essentially the same. The results by method A are believed to be near the correct values; and method B is concluded to yield results that are somewhat low. Method A requires 12 hours for the analysis of six samples, whereas method B requires only 8 hours.

CHEMICAL STATE OF RESIDUAL FLUORINE

The nature of the combinations in which residual fluorine is held in the fused phosphates bears, not only upon the treatment required for quantitative evolution of the fluorine from an analytical charge, but also upon the efficacy of the phosphates as fertilizers and upon chemical methods for their evaluation as fertilizers.

In calcium metaphosphate and fused calcium-magnesium phosphate, the residual fluorine normally appears as a component of an acid-soluble glass and seems to have no systematic effect on the solubility of the phosphates as determined by the chemical tests customarily applied in the evaluation of phosphatic fertilizers. In fused tricalcium phosphate, however, residual fluorine in the form of apatite can have a pronounced effect upon the solubility. Further tests therefore were made with the

fused tricalcium phosphates listed in Table 1 with the object of throwing some light upon the chemical state of the fluorine.

Each sample was digested for one hour with perchloric acid at 135°C. to yield 200 ml. of distillate. Fluorine in the distillate was determined by titration with thorium nitrate. The acid-insoluble portion was washed by decantation, dried at 105°C., and weighed. The fluorine content of the original samples and of the acid-insoluble residues was determined in triplicate by method A. The analytical results in Table 6 show that the proportion of fluorine in three of the acid-insoluble residues was greater than in the original samples.

TABLE 6.—*Distribution of fluorine in fused tricalcium phosphate*

SAMPLE[a]	FLUORINE BY $HClO_4$ DISTILLATION	TOTAL FLUORINE	FLUORINE RESIDUE BASIS
	per cent	*per cent*	*per cent*
Std. No. 1	0.52	0.66	0.43
FPD-6-7	0.34	0.47	0.56
8-AE	0.23	0.33	0.27
1-AE	0.17	0.31	0.40
3-AE	0.03	0.09	0.19

[a] Composition is given in Table 1.

A portion of the acid-insoluble material from each sample was studied by optical and x-ray diffraction methods. X-ray analysis indicated that the residues were essentially amorphous with small amounts of cristobalite and α-quartz. Despite the relatively high fluorine content of the acid-insoluble residues, crystalline fluorine compounds were not present in sufficient quantity for detection by the powder method of x-ray examination. Optical analysis showed that the bulk of the insoluble material was a glass of variable refractive index (1.550 to 1.450, mean near 1.520) that contained small amounts of cristobalite and fluorapatite. The glass contained numerous cavities from which apatite and other soluble phases had been leached. The amount of apatite observed as minute crystals embedded in the glass was insufficient, however, to account for more than a small part of the fluorine found by chemical analysis.

Table 7 shows chemical analyses of the acid-insoluble residues. The values found for silica represent silicic acid derived from acid-decomposable glasses and insoluble cristobalite, in addition to the silica in the insoluble glass. Despite this dilution by silica, a comparison of Table 7 with Table 1 shows that the iron and aluminum were concentrated in the insoluble residue. Evidently the iron and aluminum are major constituents in the glass phase, and it may be inferred that they contribute significantly to the retention of fluorine. The data in Table 7 also prove that much of the residual fluorine was present in some form other than apatite,

because the apatite equivalence of the fluorine exceeds that of the phosphoric oxide.

In a study of chemical methods for the evaluation of fused tricalcium phosphate fertilizer, we obtained an apparent, close correlation between the citric acid-insoluble phosphorus as determined by the A.O.A.C. method for basic slags (13), and the fluorapatite equivalence of the fluorine content as determined by method A. The present finding that much of the fluorine is a component of the acid-insoluble glass shows that the correlation was fortuitous. This finding also confirms the suggestion of

TABLE 7.—*Composition of perchloric acid-insoluble residues from fused tricalcium phosphate*

SAMPLE[a]	PERCENTAGE COMPOSITION OF RESIDUE					
	P_2O_5	CaO	SiO_2	Fe_2O_3	Al_2O_3	F
Std. No. 1	2.3	10.0	64.5	5.6	7.3	0.43
FPD-6-7	2.2	11.1	63.3	5.5	8.9	0.56
8-AE	2.6	10.7	61.6	6.4	10.0	0.27
1-AE	2.0	12.1	63.0	6.0	8.8	0.40
3-AE	1.9	14.8	58.9	8.7	9.1	0.19

[a] Composition is given in Table 1.

Jacob and co-workers (7) that some of the fluorine might be found in the glass phase.

EXPERIMENTAL STUDY OF FUSED CALCIUM-MAGNESIUM PHOSPHATE

Fused calcium-magnesium phosphate is attacked by acids with the formation of gelatinous silica, which hinders the evolution of fluorine. Tests were made to determine whether the extreme conditions required for removal of fluorine from fused tricalcium phosphate are required also for fused calcium-magnesium phosphate. The fluorine contents reported in Table 2 for the phosphate-olivine product were obtained by the lengthy fusion and double distillation procedure outlined above as method A, and were used as the basis for evaluation of the experimental methods.

The first experimental technique was a digestion with perchloric acid and collection of three successive 200-ml portions of distillate. When a 0.5-gram sample was used, significant amounts of fluorine appeared in the third distillate. Residues from single digestions contained glass in which crystal inclusions were detected by optical methods. Both the acid-resistant glass and the large amount of gelatinous silica probably were factors in the slow evolution of fluorine from 0.5-gram samples.

To lessen the effect of gelatinous silica, the weight of sample was decreased to 0.25 gram in studies of two methods for the separation of fluorine prior to its titration. In the first method the sample was digested

with perchloric acid, and three successive 200-ml portions of distillate were collected. In the second method the sample was fused with sodium hydroxide prior to the digestion with perchloric acid. Table 8 compares

TABLE 8.—*Comparison of three methods for the determination of fluorine in fused calcium-magnesium phosphate*

SAMPLE[a]	FLUORINE, PER CENT		
	$HClO_4$ DIGESTION	FUSION, $HClO_4$ DIGESTION	METHOD A
PSE-29	2.45	2.59	2.60
PSE-11	2.27	2.45	2.42
PSE-14	2.03	2.10	2.07
PSE-9	1.85	1.82	1.85
PSE-33	1.90	1.91	1.91

[a] Composition is given in Table 2.

the results of these tests with those obtained by fusion and successive digestions in sulfuric and perchloric acids (method A).

The fraction of the total fluorine appearing in the respective 200-ml. portions of distillate in the two experimental procedures is shown in Table 9.

TABLE 9.—*Stepwise distillation of fluorine from fused calcium-magnesium phosphate by two methods*

SAMPLE[a]	FLUORINE IN SUCCESSIVE PORTIONS OF DISTILLATE, PER CENT OF TOTAL F BY METHOD A					
	$HClO_4$ DIGESTION			FUSION, $HClO_4$ DIGESTION		
	FIRST	SECOND	THIRD	FIRST	SECOND	THIRD
PSE-29	89	4	2	92	7	1
PSE-11	88	4	2	95	5	1
PSE-14	84	4	2	91	8	1
PSE-9	94	4	2	89	7	2
PSE-33	94	4	2	92	7	1

[a] Composition is given in Table 2.

The data in Tables 8 and 9 show that both a fusion and a subsequent long digestion are essential for complete evolution of the fluorine from fused calcium-magnesium phosphate. A preliminary digestion of the fusion with sulfuric acid is not essential for this material. Although acid digestion without the fusion gave acceptable results for the two typical products (PSE-9 and -33), this procedure is not recommended for general application. Fusion of a 0.25-gram sample and digestion with perchloric acid to yield 500 ml. of distillate (method B, above) should be entirely adequate for separation of the fluorine from fused calcium-magnesium phosphate.

EXPERIMENTAL STUDY OF CALCIUM METAPHOSPHATE

The silica content of calcium metaphosphate being relatively low, the gelatinous form of this constituent interferes less than in the analysis of the other fused phosphate fertilizers.

Calcium metaphosphate was analyzed for fluorine by the same techniques that were applied experimentally to fused calcium-magnesium phosphate. Because of the low fluorine content of the metaphosphate, samples of 0.5 and 1.0 gram were used. The results are compared in Table 10 with those obtained by method A.

TABLE 10.—*Comparison of three methods for the determination of fluorine in calcium metaphosphate*

SAMPLE[a]	FLUORINE, PER CENT			
	$HClO_4$ DIGESTION (1.0-G SAMPLE)	$HClO_4$ DIGESTION (0.5-G SAMPLE)	FUSION, $HClO_4$ DIGESTION (0.5-G SAMPLE)	METHOD A
MP-31	0.32	0.35	0.34	0.35
MP-3	0.41	0.43	0.41	0.41
MP-6	0.39	0.40	0.41	0.40

[a] Composition is given in Table 3.

Table 11 shows the distribution of fluorine among the successive portions of distillate.

TABLE 11.—*Stepwise distillation of fluorine from calcium metaphosphate by two methods*

SAMPLE[a]	FLUORINE IN SUCCESSIVE PORTIONS OF DISTILLATE, PER CENT OF TOTAL F BY METHOD A					
	$HClO_4$ DIGESTION			FUSION, HCl_4 DIGESTION		
	FIRST	SECOND	THIRD	FIRST	SECOND	THIRD
MP-31	91	5	4	89	6	2
MP-3	86	9	8	92	6	2
MP-6	90	7	4	93	7	2

[a] Weight of sample, 0.5 gram. Composition is shown in Table 3.

It appears that the recovery of fluorine will be slightly higher in equal volumes of distillate if calcium metaphosphate is fused with sodium hydroxide prior to the digestion with perchloric acid. The difference, from 0.01 to 0.02 per cent F, is so small, however, that it seldom will justify the additional time required for a fusion.

RECOMMENDED METHODS

The proper choice of method depends upon the particular fused phosphate that is to be analyzed, and upon the accuracy that is sought.

Fused tricalcium phosphate must first be broken down with an alkaline flux and then subjected to a lengthy digestion with acid, if reproducible results approaching the true value are desired. A preliminary alkali fusion of fused calcium-magnesium phosphate prior to acid digestion is desirable also, whereas the evolution of fluorine from calcium metaphosphate usually can be attained satisfactorily in a prolonged acid digestion alone.

REAGENTS

Sodium Fluoride Soln, Buffer Soln, and Indicators.—Prepare according to the directions of Reynolds and Hill (15).

Perchloric Acid.—70 to 72%.

Thorium Nitrate Soln, 0.05 *N.*—Dissolve 13.80 g of the tetrahydrate in water and dilute to 2 liters. To standardize the soln, add freshly prepared sodium fluoride soln to a series of 125-ml Erlenmeyer flasks in increments of 0.025 mg of fluorine up to 0.200 mg, then in increments of 0.100 mg of fluorine up to 2.00 mg. Add 1 drop of phenolphthalein to each flask and make just alkaline with 0.1 *M* sodium hydroxide. Dilute to 50 ml (mark on side of flask). Add 5 drops of sodium alizarin sulfonate indicator soln, then add 0.1 *M* hydrochloric acid until the pink color of alizarin is just discharged. Add 2.5 ml of buffer soln and titrate with the thorium nitrate soln to the first definite pink color. Use a fluorescent titration illuminator and observe the end point against the white porcelain base of a buret support. It is desirable to make the titration in multiple, and to exclude titers mathematically when they are obviously incorrect. Plot the mg of fluorine against ml of thorium nitrate soln. The portion of the line below 0.2 mg of fluorine is curved, thus making impossible the subtraction of a blank in the usual sense. Above 0.2 mg of fluorine, the line is straight. The accuracy of this standardization should be checked against aliquots of the distillate from a sample of Bureau of Standards phosphate rock.

Thorium Nitrate Soln, 0.01 *N.*—Dissolve 2.76 g of the tetrahydrate in water and dilute to 2 liters. To standardize the soln, add freshly prepared sodium fluoride soln to a series of 125-ml Erlenmeyer flasks in increments of 0.01 mg of fluorine up to 0.20 mg, then in increments of 0.05 mg of fluorine up to 0.40 mg. Continue the standardization in exactly the same manner used for the 0.05 *N* soln of thorium nitrate.

DETERMINATION OF FLUORINE IN FUSED TRICALCIUM PHOSPHATE BY METHOD A

Fuse ca 4 g of sodium hydroxide in a nickel crucible. Cool to room temp. and place a 0.5-g sample on top of the melt. Add another 4 g of sodium hydroxide.

Cover the crucible and heat it gently until the alkali melts. Increase the heat gradually, and finally fuse the charge at the full heat of the Bunsen burner for about 2 min. Chill the crucible by immersing its lower half in cold water, thus causing the melt to break away from the crucible. Transfer the fusion to a distilling flask; use a minimum quantity of water for the transfer and, if necessary, a few drops of dilute sulfuric acid to remove the last adhering particles. Place the flask in a bath of ice water, and carefully neutralize its contents by dropwise addition of sulfuric acid (1+1) at temps. below 50°C. The amount of acid for neutralization should be anticipated from a prior titration of 8 g of sodium hydroxide with the acid. Add a 20-ml excess of sulfuric acid (1+1) and immediately connect the flask for steam distillation. Distill at 165° ±5°C., and collect 500 ml of distillate. Add 1 *M* sodium hydroxide until the distillate is alkaline to phenolphthalein, then evaporate to a volume of 20 ml.

While the evaporation is in progress, clean the distilling flasks with a hot 10% soln of sodium hydroxide. Then make a blank distillation of 200 ml by placing 5 ml

of water and 10 ml of perchloric acid in the flask, and distilling in the usual manner at 135° ±5°C. Discard this distillate, and empty the distilling flask. Transfer the evaporated soln to the flask, add 10 ml of perchloric acid, and distill at 135° ±5°C., catching 200 ml of distillate in a 250-ml volumetric flask. Make the distillate just alkaline to phenolphthalein by dropwise addition of 1 *M* sodium hydroxide. Cool the distillate, dilute to the mark, and mix thoroly. Transfer a 50-ml aliquot to a 125-ml Erlenmeyer flask and titrate with 0.05 *N* thorium nitrate as outlined in the section on preparation of the nitrate soln, beginning with the addition of sodium alizarin sulfonate indicator. Read the mg of fluorine from the standardization chart. Run blank determinations on equivalent quantities of each new lot of reagent used, following all the steps of the procedure, and subtract the fluorine found.

DETERMINATION OF FLUORINE IN FUSED TRICALCIUM PHOSPHATE OR FUSED CALCIUM-MAGNESIUM PHOSPHATE BY METHOD B

Fuse a 0.25-g sample, transfer the fusion to a distilling flask, and neutralize it, as described under method A; but substitute perchloric acid for the sulfuric acid. Add a 10-ml excess of perchloric acid and immediately connect the flask for steam distillation. Distill at 135° ±5°C. and catch somewhat less than 500 ml of distillate in a 500-ml volumetric flask. Make the distillate just alkaline to phenolphthalein by dropwise addition of 1 *M* sodium hydroxide. Cool the soln, dilute to the mark, and mix thoroly. Transfer a 50-ml aliquot to a 125-ml Erlenmeyer flask and titrate with 0.01 *N* thorium nitrate as outlined in the section on preparation of the nitrate soln, beginning with the addition of sodium alizarin sulfonate indicator. Read the mg of fluorine from the standardization chart. Run blank determinations on equivalent quantities of each new lot of reagents used, following all the steps of the procedure, and subtract the fluorine found.

PRECAUTIONS

A coating of silica from the hydrolysis of fluosilicic acid is deposited in the distilling flasks. It is advisable to clean the flasks with a hot 10 per cent soln of sodium hydroxide each time they are used.

When sulfuric acid is used for the isolation of fluorine, sufficient sulfate is carried over to interfere with the titration; hence an evaporation in alkaline medium and a second distillation in perchloric acid are necessary.

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NOTES ON KARL FISCHER REAGENT

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A review of the literature discloses that many formulas have been proposed for the preparation of the Karl Fischer reagent. Because of the great dissimilarity of the amounts of the ingredients proposed by the different investigators (1, 2, 3, 4, 6, 7), it is impossible to make a direct comparison of the formulas. A possible cause of this diversity may have been the example of Karl Fischer (3), who expressed the ingredients of his reagent in grams, although he used gram molecular weights. Had he stressed the gram molar ratios which he actually used, these diverse formulas might have been avoided.

Typical of these formulas are the two following preparations of the reagent: (a) Weigh into a flask 3.785 liters (1 gallon) of 2-A pyridine and

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

add 203 grams of liquid sulfur dioxide for each kilogram of pyridine. To 1359 grams of stock-pyridine-sulfur dioxide solution in a 5-liter container add 1786 grams of alcohol, mix and cool to room temperature. Add 453.6 grams (1 pound) of iodine, stopper, cool under running water before shaking, then alternately swirl and cool until the iodine is in solution (1); and (b) 502 grams anhydrous pyridine, 102.5 grams of sulfur dioxide, anhydrous; 202 grams of iodine, reagent quality; 1000 ml anhydrous methanol (4). It is not apparent that these two reagents have identical molar ratios of sulfur dioxide, iodine and pyridine, since the weights specified appear to have no relation to their gram molecular weights. Actually, if the weights of these substances in the two solutions are expressed as gram moles, with the moles of iodine taken as unity, the ratios become 1:2:8 for iodine, sulfur dioxide and pyridine, and the two solutions are identical except for the methanol used, which is 1420 and 1206 ml respectively per mole of iodine.

Table 1 gives the formulas of the six preparations of the reagent often

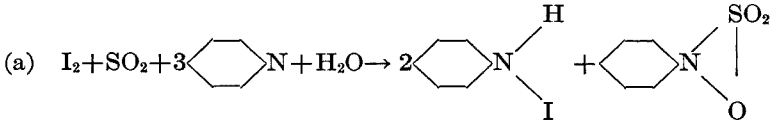
TABLE 1.—*Karl Fischer Reagent*

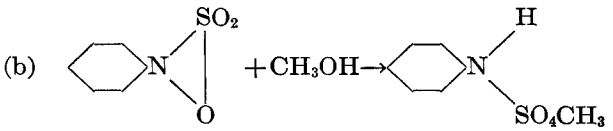
AUTHOR	FORMULAS FOR PREPARATION				MOLAR RATIOS			VOLUME PER MOLE OF IODINE
	IODINE	SULFUR DIOXIDE	PYRIDINE	METHANOL	IODINE	SULFUR DIOXIDE	PYRIDINE	METHANOL
	g	*g*						*ml*
Almy (1)		753	3.785 l		1	2	8	1420
	453.6	←To 1359 g add→		1786 g				
ERRL	238	183	752	427 ml	1	3	10	400
Fischer (2)	254	192	790 g	5000 ml	1	3	10	5000
Johnson (3)	169	128	425 ml	511 ml	1	3	8	767
Journal Amer. Oil Chem. Soc. (4)	202	102.5	502 g	1000 ml	1	2	8	1206
Smith (6)	84.7	64	269 ml	667 ml	1	3	10	2000
Wernimont (7)	500	380	1700 ml	200 ml	1	3	10	102

referred to, together with their calculated molar ratios. The table not only aids in the selection of an appropriate reagent but also serves as a guide in comparison of results obtained by the different reagents. It is easily seen that all the molar ratios show marked similarity and that the reagents differ only in concentration, which is dependent on the amount of methanol used per mole of iodine.

The methanol is not expressed on a gram molar basis because only a portion of it is used as a reactant; the remainder serves principally as a solvent and diluent. Mitchell (5) and Smith (6) have suggested that the methanol combines in a 1:1 molar ratio (eq. b), with the inner salt of the

pyridinium hydroxide-N-sulfonic acid formed in the reaction of the Karl Fischer reagent with water. One mole of the complex is formed from one mole of iodine according to reaction (a). Thus an equivalent of 1 mole of methanol is required for each mole of iodine.





Besides the 1 gram mole required in this reaction, additional methanol must be present to serve as a solvent for the products formed. However, a large excess of methanol, such as that used by Fischer (2), is to be avoided, for it sets up conditions conducive to side reactions with aldehydes and ketones, forming acetals and ketals (6). These side reactions result in high and erroneous water values. By reducing the methanol and increasing the pyridine, Smith obtained a reagent that reacted normally in the presence of aldehydes and ketones.

In titrating large amounts of water a reduction in the volumes of reagent is made possible by using a more concentrated reagent. A satisfactory reagent for 40 to 400 mg of water has 400 ml of methanol per mole of iodine. To use less than 400 ml per mole of iodine gives a reagent with such a high concentration that errors in measuring may occur when ordinary laboratory volumetric apparatus is used. A reagent with the molar ratios 1:3:10 for iodine, sulfur dioxide, and pyridine and with 400 ml of methanol will have a titer of approximately 7.8 mg of water per ml of reagent.

Of the two methods commonly used for titrations with Karl Fischer reagent—the visual and electrometric end points—the electrometric method is most applicable for general and intermittent use. Here identification of the end point is not limited to analysis of colorless or light-colored solutions, but can be used equally well with dark-colored materials. The electrometric end point is easy to detect and usually provides a warning as the end point is approached. The visual method, although limited to colorless or light-colored solutions, is well suited to routine analyses.

STANDARDIZATION

Karl Fischer reagent is standardized with a known weight of water, either added directly or contained in a methanol solution; the reaction

end point is detected visually or electrometrically. Standardizing the reagent by the visual end point method is relatively simple because it consists in only two steps (a) measuring the volume of the reagent consumed by a given volume of methanol, and (b) measuring the volume of the reagent consumed by an equal volume of methanol containing a known weight of water (5, 6). The water titer of the reagent (standardization) is obtained from the two volumes of the reagent. Since the visual method of standardization is not applicable to such strong reagents as recommended here because of the uncertain color of the end point, the electrometric method should be used. This method overcomes the problem of end-point color, but to obtain maximum sensitivity the procedure of titration should be reversed (1, 7), that is, titrate the reagent with the water-methanol solution. As used in the past, standardization of the reagent by this method was objectionable because it required (a) anhydrous methanol, (b) weighed small portions of water (8), or (c) of hydrated substances as primary standards (5).

The authors suggest an alternative method. This method avoids use of anhydrous methanol, small weighed portions of water, or hydrates of uncertain composition. The standard water-methanol solution is made by adding 4 grams of water, weighed to the nearest 0.1 mg. with a weight burette, to 200 ml of reagent-grade methanol. This solution is then made up to 2 liters with more of the methanol. It is desirable that the methanol contain less than 0.2 per cent water, a condition met by most reagent-grade methanols, so that excessive amounts of the Karl Fischer reagent will not be consumed. The water content of the prepared standard water-methanol solution is the sum of the water present as impurity, plus that added. In the proposed method, the titer of the Karl Fischer reagent is easily obtained by using only three titration steps (a, b, and c) and then substituting the volume values in a simple equation. Only the weight of the added water in the standard water-methanol solution need be known.

PROCEDURE

(a) A few milliliters, usually 5, of Karl Fischer reagent are added to the reaction vessel to provide anhydrous conditions. The excess reagent is destroyed by addition of the standard water-methanol solution.

(b) A second portion, 10–25 ml of Karl Fischer reagent, accurately measured to the nearest 0.01 ml, is added and titrated with the standard water-methanol solution. From the volume of standard water-methanol solution and the volume of Karl Fischer reagent, the ratio of standard water-methanol solution to reagent is found.

(c) A quantity of the reagent methanol and a known volume of Karl Fischer reagent, in excess of the water in the added reagent methanol, are added. The excess of Karl Fischer reagent is then back-titrated with

standard water-methanol solution. The titer of the reagent can be calculated by means of the following equation.

$$\frac{WQM}{Q + P - MR} = \text{mg of } H_2O/1 \text{ ml Karl Fischer reagent}$$

$$M = \frac{\text{volume water-methanol solution}}{\text{volume of Karl Fischer reagent}} \quad \text{(step b)}$$

W = weight of added water in mg per ml of the water-methanol solution
Q = volume of reagent methanol (step c)
R = volume of Karl Fischer reagent (step c)
P = volume of standard water-methanol solution (step c)

The analysis of a sample is performed in much the same manner.

The system is freed of water as done in step (a) of the above standardization procedure. Then (step d) an amount of the Fischer reagent is added which is known to be in excess of that required by the sample. The sample is now added and the mixture stirred, preferably by a magnetically driven "flea," and the amount of Fischer reagent that is in excess to that required by the water of the sample is determined by back titration with the standard water-methanol solution.

$$\% \text{ water in sample} = \frac{(\text{ml KF reagent added} - \text{ml. KF reagent} \Rightarrow \text{std water-methanol}) \times \text{water titer of KF reagent} \times 100}{\text{wt. sample}}$$

This method provides a simple and convenient means of standardizing the Karl Fischer reagent, avoids the preparation of absolutely anhydrous methanol and the difficulties inherent in the weighing of small amounts (100 to 200 mg) of water using ordinary laboratory apparatus.

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A METHOD FOR SEPARATING COARSE HOOF AND HORN MEAL FROM MEAT AND BONE SCRAP

By BENJAMIN L. SMITS and RICHARD COLBURN* (Kansas State College of Agriculture and Applied Science, Manhattan, Kansas)

The definition of meat and bone scrap as promulgated by the American Feed Control Officials (1) states that it is "the finely ground dry-rendered residue from animal tissue" and permits only those "traces as might occur unavoidably in good factory practice of hair, horn, hoof, blood, manure, and stomach contents." A method for the separation and identification of an excess of coarsely ground hoof and horn meal from commercial protein-concentrates such as meat and bone scrap is desired since these keratins appear as available proteins by a Kjeldahl digestion.

The "reasonableness" (2) of this definition hinges apparently on the accepted idea that these sclero-proteins are non-digestible. However, recent work has shown that the digestibility of hoof meal is a function of the particle size. Routh and Lewis (3) first pointed out that the digestibility of keratins by proteolytic enzymes is dependent upon the degree of fineness of the particles.

Wagner and Elvehjem (4, 5) obtained good growth in rats and chicks with swine hoof meal ground to pass a 60-mesh sieve. Newell and Elvehjem (6) showed that purified rations containing 30 to 40 per cent powdered hog hoofs induced substantial growth in chicks. Powdered hog hair and chicken feathers were not so effective. They stated that the rates of growth obtained with chicks and rats fed rations containing these keratins showed a positive correlation with the degree of subdivision of the keratin.

Olcott (7) found that the digestibility of hoof meal varied, not only between kinds of animals, but also in samples from the same hoof. The softer inner parts of the hoof were found to be much more easily digested than the outer horny part. His work showed that the powdered hoof material is a mixture of at least two and possibly many proteins containing different numbers of disulphide linkages.

The use of proteolytic enzymes offers the logical solution of the problem since the sclero-proteins by the classical definition are non-digestible by proteolytic enzymes. However, Sterling (8), using pepsin in 0.1 *N* hydrochloric acid at 37.5°C. on hoof material ground to pass a 40-mesh sieve, discovered that it was necessary to use a factor of 1.54 by which to multiply the weight of the digestion residue to correct for the partial digestion of the material. Perkins (9) found also, when using various proteolytic enzymes for this purpose, that the amount of the digestion residue varied, not only with the time of the digestion, but also with the degree of fineness of the hoof material. This work indicated that there was a preferential digestion of muscle fiber in the presence of hoof material.

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Olcott (7) showed that 37 per cent of dried powdered hog hoof material which passed a 40-mesh sieve and was retained on a 60-mesh sieve was digestible by pancreatin. Considerably more than 50 per cent of cattle and hog hoof ground to pass a 200-mesh sieve was digestible. Of the three kinds of hoof (hog, beef, and horse) commonly appearing in commercial meat and bone scrap, hog hoof was the most digestible and horse hoof the least.

The specific gravity of the particles of hoof meal varies, not only as to kind of animal, but also with the age of the animal. The specific gravity of a solution may be adjusted so that it will separate a given sample of ground hoof from meat particles, but it is operative for that sample only.

In making a count of the hoof particles by the use of the microscope it is difficult under ordinary illumination to distinguish definitely between hoof fragments and some of the fragments of collagen; however, under crossed nicols the brightness of the hoof particles is very characteristic. Attempts have been made to correlate specific gravity with particle counts by adding a given amount of hoof meal to a weighed sample of hoof-free meat scrap. Owing to the variation in size of the hoof particles, it was found impossible to establish a valid relationship.

The method proposed in this paper is based on the arbitrary assumption for the purpose of enforcement, that those fragments of hoof and horn which pass through a 40-mesh sieve[1] are sufficiently digestible to be significant, while those retained are so slightly digested as to have little value in nutrition. The rationale of this assumption is based on the reported work of Routh (3), Elvehjem (4, 5, 6) and Olcott (7) on the digestibility of keratins ground to different degrees of fineness.

METHOD

The essential steps in this method of separation are as follows: A 10-gram sample of the original is extracted for one hour with ether using a Soxhlet extractor. After being freed from ether, the sample is separated into the fine material which passes a 40-mesh sieve, and the coarse material which is retained. The material which passes the 40-mesh sieve is rejected, while the material retained is separated into two fractions by decantation using chloroform, following the A.O.A.C. (10) method for the separation of bone from meat scrap. Microscopic examination of the fraction lighter than chloroform shows it to be a mixture of hoof, horn, hair, stomach contents, and collagen together with a considerable number of irregular lumps. These lumps consist of an aggregation of fine bone and muscle fiber. The aggregates float or sink in chloroform, depending upon the amount of bone which they contain. In order to eliminate these aggregates, the fraction lighter than chloroform is boiled for 5 minutes[2] in a

[1] National Bureau of Standards numerical designations of sieve sizes are used throughout this work.
[2] The time of boiling should be extended to 10 minutes in the case of especially resistant aggregates.

saturated solution of calcium hydroxide (ca. 0.156 grams $Ca(OH)_2$ per 100 ml at 25°C.) containing 0.2 per cent of sodium sulphide. (The practice of using a suspension of slaked lime in water which has been "sharpened" with a small amount of Na_2S is a common procedure in preparing hides for tanning.) The action of this solution is to break up the aggregates and to soften and swell the fragments of collagen. After boiling, the material is poured onto a 40-mesh sieve and thoroughly washed while it is being rubbed with the fingers.

The residue left on the screen is transferred to a rapid filter paper and treated with 50 ml of dilute hydrochloric acid (1–19). After being washed free of acid, the material is partially dehydrated with 95 per cent alcohol and dried on the filter paper at 85°C. This treatment renders the swollen fragments of collagen very friable, enabling the analyst to make a complete separation of hoof and horn from the collagen by rubbing it through the meshes of a 40-mesh sieve. Blood, if present, occurs as small black lumps which are not appreciably affected by the limewater-sodium sulphide reagent. After being boiled 5 minutes, manure and stomach contents are eliminated to a considerable extent by the washing, and especially by the rubbing action of the fingers on the metal screen. Hair usually passes through the 40-mesh sieve during the first treatment made after the ether extraction to remove the fine material.

The hyaline fragments of hoof and horn can be easily distinguished and separated from the pieces of dried blood and stomach contents if this residue is spread out on a piece of ground glass placed over a low candle power electric light. Wetting the residue with mineral oil facilitates the separation. The fragments are of such a size that a 7× hand lens easily affords sufficient magnification for identification.

EXPERIMENTAL

In order to demonstrate that the arbitrary selection of the 40-mesh sieve size as the dividing line does not entail any considerable error, the following experiment was set up. Three samples of commercial meat and bone scrap were each divided into six sets of 10 gram duplicates. Each set of duplicates was extracted with ether until fat-free and then put through a different one of six metal sieves: 18, 20, 30, 40, 50 and 60-mesh. It was shown that the extraneous material obtained by using an 18-mesh sieve was on the average 0.65 per cent smaller than that obtained by using a 40-mesh sieve. These results are shown in Table 1. The detritus which passes the 40-mesh sieve and is retained on a 60-mesh sieve consists largely of fragments of blood, stomach contents, and hair.

A series of physical analyses of hoof meal ground in a Wiley mill to pass a 2-mm screen, also shows that over 80 per cent of the material is coarser than a 40-mesh sieve. Hoofs of steer, calf, and hog were autoclaved 15 minutes at 15 pounds pressure, and thoroughly cleaned of adhering

TABLE 1.—*Results of using different sieve sizes in the determination of extraneous material in meat and bone scrap*

	SIEVE SIZES					
	18	20	30	40	50	60
	gram	*gram*	*gram*	*gram*	*gram*	*gram*
Sample No. 1						
Ether extract	1.174	1.194	1.219	1.195	1.194	1.199
Retained	2.739	2.876	3.812	4.575	5.269	5.878
Rejected; including bone, by difference	5.813	5.645	4.677	3.909	3.147	2.448
Extraneous material	0.274	0.285	0.292	0.321	0.390	0.475
Sample No. 2						
Ether extract	1.596	1.546	1.611	1.602	1.474	1.561
Retained	1.580	1.479	2.829	3.501	4.090	4.759
Rejected; including bone, by difference	6.711	6.863	5.375	4.731	4.175	3.355
Extraneous material	0.113	0.112	0.185	0.166	0.261	0.325
Sample No. 3						
Ether extract	0.997	1.002	0.967	0.991	0.947	0.958
Retained	2.969	2.615	4.345	5.082	5.723	6.364
Rejected; including bone, by difference	5.932	6.208	4.544	3.773	3.132	2.494
Extraneous material	0.102	0.075	0.144	0.154	0.198	0.184

flesh when cool. After cleaning, the hoofs were dried at 100°C. for 24 hours and ground. It was thought that this procedure gave a higher percentage of fines than that obtained in actual practice. The results of these analyses are shown in Table 2.

TABLE 2.—*Showing the distribution of particle size in hoof material ground to pass a 2-mm sieve*

TYPE OF HOOF	MATERIAL RETAINED ON—			THROUGH 40-MESH SIEVE
	18-MESH SIEVE	30-MESH SIEVE*	40-MESH SIEVE	
	per cent	*per cent*	*per cent*	*per cent*
Calf	41.75	35.67	9.32	13.26
Steer	33.85	38.72	11.28	16.15
Hog	51.31	24.50	7.80	16.39

* The fraction retained on a 20-mesh sieve was so small in every case that it has been included with that retained on the 30-mesh sieve.

In the process of manufacture of meat and bone scrap when bone and hoof have been reduced to a size which will pass a 2-mm screen, the animal tissue has been ground to an extremely fine size. Approximately 30 per cent of the fat-free sample will pass a 60-mesh sieve. In order to secure a

product which can be handled easily, from 10 to 25 per cent of fat is usually left in it as a binding agent. Since it is essential that the sample be in its original condition, the presence of this fat makes it impossible to secure a uniform sample. It is thought that the errors of sampling offset the errors which would result from regrinding owing to the peculiar fracture of dried hoof material. Variations observed in this study are recorded in Table 3.

TABLE 3.—*Showing the variations encountered in the analyses of a single sample of commercial meat and bone scrap (10-gram samples)*

ANALYSIS NUMBER	ETHER EXTRACT	RETAINED ON 40-MESH SIEVE	REJECTED, INCLUDING BONE, BY DIFFERENCE	EXTRANEOUS MATERIAL
	grams	*grams*	*grams*	*grams*
1	0.993	5.306	3.581	0.120
2	0.927	5.333	3.576	0.164
3	0.959	5.154	3.756	0.131
4	0.956	5.154	3.729	0.161
5	0.992	5.153	3.725	0.130
6	0.993	5.083	3.802	0.122
7	0.939	5.143	3.796	0.122
8	0.996	5.158	3.695	0.151
9	0.960	5.196	3.695	0.149
10	0.975	5.259	3.614	0.152
Average	0.960	5.193	3.697	0.140

In order to test the reliability of this method of separation, two series of unknowns[3] were prepared by Dr. C. H. Whitnah of the chemistry department of Kansas State College. They were prepared by adding definite quantities of a prepared hoof meal containing equal parts of steer, calf, and hog hoof which would pass a 30-mesh sieve and were retained on a 40-mesh sieve, to two different lots of commercial meat and bone scrap. The two lots of meat and bone scrap had been freed of the major part of their extraneous material by being passed through a 20-mesh sieve. The results of this experiment are shown in Table 4.

SUMMARY

A method has been devised for the separation of gross extraneous material from meat and bone scrap; it assumes that, in order to meet the requirements of legal enforcement, all fragments of hoof, horn, and hide which are retained on a 40-mesh sieve are indigestible. The deboned and defatted sample of meat and bone scrap which is retained on a 40-mesh sieve consists of particles of hoof, horn, hide, blood, and some of the hair, together with a considerable number of aggregates, consisting of flesh

[3] Acknowledgment is made of the kindness of Wilson and Company, Chicago, Ill. in supplying the material used in this experiment.

TABLE 4.—*Showing the efficiency of recovery of known amounts of hoof meal when added to samples of meat scrap**

	HOOF MEAL ADDED	EXTRANEOUS MATERIAL FOUND	EXTRANEOUS MATERIAL CORRECTED FOR BLANK	DIFFERENCE	ERROR
	grams	*grams*	*grams*	*grams*	*per cent*
First series blank, 0.050 g					
1	0.225	0.268	0.218	0.007	3.1
2	0.520	0.523	0.473	0.047	9.0
3	0.737	0.724	0.672	0.065	8.9
4	1.459	1.417	1.367	0.092	6.3
5	1.434	1.369	1.319	0.115	8.0
6	1.914	1.772	1.722	0.192	10.0
7	1.914	1.770	1.720	0.194	10.0
8	0.302	0.317	0.267	0.034	11.6
9	0.548	0.554	0.504	0.044	8.0
10	1.222	1.173	1.123	0.099	8.1
					Av. 8.3
Second series blank, 0.009 g					
1	0.625	0.587	0.578	0.056	8.9
2	0.750	0.673	0.664	0.095	12.6
3	0.250	0.237	0.228	0.006	0.2
4	1.000	0.975	0.966	0.043	4.3
5	1.500	1.398	1.389	0.120	8.0
6	0.625	0.603	0.594	0.040	6.4
7	1.000	0.918	0.909	0.100	10.0
8	0.100	0.106	0.097	0.012	12.0
9	0.300	0.289	0.280	0.029	9.6
10	0.500	0.460	0.451	0.052	10.4
					Av. 8.24

* The scrap was first put through a 20-mesh screen to reduce extraneous material to a minimum "blank."

and bone. This residue is boiled in a saturated solution of calcium hydroxide containing 0.2 per cent of sodium sulphide. The action of boiling in this reagent is to break up the aggregates and to soften and swell the pieces of collagen. When dried, they can then be easily removed by rubbing on a 40-mesh sieve. The material retained on the 40-mesh sieve after this procedure is the gross extraneous material of the sample.

ACKNOWLEDGMENT

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EVALUATION OF THE OFFICIAL PERMANGANATE METHODS FOR THE QUALITY OF WATER-INSOLUBLE NITROGEN IN FERTILIZERS[1]

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The official procedure for estimating the activity of water-insoluble nitrogen in fertilizers involves methods that make use of both alkaline and neutral permanganate solutions (1, pp. 29–31). In interpreting the results, the alkaline and neutral permanganate activities are not regarded as indicating the percentage availability of the water-insoluble nitrogen, but rather as distinguishing between the better and poorer sources of such nitrogen. For this purpose any material, the water-insoluble nitrogen of which exhibits an activity below fifty per cent by the alkaline method and also below eighty per cent by the neutral method, is classed as inferior, with the exception that for mixed fertilizers this classification may be offset by consideration of any overrun in total nitrogen above the minimum guaranty (1, p. 902).

Haskins and co-workers (3), in the period 1925–1930, conducted an extensive series of experiments on the various sources of insoluble nitrogen available for fertilizer use. They measured both the alkaline and neutral permanganate activities for each material, and determined the availability of the nitrogen to Japanese millet in pot experiments. The permanganate tests were found to give ratings of "satisfactory" to some of the poorer materials and ratings of "inferior" to some of the better ones.

[1] Presented before the Fertilizer Section at the Sixty-Third Annual Meeting of the Association of Official Agricultural Chemists, Washington, D. C., October 10–12, 1949 under the title: Permanganate Methods for Nitrogen Availability.

Rubins and Bear (4) studied (1) the relationship between permanganate activity and recovery of the nitrogen, in the tops and roots of Sudan grass; and (2) the nitrification of the materials in soil media. In agreement with Haskins and co-workers, they found that the permanganate methods classified some of the poorer materials as satisfactory and some of the better materials as inferior. The fraction of the nitrogen converted to nitrate form in a definite period, on the other hand, was shown to be a rather reliable index of the nitrogen available for crop growth.

In view of these findings it appeared desirable to compare the permanganate activities as determined by the official methods, with the nitrification characteristics of the water-insoluble nitrogen used in commercial fertilizers in recent years. The fertilizer control officials of Florida, Georgia, North Carolina, South Carolina, and Virginia cooperated in this undertaking and supplied a total of 168 official samples of mixed fertilizers marketed in these States between July 1, 1945, and December 31, 1946, for the proposed studies. The necessary data on the alkaline and neutral permanganate activities of the water-insoluble nitrogen were supplied by the respective State laboratories.

PREPARATION OF INSOLUBLE RESIDUES

To provide adequate quantities of water-insoluble material for the nitrification tests, a 100-gram portion of each sample was mechanically stirred with 1500 ml water for 20 minutes, and the solution removed by reduced pressure through a porous alundum thimble introduced into the slurry. This operation was repeated three times, and 500 ml of water was used to transfer the insoluble residue to a Büchner funnel, and for final washing. The moist filter cake was then air-dried, crushed, and mixed.

DETERMINATION OF NITRIFICATION CHARACTERISTICS

A quantity of the washed residue of each sample containing 20 mg of nitrogen, was added to 100 grams of an air-dried soil of known nitrifying activity, in a 500 ml Erlenmeyer flask. The soil had been previously limed to an initial pH of 6.9–7.0 by the addition of calcium carbonate. After thorough mixing of the sample and the soil, the water content was adjusted to the moisture equivalent of the soil, the flask was loosely closed with a cotton plug and stored at 30°C. for the desired incubation period. During incubation, the weights of the flasks were checked at least once each week and water added to compensate for any evaporation loss. All incubations were carried out in duplicate.

At the end of 3-, 6-, and 15-week incubation periods, the soluble nitrogen was extracted from the soil by a modification of the method recommended by Harper (2). Nitrate nitrogen then was determined photometrically using the phenoldisulfonic acid method. After deducting the soil blank, nitrification was reported in terms of the percentage of the

nitrogen in the washed residue, which had been converted to nitrate form during incubation. The nitrification of ammonium sulfate was determined in parallel experiments as a standard of comparison.

SAMPLES EXAMINED

The 168 samples of mixed fertilizers examined represented products of 52 manufacturers. The total and water-insoluble nitrogen guarantees ranged from 2 to 12 per cent, and from 0 to 3 per cent, respectively. More than 50 organic materials were claimed as sources of water-insoluble nitrogen. In 51 of the mixtures, one of sixteen different materials was claimed as the sole organic constituent. Many of the other mixtures were claimed to contain as many as five organic materials.

EXPERIMENTAL RESULTS

The experimentally determined values for the alkaline and neutral permanganate activities, and for the nitrification characteristics of the water-insoluble nitrogen content of the mixtures, are presented in Table 1. This table also presents the classification of the samples into superior, average, and poor groups on the basis of permanganate activities; and into high, intermediate, and low groups on the basis of the nitrification results.

Samples classed in the superior group exhibited alkaline and neutral permanganate activities equal to or greater than 50 and 80 per cent, respectively. Samples in the average group failed to meet one of these requirements for superior classification, and those in the poor group failed to satisfy either of them.

The high group in the nitrification classification includes those samples for which the observed nitrification at at least two of three incubation periods was significantly better than the mean of all samples at odds of 99 to 1. The intermediate group contains those that were not significantly different, and the low group those that were significantly poorer under the same conditions.

The alkaline permanganate activity of 149 of the 161 samples for which permanganate values are available exceeded 50 per cent, whereas only 70 samples exhibited a neutral permanaganate activity of 80 per cent or more. Sixty-five of these 70 samples also had an alkaline activity in excess of 50 per cent and would be classed as superior sources of water-insoluble nitrogen by the permanganate tests. Seven of the 161 samples exhibited alkaline and neutral permanganate values below 50 and 80 per cent, respectively, and would be classed as inferior. Eighty-four of the 89 samples in the average classification had neutral permanganate activities of less than 80 per cent.

Positive correlation might be expected between alkaline and neutral permanganate activities. The observed correlation, although not signifi-

cant, was negative ($r = -0.13$). Figure 1 shows the scatter diagram. Negative correlations significant at odds of 19 to 1 or better, however, were observed for the Florida and Georgia samples when considered independently.

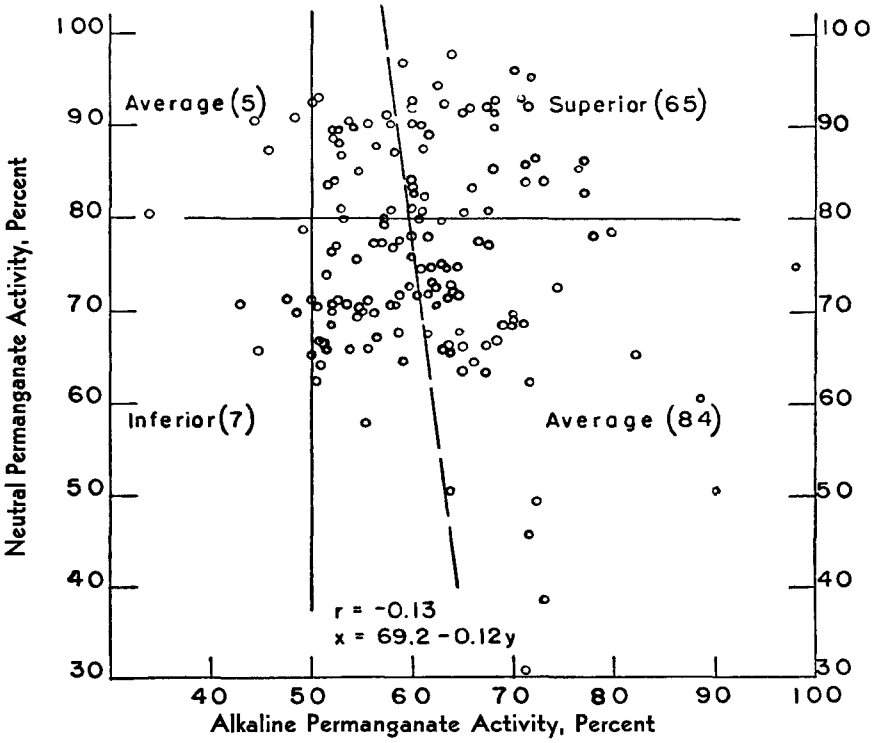

FIG. 1.—Scatter Diagram—Alkaline and Neutral Permanganate Activities of Water-Insoluble Nitrogen in Mixed Fertilizers.

Most of the samples showed some increase in nitrification between the 3- and 15-week incubation periods, but the mean nitrification increased only from 32.1 to 39.1 per cent in this interval. Thus, on the average, 82 per cent of the insoluble nitrogen which ultimately was converted to nitrate form, became available during the first 3 weeks.

The mean percentage nitrification of the nitrogen in the washed residues was less than 50 per cent of that observed for ammonium sulfate, at all incubation periods. Similarly, the mean nitrification for the low group was less than half that for the high group. Nitrification of a few samples in this group amounted to five-eighths to seven-eighths that of ammonium sulfate.

TABLE 1.—*Guaranteed nitrogen content of mixed fertilizers, and permanganate activities and nitrification characteristics of water-insoluble nitrogen*

SAMPLE NUMBER	GUARANTEED NITROGEN CONTENT		PERMANGANATE ACTIVITY[1]		WATER-INSOLUBLE NITROGEN CONVERTED TO NITRATE IN—			CLASSIFICATION OF WATER-INSOLUBLE NITROGEN BASED ON—	
	TOTAL	WATER-INSOLUBLE	ALKALINE	NEUTRAL	3 WEEKS	6 WEEKS	15 WEEKS	PERMANGANATE ACTIVITIES[2]	NITRIFICATION CHARACTERISTICS[3]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
					Florida				
1	12	0	—	—	8.5	4.3	10.9	—	L
2	12	0	5.0	98.1	47.6	53.7	70.8	A	H
3	12	0	—	—	4.9	4.2	7.7	—	L
4	8	1.50	66.2	64.2	34.3	37.1	42.3	A	I
5	8	1.40	47.7	71.4	21.2	18.5	24.2	P	L
6	5	1.71	65.0	80.8	45.2	50.0	48.0	S	H
7	5	1.60	60.6	80.0	30.8	36.8	39.0	S	I
8	5	1.46	61.0	82.5	46.2	47.9	45.3	S	H
9	5	1.35	34.0	81.0	61.0	59.8	67.5	A	H
10	5	1.35	65.5	92.3	46.2	53.5	50.8	S	H
11	5	1.30	68.8	68.8	33.0	29.8	39.2	A	I
12	5	1.30	64.6	68.1	30.9	27.9	36.5	A	I
13	5	1.30	98.0	75.0	29.5	33.7	31.5	A	I
14	5	1.30	51.9	71.3	24.1	28.3	27.0	A	L
15	5	1.25	45.0	65.5	44.7	45.7	48.3	P	H
16	5	1.20	43.0	70.8	24.8	20.5	28.5	P	L
17	5	.90	51.8	68.6	30.1	30.2	36.1	A	I
18	4	3.00	69.9	96.4	55.6	58.2	62.0	S	H
19	4	1.80	76.9	83.2	56.5	61.9	64.4	S	H
20	4	1.80	68.0	90.0	48.2	53.8	42.1	S	H
21	4	1.50	58.1	77.0	36.6	40.1	39.1	A	I
22	4	1.50	52.0	76.5	23.9	20.1	24.0	A	L
23	4	1.40	56.0	77.5	48.7	36.1	58.3	A	H
24	4	1.25	54.6	85.3	59.6	67.7	68.8	S	H
25	4	1.10	54.0	89.9	42.6	41.8	40.3	S	H
26	4	1.08	74.3	73.0	27.2	28.2	31.2	A	I
27	4	1.00	62.7	75.3	50.9	51.9	55.6	A	H
28	4	.90	71.4	46.0	9.1	8.0	10.3	A	L
29	4	.90	50.5	66.0	33.8	35.8	35.0	A	I
30	4	.90	63.5	50.4	9.4	5.8	11.1	A	L
31	4	.54	62.2	70.8	60.0	57.3	51.2	A	H
32	4	.54	70.0	70.0	58.7	54.8	44.6	A	H
33	4	.54	90.0	50.5	59.9	55.8	49.4	A	H
34	4	.54	88.6	60.7	58.1	57.4	59.6	S	H
35	4	.72	58.7	68.0	35.2	40.1	55.5	A	I

TABLE 1.—*Continued*

SAMPLE NUMBER	GUARANTEED NITROGEN CONTENT		PERMANGANATE ACTIVITY[1]		WATER-INSOLUBLE NITROGEN CONVERTED TO NITRATE IN—			CLASSIFICATION OF WATER-INSOLUBLE NITROGEN BASED ON—	
	TOTAL	WATER-INSOLUBLE	ALKALINE	NEUTRAL	3 WEEKS	6 WEEKS	15 WEEKS	PERMANGANATE ACTIVITIES[2]	NITRIFICATION CHARACTERISTICS[3]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
					Florida—Continued				
36	4	.75	51.8	70.0	22.2	22.6	36.2	A	L
37	4	.72	59.8	73.0	40.6	55.0	52.1	A	H
38	4	.54	44.6	91.0	35.6	40.0	45.5	A	I
39	4	.96	52.5	71.4	24.1	21.7	26.0	A	L
40	4	.75	48.4	70.3	45.2	38.9	49.7	P	H
41	4	.72	63.8	72.4	31.1	28.3	37.9	A	I
42	4	.74	71.3	31.0	31.8	36.1	47.6	P	I
43	4	1.65	—	—	−9.1	−6.5	7.5	—	L
44	3	.60	82.0	65.5	28.5	41.5	48.5	S	H
45	3	2.90	—	—	53.0	51.9	49.1	—	H
46	3	.21	—	—	−31.0	27.3	36.3	—	I
Mean	4.89	1.10	59.2	74.3	36.3	37.2	41.1	—	—
					Georgia				
47	3	.96	62.7	79.8	42.1	39.0	47.6	S	H
48	3	.84	56.0	72.0	32.0	40.1	54.3	A	I
49	3	.81	52.1	84.3	45.9	40.1	54.2	S	H
50	3	.72	72.8	38.4	32.7	34.7	47.1	A	I
51	3	.72	72.2	48.5	26.5	32.5	42.1	A	I
52	3	.72	63.3	71.4	27.9	30.9	36.9	A	I
53	3	1.20	64.4	72.0	40.9	40.7	55.6	A	H
54	3	1.20	58.8	64.7	49.6	41.9	59.7	A	H
55	3	1.20	57.7	70.9	44.1	44.0	60.9	A	H
56	3	.96	63.3	73.1	31.9	35.6	39.2	A	I
57	3	.96	61.6	74.8	43.6	44.2	39.5	A	H
58	3	.96	57.1	77.6	39.3	48.0	34.0	A	H
59	3	.96	59.9	83.2	29.7	32.9	38.4	S	I
60	3	.96	62.7	92.7	33.0	40.7	49.5	S	I
61	3	.96	59.9	75.9	35.4	34.8	29.1	A	I
62	3	.90	63.3	74.8	39.8	43.5	55.2	A	H
63	3	.90	58.8	77.6	35.4	44.0	49.7	A	H
64	3	.90	64.4	74.8	38.0	43.3	60.2	A	H
65	3	.90	66.6	77.6	36.8	40.8	33.8	A	I

TABLE 1.—*Continued*

SAMPLE NUMBER	GUARANTEED NITROGEN CONTENT		PERMANGANATE ACTIVITY[1]		WATER-INSOLUBLE NITROGEN CONVERTED TO NITRATE IN—			CLASSIFICATION OF WATER-INSOLUBLE NITROGEN BASED ON—	
	TOTAL	WATER-INSOLUBLE	ALKALINE	NEUTRAL	3 WEEKS	6 WEEKS	15 WEEKS	PERMANGANATE ACTIVITIES[2]	NITRIFICATION CHARACTERISTICS[3]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
					Georgia—Continued				
66	3	.90	61.6	73.1	29.9	37.2	53.9	A	I
67	3	.90	65.0	63.6	35.8	37.8	45.1	A	I
68	3	.90	65.0	66.4	36.0	34.0	50.4	A	I
69	3	.90	68.3	67.0	36.8	42.9	54.1	A	H
70	3	.90	61.6	78.2	42.5	50.3	51.7	A	H
71	3	.90	49.3	79.3	36.5	35.7	45.0	P	I
72	3	.90	70.0	69.2	43.8	40.1	61.5	A	H
73	3	.90	67.2	63.6	35.8	39.8	62.8	A	I
74	3	.75	61.0	81.0	40.6	42.5	41.7	S	H
75	3	.75	71.7	62.5	36.5	36.7	43.5	A	I
76	3	.75	63.8	65.8	32.7	27.5	34.1	A	I
77	3	.72	67.2	66.4	37.2	38.0	48.2	A	I
78	3	.72	61.6	72.0	43.1	42.2	52.4	A	H
Mean	3.00	.89	62.8	71.6	37.2	39.3	47.9	—	—
					North Carolina				
79	6	.30	58.5	71.6	15.4	20.5	20.3	A	L
80	6	.30	67.8	81.0	27.4	38.1	38.1	S	I
81	6	.30	76.2	85.4	41.3	40.9	50.0	S	H
82	5	1.00	71.7	95.5	35.0	39.2	54.9	S	I
83	5	.25	70.6	84.0	33.2	33.6	45.0	S	I
84	5	.25	61.9	75.4	19.4	19.7	31.2	A	L
85	4	.20	77.8	78.2	38.4	47.5	54.9	A	H
86	4	.20	79.5	78.7	48.2	47.0	68.5	A	H
87	4	.20	59.9	81.4	17.9	19.0	26.8	S	L
88	4	0	51.5	74.1	13.0	17.2	20.6	A	L
89	4	0	58.0	87.4	42.5	43.5	58.2	S	H
90	4	1.20	71.1	92.4	32.6	31.0	38.1	S	I
91	4	.20	72.8	84.3	46.4	53.2	58.3	S	H
92	4	.20	71.1	86.0	37.0	43.3	47.1	S	H
93	4	.20	72.2	86.8	41.7	53.5	48.9	S	H
94	4	1.00	70.6	93.3	29.0	27.5	41.3	S	I
95	4	1.00	60.8	90.2	24.5	27.5	36.0	S	I

TABLE 1.—*Continued*

SAMPLE NUMBER	GUARANTEED NITROGEN CONTENT		PERMANGANATE ACTIVITY[1]		WATER-INSOLUBLE NITROGEN CONVERTED TO NITRATE IN—			CLASSIFICATION OF WATER-INSOLUBLE NITROGEN BASED ON—	
	TOTAL	WATER-INSOLUBLE	ALKALINE	NEUTRAL	3 WEEKS	6 WEEKS	15 WEEKS	PERMANGANATE ACTIVITIES[2]	NITRIFICATION CHARACTERISTICS[3]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
					North Carolina—Continued				
96	4	1.00	65.8	83.5	23.2	18.2	20.1	S	L
97	4	1.00	76.7	86.6	24.6	25.5	29.7	S	L
98	4	.40	67.8	85.4	26.2	27.0	24.2	S	I
99	3	.15	67.5	77.4	21.3	20.5	22.2	A	L
100	3	0	60.5	73.4	22.7	26.5	36.1	A	L
101	3	.15	66.9	72.8	32.3	33.4	29.7	A	I
102	3	1.05	65.0	91.9	48.6	63.3	60.0	S	H
103	3	1.05	71.1	84.3	36.5	36.9	43.0	S	I
104	3	1.05	67.2	92.4	47.7	43.5	74.4	S	H
105	3	.90	—	—	39.4	42.8	54.6	—	H
106	3	.90	51.5	83.8	13.2	17.9	24.2	S	L
107	3	.90	68.0	93.0	38.2	35.9	43.6	S	I
108	3	.75	59.9	90.5	23.2	28.5	28.8	S	L
109	3	.90	59.6	84.3	21.1	23.7	27.5	S	L
110	3	.90	58.0	90.5	27.9	32.0	34.9	S	I
111	3	.90	68.0	91.9	29.6	25.6	23.2	S	L
112	3	.75	52.9	81.2	14.1	16.1	24.0	S	L
113	3	.75	57.7	81.2	19.0	22.0	37.4	S	L
114	2	.60	59.9	78.4	37.3	44.0	39.5	A	I
Mean	3.78	.58	65.6	84.2	30.2	32.5	39.3	—	—
					South Carolina				
115	6	1.50	60.0	92.3	16.9	17.6	20.8	S	L
116	6	1.50	63.4	95.9	19.9	19.3	23.9	S	L
117	6	1.50	53.5	90.8	57.1	59.0	65.1	S	H
118	5	.50	53.3	80.0	34.9	36.7	50.4	S	I
119	5	.50	62.2	94.6	48.2	48.7	49.5	S	H
120	5	.25	58.8	97.1	29.6	33.2	38.6	S	I
121	4	.40	57.1	79.6	48.6	59.1	66.4	S	H
122	4	1.60	—	—	37.9	48.9	53.1	—	H
123	3	1.20	52.3	88.4	48.5	53.4	56.0	S	H
124	3	1.05	61.0	87.8	22.4	18.4	27.9	S	L
125	3	.45	57.1	80.0	25.4	25.5	24.9	S	L

TABLE 1.—*Continued*

SAMPLE NUMBER	GUARANTEED NITROGEN CONTENT		PERMANGANATE ACTIVITY[1]		WATER-INSOLUBLE NITROGEN CONVERTED TO NITRATE IN—			CLASSIFICATION OF WATER-INSOLUBLE NITROGEN BASED ON—	
	TOTAL	WATER-INSOLUBLE	ALKALINE	NEUTRAL	3 WEEKS	6 WEEKS	15 WEEKS	PERMANGANATE ACTIVITIES[2]	NITRIFICATION CHARACTERISTICS[3]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
					South Carolina—Continued				
126	3	1.20	61.5	89.3	34.6	38.6	62.5	S	I
127	3	1.20	57.3	91.5	47.3	46.9	55.7	S	H
128	3	1.05	50.0	92.9	19.8	32.3	38.6	S	I
129	3	1.05	55.3	90.4	34.9	35.7	42.1	S	I
130	3	1.05	52.1	89.6	30.5	31.6	32.4	S	I
131	3	1.05	52.6	89.5	47.2	48.6	51.7	S	H
132	3	.90	52.9	87.1	41.3	39.3	38.0	S	I
133	3	.90	52.2	87.0	40.1	40.9	38.3	S	I
134	3	.90	52.9	88.2	62.2	66.4	49.5	S	H
135	3	.90	50.6	93.3	40.5	34.1	47.7	S	H
136	3	.90	48.2	91.2	51.8	50.9	46.8	A	H
137	3	.75	59.8	83.7	25.0	30.6	43.4	S	I
138	3	.75	59.2	93.0	47.1	47.1	52.9	S	H
139	3	.75	45.8	87.8	46.0	49.7	56.9	A	H
Mean	3.68	.95	55.4	89.2	38.3	40.5	45.4	—	—
					Virginia				
140	5	1.00	71.1	68.9	16.9	16.2	18.2	A	L
141	4	1.00	53.4	70.9	13.0	15.8	19.4	A	L
142	4	1.00	57.0	71.0	20.1	19.3	18.6	A	L
143	4	1.00	54.5	69.7	15.5	17.6	22.7	A	L
144	4	1.00	63.1	66.0	17.1	15.6	19.3	A	L
145	4	.80	61.8	57.9	18.7	19.9	17.9	A	L
146	3	.90	54.4	75.6	19.8	24.4	24.4	A	L
147	3	.60	56.4	67.3	4.8	7.7	13.5	A	L
148	3	.60	55.4	71.4	5.0	9.2	14.3	A	L
149	3	.60	50.0	71.4	3.8	6.5	9.2	A	L
150	3	.60	53.6	66.1	6.8	10.7	14.4	A	L
151	3	.60	55.4	66.1	6.2	11.2	12.3	A	L
152	3	.60	55.4	57.1	6.3	15.2	13.5	A	L
153	3	.60	50.8	64.4	6.9	13.1	12.9	A	L
154	3	.60	51.7	66.7	11.0	14.8	16.3	A	L
155	3	1.05	56.3	88.3	39.4	44.8	38.0	S	H

TABLE 1.—*Continued*

SAMPLE NUMBER	GUARANTEED NITROGEN CONTENT		PERMANGANATE ACTIVITY[1]		WATER-INSOLUBLE NITROGEN CONVERTED TO NITRATE IN—			CLASSIFICATION OF WATER-INSOLUBLE NITROGEN BASED ON—	
	TOTAL	WATER-INSOLUBLE	ALKALINE	NEUTRAL	3 WEEKS	6 WEEKS	15 WEEKS	PERMANGANATE ACTIVITIES[2]	NITRIFICATION CHARACTERISTICS[3]
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
					Virginia—Continued				
156	3	1.05	52.4	77.1	27.0	26.1	16.4	A	L
157	3	.90	60.2	71.6	23.9	23.2	28.2	A	L
158	3	.75	59.2	64.5	18.7	17.4	17.4	A	L
159	3	.75	56.3	70.0	19.2	22.0	19.6	A	L
160	3	.75	50.6	67.1	24.0	20.1	27.8	A	L
161	3	.75	44.7	65.9	15.1	20.3	19.2	P	L
162	3	.75	55.0	70.0	29.6	28.1	31.6	A	I
163	3	.75	50.7	70.7	15.2	9.2	19.3	A	L
164	3	.75	54.9	70.4	16.2	27.9	27.2	A	L
165	3	.60	50.0	65.5	15.5	14.2	22.0	A	L
166	3	.60	50.8	62.3	18.2	19.8	32.9	A	L
167	3	.60	63.2	66.2	12.0	20.7	20.1	A	L
168	2	.50	70.0	68.0	30.0	30.3	32.9	A	I
Mean	3.21	.76	55.8	68.6	16.5	18.3	20.6	—	—
					Florida, Georgia, North Carolina, South Carolina, and Virginia				
Mean	3.82	.87	60.1	77.1	32.1	33.8	39.1	—	—
L.S.D. P=0.01	—	—	—	—	6.1	7.3	7.9	—	—
Ammonium sulfate	—	—	—	—	77.1	76.4	81.4	—	—

[1] Analyses by (a) the Chemical Division, Florida Agricultural Department, State of Florida, (b) the Georgia Department of Agriculture, (c) the North Carolina Department of Agriculture, (d) the Department of Fertilizer Inspection and Analysis, The Clemson Agricultural College, Clemson, S. C., and (e) Division of Chemistry, Department of Agriculture and Immigration of Virginia, respectively.
[2] S, superior; A, average; P, poor.
[3] H, high; I, intermediate; L, low.

The nitrifications observed in 3-, 6-, and 15-weeks were significantly correlated at odds of 99 to 1 for all samples, and also for the samples from each State considered separately, except in two cases where the correlations for the Georgia samples were significant at odds of 19 to 1. As shown in Table 2, samples which received superior, average, or poor ratings on the basis of the permanganate activities, were rather widely distributed among the three nitrification classes. On the average, only 27 to 46 per cent of the samples exhibited comparable permanganate and nitrification

TABLE 2.—*Distribution of samples among the permanganate and nitrification classifications*

PERMANGANATE CLASSIFICATION			NITRIFICATION CLASSIFICATION AND NUMBER OF SAMPLES IN EACH CLASS		
CLASS	PER CENT AND NUMBER OF SAMPLES IN EACH CLASS		HIGH	INTERMEDIATE	LOW
	per cent	*number*			
Superior	38.6	65	30	22	13
Average	53.0	89	24	29	36
Poor	4.2	7	2	2	3
Unclassified	4.2	7	3	1	3
Total, number of samples	—	168	59	54	55
Total, per cent	100.0	—	35.1	32.1	32.8

TABLE 3.—*Correlation between permanganate activities and nitrification characteristics*

FACTOR AND STATE	NUMBER OF SAMPLES	CORRELATION REQUIRED FOR SIGNIFICANCE AT		CORRELATION OBSERVED BETWEEN THE PERMANGANATE ACTIVITY FACTOR INDICATED IN COLUMN 1 AND			
				ALKALINE PERMANGANATE ACTIVITY	PERCENTAGE NITRIFICATION IN		
		P=0.05	P=0.01		3 WEEKS	6 WEEKS	15 WEEKS
Alkaline permanganate activity							
Florida	41	0.31	0.40	—	0.09	0.01	−0.15
Georgia	32	.35	.45	—	−.28	−.24	.02
North Carolina	35	.33	.43	—	.66†	.54†	.52†
South Carolina	24	.40	.52	—	−.40*	.41*	.67†
Virginia	29	.37	.47	—	.25	.23	.17
Total	161	.16	.20	—	.14	.11	.10
Neutral permanganate activity							
Florida	41	.31	.40	−.32*	.39*	.37*	.53†
Georgia	32	.35	.45	−.65†	.23	.36*	− .26
North Carolina	35	.33	.43	.24	.39*	.31	.35*
South Carolina	24	.40	.52	.16	−.05	−.12	.15
Virginia	29	.37	.47	−.05	.53†	.54†	.37*
Total	161	.16	.20	−.13	.33†	.33†	.32†

* Significant at odds of 19 to 1 (P =0.05).
† Significant at odds of 99 to 1 (P =0.01).

ratings. The superior group was best in this respect and the average group was poorest.

The coefficients of correlation between the alkaline and neutral per-

manganate activities, and between those activities and the nitrification observed at the three incubation periods, are given in Table 3 for the samples from each State separately and for all samples.

Alkaline permanganate activity was significantly correlated at odds of 99 to 1 with nitrification behavior for the North Carolina samples at each incubation period, and for the South Carolina samples at the 15-week incubation period. The correlation was significant at odds of 19 to 1, for the South Carolina samples, at the other two incubation periods. No significant correlation was observed for the Florida, Georgia, or Virginia samples, or for the samples for all States.

The correlation between neutral permanganate activity and nitrification was found to be significant at odds of 99 to 1 only for the 3- and 6-week incubation periods for the Virginia samples, and for the 15-week incubation period for the Florida samples. When all samples were considered as a whole, however, the correlation was significant at each incubation period.

SUMMARY

The nitrification characteristics (at 30°C.) of the water-insoluble nitrogen in 161 official samples of mixed fertilizers marketed in Florida, Georgia, North Carolina, South Carolina, and Virginia between July 1, 1945, and December 31, 1946, were determined at 3-, 6-, and 15-week incubation periods; and compared with the alkaline and neutral permanganate activities of the water-insoluble nitrogen as reported by the respective State laboratories. Seven samples for which the permanganate activities were not available were included in the nitrification studies.

Less than 50 per cent of the samples which met the requirements of the official permanganate methods for classification as satisfactory sources of water-insoluble nitrogen, also exhibited nitrification characteristics placing them among the better sources. At the same time, slightly more than 25 per cent of the samples which would be classed as inferior sources by the permanganate tests, exhibited nitrification characteristics placing them among the better sources.

The lack of satisfactory correlation between alkaline and neutral permanganate activities and observed nitrification values, indicates that the official methods of analysis are inadequate for proper characterization of the insoluble nitrogen content of many organic materials.

LITERATURE CITED

(1) *Methods of Analysis, A.O.A.C.*, 1945.
(2) HARPER, H. J., *Ind. Eng. Chem.*, **16**, 180 (1924).
(3) HASKINS, H. D., and others, *Mass. Agr. Expt. Sta. Control Series Bull.* **41**, 32 (1927); *Ibid.*, **45**, 38 (1928); *Ibid.*, **51**, 52 (1929); *Ibid.*, **54**, 46 (1930).
(4) RUBINS, E. J., and BEAR, F. E., *Soil Sci.*, **54**, 411 (1942).

THE CRITICAL TEMPERATURE OF DISSOLUTION AS A RAPID TEST TO DISTINGUISH OLEOMARGARINE FROM BUTTER

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INTRODUCTION

Progress of legislation through Congress aimed to repeal the tax on oleomargarine made it evident that should a new law be enacted requiring disclosure of the fact that oleomargarine was being served in public eating places, it would create new and difficult problems for officials engaged in the enforcement of the Federal Food, Drug, and Cosmetic Act. It is estimated that there are well over 500,000 such public eating places. A great number of them will continue to serve butter, and it may be reasonably expected that those which serve oleomargarine will conform, in large measure at least, to the requirements of the law as finally enacted. Nevertheless, effective enforcement will require complete and fairly frequent coverage. To confirm compliance, or to uncover violation, will demand efficiency in control operations; and this is best accomplished by an inspection of the premises of *each individual establishment* followed immediately by an analysis of the "spread" being used. For an analysis concurrent with collection, it is obviously desirable to have a single rapid method of examination. The one chosen must be accurate and reliable in order to obtain results of evidential worth. To fulfill practical needs it must be rapid, simple, easy of manipulation, and readily interpreted.

Identification of fats and distinguishing one fat from another is often difficult; in the absence of a specific test it may be impossible. Butter fat is fortunately unlike all other fats in the proportions of the constituent fatty acid glycerides; notably it is high in the soluble volatile acid glycerides. The Reichert-Meissl number, a measure of those acids, serves positively to identify butter fat and clearly differentiate it from all other fats which might conceivably be employed in the manufacture of oleomargarine.

Conceivably, a physical measurement would be the simplest means to meet the needs. In the earlier literature it is generally stated that the refractive index is a reliable means of distinguishing oleomargarine from butter. However, in a study of present day oleomargarines, we found occasional samples that gave a reading below the value generally accepted as the maximum for butter. Numerous samples of oleomargarine were found to be at, or to lie close to, the maximum for butter. Accordingly, the refractive index is not reliable for a rapid routine testing of "spreads."

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It has long been known that oleomargarine and butter may be readily distinguished under the polarizing microscope. The fat in butter is non-crystalline while that of oleomargarine is crystalline. These differences result from the processes of manufacture, which are radically different. While most present day oleomargarines were readily recognized by this instrument, there were some that were not markedly crystalline. Possibly these exceptions were more completely emulsified by the emulsifiers now being used. With this test, process butter has even more similarity to oleomargarine than the above exceptional margarines have to butter. It was therefore decided that, although the test has real merit, it was not the method of choice if a more completely diagnostic one could be found.

HISTORICAL

It was suggested that the "critical temperature of dissolution" test (in use as an A.O.A.C. method since 1922[1] for the detection of adulteration of cacao products with foreign fat) might find application in the problem under study. The principles of this test were first enunciated by Valenta,[2] and it is often referred to as the Valenta test. Crismer[3] in 1895 proposed a procedure substituting alcohol for the acetic acid reagent of Valenta. The temperature at which turbidity reappears on cooling, after heating the oil with the solvent until the mixture clears, he designated as the "critical temperature of dissolution," abbreviated hereafter as CTD. Several months later[4] he published a complete account of his investigations—the most extensive study found in the literature on this subject.

In our preliminary application of the CTD test, glacial acetic acid was the solvent used. However, on repeating the test on a single sample, successive readings became progressively lower. From the reports by others (notably Jones[5] and Fryer and Weston[6]) this apparently is due, in large measure, to the influence of moisture on the location of the turbidity point: glacial acetic acid, of course, readily takes up moisture on exposure. The variable atmospheric conditions, which any test would necessarily be subjected to in field operations, obviously make it impractical to employ acetic acid. Fryer and Weston[6] proposed the use of a mixture of ethyl and iso-amyl alcohols. This reagent was found not to be subject to the influence of moisture changes.

DEVELOPMENT OF METHOD

In the method as finally developed, the reagent consists of two parts of ethyl, to one part of iso-amyl, alcohol. It was found that certain pre-

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[1] *This Journal*, **6**, 278 (1923).
[2] *J. Soc. Chem. Ind.* (London) **3**, 643 (1894).
[3] *Bull. l'assoc. belge des chim.*, **IX**, 71 (1895).
[4] *Ibid.*, **IX**, 143 (1895).
[5] *Analyst*, **19**, 151 (1894).
[6] *Ibid.*, **43**, 502 (1918).

cautions must be observed to insure concordant results. The alcohols should be of a specific strength, controlled by a specific gravity determination of the ethyl alcohol, and by a redistillation of the iso-amyl alcohol within a narrow boiling-point range. Further, the respective volumes used to make up the 2+1 mixture must be measured by volumetric apparatus, and *not* in graduated cylinders.

In the measuring of the volume of sample and of the reagent, rapidity can be achieved without sacrifice of accuracy. Crismer[4] showed that,

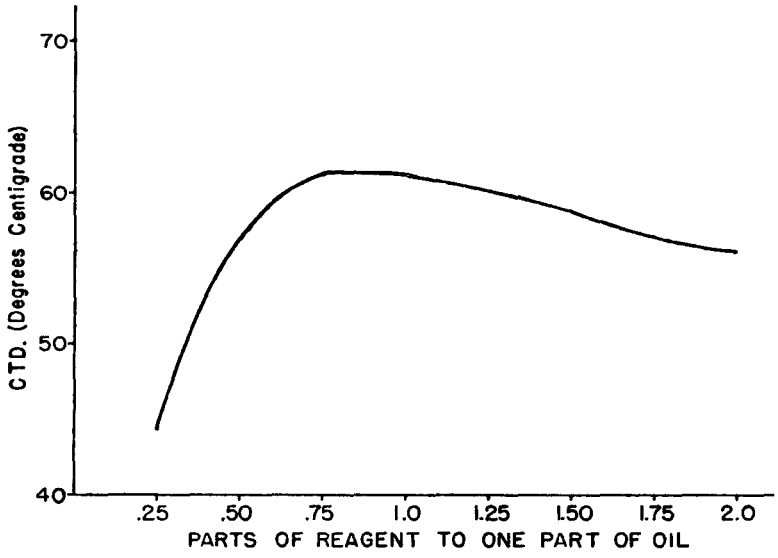

FIG. 1

beginning with high ratios of alcohol to oil, a minimum CTD was obtained increasing in magnitude as the ratio approached unity. The maximum value was reached at unity. As the ratio was progressively decreased below unity the CTD declined correspondingly. Our experiments with the mixed alcohols used in our method confirmed the observations of Crismer. Fig. 1 is typical of our results along these lines.

It is obvious from the graph that a high degree of precision in measuring the volumes of fat and alcohol reagent is not necessary. Accordingly, the method devised provides for a quick and easy measuring of volume of samples and reagent by means of calibrations on the test-tube in which the test is run.

To demonstrate the constancy of results in tubes, thus approximately calibrated, oil was added to the mark in four different tubes, reagent added,

and CTD determined. As an indication of oil variability, the four roughly measured volumes of oil were also weighed. The variation in weight between any two of the four oil volumes far exceeds the average variation of 0.0033 g found in a series of deliveries of oil by a volumetric pipette. The latter weighed, on the average, 4.4077 g. There is thus no significant influence on the CTD when the oil and reagent volumes are measured in a test tube. The results are given in Table 1.

TABLE 1.—*CTD in four different tubes*

TUBE	WEIGHT OF OIL	CTD
A	1.8665	61.2°
B	1.8900	61.3
C	1.8180	61.3
D	1.9460	61.1

METHOD

APPARATUS

(1) Pyrex test tubes (18×150 mm) calibrated by adding water from a burette at marks of 2 ml and 4 ml.
(2) Micro burner.
(3) Glass tube of about 2 to 3 ml capacity, drawn to fast-flowing tip.
(4) Thermometer covering range of 0°–100°C., graduated in degrees.

REAGENTS

Alcohol reagent.—Mix 2 vol. 95% (by vol.) ethyl alcohol (checked by sp. gr.) with 1 vol. redistilled (B.P. 128°–132°) iso-amyl alcohol, both measured with volumetric pipette or flask. Keep well stoppered.

PROCEDURE

Prepare oil from butter or oleomargarine as directed in Sec. **22.116**, *Methods of Analysis, A.O.A.C.*, 6th Ed. Oil must be clear. Fill test tube to 2 ml mark with oil, using the glass tube. Immediately add alcohol reagent to the 4 ml mark (or add 2 ml with pipette). Using thermometer as stirring rod, mix the two layers and heat in flame of micro burner. Continue stirring and heating until the mixture becomes clear and homogeneous. *Do not boil.* Remove from heat, continuing stirring until a definite turbidity appears in *the mixture proper*. Record temperature at first discernible turbidity. (Opalescence will immediately follow thruout the entire mixture with a further drop in temperature.)

ANALYSIS OF SAMPLES

Preliminary analysis of several butters and oleomargarines indicated that the method promised to be adequate for clear-cut differentiation. Five creamery, and three process, butters fell in the range of 45–48°; and ten oleomargarines fell between 69–73.5°. To test the reproducibility of the method in the hands of different analysts, suitable amounts of oil from a single butter, and also from a single oleomargarine, were analyzed

by 27 analysts, 2 located in Washington and 25 in the District laboratories of the Food and Drug Administration. The results are given in Table 2.

TABLE 2.—*CTD by 25 analysts on same samples of oil*

BUTTEROIL	OLEOMARGARINE OIL	DIFFERENCE
°C.	°C.	°C.
48.0	73.5	25.5
48.0	72.5	24.5
48.5	73.0	24.5
48.5	72.5	24.0
47.8	72.4	24.6
47.8	72.4	24.6
46.5	71.5	25.0
45.0	69.0	24.0
45.0	70.0	25.0
46.0	72.0	26.0
47.0	70.0	23.0
47.0	71.0	24.0
47.5	76.0	22.5
47.0	73.0	26.0
47.0	73.0	26.0
47.5	72.5	25.0
47.0	72.0	25.0
47.0	73.0	26.0
47.0	73.0	25.0
46.5	70.5	24.0
47.0	71.5	24.5
46.0	71.0	25.0
45.2	69.2	24.4
45.0	70.9	25.9
46.0	72.0	26.0
48.0	72.5	24.5
47.0	70.0	23.0

There is an over-all variation of only about 4 degrees in the results of all analysts on the same oil. However, each analyst obtained a substantial difference between the two oils. The range was from 22.5° to 26.5°. The duplicability between analysts on the same oil is all that could be expected with an empirical method such as this. The important consideration is the magnitude of the spread between margarine and butter, found by each individual analyst, and the narrow range in which the over-all spreads of all the analysts fall.

To establish a basis of interpretation of results in unknown samples, each District laboratory was requested to collect and analyze samples of butter manufactured in its general locality representing all grades, best, average, and poor. Similar instructions were issued for samples of oleomargarine from each manufacturer within the territory of the District's jurisdiction. Thus all oleomargarines on the market in July 1949 were

analyzed. The results on the individual butters are reported in Table 3. Table 4 shows the number of oleomargarines grouped at each CTD reading obtained.

TABLE 3.—*CTD of market butters**

AREA OF PRODUCTION	CTD °C.											MAX.	MIN.
Georgia and the Carolinas[1]	50	49	48	50	49	53	48	49	49	49		53	48
Maryland and Virginia	48	48	49	49	48	48	46	48	47	51		51	46
Vermont	48	49	50	50	45	49	49	49				50	45
Western New York, Penn., and Ohio	47	47	47	46	47	46	45	47	47	47	48	48	45
Illinois	50	48	50	48	48	48	48	48	49	48		50	48
Ohio and Tennessee	48	47	48	48	47	48	48	48	48	48	47	48	47
Colorado	47	47	47	46	47	48	46	46	49	47		49	46
Missouri	48	50	48	48	47	47	48	48	48	47		50	47
Southern California	46	47	45	42	47	47	48	48	46	46		48	42
N. and S. Dakota, Minn., Wis., and Iowa	50	51	49	53	51	53	48	50	49	50		53	48
Miss., Alabama, Louisiana, and Texas	52	48	48	47	49	51	48	49	53			53	47
Iowa, Michigan, Minnesota and Nebraska	50	46	47	49	50	47	48	47	45	48		50	45
Pennsylvania	51	52	52	50	52	49	51					52	49
Tenn., Kentucky, Missouri, Ark. and Ill.	48	47	50	48	48	48	49	49	49	49		50	47
California, N. Dakota, and Utah	48	47	46	48	49	48	48	47	50	47	48	50	46
Washington, Idaho, and Montana	45	44	44	45	44	45	44	46	45	45		46	44

* Reported readings rounded off to nearest whole degree.
[1] One sample of process butter gave a CTD of 50°.

TABLE 4.—*CTD of market oleomargarines*

CTD	NO. OF SAMPLES
°C.	
66	1
67	1
68	3
69	10
70	8
71	22
72	18
73	16
74	5
75	1

COMMENT

The results by this method, given in Tables 3 and 4, show that the method clearly distinguishes margarine from butter. The over-all CTD range for all butters was 42 to 53°. The individual CTD's showed no correlation whatever with locality of production, or with quality. The oleomargarines ran between 66° and 75°. There was no correlation between the individual CTD results and the type of oils or fats from which the margarine was made. The method has been designed for maximum simplicity and rapidity, consistent with practical requirements. Greater precision no doubt could be attained through close control of the purity of the iso-amyl alcohol, and by regulating the cooling to retard the rate. However, further refinements in the method do not appear to be needed at this time.

ACKNOWLEDGMENT

The suggestion to apply the critical temperature of dissolution was made by P. A. Clifford of the Division of Food. Appreciation is extended to those in the Food and Drug Administration who collected and analyzed the large number of market samples.

BOOK REVIEWS

Plant and Soil. Vol. 1, No. 1. January 1948. International Journal of Plant Nutrition, Plant Chemistry, Soil Microbiology and Soil-borne Plant Diseases. 119 pages. Martinus Nijhoff, Publisher, The Hague. Executive Editors: D. I. Arnon, Berkeley; J. Baeyens, Louvain; F. C. Gerretsen, Gronigen; E. G. Mulder, Groningen; A. C. Schuffelen, Wageningen.

The objectives in publishing this new Journal are well described in the Foreword: "At the present time the publishing facilities for research workers in the field of Agricultural Science are very limited; consequently the existing Journals are overcrowded and often one has to wait many months or even more than a year before papers, even of universal importance, can be published.

Moreover, in the smaller countries many scientists in this field are forced to publish the results of their investigations in national Journals of heterogenous contents and in their native language. As a consequence valuable work often remains unknown to scientists abroad. The increasing demand for intensive crop production all over the world, however, necessitates a quick exchange of the results of scientific agricultural research."

"Plant and Soil will contain original contributions, as well as letters to the editors, in English, French, or German. Four parts will be published annually. Each volume comprising about 400 pages. Annual subscription price Guilders 20."

No. 1 contains:

1. Determination of Biotin in Beet Molasses with *Neurospora crassa* Shear and Dodge as a Test Organism. By Elias Melin and Birgitta Norkrans. In English.
2. Utilisation des Engrais Phosphates par les Plantes, Apres leur Absorption dans le Sol. By G. Barbier, J. Chabannes et A. Marquis. In French.
3. Neuere Beobachtungen uber die Ursachen der Dorrfleckenkrankheit beim Hafer. By L. Gisiger und A. Hasler. In German.
4. The influence of Microorganisms on the Phosphate Intake by the Plant. By F. C. Gerretsen. In English.
5. Reaction Changes in the Life-Medium of Plants Caused by Differential Intake of Ions. By T. Menachem-Starkmann. In English.
6. Importance of Molybdenum in the Nitrogen Metabolism of Microorganisms and Higher Plants. By E. G. Mulder. In English.

This Journal is printed in large type on paper of good quality. It should prove interesting and valuable to Agricultural Scientists.

W. O. ROBINSON

Irrigated Soils: Their Fertility and Management. By D. D. THORNE and H. B. PETERSON. The Blakiston Company, Philadelphia, Pa. 1949. 288 pages. Price $5.00.

This book represents the work of two men familiar with irrigation agriculture and soils in Utah. It is neither a text book for beginners nor an advanced monograph for specialists. It is apparently intended as an intermediate text and a reference book for advisory and "action" groups. The fact that it is applicable primarily to the problems of farmers who irrigate extensively is implied by the title.

The discussion of the reclamation and management of saline and alkaline soils (alkali soils) is excellent. This reviewer is not prepared to discuss the material on irrigation practices. However, the emphasis throughout the book on the concept of the "optimum moisture level" will not be acceptable to some workers.

In view of the unavailability to most farmers of supplies of farm manure, the section devoted to this topic appears to be too long, particularly in contrast to the

discussion of green manure crops. A somewhat more adequate treatment of the relationship of organic matter to soil structure might be desirable.

This book should be found in the library of every soil scientist in the west. Its value as a text book for students of soil science is somewhat more difficult to determine. This reviewer is not prepared to discuss the requirements of teach rs of irrigation.

LANNES E. DAVIS

Outlines of Biochemistry, 3rd ed. ROSS A. GORTNER, JR., and WILLIS A. GORTNER, editors. xvi+1078 pages. John Wiley & Sons, Inc., 440 Fourth Ave., New York, N. Y. 1949. $7.50.

In contrast to that time when the regulatory chemist was primarily concerned with inorganic analyses there is now increasing emphasis on organic analysis of biochemical substances, and the trend of this emphasis is toward more specific methods. For example, in the analysis of foods for their vitamin content one does not determine vitamins as a group, but each one of interest must be measured individually; in tests for decomposition in foods individual compounds are much more desirable than analysis for a group of similar substances; *e.g.*, in a mixture of volatile acids butyric or propionic acid may be a far better criterion of decomposition than is acetic acid.

This third edition of the well known Gortner's "Outlines of Biochemistry" is not concerned primarily with methods of analysis; however, it does discuss, sometimes in detail, the fundamental principles of biochemistry in a manner which may give the analyst new ideas on possible approaches to a given analytical problem. In the words of the author of the first edition "There is a necessity for a study of the fundamental reactions underlying the broader field of biology, the primary object of which is to study and investigate the chemical and physicochemical reactions which take place in the normal biological organism, whether that organism be animal or plant." In this third edition the editors have used the same approach as in that of the original edition.

To illustrate how the subject matter of this text is divided into sections, the colloid chemistry of biological systems has 273 pages. Proteins (236 pages) are also discussed from many angles. Although the individual amino acid composition of proteins is an old problem, only in the last decade have really accurate methods become available. On the one hand these are microbiological in nature; on the other hand new and accurate chromatographic techniques have also solved that old problem.

With carbohydrates (246 pages), not only is their biochemical behavior discussed, but their structure and reactions are also included in some detail. The treatment of lipids is adequate; a chapter on "Essential Oils" is included here. "Plant Pigments" (38 pages) is a phase of biochemistry which is hardly mentioned in many texts. The section on "Biochemical Regulators" (133 pages) includes vitamins, hormones, and enzymes.

The material in this book appears to have been carefully edited, as the only errors noted were typographical in nature. Considerable historical background is included. As is usual, and probably inevitable, with books covering such a large field and written by a number of editors, much of the latest information is missing. All chemists whose work includes biochemistry will find this volume very useful.

W. I. PATTERSON

Organic Syntheses, Vol. 29. C. S. HAMILTON, Editor in Chief. vi+119 pages. John Wiley & Sons, Inc., 440 Fourth Ave., New York, N. Y. 1949. $2.50.

Volume 29 of this series of annual publications of satisfactory methods for the

preparation of organic chemicals lists 34 preparations and includes a cumulative subject index for Volumes 20 through 29. In each preparation the title is followed by detailed equations, and the names of the submitters and checkers. Then the detailed "Procedure," "Notes," a resume of "Methods of Preparation," and references are given.

Before each proposed procedure is accepted for publication it is checked in one other laboratory to assure that its details are adequate and that it works as described, especially as to the yield range. This is comparable in a limited way to the collaborative study of methods of analysis before they are accepted as official by the A.O.A.C.

W. I. PATTERSON

Nutritional Data, by HAROLD A. WOOSTER, JR., and FRED C. BLANCK, published by H. J. Heinz Company, Pittsburgh, Pa., 114 pages. Gratis.

For many years the research staff of H. J. Heinz Company has published a pamphlet called "Nutritional Charts" which was intended to provide information in a form that would be particularly useful for the physician and the medical student. This year the publication has appeared in a new form and with considerable additional material. The name has been changed to "Nutritional Data" and the format of the book, which was formerly a little unwieldy, has been redesigned so that it can readily be kept on a book shelf. There has been extensive revision of the subject matter and the order of presentation so that, in effect, the book is now a very satisfactory source of reference material relating to the subject of nutrition. About one-third of the book consists of tables of food composition, which have been revised and brought up to date. The remainder of the subject matter which covers practically the whole field of nutrition has been dealt with in such a manner that it is useful not only to the student, physician or dietitian, for whom it is primarily designed, but also for those who must deal directly with many aspects of practical nutrition. The chapter headings are:

Vitamins
The Essential Elements
Proteins and Amino Acids
The Availability of Nutrients
Signs and Symptoms of Malnutrition
The Metabolism and Action of Foods
Human Nutritive Requirements
Planning Diets for Good Nutrition
Tables of Food Composition and Nutritive Value
Nutritional Activities of H. J. Heinz Company
Suggestions for Further Reading

E. M. NELSON